

# The insecticidal activity of some essential oils and their effect on biochemical parameters of fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

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**Abstract:** Fall armyworm (FAW), *Spodoptera frugiperda* is a polyphagous insect causing damage to several crops. In the present work, the toxicity of four plant oils, namely: ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), thyme (*Thymus vulgaris*) and lemon (*Citrus limon*) against second and fourth instar larvae of FAW at different concentrations under laboratory conditions was evaluated. In each treatment, healthy sixth instar of FAW larvae that survived after treatment of fourth instar larvae with LC<sub>50</sub> concentration were taken for biochemical analyses. The results showed that lemon oil achieved superior toxic efficacy with LC<sub>50</sub> and LC<sub>25</sub> values as 0.259 and 0.096 % for second larval instar and 0.542 and 0.084 % for fourth larval instar, respectively, while the least effect was recorded with LC<sub>50</sub> and LC<sub>25</sub> values of turmeric oil as 0.941 and 0.257% for 2<sup>nd</sup> larval instar, and 1.688 and 0.38% for 4<sup>th</sup> larval instar, respectively. Regarding the effects on enzyme activity level, total proteins and total carbohydrates recorded a significant decrease in all treatments with the tested oils. The highest and lowest enzyme activity of acetylcholinesterase was observed after treated with thyme and lemon oils, respectively. While, no significant differences were observed in the activity of alpha esterase enzyme between the treatments of turmeric, ginger and thyme oils, and inhibition of glutathione S-transferase enzyme activity was occurred in all treatments. The activities of protease, amylase and lipase enzymes were decreased after using the four tested oils compared to control. Therefore, the results of this work demonstrated the effectiveness of the tested oils in combating FAW under laboratory conditions, and it can be used in field application as a safe and effective alternative and as a part of the integrated pest management program.

**Keywords:** *Spodoptera frugiperda*, essential oils, *Zingiber officinale*, *Curcuma longa*, *Thymus vulgaris*, *Citrus limon*.

## 1.Introduction:

Fall army worm (FAW), *Spodoptera frugiperda* (J.E. Smith) is an invasive damaging pest causing great losses in crops. This polyphagous pest is considered a major threat to various economically important crops, such as maize, sorghum, rice, wheat, beans, cotton, potatoes, and tomatoes, causing significant yield and quality losses. The pest's wide host range, high fecundity, and resistance to pesticides, contribute to its devastating impact on agricultural production (Cruz *et al.*, 2010 and De Almeida *et al.*, 2002). *Spodoptera frugiperda* is usually controlled by synthetic insecticides that had significant drawbacks including environmental residues, health hazards, resistance development and non-target effects (Yu, 1991). Because of these effects of the synthetic insecticides, the management of this pest with natural,

safe, effective alternative strategy is very important goal to reduce the dangerous impacts on plantations and surrounding environment.

Many plants are reportedly possessing natural substances with pest-control potential, that could form a good alternative to insecticides, therefore many researchers (Hernandez and Leon, 1994; Tembo *et al.*, 2018 and Mukanga *et al.*, 2022) reported that our nature is rich in plants possessing bioactive compounds acting as natural pesticides. Plant oils originate from the secondary metabolism of plants and consist of mixture of complex chemical substances that act as plant defense with low toxicity for animals and human, as well as had antiviral, fungicidal, bactericidal, insecticidal and repellent action. Barbosa *et al.*, 2018 demonstrated that the oils of turmeric, palmarosa and clove showed best potential control of *S. frugiperda*

from the lowest concentrations corresponding to 25  $\mu\text{L mL}^{-1}$ . Numerous researchers have demonstrated the effectiveness of various plant extracts and essential oils in combating insects (Zhao *et al.*, 1998; Isman, 2006; Sadek, 2003, and Silva *et al.* 2009).

The present work aims to evaluate the lethal and sub-lethal effects of four plant oils namely; ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), thyme (*Thymus vulgaris* L.) and lemon (*Citrus limon* L.) on the second and fourth larval instars of *S. frugiperda* under laboratory conditions, and also their biochemical effect against treated larvae.

## 2. Material and methods:

### 2.1. Insect rearing:

*Spodoptera frugiperda* (FAW) egg masses were generously provided by the Plant Protection Research Institute in Dokki, Giza, Egypt. Egg masses were kept at  $26 \pm 2^\circ \text{C}$  and  $65 \pm 5\%$  relative humidity until hatching. The newly emerged larvae were fed on fresh castor bean (*Ricinus communis*) leaves. The insect colony was sustained in the laboratory environment without the use of any insecticides according to El-Defrawi *et al.*, 1964.

