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Research Article

Identification of Volatile Compounds and Insecticidal Activity of Essential Oils from *Origanum compactum* Benth. and *Rosmarinus officinalis* L. against *Callosobruchus maculatus* (Fab.)

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This work was undertaken to investigate the volatile compounds and insecticidal activity of essential oils (EOs) from *Origanum compactum* Benth. and *Rosmarinus officinalis* L. against the crop pest *Callosobruchus maculatus* (Fab.). Essential oils of *Origanum compactum* (EOC) and *Rosmarinus officinalis L.* (EOR) were extracted by use of hydrodistillation, and their volatile compounds were profiled by gas chromatography-mass spectrometry (GC-MS). The insecticidal activity of extracted EOC and EOR was evaluated against *C. maculatus*. GC-MS analysis revealed that carvacrol (70.88%) and 1,8-cineole (62.35%) were the major constituents of EOC and EOR, respectively. EOC exhibited a potent insecticidal activity with calculated LC_{50} values of 6.77 and 3.57 μL/L air, 24 and 48 h posttreatment, respectively. Comparable LC_{50} values were obtained for EOR recording 6.25 and 3.82 μL/L, 48 h posttreatment. The effects of fumigation by the tested EOs on fertility (egg hatching) and the emergence of adult *C. maculatus* were also investigated. Notably, EOC completely abolished egg fertility judged by the abrogation of emergence of adults, regardless of the tested dose. By contrast, EOR completely inhibited the fertility and the emergence of *C. maculatus* adults at the dose of 16 μL/10 g. The outcome of the present study highlights the utility of the EOs from *O. compactum* Benth. and *R. officinalis* L. as natural sources of effective and ecofriendly pest-control agents.

1. Introduction

Nowadays, many environmental challenges influence agriculture worldwide. In addition to poor soil quality and cultivation techniques, there are problems related to insect pests. Pests attacking stored food legumes result in significant damage and loss of both quality and quantity. While losses attributed to pests are estimated to be around 40% in Africa, they do not exceed 3% in developed countries [1].

Despite the magnitude of losses caused by insect pests, a limited number of studies have shed light on pests in Africa. In Morocco, *C. maculatus* causes serious damage to stored legume foods, where *C. maculatus* larvae develop and feed on the cotyledons of legumes, particularly when no measures are taken. Pests are capable of destroying a crop within 4-5 months according to the Food and Agriculture Organization (FAO). Losses due to insect pests reached 35% of global agricultural production [2].

C. maculatus can be considered as one of the most growing challenges throughout the tropical and subtropical regions. It is a worrying pest of several pulses including *Vigna unguiculata*, *Cicer arietinum*, *Glycine max*, and *Phaseolus vulgaris*. These pulses are an important food source for millions of people based in tropical and subtropical areas. Cowpea seeds are most attacked pulses by *C. maculatus* and cause maximum damage, which could reach 2–5 kg seeds within 45–90 days when stored under optimal temperature (30 ± 10 C) and moisture conditions ($75 \pm 3\%$) [3].

Insecticides represent one of the most used control methods to manage insect pests. However, the resistance of insects to modern insecticides is still a great challenge facing chemical insecticides. In addition, these chemicals can possess risks to consumers and cause even harmful effects in the long term [4]. The plant kingdom represents a potentially effective alternative as a source of natural pest-control agents. Aromatic plants contain essential oils (EOs) that possess natural insecticidal activities. Hence, the insect-controlling potential of plant-derived EOs has been widely tested against pests attacking stored grains through their insecticidal potencies [5–8]. In this context, several researchers have reported lethal effects of EOs against plant and human pests [9, 10].

This work aimed to investigate the profile of volatile compounds and fumigant activity of EOs from *Origanum compactum* Benth. and *Rosmarinus officinalis* L. against *Callosobruchus maculatus* (Fab).

2. Materials and Methods

2.1. Insect Breeding. *C. maculatus* was obtained from a local warehouse and subsequently bred in glass jars with 500 g of *Vigna unguiculata* seeds. Jars were maintained at a temperature of 27 ± 1 °C, relative humidity of $70 \pm 5\%$, and a photoperiod of 14 h (light)/10 h (dark).

