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ORIGINAL ARTICLE

Toxicity, antifeedant and repellent activities of five essential oils on adult *Cassida vittata* Vill. (Coleoptera: Chrysomelidae)

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Abstract

In Morocco, the sugar beet crop is severely harmed by the insect pest *Cassida vittata* Vill. which affects its yield quantity and quality. Chemical pesticides are considered the most common strategy to control this pest, and their use is extremely harmful to human health and the environment. In this context, the adults of *C. vittata* were exposed to five essential oils (EOs) obtained from: *Artemisia herba alba* Asso. (Asteraceae), *Eucalyptus globulus* Labill. (Myrtaceae), *Mentha pulegium* L. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), and *Shinus terebinthifolius* Raddi. (Anacardiaciae). Their contact and fumigant activity was evaluated every 24 h for 3 days. Their repellent effect was tested by filter paper and sugar beet discs every 5 min for 30 min. Their antifeedant effect, via Relative Growth Rate (RGR), Relative Consumption Rate (RCR), Efficiency of Conversion of Ingested Food (ECI) and The Feeding Deterrence Index (FDI),was evaluated using three doses in each experiment. For the contact toxicity, *M. pulegium*, *A. herba alba* and *R. officinalis* showed the highest mortality rates with 100, 92 and 78%, respectively, after 24 h at 0.283 µl · cm^{-2}. For the fumigant toxicity, 100% mortality was observed at the highest concentration of *M. pulegium* after 24 h and from *A. herba alba* with 88 and 96 after 48 h and 72 h, respectively. Regarding the repellent effect by filter paper, the repellency of *R. officinalis* and *A. herba alba* was 82.92 and 57.85%, respectively. However, *M. pulegium* showed 63% of repellency after 5 min at 0.057 µl · cm^{-2}. In the antifeedant test, *M. pulegium* gave significant results in all nutritional indices. In conclusion, *M. pulegium* was the most effective in all tests used in this study. Our findings promote the use of these essential oils as efficient biocontrol compounds against the adults of *C. vittata*.

Keywords: bioinsecticide, *Cassida vittata*, essential oils, insect pest, toxicity

Introduction

Sugar beet beetle *Cassida vittata* Vill. (Coleoptera, Chrysomelidae) is a widespread species commonly found in temperate climates in southern Europe as well as in northern Africa. However, in Morocco this insect causes extremely severe damage (Hmimina and Bendahou 2015). It is considered to be the principal insect pest of the sugar beet. Adults feed on the lower epidermis and inner tissues of the underside of sugar beet leaves causing regular circular holes. They can also consume up to 23.5 cm^2 per leaf (Fayed *et al.* 2014; Saleh *et al.* 2019) causing a strong loss of yield and decreasing the sugar content (Saleh *et al.* 2009; El-Dessouki *et al.* 2014; Hendawy and El-Fakharany 2017).

To control this insect in Morocco, a variety of conventional pesticides are used. Based on our investigations (unpublished data), the pesticides that are most frequently used by farmers in the Larache region (northern Morocco) are Karate 5 EC against adults, and Dursban 4 EC against the larvae of *C. vittata*. The use of pesticides to eliminate this pest is extremely harmful to human health. Pesticides can lead to an increase in the risk of thyroid cancer (Norouzi *et al.* 2023), promote the occurrence and development of autoimmune diseases (Huang *et al.* 2023), negatively affect the blood-brain barrier (Cresto *et al.* 2023), raise the probability of infertility (Lahimer *et al.* 2023), increase the risk of cognitive decline (Sasaki *et al.* 2023), and can lead to cardiotoxicity (Marques *et al.* 2022). Furthermore, the toxicity of pesticides threatens aquatic environments (Eissa *et al.* 2023; Machate *et al.* 2023), leads to soil contamination (Liu *et al.* 2023) and could pose adverse risks towards honeybees (Li *et al.* 2023), sea turtles (Salvarani *et al.* 2023), birds (Cooke *et al.* 2023), fish (Wang *et al.* 2023) and freshwater (Nayak *et al.* 2023). Furthermore, 250,000 people die each year from pesticide poisoning (Pavela 2016).

These data highlight the urgent need to update the current pest control strategies, and find alternative, eco-friendly control methods to protect crops from herbivores. In this regard, natural products such as steroids, alkaloids, phenylpropanoids, phenolic compounds, terpenoids and nitrogenous compounds, as well as essential oils, emerge as great substitutes for synthetic pesticides with less to no harmful effects on human health and the environment (Gadban *et al.* 2020; Rharrabe *et al.* 2020; Kumar *et al.* 2021). More specifically, essential oils are becoming more and more popular as natural pest management solutions (Chang *et al.* 2022; Lima *et al.* 2023) since they are non-persistent in the environment and therefore less toxic to other insects and animals (Isman 2020). Among the advantages of essential oils, we may highlight the fact that they can act as repellents, attractants and antifeedants. They can also hinder insects' ability to recognize host plants and have acute toxicity (Isman 2006; Usseglio *et al.* 2023).

Based on recent studies, essential oils have been found to be effective against various species of Coleoptera, such as *Sitophilus zeamais* with essential oil from the leaves of *Schinus terebinthifolius* Raddi (Bernardi *et al.* 2024). *Eucalyptus globulus* L. (Myrtale: Myrtaceae) has been effective in the control of *Rhyzopertha dominica* (F.) (Siddique *et al.* 2022), *Callosobruchus maculatus* Fabricius has been affected by *Artemisia herba alba* Asso and *Rosmarinus officinalis L.* (Riffi *et al.* 2021; Baghouz *et al.* 2022). Furthermore, other species of insect pests such as *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and *Tuta absoluta* Meyer (Lepidoptera: Gelechiidae) were sensitive to the insecticidal effect of *Mentha pulegium* (Topuz 2023).

