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Biological Activity of *Rosmarinus Officinalis* Essential Oils against *Callosobruchus Maculatus,* (Coleoptera, Bruchinae)

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Abstract

For the purpose of finding alternative ways to synthetic insecticides to fight against pests that ravage stored pulses, rosemary's (*Rosmarinus officinalis* (L)) (Lamiaceae) essential oils have been tested against cowpea weevils (*Callosobruchus maculatus* (Fab)) (Coleoptera, Bruchinae) reared on chickpea (*Cicer arietinum* (L)) seeds. The chickpea seeds have been infested with ten newly-hatched pairs of cowpea weevils, then fumigated with increasing concentrations of 0, 4, 6, 8 or 10µl of rosemary's essential oils per liter of air. These essential oils were analyzed by GC-MS. The compound groups were predominantly monoterpenes (93.06%), 74.93% of which were oxygenated and 18.13% hydrocarbonated. The main components in the oxygenated monoterpenes are eucalyptol (1,8-cineol) (50,42%), camphor (17.73%) and borneol (5.99%), while the 3-carene (12.05%) is the most represented compound in the hydrocarbonated monoterpenes. The remaining constituents represent only 6.94% of essential oils.

Rosemary's essential oils significantly affect longevity (Treated lot: 1 – 7 days, control lot: 2 – 12 days), fecundity (10 – 48 eggs/10 females vs. 437 – 491 eggs/10 females), and fertility (66.67 – 85.00% vs. 93.75 – 95.44%). The cowpea weevil's success rates in the treated group were 0 – 60.42% compared to 86.35 – 92.33% in the control lot. The lethal concentrations at 50% (LC_{50}) and 99% (CL_{99}), for exposures between 24 and 120h , ranged from 5.51 – 2.43µl/l of air to 11.24 – 6.33µl/l of air, respectively, for males and from 6.80 – 3.04µl/l of air to 15.74 – 7.44µl/l of air for females. The demographic parameters are significantly affected. The average generation lifespan is prolonged ranging from 33.83 days for the control lots to 36.57 days for the treated lots, while the other parameters were all shortened. Rosemary's essential oils may be used in an Integrated Pest Management (IPM) of stored legumes without any health or environmental risks.

Keywords: Essential oils*, Rosmarinus officinalis, Callosobruchus maculatus,* Pulses, Fumigation.

1. Introduction

Chickpeas (*Cicer arietinum* (L.)) cultivation is one of the most important crops growing of the legumes. The legumes represent an agronomic, economic, food and environmental advantage. Unfortunately, the seeds are attacked, during storage, mainly by multivoltine weevils, including *Callosobruchus maculatus*. This species can destroy a whole stock (Boughdad 1992). According to Tingey (1992), the chemical insecticides have a very short commercial duration, which is, on average, only three years and a half. However, history also shows that overzealous use of synthetic insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, farmworkers, and even consumers; destruction of fish, birds, and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening human and environmental health; and the evolution of resistance to pesticides in pest populations (Marco 1987; Forguet 1993; Perry 1998 and Regnault-Roger 2012).

In fact, all this has sparked a renewed interest in phytosanitary alternative strategies, particularly botanical products with insecticidal properties. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment. Because of the multiple sites of action through which the plant materials can act, the probability of developing a resistant population is very low (Isman 2006). Several studies are currently being developed to isolate or identify secondary substances extracted from plants that have insecticidal, repellent or anti-feedant properties vis-à-vis the insects (Isman, 2006). Essential oils are complex mixtures of volatile compounds extracted from a large number of plants. They are endowed with insecticidal properties, some of which are the basis of various formulations for commercial repellents (Curtis et al. 1989; Nerio et al. 2010). Plants essential oils are secondary metabolites capable of an optimal potential of repulsive ability against many arthropods species. However, heir high volatility reduces the duration of protection again insects. Their efficacy and persistence can be enhanced by mixing them with binding materials. For example, citronella grass' (*Cymbopogon winteianus*) essential oils mixed with 5% of vanillin ensure 100% of protection against mosquitoes, results comparable to those of N,N-Diethyl-meta-toluamide (DEET) at 25% (Tawastin et al. 2001).

