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Chemical composition and toxicity of Moroccan *Rosmarinus officinalis* (Lamiaceae) essential oils against the potato tuber moth, *Phthorimaea operculella* (Zeller, 1873) Zeller (Lepidoptera, Gelechiidae)

K. Hannour[1*], A. Boughdad[2], A. Maataoui[3], A. Bouchelta[1]

[1]*Laboratory of Applied Entomology. Unit of Plant Protection and Environment. Department of Biology Faculty of Sciences. Moulay Ismail University. P.O. BOX 11201. 50000 Meknes. Morocco*

[2]*Department of Plant Protection and Environment. National School of Agriculture. P.O. BOX S/40. 50000 Meknes, Morocco*

[3]*Department of Agronomy. National School of Agriculture. P.O. BOX. S/40. 50000 Meknes, Morocco*

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karivision.hann@gmail.com
(K.. Hannour);
Phone: +212 671284600

Abstract

Control methods which are used to limit the losses caused by *Phthorimaea operculella* consist of treatments based on synthetic insecticides that are harmful as well for farmers, consumers and environment. To seek for alternative ways to limit the use of these insecticides, essential oils from the leaves of *Rosmarinus officinalis* collected in the Middle Atlas (RM1) and in the Loukkos (RM2) were tested in the laboratory as a fumigant on eggs, neonate larvae, pupae and adults of *P. operculella*. Five concentrations (0, 1, 2, 4, 8 µL/L of air) were applied. Essential oils were extracted by steam distillation using Clevenger distiller and the chemical composition was analysed by gas chromatography - mass spectrometry (GC / MS). The results revealed that the main components in the essential oil of RM1 are 1, 8-cineole (46.23%), camphor (17.29%), borneol (6.84%) and β-pinene (5.62%), although those of RM2 are camphor (21.33%), 1, 8-cineole (17%), α-pinene (9.19%) and β-pinene (8.58%). The toxicity of the essential oils of *R. officinalis* on adult longevity is amplified with the concentration and the exposure time. Concerning the hatchability of eggs and the survivorship of neonate larvae, the essential oil of rosemary from the Middle Atlas was more toxic than the Loukkos one. In addition, the neonate larvae have been more susceptible than the eggs; while in the pupae case, no mortality was recorded. According to the results presented in this work, essential oils tested could be exploited as alternative means against *P. operculella* during storage of potato tubers.

1. Introduction

Potato (*Solanum tuberosum*), is a product known by the general public for its undeniable agronomic interest. It constitutes one of the most important crops for human nutrition worldwide and is a healthy source of carbohydrates, high-quality protein, essential vitamins, minerals, and trace elements [1]. In Morocco, the potato occupies an area of 53.047 ha and produces 1.928.606 t per year [2]. Like the other crops, the potato is faced with different harmful biological agents, which the most redoubtable is the potato tuber moth, *Phthorimaea operculella* [3]. This moth is a widespread pest of potato throughout the tropics and subtropics zones of the world, where it causes damage to leaves, stems and tubers of the plant in the field. However, the heaviest infestations occur when potatoes are kept in stores [4, 5]. Symptoms are characterised by the presence of irregular galleries bored by the larvae, always presenting to their entry an accumulation of small blackish granules. On the same tuber, the number of galleries can be very important, and each containing no more than one larva. The larva galleries are separated between them and do not interpenetrate. Economically, the insect reduces the quality and quantity of potato yields in the pre and post-harvest stages and provides a penetration for the pathogenic microorganisms [6, 7]. The studies on the potential damages in storage indicate that a low population of *P. operculella* (60 larvae for 20 kg of potato) can infest 100% of tubers in 110 days [8]. The potato tuber moth attacks also the other Solanaceae such as tobacco, tomato and aubergine upon which damages are less important [9]. *P. operculella* performs a complete metamorphosis in four eco phases, *viz.* egg, larvae, pupa and adults. The complete life cycle ranges from 22 to 41 days; the insect develops from 2 to 12 generations per year according to nutritional and thermal conditions [10].

The control methods, used to limit the losses generated by the *P. operculella,* consist of some treatments based mainly on synthetic insecticides. In Morocco, Indoxacarb, Azinphos-methyl and Malathion are insecticides used for treatment of the potato against the potato tuber moth [11]. Despite their effectiveness, the synthetic pesticides are harmful to the humans and the environment [12]. The use of synthetic pesticides also promotes the reduction in populations of natural enemies [13] and the development of insect resistance to pesticides [14, 15]. Thus, it is necessary to seek for alternative products to fight against the potato tuber moth.
Current researches focus on the use of the medicinal and aromatic plants, which contain essential oils acting as phyto-insecticides. Due to their volatile nature, essential oils act as fumigants and can be used against the potato tuber moth during the storage [19, 20]. In Morocco, about 33.000 tons of medicinal and aromatic plants and their derivatives are produced each year [16]. The effectiveness of many essential oils and plant extracts to fight against pests and pathogens has been demonstrated by numerous researchers [e.g., 17, 18]. Current researches focus on the use of the medicinal and aromatic plants, which contain essential oils acting as phyto-insecticides. Due to their volatile nature, essential oils act as fumigants and can be used against the potato moth in the storage [19, 20].
The rosemary (*Rosmarinus officinalis*) (Lamiaceae), an evergreen shrub, is highly distributed in the Mediterranean region. In Morocco, wild rosemary is very common in the Rif, the Middle and the High Atlas. It has been used in pharmaceuticals and folk medicine [21, 22, 23] as anti-cancer [24], antifungals [25] and as insecticide [26]. The antibacterial activity of essential oils chemotype (1, 8-cineole) was also demonstrated by Fadili *et al* [27].
This study aimed to evaluate the toxicity of essential oils of *Rosmarinus officinalis* coming from two regions of Morocco (Middle Atlas and Loukkos) by fumigation against eggs, larvae, pupae and adults of the potato tuber moth, *Phthorimaea operculella*. The yield and the chemical composition of essential oils of this plant species were also determined.