The treatment of *C. vittata* by essential oil has not been documented and was one of the reasons why we started this work. We wanted to enrich the literature about the effect of essential oil on this insect, and to determine the toxicity, the repellent and the antifeedant effects of five essential oils obtained from *A. herba alba* Asso. (*Asteraceae*), *E. globulus* Labill. (*Myrtaceae*), *M. pulegium* L. (*Lamiaceae*), *R. officinalis* L. (*Lamiaceae*), and *S. terebinthifolius* Raddi. (*Anacardiaciae*) against adult beet beetles under laboratory conditions.

Materials and Methods

Essential oils

Eucalyptus globulus, *Rosmarinus officinalis*, and *Mentha pulegium* essential oils were purchased from a farm in OUJDA region which specialized in the culture of aromatic and medicinal plants where essential oils are obtained by steam distillation. *Artemisia herba alba* was obtained from a farm in Bouachta (northern Morocco).

The essential oil of *Shinus terebinthifolius* was obtained by hydro distillation using a Clevenger-type apparatus. In brief, 200 g of fresh green grains of *S. terebinthifolius* was mixed with 500 ml of distilled water and subjected to 3 hours extraction after which the essential oil was separated from the hydrolats and mixed with a pinch of anhydrous sodium to eliminate any trace of water. All essential oils were stored in dark vials and kept in a refrigerator.

The chemical composition of the EOs had been previously assessed in our laboratory using GC-MS analysis. In summary, *Mentha pulegium* EO was dominated by the presence of pulegone (83.06%) (Moullamri *et al.* 2024). *E. globulus* and *R. officinalis,* as described by Ait-Ouazzou *et al.* (2011), were characterized by a slight increase in the major compound 1.8 cineole with a respective percentage of 84% and 46.10%. As for *S. terebinthilfolius* EO, α-pinene, p-cymene and limonene were the major compounds with percentages of 32.32, 16.74, and 11.26%, respectively. In *A. herba alba* EO, Camphor Thujone and B-thujone were identified as the major compounds with respective percentages of 32.04, 19.04, and 14.22%.

Insects

Cassida vittata adults were collected from infested leaves in sugar beet fields in Ksar el Kebir city (34°59'56"north, 5°54'10" west), between January and mid-June 2023. They were kept in a climate room with controlled parameters: 25 ± 1°C, 65 ± 5% relative humidity, and 16L:8D. They were placed with fresh sugar beet leaves inside a box. For the experiment, the adults were chosen based on their age (more than 30 days). Adults were starved for 24 hours before being used in the experiment to encourage them to search for the food source.

Contact toxicity

The contact toxicity of the five essential oils was determined in an impregnated paper assay according to Obeng-Ofori *et al.* (1998) with some modifications. Three concentrations (0.6, 1.2, and 1.8%) prepared in acetone were used to screen the toxicity of the five essential oils, giving final concentrations of 0.094, 0.189, and 0.283 μl · cm^{-2}. One milliliter of each concentration was carefully distributed on filter paper (diameter 9 cm) with a micropipette. The solvent was allowed to evaporate for 3 min. The control received 1 ml of pure acetone. Filter paper was placed on the bottom of a Petri dish, then 10 adults of *C. vittata* were placed on the bottom of each Petri dish. Each treatment was replicated five times, and the mortality was recorded every 24 h for 3 days.

Fumigant toxicity

Fumigant toxicity was tested against adults of *C. vittata* according to Huang *et al.* (1997) with some modifications. The fumigant chamber consisted of 9 cm Petri dishes with a net volume of 120 ml. Two hundred and fifty μl of each concentration was distributed on the lid of a Petri dish and left to evaporate for 3 min. Ten adults were placed on the bottom of each dish and closed with the treated lid. Petri dishes were tightly sealed using parafilm. Mortality in adults was recorded every 24 h for 3 days. Three sets of acetonic concentrations (0.6, 1.2, and 1.8%) were prepared giving final concentrations of 12.5, 25, and 37.5 μl · l^{-1} of air, respectively. Five replicates were set for each concentration of essential oils and control.

Repellent activity

The repellent test was divided into two bioassays: the first one used filter paper, and the second one used leaf discs.

Filter paper bioassay

The repellent effect of the five essential oils against adult *C. vittata* was evaluated using the area preference method according to Talukder and Howse (1993) with some modifications. Filter paper circles (9 cm diameter) were cut into two halves. The first half received 300 μl of each concentration of essential oil while the second half received 300 μl of acetone. Both halves were left for 3 min at room temperature to allow the evaporation of solvent. After evaporation, the two half-circles were glued together using duct tape and placed in a Petri dish (d = 9 cm). Then, 10 adults were placed in the center of each Petri dish which was then closed to avoid the escape of insects. Finally, the number of insects present in each half was counted after 5, 10, 15, 20, 25, and 30 min. Three concentrations (0.6, 1.2, and 1.8%) were used, giving final concentrations of 0.057, 0.113, and 0.170 **μl** · cm^{-2}. Seven replicates were used for each treatment.

Leaf disc bioassay

The repellent effects of essential oils on adults were evaluated using the leaf disc test method (Cole 1994). Leaf discs, 1 cm in diameter, were treated; the first had 10 μl of essential oil, and the second had 10 μl of acetone. After 30 sec of evaporation, we placed them on two different sides of the Petri dish as illustrated in Figure 1. Thereafter, 10 adults were placed in the center of the dish and the number of insects in each zone was counted after 5, 10, 15, 20, 25, and 30 min, as described by Rharrabe *et al.* (2014). Three concentrations (0.6, 1.2, and 1.8%) were used, giving final concentrations of 0.076, 0.152, and 0.229 μl · cm^{-2}. Seven replicates were used for each treatment.