2.2. Plant Material. *O. compactum* was harvested from the region of Taounate from Morocco, whereas *R. officinalis* was harvested from the region of Taza, Morocco. Thereafter, the studied plants were identified by a botanist before being deposited at the Herbarium of Sidi Mohamed Ben Abdellah University. Next, the leaves were cleaned and dried in the shade at room temperature for 15 days.

2.3. Extraction of Essential Oils. One hundred grams of *O. compactum* and *R. officinalis* leaves were soaked in 750 mL of distilled water before being extracted at 100°C by use of a Clevenger apparatus for 4 h. The obtained EOs were dehydrated with anhydrous sodium sulfate before being stored in a refrigerator at 4°C until further use [11].

2.4. Gas Chromatography-Mass Spectrometry Analysis. The volatile compounds of the studied plants were determined by using GC-MS. Briefly, 0.1 μL of the sample was injected for analysis using a gas chromatograph coupled to a mass spectrophotometer (Agilent Technologies 5973 with an Agilent 19091S-433 HP-5MS column, 30 m long, 0.25 mm inner diameter, and 0.25 μm film thickness of the stationary phase) in positive mode. Helium was used as the carrier gas, with a typical pressure range (psi) of 0.9 mL/sec. The oven temperature program was set between 60 and 300°C for 10 min and then held at 300°C for 20 min. The detector temperature was set at 250°C, whilst the injector temperature was set at 260°C. Identification of compounds was performed by comparing retention times with standards of the database [12].

2.5. Insecticidal Activity Test. The insecticidal activity test was carried out to evaluate the activity of the essential oils in a vapor phase as reported in earlier work [11]. To achieve this goal, Whatman paper discs (3×3 cm) impregnated with different concentrations of the tested EOs (4, 8, 12, 16, and 20 μL/L of air) were attached to the inner surface of the stoppers of each jar to avoid their direct contact with the insects. Next, 10 g of cowpea seed and five pairs of *C. maculatus* aged from 0 to 48 h were separately introduced into each jar. Total mortality of insect individuals by each dose was recorded daily for 5 days. Egg-laying capacity of the females of *C. maculatus* was calculated by use of a magnifying binocular. Jars were subsequently maintained at a temperature of 27 ± 1°C, relative humidity of $70 \pm 5\%$, and a photoperiod of 14 h (light)/10 h (dark) until the emergence phase of adults.

2.6. Statistical Analysis. The results were expressed as means (±SD). A two-way analysis of variance (ANOVA) was used to analyze the effect of varying doses and exposure periods on mortality and fecundity of females and the emergence of adult *C. maculatus*. Significant differences between treatments were calculated by using Tukey's multiple range tests ($p < 0.05$). The lethal concentration LC_{50}, LC_{90}, chi-square, and 95% confidence intervals for each regression coefficient were calculated by use of probit analysis [13]. A significant difference was considered when $p < 0.05$.

3. Results and Discussion

3.1. Analysis of Essential Oil Components. The results of the volatile compounds profile of EOC are given in Figure 1 and Table 1. In this sense, the analysis showed the presence of 12 major compounds representing 99.89% of the total oil composition. EOC was majorly composed of carvacrol (70.88%) followed by caryophyllene oxide (7.97%), o-cymene (5.68%), and thymol (5.16%). Concerning the