2. **Experimental**

2.1. *Plant material*

Rosmarinus officinalis (Lamiaceae) was collected during March 2013 in the Middle Atlas (Tizi n Telghemt, 1907m above sea level, between Midelt and Imtghren, latitude: 32° 37 'N and longitude 4° 00' W) and in the Loukkos (Tatouft, a village near Ksar El Kebir, latitude: 35° 00 'N and longitude 5 ° 54' W; elevation: 16.7m). The plants were dried during ten days at an ambient temperature (22-27°C) in the laboratory until the stability of their weight and then the leaves were separated from the stems and stored away from light. The botanical identification was kindly confirmed by Professor M. Ibn Tattou at the Department of Botany and Vegetal Ecology, in the Scientific Institute of Rabat (Morocco). A voucher specimen has been deposited at the herbarium under the numbers RAB 082552 and RAB 082551 for the *Rosmarinus officinalis* (Middle Atlas) and the long leaf *Rosmarinus officinalis* (Loukkos), respectively.

2.2. *Extraction of essential oils*

The extraction of essential oils was carried out by hydro-distillation in a Clevenger type apparatus [28]. Thus, 100g of dry plant material/L of distilled water were distilled at 100°C during 3-4 hours, until the essential oil volume remained constant. Concerning the yield, a sample of 100 g of plant material was weighed which is dried in a stove set at 60°C for 48 to 60 hours to dehydrate, three repetitions were performed. Essential oils were separated by decanting; then they were dried with anhydrous sodium sulphate and stored in a refrigerator at 4°C in dark until use. The yields of essential oils are expressed in mL per 100 g of the dry matter.

2.3. *Analysis of the essential oils*

The chemical composition analysis of essential oils was carried out by a gas chromatography Agilent Technologies type (series 6890N Network GC System. USA), equipped with a capillary column HP5 of 30m in length. 0.25mm in diameter and 0.25µm in film thickness and a split-splitless injector set at 250°C, coupled to an Agilent 6890N Series Mass spectrometer Network GC (5975 Series Mass Selective Detectors). The carrier gas was the helium at 1.2 mL/min and the injection mode was of split type. The temperature gradient of the column was programed to increase from 60 to 260°C at 3°C/min and, then held at 260°C for 96.67 min. The ionization energy was set at 72eV. The device is controlled by a computer system of the type "HP ChemStation.
The apparatus is connected to a computer system managing a mass spectra library called NIST 98. The retention indices were calculated using a standard set of C_{10}-C_{30} alkanes performed under the same conditions. The identification of the components of essential oils was performed on the basis of their retention indices and their

mass spectra obtained by a chromatography coupled with a spectrometry. Then, these parameters were compared with those of the NIST database search and those of [29] and [30].

2.4. *Potato tuber moth. Phthorimaea operculella (Zeller) strain*

Potato tubers of the variety ″RUDOLPH″, infested with *P. operculella*, were collected in a storage warehouse in the Meknes province (Morocco) during August 2014. These tubers were then placed in breeding cage paper (60cm x 40cm x 30cm) covered by muslin cloth and maintained in the rearing room set at a temperature of 27 ± 1°C, relative humidity of 60 ± 5% and photoperiod of 10h light/14h dark. The newly emerged moths were placed in contact with healthy tubers of the same variety, under the same conditions of stock culture. After 48 hours, the insects were removed and tubers carrying eggs were incubated under the same previous rearing conditions, until the adult emerged. Thirty five days later, the offspring's adults aged <24 hours were used for biological tests. To ensure the development of the insect, healthy tubers were used.

2.5. *Bioassays*

To choose the appropriate concentrations of essential oils, preliminary toxicity tests were carry out and, those selected were those that caused a mortality ranging from 10 to 90% of the treated population of *P. operculella*. Thus, the concentrations used were 1, 2, 4 and 8µL essential oils/L of air. Fumigation against targeted stages (adults, eggs, neonate larva, pupa) of the potato tuber moth was carried out in hermetic transparent plastic container of 1L volume (length =20cm, width = 10cm, height =5cm). Each concentration was deposited by the micropipette on a filter paper Whatman n°4 (3cm x 3cm) inside the container. In addition, a lot without essential oils was used as a control. For each concentration, a number (see below) of the insect stage studied was added. Each concentration was repeated three times. Then all containers with insects were placed in the previously described rearing conditions.

2.5.1. *Treatment of adult*

To appreciate the effect of essential oils on the adult mortality of *P. operculella*, four potato clean tubers (variety ″RUDOLPH″, weighing about 100g) were placed in cellulose alveoli inside the containers and exposed to 10 pairs of *P. operculella*. The same procedure was followed under the same conditions described in paragraph 2.5. After 24 hours, adult mortality was recorded daily by sex until the death of all the moth adults.

2.5.2. *Treatment of eggs*

To get eggs, 10 pairs of newly emerged adults of the potato tuber moth were placed in each container presented in § 2.5 with four potato tubers (Cf. § 2.5.1). After 48 hours; the insects were removed. Tubers with eggs were separately fumigated with 0, 1, 2, 4 or 8µL essential oils/L of air. For each concentration, 30 eggs were used and three replications were carried out. After 4-6 days following oviposition, hatched and unhatched eggs were counted under binocular microscope. The percentage of hatchability was calculated by dividing the number of eggs hatched on the number of eggs used.

2.5.3. Treatment *of neonate larvae*

To evaluate the potential effect of *R. officinalis* essential oils on the neonate larvae, the same experimental protocol performed for eggs was adopted. Thirty neonate larvae were treated with 0, 1, 2, 4 or 8 µL essential oils/L of air and placed in the same previous rearing conditions. After, the number of larvae died outside of potato tuber was counted under binocular microscope and, the percentage of mortality was calculated by dividing larvae died on the used ones.

2.5.4**.** *Treatment of pupae*

In this assay, 10 male and 10 female pupae were exposed separately to 0, 1, 2, 4 or 8 µL of essential oils/L of air. Fumigation was carried in the same container cited in § 2.5. Each treatment was repeated three times. The adults emerged was recorded daily per sex untill the last ones; after the emergence, the percentage of pupa mortality was calculated by dividing the number of this stage died on the used ones.