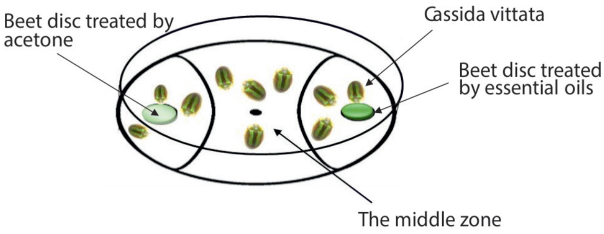

Fig. 1. Binary choices behavioral assay

For the two tests, the repulsion index was calculated according to Nerio Quintana *et al.* (2009) as follows:

$$\mathrm{PR} = \frac{N_c - N_t}{N_c + N_t} \times 100,$$

where: N_c – number of adults observed in the control area, and N_t – number of adults observed in the treated area. The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V (McDonald *et al.* 1970):

Class 0: The percentage of repulsion ≤ 0.1%: no repellency;
Class I: 0.1% < PR ≤ 20%: very poor repellency;
Class II: 20.1% < PR ≤ 40%: moderate repellency;

Class III: 40.1% < PR ≤ 60%: good repellency;
Class IV: 60.1% < PR ≤ 80%: very good repellency;
Class V: 80.1% < PR ≤ 100%: perfect repellency.

Antifeedant bioassay

The antifeedant activity was assessed using the no-choice test according to Schoonhoven (1982); discs (2–3 cm diameter) were cut from beet leaves. Discs and 10 adults were weighed separately. Next, 30 µl of each essential oil concentration were delicately added to the upper and lower sides of the discs. Leaf discs were left for 30 sec to allow the evaporation of acetone. Control discs received 30 µl of pure acetone. Finally, adults and treated discs were placed in a Petri dish and incubated at 25 ± 1°C, 65% RH and 16: 8 h (L: D). After 3 days, both discs and insects were weighed again and the mass of insects and discs before and after the experiment were used to calculate the nutritional parameters (Manuwoto and Scriber 1985; Farrar *et al.* 1989). Three concentrations (0.6, 1.2, and 1.8%) were used for each essential oil giving final concentrations of 0.18, 0.36, and 0.54 µl · disc^{-1}, and each treatment was conducted in seven replicates.

The nutritional parameters were calculated as follows:

Relative Growth Rate:

$$\mathrm{RGR} = \frac{\mathrm{A} - \mathrm{B}}{\mathrm{B}\ \times \mathrm{day}},$$

where: A – weight of live insects (mg) on the third day/number of live insects on the third day; B – original weight of insects (mg)/original number of insects.

Relative Consumption Rate (RCR)**:**

$$\mathrm{RCR} = \frac{\mathrm{D}}{\mathrm{B}\ \times \mathrm{day}},$$

where: D – biomass ingested (mg)/number of live insects on the third day.

Efficacy of Conversion of Ingested food**:**

$$\mathrm{ECI} = \frac{\mathrm{RGR}}{\mathrm{RCR}} \times 100\,.$$

The Feeding Deterrence Index (FDI):

$$\mathrm{FDI} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{T}} \times 100\,,$$

where: C – consumption of control diet and T – consumption of treated diet.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 25.0. Post-hoc testing was carried out using the Tukey's test. A significance level of 0.05 was used for all statistical tests.

Results

Contact toxicity

The insecticidal activities of *M. pulegium*, *A. herba alba*, *E. globulus*, *S. terebinthifolius* and *R. officinalis* against *C. vittata* were examined by direct contact application (Fig. 2). Toxicity increased with increasing exposure periods. The highest effect was obtained with the essential oil from *M. pulegium*, followed by *A. herba alba* and *R. officinalis*. Regarding *M. pulegium,* we found that mortality started at the low concentration of 0.094 µl · cm^{-2} after 24 h with 54%. Then, when we raised the concentration to 0.189 µl · cm^{-2}, we observed 86%, 88%, and 100% mortality after 24 h, 48 h, and 72 h, respectively. In addition, 100% mortality was recorded at the highest concentration used (0.283 µl · cm^{-2}) after 24 h. For the essential oil from *A. herba alba*, the mortality was between 52 and 58% at the lowest concentration (0.094 µl · cm^{-2}) after 48 h and 72 h, respectively, and when we increased the concentration to 0.189 µl · cm^{-2}, the mortality increased to 70%, 76% and 90% after 24 h, 48 h and 76 h, respectively. For the EO from *R. officinalis*, the most effective activity was caused by using a concentration of 0.189 µl · cm^{-2}, showing 78, 94 and 100% mortality at 24, 48, and 72 h, respectively. Both EOs of *E. globulus* and *S. terebinthifolius* showed a low mortality percentage (<50%) against *C. vittata*, as shown in Figure 2

In the fumigation bioassay (Fig. 3) there were no significant differences between time and concentrations for *S. terebinthifolius* and *E. globulus* EOs.

However, it was found that 64% mortality was due to *M. pulegium* at the concentration of 25 µl · l^{-1} of air during 24 h, 48 h and 72 h. In addition, 100% mortality was recorded at the highest concentration used (37.5 µl · l^{-1} of air) after 24 h from the same essential oil. The toxicity of *A. herba Alba* was lower than *M. pulegium* with mortality rates of 6%, 88% and 96% at the highest concentration within 24 h, 48 h and 72 h, respectively. On the other hand, we found a significant mortality rate lower than 30% for *R. officinalis.*The toxicity of essential oils decreased in the following order: *M. pulegium* > *A. herba alba* > *R. officinalis* > *E. globulus* > *S. terebinthifolius*. EOs from *M. pulegium* and *A. herba alba* showed both significant contact and fumigant toxicity. *R. officinalis* showed the highest mortality rate only in the contact toxicity.

