

Article

Toxicity of Some Essential Oils Constituents against Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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Simple Summary: The massive use of synthetic pesticides to manage agricultural pests results in environmental pollution and health hazards. The secondary plant metabolites, which are majorly dominated by terpenoids, have the potential to be developed into novel alternatives to synthetic chemicals. Therefore, the present study aimed at evaluating the toxicity, oviposition deterrence, and repellent activities of six majorly dominated essential oil constituents against adults and immature stages of oriental fruit flies, *Bactrocera dorsalis*. Our results highlight the potential of the selected essential oil constituents to be developed as a novel alternative to synthetic pesticides against *B. dorsalis*.

Abstract: The massive use of synthetic pesticides to manage agricultural pests results in environmental pollution and health hazards. The secondary plant metabolites, which are majorly dominated by terpenoids, have the potential to be developed into novel alternatives to synthetic chemicals. Therefore, in our current investigation, six majorly dominated essential oil constituents were evaluated for their toxicity against adults and immature stages of oriental fruit flies, *Bactrocera dorsalis*, a worldwide fruit pest. The results indicated that carvacrol was the most toxic essential oil constituent (EOC) to adult flies, with LC₅₀ of 19.48 mg/mL via fumigant assay, followed by thujone 75% mortality via ingestion toxicity test against adult fruit flies. Similarly, when larvae were dipped in different concentrations of EOCs, carvacrol appeared as the most toxic EOC with the lowest LC₅₀ (29.12 mg/mL), followed by (–)-alpha-pinene (26.54 mg/mL) and (R)-(+)-limonene (29.12 mg/mL). In the oviposition deterrence tests, no egg was observed on oranges seedlings treated with 5% of each EOC (100% repellency). Regarding the repellency assay, a significantly higher number of flies (77%) were repelled from the Y-tube olfactometer arm containing (–)-alpha-pinene, followed by carvacrol (76%). Our results showed that the selected essential oil constituent has the potential to be developed as an alternative to synthetic pesticides against *B. dorsalis*. However, further research is required to assess the activities of these EOCs under open-field conditions.

Keywords: oriental fruit fly; *B. dorsalis*; IPM; essential oils constituents; plant secondary metabolites; fumigation; ingestion toxicity; oviposition deterrence



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1. Introduction

Oriental fruit fly *B. dorsalis* (Hendel) (Diptera: Tephritidae) an endemic to Southeast Asia is a polyphagous and well-known insect pest of various fruits and vegetables worldwide [1]. This insect pest is also considered one of the most significant quarantine insect pests. The infestation by *B. dorsalis* poses severe harmful effects to numerous horticultural crops, rendering them unfit for human consumption due to its oviposition sting and voracious larval feeding activities [2]. For the past few decades, the management of oriental fruit flies and several other economic pests has relied heavily on the application of chemical pesticides due to their effectiveness and quick response [3,4]. However, extensive and unmonitored use of chemical pesticides has caused various insect pests to develop

resistance against many pesticides [5,6]. Many chemical insecticides have failed to control target insect pests, as these pests have evolved resistance to or neutralized the chemicals' lethal effects using their physiological and ecological functions [7]. Similarly, several cases of *B. dorsalis* developing pesticide resistance have been documented in various parts of the world [8–10].

The aforementioned research on alternative environmentally friendly management techniques for oriental fruit flies is imperative. In this scenario, several government agencies such as the United States Food and Drug Administration (USFDA), Environmental Protection Agency (EPA), and European Union (EU), under the Integrated Pest Management (IPM) standards, also promote a significant reduction in the use of chemical pesticides while encouraging the adoption of more environmentally friendly approaches [11–14].

The quest to substitute synthetic insecticides with environmentally friendly alternatives such as plant extracts and essential oils (EOs) is another step towards adopting less damaging tools in IPM techniques. In several recent studies, plant essential oils (EOs) have been shown to have antifungal, antimicrobial, cytostatic, and insecticidal properties [14,15]. Essential oils are natural combinations of chemicals with low molecular weight, volatility, and lipophilic nature found in various plant families, including Asteraceae, Apiaceae, Lamiaceae, Myrtaceae, Lauraceae, and Rutaceae [15,16]. Essential oil components (EOCs) are classified into four classes according to their chemical structure: terpenes, terpenoids, phenylpropenes, and others. They may also contain a diversity of functional groups such as hydrocarbons (monoterpenes, sesquiterpenes, and aliphatic hydrocarbons); oxygenated compounds (monoterpene and sesquiterpene alcohols, aldehydes, ketones, esters, and other oxygenated compounds); and sulphur and nitrogen-containing compounds (thioesters, sulfides, isothiocyanates and nitrile) [15–17]. Components of essential oils exert their activities (mode of action) on pests in a neurotoxic way, for example, like gamma-aminobutyric acid (GABA), insect growth regulator (IGR), octopamine synapses (OS), impending digestive enzymes, inhibition of glutathione S-transferase (GST), cytochromes P450 (CYPs), and acetylcholinesterase (AChE) inhibitors for effective control in pre-and post-harvest agricultural techniques [18–20]. Moreover, various potential essential oils and their constituents belonging to terpenes and phenylpropanoids (α -pinene, β -pinene, limonene and zingerone) showed effectiveness in integrated pest management (IPM), sterile insect technique (SIT), lure and kill techniques against many fruit flies such as *Ceratitidis capitata*, *B. dorsalis*, *B. correcta*, *B. philippinensis*, *Anastrepha* and *Zeugodacus* genera [15,16].

Essential oils or their constituents (EOCs) have been widely researched as sources of environmentally acceptable insecticides against many agricultural and public health insect pests [15,17,20,21]. During the last few decades, researchers have developed a keen interest in the research and production of botanical pesticides [15]. However, our knowledge of their effectiveness against tephritid species is still limited. Several other tephritid flies (e.g., *Ceratitidis capitata* and *B. oleae*) have been the subject of much insecticidal research on EO and their constituents [16,22]. Despite their economic importance, available literature information on EO activities on *B. dorsalis* is still very limited [23,24]. Consequently, this study intended to investigate the insecticidal properties of six EOCs, which are highly effective against insect vectors and other insect pests [12,15,25–29]. Insecticidal activities of carvacrol, (–)- α -bisabolol, thujone α , β , (R)-(+)-limonene, (–)- α -pinene, and (–)- β -pinene were tested against *B. dorsalis* immature stage and adults in laboratory conditions via fumigation, ingestion, larvicidal and pupicidal assays. Moreover, repellency and oviposition deterrence exhibited by EOCs were also determined.

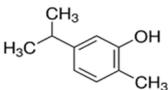
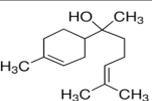
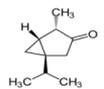
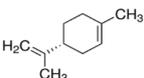
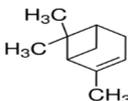
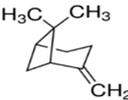
2. Materials and Methods

2.1. Essential Oil Constituents (EOCs)

Six different EOCs were used in the current study. Analytically pure carvacrol CAS-No. 499-75-2 (purity 98%) and (–)- α -bisabolol CAS-No. 23089-26-1 (purity > 93%) were purchased from, Sigma (Sigma-Aldrich Inc., St. Louis, MO, USA). Thujone (α , β -mixture)/1-Isopropyl-4-methylbicyclo [3.1.0] hexan-3-one (α - and β - mixture) CAS-No. 1125-12-8

(purity > 70.0% GC.) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) (R)-(+)-limonene, CAS-No. 5989-54-8 (purity 97%) and (–)-α-pinene CAS-No. 7785-26-4 (purity 98%) and (–)-β-pinene CAS-No. 18172-67-3 (purity 99%) were purchased from Alfa Aesar (Shanghai, China). Analytically pure dimethyl acetone was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The investigated use of EOCs properties is tabulated in (Table 1).

Table 1. List of EOCs used in the current study along with chemical structure and their sources.

