Iraqi Journal of Agricultural Sciences –2026:57(5):1443-1451
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/1za74y39

EVALUATION OF THE INSECTICIDAL ACTIVITY OF ORGANIC CHEMICALS ON PEACH FRUIT FLY, *BACTROCERA ZONATA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

This study aimed to assess the toxicity of these compounds against the third-instar larvae and pupal stages of the peach fruit fly, *Bactrocera zonata*, under laboratory conditions. Three concentrations of the above compounds (1, 3, and 5%) were prepared and evaluated. The results indicated that methyl salicylate and thymol were more effective than limonene, especially at high concentrations, with mortality reaching 100% compared to limonene's 66%. Moreover, the pupal stage was more affected by limonene compared to the third stage of *B. zonata* larvae. The results suggest that methyl salicylate and thymol compounds could be one of the safe alternatives to chemical pesticides in controlling peach fruit fly, *B. zonata*, under the integrated pest management program.

Keywords: fruit fly, essential oil, invasive pest, citrus, safe management.

Received: 16/12/2024, Accepted: 12/3/2025, Published: 30/5/2026

INTRODUCTION

Citrus fruits are among the most delectable fruits in the Rutaceae family. Orange, mandarin, lime, lemon, grapefruit, and other commercially significant species are all members of the genus Citrus (Alsabte et al., 2022). Citrus fruit infested with various pests, including the peach fruit fly (*Bactrocera zonata*, Diptera: Tephritidae), is an economically important pest at the global and local levels. It is an invasive agricultural insect pest that causes significant economic damage to more than 145 species from 50 families of commercial value among fruit tree and vegetable species, including stone fruits, apples, mangoes, figs, bananas, and pomegranates (Bakkali et al., 2008; Herrera et al., 2015; El-Sayed et al., 2022; Hidayat et al., 2023). It originated from South and Southeast Asia and has established itself in many countries throughout the Arabian Peninsula, North Africa, and parts of the Indian Ocean (Delrio et al., 2010; Deschepper et al., 2023). In the same context, more than 4,000 species have been recorded in the fruit fly family Tephritidae, of which about 500 cause significant economic damage worldwide (Han and McPheron, 1997). The peach fruit fly, *B. zonata*, is considered one of the most destructive fruit pests of the family Tephritidae worldwide. It attacks various hosts, including vegetables and fruits such as mango, guava, peach, fig, apricot, and citrus. It causes serious economic losses, either directly or indirectly. Usually, farmers rely on pesticides. As chemical pesticides pose increasing hazards to human health and the environment, it has become necessary to find safe alternatives. The most important alternatives are focused on using essential oils and their isolated chemical compounds. Methyl salicylate, thymol, and limonene are compounds produced naturally from some known plant species that have toxic effects on many insect species. Fruit flies such

