



## Effect of Some Essential Oils on the Repellence Behavior of the Peach Fruit Fly, *BACTROCERA ZONATA* (Saunders)

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### Abstract

The peach fruit fly, *Bactrocera zonata* (Saunders), is a major pest of the Tephritidae family, causing significant economic damage globally. Native to India, where it was first recorded in Kashmir, *B. zonata* has since spread to various regions, particularly Asia and the Mediterranean, affecting many crops, including peach, custard apple, guava, mango, and citrus. Infestations result in fruit damage, premature fruit drop, and the imposition of quarantine restrictions that disrupt export markets. As resistance to chemical pesticides continues to rise, the need for alternative pest control methods becomes increasingly urgent. Plant-derived insecticides are emerging as an eco-friendly and sustainable solution for pest management, as they effectively control pest populations while posing minimal risks to non-target organisms. In this study, a behavioral bioassay was performed using Petri dishes to evaluate the behavioral response to three essential oils, namely limonene, thymol, and methyl salicylate, of *B. zonata* adult females over four intervals of 15, 30, 45, and 60 minutes. The results revealed that methyl salicylate exhibited consistent repellent effects across all time intervals. In contrast, thymol showed repellent effects only after 60 minutes, while no significant differences were observed with limonene. In the field, essential oil formulations reduced the population density of *B. zonata* on citrus fruits. These findings highlight the ecological potential of methyl salicylate as a promising, eco-friendly pest control agent.

Keywords: peach fruit fly, *Bactrocera zonata*, repellent effects, behavioral bioassay, essential oils

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## Introduction

The peach fruit fly, *Bactrocera zonata* (Saunders), is among the most damaging insect pest species within the Tephritidae family worldwide. Indigenous to India, where it was first recorded in Kashmir (26), it causes significant damage, especially in Asia (6, 29, 40). It is also considered an important pest affecting a wide range of host plants, particularly peach (*Prunus persica* L. Batsch)

and custard apple (*Annona squamosa* L.) in the Mediterranean region and Egypt (2, 9). This pest significantly impacts fruit production and quality, leading to substantial economic losses. Additionally, in Pakistan, it has been reported to infest guava (*Psidium guajava* L.) and mango (*Mangifera indica* L.), further demonstrating its broad host range and economic importance (36).

Fruit fly infestations result in considerable economic losses from fruit damage, premature

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fruit drop, and quarantine restrictions impeding access to export markets. Species such as *B. zonata* are particularly harmful because of their high mobility, extensive dispersal capabilities, rapid reproduction rates, and broad feeding range. These characteristics contribute to their invasiveness and elevate their status as a priority target on quarantine lists (25, 35).

Managing fruit flies has become increasingly complex due to rising opposition from environmentalists and the general public against the extensive use of chemical pesticides (18, 39). This growing resistance underscores the urgent need to develop alternative, environmentally sustainable management strategies that minimize pesticide use while effectively controlling fruit fly populations.

The increasing demand for eco-friendly pest control solutions, coupled with growing concerns about the environmental and health risks of chemical pesticides, has led to a renewed interest in plant extracts as a viable alternative for mitigating the negative impacts of fruit flies on both yield and fruit quality (1, 19). Botanical insecticides, typically specific to the targeted pests, offer significant advantages as they pose minimal risks to non-target organisms, including humans (11, 27). These plant-based insecticides are also biodegradable, making them environmentally safe and sustainable. In contrast to conventional chemical insecticides, which often rely on a single active ingredient, plant-derived insecticides contain a wide array of chemical compounds that act synergistically. This diversity enables them to disrupt pest behavior and physiology (37, 16).

For example, extracts from the neem tree (*Azadirachta indica*) are well-documented for their broad spectrum of biological activities, including antifeedant, antioviposition, repellent, and growth-regulating effects (24). Similarly, Bošković et al. (8) demonstrated that biopesticide formulation using different essential oils exhibited both repellent and growth-inhibitory effects against the fruit fly *Drosophila suzukii*. Furthermore, monoterpenoids in essential oils have

effectively controlled stored-product insects and pests of public health significance (4, 12).

This study investigated the effects of individual essential oils, including limonene, thymol, and methyl salicylate, on *B. zonata* (peach fruit fly), which has garnered significant interest due to their potential as natural, eco-friendly pest control agents. Limonene, a monoterpene found in citrus oils, is widely recognized for its insect-repellent and toxic effects (15). Thymol, a phenolic compound derived from thyme, exhibits antimicrobial, repellent, and insecticidal properties (3). Methyl salicylate, a volatile ester, has demonstrated effectiveness as an insect repellent and disrupts the behavior of various pests (5, 22). Investigating the potential of these natural compounds could facilitate the development of more sustainable and effective pest control strategies for enhancing crop yields while reducing pesticide residues in fruits. As *B. zonata* spreads, it is essential to identify and adopt alternative control methods to manage this invasive pest, safeguarding agricultural production worldwide and promoting environmental sustainability.