CAS No	EOC Commercial Standard	Chemical Structures	Molecular Formula	Groups	Source (e.g.,)	Solubility	Toxicology	References
499-75-2	Carvacrol 98%		C ₁₀ H ₁₄ O	OM	Aromatic plants (e.g., <i>Origanum</i> spp., <i>Thymus</i> spp., <i>Thymbra capitata</i> etc.)	Ethanol, acetone, diethyl ether, carbon tetrachloride	Insecticidal activity	[25]
23089-26-1	(–)-α-Bisabolol > 93%		C ₁₅ H ₂₆ O	OS	<i>Matricaria chamomilla</i> , <i>Myoporum crassifolium</i> etc.	Slightly soluble in ethanol	Insecticidal activity	[25]
1125-12-8	Thujone (α, β-mixture) > 70.0%		C ₁₀ H ₁₆ O	OMK	<i>Salvia officinalis</i> , <i>Artemisia</i> , <i>Tanacetum vulgare</i>	H ₂ O at 20 °C	Neurotoxin, insecticide	[26]
5989-27-5	(R)-(+)-Limonene 97%		C ₁₀ H ₁₆	MH	Citrus	Benzene, chloroform, ether, CS ₂ , soluble in CCl ₄	Fumigant, antifeedant	[15]
7785-70-8	(–)-α-Pinene 98%		C ₁₀ H ₁₆	MH	Mint, holy basil, camphor, bupleurum, and Psidium, coniferous trees	Ethanol, acetone	Insecticidal activity	[27]
18172-67-3	(–)-β-Pinene 99%		C ₁₀ H ₁₆	MH	Coniferous trees and several other aromatic plants	Alcohol, chloroform, ether	Neurotoxic, insecticide	[29]

OM: Oxygenated monoterpene; OS: Oxygenated sesquiterpene; OMK: Oxygenated monoterpene ketone; MH: Monoterpene hydrocarbon.

2.2. Bioassays

The toxicity of EOCs on adults, larvae, and pupae of *B. dorsalis* was evaluated by topical, fumigants, and ingestion methods using a micro-applicator Eppendorf® 2231300002 (100–1000 µL). The formulations were prepared, following a modified protocol from Rizvi et al. 2018 [25]. Various concentrations of the treatments were prepared by adding 200 µL EOCs, 0.01% tween 80 in acetone and then mixed with a mini vortex shaker (vort: 2plus) according to the desired concentrations of 0.5, 1, 2, 3, 4, and 5% *w/v* for topical application (10 µL) and, were then prepared using the same solvent for ingestion toxicity EOCs 0.5 mL added into a sugar + water suspension, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0% (5 µL/L air) for fumigation application and the same formulation was used in mixture (synergistic) application were carvacrol, (–)-α-bisabolol, thujone (α, β-mixture), (R)-(+)-limonene, (–)-α-pinene, (–)-β-pinene with acetone at the 1:1. While in the CK (control) group, flies were treated with only 20% aqueous acetone comprising 0.1% tween 80 were applied.

2.3. *Bactrocera Dorsalis* Colonies

The culture of *B. dorsalis* was reared in the Department of Entomology, South China Agricultural University, Guangzhou, China. The *B. dorsalis* colonies were kept at 26 ± 1 °C

and $70 \pm 5\%$ RH, with a photoperiod of 10:14 (L:D). Insects were reared following the guidelines of Hassan et al. [1]. The fly eggs were harvested from insect cages without any treatment and immersed in the muslin cloths, spreading on an artificial laboratory corn-maize-based diet. Third-instar larvae and two days-old pupae were randomly selected for the larval and pupicidal toxicity bioassays. Randomly selected adult flies (12–15 days old) were used in bioassays where adult flies were tested.

2.4. Fumigation Toxicity Bioassays

Twenty adults of *B. dorsalis* of both sexes (12–15 days old) were placed in a glass jar (300 mL) and hermetically sealed with a lid. A sheet of filter paper was glued to the cap's interior. EOCs (5 μ L) in acetone at various concentrations (0.5, 1, 2, 3, 4, and 5%) were applied to the filter paper. A small layer of sterile gauze prevented the treated filter paper from direct contact with the insect. Only acetone (i.e., 100 μ L/L air) was used as a control. Before the applying EOCs treatments, the filter papers were swung for 20 s to avoid direct exposure of acetone to flies. The jars were then sealed with parafilm and kept at 26 °C, 70% RH, and a 10:14 LD photoperiod. Each test was repeated three times, and mortality was recorded in each group for 24 h following the test [30].

2.5. Ingestion Toxicity Bioassays

Following the modified protocol described by Benelli et al. [30] and Canale et al. [31], twenty mature flies (15 days old) of both sexes were placed in a 450 mL plastic container with a thin net inserted in the cap. The flies were given 200 μ L of EOCs in 800 mL clean water at various doses (0.5–5 percent). The flies were given a sugar and hydrolyze yeast ratio of 1:1 in solid form. The solution was prepared by emulsifying the EOCs in water with 2.0 percent carboxymethylcellulose sodium salt (Sigma-Aldrich®) and 12.5% sucrose [30]. The various carrier was covered with a disc of sterile gauze, allowing the flies to feed without drowning. Plastic cups were filled with sterile cotton wool and drenched with EOCs; every box has a dish inside. Each experiment was performed three times, and mortality was assessed at 96 h.

2.6. Pupal Toxicity Bioassays

The pupicidal activity of EOCs was determined by adopting the modified protocol by Zhang et al. [31], using the topical application method, and the pupae were separated by soft camel hair brush into groups of similar size and healthy. Two days-old pupae ($n = 20$) randomly selected from the cohort were then treated with 10 μ L of each concentration of specific EOCs using an Eppendorf® micropipette (10–100 μ L), and for control treatment, only acetone was applied topically. The treated and control pupae were put in the same 12 cm disposable Petri dishes with a layer of half-filled soil moisture of 70–80%. The pupae were placed in the same control conditions. After 7–10 days of EOC exposure, un-emerged or deformed treated pupae were recorded. Each treatment was replicated three times.

2.7. Larval Toxicity Bioassays

The dipping method previously described by Zhang et al. [32] was used and slightly modified to determine the toxicity of EOCs against *B. dorsalis* larvae. Each EOC was diluted to obtain different concentrations (0.5–5%) in a 10 mL glass tube. Thirty 3rd-instar larvae were immediately dipped in each dose for 1 min and transferred to the artificial rearing larval food (corn-based). For the control (CK), larvae were treated with acetone solvent only. After 48h exposure to treatment, larval mortality was recorded by touching with a soft camel hairbrush; the bioassays were replicated three times, and a total of 240 larvae per essential oil were tested.

2.8. Oviposition-Deterrent Activities

To determine the oviposition deterrent activities (ODA) of essential oil constituents, the test was performed following a modified protocol from Zhang et al. [32] and Wan-

grawa et al. [33]. Ten gravid female *B. dorsalis* were introduced in a wooden cage (30 × 30 × 30 cm) with sugar, hydrolyzed yeast, and water *ad libitum* as food. We prepared solutions of EOCs as described above, and then each concentration was applied as one puff of spray (~125 µL) on individual fruit oranges. This mechanism was offered as a two-choice test; one orange with desire treatment and the other with acetone only, provided to *B. dorsalis* adults for oviposition. The ODA bioassay replicates three times, and the number of ovipositions was counted after 24 h of exposure.

The Kramer and Mulla formula was used to determine the oviposition activity index (OAI). The index ranges from −1 to 1, with −1 indicating the most oviposition-deterrence effect and 1 indicating the lowest oviposition-deterrence effect [33,34]. The oviposition activity index (OAI) was calculated using the following formula:

$$\text{OAI} = \frac{\text{NT} - \text{NC}}{\text{NT} + \text{NC}}$$

where NT is the number (N) of eggs in treatment and NC is the number of eggs in control.

According to the oviposition activity index, three types of compounds can be identified:

I.I. No effect on oviposition OAI > 0.

II.II. Strong oviposition inhibiting substance $-1 < \text{OAI} < -0.5$.