Morocco has a great potential in the field of medicinal and aromatic plants. Currently, it is making significant productions about 33000 tons/year in medicinal and aromatic plants (MAP) and their derivatives, which gives it a significant place in the international market (Zrira, 2006).
Rosemary (*Rosmarinus officinalis* L., (Lamiaceae) is an evergreen shrub that is widely distributed in the Mediterranean region. Due to its essential oil and its biological properties, it's the herb most exported by Morocco approximately 12.70% of all the dried herbs and medicinal plants (Office EACCE, 2006 in USAID 2008).
In Morocco, wild rosemary is very common in the rif, the middle and high atlas. It has been used since ancient times for medicinal purposes and is known for its antiseptic (Bult et al. 1985), anti-rheumatic (Makino et al. 2000), anti-inflammatory (Beninca et al. 2011) anti-diabetic (Abu-Al-basal et al. 2010) and anti-ulcerogenic (Dias et al. 2000), antibacterial (Okoh, 2010 ; Jordan et al. 2013), antifungal (Soylu et al. 2010), insecticidal (Zoubiri and Baaliouamer, 2011), anti-depression (Machado et al. 2009 ; 2012) antioxidant properties (Adorjan 2010, Özcan Arslan 2011), and anti-cancer (Degner et al. 2009), In 2007, Legault and Pichette investigated the potentiating effect of ß-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene and paclitaxel against MCF-7,DLD-1 and L-929 human tumour cell lines.
In this study, we propose to present the chemical composition of rosemary's essential oils and their allelopathic effects on various biological and demographic parameters of *C. maculatus*.

2.Materials and methods

2.1Insect cultures

The strain of cowpea weevil, Callosobruchus maculatus (Coleoptera, Bruchinae) was collected from the grain wholesale market in Meknes. It was reared in the laboratory on chickpea seeds, C. arietinum, in petri dishes housed inside glass dryers with a capacity of 4.5L at 28 ± 2°C, 65% ± 5% relative humidity and 12h/12h photoperiod in an air conditioned room for several consecutive generations.

2.2Plant materials

Rosmarinus officinalis L. used is in the form of a mature, wild shrub, leaves and flowering tops were collected using in August 2009 from a mountain called jbel Mesrouh (2700m) located in the Southeast of the Meknes-Tafilalet region (Center - East of Morocco).

Extraction of essential oils

Using a Clevenger distiller, the extraction of the essential oils was performed from 100g of leaves and buds of *R. officinalis,* dried at room temperature until the weight remained constant. The hydro-distillation lasted 3 hours at a temperature of 120°C. Three repetitions were performed and the average yield was 2.68 ± 0.39 ml/100g of dried matter. The essential oils were desiccated with anhydrous sodium sulfate and stored in a refrigerator at 4°C until use.
Analysis of the essential oils was performed using a gas chromatograph (Trace GC Ultra), connected to an ion trap mass spectrometer (MS), Polaris Q, by electron-impact ionization (EI) at an ionization voltage of 70eV, mass range with a column VB5 (5% phenyl, 95% methylpolyxiloxane, 30 m with 0.25 mm i.d, film thickness 0.25 µm). The scanning range was from 10 to 350 m/z. The oven temperature was programmed as follows: 50°C raised to 250°C at a rate of 5°C/min and from 250°C to 300°C. Helium was used as the carrier gas at 1.4 ml/min. The injection temperature was 220°C. The sample (1µL) of essential oils, diluted to 1:10 in hexane, was injected manually in the split mode. The identification of the constituents of essential oils was performed using the NIST MS Search database and compared to Adams (2007). The retention indices were calculated using a standard series of C_9-C_{30} n-alkanes performed in the same conditions.