## Materials and Methods

**Insect Rearing:** Peach fruits infected with *B. zonata* were collected from an orchard near Baghdad and transported to the Economic Insects Laboratory at the College of Agricultural Engineering Sciences, University of Baghdad, for identification and further experiments. The infested fruits were placed in plastic containers, with a 2 cm thick layer of soil at the base and covered with tulle to prevent larvae from emerging. Periodic observations were made to monitor their development. Once the larvae entered the pupal stage, the soil was sieved using a 1.5 mm wire mesh to separate the pupae, which were then isolated and placed in circular plastic dishes (18 cm diameter) containing 2 cm of sieved soil at the base. The dishes were subsequently transferred into rearing cages (30

× 30 × 30 cm) (Fig. 1), which were maintained under controlled laboratory conditions at  $25 \pm 2$  °C and 70-80% humidity. A 12-hour light/dark cycle was provided, and daily

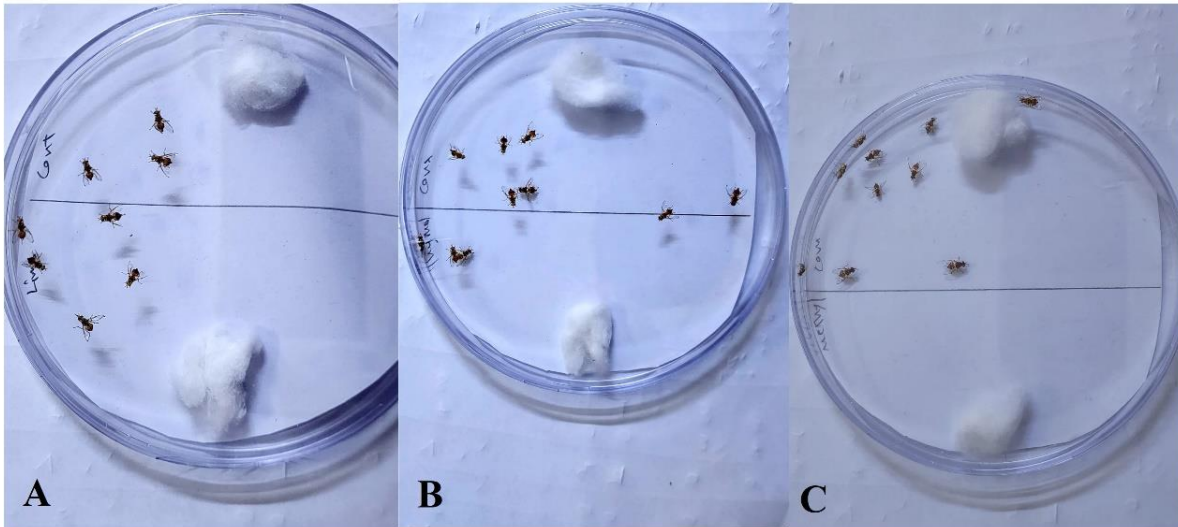
monitoring was conducted to ensure the emergence of adult insects for subsequent laboratory experiments.



**Fig. 1.** *Bactrocera zonata* rearing cages in the laboratory

Laboratory Bioassay: Glass Petri dishes (15 cm in diameter) were used to determine the behavioral selection of *B. zonata* females toward some selected compounds. The dishes were divided into two parts (left and right). In brief, 100  $\mu$ L of the tested compound was placed on 1 × 1 cm filter paper pieces (Whatman No. 1), allowing the solvent to evaporate for 60 s. An equivalent volume of solvent (ethanol) was used in control treatments. Each piece of filter paper was randomly placed at the end of the Petri dish (left or right part) (Fig. 2), and the dish was closed in each bioassay. Observation response

times of 15, 30, 45, and 60 minutes were recorded. A total of 10 individual bioassays were conducted for each replicate in each corresponding observation. After each replicate, the Petri dish was washed in water, followed by acetone, and kept in the oven at 180 °C. The compounds tested were limonene, methyl salicylate, and thymol at 3% concentration each (purity of >98% obtained from Sigma-Aldrich). Bioassays were performed under laboratory conditions ( $25 \pm 2$  °C and  $65 \pm 10\%$  RH) between 8:00 a.m. and 2:00 p.m.



