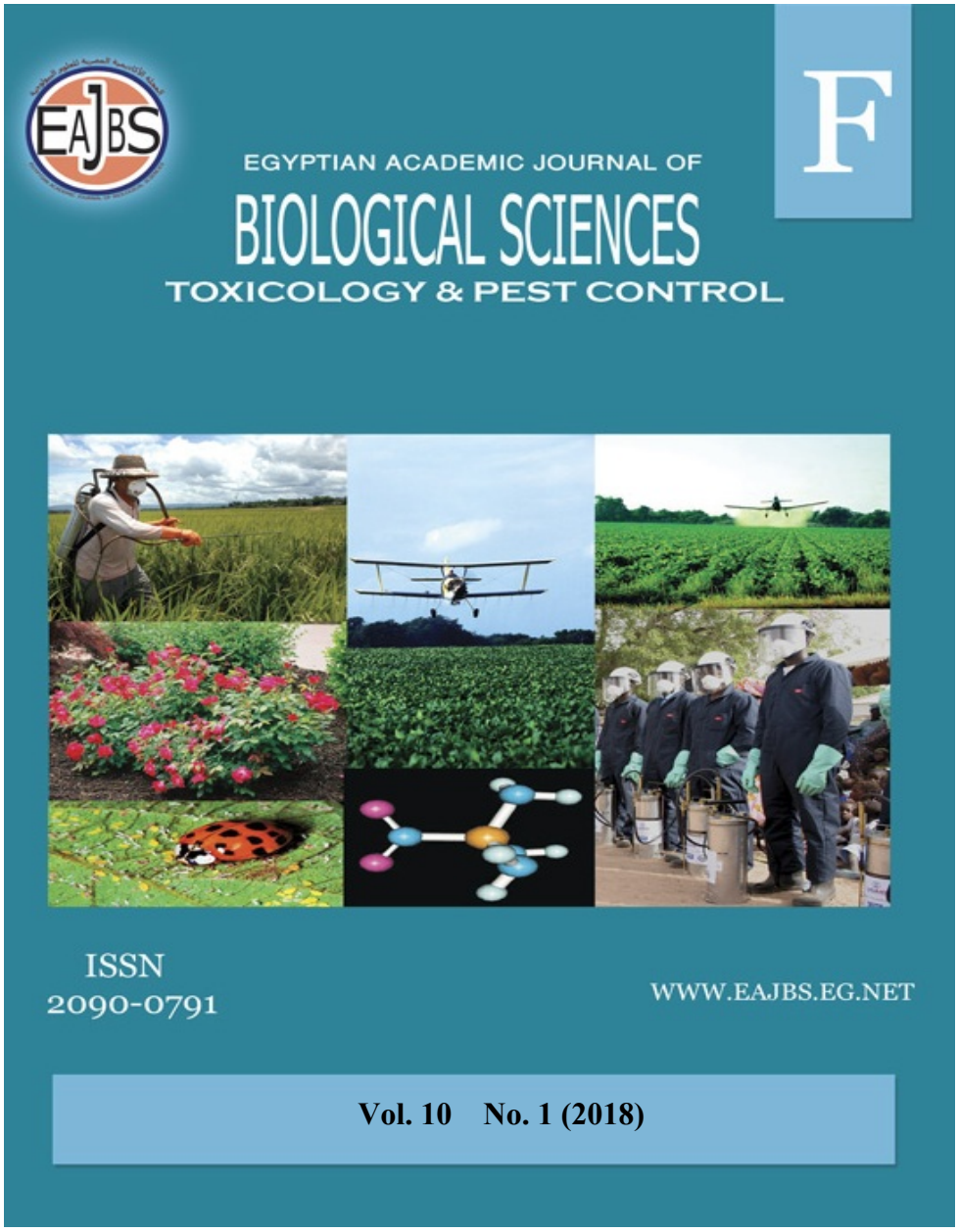


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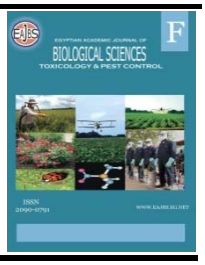