III.III. Moderate oviposition-inhibiting substance $-0.5 < \text{OAI} < 0$

The percentage of effective repellency (ER) was calculated using the following formula:

$$\text{ER} = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100.$$

2.9. Repellency Test for the EOCs

We followed the procedures of that Zaka et al. [35] and Jaleel et al. [36] used to test the repellency of different EOCs. A Y-tube olfactometer glass tube, with central and two lateral arm sizes of 20 cm × 5 cm, was used in this experiment. Sources of EOCs or control were placed in lateral arms connected with the glass chamber. To ensure delivery of odor-free air, both arms of the olfactometer obtained charcoal purified by a humidifier operating at a rate of 1.3 L/min. EOCs (0.5 µL, 3%) were placed inside the treatment glass chamber to test the attractancy or repellency. CK-acetone was placed in another chamber at the same amount. The virgin female and unmated males were individually released at the entry point of the olfactometer and allowed for 5 min toward selective responses. Each response was recorded when the fly movement into one arm was >3 cm and stayed for more than 1 min. The chamber was shuffled to the opposite side after every ten individual fly tests. The experiment was repeated three times. Furthermore, the Y-tube olfactometer and their accessory instruments were washed with 70% alcohol followed by distilled water and sterilized at 200 °C for 30 min after every investigation. Teflon tubing was used to connect spare parts. The olfactometer was set up in a wooden box that measured 100 × 80 × 80 cm in size, with a light intensity of 5000 lx and a temperature set at 27 ± 1 °C to measure the responses. Sixty mature adult male and female flies were used once for each odor and replicated three times.

2.10. Data Analysis

Mortality data obtained in fumigation, larval, pupal, and ingestion toxicity bioassays were analyzed with a general linear model followed by Tukey's HSD test. A probability level of $p < 0.05$ was used for the significance of differences between means. The experimental mortality was corrected with Abbott's formula (Abbott, 1925) before the calculation of lethal concentrations LC₅₀ and LC₉₀ with an associated 95% confidence interval (CL), and chi-squares were estimated using Probit analysis (Finney, 1978) in Minitab 7. Data obtained from olfactometer bioassays were analyzed using the chi-square test and Kruskal-Wallis one-way analysis of variance. GraphPad Prism Version 7 and Jamovi software were used to conduct the biological meaning of the statistical analysis.

3. Results

3.1. Fumigation Toxicity Bioassays

In fumigation assays, significant effects of EOCs ($F_{6,112} = 200.08$, $p < 0.001$), their concentrations ($F_{7,112} = 503.01$, $p < 0.001$) and the interaction (EOCs \times concentrations) ($F_{42,112} = 12.38$, $p < 0.001$) was observed. At a concentration of 5%, maximum mortality (80%) of adult *B. dorsalis* was observed after treatment with carvacrol, and similar toxicity was observed after treatment with (–)-alpha-pinene with a 78% mortality rate. At 5% concentration, (–)-β-pinene showed 67% followed by (R)-(+)-limonene (55%) and (–)-α-bisabolol (47%). Minimum mortality (35%) at the highest concentration was observed in files fumigated with thujone and mixture. Overall, at all concentrations, mortality in *B. dorsalis* adults was higher after fumigation with carvacrol, followed by (–)-alpha-pinene. However, mortality caused by (–)-β-pinene fumigation was not significantly different from mortality caused by (–)-alpha-pinene at 1, 2, and 3% concentrations. Overall, the lowest mortality at all concentrations was observed after fumigation with thujone, followed by mixtures. Results showed that carvacrol was the most toxic essential oil constituent with the lowest LC₅₀, 19.48 mg/mL, followed by (–)-alpha-pinene (27.94 mg/mL), (–)-β-pinene (29.0 mg/mL), and (R)-(+)-limonene (42.24 mg/mL). Thujone was the least toxic essential oil constituent with the highest LC₅₀ (66.25 mg/mL) in the fumigation bioassay (Table 2).

Table 2. Toxicity of different EOCs in fumigant toxicity bioassay against *B. dorsalis* adults.

EOCs ^a	LC ₅₀ (95% CL) ^b	LC ₉₀ (95% CL) ^b	Slope \pm SE ^c	χ^2 (d.f.) ^d	p-Value
(–)-alpha-Pinene	27.94 (24.96–31.20)	56.28 (50.55–64.31)	1.26 \pm 0.12	28.36 (5)	0.00
(–)-β-Pinene	29.0 (25.66–34.25)	71.6 (61.0–83.44)	0.96 \pm 0.11	20.76 (5)	0.001
(–)-α-Bisabolol	47.31 (40.58–58.29)	95.84 (79.09–126.47)	1.24 \pm 0.004	12.64 (5)	0.02
Carvacrol	19.48 (15.23–23.49)	59.74 (51.47–72.75)	0.62 \pm 0.003	35.77 (5)	0.00
Thujone	66.25 (55.16–88.03)	115.25 (92.09–163.04)	1.71 \pm 0.15	4.56 (5)	0.47
(R)-(+)-Limonene	42.24 (37.07–49.73)	83.52 (71.09–104.21)	1.31 \pm 0.12	16.13 (5)	0.006
Mixture	57.52 (49.30–71.87)	101.63 (85.36–137.86)	1.67 \pm 0.15	8.42 (5)	0.13

a: essential oil constituents; b: lethal concentration (mg/mL) killing 50% (LC₅₀) or 90% (LC₉₀) of the exposed population, CL, confidence limit; c: SE, standard error; d: χ^2 , chi-square; d.f., degrees of freedom.

3.2. Ingestion Toxicity Bioassays

All EOCs investigated in this study showed insecticidal activity against adults of *B. dorsalis* when incorporated into the diet. A significant effect of the tested EOCs ($F_{6,112} = 10.27$, $p < 0.001$), their concentration ($F_{7,112} = 391.01$, $p < 0.001$) and the interaction (EOCs \times concentrations) ($F_{42,112} = 4.50$, $p < 0.001$) were observed. Concentration-dependent mortality of adult flies was observed for all tested EOCs. Maximum mortality (75%) was observed in flies feeding on a thujone-incorporated diet followed by (R)-(+)-limonene with 65% mortality in adult flies ($p = 0.94$). The lowest mortality (45%) at 5% concentration was observed in flies feeding on a (–)-α-bisabolol-incorporated diet. However, mortality was not significantly different from mortality caused by (–)-alpha-pinene (51.6%) at the highest concentration. In contrast, at 4% concentration, the lowest mortality was observed in flies feeding on a diet containing thujone and (–)-α-bisabolol (~38%). (R)-(+)-limonene caused maximum mortality at this concentration (55%) which was not significantly different from mortality caused by (–)-β-pinene, mixture, and (–)-alpha-pinene. At the lowest concentration (0.5%), thujone and carvacrol caused the lowest mortality (20–22%) of adult flies and no mortality by the mixture. To determine the effect of essential oil constituents on the mortality of adult flies, we also calculated lethal concentrations (LC₅₀ and LC₉₀) of each EOC using Probit analysis (Table 3). Overall, the highest toxicity was observed when flies fed on a diet containing (R)-(+)-limonene with the lowest LC₅₀ (37.89 mg/mL), followed by (–)-β-pinene (37.89 mg/mL) and thujone (38.85 mg/mL). (–)-α-bisabolol was the least toxic essential oil constituent with the highest median lethal concentration (50.78 mg/mL).

Table 3. Toxicity of different EOCs in ingestion toxicity bioassay against *B. dorsalis* adults.

EOCs ^a	LC ₅₀ (95% CL) ^b	LC ₉₀ (95% CL) ^b	Slope ± SE ^c	χ ² (d.f.) ^d	p-Value
(−)-alpha-Pinene	43.65 (38.11–51.92)	86.61 (73.19–109.43)	1.30 ± 0.12	14.55 (5)	0.034
(−)-β-Pinene	37.89 (33.24–44.24)	78.86 (67.48–97.41)	1.18 ± 0.12	11.89 (5)	0.00
(−)-α-Bisabolol	50.78 (43.77–62.31)	96.35 (79.98–125.87)	1.42 ± 0.13	7.75 (5)	0.17
Carvacrol	41.97 (35.84–51.59)	92.81 (76.35–123.02)	1.05 ± 0.11	17.33 (5)	0.004
Thujone	38.85 (34.38–44.89)	76.56 (66.17–93.02)	1.32 ± 0.12	20.11 (5)	0.001
(R)-(+)-Limonene	37.08 (33.50–41.53)	67.73 (60.18–78.83)	1.55 ± 0.13	8.77 (5)	0.11
Mixture	41.36 (37.74–46.02)	69.37 (61.96–80.35)	1.89 ± 0.16	19.22 (5)	0.002

a: essential oil constituents; b: lethal concentration (mg/mL) killing 50% (LC₅₀) or 90% (LC₉₀) of the exposed population, CL, confidence limit; c: SE, standard error; d: χ², chi-square; d.f., degrees of freedom.

