

Research Article

Essential Oils from *Artemisia herba alba* Asso., *Maticaria Recutita* L., and *Dittrichia Viscosa* L. (Asteraceae): A Promising Source of Eco-Friendly Agents to Control *Callosobruchus maculatus* Fab. Warehouse Pest

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Callosobruchus maculatus (Fab.) (*C. maculatus*) is one of the major pests of legume seeds in storage causing significant damage, leading to food insecurity and low income for farmers. This work was planned to develop eco-friendly agents from essential oils of *Artemisia herba alba* Asso. (AEO), *Maticaria Recutita* L. (MEO), and *Dittrichia Viscosa* L. (DEO) to control *C. maculatus*. To achieve this goal, essential oils (EOs) were extracted by hydro-distillation using Clevenger apparatus before being characterized by GC-MS. EOs were used for testing purposes using three different tests, namely, inhalation toxicity, contact toxicity, and repellency tests. GC-MS analysis of EOs showed the presence of 16 potentially active compounds in AEO and 38 in MEO, whilst 15 compounds were identified in DEO. AEO was higher in thujone (57.6%) and chrysanthenone (11.8%). Santolina alcohol (40.7%) and germacrene D (8.9%) were the major compounds identified in MEO, whereas isocostic acid (72.3%) was the chief compound of DEO. The obtained findings showed that the studied EOs showed considerable insecticidal activity against *C. maculatus* with a lethal dose (LC₅₀) of 3.78, 8.86, and 14.34 $\mu\text{L}/1$ liter of air by AEO, MEO, and DEO, respectively. At 1 $\mu\text{L}/1$ liter of air, the oviposition reduction rate was 90.02%, 70.65%, and 48.23% by AEO, MEO, and DEO, respectively, whereas the emergence reduction rate was 87.32%, 60.08%, and 32.24% by AEO, MEO, and DEO, respectively. With increasing doses up to 20 $\mu\text{L}/\text{L}$, the reduction of individual emergence reached 98.8% by AEO of 24 h after treatment. AEO, MEO, and DEO showed significant repellent effects against adults of *C. maculatus* with repulsion percentages of 60.83%, 50.83%, and 72.5%, respectively. The outcome of this work suggests that the essential oils of the studied plants, particularly *Artemisia herba alba* Asso. oils, can constitute a natural and environmentally friendly alternative to develop new bioinsecticides for the control of *C. maculatus*.

1. Introduction

Aromatic and medicinal plants and their extracts have been traditionally used as plant-protection agents in various ancient cultures history recorded. However, chemically synthesized insecticides have gradually replaced natural insecticides during the 20th century. With the massive and sometimes irrational use of chemically synthetic insecticides generated in recent years, people are becoming increasingly aware of their harmful effects on human health and the environment besides their effectiveness in crop protection. Most of these synthetic insecticides are nondegradable and accumulate in the environment or the human body through food chains, often causing chronic diseases and other severe physiological disorders [1–5]. Although these chemicals have been more strictly regulated, it is necessary to explore new, safe, and environmentally friendly alternatives with considerable insecticidal efficacy.

Plant EOs are mixtures of bioactive, fragrant, volatile, and lipophilic secondary metabolites that are also synthesized to defend against various plant pathogens. Essential oils have been scientifically studied worldwide to control postharvest pests of cereals, legumes, citrus, tomatoes, and food-borne microorganisms [6–9].

Several studies on natural bioinsecticides have revealed that essential oils along with other secondary plant metabolites (terpenoids, polyphenols, steroids, and alkaloids) synthesized by aromatic and medicinal plants are responsible for several biological activities against plant pests, especially essential oils which have shown important insecticidal, nematocidal, acaricidal, and larvicidal properties [10–20]. Essential oils not only act as poisons or neurotropic on the nervous system of insects [21–23] but also can affect cytochrome P450, which plays a key role in the insect detoxification system, alter insect metabolism (e.g., ATP synthesis, Krebs cycle, oxidative phosphorylation, and others) [24]. Overall, the final action of EOs may be the result of more than one specific interaction with the targets, so that the possibility of insects developing resistance is greatly reduced [25].

Callosobruchus maculatus (Fab.) (Chrysomelidae), known as cowpea weevil, is one of the most well-known storage pests causing damage to chickpea (*Cicer arietinum*) and other leguminous crops, particularly in Africa and tropical and South American regions [26–28]. The negative economic impacts caused by this insect are related to the penetration of the larvae to feed inside the grain causing a significant loss of weight and a reduction in nutritional values and germination potential [29–32].

The *Asteraceae* family contains a large number of plant species, with more than 1600 genera and over 23,000 species. Some of these species, such as *Matricaria recutita* L., *Artemisia herba alba* Asso., and *Dittrichia viscosa* L. have already been reported to have important medicinal and nutraceutical applications. Plants belonging to the family *Asteraceae* can be used as insecticides thanks to the variety of bioactive molecules present in the EOs of several species.

Therefore, this study aimed to evaluate the efficacy of EOs from *Artemisia herba alba* Asso. and *Matricaria Recutita* L. and *Dittrichia Viscosa* L. in the control of *C. maculatus* because no similar has been done up to date.

2. Materials and Methods

2.1. Animal Material. For testing purposes, *C. maculatus* was isolated from a sample of chickpeas stored in the Fez. city of Fez-Morocco. Mass rearing of *C. maculatus* was performed in glass jars containing *Cicer arietinum* (chickpea) seeds. The jars were maintained at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity, and a photoperiod of 14:00 (light)/10:00 (dark) to obtain several successive generations of the insect. The nonwinged form of the adults with greater reproductive capacity was selected for testing. The active/inactive form was determined both by the presence of flight activity, the size of the elytra, and the intensity of pigmentation on the elytra [33].

2.2. Plant Material. In this work, three medicinal and aromatic plants growing in different regions in Morocco were collected for testing purposes (Table 1).

2.3. Extraction of Essential Oils. The aerial parts of the plants were dried in the shade and ventilated area with a temperature of about 25°C for 5 days before distillation. The extraction of EOs from different aromatic and medicinal plants was conducted using a Clevenger apparatus [34]. Briefly, 200 g of each plant aerials part previously crushed was introduced into a flask with 1200 mL of distilled water. Next, the whole was boiled for 3 hours until complete extraction. The obtained essential oils were dehydrated on anhydrous sodium sulfate and stored at 4°C until further use.