### 2.2. Essential oils used:

Four plant oils; ginger (*Zingiber officinale* Rosco, Fam.: Zingiberaceae), turmeric (*Curcuma longa*, Fam.: Zingiberaceae), thyme (*Thymus vulgaris* L., Fam.: Lamiaceae) and lemon (*Citrus limon* L., Fam.: Rutaceae) were sourced from National Research Center, Dokki, Giza, Egypt. These oils were extracted from the leaves, flowers and stems of these plants as 100% concentration.

### 2.3. Bioassay experiments:

The toxicity of *Z. officinale*, *C. longa*, *T. vulgaris* and *C. limon* oils against the 2<sup>nd</sup> and 4<sup>th</sup> larval instars of *S. frugiperda* was determined using the leaf-dipping method. Four concentrations of each oil (10%, 5%, 1.25%, & 0.625%) were prepared in distilled water with a small amount of Tween-20 (10 mg/l) (as surfactant agent). Fresh castor bean leaves were dipped in each concentration for 30 seconds and air-dried for half an hour at room temperature. The control leaves were immersed in water containing tween-20. Treated leaves were placed in jars (500 ml capacity). Ten larvae of *S. frugiperda* were transferred to each jar, and each treatment were replicated three times. The percentage of mortality was recorded after 3 and 7 days of

exposure. The sublethal concentrations ( $\text{LC}_{50}$  &  $\text{LC}_{25}$ ) were estimated by probit analysis methods.

### 2.4. Biochemical assays:

The 4<sup>th</sup> larval instar of *S. frugiperda* treated with  $\text{LC}_{50}$  of ginger, turmeric, thyme and lemon oils using dipping technique (Abo El-Ghar *et al.* 1994) of castor bean leaves and feeding for 48 h. then the larvae feed on untreated leaves; another group of larvae fed on untreated leaves (dipped in distilled water containing tween-20). Sixth instars larvae of healthy collected from the surviving treated and untreated larvae for biochemical studies.

Analysis of enzymes, proteins and carbohydrates:

#### 2.4.1. Acetylcholinesterase (AChE) activity:

The measurement of AChE activity was conducted using the methodology outlined by Simpson *et al.* (1964), with acetylcholine bromide (AChBr) serving as the substrate.

#### 2.4.2. Amylase activity:

The measurement of digestive enzymes followed a modified version of Ishaaya and Swirski's (1976) method, as adapted by Amin (1998).

#### 2.4.3. Lipase activity:

Lipase activity is measured using the Spectrum diagnostic kit, available at [www.spectrum-diagnostics.com](http://www.spectrum-diagnostics.com). The process involves the enzymatic breakdown of a synthetic substrate (DGMRE) by lipase, resulting in the formation of a colored product, methylresorufin. The rising absorbance of the red methylresorufin is then monitored photometrically.

#### 2.4.4. Total proteins:

Total proteins were determined by the method of Bradford (1976).

#### 2.4.5. Total carbohydrates:

Total carbohydrates determined according to Crompton and Birt (1967).

#### 2.4.6. Glutathione S-transferase (GST):

GST enzyme activity determined according to Habig *et al.* (1974).

### 2.5. Statistical analysis:

Percentage mortality of larvae *S. frugiperda* corrected using Abbott's formula (Abbott, 1925) and then using software LDP Line to calculate  $\text{LC}_{50}$  values by using probit-analysis Finney (1971). Data of the activity enzymes were analysis using one-way ANOVA by software Costat system, Version 6.311 (Costat, 2006) followed by duncan's multiple range (Duncan, 1955).

### 3.Results and discussion:

#### 3.1.Effect of tested oils on second and fourth larval instars of *S. frugiperda*:

The data in Tables (1 and 2) show the efficacy of the tested compounds represented by  $LC_{50}$  and  $LC_{25}$  against the second and fourth larval instars of *S. frugiperda*. From the obtained results, it can be proven that lemon oil showed great success as the best toxicant compared to other tested oils, where  $LC_{50}$  and  $LC_{25}$  values were 0.259 and 0.096 % on second larval instar and 0.542 and 0.084 % on fourth larval instar, respectively, followed by thyme oil then ginger oil and finally turmeric oil with  $LC_{50}$  of 0.349, 0.702 and 0.941% for second larval instar and 0.686, 1.521 and 1.688% for fourth larval instar, respectively. Thus, these results revealed that the least effective oil against the larvae of the two tested larval instars (second and fourth) is turmeric oil.