3.3. Pupal Toxicity Bioassays

From the two-way analysis of variance, the essential oil constituents had a significant effect on pupal mortality ($F_{6,112} = 75.1, p < 0.001$) as did concentrations ($F_{7,112} = 175.8, p < 0.001$) and their interaction ($F_{42,112} = 6.20, p < 0.001$). Maximum pupal mortality (97%) was observed at a higher concentration (5%) when pupae were treated with carvacrol and thujone (96%), followed by (R)-(+)-limonene (93%) and mixture (90%). Lower pupal mortality was observed after treatment with (−)-α-bisabolol and (−)-β-pinene at 1–5% concentrations compared to the rest of the essential oil constituents. At the lowest concentration, mortality caused by all EOCs was less than 40% and not significantly different from each other except (−)-alpha-pinene, which showed significantly higher mortality (53%) compared to the rest of the EOCs. Probit analysis results after the toxicity bioassay are shown in Table 4. Overall, (−)-alpha-pinene was the most toxic EOC with the lowest LC₅₀, (11.40 mg/mL), followed by carvacrol with (14.89 mg/mL) LC₅₀. Median lethal concentrations for thujone and (R)-(+)-limonene were very similar to each other (16.21 and 16.34 mg/mL), respectively. Overall, (−)-β-pinene was the last toxic essential oil constituent with a higher LC₅₀ (27.62 mg/mL).

Table 4. Toxicity of different EOCs against *B. dorsalis* pupa.

EOCs ^a	LC ₅₀ (95% CL) ^b	LC ₉₀ (95% CL) ^b	Slope ± SE ^c	χ ² (d.f.) ^d	p-Value
(−)-alpha-Pinene	11.40 (7.50–14.50)	40.80 (36.0–47.60)	0.49 ± 0.10	40.59 (5)	0.45
(−)-β-Pinene	27.62 (23.85–31.94)	65.40 (56.91–78.51)	0.92 ± 0.11	16.84 (5)	0.34
(−)-α-Bisabolol	23.89 (20.11–27.91)	62.02 (53.23–74.52)	0.80 ± 0.10	16.22 (5)	0.33
Carvacrol	14.89 (12.11–17.52)	38.52 (34.5–43.48)	0.71 ± 0.11	17.94 (5)	0.23
Thujone	16.21 (13.50–18.71)	39.29 (35.39–44.34)	0.89 ± 0.11	25.22 (5)	0.32
(R)-(+)-Limonene	16.34 (13.29–19.16)	43.16 (38.50–49.35)	0.77 ± 0.13	27.33 (5)	0.12
Mixture	16.56 (13.47–19.34)	43.88 (39.29–52.12)	0.78 ± 0.15	29.13 (5)	0.23

a: essential oil constituents; b: lethal concentration (mg/mL) killing 50% (LC₅₀) or 90% (LC₉₀) of the exposed population, CL, confidence limit; c: SE, standard error; d: χ², chi-square; d.f., degrees of freedom.

3.4. Larval Toxicity Bioassays

All EOCs investigated in this study showed insecticidal activity against *B. dorsalis* larvae. A significant effect of the tested EOCs ($F_{6,112} = 33.54, p < 0.001$), their concentration ($F_{7,112} = 1042, p < 0.001$) and the interaction (EOCs × concentrations) ($F_{42,112} = 7.27, p < 0.001$) were observed. Maximum larval mortality (85%) was observed after treatment with thujone at a 5% concentration. However, it was not significantly different from mortalities after treatment with (−)-β-pinene, (R)-(+)-limonene, and carvacrol at this concentration. (−)-alpha-pinene and (−)-α-bisabolol caused significantly lower mortality (73%) compared

to all other tested EOCs except the EOC mixture, which caused 58% larval mortality at 5% concentration. Larval mortality was concentration-dependent for all EOCs. Overall, carvacrol was the most toxic essential oil component with the lowest LC₅₀ (26.19 mg/mL), followed by (–)-alpha-pinene (26.54 mg/mL) and (R)-(+)-limonene (29.12 mg/mL). The lowest toxicity was observed when essential oil as a mixture was applied to larvae with the highest LC₅₀ (38.56 mg/mL) (Table 5).

Table 5. Toxicity of different EOCs against *B. dorsalis* larvae.

EOCs ^a	LC ₅₀ (95% CL) ^b	LC ₉₀ (95% CL) ^b	Slope ± SE ^c	χ ² (d.f.) ^d	<i>p</i> -Value
(–)-alpha-Pinene	26.54 (22.90–30.52)	62.81 (54.92–74.74)	0.93 ± 0.11	24.68 (5)	0.00
(–)-β-Pinene	31.91 (29.03–35.19)	57.69 (52.20–65.26)	1.58 ± 0.13	6.45 (5)	0.14
(–)-α-Bisabolol	33.12 (29.51–37.24)	69.41 (56.92–75.59)	1.33 ± 0.12	12.52 (5)	0.15
Carvacrol	26.19 (23.39–29.21)	53.31 (48.08–66.03)	1.23 ± 0.12	20.69 (5)	0.32
Thujone	31.12 (28.61–33.73)	51.77 (47.62–57.15)	1.93 ± 0.15	6.35 (5)	0.32
(R)-(+)-Limonene	29.12 (26.41–32.23)	54.23 (49.21–60.92)	1.49 ± 0.13	13.43 (5)	0.19
Mixture	38.56 (35.09–42.95)	67.56 (60.32–78.07)	1.70 ± 0.14	17.23 (5)	0.24

a: essential oil constituents; b: lethal concentration (mg/mL) killing 50% (LC₅₀) or 90% (LC₉₀) of the exposed population, CL, confidence limit; c: SE, standard error; d: χ², chi-square; d.f., degrees of freedom.

3.5. Oviposition-Deterrent Activities

Egg-laying by *B. dorsalis* was significantly reduced on the treated oranges compared to oranges treated with acetone. The inhibition of egg-laying by *B. dorsalis* depended on EOC concentrations ($p < 0.05$). The ODA gradually decreases with increasing concentrations for all the essential oils tested. All EOCs showed a stronger correlation between concentration and oviposition deterrence. At the maximum concentration, all EOCs strongly inhibited the oviposition of *B. dorsalis* gravid females. No eggs were observed on oranges treated with 5% of each EOC ($p < 0.05$) (100% repellency). However, at 4% concentration, the lowest oviposition deterrence was observed in fruits treated with (R)-(+)-limonene compared to fruits treated with all other EOCs. At the lowest concentration (0.5%), the minimum activity was observed in fruits treated with carvacrol (12.5%), followed by (R)-(+)-limonene (16.98%). Thujone and mixture showed 31–32% repellency at the lowest concentration, which was significantly less than the rest of the ECOs, which showed slightly higher repellency (37–40%) to oviposition on treated oranges. Overall, all ECOs showed very weak or moderate oviposition deterrence at 1 and 2% concentration and high oviposition deterrence at 3, 4, and 5% of each ECOs except (R)-(+)-limonene which moderate deterrence at 3% concentration (Table 6).

3.6. Repellency Test

The responses of *B. dorsalis* in the Y-tube olfactometer at the tested concentration of EOCs are shown in (Figure 1). Essential oils constituents showed a repellency in varying degrees against *B. dorsalis* adult flies compared to control treatment in olfactometer Bioassays. All EOCs showed significantly higher repellency at the tested concentration than the control treatment determined by χ² analysis at $p < 0.05$. Similarly, a significant difference in repellence was also observed among different essential oil constituents determined by the Kruskal-Wallis statistic ($H = 23.55$; $p = 0.006$). A higher number of flies (77%) were repelled from the Y-tube olfactometer arm containing (–)-alpha-pinene, followed by carvacrol (76%). The lowest repellence was observed in arms containing mixture (R)-(+)-limonene and thujone (59, 61, and 65%, respectively).

Table 6. The oviposition-deterrent activities of different EOCs against *B. dorsalis* adults.