**Fig. 2.** Testing the behavioral response of *Bactrocera zonata* female adults to (A) limonene, (B) thymol, and (C) methyl salicylate at 3% concentrations against the solvent (control) at different observation times

**Preparation of the Methyl Salicylate Formulation:** A 20% (v/v) emulsion of the essential oil methyl salicylate was prepared for application in field research in an emulsion concentrate (EC) formulation. The formulation comprised 40 mL methyl salicylate, 20 mL Tween 20% (as an emulsifying agent), and 140 mL distilled water. The components were added gradually into a glass beaker placed on a magnetic stirrer, with continuous stirring to ensure thorough incorporation. After complete addition, the mixture was stirred continuously for an additional 20 minutes to promote homogeneity and stable emulsion formation at the target concentration (10, 28). Treatments were applied at 3, 5, and 10 mL/L.

**Field Evaluation:** The field study was conducted in a citrus orchard in Baghdad province, covering an area of 7 dunums. The orchard was planted with three citrus cultivars, i.e., mandarin (*Citrus reticulata*), sweet lemon (*Citrus limetta*), and sweet orange (*Citrus sinensis*). Pest monitoring was conducted using species-specific baited traps distributed throughout the orchard. Data on adult fly captures were recorded regularly to assess infestation levels. Treatments were applied over five weeks, from early October to mid-November 2024, during which climatic

conditions remained suitable for fruit fly activity.

**Statistical Analysis:** The data obtained from the laboratory bioassay experiments were analyzed using the Chi-Square ( $\chi^2$ ) test. The data from the field were expressed as percentage values, representing the infestation levels recorded during the study period, and the results were compared to the P-value. The statistical analyses were performed using SPSS, version 26.

## Results and Discussion

The behavioral response of female *B. zonata* adults to methyl salicylate provides significant implications for pest management strategies, as the compound acted as a repellent for the insect. Their response to 3% methyl salicylate concentration at the different observation times is reported in Fig. 3. Overall, *B. zonata* showed repellent behavior to methyl salicylate for all observation times. Specifically, statistical differences were reported after 15 min observation ( $p < 0.01$ ), where 86 % of the flies were attracted to the control treatment, while only 13% were attracted to methyl salicylate. Moreover, after 30 minutes of observation, statistical

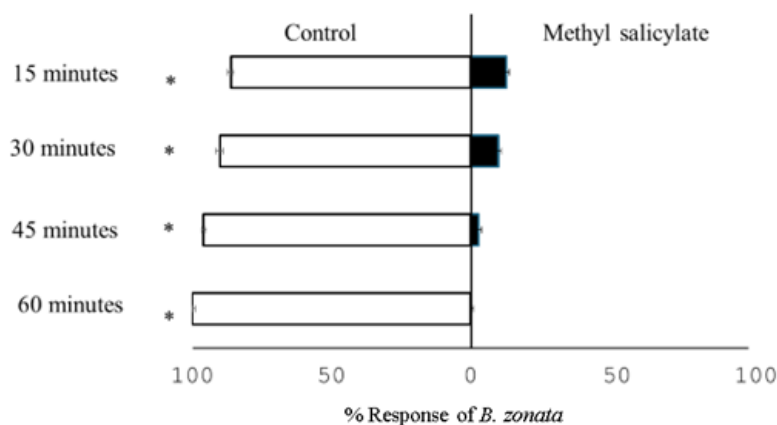
differences were reported ( $p < 0.004$ ), where 90 % of the flies were attracted to the control treatment and only 10% to methyl salicylate. After 45 min, statistical differences were also reported ( $p < 0.004$ ), with 96 % attracted to the control treatment. In the same context, after 60 minutes of observation, 100% of *B. zonata* tested were attracted to the control treatment ( $p < 0.0005$ ).

Thymol, derived from thyme oil, exhibits toxic and repellent properties against many pests, including *B. zonata*, essential for integrated pest management strategies. The responses of *B. zonata* female adults to thymol at 3% concentration at different observation times are reported in Fig. 4. Specifically, statistical differences were noted only after 60 min observation ( $p < 0.05$ ), with 80% of the flies attracted to the control treatment and only 20% to thymol. No statistical differences were reported after 15, 30, and 45 minutes of observation. Based on the results, thymol has been identified as a repellent, influencing the behavior of *B. zonata* females. Its presence can deter these flies from settling on treated surfaces, reducing their reproductive success.