2.6. *Data analysis*

To compare the yields of essential oils (expressed in mL/100 g of dry material (DM)), an analysis of variance of the quantitative raw data followed by Newman-Keuls test at 5% was performed using SPSS 20. To compare the average mortality of eggs, neonate larvae and pupae, an analysis of variance in two factors followed by Scheffe

test at 5% was realised using SPSS 20 a transformed Arcsin square root of the mortality. To reveal eventual effects of essential oils tested on adults, the survival curves obtained for each concentration were compared with each other using the Logrank test [31] by Excel 2007. The LC_{50} and LC_{99} were determined by the probit method according to Finney [32] using "Probit analysis program EPAVersion 1.5". Mortalities were adjusted using Abbott's formula [33]. The lethal time, required for 50% mortality (LT_{50}) and 99% (LT_{99}) of adults exposed to different concentrations of the essential oil was calculated from the regression equations between adult mortality of *P. operculella* and the duration of exposure to essential oils.

3. **Results and discussion**

3.1. *Chemical composition of essential oil*

The average yields of essential oils extracted from the *R. officinalis* collected in Loukkos (2.73 ± 0.12%) were superior to those from the Middle Atlas (2.3 ± 0.17%) ($F = 13$; $df = 1, 5$; $P<0.05$). These yields are relatively close to those obtained by Douiri et al. [34] and Fadili et al. [27] of the same species from the Middle Atlas, but are better than those obtained in Algeria (0.8%) [35], in Morocco (0.54%) [36] and in Tunisia (0.71%) [37]. Furthermore, in Argentina, Adriana et al. [38] got yields of the oil extracted from rosemary close to 2.28-2.58%. The chromatograms of these essential oils are shown in Figure 1; while the essential oil constituents and its retention index are presented in Table 1.

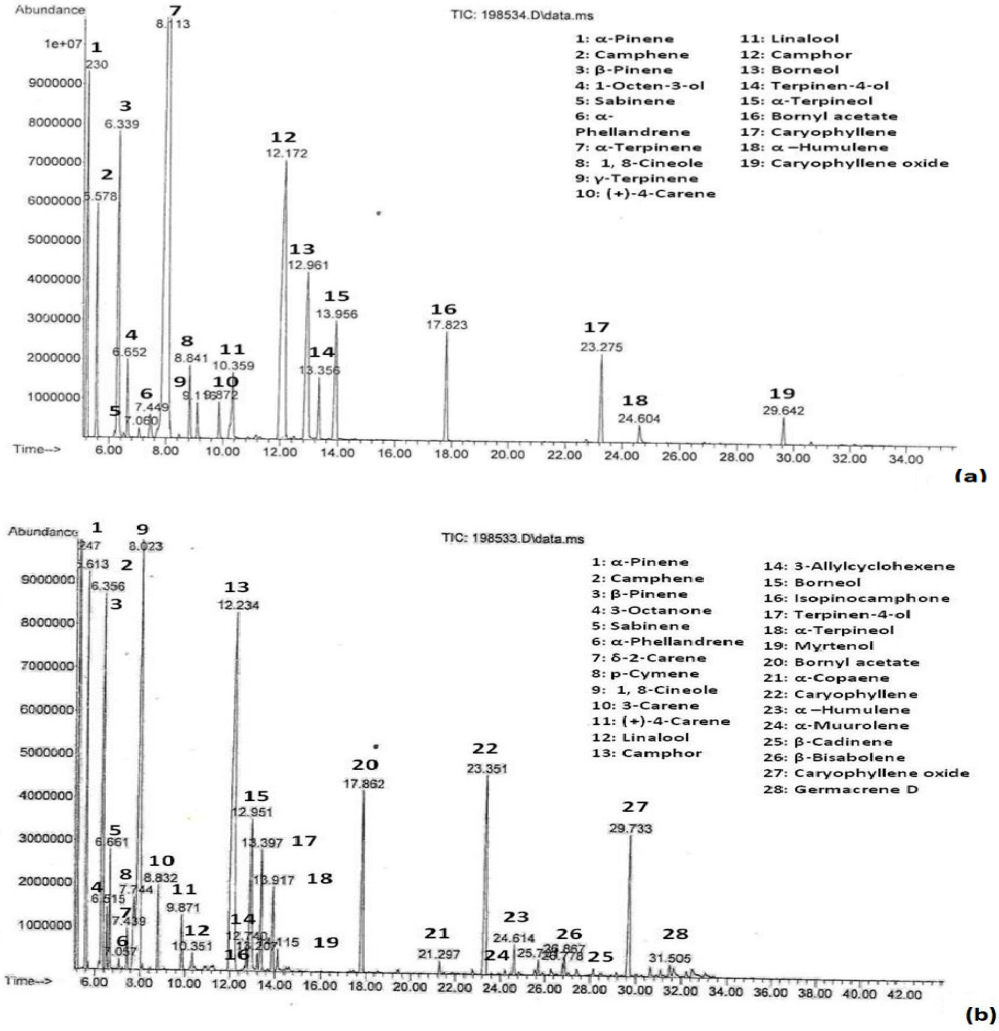

Figure 1: Chromatograms of *Rosmarinus officinalis* essential oil of the Middle Atlas (a) and that of Loukkos (b)

The chemical composition of the essential oils of both regions is different in quantity and in quality. Nineteen compounds of the *R. officinalis* oil collected from the Middle Atlas and twenty-eight components of that from Loukkos were identified, representing 100% of the oils. They appeared between 5.23 and 29.64 min in the rosemary oils collected from Middle Atlas and between 5.28 and 31.50 min in the Loukkos case (Fig. 1); their RI ranges from 953-1583 for *R. officinalis* in Middle Atlas and from 953-1631 in the Loukkos one (Table 1).