# Enzymatic Changes and Toxic Effect of Some Aromatic Plant Oils on The Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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**ABSTRACT**

In order to evaluate the larvicidal and biochemical effects of some aromatic plant oils against the cotton leafworm larvae, *Spodoptera littoralis* (Boisduval), the present investigation was carried out. Four commercial aromatic oils; garlic, mint, eucalyptus, and lavender oils, were tested against 2nd and 4th instar larvae. Results showed that all tested oils were so efficient, especially; the garlic oil as it exhibited the least $LC_{50}$ value for both 2nd and 4th instar larvae. Results also revealed significant disruption (either reduced or enhanced) enzyme activity. In conclusion, tested oils proved to have insecticidal, antifeedant, and inhibitory enzymatic activity effects. So that, tested oils can be applied safely in IPM program for the cotton leafworm, *Spodoptera littoralis* control.

## INTRODUCTION

The unwise use of synthetic insecticides gives rise to high resistance to many chemical pesticides, resurgence, residual toxicity, widespread chemical hazards and increasing costs of application of chemical insecticides have directed the need for effective biodegradable pesticides (Abd El-Aziz and Ezz El-Din, 2007). Plant-derived products have received increasing attention from the scientists and recently more and more plants have been screened for their insecticidal properties. Botanical insecticides have been used in agriculture for at least two thousand years in Asia and the Middle East (Farag *et al*., 2011). The interest in new botanical compounds for pest control is based on their bio-efficiency, biodegradability, and physiological activity (Rodríguez, 1998 and Isman, 1999). There are four major types of botanical insecticides used for insect control including pyrethrum, rotenone, neem, and essential oils (Isman, 2006). Generally essential oils are secondary metabolites, volatile, with strong odor and are formed of mixture of several up to dozens of mono- and di-, sesqui-terpenes (Pavela, 2005). The composition of essential oils varies with every plant species and also on the growth stage of the plant. Different essential oils have been used as repellent, fumigant, larvicidal, ovicidal and adulticidal against different insect orders (Isman, 2000 and Mossa, 2016). Many researchers have documented the larvicidal, antifeedant and physiological effects of several essential oils such as neem oil, jojoba

oil, peppermint oil, and ginger oils against the *S. littoralis* larvae (Ismail and Shaker, 2014). The major bioactive components responsible for the benefits of garlic are assumed to be allylic sulfur compounds (Pavela, 2008; Klevenhusen *et al*., 2011; and Karamaouna *et al*., 2013; and Ali *et al*., 2017). Several studies showed that garlic plant is not only beneficial as medicinal plants, but they can be used also as insecticidal, acaricides and as insect repellent to some plant pests (Klevenhusen *et al*., 2011). Eucalyptus oil has been known for hundreds of years as having antibacterial, antifungical and antiseptic properties (Brooker and Kleinig, 2006). Eucalyptus essential oils have a wide spectrum of biological activity against fungi, bacteria, insects, mites and weeds and provide a simple, inexpensive and environmentally friendly (nonpolluting and less or no toxicological concerns) alternative to pest control (Mossi *et al*., 2011). Lavender oil is useful for use in nervous system stimulants, hypnotics, sedatives, tranquilizers and stress repellents. In addition, it has useful dermatological uses in the treatment sunburn and skin rashes, as well as strong antiseptic and antibiotic effects (Shalaby *et al*., 2016). The genus *Mentha* belongs to the family Lamiaceae (Labiatae), and consists of about 25-30 species, most of which are found in temperate regions of Eurasia, Australia and South Africa (Lange and Croteau, 1999). Various biological activities have been reported for some species of *Mentha*, such as antibacterial (Hajlaoui *et al*., 2008), antifungal (Bouchra *et al*., 2003), and insecticidal properties (Franzios *et al*., 1997; Lamiri *et al*., 2001; Pavela, 2005; and Saljoqi *et al*., 2006). In context, the present study aims to evaluate the larvicidal effects of garlic, mint, eucalyptus, and lavender essential oils against *S. littoralis* 2$^{nd}$ and 4$^{th}$ instar larvae. Also the effects of the sub-lethal concentration ($LC_{50}$) of each essential oil on the digestive enzymes activity (α- and β-esterases, and Acid and Alkaline phosphatases) as well as the detoxification enzymes (Mixed function oxidase and glutathione s-transferase) were evaluated.