The essential oil yield in percentage was calculated by the following formula:

$$Y_{\text{HE}} = \frac{M_{\text{HE}}}{M_{\text{D}}} \times 100. \quad (1)$$

Here, Y_{HE} is the yield of essential oil (%), M_{HE} is the mass of the EO (g), and M_{D} is the mass of dry plant matter (g).

2.4. Analysis of the Chemical Composition of Essential Oils. The analysis of the essential oils was performed by gas chromatography-mass spectrometry (GC-MS). The coupling was performed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and an HP-5MS capillary column (30 m \times 0.25 mm, film thickness 0.25 μm). Temperature programming from 35°C to 250°C , with a gradient of $5^\circ\text{C}/\text{min}$. Retention indices were determined by gas chromatography on two fused silica capillary columns (30 m \times 0.25 mm) from Agilent Technologies, Little Falls, CA, USA, with temperature programming from 35°C to 250°C at a rate of $5^\circ\text{C}/\text{min}$, with the lower and upper temperatures maintained for 3 and 10 minutes,

TABLE 1: Plant species used for the extraction of essential oils.

Species studied	Voucher specimen ID	Family	Sample site	Harvest period	Parts used
<i>A. herba alba</i>	BA22/13009	Asteraceae	Boulemane	June 2019	Leaves
<i>M. recutita</i>	BM19/09549	Asteraceae	Taounate	April 2019	Leaves
<i>D. viscosa</i>	BD01/00282	Asteraceae	Fez	August 2019	Leaves

respectively. The carrier gas (helium) flow rate was 1.0 mL/min. A 1.0 μ L sample was injected in splitless mode. The essential oil constituents were identified by comparing their mass spectra with those of the NIST02GC/MS library data and the Adams.

2.5. Toxicity of Essential Oils

2.5.1. Contact Toxicity. To evaluate the effect of each EO, 100 g of seed infested by five pairs of insects (male and female) aged 0 to 48 h were used. The tests were packaged in glass containers (1 L) duly closed with a perforated lid and covered with a thin transparent cloth. The essential oils were added directly to the seeds with a pipette, followed by manual shaking for 2 minutes. After 48 h of confinement, adult mortality was assessed [35]. A control for each test was performed under the same conditions without the EOs. Based on the results obtained in the preliminary tests, treatments with increasing concentrations (1, 5, 10, 20 μ L/1 liter of air) were performed. After 24 hours, mortality was assessed, and dead insects were removed. Eggs deposited in the grains were also counted after 12 days from the start of the tests, followed by a regular count of emerged insects from Day 28 after confinement.

The observed mortality rate was corrected by the Abbott formula as follows:

$$Pc = 100 \times \frac{Po - Pt}{100 - Pt} \quad (2)$$

Here, **Pc** is the corrected mortality percentage (%), **Po** is the mortality observed in the test, and **Pt** is the mortality observed in the control.

The reduction percentage in the number of eggs and adults emerging in each concentration of essential oil was calculated using the following formula:

$$PR = \frac{NC - NT}{NC} \times 100. \quad (3)$$

Here, **PR** is the percentage of oviposition (%), **NC** is the number of eggs laid in the control, and **NT** is the number of eggs in the experiment.

2.5.2. Inhalation Toxicity. In 1 L glass jars with tightly closed lids, each containing 10 *C. maculatus* bruchids (male and female) ranging in age from 0 to 48 h, small cotton balls were suspended with a thread attached to the inside of each lid. Using a micropipette, doses of 1 μ L, 5 μ L, 10 μ L, and 20 μ L/L of each essential oil were each deposited into the above cotton balls. For each dose, three replicates were performed. The comparison was made to a control sample (cotton without essential oil) [36].

The observed mortality rate is corrected by the Abbott formula and calculated as follows:

$$Pc = 100 \times \frac{Po - Pt}{100 - Pt} \quad (4)$$

Here, **Pc** is the corrected mortality percentage (%), **Po** is the mortality observed in the test, and **Pt** is the mortality observed in the control.

2.6. Repulsion Test. The repellent effect of the studied essential oils on *C. maculatus* adults was evaluated using the preferential area method on filter paper described by McDonald et al. [37]. Briefly, 9 cm diameter filter paper discs used for this purpose were divided into two halves with 31.80 cm² area in each. Next, 0.5 mL of each EOs solutions previously prepared in acetone (1, 5, 10, and 20 μ L/mL of acetone) was uniformly spread on one of the two disc halves in order to obtain doses such as 0.016, 0.079, 0.157, and 0.315 μ L/cm² per disc. While the other half received only 0.5 mL of acetone. The Petri dishes were then sealed with Parafilm. After 30 minutes, the number of bruchids presents on the half of the disc treated with the essential oil was counted against the number on the untreated part. Three replicates for each experiment were done under the same environmental conditions as the insect breeding.

The percentage of repulsion (**PR**) was calculated according to the following formula [38]:

$$PR = \frac{NC - NT}{NC + NT} \times 100. \quad (5)$$

Here, **PR** is the percentage of repulsion (%), **NC** is the number of insects in the control area, and **NT** is the number of insects in the treated area.

The average percentage of repulsion was calculated for each EO assigned to one of the different repulsive classes ranging from 0% to 100% [39].

2.7. Data Analysis. SPSS for Windows[®] (version 21.0) statistical software was used to perform analysis. Data were treated with a one-way analysis of variance (ANOVA) to determine the difference between the extreme values of the group. Fisher's minimum significant difference (LSD) test was used to separate significant from nonsignificant means at $\alpha = 0.05$. Lethal concentrations LC_{50} and LC_{95} with their confidence intervals were determined using the probit method [40].

3. Results

3.1. Essential Oil Yield. The extracted EOs were found to be different in terms of specific characteristics, especially in odor and color. The values of the essential oil yields varied

from one species to another with highly significant differences ($F = 1179.71$; $P < 0.001$). From Figure 1, it can be seen that the highest yield of EOs was recorded for *A. herba alba* with a value of $1.18 \pm 0.15\%$, followed by *M. recutita* ($0.45 \pm 0.12\%$) and *D. viscosa* ($0.23 \pm 0.06\%$).

3.2. Chemical Composition of Essential Oils. The compounds identified in the EOs of the three studied plants along with their retention index and percentages are figured in Figures 2–4 and Table 2, whereas the chemical formulas of the chief compounds are presented in Table 3. The obtained results revealed the presence of 16 potentially bioactive compounds in AEO and 38 in MEO, whilst 15 compounds were identified in DEO. The main compounds identified in AEO were thujone (57.6%) and chrysanthenone (11.8%). Santolina alcohol (40.7%) and germacrene D (8.9%) were the major compounds identified in MEO, whereas, isocostic acid (72.3%) was the chief compound of DEO.

