INSECTICIDAL ACTIVITY ON *CALLOSOBRUCHUS MACULATUS OF* ESSENTIAL OILS EXTRACTED FROM NATURALLY GROWN AND CULTIVATED ROSEMARY

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ABSTRACT

Bouchikhi Tani, Z., Kechairi, R., Chemouri, S. (2022). Insecticidal Activity on *Callosobruchus Maculatus of* Essential Oils Extracted from Naturally Grown and Cultivated Rosemary. *Lebanese Science Journal*, 23(2): 134-145.

The biological activity of the essential oils extracted from the leaves of Rosemary, Rosmarinus officinalis L. growing naturally (Honaine region, Algeria), and cultivated Rosmarinus tournefortii de Noé (Tlemcen region, Algeria) was tested under laboratory conditions against the chickpea bruchid Callosobruchus maculatus (F.) which causes losses in the field and during storage. The essential oils were tested at three different doses 1, 3, and 5 µL/30 g of seeds, on different biological parameters: adult mortality, female fecundity, and fertility of laid eggs. Results indicated that these essential oils exert varying toxicity levels on the adults of C. maculatus, with a decrease or even inhibition of the fecundity of the females and the fertility of the laid eggs at high doses. However, the essential oils of R. officinalis were more effective than that of R. tournefortii with LD50 of 2.97 and 4.74 µL/30 g of seeds and LT50 of 2.10 and 3.14 days, respectively. Thus, the chemical composition of the major toxic components in the two essential oils, like α-pinene, camphor, β-pinene, cineole, and borneol, extracted from the two national regions is essential to make use of these products as botanical insecticides.

Keywords: *Callosobruchus maculatus, Rosmarinus officinalis, Rosmarinus tournefortii,* Essential Oils, Biocides.

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INTRODUCTION

Fabaceae crops, known as legumes, have been cultivated for a very long time in the world and are an important part of the human diet in many developing countries. They are rich in proteins and can to some extent correct the animal protein deficiencies of a population whose diet is exclusively based on cereals. In Algeria, chickpeas are second only to broad beans. Most of the cultivated area of this species is concentrated in the west of the country (MADR, 2014).

Many plants and their secondary metabolites have physiological and behavioural effects, such as repellence and inhibitory on insect pests (Morallo-Rejesus, 1984). Essential oils extracted from rosemary (Lamiaceae) are already used by researchers in the context of biological control against insect pests which have a remarkable toxic effect (Bouchikhi Tani, 2011; Khoobdel et al. 2017). Essential oil from *Rosmarinus officinalis* (Linnaeus, 1753) is one of the most common EOs and has been extensively used for different purposes. It is currently available for consumers and is exempted from Environmental Protection Agency (EPA) regulation because it is safe for humans and the environment (Waliwitiya et al., 2005).

Callosobruchus maculatus (Fabricius, 1775) (Coleoptera: Bruchidae) is an oligophagous species, whose post-embryonic development takes place in the seeds of different Fabaceae species (Kellouche & Soltani, 2004). The weight losses caused in the stocks can be estimated at more than 800 g/kg of seeds after seven months of storage and these are inedible (Ouedraogo et al., 1996).

Global pesticide sales are expected to exceed US$40 billion (Rana, 2010). Excessive use of pesticides to control pests undermines the natural balance of the agricultural ecosystem; it disrupts parasitoid and predator populations and leads to infestations of secondary pests. It also creates a vicious cycle of pest resistance, which will require new investments in pesticide development (Wood, 2002).

Biopesticides are living organisms or products derived from them, which can suppress or reduce pests. Biopesticides have been used for centuries by farmers and these products offer many advantages. Today, biopesticides are classified into three groups according to their origin (microbial, plant, or animal), Although they often have the reputation for being less effective than chemical pesticides, biopesticides are the subject of growing interest among farmers, particularly in the context of Integrated Pest Management strategies. The marketing of biopesticides is facilitated in some regions, such as the USA, while in others, such as Western Europe, the approval process is long and expensive, the future development of biopesticides depends on many factors, such as government policies both in terms of research support and in regulating agribusiness strategies, and the evolution of consumer choice (Jovana et al., 2014).