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as *B. zonata* can cause direct or indirect losses to crops, thereby reducing trade and export opportunities (Clarke and Measham, 2022). Direct damage can be caused by mated female fruit flies laying their eggs in ripe citrus fruits, followed by larvae that feed on the citrus fruit pulp, and indirect damage can be caused by the development of fungi and bacteria on the fruits after infection, which causes spoilage and reduced yield (Bakkali et al., 2008; Alsabte et al., 2022). Control programs mostly rely on synthetic pesticides as a quick option to reduce the damage caused by this pest (Nazir et al., 2022). However, their widespread use has led to the emergence of resistance to these pesticides, effects on natural enemies, and many negative impacts, especially on human health and the environment (Park et al., 2017). Hence, there is a need to identify appropriate alternatives to address this problematic pest within an integrated pest management approach that ensures effective pest control and is safe for human health and the environment (Hossain et al., 2017; Isman, 2020). Plant extracts are among the proposed alternatives for controlling fruit flies, as they have been used as attractants or repellents, as growth inhibitors, and as safe for natural enemies (Sharma and Sohal, 2015). Plant-based insecticides are more dependable, biodegradable in the environment, and less likely to cause resistance to fruit flies. Economically, plant-based insecticides are less expensive to produce than other insecticides (El-Maghraby et al., 2023). Most plant-based insecticides are specific and have negligible effect on the survival of natural enemies (Rani et al., 2021). Therefore, plant-based insecticides are more effective in controlling several pests in an integrated pest management program. Furthermore, limonene, thymol, and methyl salicylate are naturally occurring compounds found in various plants and have received significant attention for their exploitation as biopesticides (Arif and Guarino, 2023). For example, thymol derived from *Thymus vulgaris* showed insecticidal toxicity against *Pochazia shantungensis* (Papachristos and Stamopoulos, 2002). In addition, Limonene, a citrus extract, showed effectiveness in controlling mealybugs and scale insects (Hollingsworth, 2005). Moreover, recent studies by Arif and Guarino (2024) have demonstrated the effectiveness of methyl salicylate against several stored-product pests, particularly against *T. granarium* larvae, highlighting its potential as a fumigant in pest management strategies .The objective of this study is to evaluate the toxic effects of thymol, limonene, and methyl salicylate against *B. zonata*. A set of laboratory experiments was conducted to quantify the mortality rates of third-instar larvae and pupae. The findings are expected to enhance understanding of the activities of thymol, limonene, and methyl salicylate as alternatives to chemical pesticides. They may provide insights into their promising role in integrated pest management strategies (IPM) and organic farms.

MATERIALS AND METHODS

Insects culture: Infested citrus fruits by *B. zonata* were collected from one of the orchards of Baghdad Governorate from Baghdad GPS Coordinate (Latitude, Longitude) shown below, in 2024: 33.187881; 43.995995; 33.198491, 43.981983; 33.194906, 44.007191; 33.197572, 43.994949; and then transferred to the insect laboratory, College of Agricultural Engineering Sciences, University of Baghdad, Iraq. The infested citrus fruits were placed in plastic containers containing a 2 cm layer of soil at the base and covered with a piece of cloth to prevent the larvae from emerging. Periodic examinations were conducted to monitor their development. After that, when the larvae entered the pupal stage, the soil was sieved through a 1.5 mm wire mesh, and the resulting pupae were isolated in circular plastic dishes with a diameter of 18 cm, with sieved soil at the base, 2 cm thick. The dishes were transferred to the rearing cages measuring 30 × 30 × 30 cm. The rearing boxes were placed in laboratory conditions at a temperature of 25 ± 2 °C with a humidity of 70 – 80 % by placing wet cotton inside each cage and providing a light source at a rate of 12 hours of light and 12 hours of darkness with daily monitoring to ensure the emergence of the adult insect. The third instar larvae and pupal stage were isolated using a fine brush and placed in 9 cm Petri dishes for experiments. The third instar

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larva of *B. zonata* is yellowish-white and 8 to 10 mm long.

Laboratory experiments

1 .Evaluation of the effectiveness of essential oils on the third instar larvae: To evaluate the effectiveness of the essential oils of limonene, methyl salicylate, and thymol (Sigma-Aldrich) and their effect on the third instar larvae of *B. zonata*, three concentrations of each of the above essential oils were prepared 1%, 3%, and 5% using ethanol alcohol for dilution. The slice dipping method (Ling et al., 2009) was used. Slices of apple fruit were cut to a thickness of 2 cm, then each piece was dipped in one of the above concentrations of essential oils separately and left for 10-15 seconds. After that, the surfaces were dried with paper towels; then, the treated slices were placed in a petri dish with filter paper at the bottom. Ten larvae of the third instar of *B. zonata* were released. As for the comparison treatment, the apple slices were dipped in ethanol and treated as described above. Three replicates were performed for each concentration, and the dishes were tightly sealed to prevent larvae from emerging, then placed under observation in the laboratory at 25 ± 2 °C, 70-80% humidity, and 12:12 hours of light. After 24 hours, the mortality rate of third-instar larvae was determined by gently brushing them; larvae were considered dead if they did not move. The mortality rates were corrected according to the Abbott equation (Ahmed and Ren, 2023).