Repellent activity

In the filter paper test (Fig. 4), of the four EOs tested, *R. officinalis* exhibited the best levels of repellency at all the concentrations used with a percentage of repellency (PR) belonging to Class V of more than

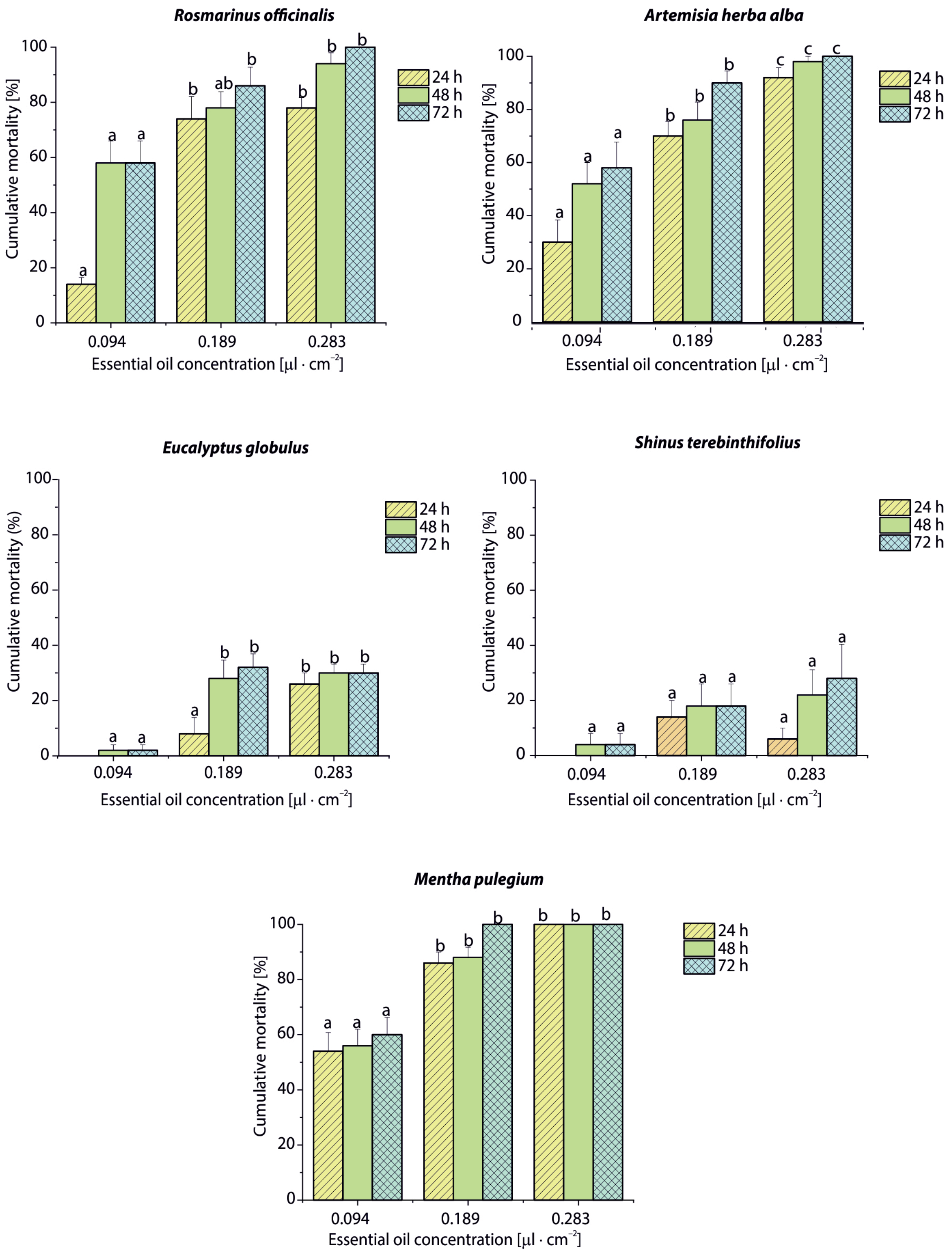

Fig. 2. Contact toxicity of *R. officinalis, A. herba alba, E. globulus, S. terebinthifolius* and *M. pulegium* EOs against *C. vittata* adults. Each point represents the mean ± SEM of five replicates. Different letters indicate statistical differences between the time and the concentrations among treatments while the same letter indicates no differences

80%. For *A. herba alba*, PR exceeded 60% at the lowest and the highest concentrations used, while 42.37% of repellency has been registered at the dose of 0.113 µl · cm^{-2} (hormetic effect), which demonstrated that this essential oil was a good repellent showing Class III repellency status. Furthermore, the repellency of *M. pulegium* was more than 60% after 5 min at the lowest concentration (0.057 µl · cm^{-2}), and the repellency index decreased as we increased the concentration from 0.057 µl · cm^{-2} to 0.170 µl · cm^{-2}. On the other hand, both EOs of *E. globulus* and *S. terebinthifolius* showed a low repellent effect (<20%) against *C. vittata.*