The use of bio-pesticides, as plants with insecticidal properties, in some developing countries represents an alternative to chemical control for crop protection (Hall & Menn, 1999). The objective of this study was to evaluate the biological activity of essential oils extracted from the leaves of Rosemary, *Rosmarinus* spp. against the economic pest chickpea bruchid *Callosobruchus maculatus* under laboratory conditions.

MATERIALS AND METHODS

The experimental work was performed at the Laboratory of valorisation of human actions for the protection of the environment and application in public health, Department of ecology and environment, Tlemcen University, Algeria, for 2 months in 2020.

A. Insect and Plant material

1. Mass rearing

The mass rearing of *Callosobruchus maculatus* (Fabricius, 1775) was carried out in three glass jars. Each jar contained 500 g of chickpeas (*Cicer arietnum* L.) grains never treated with insecticides. The chickpea seeds are already infected from a storage warehouse located in Ouled Mimoun Tlemcen. The bruchids directly emerged in the jars, from infested chickpeas. The rearing was performed under controlled conditions of 26°C temperature and 70% relative humidity, in an incubator.

2. Extraction of essential oils

Leaves of rosemary were harvested in March 2020 from Tlemcen region for the cultivated Rosemary species *R. tournefortii* (Noë ex Jord. & Fourr) (Jahand. & Maire, 1934) and from the Honaine region for the naturally grown rosemary (*R. officinalis L.*). At the laboratory, the collected leaves were spread out on paper and left to dry for 10 days at an ambient temperature of 20-23°C.

The extraction of essential oils from the dried Rosemary leaves was carried out at the Applied Biochemistry Laboratory, Biology Department, Abou BekrBelkaid University, Tlemcen Algeria, using a hydro-distillation device assembly (Composed of a round bottom baloon 6 Litre reference: 0925006; 6 litre tank heater Witeg reference DH. WHM12028; Clevenger for 6 Litre balloon with central tube 29/32).

For oil collection, 500g of dry leaves were mixed with 2 L of distilled water and then boiled for about 5 h.

B. Experimental set-up

1. Bio-efficacy of the essential oils

The insecticidal activity of the essential oils extracted from Rosemary has been tested on different biological parameters of the bruchid *C. maculatus.* The collected essential oils were tested at three different doses: 1, 3, and 5µl per 30g of chickpea seeds versus untreated control, with 1ml of acetone, added for the homogeneous dispersion of the essential oil over the entire surface of the chickpeas in the Petri dish. After the total evaporation of the solvent three pairs of *C. maculatus*, aged less than 24h, were introduced. Four replicates for each of the 4 treatments were executed for testing the following biological parameters:

a. Insect mortality

To estimate adult mortality, dead bruchids were counted every 24h for a period of 4 days. The observed mortality was determined after correction by Abbott's formula (Abbott, 1925), on the condition that the mortality in the control is less than 20%, using equation 1 as follows:

$$Pc = \frac{(Po - Pt)}{(100 - Pt)} \times 100 \qquad (1)$$

Where, Pc = corrected mortality in %, Pt: mortality observed in the control, Po: mortality observed in the treatment with essential oil.

b. Fecundity

The eggs laid by the Bruchid adults were counted using a binocular magnifier. The eggs were counted after the total mortality of the adult female Bruchids.