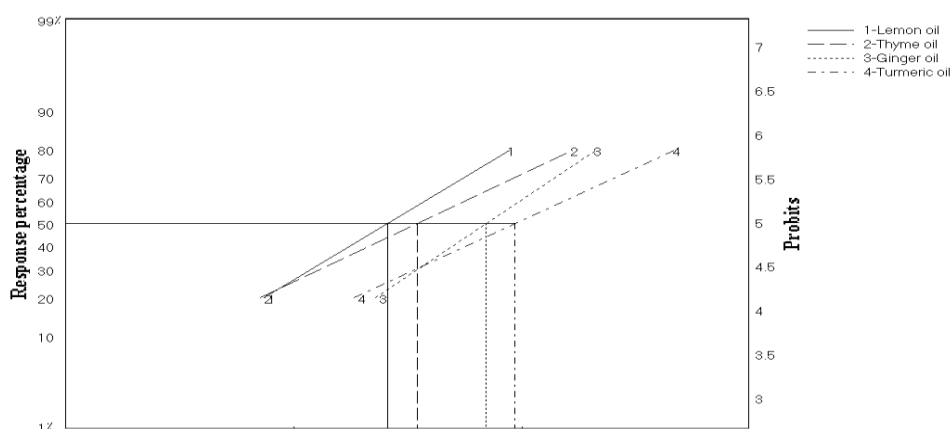
The current results align with those reported by (Moustafa *et al.*, 2023) who reported that *Cymbopogon citratus* essential oil showed high larvicidal activity against *Spodoptera littoralis*, and (Knaak *et al.*, 2013) found that *C. citratus* and *Zingiber officinale* oils have value of  $LC_{50}$  = 0.19 and 0.25  $\mu\text{L}/\text{cm}^2$ , respectively, furthermore, (El-Aw *et al.*, 2021) who recommended that the most effective essential oils in controlling *S. frugiperda* larvae are *Z. officinale* and *C. citratus* and reported that  $LC_{50}$  value for the cotton leafworm was 0.108% after 72 hours of treatment of fourth instar larvae with thyme oil (*Thymus vulgaris*). Recently, (Abdullah *et al.*, 2024 and Amadi *et al.*, 2024) reported that *Z. officinale* oil can serve as insecticides to control *S. littoralis* and *S. frugiperda*, especially when mixed with paraffin oil. Also, (Passara *et al.*, 2021) who reported that *Curcuma longa* oil registered the highest anti-feedant effect for *Spodoptera litura* larvae at 0.25% concentration.

**Table 1. Toxicity of the essential tested oils against second-instar larvae of *S. frugiperda***

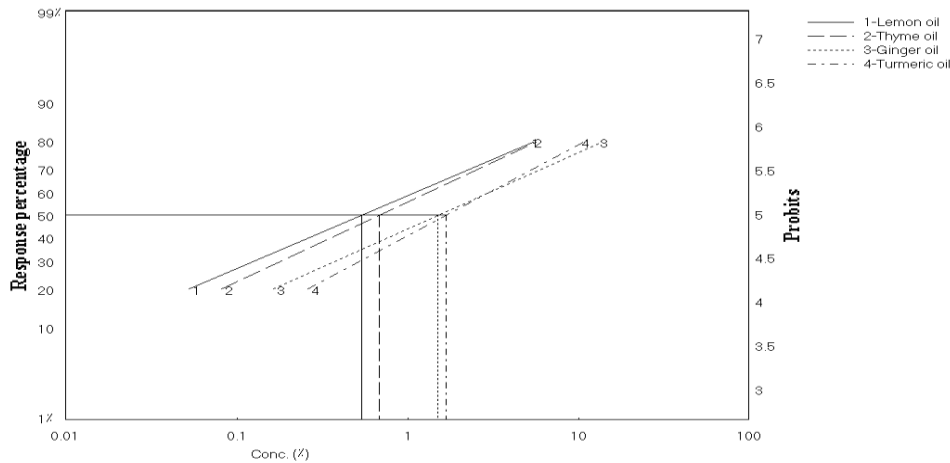
| Treatments (oils) | $LC_{50}$ % | Lower limit | Upper limit | Slope $\pm$ SE    | $LC_{25}$ % | Index  |
|-------------------|-------------|-------------|-------------|-------------------|-------------|--------|
| Lemon             | 0.259       | 0.079       | 0.417       | 1.56 $\pm$ 0.316  | 0.096       | 100    |
| Thyme             | 0.349       | 0.069       | 0.667       | 1.229 $\pm$ 0.237 | 0.099       | 74.212 |
| Ginger            | 0.702       | 0.494       | 0.939       | 1.742 $\pm$ 0.205 | 0.288       | 36.895 |
| Turmeric          | 0.941       | 0.418       | 1.508       | 1.195 $\pm$ 0.216 | 0.257       | 27.524 |