## MATERIALS AND METHODS

**Insect rearing:**

A laboratory strain of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), was obtained and reared in the cotton leafworm Department, Plant Protection Research Institute, Dokki, Giza under constant laboratory conditions as described by El-Defrawi *et al*. (1964). Rearing conditions were: a 12 h photo regime at 25±1°C and 65±5% relative humidity (RH).

**Applied essential oils:**

Four Egyptian oils, obtained from El-Captain Co., Al-Obor city, Cairo, Egypt, approved for human use from the Egyptian Ministry of Health. Such oils include the following: garlic oil, *Allium sativum*; peppermint, *Mentha piperita*; eucalyptus oil, *Eucalyptus camaldulensis*; and lavender, *Lavandula angustifolia*.

**Insecticidal activity of essential oils:**

The leaf-dipping technique, similar to that described by Tabashink *et al*. (1991), was used to determine the toxicity of essential oils against the 2$^{nd}$ and 4$^{th}$ instar larvae using concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% for 2$^{nd}$ instar larvae and 1, 1.5, 2, 2.5, and 3 % for 4$^{th}$ instar larvae. Castor leaves were dipped for 5 s in each solution, and then the treated leaves were left for natural air-drying. Three replicates each with 20 larvae of both 2$^{nd}$ and 4$^{th}$ instar larvae and were allowed to feed on treated leaves for 24 h. Three replicates of 20 larvae were fed on water-treated leaves for 24 h to serve as control. Larval mortality was recorded after 24 h. Mortality

was calculated using the Abbott formula (**Abbott, 1925**) and subjected to probit analysis according to Finney (1971) using "LdPLine®" software.

**Biochemical effect:**

**1.Sample preparation:**

Larvae were treated as 4th instar with tested oils at (concentration). After 24h treatment, one gram of treated larvae was weighed. Larvae were then homogenized on ice in ice-cold 100 homogenization buffer (0.1 M phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 1 mM PTU and 20% glycerol). The homogenate was centrifuged at 4°C, 10,000 g for 30 min using sigma 3K 30 rotors NO. 12158, sigma laboratories centrifuge 3K30, the solid debris and cellular material were discarded. The supernatant was transferred into a clean Eppendorf tube, placed on ice and used immediately for assaying mixed function oxidase (MFO), glutathione S-transferase (GST), Acid and Alkaline Phosphatases (ACP and ALP) and Non-specific esterases (α- and β- esterases).

**2.Enzymes assay:**

The assay of MFO was conducted using the procedures developed by Rose *et al*. (1995) with slight modification (Letelier *et al*., 2009). GST activity was measured according to the method of Asaoka and Takahashi (1983) using ethanolic solution of DNB as a substrate with slight modification as done with El-Shahawi and Al Rajhi (2000). Alpha esterase (α-esterase) and beta esterase (β-esterase) were determined according to Van Asperen (1962) using α- and β-naphthyl acetate as substrates, respectively. Acid and alkaline phosphatases were determined according to the method described by Powell and Smith (1954).

## RESULTS AND DISCUSSION

**Bioassay test:**

**1.Morphological changes:**

Several morphological abnormalities were observed. Larval body becomes very thin and stretched. Darkening of larval body to uniform dark grey color and disappearance of spotted characteristic pattern found in normal larvae. Abnormalities like darkening of cuticle and different levels of cuticular melanization have been reported earlier by Koul (1987) in azadirachtin treated *Bombyx mori* and they associated these symptoms to changes in the levels of ecdysteroids and juvenile hormones (Hori *et al*., 1984). Results also agreed with Summarwar *et al*. (2016) who associated the abnormalities signs to defective moulting and included death during moulting, incomplete removal of old cuticle or exuviae, abnormal stretching of body, and rupturing of cuticle.