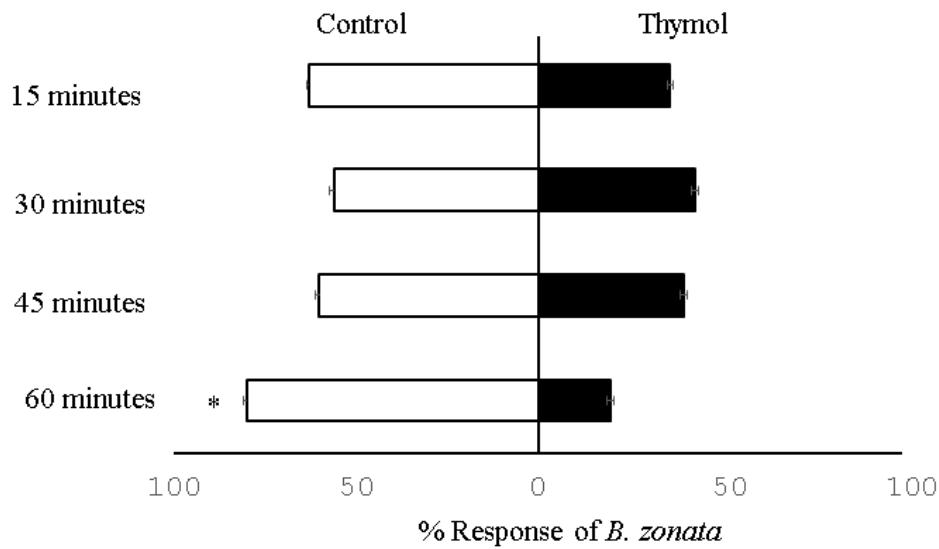
The behavioral response of female *B. zonata* adults to limonene has gained interest due to its potential as a natural insect repellent and attractant. Limonene, a monoterpene found in citrus oils, exhibits various biological

activities, including insecticidal and repellent properties, which may influence the behavior of the adult female flies. Their response to limonene at 3% concentration at different observation times is reported in Fig. 5. Overall, they had no statistical differences in behavioral response among the different observation times. Specifically, after 15 minutes of observation, 36% were attracted to the control treatment, while 56% showed attraction to limonene. Moreover, after 30 minutes of observation, 26% were attracted to the control treatments, while 73 % were attracted to limonene. After 45 minutes of observation, 30% responded to the control treatment, while 70 % of *B. zonata* were attracted to limonene. The results indicate that limonene can also act as a repellent against *B. zonata* adults. The dual nature of limonene, acting as both an attractant and repellent, suggests that its effects depend on environmental conditions and concentration.

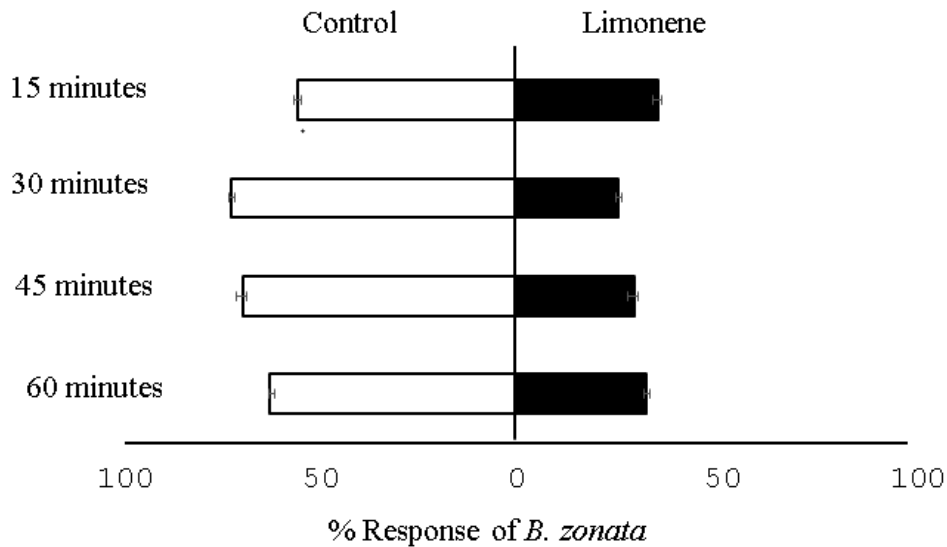
Olfaction plays a significant role in allowing the insect to recognize host fruits or chemical compounds, as shown in Figures 1 to 3. Therefore, the chemical compounds in this study, methyl salicylate, thymol, and limonene, can affect peach fruit fly behavior positively or negatively by attracting them to the essential oils.



