

Laboratory evaluation of insecticidal and repellent activity of essential oils against the Mediterranean fruit fly

Original Article

Abstract:

The Mediterranean fruit fly (medfly) is a major pest affecting fruit crops worldwide. Growing concerns over insecticide resistance and environmental impacts have increased interest in botanical alternatives such as essential oils (EOs). This study evaluated the insecticidal and repellent activities of EOs from *Rosmarinus officinalis*, *Lavandula officinalis*, and *Eucalyptus globulus* against adult medflies under laboratory conditions. Oils were tested at concentrations of 0.08%, 0.16%, 0.32%, and 0.64% (v/v) using topical application and filter paper bioassays. Mortality was recorded at 24, 48, 72, and 96 h, while repellency was assessed after 2 h. All treatments showed significant insecticidal and repellent effects compared to controls ($p < 0.05$). *Eucalyptus globulus* exhibited the highest mortality and repellency, particularly at 0.64%. These findings highlight the potential of EOs as effective alternatives to synthetic insecticides.

Key words:

Essential oils, repellency, *Eucalyptus globulus*, *Lavandula officinalis*, *Rosmarinus officinalis*, Mediterranean fruit fly

Apstrakt:

Laboratorijska procena insekticidne i repelentne aktivnosti etarskih ulja protiv mediteranske voćne mušice

Mediteranska voćna mušica predstavlja značajnog štetočinu koji utiče na voćarske kulture širom sveta. Sve veća zabrinutost zbog otpornosti na insekticide i negativnih uticaja na životnu sredinu povećala je interesovanje za biljne alternative kao što su etarska ulja (EOs). U ovoj studiji procenjene su insekticidne i repelentne aktivnosti etarskih ulja *Rosmarinus officinalis*, *Lavandula officinalis* i *Eucalyptus globulus* protiv odraslih jedinki mediteranske voćne mušice u laboratorijskim uslovima. Ulja su testirana u koncentracijama od 0,08%, 0,16%, 0,32% i 0,64% (v/v) primenom topikalne aplikacije i bioeseja na filter papiru. Mortalitet je praćen nakon 24, 48, 72 i 96 h, dok je repelentni efekat procenjen nakon 2 h. Svi tretmani su pokazali značajne insekticidne i repelentne efekte u poređenju sa kontrolom ($p < 0,05$). *Eucalyptus globulus* je pokazao najveći mortalitet i repelentnost, naročito pri koncentraciji od 0,64%. Ovi rezultati ukazuju na potencijal etarskih ulja kao efikasne alternative sintetičkim insekticidima.

Кључне речи:

etarska ulja, repelentnost, *Eucalyptus globulus*, *Lavandula officinalis*, *Rosmarinus officinalis*, mediteranska voćna mušica

Introduction

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae), commonly known as the Mediterranean fruit fly (medfly), is one of the most economically damaging pests of fruit crops worldwide. Current control strategies rely heavily on broad-spectrum synthetic insecticides, primarily targeting the adult stage of the insect (Boulahia-Kheder, 2021). However, the indiscriminate use of these chemicals has led to several negative consequences, including the development of insecticide resistance,

environmental contamination, and harm to non-target organisms, such as natural enemies (Elfekih et al., 2014; Yadav et al., 2017).

Sustainable control of *C. capitata* remains a major challenge due to its polyphagy, high reproductive capacity, invasive nature, and the significant costs associated with eradication and containment efforts (Dominiak & Daniels, 2012; Szyniszewska & Tatem, 2014; Pinto Dias et al., 2022; Giunti et al., 2023). In response to these challenges, plant-derived compounds, particularly EOs, have gained increasing

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attention as environmentally friendly alternatives to conventional insecticides. EOs from various botanical families have demonstrated insecticidal, repellent, and fumigant activities against a wide range of insect pests through contact, ingestion, and vapor-phase exposure (Oviedo et al., 2020). Moreover, EOs or their chemical constituents may attract beneficial insects or repel harmful ones (Liu et al., 2019). Their low persistence, biodegradability, and reduced toxicity to non-target organisms make them promising candidates for use in integrated pest management (IPM) programs (López et al., 2011). Some EOs have also shown potential in preserving fruit quality during postharvest storage (Sivakumar & Bautista-Baños, 2014; Botelho et al., 2016).