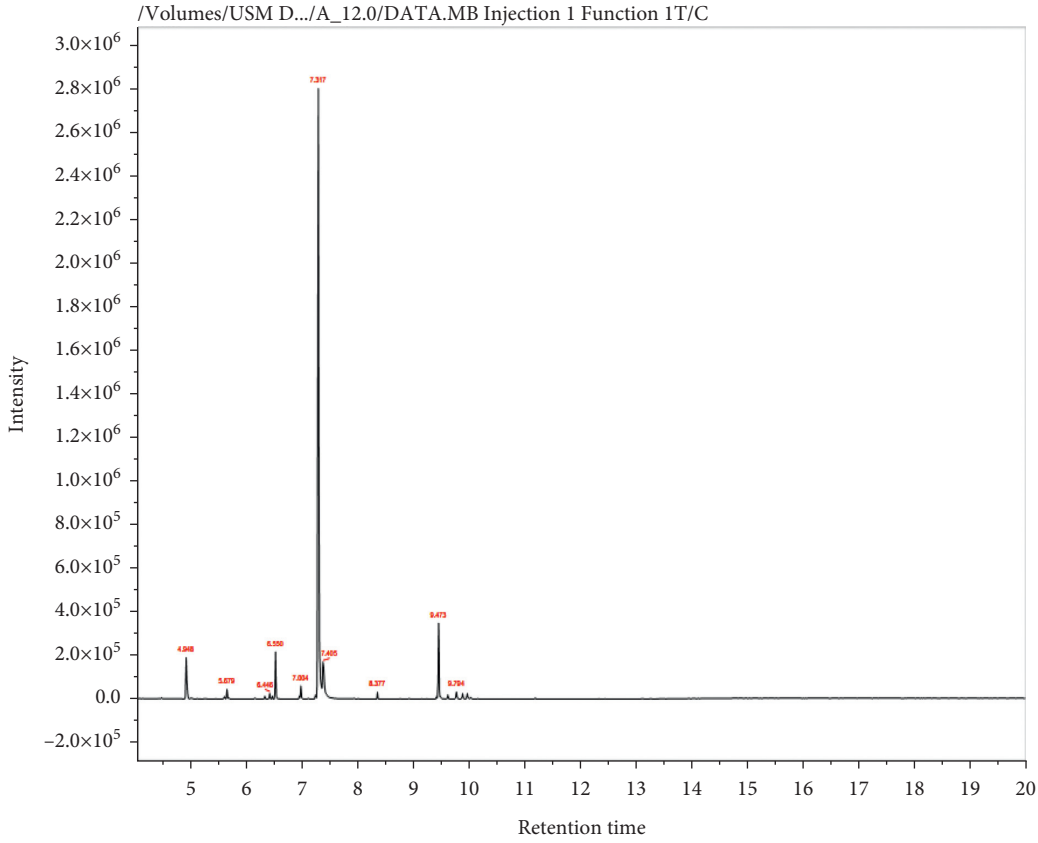

Figure 1: Chromatographic profile of EOC identified by GC-MS.

Table 1: Volatile compounds of EOC identified by GC-MS.

Peak	RT	Compound	Chemical formula	Chemical class	RI		Area (%)
					Cal	Lit	
1	4.94	o-Cymene	C10H14	MO.H	1024	1024	5.68
2	5.67	Terpinolene	C10H16	MO.H	1280	1282	0.93
3	6.44	β-Terpineol	C10H18O	MO.O	1143	1144	0.53
4	6.55	α-Terpineol	C10H20O	MO.O	1163	1164	4.58
5	7.00	Piperitenone	C10H14O	MO.O	1341	1343	1.34
6	7.31	Carvacrol	C10H14O	MO.O	1297	1299	70.88
7	7.40	Thymol	C10H14O	MO.O	1290	1290	5.16
8	8.37	Trans-Caryophyllene	C15H24	ST.H	1594	1598	0.60
9	9.47	Caryophyllene oxide	C15H24O	ST.O	1986	1986	7.97
10	9.79	Adamantanone	C10H14O	MO.O	1309	1311	0.86
11	9.90	Naphthalene	C11H10O	O	1445	1447	0.65
12	9.98	Camphene	C10H16	MO.H	1065	1028	0.81
			Monoterpene hydrate (MO.H)				7.42
			Monoterpene oxygenated (MO.O)				83.25
			Sesquiterpenes hydrate (ST.H)				0.60
			Sesquiterpenes oxygenated (ST.O)				7.97
			Others (O)				0.65
			Total identified (%)				99.89

RI, retention indices; Lit, literature; Cal, calculate; RT, retention time in minutes.

volatile compounds profile of EOR, GC-MS analysis revealed the presence of nine major compounds representing 99.89% of the total oil composition. EOR was mainly composed of 1,8-cineole (62.35%), camphor (23.14%), borneol (5.51%), and camphene (4.10%) (Figure 2 and Table 2).