3.3. Inhalation Toxicity of Essential Oils. In this test, EOs at different doses (0, 1, 5, 10, and 20 $\mu\text{L}/1$ litre air) were used to evaluate their toxicity against *C. maculatus* through inhalation. Adult mortality was recorded every 24 hours for 4 days. The obtained results are illustrated in Figures 5–7.

According to the results obtained, most of the essential oils studied showed a considerable insecticidal effect with a significant difference ($P < 0.001$) in the longevity of the treated adults depending on the dose and duration of exposure. The mortality of *C. maculatus* adults increased with increasing doses and duration of exposure to essential oils. A reduction in mortality was observed in chickpea bruchid adults treated with the lowest dose (1 μL) of *A. herba alba* essential oils causing total mortality after 96 h of exposure, thus reflecting their powerful insecticidal effect. At higher doses, and after 96 h of exposure, all three essential oils show a total insecticidal effect at the 20 μL dose.

The probit method (Table 4) allows calculating the lethal concentrations (LC50) which cause the mortality of 50% of the adults of *C. maculatus* treated with essential oils. The results obtained (Table 3) allow us to classify the tested EO from the most toxic to the least toxic according to the following order: *A. herba alba*, *M. recutita*, and *D. viscosa* whose LC50 are, respectively, 2.18, 4.09, and 19.96 $\mu\text{L}/1$ litre of air volume.

3.4. Contact Toxicity of Essential Oils. The action of essential oils of different aromatic plants by direct contact on the biology of the *C. maculatus* insect was also studied. In this regard, the tests performed concerned the longevity of the adults, the fecundity of the females, and the viability of the eggs. In view of the results illustrated in Figures 8 to 10, all the essential oils tested showed a more or less significant insecticidal effect depending on the dose and duration of exposure.

In general, mortality of *C. maculatus* adults increased significantly ($P < 0.001$) with increasing EO doses, or when contact time with the oil was extended to nearly 96 h. EOA

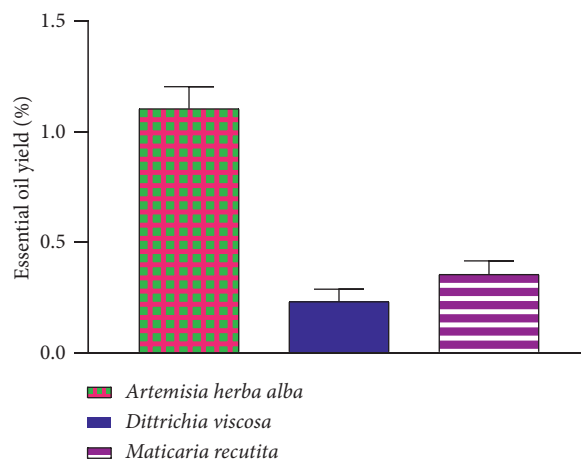


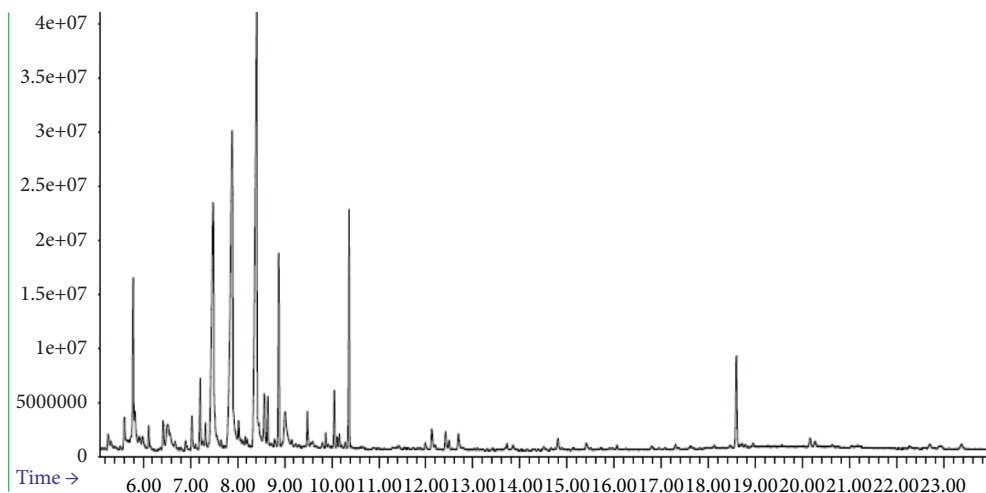
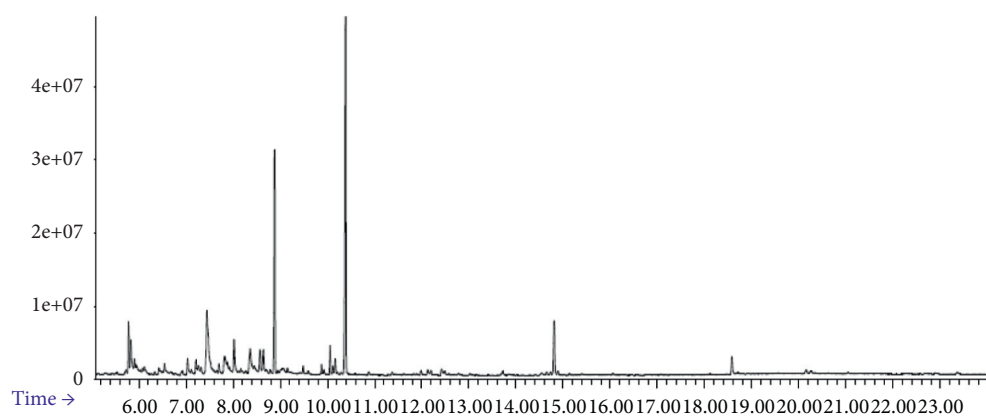
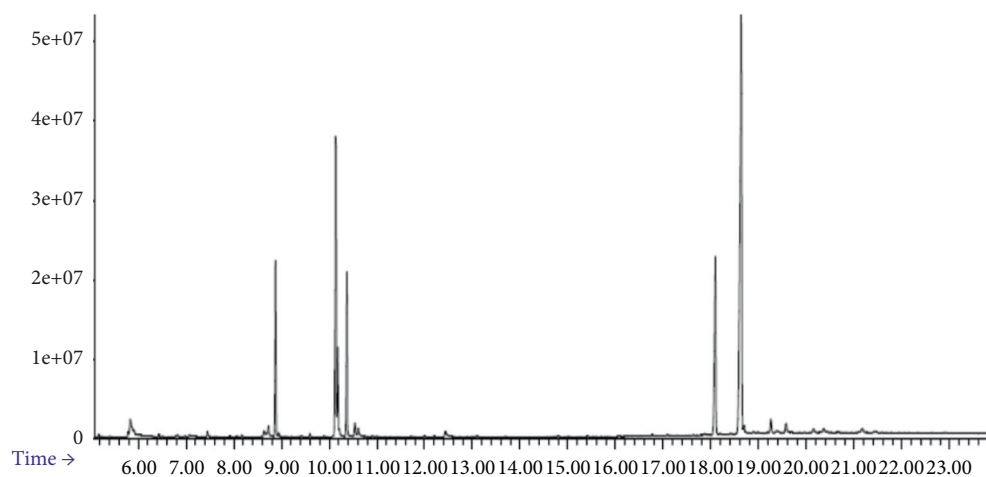
FIGURE 1: Essential oil yield of the studied plants.