2.3Bioassay with adults of C. maculatus

The experiment was carried out in Petri dishes of 9cm in diameter. 50 seeds of chickpea (about 28g) were taken at random and exposed to 10 pairs of *C. maculatus*. Using a micropipette, volumes of 4µl, 6µl, 8µl or 10µl of essential oil of *R. officinalis* were placed separately in watch glasses. Then, each concentration was placed in a glass desiccator of 4.5L-capacity with three Petri dishes each containing 50 chickpea seeds infested with ten pairs of weevils. Meanwhile, a lot of 50 chickpea seeds, untreated, was also exposed to ten couples of weevils in another desiccator; and was used as a control. For each tested concentration, three replicates were performed.
After 24 hours, adult mortality was recorded daily by sex until the death of all adults, whereas the numbers of hatched and unhatched eggs were counted ten days later. Then, as soon as adult emergincy began (27 days after laying), the number of emerged insects was counted every day until the end of the hatch. The parameters thus measured were the weevil's longevity, fecundity, fertility of eggs = (Number of hatched eggs / number of eggs laid) * 100, embryonic mortality = ((Number of eggs laid - number of eggs hatched) / number of eggs laid) * 100, the success rate = (number of insects emerged / number of eggs laid) * 100, the mortality rate in the seed = ((number of hatched eggs - insects emerged) / number of hatched eggs) * 100.
The demographic parameters were calculated according to Carey (1993), so the net reproduction rate R0 = (total

number of females hatched / initial number of females used). The average generation lifespan in days: T = (Σ (frequency of adults * development time)) / total number of adults obtained, the intrinsic rate of increase, r_m = (ln(R0)) / T, and the finite rate of increase, $\lambda = e^{rm,}$ and the doubling time, DT = Ln(2) / r_m, and the Sex ratio = Number of males / number of females.

2.4Statistical analysis

To detect any significant differences between the effects of rosemary's essential oils on *C. maculatus*, analysis of variance, followed by Scheffé's test at 5%, has been conducted. Statistical analyses were performed on raw data, in the case of quantitative variables (longevity, fecundity), and on data transformed by Arcsin(square root(%)), in the case of proportions (fertility, success rate). The software used is Excel 2007.

For the lethal concentrations of 50% (LC_{50}) or 99% (LC_{99}) of individuals exposed to different concentrations tested, the line slopes and confidence intervals were determined by the probit method (Finney, 1971) using the EPA Probit Analysis Program, v. 1.5. They are expressed in µL of rosemary's essential oils per L of air. Mortalities were adjusted using Abbott's formula (1925). Lethal times (LT) of 50% or 99% of adults exposed to different concentrations studied were calculated from the regression lines contrasting the mortalities recorded per concentration to the duration of exposure of the insects. The statistical comparison of the demographic parameters was performed using the analysis of variance.

3. Results

3.1Chemical Composition of R. officinalis essential oils

The yield obtained in essential oils is 2.68 ± 0.39 ml/100g of the dry weight of leaves and buds. Rosemary's essential oils are composed of 25 compounds covering 99.61% of peak areas and appearing between 7.77 and 44.73 min. Their relative importance varies from 0.01 to 50.42% in terms of peak areas (Table 1). The main components of rosemary essential oils are 93.06% monoterpenes, 74.93% of which are oxygenated and 18.13% hydrocarbonated. The major constituents of the oxygenated monoterpenes are 1,8-Cineole (50.42%), camphor (17.73%), and borneol (5.99%); while the 3-carene (12.05%) is the most prevalent element of the hydrocarbonated monoterpenes. The remaining constituents represent only 6.94% of the total (Table 1).

3.2Rosemary's essential oils effects on C. maculatus

Effects on longevity

The lifecycle of *C. maculatus* adult males placed in contact with the different concentrations of rosemary's essential oils is 1 to 6 days after treatment compared to 1 to 11 days for males in the control lots. In addition. the longevity of the treated adult females is 1 to 6 days and that of the females in the control lots ranges from 1 to 12 days (**Fig 1**). The essential oils, thus, significantly affect the longevity of the cowpea weevils.

Furthermore, the lethal times (LT) of 50% and 99% of adults fumigated with rosemary's essential oils vary from about 1 to 12 days depending on the gender and concentration used. They are negatively correlated with the concentrations of the essential oils (**Table 2**).