On the other hand, egg fecundity by adult female Bruchid under the experimental conditions was determined, after the total mortality of the females, by the following equation 2:

$$\text{Fecundity of the pest} = \frac{\text{Total number of eggs laid}}{\text{Number of female insects in one Petri dish}} \qquad (2)$$

c. Fertility

Egg fertility was determined based on the number of eggs hatching from laid eggs per Petri dish after about 10 days of treatment. However, egg fertility was determined based on the number of eggs hatching after about 10 days of the test using the following equation 3 (Hamdani, 2012):

$$\text{Egg fertility} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs laid}} \times 100 \qquad (3)$$

2. Statistical analysis

We used the analysis of variance with two classification criteria ANOVA -2 (Dagnelie, 1975); useful for the study of the action of two factors, treatment (the dose used), and time of exposure, on the pest mortality, fecundity, and egg fertility. For comparison of the toxicity of the two essential oils tested, the LD_{50} values were calculated after two days of exposure, using the probit method (Finney, 1971). For this purpose, the mortality percentages were transformed into probits, and the regression of the logarithm of the dose according to the probits of mortality using MINITAB software (version 18) made it possible to determine the lethal dose for 50% of the insect population for each essential oil. Furthermore, to confirm the results obtained, in addition to the LD_{50} we had calculated the lethal exposure time for 50% of the insect population (TL_{50}) using the average dose (3 µl) for the two essential oils tested, by applying the probit method (Finney, 1971). According to previous works, to calculate the LD50 and the TL50, an average dose is always used (Bouchikhi Tani, 2011; Kellouche & Soltani, 2004), in the case of this study this average dose is 3 µl.

RESULTS

Mortality of *C. maculatus* adults in presence of rosemary essential oils is shown in Figure 1. Data analysis for the biological activity of the two essential oils tested indicated that there was no significant difference in % mortality of the Bruchids ($F = 2.48$; $df = 2$; $P = 0.16$) among different doses of the essential oil extracted from *R. Tournefortii* (Figure 1). However, there was a significant difference in % mortality of the Bruchids (F = 40.2; df= 2; P = 0.00) among different doses of the essential oil extracted from *R. officinalis* (Figure 2).

Our results clearly showed that the essential oils of *R. officinalis* and *R. Tournefortii* had a biological effect on adult mortality, fecundity, and egg fertility of the bruchid *C. maculatus*. These essential oils had a remarkable insecticidal (biocidal) effect compared to the control. In fact, the mortality rate in the control under laboratory conditions did not exceed 25% (please note that to apply Abbott's formula, mortality in the control should be between 5 and 20%); whereas using the highest essential oil dose of 5µL/30g of seeds caused adult Bruchid mortality of 66.66 and 100% with the essential oil extracted from *R. Tournefortii* and *R. officinalis*, respectively, after 4 days of exposure.

The statistical analysis showed further that there was no significant difference among the mortality rates according to the two factors oil dose and exposure time to the essential oil of the plant *R. tournefortii*. However, there was a significant difference in the mortality rates for the same mentioned two factors to the essential oil of *R. officinaalis*.

Furthermore, LD_{50} values calculated after two days of exposure showed that the essential oil extracted from *R. officinalis* was more toxic than that extracted from *R. tournefortii* with LD_{50} values of 2.97 and 4.74 µL/30 g of seeds, respectively. Similarly, the TL_{50} values using an average dose of 3µL/30g of seeds confirm that *R. officinalis* essential oil is the most toxic compared to *R. tournefortii* essential oil with TL_{50} values of 2.10 and 3.14 days, respectively.

On the other hand, there was no significant difference in % mortality of the Bruchids (F = 4.66; df= 3; P = 0.05) among different exposure periods to the essential oil extracted from *R. tournefortii* (Figure 1). However, there was a significant difference in % mortality of the Bruchids (F = 40.95; df= 3; P = 0.00) among different exposure periods to the essential oil extracted from *R. officinalis* (Figure 2).