3.2. Insecticidal Activity Test

3.2.1. Effect on Adult Mortality. Insecticidal activity of EOC and EOR against the adults of *C. maculatus* is given in Table 3. Statistical analysis revealed that the observed insecticidal effect is both time and dose-dependent. EOC exhibited significantly

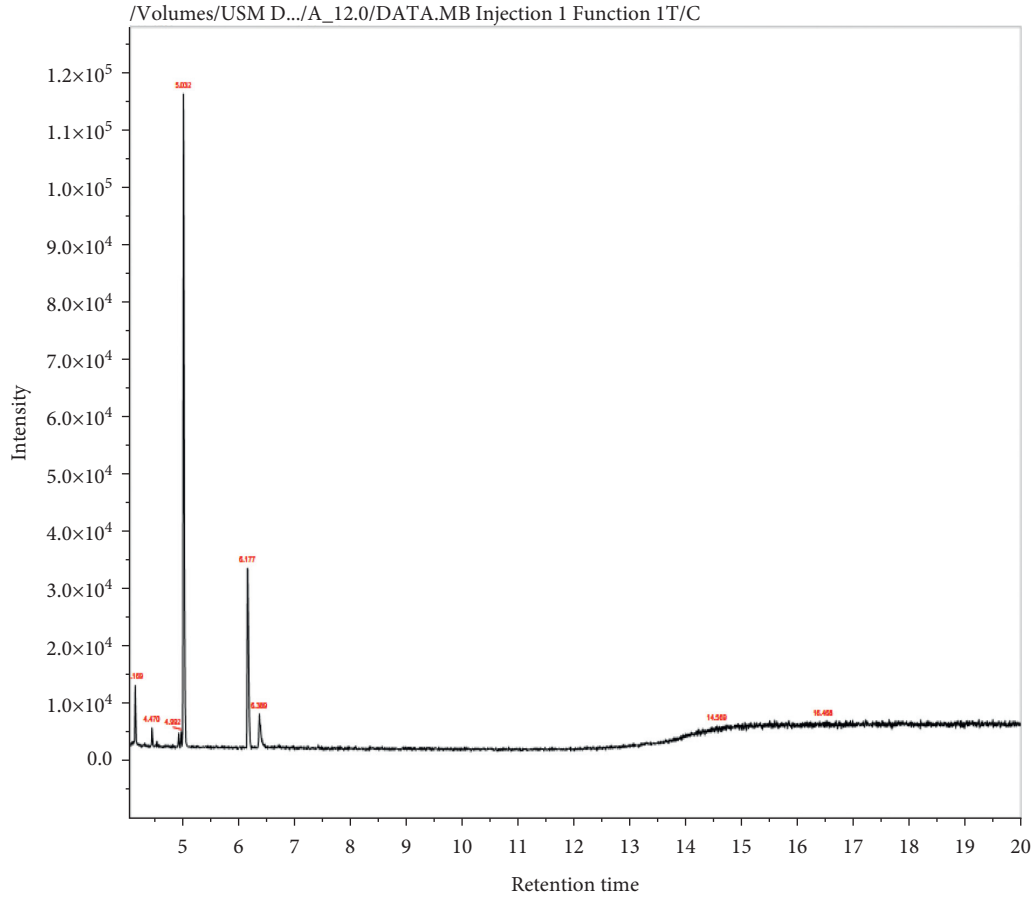

Figure 2: Chromatographic profile of EOR volatile compounds identified by GC-MS.

Table 2: Volatile compounds of EOR identified by GC-MS.

Peak	RT	Compound	Chemical formula	Chemical class	RI		Area (%)
					Cal	Lit	
1	4.16	Camphene	C10H16	MO.H	1085	1068	4.10
2	4.47	Cis-Ocimene	C10H16	MO.H	1037	1037	1.30
3	4.94	o-Cymene	C10H14	MO.H	1024	1024	1.07
4	4.99	Limonene	C10H16	MO.H	1028	1029	0.91
5	5.03	1,8-Cineol	C10H18O	MO.O	1186	1186	62.35
6	6.17	Camphor	C10H16O	MO.O	1146	1146	23.14
7	6.38	Borneol	C10H18O	MO.O	1169	1169	5.51
8	14.56	Santolinyl acetate	C12H20O2	O	1172	1174	0.80
9	16.46	Butanoic acid	C11H22O2	O	1196	1197	0.72
			Monoterpene hydrate (MO.H)				7.38
			Monoterpene oxygenated (MO.O)				91
			Sesquiterpenes hydrate (ST.H)				0
			Sesquiterpenes oxygenated (ST.O)				0
			Others (O)				1.52
			Total identified (%)				99.90

RI, retention indices; RT, retention time in minutes.