2 .Evaluation of the effectiveness of essential oils on the pupal stage: The effects of essential oils limonene, methyl salicylate, and thymol on the pupal stage of the peach fruit fly *B. zonata* were assessed under laboratory conditions. Petri dishes of 9 cm were prepared. Three dishes were used for each concentration, and each was treated with 1 cm^3 separately. The other three dishes were also treated with ethanol alcohol only as a control treatment. After the ethanol evaporated, 10 3- to 4-day-old pupae were placed in each dish and left until the adults emerged. The pupa that failed to develop into an adult was reported as a dead pupa. The experiment was conducted under laboratory conditions at 25 ± 2 °C, 70-80% humidity, and 12:12 h (light/dark). The mortality rates were corrected using the Abbott equation (Ahmed, 2022).

Statistical analysis: All experiments were conducted using a completely randomized design (CRD). After the end of each experiment, the mean number of dead individuals was compared using one-way ANOVA followed by the Tukey test. All the statistics were performed using SPSS version 20 (IBM, USA).

RESULTS AND DISCUSSION

Laboratory evaluation: The effects of limonene, methyl salicylate, and thymol assessed at different concentrations on the mortality of the third instar larvae and pupal stage of *B. zonata* are shown in Figures 1 and 2. In general, methyl salicylate and thymol showed higher insect mortality than limonene. Specifically, the percentage of insect mortality of the essential oils of limonene, methyl salicylate, and thymol at three concentrations of 1, 3, and 5% on the third instar larvae of the peach fruit fly *B. zonata* after 24 hours of treatment is shown in Table 1. Results indicated significant differences among treatments ($f = 7.9$, $df = 9$, $p < 0.05$). However, the highest mortality rate was observed with both 3 and 5 % of methyl salicylate. Moreover, thymol also showed a high mortality rate with only 5% concentration. Meanwhile, limonene showed a lower mortality rate than other compounds (Figure 1) .Furthermore, the mortality percentage of *B. zonata* insects after 12 days of treatment with essential oils limonene, methyl salicylate, and thymol at three concentrations of 1, 3, and 5% in the pupal stage is shown in Table 2. The tested oils showed significant differences between the treatments ($f = 16$, $df = 9$, $p < 0.05$). However, the pupal stage was more sensitive to methyl salicylate at 3% and 5%, and to thymol at 1%, 3%, and 5%, with mortality reaching 100%. While the lowest mortality percentage was observed with limonene at 1, 5, and 3%, reaching 26.66%, 53.33%, and 66.66%, respectively (Figure 2).

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Table 1. Treatments of limonene, methyl salicylate, and thymol on *Bactrocera zonata* third instar larvae

Treatments	Mortality %	SE	95% Confidence Interval for Mean		Mean composition*
			Lower Bound	Upper Bound	
Control	10.00	5.77	-14.84	34.84	a
Limonene 1%	13.33	8.81	-24.61	51.27	a
Limonene 3%	26.66	6.66	-2.01	55.35	b
Limonene 5%	33.33	8.81	-4.61	71.27	b
Methyl salicylate 1%	43.33	28.48	-79.20	165.87	b
Methyl salicylate 3%	100.00	0.00	100.00	100.00	c
Methyl salicylate 5%	96.66	3.33	82.32	111.00	c
Thymol 1%	30.00	5.77	5.15	54.84	b
Thymol 3%	56.66	13.33	-0.70	114.03	c
Thymol 5%	83.33	12.01	31.62	135.04	c