**Fig. 3.** Behavioral response of *Bactrocera zonata* female adults to methyl salicylate at 3% concentration against the solvent (control) at different observation times



**Fig. 4.** Behavioral response of *Bactrocera zonata* female adults to thymol at 3% concentration against the solvent (control) at different observation times



**Fig. 5.** Behavioral response of *Bactrocera zonata* female adults to limonene at 3% concentration against the solvent (control) at different observation times

The results of this study suggest that methyl salicylate plays a significant role as a repellent against *B. zonata*, demonstrating stronger efficacy than thymol and limonene at 3% concentration. This finding supports previous research showing the organic compound's broad insect-repellent properties. For instance, a study reported that methyl eugenol volatiles can reduce the male of *B. zonata* attracted (13).

At the same time, Arif and Guarino (5) observed its ability to reduce the population density of *Trogoderma granarium* and cause a high mortality rate. Koschier et al. (19) also found that a 1% methyl salicylate solution significantly deterred female thrips from ovipositing and reduced feeding damage on bean and cucumber plants within 24 hours. During the experiments, the environmental

conditions ranged between 25 -28 °C with a relative humidity of 60 -70%.

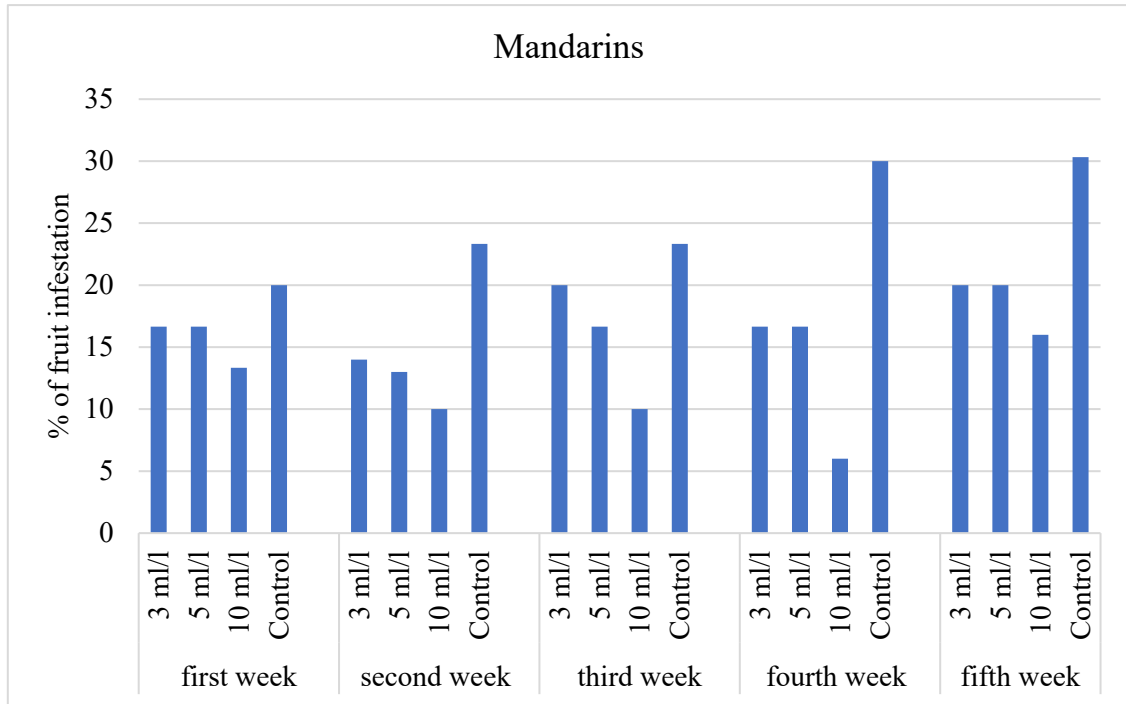
Methyl salicylate's insecticidal and repellent effects are attributed to its phenolic hydroxyl group, shared with other insecticidal compounds like thymol and carvacrol. These compounds have been shown to control pests (33, 34) effectively. The strong efficacy of methyl salicylate can be explained by its chemical structure. The positioning of the hydroxyl group on the benzene ring is important in influencing both antifungal and insecticidal activities, as highlighted by studies from (8, 31). This structural feature enhances the bioactivity of methyl salicylate and other natural insecticides.