high mortality rate as a function of increasing concentrations ($F = 156.60$; df = 5,48; $p < 0.0001$) and exposure time ($F = 102.25$; df = 2,48; $p < 0.0001$), whereas EOR showed significant variation in *C. maculatus* mortalities at different concentrations ($F = 348.49$; df = 5, 36; $p < 0.0001$) and was highly significant with increasing exposure time ($F = 229.8$; df = 2, 36; $p < 0.0001$). The LC_{50} value for EOC was 6.77 and 3.57 μL/L air 24 h and 48 h postexposure, respectively; whereas, the LC_{90} ranged from 35.90 to 15.17 μL/L, respectively. The LC_{50} value for EOR ranged from 6.25 to 3.82 μL/L 48-hour post-exposure, whereas the LC_{90} ranged from 20.70 to 12.40 μL/L (Table 3).

Table 3: Lethal concentrations (μL/L) and chi-square (χ^2) values for EOC and EOR against adult *C. maculatus*.

Essential oils	Days	LC_{50} (μL/L)	95% CI	LC_{90} (μL/L)	95% CI	d*f*	χ^2
EOC	1	6.77	0.58–10.99	35.90	17.98–50242.031	3	1.23
	2	3.57	0.039–6.20	15.17	9.39–302.76	3	1.32
EOR	1	6.25	2.39–8.99	20.70	13.41–103.89	3	2.28
	2	3.82	0.42–6.05	12.40	8.23–51.18	3	2.66

Table 4: Effect of essential oils on mortality of *C. maculatus* as a function of concentrations and exposure times.

Essential oils	Doses (μL/L of air/10 g)	Exposure time (h)			
		24 h	48 h	72 h	96 h
EOC	4	36.66±5.77	66.66±5.27	90±1.0	100±0
	8	53.33±5.77	73.33±6.54	96.66±5.70	100±0
	12	63.33±5.77	86.66±6.54	100±0	100±0
	16	73.33±5.27	96.66±5.70	100±0	100±0
	20	93.33±3.74	100±0	100±0	100±0
	Control	0±0	0±0	0±0	0±0
EOR	4	36.66±3.35	63.33±3.11	100±0	100±0
	8	46.66±5.77	70±0.0	100±0	100±0
	12	63.33±3.07	86.66±6.54	100±0	100±0
	16	76.66±2.01	100±0	100±0	100±0
	20	100±0	100±0	100±0	100±0
	Control	0±0	0±0	0±0	0±0

As given in Table 4, both EOC and EOR showed dose and exposure time-dependent insecticidal activities, leading to100% of adult mortality 72 h postexposure. At the highest dose, EOC induced 90.0% of adult mortality (20 μL/L air/10 g) 24 and 48-hour posttreatment, whereas at the highest dose, EOR exhibited 100% of adult mortality 24-hour posttreatment. No mortalities were recorded in control groups.

3.2.2. Effect on Fecundity. The fecundity of *C. maculatus* females was strongly affected by the insecticidal effects of EOs tested. The obtained results showed a significant decrease in the number of eggs laid by females after being exposed to the vapor of EOC and EOR relative to the control (Figure 3 and Table 5). At the highest dose used for testing (20 μL/10 g seeds), the two EOs completely inhibited the fecundity of females relative to the control value of 196.66 ± 11.54. ANOVA analysis indicated that the EO-mediated toxicity against *C. maculatus* fecundity was highly significant as a function of increasing concentrations ($F = 1123.48$; df = 5, 24; $p < 0.0001$). Moreover, there is no significant difference between EOC and EOR towards the fecundity of females ($F = 6.31$; df = 1, 24; $P = 0.0191$). This can be explained by the fact that *C. maculatus* has sensitivity towards EOs of the tested aromatic plants.

3.2.3. Effect on Fertility. The obtained results showed that both EOC and EOR significantly reduced the egg hatchability when compared to the control in a dose and time-dependent manner (Figure 4 and Table 5). For all tested EOC doses, egg hatching was not recorded compared to the control fecundity rate of 94.02 ± 4.08.