On the other hand, the toxicological parameters of rosemary's essential oils are summarized in **Table 3**. They allow to assess the toxicity capability of these compounds according to the weevil's sex and the duration of fumigation. Indeed, after 24 hours of exposure of adult weevils to the different concentrations tested, the LC_{50} and LC_{99} / L of air (24h to 120h) range from 5.51 to 2.43 µL / L of air and 11.24 to 6.33 µL / L of air, respectively, for males and 6.80 to 3.04 and 15.74 to 7.44 µL / L for females. *C. maculatus* mortality increases linearly with the oils concentrations used (**Table 3**).

For both sexes of *C. maculatus,* the lethal concentrations LC_{50} and LC_{99} of *R. officinalis* essential oils correlate linearly with the duration of exposure. They gradually decrease with time of exposure to the fumigant (Fig 2).

The toxicity parameters study has permitted us to conclude that the more the bruchids time of exposure to the various concentrations of the essential oils increases, the more LC_{50} and LC_{99} decreases. for both males and females.

Effects on fecundity, fertility, eggs mortality, in-seed mortalty and success rate

The fecundity of *C. maculatus* upon the chickpea seeds, fumigated with the different concentrations of rosemary's essential oils, is strongly affected. The number of eggs laid over the fumigated seeds is significantly lower compared to that observed on untreated seeds ($F_{calculated} = 682.45 > F_{(.05.\ 4\text{-}14)} = 3.48$). It ranges from 10 – 48 eggs/10 females for the treated lots, based on the concentration used, compared to 437 – 491 eggs/10 females in the control group (**Table 4**). The number of eggs laid is negatively correlated with the concentration (r = -0.96). Moreover, the fecundity of *C. maculatus* is directly related to the longevity of females ($R^2 = 0.996$). This suggests that the essential oils of rosemary exert a toxic effect on the weevil.

The fertility of *C. maculatus* eggs laid upon the seeds fumigated with rosemary's essential oils is slightly lower than that obtained with the control lots, it varies from 66.67 to about 85.00% of eggs laid, for the treated lots, versus 93.75-95.44%, for the control lots ($F_{calculated} = 8.77 > F_{(0.05.\ 4\text{-}14)} = 3.48$).

In this experiment, rosemary's essential oils have shown to be toxic to the embryo stage, eggs mortality increased from 4.56 - 6.44% for the control lots to 15.56 - 50.00% for the treated lots. In-seed mortality varies from to 25-100% according to concentration, for the fumigated batches against only 3.27-8.23% for the control. The number of adults emerged from the seeds fumigated with the different concentrations of rosemary's essential oils, varies from 0 to 29 adults. In contrast, from the control seeds 385-419 adults have emerged resulting in a high success rate for the control lots compared to the fumigated lots, it is statistically different (**Table 4**).

Effects on the demographic parameters of C. maculatus

Rosemary's essential oils also affect the monitored biodemographic parameters of the weevil populations. They change the generation time and reduce the net reproduction rate and the intrinsic rate of increase. This is even at the lowest concentration tested (4µl) (**Table 5**).

4. Discussion

In this study, the yield of rosemary's essential oils is 2.68 ± 0.39 ml/100g of the dry weight of leaves and buds. Jordan et al. (2013) found that in warm Mediterranean areas, the yields in essential oils of rosemary vary respectively from 2.44 ± 1.26% to 2.58 ± 0.75% of dry weight. On their part, Adriana et al. (2013) obtained a yield ranging from 2.28 to 2.58% of dry weight in Argentina. The variability in yields of essential oils may derive from the place of origin (Jamshidi et al. 2009), the environmental and agronomic conditions (Moghtader and Afzali. 2009), the time of harvest (Celiktas et al.. 2007), the development stage of the plants (Ruberto and Barata 2000) and the method of extraction (Okoh et al. 2010).

The main groups of chemical compounds of rosemary's essential oils are monoterpenes (93.06%). 74.93% of which are oxygenated and 18.13% hydrocarbonated. The principal compounds in oxygenated monoterpenes are 1.8-cineole (50.42%), camphor (17.73%) and borneol (5.99%); whereas 3-carene (12.05%) constitutes the main compound of the hydrocarbonated monoterpenes. Zouali et al. (2013) also found that the oxygenated monoterpenes are the major compounds (67.8%) of the essential oils extracted from the leaves of rosemary.