Thymol displayed a delayed repellency effect on adult female *B. zonata*, and became significant only after 60 min observation, with no significant response observed at shorter time intervals. Its delayed response effect may be related to the low volatility released compared to methyl salicylate. This indicates that thymol's repellent efficacy is time-dependent, requiring a prolonged exposure period to manifest its deterrent properties fully. Several studies have demonstrated the repellent activity of thymol against various insect species, suggesting its potential as a natural alternative to synthetic insecticides (31). Notably, it has been found effective against *Culex pipiens pallens*, a vector for the West Nile virus (30), as well as against *Solenopsis richteri* ants and *Riptortus clavatus* bean bugs (20, 32). Despite the delayed response observed in *B. zonata*, thymol has broad-spectrum repellency, underscoring its potential for pest control applications. Higher

concentrations of thymol should be tested to achieve a more immediate effect.

Limonene at 3% concentration did not exhibit a statistical repellent effect on *B. zonata*; at lower concentrations, it may act as an attractant. For instance, D-limonene is the primary olfactory attractant in orange juice for *B. dorsalis* (23). Limonene might influence fruit fly behavior differently depending on its concentration. At higher concentrations, however, limonene could act as a repellent, similar to other compounds that exhibit concentration-dependent effects on insect behavior. Low concentration of limonene can be an attractant, while high concentration can act as a repellent. Further studies are required to determine the exact threshold at which this shift occurs.

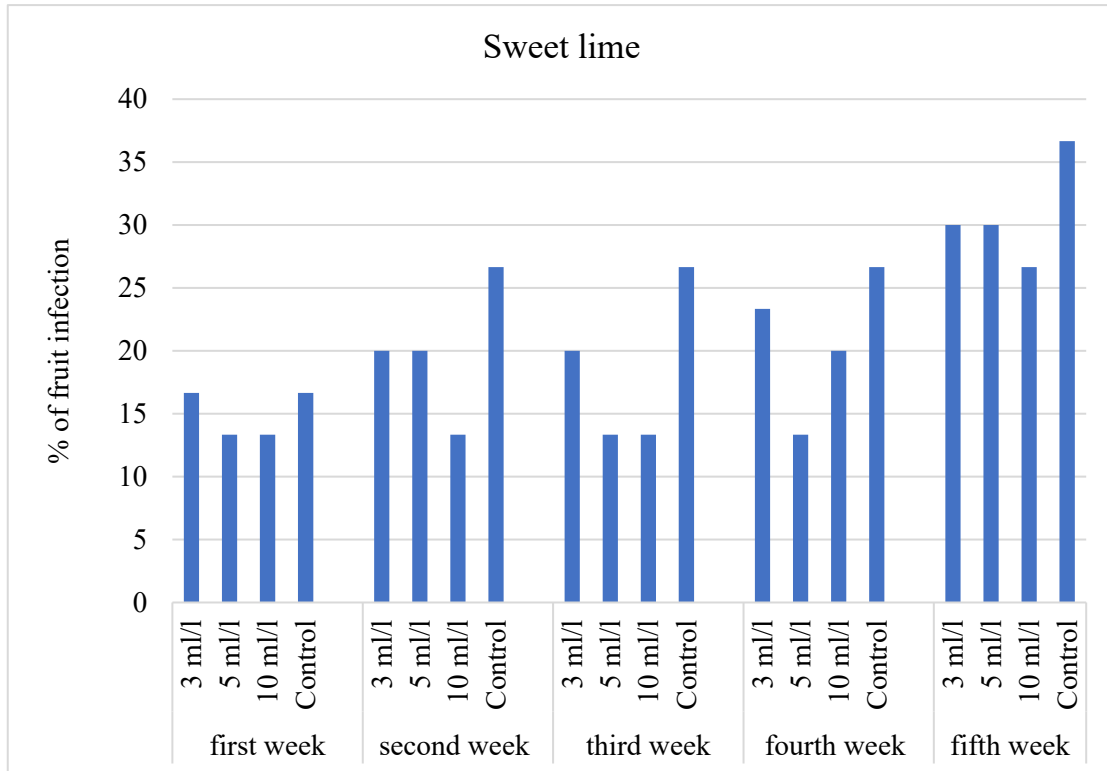
Moreover, the results of field evaluations of methyl salicylate applied to mandarin fruit demonstrated a differential effect based on concentration levels. The 10 mL/L concentration exhibited superior efficacy compared to the other treatments throughout the duration of the study. In contrast, the effects observed at 3 mL/L and 5 mL/L concentrations were comparable. Notably, the lowest incidence of infestation was recorded during the second, third, and fourth weeks of observation, with infestation rates of 10, 10, and 6%, respectively, at the 10 mL/L concentration (Fig. 6). From the results, utilizing methyl salicylate in pest management strategies could provide an eco-friendly alternative to synthetic pesticides, balancing attraction and repulsion to manage *B. zonata* populations effectively in mandarin fruit infestations.