Table 1: Composition of *Rosmarinus officinalis* essential oils collected from Middle Atlas and Loukkos in Morocco

Compounds	RI	Content (%)	
		***Rosmarinus officinalis* Middle Atlas**	***Rosmarinus officinalis* Loukkos**
α-Pinene	953	1.85	9.19
Camphene	964	2.63	7.44
β-Pinene	984	5.62	8.58
1-Octen-3-ol	986	0.17	-
3-Octanone	990	-	0.83
Sabinene	995	1.51	1.56
α-Phellandrene	1006	0.23	0.14
δ-2-Carene	1017	-	0.68
α-Terpinene	1018	0.79	-
p-Cymene	1027	-	2.44
1, 8-Cineole	1035	46.23	17
3-Carene	1058	-	1.32
γ-Terpinene	1059	1.41	-
(+)-4-Carene	1089	0.67	0.98
Linalool	1103	1.54	0.39
Camphor	1149	17.29	21.33
3-Allylcyclohexene	1163	-	0.46
Borneol	1168	6.84	4.75
Isopinocamphone	1175	-	0.28
Terpinen-4-ol	1179	2.19	2.8
α-Terpineol	1194	5.31	1.85
Myrtenol	1198	-	0.38
Bornyl acetate	1288	1.97	4.76
α-Copaene	1370	-	0.24
Caryophyllene	1419	2.3	6.43
α –Humulene	1452	0.53	0.64
α-Muurolene	1479	-	0.3
β-Cadinene	1506	-	0.26
β-Bisabolene	1509	-	0.39
Caryophyllene oxide	1583	0.92	4.34
Germacrene D	1631	-	0.24
Monoterpenes oxygenated		**79.4**	**48.78**
Monoterpenes hydrocarbonated		**14.71**	**32.33**
Sesquiterpenes oxygenated		**0. 92**	**4.34**
Sesquiterpenes hydrocarbonated		**2.83**	**8.5**
Others		**2.14**	**6.05**
Total		**100**	**100**
Yield (%)		**2.3± 0.17**	**2.73±0.12**

-: Not detected
RI: Retention index on HP5-MS capillary column

The both essential oils consisted mainly of monoterpene fraction, which accounted for 94.11 and 81.11% of the overall essential oils, respectively. Among this fraction, the oxygenated monoterpenes (7 and 8 compounds) represent 79.4 and 48.78%; the hydro-carbonated monoterpenes (8 and 9 compounds) represent 14.71 and 32.33% of oil rosemary from the Middle Atlas and that from Loukkos, which the main constituents found in both oil were 1, 8-cineole (46.23 and 17%) and camphor (17.29 and 21.33%), respectively. In rosemary of Middle Atlas, *β*-pinene (5.62%) and camphene (2.63%) are the most common elements in the hydro-carbonated group; whereas those of Loukkos, there are the *α*-pinene (9.19%) and *β*-pinene (8.58%). The sesquiterpene fraction occurs in low content (3.75%) in oil from the Middle Atlas; it is composed of oxygenated

sesquiterpenes (0.92%) and hydro-carbonated sesquiterpenes (2.83%), where the *β*-caryophyllene (2.3%) is the main component of this fraction. In Loukkos, this essential oil also contains 4.34% of oxygenated sesquiterpenes and 8.5% of hydro-carbonated sesquiterpene, whose major constituents are *β*-caryophyllene (6.43%) and caryophyllene oxide (4.34%) (Table 1).

Compared to the studies conducted in different regions of Morocco; 1, 8-cineole (43.99%), camphor (12.41%) and *α*-pinene (10.09%) were the major components of *R. officinalis* in Oujda region [39] or Meknes-Tafilalet where the levels of 1, 8-cineole and camphor raised to 50.42% and 17.73%, respectively [34]. On the contrary, [36] found that the rosemary from the Fès-Boulemane region is rich in *α*-pinene (18.25%), camphor (6.02%), 1, 8-cineole (5.25%) and camphene (5.02%).

On the other hand, the essential oil of *R. officinalis* taken from Spain consists of camphor (32.33%) and *α*-pinene (11.56%) [40]; whereas, the essential oil of *R. officinalis* from Italy consists mainly of verbanone (20.3%), *α*-pinene (13.7%), 1, 8-cineole (3.4%) and camphor (2.9 %) [41]; while the essential oil of *R. officinalis* from Portugal consists of verbanone (35.4%), camphor (5.5%) and 1, 8-cineole (3.1%) [42]. In Turkey, the oil obtained from *R. officinalis* is rich in 1, 8-cineole (60.9%), *α*-pinene (7.8%) and camphor (7.1%) [43]. For their part, [44] found that the rosemary in India is rich in camphor (26.40%), the 1, 8-cineole (23.40%) and *α*-pinene (9.94 %). In Tunisia, the rosemary essential oils consist primarily of 1, 8-cineole (47.5%), camphor (14.9%), *α*-pinene (14.1%) and borneol (13.15%) [37].

According to [45], the essential oils of rosemary can be classified into three chemotypes, in the case of the cineoliferum (High in 1, 8-cineole), camphoriferum (camphor > 20%) and verbenoniferum (verbenone > 15%). According to this classification, the rosemary of Middle Atlas can be a cineoliferum chemotype and that of the Loukkos camphoriferum one.

The variability of yields and chemical compositions of our samples can be attributed to many factors such as the geographical origin, the environmental conditions and the extraction methods [46, 47] and the phenological stage of the plant [48].

3.2. *Toxicity of essential oil of Rosmarinus officinalis vis-a-vis Phthormaea operculella*

3.2.1. *Effect on adults*

All applied concentrations showed a toxic effect on the adults of *P. operculella*; this toxicity is increasing progressively as the concentration increases. Indeed and compared to control corresponding, except with 1µL/L of air, essential oils of Middle Atlas rosemary, which has a similar effect on males than the control, all other concentrations significantly shorten the longevity of adult potato tuber moth (Fig. 2a). In addition, for each concentration tested the probability of survival adult decreases as the exposure duration increases (Fig. 2a). On the other hand, the responses of both sexes are statistically comparable, whatever the provenance of the rosemary, when the insects were fumigated with 1 or 2µL/L air ($\chi^2_{\text{calculated}}$ = 0.20-2.87 < $\chi^2_{(0.05;\ 1)}$ = 3.841). On the contrary, with both oils applied at 4 or 8 µL/L air, males were more susceptible than female ($\chi^2_{\text{calculated}}$ = 5.86-5.33 > $\chi^2_{(0.05;\ 1)}$ = 3.841).