EOCs	Doses											
	5%		4%		3%		2%		1%		0.5%	
	EOD (%)	OAI	ER (%)	OAI	ER (%)	OAI	ER (%)	OAI	ER (%)	OAI	ER (%)	OAI
(-)-alpha-Pinene	100 ^{a, A}	-1	93.17 ^{b, A}	-0.87	75.03 ^{c, A}	-0.60	63.64 ^{d, B}	-0.46	47.74 ^{e, AB}	-0.31	36.75 ^{f, B}	-0.22
(-)-β-Pinene	100 ^{a, A}	-1	97.58 ^{a, A}	-0.95	78.03 ^{b, A}	-0.63	60.98 ^{c, BC}	-0.43	46.33 ^{d, B}	-0.30	36.60 ^{e, B}	-0.22
(-)-α-Bisabolol	100 ^{a, A}	-1	97.32 ^{a, A}	-0.94	78.42 ^{b, A}	-0.64	67.55 ^{c, AB}	-0.51	54.09 ^{d, A}	-0.37	40.55 ^{e, A}	-0.25
Carvacrol	100 ^{a, A}	-1	95.87 ^{a, A}	-0.92	68.75 ^{b, B}	-0.52	43.75 ^{c, D}	-0.28	22.93 ^{d, E}	-0.12	12.5 ^{e, E}	-0.043
Thujone	100 ^{a, A}	-1	94.78 ^{b, A}	-0.90	84.20 ^{c, A}	-0.72	73.69 ^{d, A}	-0.58	39.49 ^{e, C}	-0.24	31.59 ^{f, C}	-0.18
(R)-(+)-Limonene	100 ^{a, A}	-1	81.14 ^{b, B}	-0.68	62.28 ^{c, C}	-0.45	47.16 ^{d, CD}	-0.30	26.38 ^{e, D}	-0.15	16.98 ^{f, D}	-0.092
Mixture	100 ^{a, A}	-1	97.32 ^{a, A}	-0.94	72.99 ^{b, AB}	-0.57	54.09 ^{c, C}	-0.37	40.55 ^{d, C}	-0.25	32.44 ^{e, C}	-0.19

Small letters = Different letters show significant differences between different concentration of EOC by Tukey HSD test ($p < 0.05$). Capital letters = Different letters show significant differences between different EOCs at the same concentration level by Tukey HSD test ($p < 0.05$). OAI = Oviposition Active Index; EOCs = Essential oil constituents; EOD = effective oviposition deterrent.

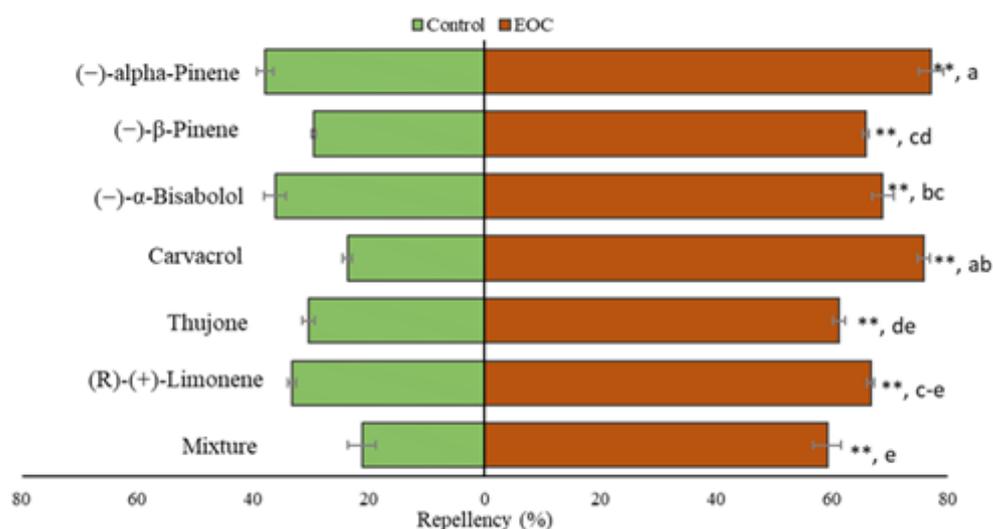


Figure 1. Response of *B. dorsalis* adults to the EOCs against control in a Y-tube olfactometer study. Bars marked with ‘***’ represent significantly greater repellency by EOC than control by χ^2 analysis at $p < 0.05$. ‘‘Alphabets’’ on each bar represent significantly different responses between the different EOCs at the same concentration by Kruskal-Wallis H-test (at $p < 0.05$).

4. Discussion

The result of the current study implies that the EOCs could be utilized to control this pest because they were poisonous and repulsive to *B. dorsalis* to varying degrees. The toxicity and repellency of these tested EOCs have been reported against other fruit flies and insect pests in previous research [16,22]. The constituents of essential oils studied in the current study belong to several chemical classes that can be acquired from various sources. Therefore, they may have a distinct mode of action against insect pests.

Moreover, the biological activities of essential oils are strongly influenced by the insect species studied. The findings may vary when different insect species are employed in experiments using the same essential oil. Moreover, the disparity in toxicity across the tested compounds on fruit flies could be due to the various means of EO chemical

application (contact ingestion vs. topical application) and the different developmental phases of the flies studied (larvae vs. adults) [37].

In our fumigation, pupicidal, and larvicidal toxicity tests, carvacrol was the most toxic EOC against *B. dorsalis*. This could be due to its interaction with tyramine, a precursor of octopamine in insect tissues, including the nervous system [38]. Carvacrol also has a cytotoxic effect when absorbed and stored in the tissues [16]. The essential oil extracted from the genera *Origanum*, *Thymus*, *Coridothymus*, *Thymbra*, *Satureja* and *Lippia* are rich sources of carvacrol [39]. Fumigation activities of carvacrol against tephritid flies have not been studied previously except for *C. capitata* [40]. However, carvacrol had good fumigant efficacy against *Lycoriella ingenua* larvae, *Culex pipiens* adults ($LC_{50} = 0.26$ mg/L air) and *Reticulitermes speratus* workers ($LC_{50} = 0.34$ mg/Petri dish) [41–43]. Carvacrol fumigant toxicity against adults of *Tenebrio molitor* had LC_{50} values of 5.53 and 4.77 $\mu\text{L/L}$ after 24 and 48 h, respectively [44]. The larvicidal potential of carvacrol has been observed in several insect pests, including *Anopheles stephensi*, *Anopheles subpictus*, *C. quinquefasciatus*, and *C. tritaeniorhynchus* with LC_{50} values ranging from 21.15 to 27.95 $\mu\text{g/mL}$ [45]. LC_{50} of carvacrol using the leaf dipping bioassay against *Pochazia shantungensis* nymphs was 56.74 mg/L [46]. Carvacrol was very toxic to *Alphitobius diaperinus* larvae at 1% concentration and caused more than 85% mortality in laboratory bioassays [47]. Contact toxicity LD_{50} values of carvacrol were 1.30–2.60 $\mu\text{g/fly}$ for male and female *Drosophila suzukii* [48], 15 $\mu\text{g/larva}$ against third instar *Spodoptera littoralis* [49]. Carvacrol significantly reduced the adult emergence rate in *Musca domestica* in contact toxicity assays [50]. On the other hand, the fumigant and larval toxicities of essential oils and their compounds were much lower compared to phosphine against *B. tau* ($LD_{50} = 0.40$ $\mu\text{g/insect}$) [51] and Emamectin benzoate ($LC_{50} = 0.943$ mg/L) [52] or Spinetoram ($F_{4,20} = 67.569$, $p < 0.01$) against *C. capitata* [53] in positive control.

In the ingestion toxicity bioassay, (R)-(+)-limonene was the most toxic EOC, followed by (–)- β -pinene and thujone. Essential oils obtained from many plants of the family Rutaceae are good sources of limonene and other monoterpene compounds [54]. Essential oil components of limonene and pinene, though the mode of action remains primarily unknown, on the other hand, interact with the acetylcholinesterase (AChE) and octopaminergic systems (OS). The mechanism of action of *Melaleuca alternifolia* essential oil against *C. capitata* adults has also been proposed as an anti-AChE impact [16]. Our results support the previous study in which, compared to controls, essential oils from *Baccharis dracunculifolia* and *Pinus elliottii*, which contain large quantities of α -pinene and limonene, caused 58–70% mortality in *C. capitata* adults in ingestion bioassay [55]. Similarly, despite the diet, limonene was one of the most toxic EOCs that caused substantial toxicity in adult medflies, with males being more sensitive than females [40]. However, the toxicity of EOCs was less than acetamiprid ($LC_{50} = 0.448$ mg/L) or chlorpyrifos ($LC_{50} = 0.957$ mg/L) against *B. dorsalis* [52] in positive control.