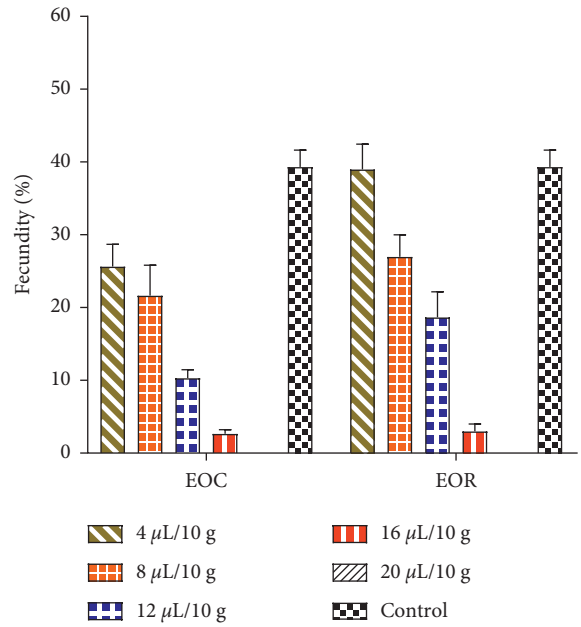

Figure 3: Effects of essential oil of EOC and EOC on the fecundity of *C. maculatus* (mean ± SD).

Similarly, EOR exhibited a potent egg hatching inhibitory effect, wherein the dose of 16 μL/10 g, resulted in complete abrogation of egg hatchability (Figure 4; Table 5). EOC possessed a toxic effect on the fertility of *C. maculatus* eggs irrespective of the tested dose, whereas EOR inhibited the fertility at the highest tested dose. Therefore, EOC exhibited a far more potent inhibitory effect on egg hatchability than EOR.

Table 5: Effects of EOC and EOR on fecundity, fertility, and emergence of *C. maculatus*.

Dose (μL/L)	Fecundity		Fertility (%)		Adult emergence (%)	
	EOC	EOR	EOC	EOR	EOC	EOR
4	23.66 ± 5.03	37.33 ± 5.50	0 ± 0	53.03 ± 7.27	0 ± 0	39.03 ± 4.69
8	21.66 ± 4.16	27 ± 3	0 ± 0	41.63 ± 4.64	0 ± 0	22.12 ± 1.25
12	10.33 ± 1.15	18.66 ± 3.51	0 ± 0	22.77 ± 3.92	0 ± 0	14.24 ± 1.33
16	2.66 ± 0.57	3 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Control	196.66 ± 11.54	196.66 ± 11.54	94.02 ± 4.08	94.02 ± 4.08	89.97 ± 0.89	89.97 ± 0.89

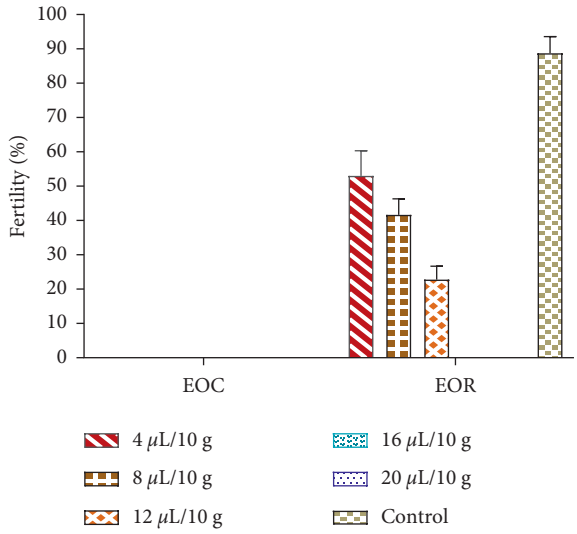

Figure 4: Effects of essential oil of EOR on the fertility of *C. maculatus* (mean ± SD).

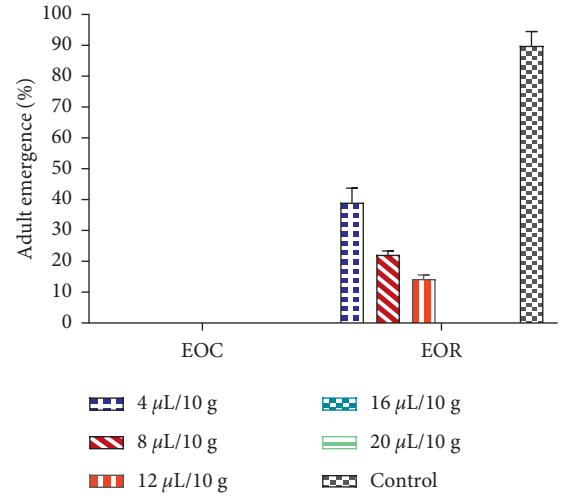

Figure 5: Effects of EOC and EOR on the emergence rate of *C. maculatus* adults.