**Table 2. Toxicity of the essential tested oils against fourth-instar larvae of *S. frugiperda***

|          | $LC_{50}$ % | Lower limit | Upper limit | Slope $\pm$ SE    | $LC_{25}$ % | Index  |
|----------|-------------|-------------|-------------|-------------------|-------------|--------|
| Lemon    | 0.542       | 0.105       | 1.075       | 0.833 $\pm$ 0.171 | 0.084       | 100    |
| Thyme    | 0.686       | 0.152       | 1.282       | 0.912 $\pm$ 0.188 | 0.125       | 79.009 |
| Ginger   | 1.521       | 0.694       | 2.732       | 0.874 $\pm$ 0.164 | 0.257       | 35.634 |
| Turmeric | 1.688       | 0.934       | 2.785       | 1.042 $\pm$ 0.167 | 0.38        | 32.109 |



**Fig. (1): The effectiveness of the tested oils on the second larval instar of FAW**



**Fig. (2): The effectiveness of the tested oils on the fourth larval instar of FAW**

### 3.2.Biochemical changes:

Impact of  $LC_{50}$  values of the tested oils on total protein and carbohydrate levels, in addition to acetylcholinesterase and alpha-esterase activities, in sixth instar larvae derived from treated fourth instar larvae of *S. frugiperda* as shown in Table (3).

### 3.3.Total proteins:

The content of total proteins in *S. frugiperda* larvae were analyzed. The results in Table (3) indicated

that total proteins were significantly increased in untreated larvae as 54.87 mg/ g.b.wt , compared with other treatments. There is no significant between turmeric and ginger oils and showed the lowest activity were 43.10 and 39.80 mg/ g.b.wt, respectively followed by thyme and lemon oils were 49.90 and 47.23 mg/ g.b.wt, respectively. Moreover, (Abdullah *et al.*, 2024) reported that total protein was increased when treated larvae of *S. littoralis* was treated with ginger oil.

**Table 3: Impact of  $LC_{50}$  concentrations of the tested oils on total protein, total carbohydrate, AchE activity, and alpha-esterase activity in *S. frugiperda***

| Treatments (oils) | Total proteins<br>(mg/ g.b.wt) | Total carbohydrates<br>(mg/ g.b.wt) | AchE<br>( $\mu$ g AchBr /min/ g.b.wt) | Alpha esterase<br>( $\mu$ g $\alpha$ -naphthol/min/ g.b.wt) |
|-------------------|--------------------------------|-------------------------------------|---------------------------------------|---|
| Turmeric          | 43.10 $\pm$ 1.12 c             | 24.70 $\pm$ 0.42 bc                 | 155.33 $\pm$ 1.20 bc                  | 1084.00 $\pm$ 8.33 b  |
| Ginger            | 39.80 $\pm$ 0.99 c             | 25.67 $\pm$ 0.18 ab                 | 152.00 $\pm$ 1.53 c                   | 1044.67 $\pm$ 5.78 bc                                       |
| Thyme             | 49.90 $\pm$ 0.38 b             | 22.90 $\pm$ 0.26 c                  | 179.33 $\pm$ 4.91 a                   | 1090.00 $\pm$ 15.28 b                                       |
| Lemon             | 47.23 $\pm$ 0.61 b             | 20.77 $\pm$ 1.04 d                  | 84.33 $\pm$ 1.86 d                    | 1236.00 $\pm$ 22.72 a                                       |
| Untreated         | 54.87 $\pm$ 1.88 a             | 27.70 $\pm$ 0.91 a                  | 162.33 $\pm$ 1.45 b                   | 1011.67 $\pm$ 14.24 c                                       |

Significant differences do not exist between means that have the same letter in the same column.

### 3.4.Total carbohydrates:

Data show that, larvae in every treatment were lower in total carbohydrates compared to untreated which amounted to 27.70 mg/ g.b.wt , although there was no significant difference between untreated and treated larvae with ginger oil & lemon oil treatment for larvae which has significant decrease in total carbohydrates, compared to other oils as 20.77 mg/ g.b.wt Table (3).