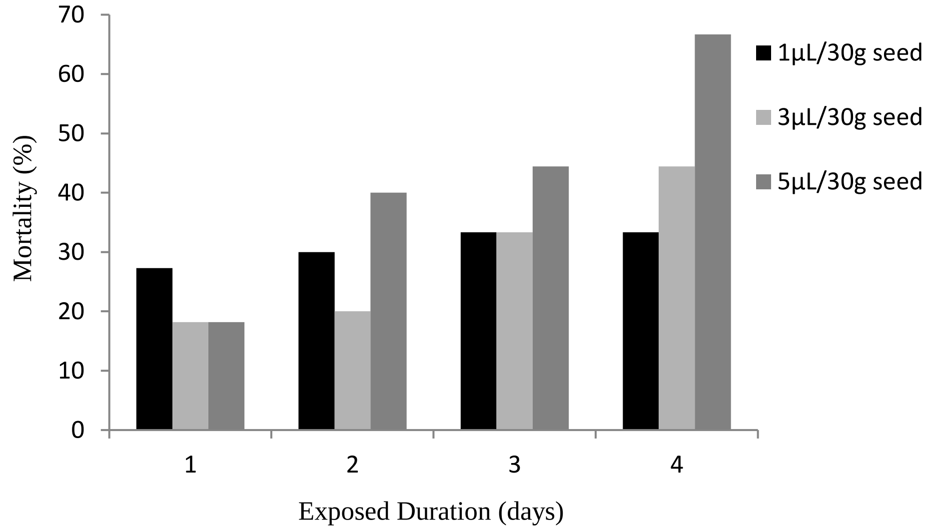

Figure 1. Adult mortality of *Callosobruchus maculatus* in chickpea seeds treated with essential oil of *Rosmarinus tournefortii*.

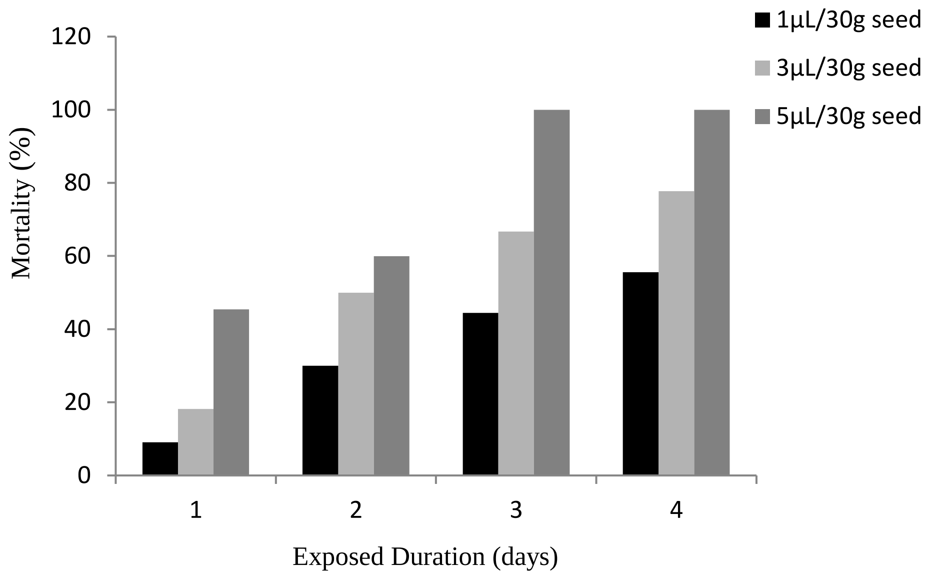

Figure 2. Adult mortality of *Callosobruchus maculatus* in chickpea seeds treated with essential oil of *Rosmarinus officinalis*.

Furthermore, a comparison of the toxicity level of Rosemary essential oils on adults of *C. maculatus* indicated that *R. officinalis* oil is more toxic to the pest at LD_{50} value of 2.97 µL/30 g of seeds compared to the other oil (Table 1). This result is confirmed by the TL_{50} value being 2.10 days for *R. officinalis* oil less than the value of the other tested oil (Table 2).

Table 1. LD_{50} values two days after exposure of *Callosobruchus maculatus* to the essential oils tested.

Plant Species	Regression Equation	LD_{50}
Rosmarinus tournefortii	Y = 4.374 + 0.9267 X	4.74 µL/30 g of seeds
Rosmarinus officinalis	Y = 4.479 + 1.101 X	2.97 µL/30 g of seeds

Table 2. TL_{50} values using the 3 µL/30 g dose of seeds of the two essential oils tested against *Callosobruchus maculatus*.