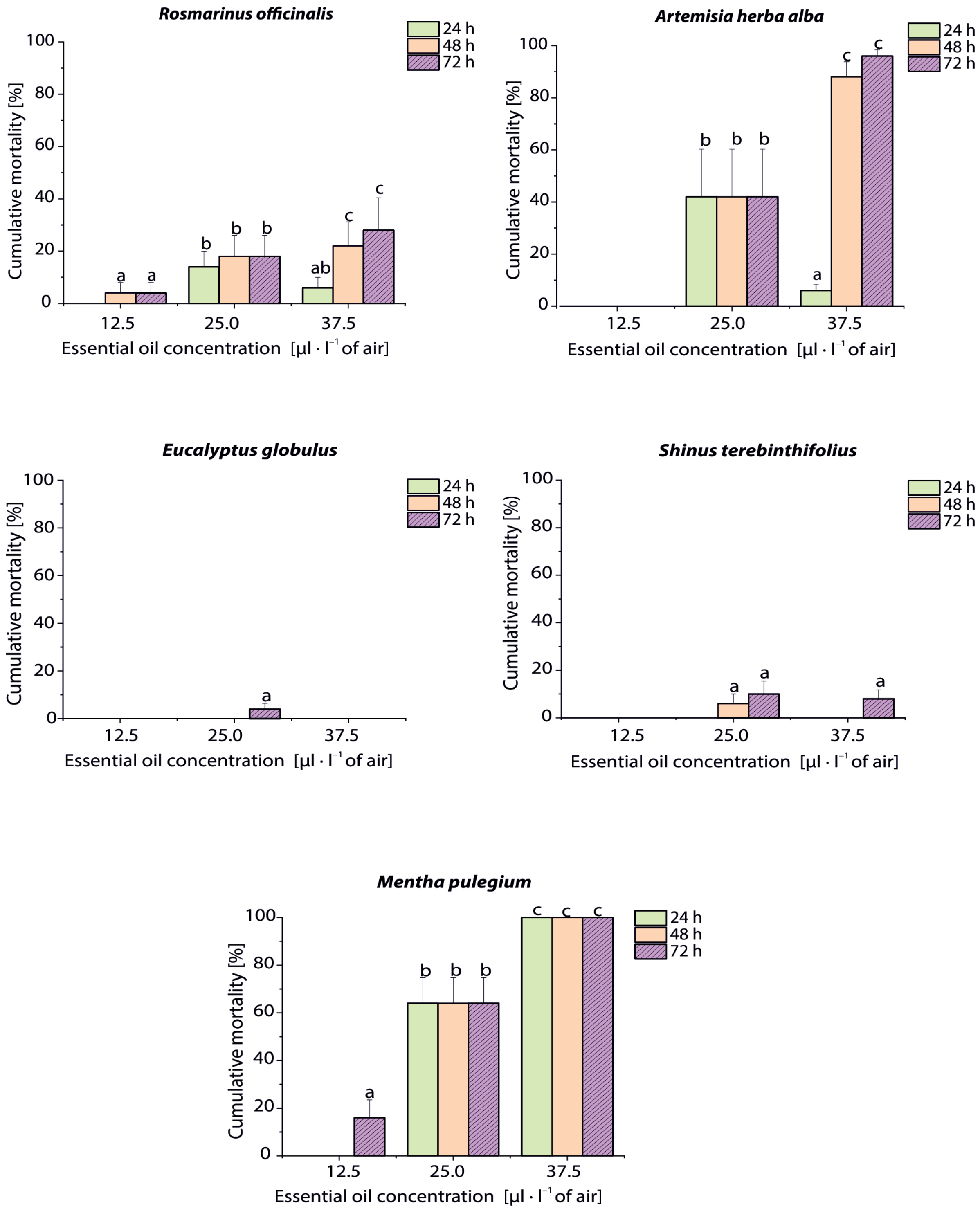

Fig. 3. Fumigant toxicity of *M. pulegium, A. herba alba, E. globulus, S. terebinthifolius* and *R. officinalis* EOs against C. vittata adults. Each point represents the mean ± SEM of five replicates. Different letters indicate statistical differences between the time and the concentrations among treatments while the same letter indicates no differences

Regarding the repellent effect of the disc (Fig. 5), *M. pulegium* essential oil was very effective as a repellent against *C. vittata* adults with 65% repellency after 5 min exposure at 0.076 µl · cm^{-2} doses, thereby being in Class IV. However, Classes II and III repellency status were observed at concentrations of 0.152 µl · cm^{-2} and 0.229 µl · cm^{-2}, respectively. On other hand, the repellency potential of *A. herba alba* corresponded to Class IV at the highest concentration used against the beetle. We noted an attractive effect (<20%) from the same EO at doses of 0.076 µl · cm^{-2} and 0.152 µl · cm^{-2}. Meanwhile, *R. officinalis, S. terebinthifolius* and *E. glo-*