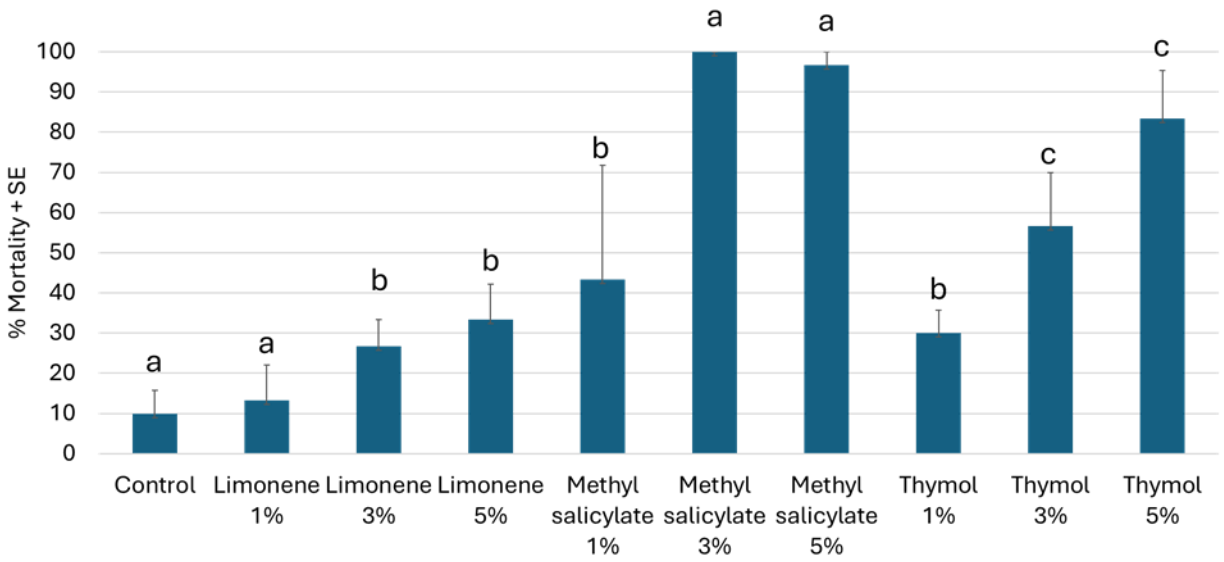

Figure 1. Mean mortality (%) ± SE of *Bactrocera zonata* on third instar larvae after 24 hours of treatments with limonene, methyl salicylate, and thymol at different concentrations (P < 0.05)

Table 2. Treatments of limonene, methyl salicylate, and thymol on the *Bactrocera zonata* pupal stage

Treatments	Mortality %	SE	95% Confidence Interval for Mean		Mean composition*
			Lower Bound	Upper Bound	
Control	3.33	3.33	-11.00	17.67	a
Limonene 1%	26.66	13.33	-30.70	84.03	a
Limonene 3%	66.66	3.33	52.32	81.00	b
Limonene 5%	53.33	20.27	-33.90	140.57	b
Methyl salicylate 1%	53.33	13.33	-4.03	110.70	b
Methyl salicylate 3%	100.00	0.00	100.00	100.00	c
Methyl salicylate 5%	100.00	0.00	100.00	100.00	c
Thymol 1%	100.00	0.00	100.00	100.00	c
Thymol 3%	100.00	0.00	100.00	100.00	c
Thymol 5%	100.00	0.00	100.00	100.00	c