3.2.4. Effect on Adult Emergence. The obtained results showed no *C. maculatus* adults' emergence in *Vigna unguiculata*. Seeds provisory treated with EOC regardless of the used dose; meanwhile, the total inhibition of *C. maculatus* adults' emergence by EOR was recorded for the hastiest dose used for testing 16 μL/10 g (Figure 5 and Table 5). Therefore, EOC exhibited higher inhibitory activity on the emergence rate of *C. maculatus* adults as compared to that of EOR.

4. Discussion

The obtained results showed that EOC was higher in carvacrol (70.88%), which is in agreement with a previously published study [14], reporting that carvacrol was the major constituent of EO extracted from *Origanum compactum* Benth. (43.97%). Additionally, other studies reported variable concentrations of carvacrol present in EO of *O. compactum* of 47.85% and 31.22% [15]. The current study, the result of which are reported here, found that 1,8-cineole (62.35%) was the major compound of EOR. Similarly, these findings were conforming to those reported by Ait-Ouazzou and co-workers [16], who showed that 1,8-cineole was the main constituent of the EO extracted from *R. officinalis* (43.99%).

Considering the insecticidal activity of the tested EOs against *C. maculatus*, the obtained results clearly indicated that EOC efficiently controlled *C. maculatus*, which is in agreement with previous reports [17], which revealed that EO of *O. compactum* possessed high insecticidal activity against *Spodoptera littoralis* larvae, with an LD_{50} of 0.05 mL/larva. Similarly, *O. compactum* possessed the insecticidal effect against adults of *Musca domestica* and *Mayetiola destructor* [17–19]. In this study, EOR was shown to be effective against *C. maculatus*, which is corroborated by findings reported by another previous work [20]. In that study, authors reported that EO of *R. officinalis* was bioactive against *C. maculatus*. Accordingly, Douiri and co-workers reported the insecticidal effect for EO of *Rosmarinus species* on *C. maculatus* males and females with LC_{50} varying from 5.51 to 2.43 μL/L air and 6.80 to 3.04 μL/L air, respectively [21].

In the present work, the insecticidal effect of the studied oils resulted in a significant reduction in the number of eggs laid per female. It is thus fitting to conclude that our results were comparable with those reported by Douiri and co-workers [22], who showed that EOs from *Asteraceae* species efficiently controlled *C. maculatus* potently impacting their fecundity, longevity, fertility (89.03–93.40%), and success rate (80–90%). In addition, LC_{50} was determined to be 2.5 and 23.3 μL/L of air for females and 2.56 and 46.07 μL/L for males. In this context, Bounechada et al. stated that the leaf powder of *Ocimum basilicum* completely abrogated the emergence of *Trogoderma granarium*, which

indicated that *Ocimum basilicum* might serve as an ecofriendly control agent specifically for this pest species [2].

Furthermore, it was reported that at a dose of 33.3 μL/L, the essential oils of *Melaleuca quinquenervia* and *Ocimum gratissimum* significantly reduced the oviposition of the *C. maculatus* females by 98.78% ± 0.87 and 99.94 ± 0.35%, respectively [23, 24]. In this study, the obtained results showed that EOs efficiently controlled the fertility of *C. maculatus* (hatching eggs). In this case, EOC completely inhibited the hatching of eggs laid by the female *C. maculatus*, regardless of the used dose. Meanwhile, the total inhibition of hatching eggs by EOR was obtained by the highest dose used. Specifically, at the dose of 400 μL, EOs extracted from *O. basilicum* and *O. gratissimum* inhibited the hatching of eggs of *C. maculatus* [25]. Similarly, Ketoh et al. stated that *C. schoenanthus* EO inhibited hatching egg and development of neonate *C. maculatus* larvae at the dose of 33.3 μL/mL [26]. In addition, EO of *Z. multiflora* has been previously reported to exhibit a strong insecticidal effect against eggs, larvae, and adults of *C. maculatus* [27].