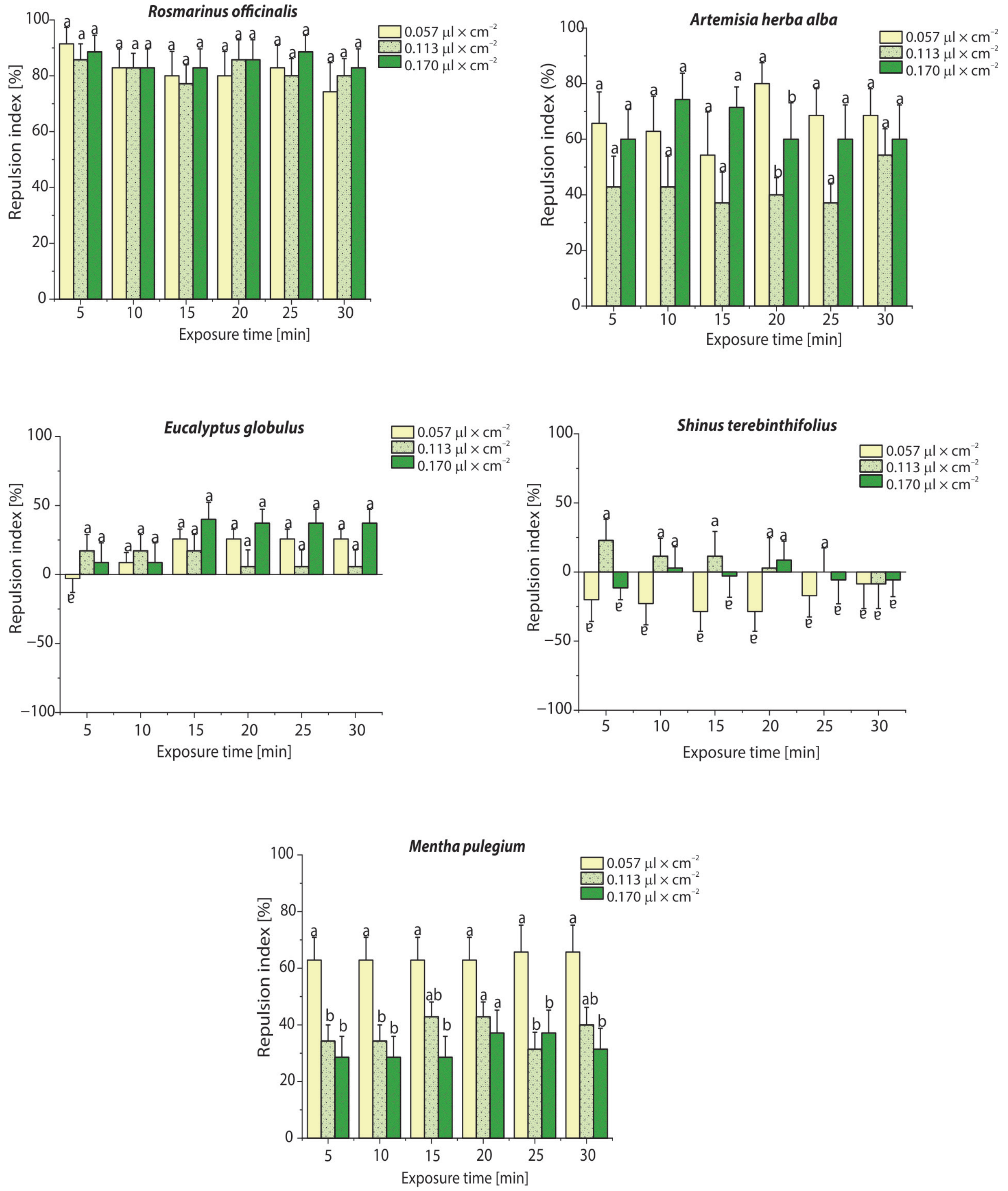

Fig. 4. Repellent activity of *R. officinalis, A. herba alba, E. globulus, S. terebinthifolius* and *M. pulegium* EOs on C. vittata adults after different exposure times using a filter paper. Different letters indicate statistical differences between the time and the concentrations among treatments while the same letter indicates no differences

bulus were the least repellent EOs showing Classes I and II repellency status for most doses against the beetle *C. vittata*

Antifeedant activity

No significant differences in nutritional parameters were observed during the treatment with *E. globulus* (Table 1). However, *S. terebinthifolius* had a significant reduction in RCR index compared to the control value at all concentrations used. Then, 56.4% of FDI occurred at 0.18 µl × disc^{-1} of concentration followed by 48.5 and 48% at 0.36 µl · disc^{-1} and 0.54 µl · disc^{-1} of concentration, respectively. For *R. officinalis,* we recorded a phagostimulant effect with −22.2% of the FDI at the two concentrations (0.18 and 0.36 µl · disc^{-1})