Limonene was more toxic to medfly larvae as compared to α -pinene [56]. Moreover, Papanastasiou et al. [40] evaluated the impact of sub-lethal doses of limonene on medflies, suggesting that, depending on the dosage, this molecule may have a hormetic-like or insecticidal effect. Essential oils containing α -pinene and α -thujone have already been revealed to be toxic to adults *Drosophila* [57] and *C. capitata* via ingestion toxicity > 50% mortality at the dose of 1.5% (w/v) and contact toxicity LD_{50} of 0.024 $\mu\text{L/fly}$ after 96 h [58] and widely recognized toxic to spider mite, *Oligonychus ununguis* [59]. In addition, the adults of *M. domestica* were more susceptible to the increased contact toxicity of *Thuja occidentalis* containing (thujone and pinene) EO ($LD_{50} = 33$ mg fly^{–1}, after 24 h of exposure) [60]. However, the toxicity of EOCs was less toxic to pupae than *Escherichia coli*-based dsRNA (87.8% 700 μL of $\times 200$ of 3.5×10^8 cells) [61] or *Metarhizium anisopliae* ($LC_{50} = 1.0 \times 10^{10}$ conidia/mL, $F_{4,10} = 251.7$, $p < 0.001$) [62] in positive control.

Essential oils are responsible for the distinctive odor of plants. Therefore, essential oils or their components also act as repellents and alter insect behavior by preventing them from flying to, landing/walking on, or ovipositing on a specific source [60]. In the current

study, all EOCs showed concentration-dependent antifeedant activity and were repellent to adults *B. dorsalis*. A large group of EO extracted or their components from different families have been shown to have high repellency against many arthropod species [63]. Only a few studies have examined the repellent and oviposition-deterrent properties of essential oils and their major constituents against tephritid fruit flies [16,64,65]. However, previous studies have reported the repellent, antifeedant and biological activities of tested compounds, and other IPM techniques against various insect pests [29,66–72]. The Y-tube olfactometer bioassay used in the current investigation demonstrated the tested chemicals' repellent properties. However, some essential oils or their constituents' repellent activity is typically short-lived and only works when applied freshly. Therefore, field or semi-field testing is required to determine the repellent effectiveness of these chemicals. Moreover, adding some fixative agents to retain their repellent activity warrants further studies.

5. Conclusions

This study adds to our understanding of six essential oil constituents' insecticidal and repellent activities against larvae and adults of the oriental fruit fly, *B. dorsalis*. Regardless of the bioassay employed, the results demonstrated that all EOCs showed insecticidal and repellent activities to *B. dorsalis* in a concentration-dependent manner. To better analyze the biological activity of these chemicals, more research is needed, including field studies to test their oviposition deterrent efficacy and field trials to examine their influence on fruit infestation. The essential oil constituents have high volatility for open field application—appropriate formulations, i.e., nano-formulation, cyclodextrin, zein nanoparticles, etc., should be prepared to enhance their persistence.

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References

1. Hassan, B.; Siddiqui, J.A.; Xu, Y. Vertically transmitted gut bacteria and nutrition influence the immunity and fitness of *Bactrocera dorsalis* larvae. *Front. Microbiol.* **2020**, *11*, 596352. [\[CrossRef\]](#)
2. Chang, C.; Huang, C.-Y.; Dai, S.-M.; Atlihan, R.; Chi, H. Genetically Engineered Ricin Suppresses *Bactrocera dorsalis* (Diptera: Tephritidae) based on Demographic Analysis of Group-Reared Life Table. *J. Econ. Entomol.* **2016**, *109*, 987–992. [\[CrossRef\]](#)
3. Perry, A.S.; Yamamoto, I.; Ishaaya, I.; Perry, R.Y. *Insecticides in Agriculture and Environment: Retrospects and Prospects*; Springer Science & Business Media: Berlin, Germany, 2013; ISBN 13.978-3540627296.
4. Schreinemachers, P.; Tipraqsa, P. Agricultural pesticides and land use intensification in high, middle and low income countries. *Food Policy* **2012**, *37*, 616–626. [\[CrossRef\]](#)
5. Sparks, T.C.; Nauen, R. IRAC: Mode of action classification and insecticide resistance management. *Pestic. Biochem. Physiol.* **2015**, *121*, 122–128. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Pan, H.; Preisser, E.L.; Chu, D.; Wang, S.; Wu, Q.; Carrière, Y.; Zhou, X.; Zhang, Y. Insecticides promote viral outbreaks by altering herbivore competition. *Ecol. Appl.* **2015**, *25*, 1585–1595. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Dunn, P.E. Biochemical aspects of insect immunology. *Annu. Rev. Entomol.* **1986**, *31*, 321–339. [\[CrossRef\]](#)
8. Hsu, J.-C.; Feng, H.-T.; Wu, W.-J. Resistance and synergistic effects of insecticides in *Bactrocera dorsalis* (Diptera: Tephritidae) in Taiwan. *J. Econ. Entomol.* **2004**, *97*, 1682–1688. [\[CrossRef\]](#)