In addition, the lethal times, LT_{50} and $LT_{99,}$ of *P. operculella* adults exposed to different concentrations of essential oils vary according to sex and the concentration used, they range from 1 to 8 and from 1 to 7 days for the *R. officinalis* of Middle Atlas and that of Loukkos, respectively; whereas in the control group, the adults live between 3 and 11 days. These lethal times are negatively correlated to the essential oil concentrations tested (Table 2).

Furthermore, toxicological parameters of the tested essential oils are grouped in Table 3. As shown in Table 3, the toxicity of essential oils tested varies according to their origin and the moth sex. Indeed, in terms of LC_{50} or LC_{99} and line slopes, males seem to be more susceptible than females and, the essential oils extracted from rosemary collected in the Middle Atlas was more toxic than that taken in the Loukkos; against males, LC_{50} or LC_{99} vary from 4.55 to 2.08 or from 25.37 to 11.37 µL of essential oil/L of air for Middle Atlas rosemary and from 7.62 to 0.82 or from 77.29 to 4.26 µL of essential oil/L of air for Loukkos one. Vis-a-vis the females, LC_{50} or LC_{99} range from 6.54 to 1.07 or from 77.47 to 3.89µL of essential oil/L of air for *R. officinalis* of Middle Atlas, and, from 7.73 to 0.33 or from 62.85 to 5.48 µL of essential oil /L of air for *R. officinalis* of Loukkos (Table 3). In both sexes, the LC_{50} or LC_{99} decrease gradually as the fumigation time is extended following linear models (Fig. 2b). However, for LC_{99}, it is worth to be noted that their maximum values exceed the range of concentrations tested; then, to kill all the population just after the start of fumigation, the concentration must be increased, or to wait at least six days after the essential oil application if we use the same concentration tested in this work.

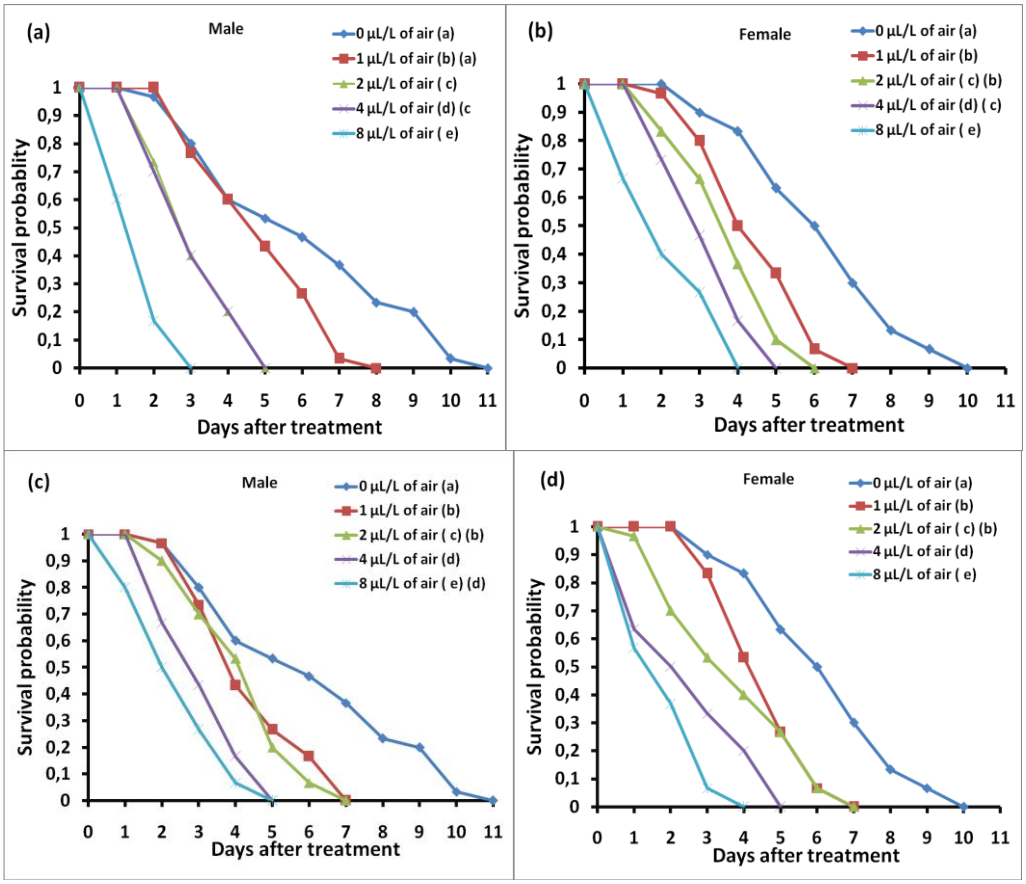

Figure 2a: Survival curves of *Phthorimaea operculella* adults exposed to *Rosmarinus officinalis* essential oils of the Middle Atlas ((a) and (b)) and that of Loukkos ((c) and (d)) (Concentrations affected by the same letter do not differ statistically (logrank test compared to $\chi^2_{(0.05,\ 1)} = 3.841$)

Table 2: LT_{50} and LT_{99} of *Phthorimaea. operculella* adults exposed to *Rosmarinus officinalis* essential oils collected from Middle Atlas or from Loukkos

Regions of *R. officinalis*	**Sex**	**Concentrations (µL/L of air)**	**LT_{50} (Days)**	**r ($r_{(0.05\ ;\ 3)}$= - 0.87)**	**LT_{99} (Days)**	**r ($r_{(0.05\ ;\ 3)}$= - 0.87)**
Middle Atlas	Males	0	5.67		10.57	
		1	4.46		7.83	
		2	2.75	- 0.97	4.96	- 0.96
		4	2.73		4.95	
		8	1.33		2.76	
	Females	0	5.67		9.85	
		1	4.01		6.98	
		2	3.36	- 0.97	5.96	- 0.96
		4	2.78		5.00	
		8	1.86		3.90	
Loukkos	Males	0	5.67		10.57	
		1	3.95		6.99	
		2	3.82	- 0.96	6.79	- 0.93
		4	2.70		4.91	
		8	2.21		4.52	
	Females	0	5.67		9.85	
		1	4.03		6.95	
		2	3.44	- 0.98	6.62	- 0.97
		4	2.21		4.87	
		8	1.60		3.56	