was found to be the most toxic against *C. maculatus* with total mortality (100%) recorded for a dose of (5 μL) 4 days postcontact. In the case of EOM and EOD, total mortality (100%) was achieved by increasing the doses up to 10 μL .

Based on the results of the contact test with EOs at different doses, the lethal concentrations LC₅₀ and LC₉₅ causing mortality of 50% and 95% of *C. maculatus* adults respectively were calculated according to the Probit method (Table 5), and the obtained values are shown in Table 4. This allowed us to classify the EOs tested by contact according to their degree of toxicity from the most toxic to the least toxic using the following classification: EMA, EOM, and EOD whose LC50 are respectively 3.78, 8.86, and 14.17 $\mu\text{L}/1$ litre of air.

3.5. Effect of Direct Contact with Essential Oils on Fecundity and Emergence of *Callosobruchus maculatus* Fab. Individuals. Fecundity in *C. maculatus* occurred within 24 h after mating. In this contact test, the fecundity process was evaluated in the presence of EOs of different aromatic plants. The obtained results are displayed in Figure 11. From this figure, it can be seen that the number of eggs laid varies according to the EOs and the doses used. Thus, the average fecundity in the control batches was 184.67 ± 17.78 eggs/five females. This process decreased significantly ($P < 0.001$) with increasing doses of EOs. Indeed, at the highest dose (20 μL), EOA, EOD, and EOM significantly affect the fertility of *C. maculatus*, which was reduced to 6 ± 2 , 19.33 ± 7.50 , and 57.66 ± 12.58 eggs/female, so that the percentage of reduction was of 96.79%, 89.69%, and 68.95%, respectively (Figure 12).

After the emerged individuals of *C. maculatus* completed their developmental cycle, they were subjected to evaluation of emergence under different doses of EOs and the obtained results of the test are presented in Figures 13 and 14. In this regard, EOs significantly reduced number of *C. maculatus* emergences when compared to the control batches (111.67 ± 4.44) ($P < 0.001$). Moreover, at 20 $\mu\text{L}/1$ litre of air, only 1.33 ± 0.57 , 9.67 ± 2.31 , and 21.67 ± 2.03 individuals emerged in the presence of EOA, EOM, and EAD respectively.

FIGURE 2: Chromatogram of *Artemisia herba alba* Asso.essential oil.FIGURE 3: Chromatogram of *Matricaria recutita* L. essential oil.FIGURE 4: Chromatogram of *Dittrichia viscosa* L. essential oil.

3.6. *Repulsive Activity of Essential Oils.* The results of the repulsion test are given in Figure 15. Overall, the EOs were found to be repulsive to adults of *C. maculatus*. The obtained results showed that the repulsive activity of the studied EOs was in a dose-dependent manner. From Table 5, it can be

seen that with increasing concentration up to 20 μ L, the mean of repulsive activity by EOD reached 72.5%. Regarding the repulsive activity, the analysis of variance showed significant differences ($P < 0.001$) in EOM and EOD towards *C. maculatus* (Figure 15).

TABLE 2: Chemical composition of essential oils from *Artemisia herba alba* Asso., *Matricaria recutita* L., and *Dittrichia viscosa* L.

Compounds	RI	Relative percentage		
		<i>A. herba alba</i>	<i>M. recutita</i>	<i>D. viscosa</i>
α -Pinene	937	0.6	5.7	–
Camphene	954	2.3	0.2	–
Sabinene	972	0.7	0.1	–
b-Pinene	974	–	0.3	–
Myrcene	992	–	0.5	–
Yomogi alcohol	986	–	3.0	–
p-Cymene	1025	0.2	0.1	–
Limonene	1028	–	3.1	–
1,8-Cineole	1030	11.7	0.3	–
Santolina alcohol	1037	–	40.7	–
g-Terpinene	1072	0.3	–	–
Artemesia alcohol	1080	–	4.3	–
a-Thujone	1109	29	–	–
b-Thujone	1112	28.6	–	–
Campholenal	1121	–	0.1	–
(E)-Pinocarveol	1139	–	1.3	–
Camphor	1146	0.2	–	–
Chrysanthenone	1158	14.8	–	–
Borneol	1164	0.1	0.3	–
Pinocarvone	1166	–	1.9	–
Santolinaacetate	–	–	2.2	–
Terpinen-4-ol	1173	–	0.3	–
trans-Pinocarveol	1178	1.9	–	–
a-Terpineol	1199	–	0.2	0.1
Hexylisovalerate	–	–	0.1	–
Acetate de bornyl	1289	0.1	1.0	–
Phenethylacetate	–	–	0.1	–
trans-Sabinyacetate	1299	0.3	–	–
α -Copaene	1377	–	0.1	–
b-Bourbonene	1380	0.2	–	–
β -Maaliene	*	–	0.4	–
β -Elemene	1392	–	0.6	–
B-Caryophyllène	1419	–	1.7	1.8
(E)- β -Farnesene	1456	–	4.0	–
γ -Curcumene	1479	–	–	0.3
α -Humulene	1480	–	0.4	–
Germacrene D	1492	4.3	8.9	–
δ -Selinene	1493	–	–	0.2
α -Muurolene	1503	–	1.0	0.7
δ -Cadinene	1520	–	1.1	–
trans- γ -Cadinene	1513	–	–	0.1
(Z)-Nerolidol	1536	–	0.3	–
Germacrene D-4-ol	1570	–	1.6	–
Caryophylleneoxide	1579	–	0.9	–
Caryophylladienol	–	–	2.8	–
10-Epi- γ -eudesmol	1623	–	–	3.4
Caryophylla-4(14),8(15)-dien-5-ol	1636	–	–	0.6
Epi-a-cadinol	1639	–	1.2	–
Epi- α -muurolol	1640	–	1.6	–
α -muurolol	1641	–	0.6	–
a-Cadinol	1643	–	1.4	–
Cedr-8(15)-en-9- α -ol	1650	–	–	0.4
Selin-11-en-4- α -ol (=Kongol)	1653	–	–	2.5
Ylangenal	1675	–	–	2.4
Junipercamphor	1692	–	–	5.1
Isocosticacid	1890	–	–	72.3
Phytolacetate	2223	–	–	0.9
Monoterpenes		90.4	62.5	0.1
Sesquiterpenes		4.5	30.8	89.8

TABLE 2: Continued.