Plant Species	Regression Equation	TL_{50}
Rosmarinus tournefortii	Y = 4.518 + 0.9690 X	3.14 days
Rosmarinus officinalis	Y = 4.108 + 2.767 X	2.10 days

a. Fecundity of the pest

As the fecundity of the adult female Bruchid was determined after the total mortality of the adults in each replicate, data analysis showed a significant difference in the number of eggs laid per female among doses of the tested essential oils (F = 14.47; df=3; P = 0.02). However, there was no significant difference in the number of eggs laid among doses of each of the tested essential oils (F = 4.7; df= 1; P = 0.12) from the two Rosemary spp. against the adult female insects (Figure 3).

It is important to note that under laboratory conditions the average fecundity of *C. maculatus* females in the control (acetone only) was 32.33 ± 8.01 eggs /female. Under the same conditions, as the seeds were treated with the essential oils extracted from the two aromatic plants, the average fecundity decreased. Thus, the essential oil from *R. officinalis* was more effective than that of *R. tournefortii*; it completely inhibited the fecundity of females at the dose of 5µL/30 g of seeds.

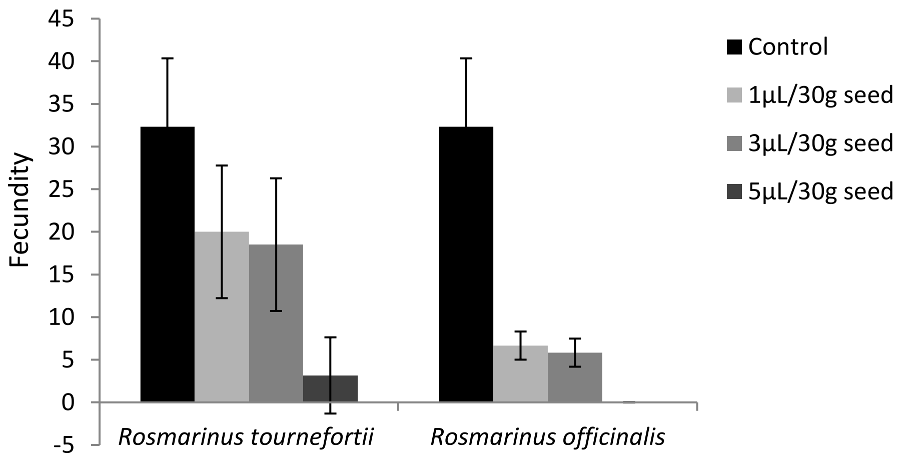

Figure 3. Influence of essential oils on the fecundity of *Callosobruchus maculatus* in presence of chickpea seeds treated with essential oils of two rosemary species (values are average number of eggs±Std. Error).

b. Fertility

The biological activity of the tested essential oils was further determined by the fertility of laid eggs by the adult female Bruchids after the total mortality of the adults in each replicate. Data analysis had shown a significant difference in the % fertility of laid eggs per female among doses of each of the tested essential oils (F = 12.29; df= 3; P = 0.034). Similarly, there was no significant difference in the % fertility of eggs among the essential oils tested from the two Rosemary species (F = 5.62; df=1; P = 0.01) (Figure 4).

Furthermore, in the control, the fertility of the eggs was 25,27± 8,46%, under the laboratory rearing conditions. However, in the presence of essential oils the fertility of the eggs decreased; the essential oil extracted from *R. officinalis* decreased fertility up to 4.76 ± 7.3% at the dose of 3µL/30 g seeds, but totally inhibited fertility at the highest tested dose of 5µL/30 g of seeds.

Thus, it can be concluded that even if Bruchid adults escape the lethal effect of the oils and succeed in laying their eggs, the essential oils can influence egg fertility by reducing the number of hatching eggs, which consequently reduces the damage caused by these insect pests.