Table 3: Toxicity parameters of *Rosmarinus officinalis* essential oils coming from Middle Atlas or from Loukkos against *Phthorimaea operculella* adults

Plant	Sex	Days after treatment	Slopes ± SE	χ^2 calculated (χ^2 dl (2) ; p (0.05))	LC_{50} (µL/L of air) [Confidence interval]	LC_{99} (µL/L of air) [Confidence interval]
R. officinalis of Middle Atlas	Male	1	-	-	-	-
		2	3.12±1.23	7.31	4.55*	25.37*
		3	3.18±1.58	8.31	2.73*	14.74*
		4	3.15±0.90	5.57	2.08 [0.89; 3.04]	11.37 [6.74; 56.08]
	Female	1	-	-	-	-
		2	2.17±0.46	0.82	6.54 [4.84; 11.05]	77.47 [31.07; 665.12]
		3	1.95±0.53	0.001	4.25 [2.61; 6.81]	66.06 [24.19 ; 1591.41]
		4	2.46±0.56	2.15	1.53 [0.84; 2.16]	13.46 [7.56; 54.78]
		5	4.16±1.31	0.32	1.07 [0.49; 1.46]	3.89 [2.55; 18.19]
R. officinalis of Loukkos	Male	1	-	-	-	-
		2	2.31±0.68	0.75	7.62 [5.44; 15.63]	77.29 [27.77; 3147.64]
		3	2.34±0.85	0.53	5.04 [2.40; 8.44]	49.71 [19.00; 10479.98]
		4	3.63±1.34	2.98	3.47 [1.03; 5.00]	15.13 [8.83; 278.53]
		5	3.47±1.13	2.69	1.27 [0.42; 1.89]	5.93 [3.55; 40.45]
		6	3.25±1.38	0.45	0.82 [0.03; 1.32]	4.26 [2.41; 596.94]
	Female	1	2.56±0.55	4.73	7.73 [5.80; 13.22]	62.85 [27.59; 462.46]
		2	2.28±0.49	5.14	4.80 [3.56; 7.00]	50.24 [22.74; 336.23]
		3	2.89±0.59	0.90	2.72 [1.87; 3.58]	17.40 [10.45; 53.14]
		4	2.53±0.57	2.61	1.72 [0.98; 2.41]	14.32 [8.06; 55.80]
		5	2.97±0.86	5.47	1.16 [0.44; 1.73]	7.07 [4.11; 39.76]
		6	1.90±1.09	1.54	0.33*	5.48*

- : No mortality was observed in this time,
*: The confidence intervals are too large.

The analysis of the chemical composition of the *R. officinalis* essential oils of the two Moroccan regions shows the presence of some compounds known for their insecticidal properties; it is the case, for example, of the *α*-terpineol, 1, 8-cineole and camphor against the *T. confusum* [49] but also the *α*-pinene [50], *β*-pinene, *α*-terpinene and terpeneol-4-ol against *S. oryzae* [51].

The compounds of both essential oils studied in this work have an insecticidal effect on the adults of *P. operculella*, which varies depending on the plant material, the concentration of oils and the duration of exposure. Considering the lethal concentration values and lethal time, *R. officinalis* essential oils of the two regions are shown toxic. Among the essential oils, extracted from 24 botanical species and tested against *Acanthoscelides obtectus*, 7 are shown very toxic [52]; these latter include *T. serpyllum*, *O. vulgare*, *S. hortensis*, *L. angustifolia*, *R. officinalis*, *O. majorana* and *O. basilicum*, and the *P. sativum*, they killed all test insects fumigated after 1-4 days of exposure at 2 to 10µL/L air. For their part, [53] showed that after 24 hours, the essential oil of *R. officinalis*, applied at 0.93 µL/L air, was more toxic to adults of *Plodia interpunctella* than that extracted from *Z. multiflora* and used at 1.75 µL/L air. Amri *et al*. [37] also observed that the essential oils of *R. officinalis* were very toxic as fumigant vis-a-vis *Ectomyelois ceratoniae*, they caused the death of 100% of individuals treated with 20µL/mL in 6h exposure.

3.2.2. *Effect on eggs, neonate larvae and pupae*

Vis-a-vis the *P. opercullela* eggs, effect of the *R. officinalis* essential oils on their hatchability varies statically according to rosemary's origin ($F = 27.67$; $df = 1, 29$; $P < 0,05$) and for a same provenance, according to the concentration of the products used ($F = 361.94$; $df = 4, 29$; $P < 0,05$). Except with 1uL of essential oils tested /L air, that does not significantly affect the potato tuber moth egg's hatchability compared to controls, all other concentrations reduce drastically and gradually the egg eclosion rate following the models $Y_{(R.\ officinalis\ \text{of the Middle Atlas})} = 6.968x + 7.093$ - $R^2 = 0.87$ and $Y_{(R.\ officinalis\ \text{de Loukkos})} = 6.\ 578x + 1.315$ - $R^2 = 0.78$. Essential oils extracted from rosemary of the Middle Atlas were more harmful to the eggs of the insect than the Loukkos ones (Fig. 3).