Similar results were also obtained by (Ayadi et al. 2011 and Benelli et al. 2012). The compounds of the essential oils of rosemary from Sidi Bouzid in Tunisia are 1.8-cineole (58.1%). α-pinene (11.5%) and camphor (7.8%). The variation in content of the constituents of essential oils depends on the bioclimate. The content on 1.8-cineole varies from 47.2 to 27.5% depending on weather conditions (Zaouali. 2010). According to Napoli et al. (2010), rosemary's essential oils can be classified into three chemotypes:

- cineoliferum (high in 1.8-cineol)
- camphoriferum (camphor > 20%) and
- verbenoniferum (verbenone > 15%).

In our case, the chemotype of the rosemary studied would be the cineoliferum type.

Concerning the biocidal effects of rosemary's essential oils, the fumigation of chickpea seeds by these compounds has revealed some harmful effects at all stages of development of the weevil. These oils have proven toxic to adults and pre-imaginal stages. With reference to fumigation toxicity trials, a previous study on Lamiaceae essential oils against *Ceratitis capitata* adults was recently published by Chang et al. (2009), who reported that *Ocimum basiculum* L, essential oils had a toxic action on medflies. Our data also show fumigation toxicity could be due to 1,8cineole, a major component in both Essential oils and This chemical was already acknowledged as a toxic fumigant molecule against adults of *Sitophilus orysae* (L.) experting more than 95% of fumigation toxicity of Mortality after 24h exposure at 42mg m^{-3} (Lee et al. 2004). Similarly, Papachristos et al. (2002) demonstrated the toxicity of Lavandula *hybrid*, *Rosmarinus officinalis* and *Eucalyptus globulus* on the eggs of *Acanthoscelides obtectus* with a difference of sensitivity significantly correlated with age. Similarly, Pavela (2008) reported that the fumigation toxicity of *R. officinalis* essential oils was also shown in fumigation assays against adults of the house fly, *Musca domestica*. The longevity of *C. maculatus* adult males and females in contact with the different concentrations of rosemary's essential oils is shorter compared to the control group. Rosemary's essential oils proved toxic to the cowpea weevils. The lethal concentrations LC_{50} after 24, 48, 72, 96 and 120 hours of exposure, are respectively 6.80 to 3.04 µl/28g of seeds for females and from 5.51 to 2.43 µl/28g of seeds for males. In turn, Bouchikhi Tani et al. (2008) showed that essential oils extracted from *R. officinalis* also exert a toxic effect on A. *obtectus* after 48 hours of exposure. Their side, Benelli et al. 2012 found that *R. officinalis* essential oils caused mortality rates higher than 70% at 24h of *Ceratitis capitata.*

As for the fecundity it is strongly affected. Similar results were also found by Bouchikhi Tani et al. (2008), at 5µL/30g of seeds of *Phaseolus vulgaris* L. Essential oils of *R. officinalis* and *Thymus vulgaris* completely inhibit the fecundity of the weevil *A. obtectus*. Bouchikhi Tani et al. (2010), also note that *Cymbopogon schoenanthus* completely inhibits the fecundity of *A. obtectus* beginning with the dose of 4µl/30g of seeds and *Artemisia herba-alba* completely inhibits the fecundity of *T. bisselliella* from a dose of 3µL/30g of seeds. The fertility of *C. maculatus* eggs laid on seeds fumigated with rosemary's essential oils is slightly lower than that obtained with the control, while the success rate is significantly affected that the eggs mortality and embryonic mortality are high. Similar effects were observed by Douiri et al. (2013) in the case of chickpea seeds treated with essential oils of *Allium sativum* against *C. maculates*, the interesting insecticidal activity was noted and no adults emerged.

The weevil populations demographic parameters monitored are significantly affected. In fact, the generation time was changed, while the net reproduction rate and the intrinsic rate of increase were reduced. Similarly, in studying the demographic parameters of *C. maculatus* under natural conditions in Burkina Faso, Sanon (1997) showed that the increase in temperature and relative humidity causes, in this species, an increase in the intrinsic rate of increase and a decrease in the generation time.