The present study aims to evaluate the insecticidal and repellent activities of commercial EOs extracted from *Rosmarinus officinalis* L. (Lamiaceae), *Lavandula officinalis* Chaix (Lamiaceae), and *Eucalyptus globulus* Labill. (Myrtaceae) against adult *C. capitata* under laboratory conditions. This study aims to assess the potential of these botanical products as eco-friendly alternatives for the management of tephritid fruit flies.

Material and methods

Insects

The *C. capitata* colony used in this study was obtained from the insect-rearing facility of the Specialized Institute of Agricultural Training (ITSA), Heraoua, Algeria. The colony was maintained under controlled laboratory conditions at 25 ± 1 °C, $65 \pm 5\%$ relative humidity (RH), and a 16:8 h (L:D) photoperiod. Insects were reared following the procedure described by Canale & Benelli (2012). Adult flies of mixed sex, aged 10–12 days, were used in all bioassays.

Plant material

Three commercial EOs: rosemary (*R. officinalis*), lavender (*L. officinalis*) and eucalyptus (*E. globulus*), were obtained in pure form (100%) from Eva Pharma company. The oils were stored at room temperature, protected from light and humidity, following the manufacturer's recommendations. The chemical composition of these oils is well documented in the literature. EOs of *R. officinalis* and *E. globulus* are particularly rich in bioactive compounds such as 1,8-cineole, α -pinene, camphor, and linalool (Mimica-Dukić et al., 2003; Batish et al., 2008; Koutsaviti et al., 2020). According to Canale et al. (2013), *R. officinalis* oil typically contains 1,8-cineole (18.6%), α -pinene (15.6%), and camphor (15.3%) as its main constituents, with a significant proportion of monoterpene hydrocarbons. In *L. angustifolia* oil,

linalool (39.5%), linalyl acetate (18.2%), camphor (9.7%), and 1,8-cineole (6.5%) are predominant (Canale et al., 2013). For *E. globulus*, the dominant components include 1,8-cineole (63.1%), p-cymene (7.7%), α -pinene (7.3%), and α -limonene (6.9%), with monoterpenes accounting for up to 99.2% of the total composition (Čmíková et al., 2023).

Bioassays

Topical application

To evaluate the insecticidal activity of EOs, a topical application bioassay was conducted under controlled laboratory conditions. Four concentrations were prepared for each EO by diluting 4, 8, 16, and 32 μ L of oil in acetone and adjusting the final volume to 5 mL, corresponding to 0.08% (v/v), 0.16% (v/v), 0.32% (v/v), and 0.64% (v/v), respectively. From each dilution, 0.5 μ L was topically applied to the thorax of adult *C. capitata* using a 100 μ L Hamilton microsyringe.

For each concentration, three replicates of 20 adult flies were performed. Control individuals received 0.5 μ L of acetone alone under identical conditions. After treatment, the flies were transferred to ventilated plastic containers covered with fine mesh and maintained at 25 ± 1 °C, 75% relative humidity, and under a natural photoperiod. All insects were provided with a dry diet composed of sugar and brewer's yeast (3:1) and supplied with water ad libitum. Mortality was recorded at 24, 48, 72, and 96 h post-treatment. Insects were considered dead if they exhibited no movement when gently probed with a soft brush. Mortality data were corrected using Abbott's formula. Probit analysis was used to estimate LD_{50} and LD_{95} values.

Repellency test of essential oils

The repellent activity of EOs against adult *C. capitata* was evaluated using a filter paper arena assay. Circular filter papers (11 cm diameter) were cut into two equal semicircles. One half was treated with 0.5 mL of EO solution, prepared by diluting 4, 8, 16, or 32 μ L in 0.5 mL of acetone to obtain final concentrations of 0.08%, 0.16%, 0.32%, and 0.64% (v/v), respectively. The other half was treated with 0.5 mL of pure acetone, serving as the control. The solvent was allowed to evaporate completely for 15 minutes at room temperature before the assay commenced. The two halves were then rejoined using adhesive tape and placed inside a clean Petri dish.