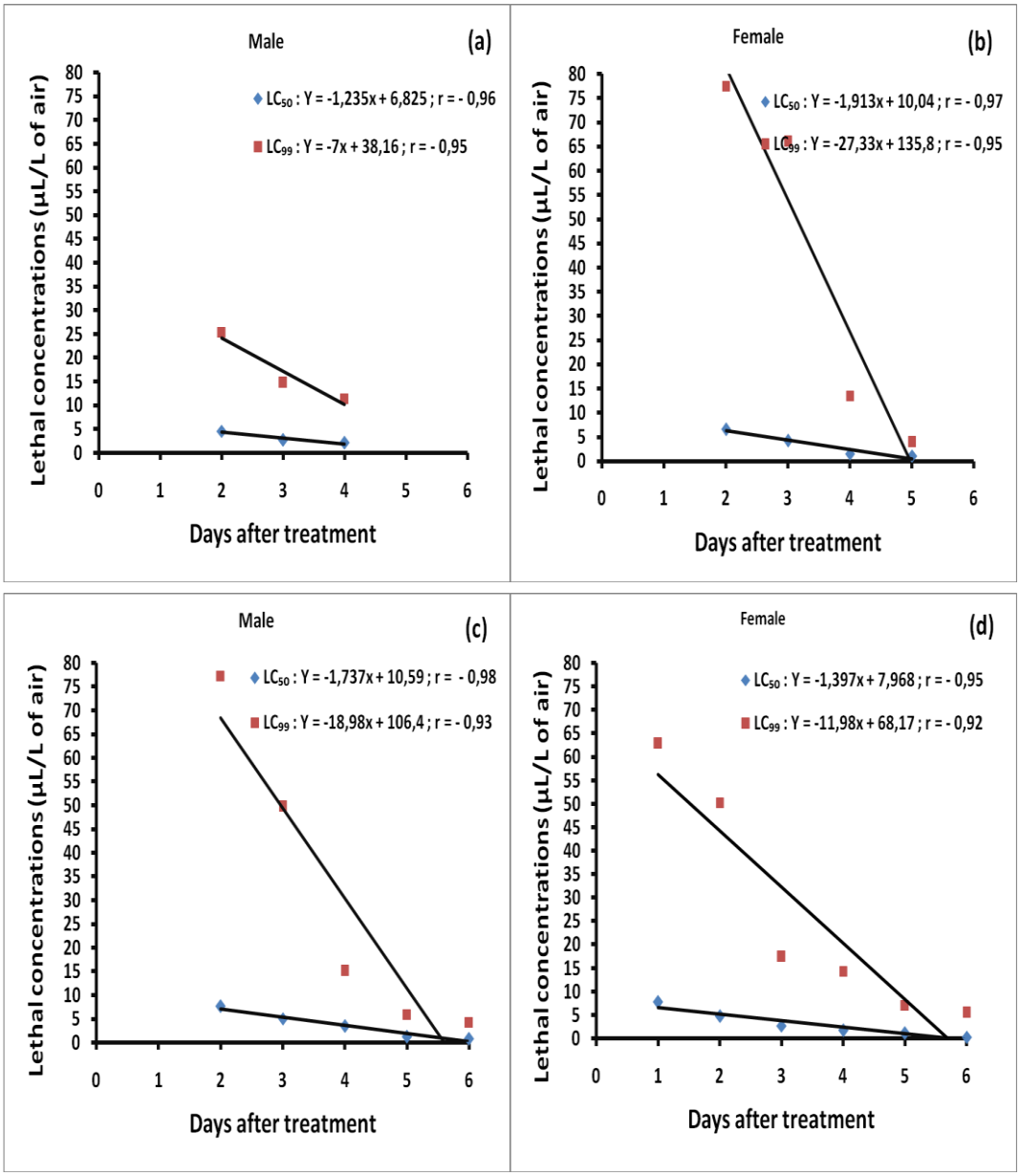

Figure 2b: Relationship between the lethal concentrations and the exposure duration of *Phthorimaea operculella* adults to *Rosmarinus officinalis* essential oils coming from Middle Atlas (a and b) and that from Loukkos (c and d)

In previous studies, [54] showed that the essential oil of rosemary has a toxicity on the eggs hatching of *A. obtectus*. Also, [37] showed that essential oil of *T. capitatus* and *R. officinalis* cause the total inhibition of the eggs' fertility of *Ectomyelois ceratoniae*. Indeed, the ovicidal activity of the tested essential oil is probably due to the blocking of embryo genesis following the penetration of the oil vapours in the eggs by the respiratory tube as described at *C. maculatus* by Credland [55]; while according to [56], essential oils have a sterilizing action on eggs. The high toxicity of rosemary essential oils coming from the Middle Atlas may be due to their high content of 1, 8-Cineole (op. cit.). Against *P. operculella* neonate larvae, rosemary essential oils tested also shown toxic; their toxicity was stronger with the Middle Atlas rosemary than that of ($F = 36.82$; $df = 1, 29$; $P < 0.05$). Here also, the toxicity of these oils is increasing with concentration ($F = 367.81$; $df = 4, 29$; $P < 0.05$) (Fig. 4). Mortality of treated larvae increases gradually as the concentration increases according to the linear models: $Y_{(R.\ officinalis\ \text{of the Middle Atlas})} = 8.513x + 16.53$- $R^2 = 0.833$ and $Y_{(R.\ officinalis\ \text{of Loukkos})} = 7.235x + 10.52$- $R^2 = 0.841$; thus, with 1-8 µL of essential oils /L of air, the mortality of neonate larvae grew up from 11.11 to 79.01% and from 7.41 to 62.69% for the rosemary in Middle Atlas and in Loukkos, respectively.

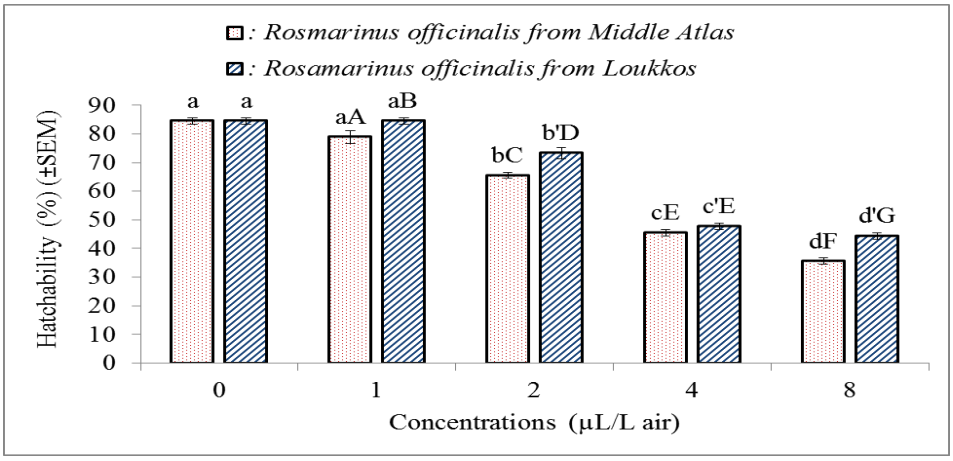

Figure 3: Effect of *Rosmarinus officinalis* essential oils on the egg's hatchability of *Phthorimaea operculella* eggs [The histograms affected by the same letter do not differ statistically between them (ANOVA and Scheffe's multiple comparison tests at 5 %; (SEM: Standard error of the mean); the small letters indicate comparison between the concentrations within the same origin of the rosemary and, the capital letters between plant's origin at a same concentration]