In general, essential oils are known, nowadays, as acute neurotoxins interfering with the Arthropods' octopaminergic transmitters. These oils are less toxic to warm-blooded animals and are volatile and highly toxic to insects. In addition, essential oils are highly active insecticides on insects but do not affect the germination of treated seeds (Keita et al. 2001). EOs such as oil of thyme, rosemary (*Rosmarinus officinalis*) and eucalyptus have antifeedant (Regnault-Roger, Hamraoui 1994) or repellent activity (Nerio et al. 2010*).* Koschier and Sedy, (2001) studied the antifeedant effect of essential oil of majoram and rosemary oil (*Rosmarinum officinalis*) at 0.1–1.0% concentration against onion thrips, *Thrips tabaci* Lindeman.

The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (Enan 2001, Kostyukovsky 2002) by some oils and with GABA-gated chloride channels by others (Priestley 2003 in Geoff 2012).

Furthermore, essential oils are derived from botanical, biodegradable products of renewable sources and act at low doses. They are economical and their environmental impact is low and often undetectable. However, considering their high volatility, they are not recommended for long-term protection of stored legumes, hence the need to develop methods of stabilization.

5.Conclusion

The essential oils extracted from the rosemary used in our work have a high content of 1.8-cineole (50.42%) Therefore, from the point of view of Chemistr, it is a plant of cineoliferum chemotype.

These essential oils demonstrated an insecticidal activity vis-à-vis the parameters of the weevil. Their use in stored legumes protection is a promising alternative to synthetic pesticides without adverse effects on the environment and consumers, their constituents are biodegradables with short half-lives.

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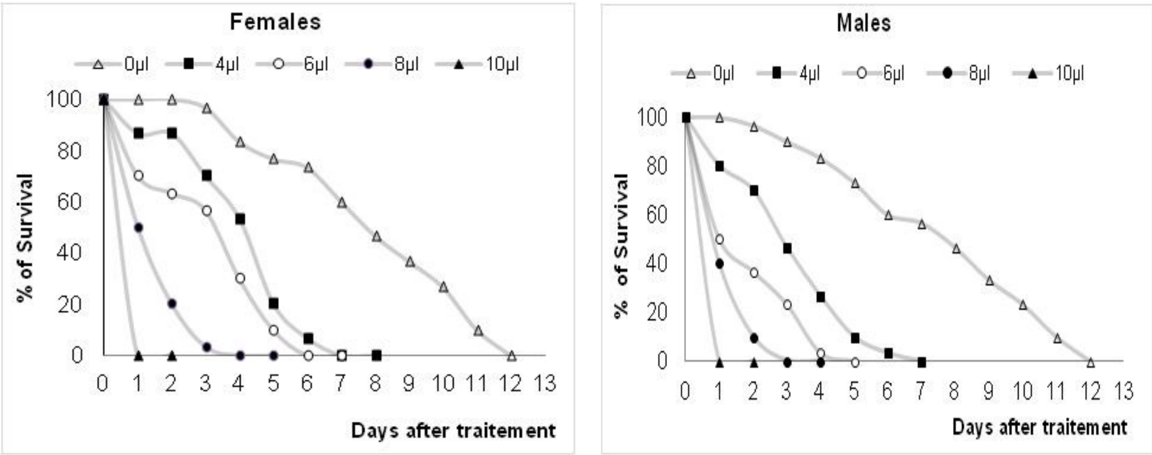

Figure1 Survival curves of *Callosobruchus maculatus* adults fumigated with *Rosmarinus officinalis* essential oils