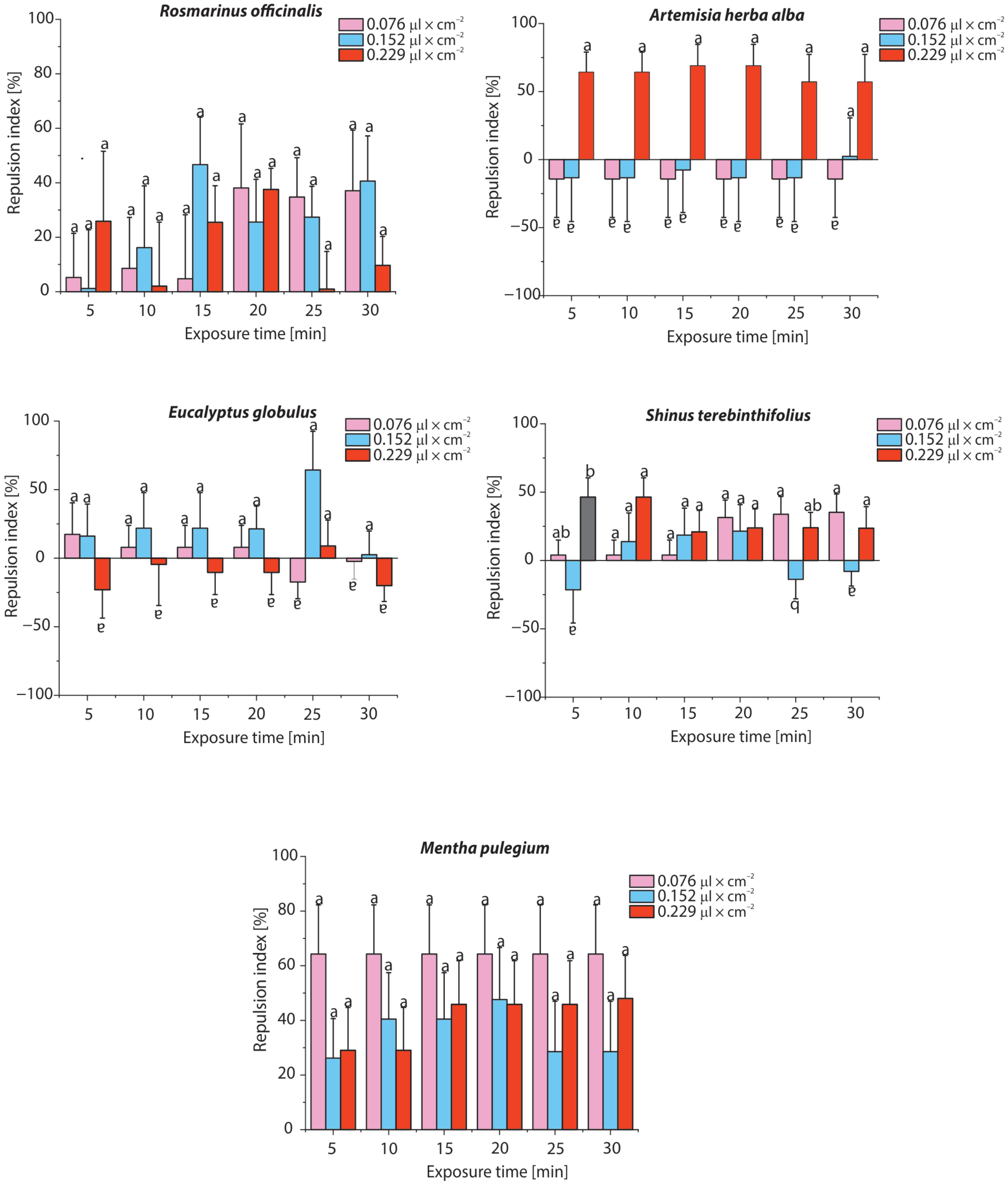

Fig. 5. Repellent activity of *R. officinalis, A. herba alba, E. globulus, S. terebinthifolius* and *M. pulegium* EOs on C. vittata adults after different exposure times using a disc of sugar beet. Each point represents the mean ± SEM of seven replicates. Different letters indicate statistical differences between the time and the concentrations among treatments while the same letter indicates no differences

followed by –29.2% at the highest concentration used. On the other hand, *M. pulegium* registered a significant effect on all nutritional parameters studied with a FDI value of 22.1% at the highest concentration used (0.54 µl · disc^{-1}). Concerning *A. herba alba*, a significant difference was recorded only at the highest concentration used (0.54 µl · disc^{-1}) for the RCR parameter.

Discussion

The present study was the first attempt to test the toxicity by contact and fumigation as well as the repellent and antifeedant effects of five essential oils on *C. vittata* adults. Our results align with previous

Table 1. Antifeedant activity of EOs obtained from *R. officinalis, A. herba alba, S. terebinthifolius, M. pulegium* and *E. globulus* ± SEM of seven replicates, at various concentrations on nutritional indices of *C.vittata* adults. Means within a column followed by the same letter are not significantly different (Tukey's HSD test, $p < 0.05$)

Plant Specie	Concentration [µl × disc^{-1}]	RGR [mg/mg/day]	RCR [mg/mg/day]	ECI [%]	FDI [%]
Rosmarinus officinalis	0	−0.002 ± 0.002 a	0.571 ± 0.027 a	−0.2 ± 0.3 a	0.0 a
	0.18	0.005 ± 0.004 a	0.721 ± 0.030 a	0.7 ± 0.5 a	−22.2± 6.0 b
	0.36	0.005 ± 0.004 a	0.728 ± 0.050 b	0.6 ± 0.5 a	−22.1± 4.1 b
	0.54	−0.001 ± 0.005 a	0.784 ± 0.046 b	−0.2 ± 0.7 a	−29.2± 7.3 b
Artemisia herba alba	0	−0.002 ± 0.002 a	0.571 ± 0.027 a	−0.2 ± 0.3 a	0.000 a
	0.18	−0.001 ± 0.006 a	0.656 ± 0.047 a	−0.4 ± 0.9 a	−19.6 ±6.4 a
	0.36	0.036± 0.021 a	0.723 ± 0.075 a	4.4 ± 2.7 a	−22.0±8.3 a
	0.54	−0.017 ± 0.020 a	0 .750 ± 0.055 b	−3.0 ± 2.7 a	−38.4± 6.4 a
Schinus terebinthifolius	0	−0.002 ± 0.002 a	0.571 ± 0.027 a	−0.2 ± 0.3 a	0.0 a
	0.18	0.015 ± 0.009 a	0.269 ± 0.017 b	4.7 ± 3.0 a	56.4 ± 3.1 b
	0.36	−0.001 ± 0.005 a	0.269 ± 0.015 b	0.7 ± 1.9 a	48.5 ± 4.2 b
	0.54	0.006 ± 0.009 a	0.288 ± 0.019 b	0.8 ± 3.8 a	48.0 ± 2.2 b
Mentha pulegium	0	−0.002 ± 0.002 a	0.571 ± 0.027 a	−0.2 ± 0.3 a	0.0 ab
	0.18	−0.005 ± 0.002 a	0.576 ± 0.029 a	−0.8 ± 0.3 a	−6.0 ±4.0 b
	0.36	−0.010 ± 0.005 ab	0.463 ± 0.01 b	−2.4 ± 1.1 ab	12.8 ± 3.7 ac
	0.54	−0.060 ± 0.040 b	0.349 ± 0.05 b	−4.7 ± 0.5 b	22.1 ± 4.5 cd
Eucalyptus globulus	0	−0.002 ± 0.002 a	0.571 ± 0.027a	−0.2 ± 0.3 a	0.0 a
	0.18	−0.011 ± 0.005 a	0.543 ± 0.014a	−2.0 ± 2.0 a	−3.6 ± 2.8 a
	0.36	−0.006 ± 0.002 a	0.523 ± 0.021a	−1.1 ± 0.3 a	−5.4 ± 2.6 a
	0.54	−0.040 ± 0.046 a	0.504 ± 0.047a	−13.1 ± 9.5 a	2.3 ± 9.1 a