**Fig. 6.** Peach fruit fly infestation levels after 3, 5, 10 ml/l treatments with 20% methyl salicylate formulation on mandarin trees

Applying methyl salicylate formulation at concentrations of 3 mL/L, 5 mL/L, and 10 mL/L on sweet lemon trees yielded comparable results across the first to fourth weeks, with the lowest infestation incidence recorded at 13%. However, the infestation rate increased during the fifth week following treatment (Fig. 7). Meanwhile, methyl salicylate shows promise as a natural pesticide because it has demonstrated higher mortality rates in *B. zonata*, especially at the 10%

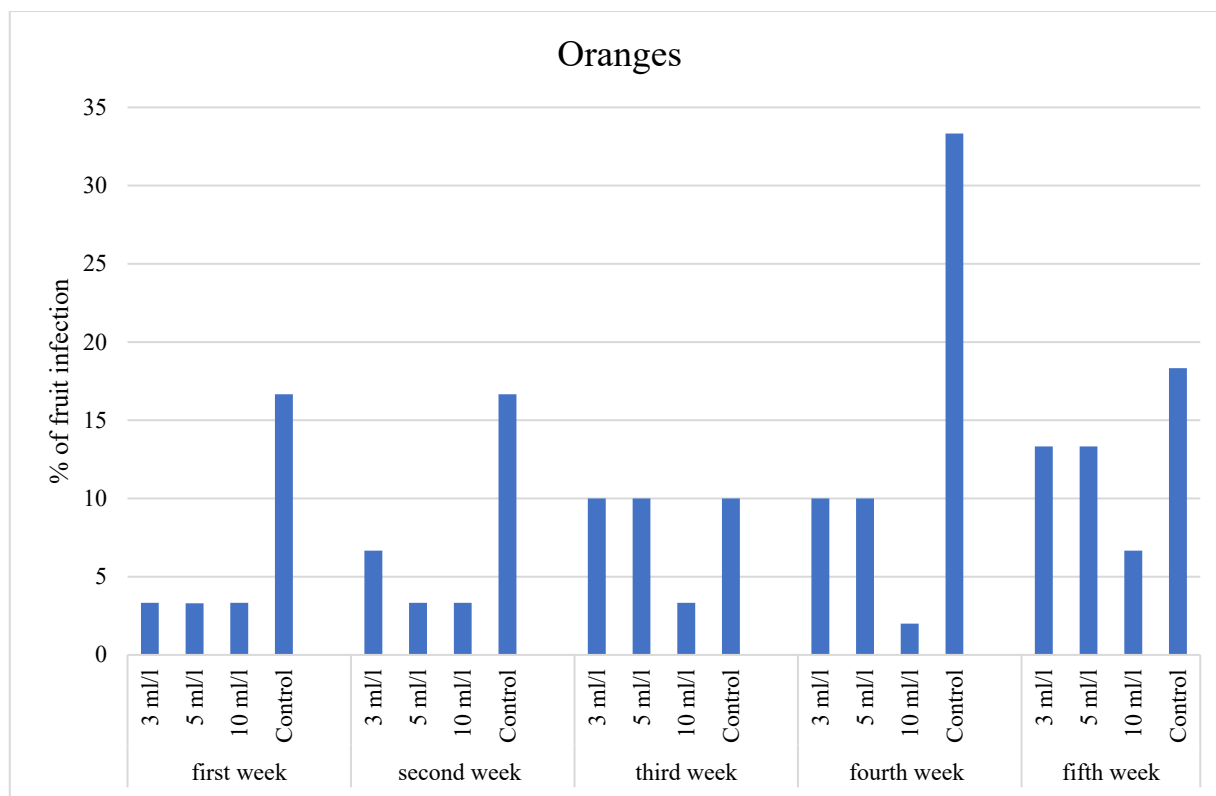
concentration, indicating that it may be more effective if used in combination with other natural insecticides. However, the reliance on synthetic insecticides remains prevalent due to their immediate effectiveness, despite the growing interest in botanical alternatives like methyl salicylate. This highlights the ongoing need for research into optimizing natural compounds for pest control while considering environmental impacts.