### 3.5.Acetylcholinesterase:

Thyme oil treatment for larvae recorded the highest enzyme activity of acetylcholinesterase as 179.33  $\mu$ g AchBr /min/ g.b.wt, while lemon oil recorded the lowest enzyme activity as 84.33  $\mu$ g AchBr /min/ g.b.wt. No significant difference in enzyme acetylcholinesterase activity between turmeric oil & untreated , as well as between turmeric oil & ginger oil . The obtained data are consistent with (El-Aw *et al.*, 2021) who found that addressing cotton leafworm's fourth larval instar with thyme oil led to the suppression of the enzyme acetylcholinesterase. In addition , (Tharamak *et al.*, 2020) reported that, treated larvae of *Spodoptera litura* by thymol led to increase of

acetylcholinesterase activity, while glutathione S-transferase enzyme showed no significant difference.

### 3.6. Alpha esterase:

Results presented in Table (3) show that treatment with LC<sub>50</sub> of lemon oil recorded a substantial increase in alpha esterase enzyme activity as 1236.00 µg α-naphthol/min/g b.wt., while there were no

significant in alpha esterase activity between the three treatments of turmeric oil, ginger oil and thyme oil (1084.00, 1044.67 and 1090.00 µg α-naphthol/min/ g.b.wt, respectively) and the control (101.67 µg α-naphthol/min/ g.b.wt). Recently, (Moustafa *et al.*, 2023) reported that *Cymbopogon citratus* essential oil was significantly stimulates the activity of α- and β-esterase.

**Table 4: Impact of LC<sub>50</sub> concentrations of the tested essential oils on the activities of GST, proteases, amylase, and lipase enzymes in *S. frugiperda*.**

| Treatments (oils) | GST (mmol sub.conjugated/m g.b.wt) | Proteases (µg alanine /min / g.b.w) | Amylase (µg glucose / min / g.b.wt) | Lipases (mU/ g.b.wt) |
|-------------------|------------------------------------|-------------------------------------|-------------------------------------|----------------------|
| Turmeric          | 44.23 ± 0.65 b                     | 97.97 ± 2.55 c                      | 93.90 ± 0.78 a                      | 124.00 ± 2.65 a      |
| Ginger            | 39.37 ± 0.88 c                     | 81.93 ± 3.55 d                      | 91.50 ± 0.76 a                      | 119.33 ± 1.53 a      |
| Thyme             | 53.70 ± 1.48 a                     | 135.33 ± 1.20 a                     | 87.87 ± 0.41 b                      | 91.83 ± 1.76 c       |
| Lemon             | 34.67 ± 0.80 d                     | 122.00 ± 1.53 b                     | 78.73 ± 1.83 c                      | 107.00 ± 2.65 b      |
| Untreated         | 36.53 ± 1.03 cd                    | 136.00 ± 3.21 a                     | 94.70 ± 0.89 a                      | 126.67 ± 7.64 a      |

Significant differences do not exist between means that have the same letter in the same column.

Glutathione S-transferase (GST), proteases, amylase and lipase activity were determined after 48 hours of treatment with the four tested oils and the data are shown in Table 4.

### 3.7. Glutathione S-transferase (GST):

The activity of GST enzyme (Table 4) showed that, thyme oil has significant increase of enzyme activity followed by turmeric oil (53.70 and 44.23 mmol sub.conjugated / min/ g.b.wt, respectively). On the contrary, no significance differences were observed post exposure treatments with ginger oil and the untreated, or between lemon oil and the untreated. The obtained results are in agreement with those of (Moustafa *et al.*, 2021 & Moustafa *et al.*, 2023) who reported that GST activity was decreased post-exposure the second-instar larvae of *S. littoralis* to *Cymbopogon citratus* oil and showed significantly effect on detoxification enzymes. (Abdullah *et al.*, 2024) who found that use *Z. officinale* oil against *S. frugiperda* caused inhibition of GST activity.

### 3.8. Proteases:

Regarding to Protease activity (Table 4) there were notable variations between the four oils that were examined and the highest and lowest enzyme activity after treating with thyme oil and ginger oil as 135.33 and 81.93 µg alanine/min/ g.b.wt, respectively, while no significant variation was recorded between thyme oil and untreated.

### 3.9. Amylase:

As for Amylase enzyme activity (Table 4) it was decreased after the treatment with the four tested oils compared to the untreated ones, although there were no clear differences between treatment with turmeric oil, ginger oil and untreated (93.90, 91.50 and 94.70 µg glucose / min/ g.b.wt, respectively) and the enzyme's lowest activity, following treatment with lemon oil, was 78.73 µg glucose/min/g.b.wt. These findings are supported by (Yacoub, 2013) who reported that the enzymes trehalse and amylase were significantly impacted by plant extracts.