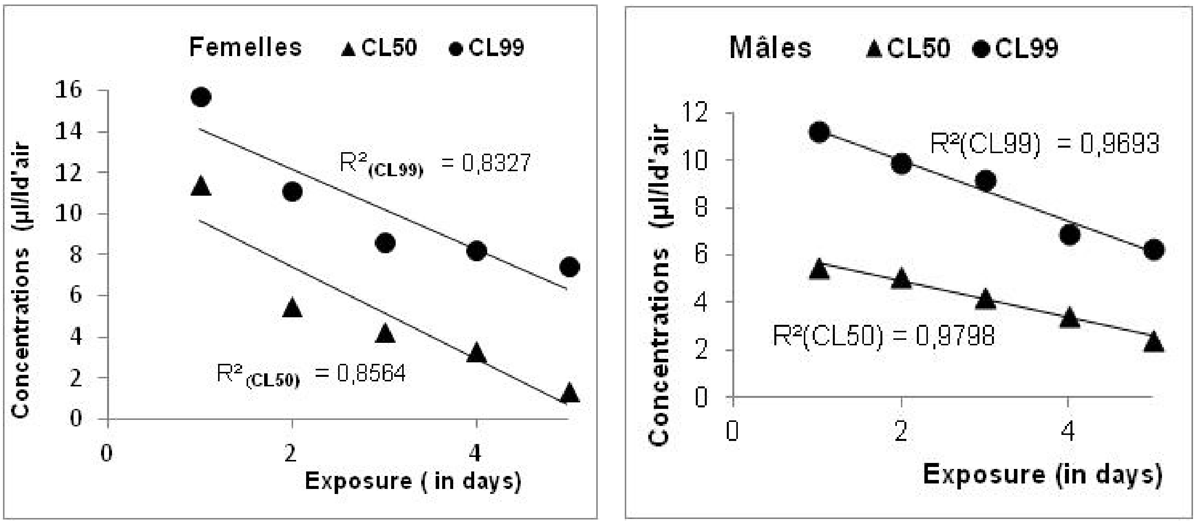

Figure 2 Relationship betweeen the lethal concentrations and the time of exposure of adult *Callosobruchus maculatus* fumigated with *Rosmarinus officinalis* essential oils

Table 1 Compounds of rosemary *(Rosmarinus officinalis)* essential oils

Compounds	RI	Peak Area (%)
α-Pinene	884	0.14
3-Carene	898	12.05
Camphene	1 012	4.83
Sabinene	1 016	0.09
β-Pinéne	1 041	0.39
Limonène	1 049	0.05
Alloocimène	1 054	0.02
Cyclofenchene	1 065	0.09
Limonène	1 069	0.08
Verbenone	1 089	0.05
Camphor oxime	1 095	0.02
1,8 Cinéole	1 099	50.42
Carveol	1 187	0.07
Camphor	1 215	17.73
Borneol	1 240	5.99
Bicyclo[2.2.1]heptanes -2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	1 259	0.11
Géraniol	1 279	0.26
α -Terpineol	1 289	0.35
Linalyl acetate	1 368	0.48
Bornyl acétate	1 508	0.03
Caryophyllene	1 545	0.22
Elemene	1 604	1.95
α –Humulene	1652	1.34
Cedrenol	1770	2.89
Caryophyllene oxide	2138	0.02
Monoterpenes oxygenated		74.93
Monoterpenes carbonated		18.13
Sesquiterpenes oxygenated		2.91
Sesquiterpenes carbonated		3.51
Other		0.13
Total		99.61
Yield (%)		2.68 ± 0.39

Table 2 Lethal times (LT) of *Callosobruchus maculatus* adults fumigated with *Rosmarinus officinalis* essential oils

Sex	Concentration (µL/L air)	LT_{50}	r	LT_{99}	r
Females	**0**	7.62 ± 0.28	-0.89	13.32 ± 0.57	-0.97
	4	3.64 ± 0.26		6.61 ± 0.51	
	6	2.47 ± 0.17		5.35 ± 0.43	
	8	1.37 ± 0.25		4.21 ± 0.48	
	10	0.5 ± 0		2.5 ± 0.20	
Males	**0**	7.59 ± 0.39	-0.95	13.2 ± 0.09	-0.98
	4	2.89 ± 0.42		6.21 ± 0.29	
	6	1.69 ± 0.21		4.13 ± 0.45	
	8	0.97 ±0.11		1.88 ± 0.03	
	10	0.5 ± 0		0.99 ± 0	

Table 3 Toxicity parameters of rosemary essential oils against adults *Callosobruchus maculatus*