Compounds	RI	Relative percentage		
		<i>A. herba alba</i>	<i>M. recutita</i>	<i>D. viscosa</i>
Others		0.4	3.3	0.9
Total identified		95.3%	96.6%	90.8%

TABLE 3: Major compounds of essential oils of *Artemisia herba alba* Asso., *Matricaria recutita* L., and *Dittrichia viscosa* L.

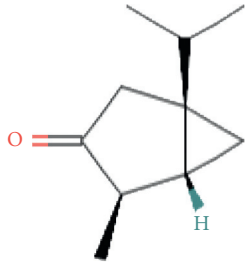
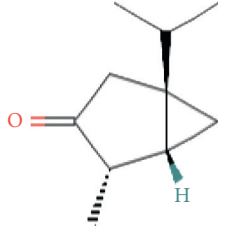
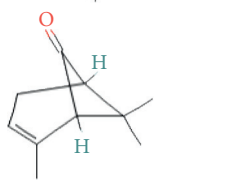
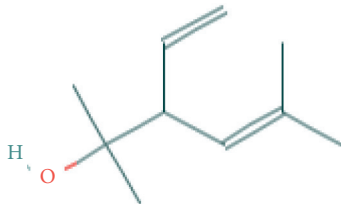
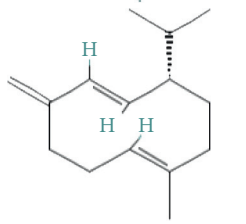
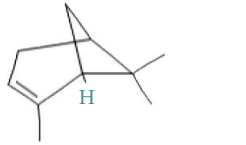
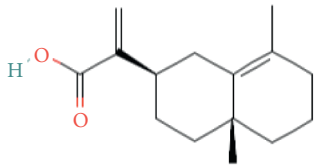
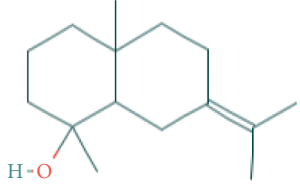
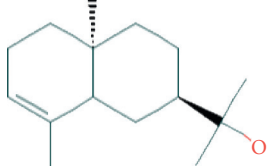
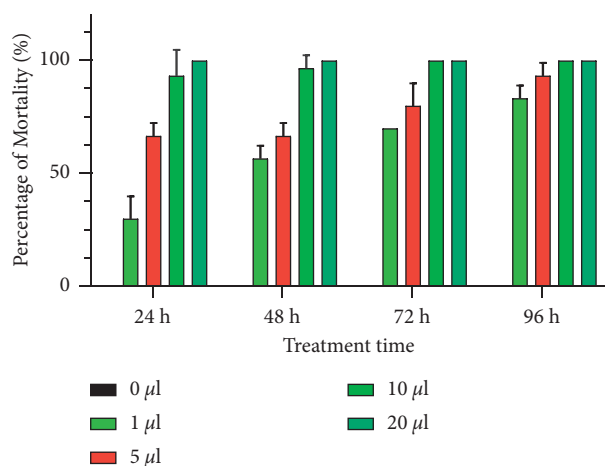
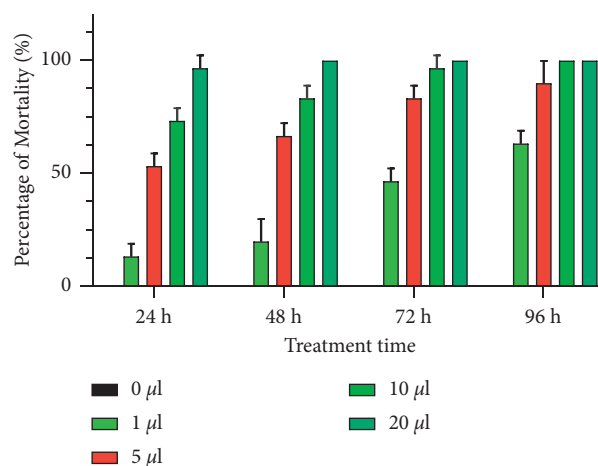
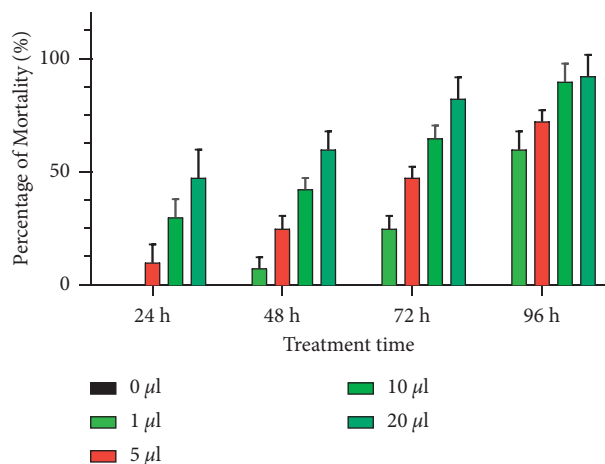
Plant species	Major compounds	Relative percentage	Chemical formula	Chemical structure
<i>A. herba alba</i>	a-Thujone	29	C ₁₀ H ₁₆ O	
	b-Thujone	28.6	C ₁₀ H ₁₆ O	
	Chrysanthenone	14.8	C ₁₀ H ₁₄ O	
	Santolina alcohol	40.7	C ₁₀ H ₁₄ O	
<i>M. recutita</i>	Germacrene D	8.9	C ₁₅ H ₂₄	
	Alpha-pinene	5.7	C ₁₀ H ₁₆	

TABLE 3: Continued.