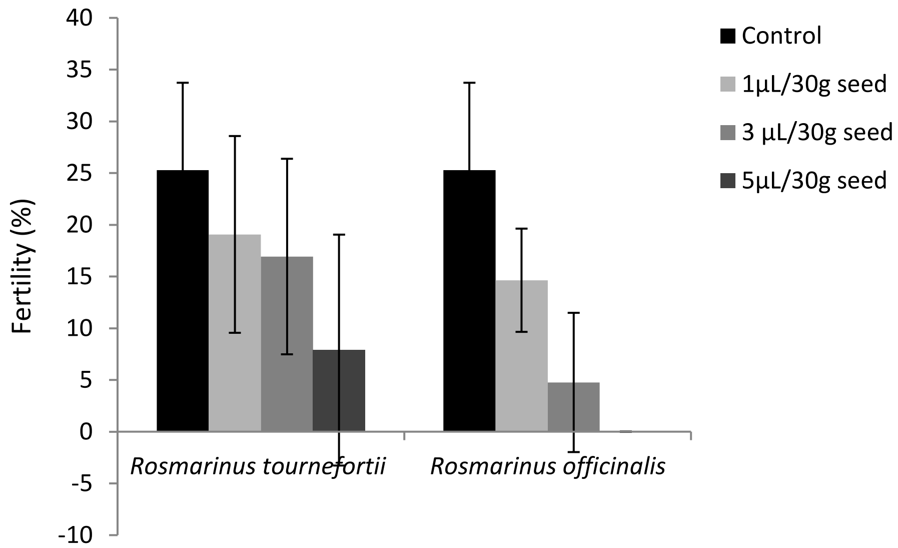

Figure 4. Influence of essential oils on egg fertility of *Callosobruchus maculatus* in presence of chickpea seeds treated with essential oils of two rosemary species (values are % hatched eggs± Std. Error).

DISCUSSION

Our study showed that the fecundity of female Bruchids had decreased when using essential oils compared to that of the control, 32.33± 8.01 laid eggs/female. Moreover, the extracted oil *R. officinalis* was more effective than *R.tournefortii because* it completely inhibited the fecundity of females at the dose of 5µL/30 g of seeds. The statistical analysis confirmed further a significant difference between the average fecundity rates according to the oil dose factor, but there was no significant difference according to the second factor, which is the plant species used. Similarly, Kellouche and Soltani (2004) found that essential oils extracted from cloves (Eugenol) inhibit the fecundity of C. *maculatus* females at a dose of 5 µL.

In comparison with other Bruchid species, Bouchikhi-Tani (2011), tested the effectiveness of ten essential oils extracted from aromatic plants in the Tlemcen region on the dried bean beetle, *Acanthoscelides* obtectus Say (Coleoptera: Chrysomelidae, Bruchinae). Examination of the LD_{50} values after two days of exposure allowed him to deduce that LD_{50} values for *A. obtectus* were lower than that of *C. maculatus*. The most effective essential oils are those extracted from *R. officinalis, Origanum glandulosum, and Artemisia* herba-alba with LD_{50} values of 0.59, 1.44, and 1.69µL/30g of seeds, respectively. Hamdani (2012) also tested the effectiveness of oils extracted from the leaves of lemon, orange, grapefruit, and sour orange; these oils were found to affect the longevity of adults of *A. obtectus* at the lowest dose of 2 µL, but the oil extracted from sour orange was the most toxic to this pest.

For other Bruchid spp., Hamdani (2012) found that the fecundity of female *A. obtectus* declines to 20 eggs/5 females using the 8 µL dose of lemon, orange, and grapefruit oil, and 12 eggs/5 females using only the 4 µL dose of sour orange oil as the latter completely inhibits egg laying with the 6 µL dose. Bouchikhi-Tani (2011) found further that the fecundity of female *A. obetctus* was completely inhibited by essential oils extracted from *R. officinalis* and *Artemisia herba-alba* with a dose greater than or equal to 5 µL / 30 g of seeds. Regnault-Roger & Hamraoui (1993) indicated that aromatic plants of the genera: Mentha, Origanum, Rosmarinus, *Thymus,* cause a significant decrease in oviposition of the females of the bruchid A. *obtectus.*