**2.Virulence of the tested compounds against the cotton leaf worm:**

The insecticidal activity of four essential oils obtained from garlic oil, *Allium sativum*; peppermint, *Mentha piperita*; eucalyptus oil, *Eucalyptus camaldulensis*; and lavender, *Lavandula angustifolia* against 2nd and 4th instar larvae of *Spodoptera littoralis* were summarized in tables (1) and (2). All essential oils applied, proved to be toxic to the cotton leafworm, although they differed in their efficacy. The percent mortality of treated larvae was increased by increasing the concentration. Essential oils of garlic and peppermint exhibited the highest efficiency followed by eucalyptus and lavender oils. In addition results showed that the 2nd instar larvae were more susceptible to tested oils than the 4th instar larvae. These results of the effectiveness of tested essential oils coincide with those obtained by other authors (Abd El-Aziz and Ezz El-Din, 2007; Marei *et al*., 2009; Abd El-Mageed and Shalaby, 2011; Shabnum

and Wagay, 2011; Hassan, 2012; Khedr and El-Kawas, 2013; Abdel-Aziz *et al*., 2013; and Ali *et al*., 2017). The efficacy of the applied oils met these criteria for the applied dipping assays. Similarly, garlic extracts have shown a considerable toxicity to a number of species of different insect orders and to different developmental stages (Ismail *et al*., 2011; Meriga *et al*., 2012; Wang *et al*., 2014; and Ali *et al*., 2017). Furthermore, lavender and eucalyptus oils were used efficiently to control other species (Khater *et al*. 2009; Khater *et al*. 2013; and Shalaby *et al*., 2016)

**Table (1): Toxicity of tested essential oils against 2nd instar larvae of *S. littoralis***

| Essential oils | Lethal concentration (%) | | 95% Confidence limits | | Slope ± S. E. |
|---|---|---|---|---|---|
| | | | Upper | Lower | |
| Garlic | $LC_{50}$ | 0.32 | 0.45 | 0.25 | 1.01±0.23 |
| | $LC_{90}$ | 5.91 | 79.54 | 2.19 | |
| Peppermint | $LC_{50}$ | 0.33 | 0.46 | 0.26 | 1.07±0.23 |
| | $LC_{90}$ | 5.25 | 51.67 | 2.09 | |
| Eucalyptus | $LC_{50}$ | 0.34 | 0.46 | 0.27 | 1.21±0.24 |
| | $LC_{90}$ | 3.90 | 21.34 | 1.81 | |
| Lavender | $LC_{50}$ | 0.39 | 0.59 | 0.31 | 1.13±0.24 |
| | $LC_{90}$ | 5.39 | 45.60 | 2.19 | |

**Table (2): Toxicity of tested essential oils against 4th instar larvae of *S. littoralis***

| Essential oils | Lethal concentration (%) | | 95% Confidence limits | | Slope ± S.E. |
|---|---|---|---|---|---|
| | | | Upper | Lower | |
| Garlic | $LC_{50}$ | 1.62 | 1.88 | 1.32 | 1.68±0.34 |
| | $LC_{90}$ | 9.34 | 27.57 | 5.79 | |
| Peppermint | $LC_{50}$ | 1.96 | 2.35 | 1.67 | 1.65±0.34 |
| | $LC_{90}$ | 11.70 | 41.49 | 6.79 | |
| Eucalyptus | $LC_{50}$ | 1.85 | 2.18 | 1.56 | 1.67±0.34 |
| | $LC_{90}$ | 10.79 | 35.31 | 6.43 | |
| Lavender | $LC_{50}$ | 2.08 | 2.56 | 1.77 | 1.61±0.34 |
| | $LC_{90}$ | 13.07 | 53.25 | 7.27 | |