Five adult pairs (5 males and 5 females) of *C. capitata*, from the same laboratory generation, were introduced into each dish. The test was conducted with four replicates per concentration. After 2 h of

exposure under controlled conditions (25 ± 1 °C, 75% relative humidity, natural photoperiod), the number of flies present on the EO-treated zone (TR) and on the acetone-treated control zone (NT) was recorded. The repellency index (RI) was calculated using the formula (Isman, 2006; Kumar et al., 2011).

$$RI(\%) = \frac{(NT-TR)}{(NT+TR)} \times 100$$

The repellency percentage (RI%) was calculated based on the distribution of insects between the treated and untreated zones. Repellency levels were classified according to McDonald et al. (1970) as follows:

- 0–20%: Class I – No or low repellency
- 21–40% : Class II – Slight repellency
- 41–60% : Class III – Moderate repellency
- 61–80% : Class IV – High repellency
- 81–100% : Class V – Very high repellency

Data analysis

The normality of the data was assessed using the Shapiro–Wilk test, and the homogeneity of variances was verified with Levene’s test. As both assumptions were satisfied ($p > 0.05$), mortality data were analyzed using a one-way ANOVA, while repellency indices were evaluated with a two-way ANOVA (factors: EO type and concentration). When significant effects were detected, Tukey’s HSD post hoc test was applied to compare group means. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics version 27.0.1 (IBM Corp., Armonk, NY, USA).

Results

Insecticidal effect of essential oils (Topical application)

The topical application of three EOs (*R. officinalis*, *L. officinalis*, and *E. globulus*) to *C. capitata* adults resulted in dose- and time-dependent mortality.

As shown in **Tab. 1**, mortality rates increased with both the concentration of the EO and the duration of exposure (24, 48, 72, and 96 h). At the highest concentration (0.64% v/v), *E. globulus* EO induced a mortality rate of $86.25 \pm 1.25\%$ (mean $\pm$ SE, $n = 4$) after 96 h of exposure. Mortality was significantly lower at lower concentrations, with $2.5 \pm 1.44\%$ mortality observed at 0.08% concentration after 24 h. Similarly, *R. officinalis* and *L. officinalis* caused mortalities of $71.67 \pm 4.56\%$ and $67.25 \pm 1.44\%$, respectively, at 0.64% concentration after 96 h. No mortality was observed in the acetone control group.

One-way ANOVA revealed a highly significant effect of treatment on mortality ($F(3, 230) = 25.78$, $p < 0.0001$). This indicates that the type of EO used

significantly influenced insect mortality. Tukey’s HSD post hoc test confirmed that all three EO treatments caused significantly higher mortality than the control ($p < 0.0001$). However, no significant differences were observed between *E. globulus*, *R. officinalis*, and *L. officinalis* treatments ($p > 0.05$).

The dose–response curve, fitted using a three-parameter logistic model based on mortality data at 96 h post-treatment (with Bottom = 0 and Top = 100), estimated a LD_{50} of 0.27% (v/v), with a 95% confidence interval ranging from 0.21% to 0.36%. The model provided a good fit to the data ($R^2 = 0.85$), confirming the dose-dependent insecticidal activity of *E. globulus* EO.

For *R. officinalis*, the dose–response curve at 96 h post-treatment yielded a DL_{50} value of 0.17% (v/v), with a 95% confidence interval ranging from 0.02% to 0.77. The model also exhibited a satisfactory fit ($R^2 = 0.90$), indicating a strong dose-dependent insecticidal activity.

For *L. officinalis*, the dose–response curve at 96 h post-treatment estimated a DL_{50} of 0.236% (v/v), with a 95% confidence interval of 0.208 to 0.267%. The model showed an excellent fit to the data ($R^2 = 0.91$), supporting the strong dose-dependent toxicity of this EO against *C. capitata*.