findings by other researchers who have also tested the effects of the studied EOs on various Coleoptera species; *A. herba alba* demonstrated an important contact toxicity and fumigant toxicity against the adults of *Callosobruchus maculatus* Fab. (Aimad *et al.* 2022). Additionally, *M. pulegium* and *A. herba alba* showed the highest fumigant toxicity against *Tribolium confusum* (Abbad *et al.* 2014; Amoura *et al.* 2021), while *R. officinalis* exhibited contact and fumigant toxicity against adults of *Ephestia kuehniella* (Rekioua *et al.* 2022) and contact toxicity against *Sitophilus oryzae* (El-Bakry *et al.* 2019).

The results also showed that *M. pulegium* and *A. herba alba* EOs were the most repellent against *C. vittata* via the filter paper test and the disc test, respectively. According to the results found in the two tests, we noticed that the effectiveness of the essential oil was much stronger in the filter paper test than in the disc test. This result can be explained by the interaction of the odor given off by the beet plant, which may have weakened the effect of the essential oil used. This was shown by the insects' attraction to the beet disc, either by smell or color (Turlings and Ton 2006; Rharrabe *et al*. 2014; Arnold *et al*. 2015; Zhang *et al.* 2016; Webster and Card 2017). Moreover, *M. pulegium* registered an indirect dose-response correlation against *C.vittata*. We found that the repellency index of *M. pulegium* decreased as we increased the concentration. This may have been due to lateral inhibition within the antennal lobe, or to over-saturation of the receptor neurons of the insect (Skiri *et al.* 2004). The repellent effect of *M. pulegium* has been demonstrated on other insects, either as essential oils, against adult *Tribolium confusum* (Amoura *et al.* 2021) or as an extract against the two beetle pests *Tribolium castaneum and Lasioderma serricorne* (Salem *et al.* 2018) or as a powder of the aerial part against *Sitophilus oryzae* (Lougraimzi *et al.* 2018).

In the antifeedant test, we recorded a negative percentage of the FDI at the lowest concentration used by *R. officinalis*, which meant that the latter was a phagostimulant for *C. vittata*. In a different study, the extracts of *R. officinalis* had potent antifeedant activity against *Leptinotarsa decemlineata* Say (Sanchez-Vioque *et al.* 2015). Along the same lines, Kiran and Prakash (2015) saw that the essential oil of *R. officinalis* showed an antifeedant activity against *Sitophilus oryzae* L. (rice weevil) and *Oryzaephilus surinamensis* L. (sawtoothed grain beetle). For *M. pulegium*, we found a significant effect on all indices studied against *C. vittata*. Furthermore, an antifeedant effect was seen by the same EO against *Tribolium castaneum* (Herbst) in another study by Heydarzade *et al.* (2019).

Among the EOs used, we found that *M. pulegium* was the most effective in all tests used against *C. vittata* adults. To explain the efficacy of this essential oil, numerous studies have shown that the most abundant compounds in *M. pulegium* are pulegone, menthone, and limonene (Herrera *et al.* 2014; Mejdoub *et al.* 2019; Candy *et al.* 2020; El Hassani 2020; Bachrouch *et al.* 2023). In keeping with that, numerous EOs rich in pulegone and menthone as the major compounds had insecticidal effects on other coleopters, such as *T. confusum, Rhyzopertha dominica, Sitophilus zeamais* and *T. castaneum* (Liu *et al*. 2011; Kasrati *et al.* 2015; Abbad *et al*. 2023). Also, they demonstrated both their contact and fumigant toxicity as well as a repellent effect against adults of *Rhyzopertha dominica* (Brahmi *et al.* 2016). Furthermore, the essential oil of *Mentha piperita* L.*,* was also rich in menthone, menthol, and pulegone. These major compounds had a strong antifeedant effect against *Callosobruchus maculatus (*Saeidi and Mirfakhraie 2017).

The insecticidal property of *M. pulegium* EO is mainly attributed to major monoterpenoid compounds such as pulegone which are generally volatile and rather lipophilic and can penetrate rapidly into insects and interfere with their physiological functions (Bachrouch *et al.* 2015). Major compounds, such as pulegone and menthol can destroy insects by dysfunctioning the nervous system (Sánchez-Borzone *et al*. 2017; Jankowska *et al.* 2019a, b; Candy *et al.* 2020; Boulamtat *et al.* 2020), or through neuromuscular action (Ramdani *et al.* 2021). They can also affect cytochrome P450, which plays a key role in the detoxification system of insects (Rossi et *al.* 2012).

In the current literature there are no reports on the activity of EOs on *C. vittata*, even though the effectiveness of these essential oils on other insects has been extensively studied. It is necessary to conduct additional experiments to confirm the efficiency of the chosen EOs *on C. vittata* larvae stages and to test their effectiveness in sugar beet fields against the *C. vittata* beetle.

Conclusions

The findings of the current study demonstrated that all the studied EOs had insecticidal activity in at least one test, especially *M. pulegium*, *A. herba alba* and *R. officinalis*. However, *M. pulegium* was found to be the most effective essential oil since it showed significant efficacy in all of the tests conducted. Using these products to reduce the damage caused by this insect, controlling it through their antifeedant and repellent effects, and reducing insect populations through their toxicity can be remarkably advantageous. However, further studies are required to assess their safety for humans and the environment and to develop practical large-scale applications under field conditions against *C.vittata*.

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