9. Jin, T.; Zeng, L.; Lin, Y.; Lu, Y.; Liang, G. Insecticide resistance of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), in mainland China. *Pest Manag. Sci.* **2011**, *67*, 370–376. [[CrossRef](#)] [[PubMed](#)]
10. Khan, H.A.A.; Akram, W. Trichlorfon and spinosad resistance survey and preliminary determination of the resistance mechanism in Pakistani field strains of *Bactrocera dorsalis*. *Sci. Rep.* **2018**, *8*, 11223. [[CrossRef](#)] [[PubMed](#)]
11. Wadhwa, G.; Kumar, S.; Chhabra, L.; Mahant, S.; Rao, R. Essential oil–cyclodextrin complexes: An updated review. *J. Incl. Phenom. Macrocycl. Chem.* **2017**, *89*, 39–58. [[CrossRef](#)]
12. Rizzo, R.; Verde, G.L.; Sinacori, M.; Maggi, F.; Cappellacci, L.; Petrelli, R.; Vittori, S.; Morshedloo, M.R.; Benelli, G. Developing green insecticides to manage olive fruit flies? Ingestion toxicity of four essential oils in protein baits on *Bactrocera oleae*. *Ind. Crops Prod.* **2020**, *143*, 111884. [[CrossRef](#)]
13. Hillocks, R.J. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Prot.* **2012**, *31*, 85–93. [[CrossRef](#)]
14. Kalemba, D.; Kunicka, A. Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.* **2003**, *10*, 813–829. [[CrossRef](#)] [[PubMed](#)]
15. Isman, M.B. Botanical insecticides in the twenty-first century—fulfilling their promise? *Annu. Rev. Entomol.* **2020**, *65*, 233–249. [[CrossRef](#)] [[PubMed](#)]
16. Zeni, V.; Benelli, G.; Campolo, O.; Giunti, G.; Palmeri, V.; Maggi, F.; Rizzo, R.; Lo Verde, G.; Lucchi, A.; Canale, A. Toxics or lures? Biological and behavioral effects of plant essential oils on tephritidae fruit flies. *Molecules* **2021**, *26*, 5898. [[CrossRef](#)] [[PubMed](#)]
17. Isman, M.B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochem. Rev.* **2020**, *19*, 235–241. [[CrossRef](#)]
18. Burčul, F.; Blažević, I.; Radan, M.; Politeo, O. Terpenes, phenylpropanoids, sulfur and other essential oil constituents as inhibitors of cholinesterases. *Curr. Med. Chem.* **2020**, *27*, 4297–4343. [[CrossRef](#)] [[PubMed](#)]
19. Abdel-Kader, M.; El-Mougy, N.; Lashin, S. Essential oils and *Trichoderma harzianum* as an integrated control measure against faba bean root rot pathogens. *J. Plant Prot. Res.* **2011**, *51*, 3. [[CrossRef](#)]
20. Regnault-Roger, C.; Vincent, C.; Arnason, J.T. Essential oils in insect control: Low-risk products in a high-stakes world. *Annu. Rev. Entomol.* **2012**, *57*, 405–424. [[CrossRef](#)] [[PubMed](#)]
21. Ebadollahi, A.; Jalali Sendi, J. A review on recent research results on bio-effects of plant essential oils against major Coleopteran insect pests. *Toxin Rev.* **2015**, *34*, 76–91. [[CrossRef](#)]
22. Segura, D.F.; Belliard, S.A.; Vera, M.T.; Bachmann, G.E.; Ruiz, M.J.; Jofre-Barud, F.; Fernández, P.C.; López, M.L.; Shelly, T.E. Plant chemicals and the sexual behavior of male tephritid fruit flies. *Ann. Entomol. Soc. Am.* **2018**, *111*, 239–264. [[CrossRef](#)]
23. Diongue, A.; Yen, T.B.; Lai, P.-Y. Bioassay studies on the effect of essential oils on the female oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Pest Manag. Hortic. Ecsyst.* **2010**, *16*, 91–102.
24. Akter, M.; Theary, K.; Kalkornsurapranee, E.; Prabhakar, C.S.; Thaochan, N.M. The effects of methyl eugenol, cue lure and plant essential oils in rubber foam dispenser for controlling *Bactrocera dorsalis* and *Zeugodacus cucurbitae*. *Asian J. Agric. Biol.* **2021**, *9*, 356–367. [[CrossRef](#)]
25. Rizvi, S.A.H.; Ling, S.; Tian, F.; Xie, F.; Zeng, X. Toxicity and enzyme inhibition activities of the essential oil and dominant constituents derived from *Artemisia absinthium* L. against adult Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Ind. Crops Prod.* **2018**, *121*, 468–475. [[CrossRef](#)]
26. Rizvi, S.A.H.; Ling, S.; Zeng, X. *Seriphidium brevifolium* essential oil: A novel alternative to synthetic insecticides against the dengue vector *Aedes albopictus*. *Environ. Sci. Pollut. Res.* **2020**, *27*, 31863–31871. [[CrossRef](#)] [[PubMed](#)]
27. Langsi, J.D.; Nukene, E.N.; Oumarou, K.M.; Moktar, H.; Fokunang, C.N.; Mbata, G.N. Evaluation of the insecticidal activities of α -Pinene and 3-Carene on *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Insects* **2020**, *11*, 540. [[CrossRef](#)] [[PubMed](#)]
28. Sun, J.; Feng, Y.; Wang, Y.; Li, J.; Zou, K.; Liu, H.; Hu, Y.; Xue, Y.; Yang, L.; Du, S. Investigation of pesticidal effects of *Peucedanum terebinthinaceum* essential oil on three stored-product insects. *Rec. Nat. Prod.* **2020**, *14*, 177–189. [[CrossRef](#)]
29. Pajaro-Castro, N.; Caballero-Gallardo, K.; Olivero-Verbel, J. Neurotoxic effects of linalool and β -pinene on *Tribolium castaneum* Herbst. *Molecules* **2017**, *22*, 2052. [[CrossRef](#)]
30. Benelli, G.; Flamini, G.; Canale, A.; Cioni, P.L.; Conti, B. Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitidis capitata* (Wiedemann)(Diptera Tephritidae). *Crop Prot.* **2012**, *42*, 223–229. [[CrossRef](#)]
31. Canale, A.; Benelli, G.; Conti, B.; Lenzi, G.; Flamini, G.; Francini, A.; Cioni, P.L. Ingestion toxicity of three Lamiaceae essential oils incorporated in protein baits against the olive fruit fly, *Bactrocera oleae* (Rossi)(Diptera Tephritidae). *Nat. Prod. Res.* **2013**, *27*, 2091–2099. [[CrossRef](#)] [[PubMed](#)]
32. Zhang, Y.-N.; He, P.; Xue, J.-P.; Guo, Q.; Zhu, X.-Y.; Fang, L.-P.; Li, J.-B. Insecticidal activities and biochemical properties of *Pinellia ternata* extracts against the beet armyworm *Spodoptera exigua*. *J. Asia Pac. Entomol.* **2017**, *20*, 469–476. [[CrossRef](#)]
33. Wangrawa, D.W.; Badolo, A.; Guelbéogo, W.M.; Nébíé, R.C.H.; Sagnon, N.F.; Borovsky, D.; Sanon, A. Larvicidal, oviposition-deterrence, and excito-repellency activities of four essential oils: An eco-friendly tool against malaria vectors *Anopheles coluzzii* and *Anopheles gambiae* (Diptera: Culicidae). *Int. J. Trop. Insect Sci.* **2021**, *41*, 1771–1781. [[CrossRef](#)]
34. Kramer, W.L.; Mulla, M.S. Oviposition attractants and repellents of mosquitoes: Oviposition responses of *Culex* mosquitoes to organic infusions. *Environ. Entomol.* **1979**, *8*, 1111–1117. [[CrossRef](#)]
35. Zaka, S.M.; Zeng, X.N.; Holford, P.; Beattie, G.A.C. Repellent effect of guava leaf volatiles on settlement of adults of citrus psylla, *Diaphorina citri* Kuwayama, on citrus. *Insect Sci.* **2010**, *17*, 39–45. [[CrossRef](#)]