### 3.10. Lipases:

Finally as for the results of lipase activity (Table 4) it was similar to that of amylase activity, there were non-significant differences between treatment with turmeric oil, ginger oil and untreated (124.00, 119.33 and 126.67 mU/ g.b.wt, respectively) while, the lowest enzyme activity was recorded with larvae treated with thyme oil, followed by lemon oil as 91.83 and 107.00 mU/ g.b.wt, respectively.

## Conclusion

The toxicity of four plant oils was examined (ginger (*Z. officinale*), turmeric (*C. longa*), thyme (*T.*

*vulgaris*) and lemon (*C. limon*)) on second and fourth instar larvae of *Spodoptera frugiperda* was evaluated in this study. The LC<sub>50</sub> values of each oil were presented to the larvae in their fourth instar. then, after larvae reached the sixth instar, healthy larvae were taken for biochemical analysis. The findings indicated that lemon oil achieved superior toxic efficacy for second and fourth larval instar with LC<sub>50</sub> values as 0.259 and 0.542% respectively, On the contrary, turmeric oil had the least effective with LC<sub>50</sub> values as 0.941 and 1.688% , respectively. Also, biochemical analysis results revealed varying differences between the treatments and the control. Therefore, the results demonstrated the efficacy of tested oils in controlling fall army worm under laboratory conditions, and has the potential for application in field as a safe and effective alternative strategy.

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## النشاط الإبادي الحشري لبعض الزيوت العطرية وتأثيرهم على الدلائل البيوكيميائية لدودة الحشد الخريفية

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### الملخص العربي:

دودة الحشد الخريفية هي حشرة متعددة التغذية تسبب أضرارًا للعديد من المحاصيل. في الدراسة الحالية، تم تقييم سمية أربعة زيوت عطرية نباتية، وهي الزنجبيل (*Zingiber officinale*)، والكرم (*Curcuma longa*)، والزعتر (*Thymus vulgaris*)، والليمون (*Citrus limon*) ضد يرقات العمرين الثاني والرابع من دودة الحشد الخريفية بتركيزات مختلفة تحت الظروف المعملية. في كل معاملة تم اخذ يرقات العمر السادس السليمة لدودة الحشد الخريفية التي نجت بعد معاملة يرقات العمر الرابع بتركيز  $LC_{50}$  لإجراء التحاليل البيوكيميائية. اوضحت النتائج أن المعاملة بزيت الليمون حققت أعلى سمية حيث بلغت قيم  $LC_{50}$  و  $LC_{25}$  ٠,٢٥٩ و ٠,٠٩٦٪ للعمر اليرقي الثاني و ٠,٥٤٢ و ٠,٠٨٤٪ للعمر اليرقي الرابع على التوالي بينما كانت المعاملة بزيت الكرم الأقل فعالية حيث بلغت قيم  $LC_{50}$  و  $LC_{25}$  ٠,٩٤١ و ٠,٢٥٧٪ للعمر اليرقي الثاني و ١,٦٨٨ و ٠,٣٨٪ للعمر اليرقي الرابع على التوالي. أما فيما يتعلق بالتأثيرات على مستوى نشاط الإنزيمات، فقد سجلت البروتينات الكلية والكربوهيدرات الكلية انخفاضًا معنويًا في جميع المعاملات بالزيوت العطرية المختبرة. وكان أعلى وأدنى نشاط إنزيمي لأستيل كولين استريز عند معاملة اليرقات بزيت الزعتر والليمون على التوالي. بينما لم يتم ملاحظة أي اختلافات معنوية في نشاط إنزيم ألفا استريز بين زيت الكرم والزنجبيل والزعتر وحدث تثبيط لنشاط إنزيم GST في جميع المعاملات. كما انخفضت أنشطة إنزيم البروتيناز والأميليز والليباز بعد المعاملة بالزيوت الأربعة المختبرة مقارنة باليرقات غير المعاملة. لذلك، أثبتت نتائج هذا العمل فعالية الزيوت العطرية المختبرة في مكافحة دودة الحشد الخريفية في ظل الظروف المعملية، ويمكن استخدامها في التطبيق الحقل كبدائل آمنة وفعالة وتدخل ضمن برنامج مكافحة متكاملة لمكافحة الآفة.