Repellent effect of essential oils

As shown in **Tab. 2**, the repellent activity of the three EOs varied depending on both the concentration and the type of oil tested. *Eucalyptus globulus* demonstrated the highest repellency overall, reaching an RI of 80% at the highest concentration (0.64%), corresponding to Class IV – High repellency. Even at lower concentrations, it exhibited consistent activity, moving from slight to moderate repellency.

Rosmarinus officinalis showed moderate to high repellency, with a maximum RI of 70% at 0.64%, also falling into Class IV. At intermediate concentrations, it maintained moderate repellency, indicating a strong potential as a natural repellent. However, *L. officinalis* was the least effective of the three oils. It only reached an RI of 60% at the highest concentration, corresponding to Class III – Moderate repellency. At lower concentrations, its effect was minimal, falling within Class I or Class II.

Overall, the results indicate that *E. globulus* is the most potent repellent, followed by *R. officinalis*, while *L. officinalis* exhibits significantly lower repellent activity against *C. capitata*.

A two-way ANOVA was conducted to evaluate the effects of EO type and concentration on the repellency index (RI%). The analysis revealed that concentration had a highly significant effect, accounting for 62.58% of the total variation in repellency ($F(15, 30) = 4.185$, $p = 0.0004$).

Table 1. Mortality rates (%) $\pm$ SE of *Ceratitis capitata* adults after topical application of essential oils at different concentrations and exposure times

Essential Oil	Concentration (% v/v)	Exposure Time			
		24 h	48 h	72 h	96 h
<i>Eucalyptus globulus</i>	0.08%	02.5 $\pm$ 1.44	6.25 $\pm$ 1.25	11.25 $\pm$ 1.25	13.75 $\pm$ 1.25
	0.16%	5.00 $\pm$ 2.04	12.25 $\pm$ 2.5	23.75 $\pm$ 2.5	27.5 $\pm$ 1.44
	0.32%	11.25 $\pm$ 2.3	28.75 $\pm$ 1.25	41.25 $\pm$ 3.15	55.00 $\pm$ 3.54
	0.64%	17.5 $\pm$ 3.23	33.75 $\pm$ 2.39	55.00 $\pm$ 3.54	86.25 $\pm$ 1.25
<i>Rosmarinus officinalis</i>	0.08%	1.25 $\pm$ 1.25	3.75 $\pm$ 1.25	7.5 $\pm$ 2.5	13.75 $\pm$ 1.25
	0.16%	10.00 $\pm$ 00	12.5 $\pm$ 1.44	21.25 $\pm$ 1.25	31.25 $\pm$ 1.25
	0.32%	22.5 $\pm$ 6.61	35.00 $\pm$ 2.04	42.5 $\pm$ 2.5	56.25 $\pm$ 6.57
	0.64%	25.00 $\pm$ 2.89	45.00 $\pm$ 00	63.75 $\pm$ 2.39	71.67 $\pm$ 4.56
<i>Lavandula officinalis</i>	0.08%	1.25 $\pm$ 1.25	11.25 $\pm$ 1.25	18.75 $\pm$ 1.25	23.25 $\pm$ 1.25
	0.16%	6.25 $\pm$ 2.39	22.5 $\pm$ 1.44	32.5 $\pm$ 1.44	45.00 $\pm$ 2.04
	0.32%	20.00 $\pm$ 2.04	32.00 $\pm$ 4	48.75 $\pm$ 1.25	58.00 $\pm$ 2.39
	0.64%	31.25 $\pm$ 1.25	46.25 $\pm$ 1.25	56.25 $\pm$ 1.25	67.25 $\pm$ 1.44

Table 2. Repellency index (RI%) and repellency class of essential oils at different concentrations

Essential oil	Concentration (%)	RI (%)	Repellency class
<i>Eucalyptus globulus</i>	0.08%	25%	Slight repellency
	0.16%	45%	Moderate repellency
	0.32%	60%	Moderate repellency
	0.64%	80%	High repellency
<i>Rosmarinus officinalis</i>	0.08%	20%	low repellency
	0.16%	30%	Slight repellency
	0.32%	60%	Moderate repellency
	0.64%	70%	High repellency
<i>Lavandula officinalis</i>	0.08%	10%	low repellency
	0.16%	20%	low repellency
	0.32%	40%	Slight repellency
	0.64%	60%	Moderate repellency

Additionally, the type of EO significantly influenced repellency, explaining 7.52% of the variation ($F(2, 30) = 3.769$, $p = 0.0346$). These results indicate that both factors play important roles in determining the repellent efficacy, with concentration showing a stronger impact than oil type. Post hoc comparisons were subsequently performed using Tukey's HSD test.