Our results also showed that the tested EOs efficiently controlled the emergence of *C. maculatus* adults. EOC has completely prevented the total emergence of adults irrespective of the concentration used, whereas EOR prevented the emergence of *C. maculatus* when applied at the highest dose. Moussa Kéïta et al. [25] with a drop in the emergence of *C. maculatus* adults to 0 and 4% follow exposure to EOs of *Ocimum basilicum* and *Ocimum gratissimum*. It was also reported that the emergence of *C. maculatus* F_1 adults was significantly inhibited by EO of *Alpinia calcarata* at concentrations of 0.80 g/L using fumigant toxicity [28]. Similarly, the emergence of *C. maculatus* has been previously reported to be also controlled by *Allium sativum* [21].

EOs from aromatic plants exhibit a potent insecticidal effect by fumigation, contact, and repulsion assays [5, 8, 29]. EOs are known for their ovicidal, repellent, and insecticidal activities against various insects attacking stored products [29]. The mechanism of action (MOA) of EOs against insects was investigated by Renoz and co-workers who reported that EOs resulted in *Sitophilus granarius* death by altering a variety of key biological processes and activities, namely, muscular and neurological systems, cellular respiration, protein synthesis, development, reproduction, and insects' behavior [30]. Rajendran et al. reported that terpenoids have gained particular attention among other constituents of EOs because of their potent fumigant effect against stored grain insects [31]. In this context, it has been postulated that *C. maculatus* could absorb EOs along with their components, for example, terpenoids. Consequently, the toxicity of the tested EOs in the current study is hypothesized to be attributed to the presence of carvacrol [32, 33].

In the present work, the insecticidal activity of both EOC and EOR could be due to bioactive compounds identified in the oils, particularly carvacrol, which is known for its bioactivity including the insecticidal effect [34]. In the current study, carvacrol was found to be the major component in EOC with 70.88%, whereas 1,8-cineole was reported to be the dominant constituent in EOR with 62.35%. Taken together, these monoterpene compounds could be responsible for the biological activity of these EOs. Additionally, 1,8-cineole, borneol, and thymol have been previously reported to exert adverse toxicities against *S. oryzae* adults at the lowest dose (0.1 μL/720 mL volume), 24 h posttreatment by fumigation [35]. Camphor and linalool caused 100% mortality for *R. dominica* adults, and this has been attributed to monoterpenoids contained in EOs accounting for the observed insecticidal activity [4]. It was also reported that carvacrol, linalool, thymol, terpineol, and eugenol inhibited the emergence of *A. obtectus* adults [36, 37]. Citral and 1,8-cineole contained in EO were found to be ovicides and strong inhibitors of the emergence of adult houseflies. Eugenol and (−)-menthone powerfully inhibited adult emergence *C. maculatus* adults [38]. The MOA by which terpenes can exert this insecticidal effect have been reported in an earlier work [28]. EO constituents can operate synergistically or individually depending on which insect pest is being targeted. For example, the two components D-limonene and α-terpineol showed a synergistic toxicity against *Trichoplusia ni*, whereas no correlation was found with toxicity against *Spodoptera frugiperda*. The MOA underlying the synergistic interaction of 1,8-cineole and camphor, the major constituent of the EO of *R. officinalis* against *Trichoplusia ni*, has already been reported by Tak et al. [39], who showed that 1,8-cineole enhances the penetration of camphor into the blood circulation through the insect's body wall referred to as integument. The MOA of the reported insecticidal activity of EOs has been thoroughly investigated by Rattan and co-workers [40], who reported that EOs and their components, particularly thymol, result in insect death through the inhibition of acetylcholinesterase, thereby leading to its accumulation eventually causing hyperstimulation of nicotinic and muscarinic receptors and disrupted neurotransmission. Moreover, it might act by blocking the octopamine receptors through tyramine receptors cascade or by disruption of the octopaminergic system [40].

5. Conclusion

The obtained results revealed that the studied EOs efficiently controlled the insect life cycle, which could be attributed to its richness in specific bioactive monoterpenoid alcohols such as carvacrol. Taken together, the outcome of the present study highlights the benefits of the EOs extracted from *Origanum compactum* Benth. and *Rosmarinus officinalis* L. as effective ecofriendly pest-control agents. Further investigation is therefore warranted to evaluate the safety of these EOs and their nontarget toxicities against mammals and humans.

Data Availability

The data used to support the findings are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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