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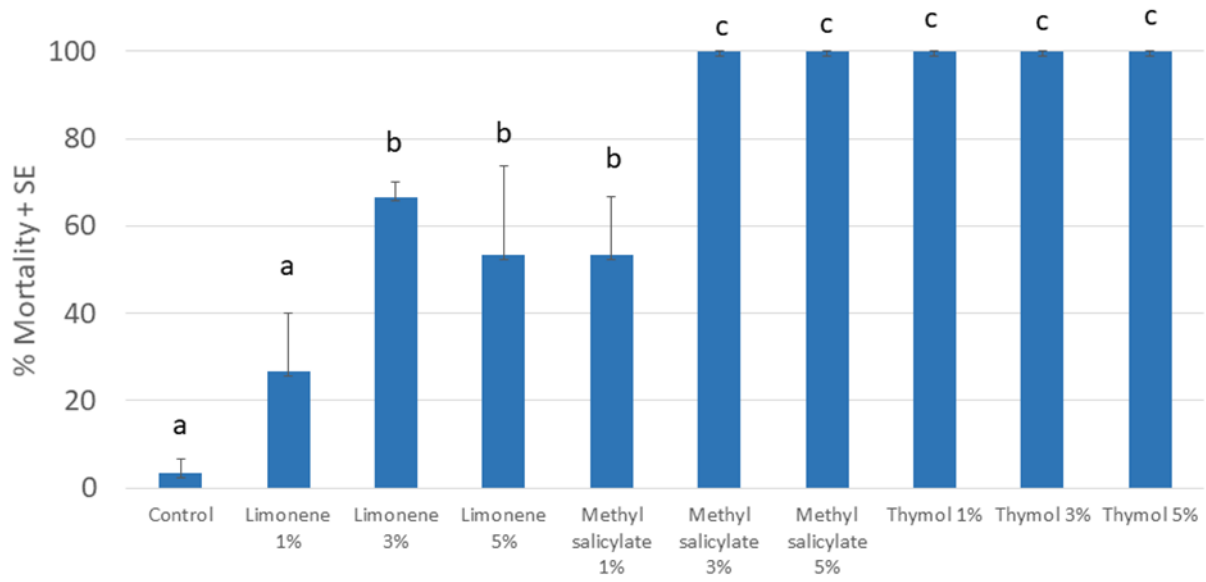

Figure 2. Mean mortality (%) ± SE of *Bactrocera zonata* in the pupal stage after 24 hours of treatments with limonene, methyl salicylate, and thymol at different concentrations (P < 0.05).

Plant-based natural solutions, especially essential oils, are widely used as biopesticides in agriculture, providing effective alternatives to synthetic chemical pesticides for controlling various pests. The results of this study showed significant differences in the biocidal activity of limonene, methyl salicylate, and thymol against *B. zonata* third-instar larvae and pupae, with varying effects depending on growth stage and chemical concentration .In detail, methyl salicylate and thymol exhibited concentration-dependent effects on both third-instar larvae and the pupal stage of *B. zonata*. Both higher concentrations of methyl salicylate and thymol (3 and 5 %) showed higher mortality levels, while lower concentrations (1 %) showed lower mortality levels, specifically for third-instar larvae. These results suggest that methyl salicylate and thymol exhibit clear insecticidal activity against *B. zonata* third-instar larvae and pupae. In other studies, methyl salicylate was more effective against adults of *T. granarium* than limonene (Arif and Guarino, 2023). The use of methyl salicylate and limonene increases the attraction of predators to infested citrus (Alsabte et al., 2022). However, the 5 % limonene concentration was reported to yield 66 % mortality, which may not be sufficient to recommend, as observed with methyl salicylate and thymol, which reached 100 %. Moreover, essential oils have demonstrated many effects, such as repellent effects, antifeedant activity, oviposition deterrence, attraction properties, growth inhibition, and suppression of reproductive behavior as documented in various studies, for example, the study by (Jaffar and Lu, 2022) tested the insecticidal, repellent activities and oviposition deterrence of six essential oil constituents toward adults and immature stages of oriental fruit flies, *B. dorsalis* with promising results. Moreover, Basil oil and its three major active constituents (linalool, estragole, and trans-anethole) obtained from basil (*Ocimum basilicum* L.) were tested for their insecticidal activity on three different tephritid fruit fly species, such as *Ceratitis capitata*, *B. dorsalis*, and *B. cucurbitae*; however, all chemicals tested showed positive results with a dose-response relationship (Al Kostyukovsky et al., 2002). Moreover, in another study, larvicidal activity and the biochemical effects of selected essential oils and biopesticides against two fly species, *Dacus ciliates* and *B. zonata*, were shown to be effective and suitable control options (Deschepper et al., 2023; Al-Behadili et al., 2024). Thymol is one of the main compounds found in thyme, *Thymus vulgaris*, and was selected due to bioactivity against several pests, such as the aphid *Myzus persicae* and the larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) (Nkechi et al., 2018; Seni, 2019; Ukoroije and