36. Jaleel, W.; He, Y.; Lü, L. The response of two *Bactrocera* species (Diptera: Tephritidae) to fruit volatiles. *J. Asia Pac. Entomol.* **2019**, *22*, 758–765. [[CrossRef](#)]
37. Lima, A.; Arruda, F.; Medeiros, J.; Baptista, J.; Madruga, J.; Lima, E. Variations in Essential Oil Chemical Composition and Biological Activities of *Cryptomeria japonica* (Thunb. ex L.f.) D. Don from Different Geographical Origins & mdash; A Critical Review. *Appl. Sci.* **2021**, *11*, 11097. [[CrossRef](#)]
38. Enan, E.E. Molecular response of *Drosophila melanogaster* tyramine receptor cascade to plant essential oils. *Insect Biochem. Mol. Biol.* **2005**, *35*, 309–321. [[CrossRef](#)] [[PubMed](#)]
39. Can Baser, K. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. *Curr. Pharm.* **2008**, *14*, 3106–3119. [[CrossRef](#)] [[PubMed](#)]
40. Papanastasiou, S.A.; Bali, E.-M.D.; Ioannou, C.S.; Papachristos, D.P.; Zarpas, K.D.; Papadopoulos, N.T. Toxic and hormetic-like effects of three components of citrus essential oils on adult Mediterranean fruit flies (*Ceratitidis capitata*). *PLoS ONE* **2017**, *12*, e0177837. [[CrossRef](#)] [[PubMed](#)]
41. Park, I.-K.; Kim, J.N.; Lee, Y.-S.; Lee, S.-G.; Ahn, Y.-J.; Shin, S.-C. Toxicity of plant essential oils and their components against *Lycoriella ingenua* (Diptera: Sciaridae). *J. Econ. Entomol.* **2008**, *101*, 139–144. [[CrossRef](#)]
42. Ma, W.-B.; Feng, J.-T.; Jiang, Z.-L.; Wu, H.; Ma, Z.-Q.; Zhang, X. Fumigant activity of eleven essential oil compounds and their selected binary mixtures against *Culex pipiens pallens* (Diptera: Culicidae). *Parasitol. Res.* **2014**, *113*, 3631–3637. [[CrossRef](#)]
43. Sekine, N.; Shibutani, S. Chemical structures of p-menthane monoterpenes with special reference to their effect on seed germination and termite mortality. *J. Wood Sci.* **2013**, *59*, 229–237. [[CrossRef](#)]
44. Lima, R.K.; Cardoso, M.D.G.; Moraes, J.C.; Carvalho, S.M.; Rodrigues, V.G.; Guimarães, L.G.L. Chemical composition and fumigant effect of essential oil of *Lippia sidoides* Cham. and monoterpenes against *Tenebrio molitor* (L.)(Coleoptera: Tenebrionidae). *Cienc. Agrotecnol.* **2011**, *35*, 664–671. [[CrossRef](#)]
45. Govindarajan, M.; Rajeswary, M.; Hoti, S.; Benelli, G. Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). *Res. Vet. Sci.* **2016**, *104*, 77–82. [[CrossRef](#)]
46. Park, J.-H.; Jeon, Y.-J.; Lee, C.-H.; Chung, N.; Lee, H.-S. Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu., newly recorded pest. *Sci. Rep.* **2017**, *7*, 40902. [[CrossRef](#)] [[PubMed](#)]
47. Szczepanik, M.; Zawitowska, B.; Szumny, A. Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). *Allelopathy J.* **2012**, *30*, 129–142.
48. Park, C.G.; Jang, M.; Yoon, K.A.; Kim, J. Insecticidal and acetylcholinesterase inhibitory activities of Lamiaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). *Ind. Crops Prod.* **2016**, *89*, 507–513. [[CrossRef](#)]
49. Pavela, R. Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Boisid.(Lep., Noctuidae) larvae. *Ind. Crops Prod.* **2014**, *60*, 247–258. [[CrossRef](#)]
50. Xie, Y.; Huang, Q.; Rao, Y.; Hong, L.; Zhang, D. Efficacy of *Origanum vulgare* essential oil and carvacrol against the housefly, *Musca domestica* L. (Diptera: Muscidae). *Environ. Sci. Pollut. Res.* **2019**, *26*, 23824–23831. [[CrossRef](#)]
51. Li, L.; Liu, T.; Li, B.; Zhang, F.; Dong, S.; Wang, Y. Toxicity of Phosphine Fumigation against *Bactrocera tau* at Low Temperature. *J. Econ. Entomol.* **2014**, *107*, 601–605. [[CrossRef](#)]
52. Li, J.; Liu, J.; Chi, B.; Chen, P.; Liu, Y. Sublethal and transgenerational effects of six insecticides on *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *J. Asia Pac. Entomol.* **2021**, *24*, 14–23. [[CrossRef](#)]
53. Lin, J.; Hao, X.; Yue, G.; Yang, D.; Lu, N.; Cai, P.; Ao, G.; Ji, Q. Efficacy of wax-based bait stations for controlling *Bactrocera dorsalis* (Diptera: Tephritidae). *Pest Manag. Sci.* **2022**, *78*, 3576–3586. [[CrossRef](#)] [[PubMed](#)]
54. Eddin, L.B.; Jha, N.K.; Meeran, M.N.; Kesari, K.K.; Beiram, R.; Ojha, S. Neuroprotective potential of limonene and limonene containing natural products. *Molecules* **2021**, *26*, 4535. [[CrossRef](#)] [[PubMed](#)]
55. Hamraoui, A.; Regnault-Roger, C. Comparaison des activités insecticides des monoterpènes sur deux espèces d’insectes ravageurs des cultures: *Ceratitidis capitata* et *Rhopalosiphum padi*. *Acta Bot. Gall.* **1997**, *144*, 413–417. [[CrossRef](#)]
56. Papachristos, D.; Kimbaris, A.; Papadopoulos, N.; Polissiou, M. Toxicity of citrus essential oils against *Ceratitidis capitata* (Diptera: Tephritidae) larvae. *Ann. Appl. Biol.* **2009**, *155*, 381–389. [[CrossRef](#)]
57. Höld, K.M.; Sirisoma, N.S.; Ikeda, T.; Narahashi, T.; Casida, J.E. α -Thujone (the active component of absinthe): γ -aminobutyric acid type A receptor modulation and metabolic detoxification. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3826–3831. [[CrossRef](#)]
58. Kurtca, M.; Tumen, I.; Keskin, H.; Tabanca, N.; Yang, X.; Demirci, B.; Kendra, P.E. Chemical Composition of Essential Oils from Leaves and Fruits of *Juniperus foetidissima* and Their Attractancy and Toxicity to Two Economically Important Tephritid Fruit Fly Species, *Ceratitidis capitata* and *Anastrepha suspensa*. *Molecules* **2021**, *26*, 7504. [[CrossRef](#)]
59. Ellis, M.D.; Baxendale, F.P. Toxicity of Seven Monoterpenoids to Tracheal Mites (Acari: Tarsonemidae) and Their Honey Bee (Hymenoptera: Apidae) Hosts When Applied as Fumigants. *J. Econ. Entomol.* **1997**, *90*, 1087–1091. [[CrossRef](#)]
60. Pavela, R. Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). *Phytother Res.* **2008**, *22*, 274–278. [[CrossRef](#)]
61. Mohanpuria, P.; Govindaswamy, M.; Sidhu, G.S.; Singh, S.; Kaur, S.; Chhuneja, P. Ingestion of bacteria expressing dsRNA to maggots produces severe mortality and deformities in fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Egypt. J. Biol. Pest Control* **2021**, *31*, 1. [[CrossRef](#)]

62. Wang, D.; Liang, Q.; Chen, M.; Ye, H.; Liao, Y.; Yin, J.; Lü, L.; Lei, Y.; Cai, D.; Jaleel, W.; et al. Susceptibility of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) pupae to entomopathogenic fungi. *Appl. Entomol. Zool.* **2021**, *56*, 269–275. [[CrossRef](#)]
63. Nerio, L.S.; Olivero-Verbel, J.; Stashenko, E. Repellent activity of essential oils: A review. *Bioresour. Technol.* **2010**, *101*, 372–378. [[CrossRef](#)] [[PubMed](#)]
64. Brilinger, D.; Wille, C.L.; Da Rosa, J.M.; Franco, C.R.; Boff, M.I.C. Mortality Assessment of Botanical Oils on *Anastrepha fraterculus* (Wiedemann, 1830) Applied in Fruits Under Laboratory Conditions. *J. Agric. Sci.* **2019**, *11*, 287. [[CrossRef](#)]
65. Niassy, S.; Murithii, B.; Omuse, E.R.; Kimathi, E.; Tonnang, H.; Ndlela, S.; Mohamed, S.; Ekesi, S. Insight on fruit fly IPM technology uptake and barriers to scaling in Africa. *Sustainability* **2022**, *14*, 2954. [[CrossRef](#)]
66. Sedy, K.; Koschier, E. Bioactivity of carvacrol and thymol against *Frankliniella occidentalis* and *Thrips tabaci*. *J. Appl. Entomol.* **2003**, *127*, 313–316. [[CrossRef](#)]
67. Malacrino, A.; Campolo, O.; Laudani, F.; Palmeri, V. Fumigant and repellent activity of limonene enantiomers against *Tribolium confusum* du Val. *Neotrop Entomol.* **2016**, *45*, 597–603. [[CrossRef](#)] [[PubMed](#)]
68. Hebeish, A.; Fouda, M.M.; Hamdy, I.; El-Sawy, S.; Abdel-Mohdy, F. Preparation of durable insect repellent cotton fabric: Limonene as insecticide. *Carbohydr. Polym.* **2008**, *74*, 268–273. [[CrossRef](#)]
69. Guo, S.; Zhang, W.; Liang, J.; You, C.; Geng, Z.; Wang, C.; Du, S. Contact and repellent activities of the essential oil from *Juniperus formosana* against two stored product insects. *Molecules* **2016**, *21*, 504. [[CrossRef](#)]
70. Usha Rani, P.; Madhusudhanamurthy, J.; Sreedhar, B. Dynamic adsorption of α -pinene and linalool on silica nanoparticles for enhanced antifeedant activity against agricultural pests. *J. Pest Sci.* **2014**, *87*, 191–200. [[CrossRef](#)]
71. Tabari, M.A.; Youssefi, M.R.; Benelli, G. Eco-friendly control of the poultry red mite, *Dermanyssus gallinae* (Dermanyssidae), using the α -thujone-rich essential oil of *Artemisia sieberi* (Asteraceae): Toxic and repellent potential. *Parasitol. Res.* **2017**, *116*, 1545–1551. [[CrossRef](#)]
72. Mudereri, B.T.; Abdel-Rahman, E.M.; Ndlela, S.; Makumbe, L.D.M.; Nyanga, C.C.; Tonnang, H.E.Z.; Mohamed, S.A. Integrating the Strength of Multi-Date Sentinel-1 and -2 Datasets for Detecting Mango (*Mangifera indica* L.) Orchards in a Semi-Arid Environment in Zimbabwe. *Sustainability* **2022**, *14*, 5741. [[CrossRef](#)]