Tukey's multiple comparisons test revealed a significant difference in repellency between

L. officinalis and *E. globulus* ($p < 0.05$), with *Lavandula* showing a lower repellency index. No significant differences were found between *R. officinalis* and *E. globulus* ($p > 0.05$), nor between *L. officinalis* and *R. officinalis* ($p > 0.05$). These results indicate that *L. officinalis* exhibited significantly lower repellency than *E. globulus*, whereas the difference between *R. officinalis* and *E. globulus* was not statistically significant.

Discussion

The present study demonstrates the promising insecticidal and repellent potential of *E. globulus*, *R. officinalis*, and *L. officinalis* EOs against adults of *C. capitata*. Among the tested oils, *E. globulus* exhibited the highest efficacy in terms of both mortality and repellency, particularly at the highest concentrations tested. This finding is consistent with previous studies linking the bioactivity of *E. globulus* to its major constituent 1,8-cineole, a monoterpene well known for its neurotoxic and fumigant properties against insect pests (Isman, 2000; Nerio et al., 2010). Mechanistically, 1,8-cineole has been reported to interfere with octopamine receptors, GABA-gated chloride channels, and acetylcholinesterase (AChE) activity, thereby disrupting neural transmission and leading to insect paralysis and death (Kostyukovsky et al., 2002; Pavela, 2015).

Although the biocidal mechanism of EOs and their components is still not fully elucidated, the rapid action of EOs on insects and behavioral patterns observed in treated individuals have pointed to a neurotoxic mode of action. It has been proposed that there are multiple mechanisms underlying EO insecticidal activity, including AChE inhibition, GABA receptor modulation, and octopamine receptor activation. Acetylcholinesterase, an essential enzyme in cholinergic synapses and neuromuscular junctions of both vertebrates and invertebrates, is a frequent target of synthetic insecticides such as organophosphates and carbamates. However, resistance development in pest populations (Lee et al., 2015) has motivated the search for botanical alternatives. Several EOs, including *R. officinalis*, *Salvia officinalis* L. (Lamiaceae), and *Lippia origanoides* Kunth (Verbenaceae), as well as individual monoterpenes such as α -pinene, β -pinene, limonene, linalool, eugenol, pulegone, and 1,8-cineole, have demonstrated strong AChE inhibitory activity (Bhavya et al., 2018; Abdelgaleil et al., 2019; Oyedele et al., 2020).

In addition to their effects on the cholinergic system, some EO constituents modulate GABAergic transmission, a key inhibitory pathway in invertebrate nervous systems. Monoterpenic compounds such as thymol, carvacrol, and pulegone have been reported as positive allosteric modulators of GABA receptors in insects (Gashout et al., 2020). Likewise, linalool has shown synergistic interactions with methyl eugenol, enhancing GABA-mediated effects (Li et al., 2020). These interactions with GABA-gated chloride channels may contribute to the neuroexcitation and paralysis typically observed following EO exposure.

Another important target of EO constituents

is the octopaminergic system, which regulates physiological and behavioral functions in invertebrates, including locomotion, reproduction, and feeding (Enan, 2001; Hana & Lange, 2017). Octopamine receptor interference has been associated with hyperactivity, uncoordinated movement, and eventual immobilization in insects (Enan, 2001). Components such as eugenol, α -terpineol, and cinnamic alcohol have been shown to induce similar neurotoxic effects (Price & Berry, 2006; Jankowska et al., 2017), supporting the hypothesis that EO-induced mortality results from combined disruptions of multiple neural pathways rather than a single mechanism.