Time (days)	Adults (N = 120/genre)	Slopes ± Standard Error	$\chi^2 < \chi^2_{(0.05,\ 2)}$ = 5.991	LC_{50}[CI]* (µL/L d'air)	LC_{99}[CI]* (µL/L d'air)
1	Females	6.38 ± 2.47	11.44	6.80**	15.74**
	Males	7.50 ± 1.13	2.99	5.51[4.96;6.01]	11.24[9.54;14.91]
2	Females	8.51 ± 1.24	5.48	5.92[5.40;6.41]	11.10[9.61; 14.20]
	Males	6.78 ± 1.42	3.71	5.11[4.42;5.69]	9.91[9.79; 12.55]
3	Females	7.74 ± 1.75	4.23	4.84[3.90;5.50]	8.61[7.82;11.09]
	Males	6.33 ± 1.55	2.81	4.25[3.25;4.88]	9.20[7.85;18.19]
4	Females	6.71 ± 1.41	3.32	4.33[3.80;5.14]	8.23[7.80;14.61]
	Males	5.60 ± 1.90	0.10	3.50[2.04;4.08]	6.94[5.67;16.13]
5	Females	4.73 ± 1.89	1.34	3.04[0.48;4.02]	7.44[6.85;92.93]
	Males	4.85 ± 2.4	0.413	2.43[0.0;3.57]	6.33[5.48;9.38]

*: Confidence Intervals * *: Confidence intervals could not be calculated

Table 4 Biological parameters of *Callosobruchus maculatus* fumigated with *Rosmarinus officinalis* essential oils

Biological Parameters	Concentrations (in µl/l air) 0	4	6	8	10
Fecundity	469.33^{a}*±28.54	44.67^{b}±3.51	36.00^{c}±3.61	23.33^{d}±3.05	12.33^{e}±2.52
Fertility	94.09^{a} ±0.90	81.94^{b}± 3.42	78.80^{b}± 2.85	77.69^{b}± 6.40	76.67^{b} ± 8.82
Eggs Mortality	7.03^{a}±1.65	18.06^{b} ±3.42	21.20^{b} ±2.85	22.31^{b} ±6.40	23.33^{b} ± 8.82
Descendants	409.33^{a} ± 21.22	26.33^{b}± 2.52	18.67^{b} ± 1.53	10.33^{c}± 0.58	2.33^{d}±2.08
Success Rates	88.66^{a}± 3.21	58.91^{b}± 1.36	51.92^{c}± 1.04	44.76^{c}±5.84	26.67^{d}±9.78
In-seed Mortality	6.13^{a} ± 2.57	28.03^{b} ±3.32	34.708^{b} ±1.73	42.48^{c} ± 4.39	76.67^{d} ± 20.82

*: At the row level, the means labelled with the same letter do not differ statistically between themselves (One-Factor ANOVA followed by Scheffé's test at 5%)

Table 5 Biodemographic parameters of *Callosobruchus maculatus* fumigated with *Rosmarinus officinalis* essential oils

Concentrations in µL/L air	Net Reproduction Rate/ generation (R_0)	Average Generation Time in days (T)	Intrinsic Rate of Increase r_m (Days)	Lambda λ	Sex Ratio	Doubling Time DT
0	6.82^{a}±0.25	33.83^{a}±0.32	0.02^{a}±0	1.02^{a} ± 0	1.00^{a}±0.03	26.96^{a}± 6.67
4	0.42^{b}±0.05	36.57^{b}±0.45	-0.01^{b}±0	0.99^{a}±0	1.13^{a} ±0.22	**
6	0.34^{b}±0.04	35.86^{b}±0.29	-0.01^{b}±0	0.99^{a}±0	0.81^{a} ±0.17	**
8	0.18^{c}±0.02	34.68^{a}±1.49	-0.02^{c}±0	0.98^{a}±0	1.10^{a} ±0.19	**
10	0.07^{d}±0.0	**	-	-	-	**

*: At de same colon, the means labelled with the same letter do not differ statistically between themselves (One-Factor ANOVA followed by Scheffé's test at 5%)

**: Parameters could not be calculated