Against *Ectomyelois ceratoniae*, the essential oils of *T. capitatus* or that of *R. officinalis* at 20µL/mL causes a mortality of 100% and 90%, respectively, after 24 h exposure of the treated population [37]. The larvicidal activity observed may be due to the inhibition of the production of certain substances, such as growth regulators; thus, [57] attributed this inhibition to juvocimenes substances similar to juvenile hormone of insects present in some certain oils as that of *Ocimum basilieum* L. The action of essential oils tested in our work may also be related to the richness of this essential oil in terpenes which are compounds similar a juvenile hormone.

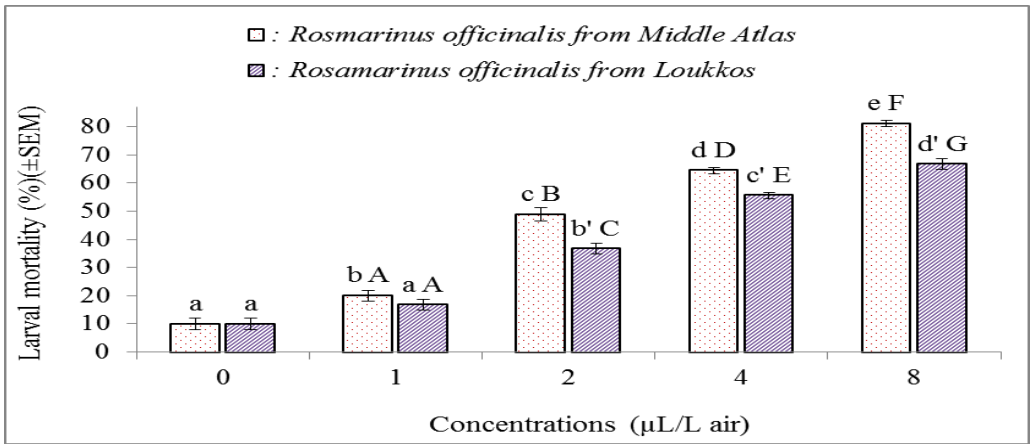

Figure 4: Effect of *Rosmarinus officinalis* essential oils on the mortality on neonate larvae of *Phthorimaea operculella* [The histograms affected by the same letter do not differ statistically between them (ANOVA and Scheffé's mlultiple comparison tests at 5 %) (SEM: Standard error of the mean); the small letters indicate comparison between the concentrations within the same origin of the rosemary and, the capital letters between different rosemary's origin at a same concentration]

Considering the LC_{50} and LC_{99} values in our experimental conditions, the toxicity of rosemary varies according to the plant material and the stage of *P. operculella*. The essential oil of rosemary collected from the Middle Atlas appears more toxic than that originated from Loukkos. In addition, the eggs require two-fold concentration than those neonate larvae to affect 50% or 99% of the embryonic population; then, the neonate larvae showed to be more vulnerable than eggs (Table 4). Vis-a-vis the pupae, the tested rosemary essential oils do not affect their survival; all pupae used have turned into adults.

In agreement with [58] and [59], the toxicity of essential oils of *R. officinalis* towards adults, larvae and eggs can be explained by the high respiratory activity of these stages compared to pupae which have shown tolerance to these compounds. The insecticidal activity of essential oil is mainly due to monoterpenoids [60, 61]. These compounds can inhibit the activity of the acetylcholinesterase enzyme [62], that of the octopamine [63] or that of the monooxygenases in the dependent P450 cytochrome [64]. These findings agree those obtained recently [65-67]

Table 4: Toxicity parameters of *R. officinalis* essential oils of Middle Atlas and that of Loukkos against eggs and neonate larvae of *P. operculella* after 5 days exposure

Regions of *R. officinalis*	Stages	Slopes ± SE	χ^2 calculated (χ^2 dl (2) ; p (0.05))	LC_{50} (µL/L of air) [Confidence interval]	LC_{99} (µL/L of air) [Confidence interval]
Middle Atlas	Eggs	1.72±0.35	1.40	5.40 [3.93; 7.67]	121.41[47.36; 1006.89]
	Larvae	2.03±0.28	2.78	2.95 [2.32; 3.63]	41.14 [23.84; 102.85]
Loukkos	Eggs	1.78±0.40	5.37	6.91[5.05;10.54]	139.40 [50.18;1829.53]
	Larvae	1.75±0.29	2.37	4.49 [3.45; 5.94]	95.25 [43.10; 433.10]

Conclusion

The essential oils extracted from *R. officinalis* collected from the Middle Atlas and Loukkos are characterised by their high content of 1, 8-cineole (46.23%; 17%) and camphor (21.33%; 17.29%), respectively. As a result, the rosemary of Middle Atlas is chemotype 1, 8-ceniole and that of Loukkos is camphor chemotype. The study of the action of these essential oils on eggs, neonate larvae, pupae and adults of *P. operculella* revealed that they have ovicid, Larvicid and adulticid properties; their toxicity may be linked to their high content of oxygenated monoterpenes, including 1, 8-cineole, as it was reported towards others insect species [58, 59]. The essential oils of *R. officinalis* may be therefore used in the sustainable management of the potato tuber moth in storage. This approach may be beneficial because it is respectful of the environment and socially acceptable. However, other studies must be carried out to evaluate the effect of individual essential oil compounds and to develop formulations improving the efficiency and the stability of the tested oils. The cost of the use of essential oils during storage should be also evaluated on a commercial scale.

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