Our results also agree with earlier investigations on the insecticidal potential of other plant-derived products. Oviedo et al. (2020), for example, demonstrated that the EOs of *Baccharis dracunculifolia* DC (Asteraceae), and *Pinus elliottii* Engelm. (Pinaceae), as well as ethanol extracts of *Solanum granulosoleprosum* Dunal (Solanaceae) and *Ricinus communis* L. (Euphorbiaceae), exerted significant toxicity against *C. capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae). These findings reinforce the idea that botanically derived volatiles can provide viable alternatives to synthetic insecticides in tephritid management programs.

In our study, *R. officinalis* also displayed considerable insecticidal and repellent activity, especially at moderate to high doses. This bioactivity is likely related to the presence of camphor and α -pinene, known for their neurotoxic and fumigant activities against insects (Kumar et al., 2011). Interestingly, the probit analysis revealed that *R. officinalis* exhibited the lowest LD₅₀ value, suggesting higher potency compared to *E. globulus* and *L. officinalis*. However, the wide and overlapping confidence intervals observed among the tested oils suggest variability in efficacy and highlight the need for additional experiments with larger sample sizes. Although *L. officinalis* showed the weakest effect among the oils, it still induced significant repellency and moderate mortality relative to the control. This indicates that even less potent oils may play a role in integrated pest management (IPM), particularly when included in multi-component formulations that exploit synergistic or additive effects.

The dose-dependent responses observed for all oils highlight the importance of precise formulation and concentration optimization for practical use. However, the maximum mortality recorded (86.25%) precluded the estimation of reliable LD₉₀ values, pointing to a limitation in the current dataset. Broader concentration ranges and longer observation periods may help address this constraint in future studies. Additionally, formulation improvements, such as

encapsulation, emulsification, or nanoemulsions, have been shown to enhance EO stability, persistence, and insecticidal performance (Campos et al., 2019).

From an applied perspective, the demonstrated efficacy of these oils against *C. capitata* is highly relevant given the pest's destructive impact on fruit crops worldwide, particularly citrus, peaches, and olives, with annual losses estimated in billions of dollars (He et al., 2023). Furthermore, the increasing occurrence of resistance to conventional insecticides in *C. capitata* populations (Magaña et al., 2008) underscores the urgent need for alternative control methods. In this regard, the repellency effects of *E. globulus* and *R. officinalis* are especially promising, as they could be integrated into push–pull or attract-and-kill strategies to reduce fruit infestation while minimizing reliance on lethal action.

Despite their potential, some limitations must be acknowledged. Laboratory conditions provide direct and confined exposure that may overestimate efficacy compared to field settings, where factors such as volatility, photodegradation, and environmental interactions can reduce bioactivity. Moreover, the potential non-target effects of EOs on beneficial organisms, including pollinators like *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) and parasitoids used in biological control, were not assessed and should be carefully examined prior to large-scale deployment. Another challenge lies in the variability of EO composition, which can be influenced by plant origin, chemotype, extraction methods, and seasonal variation, thereby affecting reproducibility and consistency of results.

Future studies should therefore focus on semi-field and field trials to evaluate the persistence and practical applicability of these EOs under realistic agricultural conditions. Development of advanced formulations, such as microencapsulation or controlled-release systems, may improve stability and prolong insecticidal activity. Additionally, exploring synergistic interactions among different EOs or with other biocontrol agents could enhance efficacy while reducing application rates. Finally, assessing compatibility with natural enemies and pollinators will be essential to ensure safe integration into IPM programs.

Conclusion

This study demonstrates that *E. globulus*, *R. officinalis*, and *L. officinalis* EOs exhibit strong insecticidal and repellent activities against *C. capitata* adults, with *E. globulus* and *R. officinalis* showing the highest efficacy. These EOs demonstrated significant bioactivity that, combined with their biodegradability and low environmental

persistence, supports their potential as eco-friendly alternatives to conventional synthetic insecticides. Their incorporation into integrated pest management (IPM) programs could contribute to reducing reliance on chemical pesticides while maintaining effective fruit fly control. However, further work is needed to optimize formulation stability, assess selectivity toward non-target organisms, and validate their performance under field conditions. Addressing these challenges will be essential for the successful transition of EO-based formulations from laboratory research to practical applications in agriculture.

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