Under the same rearing conditions and in presence of the essential oils of the two aromatic plants tested *R. tournefortii* and *R. officinalis*, the fertility of the eggs decreased to 7.89 ± 11.16and 0.00 ± 0.00 %, respectively, using the dose of 5 µL/30g of seeds; in comparison to the control which had shown an average egg fertility of 25.27 ± 8.46%. These results were confirmed further by the analysis of variance with two classification criteria; a significant difference between the egg fertility averages according to the dose factor of essential oils was detected, whereas there was no significant difference according to the plant factor. Similarly, Aiboud (2011) found that essential oils extracted from myrtle, oregano, eucalyptus, and clove closes were effective against the hatching of *C. maculatus* eggs; analysis of variance with two classification criteria revealed a highly significant difference for the plant factor and not significant for the dose factor.

On the other hand, for the Bruchid sp. *A. obtectus*, Hamdani (2012) found that the number of hatching eggs decreased as the oil dose increased; with 129.68 ± 0.36 eggs/5 females of *A. obtectus*in the control versus the decrease to an average of 78.75±21.35 eggs/5 females for all the oils tested namely lemon, orange and grapefruit at a dose of 2 µL. Whereas oil extracted from sour orange reduced the hatching of eggs to less than 5 eggs / 5 females at the 4 µL dose, and the oil extracted from lemon reduced hatching to less than 15 eggs/5 females at the 6 µL dose.

According to the study carried out by Khoobdel et al. (2017), the opportunity to enhance the insecticidal activity of Rosmarinus officinalis essential oil was studied for effective management of the red flour beetle, Tribolium castaneum, the fumigant toxicity of the non-formulated oil and nanocapsules of R. Officinalis were investigated at 13.20, 15.92, 19.12, 23.04, and 27.76 µL/L air after 24 and 72 h exposure, and the contact toxicity of the non-formulated oil and nanocapsules were investigated at 4.28, 3.55, 2.95, 2.45 and 2.36µL/cm2 after 24 h exposure.

Among aromatic plants, the Lamiaceae (mint Family) remains the most insecticidal plant family. These species are well known for their composition of essential oils and polyphenolic substances capable of protecting plants against attacks by insect pests (Regnault-Roger & Hamraoui, 1994). Bouchikhi-Tani (2011) indicated that the effectiveness of essential oils extracted from aromatic plants is due to the presence of the major components known for their insecticidal properties and their egg-laying inhibiting effects, such as 1,8-cineole, camphor, thymol, α-pinene, β-pinene, α-terpineol, carvacrol, and limonene.

Thus, the detected biocidal activities in our study would be related to the major compounds in Rosemary spp. Especially that Atik-Bekkara et al. (2007) who conducted a study in the same harvest regions (Tlemcen and Honaine regions) found that the main compounds in *R. officinalis* (in decreasing order) are: α-pinene (23.1%), camphor (15.3%) and β-pinene (12.2%); whereas the main compounds in *R. tournefortii* (in decreasing order) are: camphor (13.8%), α-pinene (12.6%), cineole (11.8%) and borneol (10.8%). However, Outaleb (2016) found that the major compound in *R. officinalis* and *R. tournefortii*is camphor with 41.6 and 40.7%, respectively in the Mostaganem region, but the major compound was α-pinene with 51.8 and 24.7%, respectively in the Beni Yenni region. These results confirm the fact that botanical components of the same plant species might change between locations and harvesting seasons in the same country as well as among different countries in the world (Outaleb, 2016). Knowing that oils are secondary plant metabolites whose composition and yield will depend on climate and habitat conditions, planting and harvesting methods, plus genetics and plant age (Taiz & Zeiger, 2010; Derwich et al., 2011). Hence, further research studies with Rosemary essential oils against storage pests are needed to investigate the level of health safety if treated seeds with these oils are to be consumed by humans and other mammals.

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