Plant species	Major compounds	Relative percentage	Chemical formula	Chemical structure
	Isocostic acid	72.3	$C_{15}H_{22}O_2$	
<i>D. Viscosa</i>	Juniper camphor	5.1	$C_{15}H_{26}O$	
	10-Epi- γ -eudesmol	3.4	$C_{15}H_{26}O$	

FIGURE 5: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to the inhalation test of *Artemisia herba alba* L. essential oils.FIGURE 7: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to the contact test of *Matricaria recutita* essential oils.FIGURE 6: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to the inhalation test of *Dittrichia viscosa* L. essential oils.

The percentage of repellency calculated by the method of McDonald et al. [37] showed that the EOA and EOD were repulsive with respective rates of 60.83% and 72.5%, whereas, EAM was moderately repulsive (50.83%).

4. Discussion

This study aimed to investigate the chemical composition and insecticidal activity of EOs from *A. herba alba*, *M. Recutita.*, and *D. Viscosa* on *C. maculatus*. The obtained results in this context showed that EOs yields in these plants ranged from $0.23 \pm 0.06\%$ to $1.18 \pm 0.15\%$. It is thus fitting that these yields were relatively low when compared to some industrially exploited plants as sources of essential oils [41]. Many factors influence the yield, content, physicochemical characteristics, and chemical composition of essential oils such as plant species, environmental conditions, extraction technique, drying, harvesting time and environment, cultivation practices, and age of plant material [39].

TABLE 4: LC50 and LC95 values ($\mu\text{L}/1$ litre of air) calculated based on the mortality of *C. maculatus* adults after 24 h exposure to various concentrations of essential oils in the inhalation test.

Treatment	DF	Slope + ES	LC ₅₀ (IC 95%)	LC ₉₅ (IC 95%)	χ^2
<i>A. herba alba</i>	2	1.91 ± 0.17	2.18 (0.12; 5.26)	15.82 (6.22; 7720.86)	8.46
<i>M. recutita</i>	2	2.02 ± 0.18	4.09 (1.42; 7.99)	26.73 (11.99; 519.77)	5.81
<i>D. viscosa</i>	2	2.09 ± 0.3	19.96 (16.27; 27.09)	14.66 (12.95; 17.43)	1.24

LC50 and LC90 were the concentration of causing 50% and 90% mortality against larvae after 24 h; slope: measures of inclination of the line probit (p) = constant + Bx (covariates x transformed using log base 10; SE = standard error; 95% CI: 95% confidence interval; χ^2 = chi-square.

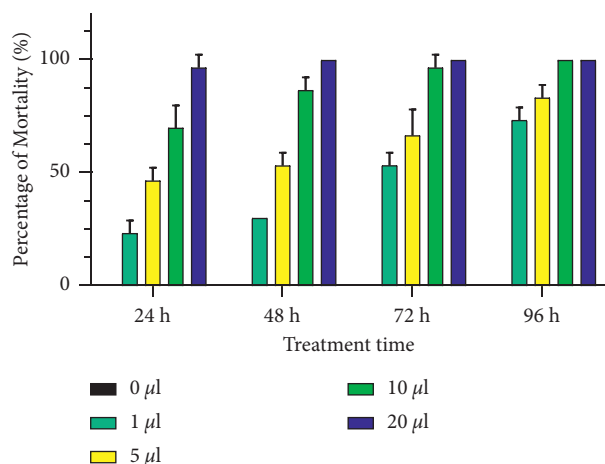


FIGURE 8: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to the contact test of *Artemisia herba alba* L. essential oils.

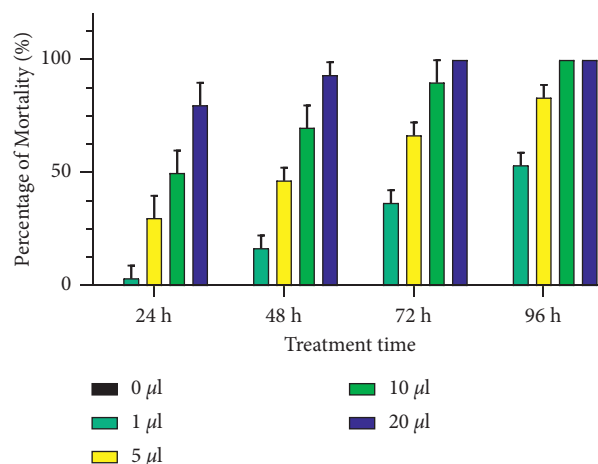


FIGURE 10: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to a contact test of *Matricaria recutita* L. essential oils.

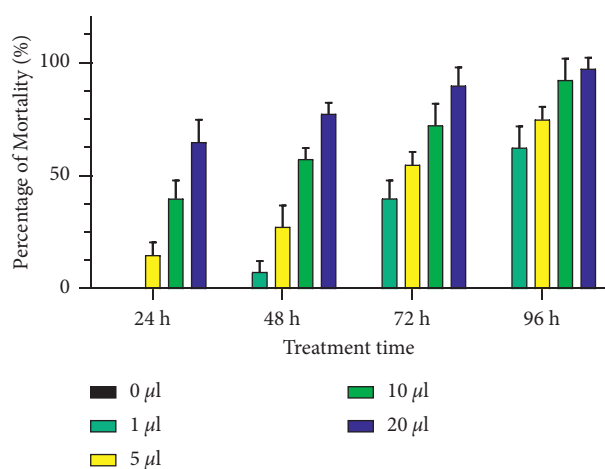


FIGURE 9: Mortality rate of *Callosobruchus maculatus* Fab. adults exposed to a contact test of *Dittrichia viscosa* L. essential oils.

The chemical composition of EOs investigated here was similar to that described in previous investigations. Our findings revealed that AEO was higher in thujone (57.6%) and chrysanthenone (11.8%), whilst MEO was higher in santolina alcohol (40.7%) and germacrene D (8.9%). Iso-costic acid (72.3%) was the chief compound of DEO so that these results were consistent with prior works [41–43].

This study evaluated the insecticidal activity of EOs by inhalation on adult individuals of *C. maculatus* using multiple doses and exposure times. The obtained results indicated that inhalation treatments with EOs from the studied aromatic plants considerably affect the longevity of

C. maculatus adults. Overall, the mortality averages of *C. maculatus* adults became increasingly important with increasing dose and duration of exposure to EOs. Our results were in harmony with those obtained by many authors [36, 44–46], who reported the insecticidal effect of EOs of many aromatic plants. The studied EOs here provide new perspectives in the control of *C. maculatus*. For comparison purposes, Mahmoudvand et al. [47] observed that EOs of *Lippia citrodora* L., *Rosmarinus officinalis* L., *Mentha piperita* L., and *Juniperus Sabina* L. caused mortality of genus *Callosobruchus*. Aboua et al. [48] showed that EOs of *Melaleuca quinquenervia* L., *Citrus aurantifolia* (Christm.), and *Ageratum conyzoides* L. possessed fumigation toxicity effects against this pest. *Carum copticum* and *Vitex pseudo-negundo* oils also posed effects on the genus *Callosobruchus* at different stages of insect development [49].