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Otayor, 2020; Ahmed, 2022) .Moreover, the toxicity of the molecule derived from essential oil could be explained by the presence of monoterpenes and sesquiterpenes, as well as other active groups, which can interact with the insect nervous system, inhibiting acetylcholinesterase or antagonizing octopamine receptors (Oulebsir-Mohandkaci et al., 2015; Herrera et al, 2015; Khursheed et al., 2022). These compounds are volatile and lipophilic and can interact rapidly with insect bodies, affecting their physiological functions and growth processes (Szczepanik et al., 2012; Isman, 2020; Arif and Guarino, 2023). This study suggests that methyl salicylate and thymol, being naturally occurring compounds, may provide a more environmentally friendly alternative for managing B. zonata while minimizing adverse effects on natural enemies and the environment. Therefore, more studies should be conducted under open-field conditions to determine the effectiveness of these compounds, especially in combination with other integrated pest management tools.

CONCLUSION

The experiment found that methyl salicylate and thymol were highly effective against the third instar larvae and pupal stages of *B. zonata* under laboratory conditions. There was a concentration-dependent effect, with increasing mortality at higher concentrations. Methyl salicylate at 3 and 5%, and thymol at 5%, were the most effective in killing larval stages. However, both compounds resulted in complete (100%) mortality at most of the tested concentrations in the pupal stage. By contrast, limonene was less toxic to the insect stages tested but caused moderate mortality at high concentrations. The pupal stage was more susceptible to limonene than third instar larvae, while methyl salicylate and thymol were highly effective against both stages of development. The results confirm the promising potential of naturally derived compounds, especially methyl salicylate and thymol, as safer alternatives to conventional synthetic insecticides for managing *B. zonata* populations. These compounds can be effective participants in IPM programs, reducing reliance on chemical pesticides and minimizing negative environmental effects. However, more field-based research is needed to confirm their effectiveness in open-field conditions and to determine their compatibility with other pest control strategies.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Plant Protection staff of the College of Agriculture, University of Baghdad, Iraq, for their assistance. We would also like to thank the Plant Protection Directorate, Ministry of Agriculture, in Abu-Ghraib, Baghdad, for its valuable assistance and technical support in conducting this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal Welfare

Funds: There were no funds for this study

AUTHOR'S CONTRIBUTION STATEMENT

Taghreed and Qasim: methodology, writing original draft preparation; Qasim and Mokhtar: writing review and editing. All authors have read and agreed to the published version of the manuscript

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Iraqi Journal of Agricultural Sciences –2026:57(5):1443-1451
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/1za74v39

تقييم فعالية الليمونين ومثيل السالسليت والثيمول على ذبابة فاكهة الخوخ *Bactrocera zonata* (Diptera: TEPHRITIDAE)

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المستخلص

هدفت هذه الدراسة إلى تقييم التأثيرات السمية لهذه المركبات ضد يرقات الطور الثالث والعذراء لذبابة ثمار الخوخ (*B. zonata*) تحت ظروف المختبر. حضرت ثلاث تركيزات 1 و3 و5% من المركبات المذكورة أعلاه وقيمت، أشارت النتائج إلى أن مركبات ميثيل الساليسليت والثيمول وخاصة عند التركيزات العالية كانت أكثر فعالية من الليمونين اذ بلغت نسبة الوفيات 100% مقارنة بالليمونين والتي بلغت 66%، كما أثر الليمونين على مرحلة العذراء أكثر من تأثيره على اليرقات. تشير النتائج إلى أن مركبات ميثيل الساليسليت والثيمول يمكن أن تكون أحد البدائل الآمنة للمبيدات الكيميائية في مكافحة ذبابة ثمار الخوخ في إطار برنامج الإدارة المتكاملة للآفات.

الكلمات المفتاحية: ذبابة الفاكهة، الزيوت العطرية، الآفات الغازية، الحمضيات، الإدارة الأمنة.