**Fig. 7.** Peach fruit fly infestation levels after 3, 5, 10 ml/l treatments with 20% methyl salicylate formulation on sweet lime trees

Similarly, applying methyl salicylate at concentrations of 3 mL/L, 5 mL/L, and 10 mL/L on orange trees demonstrated that the latter two treatments exhibited superior efficacy across all evaluation periods. The lowest incidence of infestation, recorded at 2%, was observed during the first through fourth weeks. However, an increase in infestation rate to 6.6% was noted in the fifth week following treatment with the 10 mL/L concentration (Fig. 8). Based on the results, the repellent nature of methyl salicylate could be

harnessed in integrated pest management (IPM) strategies, potentially reducing *B. zonata* populations in agricultural settings. Utilizing it in a "push-pull" strategy could enhance the effectiveness of pest control by repelling *B. zonata* while attracting natural enemies to the area. Conversely, while methyl salicylate shows promise as a repellent, its effectiveness may vary based on environmental conditions and the concentrations, necessitating further research to optimize its application in pest management.



**Fig. 8.** Peach fruit fly infestation levels after 3, 5, 10 ml/l treatments with 20% methyl salicylate formulation on orange trees

The findings also revealed variability in the behavioral response of the peach fruit fly depending on the host plant species. The repellent effect of methyl salicylate was more pronounced on orange trees compared to mandarin and sweet lemon trees. This variation in response may be attributed to differences in the chemical profiles of the host plants, both qualitatively and quantitatively. Certain volatile compounds may function as either attractants or repellents, depending on their chemical nature and concentration. Specifically, higher concentrations may exert a repellent effect, while lower concentrations of the same substances may attract the insect (7, 21, 38). Visual cues such as differences in fruit or foliage coloration may also modulate the insect’s host selection behavior (14, 17).

### Conclusion

The findings highlight the ecological and particulate significance of *B. zonata* repellency to methyl salicylate, offering valuable insights

into eco-friendly pest control strategies. These approaches reduce reliance on chemical pesticides and particularly benefit organic farming systems. The laboratory and field results consistently demonstrate the efficacy of the target essential oils, especially at high concentrations. By minimizing pesticide use, such methods support biodiversity, protect non-target species, and promote sustainable agricultural practices, providing growers with effective, environmentally responsible alternatives for pest management. Future studies should be conducted on methyl salicylate in a push-pull strategy, combining with other peach fruit fly control methods as IPM strategies.

### Supplementary Materials:

None.

### Author Contributions:

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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The authors declare no conflict of interest.

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## تأثير بعض الزيوت العطرية على السلوك الطارد لحشرة ذبابة ثمار الخوخ *BACTROCERA ZONATA* (Saunders)

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### الخلاصة

ذبابة ثمار الخوخ، *Bactrocera zonata*، آفة رئيسية من عائلة Tephritidae، تُسبب أضراراً اقتصادية كبيرة عالمياً. موطنها الأصلي الهند، إذ سُجلت لأول مرة في كشمير، وقد انتشرت منذ ذلك الحين إلى مناطق مختلفة من العالم، وخاصة آسيا والبحر الأبيض المتوسط، مُلحقة أضراراً بالعديد من المحاصيل، بما في ذلك الخوخ والجوافة والمango والحمضيات والفاكهة ذات النواة الحجرية. تُؤدي الإصابة إلى تلف الثمار، وتساقطها المبكر، وفرض قيود الحجر الصحي التي تعطل التصدير إلى الأسواق العالمية. مع استمرار تزايد مقاومة المبيدات الكيميائية، تزداد الحاجة إلى طرق بديلة لمكافحة الآفات ضرورة مهمة. أصبحت المبيدات الحشرية ذات الأصل النباتي كحلّ صديق للبيئة ومستدام لإدارة الآفات، إذ تُسيطر بفعالية على أعداد الآفات مع الحد الأدنى من المخاطر على الكائنات الحية غير المُستهدفة. في هذه الدراسة، أُجري اختبار حياتي سلوكي باستخدام أطباق بتري لتقييم الاستجابة السلوكية لثلاثة زيوت عطرية: الليمونين، والثيمول، وساليسيلات الميثيل، تجاه بالغات *B. zonata* على مدى أربع فترات زمنية (١٥، ٣٠، ٤٥، و ٦٠ دقيقة). أظهرت النتائج أن سالييلات الميثيل أظهرت تأثيرات طاردة ثابتة على مدار جميع الفترات الزمنية. في المقابل، لم يُظهر الثيمول تأثيرات طاردة إلا بعد ٦٠ دقيقة، بينما لم يلاحظ أي فروق معنوية مع الليمونين. حقلًا، قللت مستحضرات الزيوت العطرية من الكثافة لحشرة *B. zonata* على ثمار الحمضيات. تُبرز أهمية هذه النتائج إلى ان سالييلات الميثيل كعامل واعد وصديق للبيئة لمكافحة الآفات.

**كلمات مفتاحية:** ذبابة ثمار الخوخ، *Bactrocera zonata*، التأثير الطارد، الاختبار السلوكي والزيوت العطرية

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