In this work, three EOs extracted from Moroccan aromatic plants were tested by direct contact at different doses in order to evaluate their insecticidal effects on *C. maculatus* adults along with their reproduction (fecundity and emergence). In this instance, the results showed that the EOs of the studied aromatic plants had well-controlled the longevity of the insect and clearly limited its fecundity and emergence in a dose- and time-dependent manner. In this sense, the EOA was the most effective, whereas, EOM was the least effective in terms of toxicity against *C. maculatus*.

Numerous studies have demonstrated the action of EOs on the longevity of adults of different species of stored grain pests. Due to their high volatility, EOs and their constituents, particularly monoterpenes, exert insecticidal effects, and reduce or disturb the insect growth at different stages of their

TABLE 5: LC50 and LC95 values ($\mu\text{L}/1$ litre of air) calculated for mortality of *C. maculatus* adults after 24 h of postcontact with various concentrations of essential oils.

Treatment	DF	Slope + ES	LC ₅₀ (IC 95%)	LC ₉₅ (IC 95%)	χ^2
<i>A. herba alba</i>	2	1.62 + 0.15	3.78*	39.24*	16.15
<i>M. recutita</i>	2	2.06 + 0.21	8.86 (7.55, 10.48)	55.62 (39.22, 92.19)	1.63
<i>D. viscosa</i>	2	2.47 + 0.30	14.17 (12.24, 16.99)	65.31 (44.98, 193.93)	0.35

LC50 and LC90 were the concentration causing 50% and 90% mortality against larvae after 24 h of posttreatment; slope: measures of inclination of the line probit (p) = constant + Bx (covariates x transformed using using log based 10; SE = standard error; 95% CI: 95% confidence interval; χ^2 = chi-square. *Wide confidence intervals were excluded from the calculation.

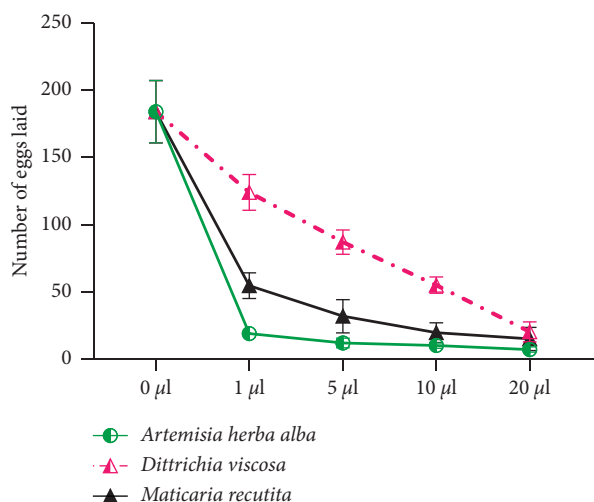


FIGURE 11: Effects of essential oils on the number of eggs laid by females of *Callosobruchus maculatus* Fab.

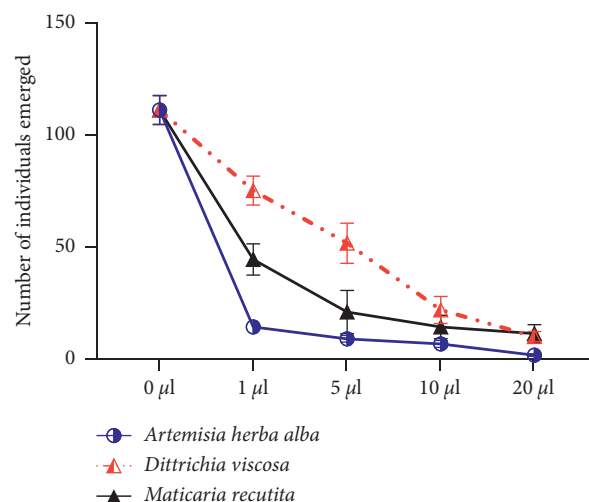


FIGURE 13: Effects of essential oils on the number of individuals emerging after the completion of life cycle.

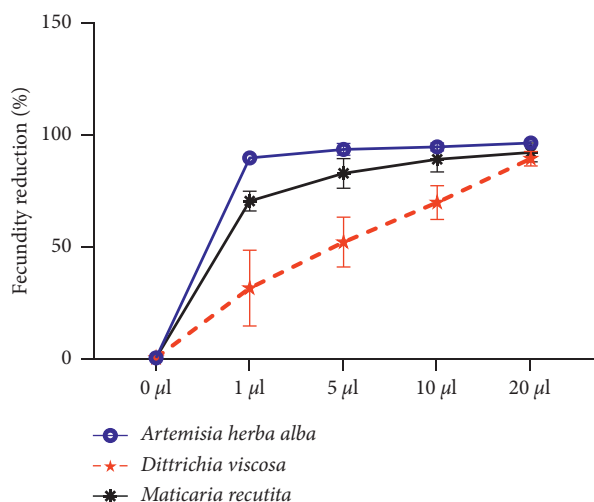


FIGURE 12: Rate of the fertility reduction in females under the effect of EOs.

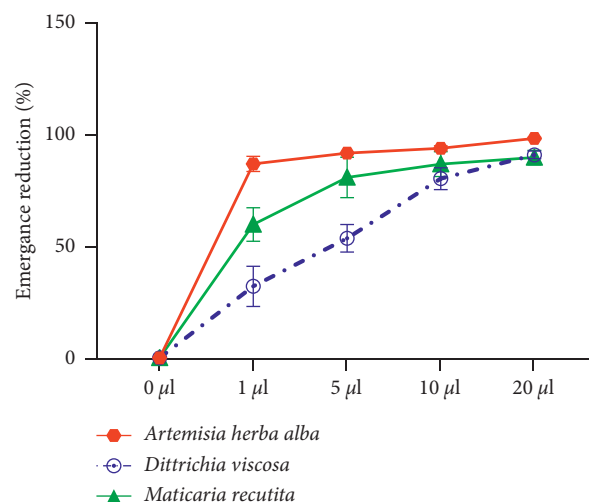


FIGURE 14: Rate of the reduction of *Callosobruchus maculatus* Fab. emergence under the effect of essential oils.

life [45, 50–53]. It was reported that EOs' efficiencies are closely related to the phytochemical profile and the entomological target [54].

A. herba alba Asso. is among the most important species of the family Asteraceae, which has been the subject of various chemical and biological studies, including insecticidal properties [55, 56]. It is thus fitting that our results are in agreement with those reported by Hussain [57], who

investigated the insecticidal properties of *A. herba alba* Asso on other insects. In addition, Abdelgaleil et al. [58] and Aggarwal et al. [59] reported that the insecticidal properties of many *Artemisia* species are due to the presence of 1,8-cineole. Furthermore, Bachrouch et al. [60] reported that the high percentages of α Thujone, 1,8-cineole, and norbornan-2-one in the essential oil of *A. herba alba* conferred it the best insecticidal potential against two stored commodity insects

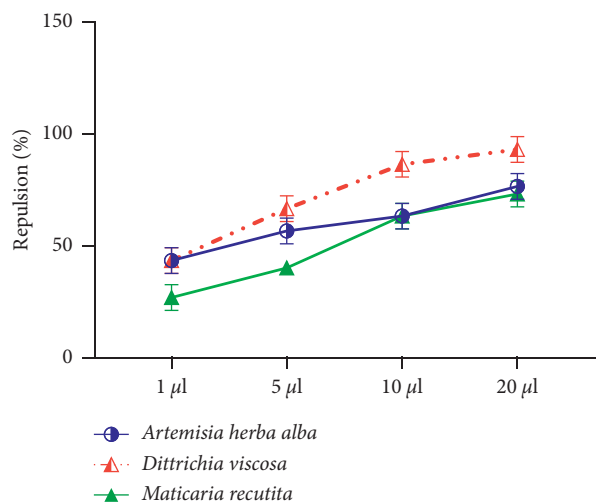


FIGURE 15: Repulsive effects of essential oils against *Callosobruchus maculatus* Fab.

including *Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* (Herbst). Therefore, we can confirm that the insecticidal effects of the studied EOs in this study could be due to the thujone identified by GC-MS in EOA.

For better understating this insecticidal effect of EOs, the study conducted by Senthil-Nathan [61] revealed that botanical chemicals affect the metabolism and growth of insects through physiological processes and enzymatic activities. In this way, understanding the biochemical effects of pesticides on insects can certainly provide safe pest control strategies [62]. In this sense, and according to França et al. [63], botanical insecticides can act on insects as acetylcholinesterase (AChE) inhibitors. So that the inhibition of AChE by phytochemicals, especially monoterpenoids, causes blockage of the neurotransmitter acetylcholine at the synaptic cleft [64], which is the case for the EOs tested in our study, whose essences are rich in compounds with a strong insecticidal effect against *C. maculatus*. Indeed, the pharmacological effect occurred at the level of chloride channels, octopamine receptors, tyramine receptors, acetylcholine esterase, nicotinic acetylcholine receptors (nAChR), and sodium channels in *C. maculatus* treated with EOs may be due to the individual activity of single compound or due to synergistic relationship of several chemical compounds because EOs contain a complex mixture of constituents that can interact with many target molecules [22]. EO compounds can also act with a variety of other targets including g-aminobutyric acid (GABA) gated.

A study was carried out by François Renoz et al. [65] on the mode of action of the EO of *M. arvensis* tested by contact on adult *Sitophilus granarius*, leading to rapid paralysis and alteration of walking behavior. This study revealed that the EOs of *Mentha arvensis* induced dramatic physiological changes in the exposed insects. In this case, the majority of differentially expressed proteins (DEPs) related to the muscle and nervous system were dysregulated along with serious problems affecting cellular respiration, protein synthesis, and detoxification process.

These results suggest that EOs are capable of affecting a variety of biological processes.

The obtained findings showed that a significant reduction in fecundity and emergence rate was recorded. This could be explained by ovicidal and larvicidal activities of the tested EOs that potentially creep into eggs, which in turn result in the blockage of embryogenesis [65, 66]. The ovicidal activity of the EOs would be due to the direct effects of their compounds leading to the inhibition of the metabolic activity of the eggs. This is the case of piperitone isolated from the EOs of *Cymbopogon schoenanthus* L. on the eggs of *Callosobruchus maculatus*. Moreover, β -ararone identified in the EOs of *Acorus calamus* L. showed toxicity on the eggs of *Callosobruchus chinensis*, *Sitophilus oryzae* L., and *Sitophilus granarius* L. [67]. In addition, according to Schmidt et al. [67], EOs have a sterilizing effect on eggs. Previous works carried out on *Acanthoscelides obtectus* belonging to the family Bruchidae, showed high sensitivity of eggs to the vapors of EOs from *Lavandula hybrid* L., *Rosmarinus officinalis* L., and *Eucalyptus globules* L. [68].

The percentage of repulsion effect evaluated by the method of McDonald et al. [37] showed that the EOA, EOD, and EOM were repulsive towards *C. maculatus* with a respective rate of 60%. In this context, Papachristos and Stamopoulos [69] showed that EOs of 13 aromatic plants, five of them *Monardella viridis* L., *Eucalyptus globulus* L., *Melaleuca microphylla* L., *Rosmarinus officinalis* L., and *Lysimachia hybrid* L. showed significant repulsive effects against *Theileria orientalis* L. and *Clonorchis sinensis* L. Furthermore, these authors reported that the most toxic EO exhibited simultaneously repulsive and reproductive inhibitory effects on bean bruchid, so that our results were in agreement with this literature.

Further works aim to evaluate potential toxicities of the studied EOs on nontarget organisms, and humans will be more appreciated prior to developing insecticide-based essential oils.

5. Conclusion

This study concluded that the essential oils from *Artemisia herba alba* Asso. and *Maticaria Recutita* L. and *Dittrichia Viscosa* L. well controlled the development of *Callosobruchus maculatus* with more attention can be paid to *Artemisia herba alba* Asso. essential oil rich in alpha thujone, beta-thujone, and chrysanthenone. The obtained results in this study showed that the use of essential oils as a biological pesticide could be incorporated in the management program for the control of chickpea pests as a safe alternative form.

Data Availability

Data used to support the findings are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

A. A., M. B., H. H., R. S., and A. M. S. contributed to original draft preparation and review and editing. W. S., H. Z. R., L. O., and E. A. Y. carried out formal analysis and review and editing. E. N. and F. M. performed conceptualization and supervision.

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