**Biochemical effect:**

Data in table (3) showed the effect of sub lethal concentration ($LC_{50}$) of garlic, peppermint, eucalyptus, and lavender oils on the activity of α- and β- esterases in treated 4th instar larvae of *S. littoralis* for 24-h. Obtained results revealed significant decrease in the both enzyme activity compared to untreated group. The lowest α-esterase activity was observed when peppermint oil $LC_{50}$ was used. In addition, the lowest β-esterase activity was acquired when lavender oil was applied. Furthermore, results in table (4) showed the effect of $LC_{50}$ of tested oils on acid (ACP) and alkaline (ALP) phosphatases in 4th instar larvae after 24-h application. Results revealed significant decrease in ACP activity when larvae were treated with tested oils. The most effective oil was eucalyptus and peppermint oils. In addition, significant increase in ALP activity was obtained when larvae were treated with peppermint and eucalyptus oils while the ALP activity was decreased when larvae were treated with garlic and lavender oils. Data presented in table (5) illustrated changes in mixed function oxidase (MFO) and glutathione s-transferase (GST) enzyme activity in treated 4th instar larvae with $LC_{50}$ of tested oils. Obtained results showed significant increase in MFO enzyme activity when larvae were treated with garlic and peppermint oils, while slight increase was obtained in case of eucalyptus oil and no effect obtained when lavender oil was applied. Results also revealed significant

decrease in GST enzyme activity compared to control. Garlic and eucalyptus oils exhibited the most effect on GST activity followed by lavender and peppermint oils.

Detoxification enzyme in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li and Liu, 2007). The detoxifying enzymes react against insecticides, or compounds exhibiting insecticidal activities. They include general esterases, glutathione S-transferase and phosphatases (Zibaee *et al*., 2011). Esterase (EST) is an important detoxifying enzyme which hydrolyzes the esteric bond in synthetic chemicals. Also, esterase is one of the enzymes showing the strongest reaction to environmental stimulation (Hemingway and Karunatne, 1998). In this study EST significantly decreased compared to control 24 h after treatment. Similar results were reported by Liu *et al*. (1990) and Nasr *et al*. (2017). In the present study, acid phosphatase (ACP) activity in treated larvae was found to be less than in the control. This inhibitory effect agrees, to some extent, with some reported results for various insect species by other plant extracts, such as *M. domestica* by azadirachtin (Saeed *et al*., 1987) and Margosan-O (a neem preparation) or Jojoba oil (Ghoneim *et al*., 2008); *S. littoralis* (Ayyangar and Rao, 1990) by azadirachtin; *Euprepocnemis plorans* by some neem limonoids (Al-Dali, 2007); *Rhizopertha dominica* by hexane extract of *Capparis deciduas* (Upadhyay, 2013); *Tribolium castaneum* by various doses of different extracts of *Melia azedarach, Nicotiana tabacum, Azadirachta indica* and *Colosynthus citrullus* (Ali *et al*., 2015) or by $LC_{50}$ of the garlic oil (Beltagy and Omar, 2016). So many controversial effects of several botanicals on ALP activity are available in the literature (Senthil-Nathan *et al*., 2005, 2006; Basiouny *et al*., 2010; and Ghoneim *et al*., 2016). In the current study used oils exhibited induction and reduction effect. The inhibitory effect of plant extracts on ALP activity are in agreement with similar inhibitory effects of some plant extracts on various insects, such as hexane extract of *C. deciduas* on *R. dominica* (Upadhyay, 2013); different extracts of *Curcuma longa* on *T. castaneum* (Uma devi and Sujatha, 2013); *A. visnaga* seed extracts on last instar nymphs of *S. gregaria* (Ghoneim *et al*., 2014); different extracts of *M. azedarach, N. tabacum, A. indica* and *C. citrullus* on *T. castaneum* adults (Ali *et al*., 2015); $LC_{50}$ of *Acorus calamus* (essential oil) extracts or Biosal (a neem preparation) on *Callosobruchus analis* (Arif *et al*., 2015). The reduced ALP activity induced by tested oils in the present study may be explicated by some developmental disturbance as an appreciated suggestion of Wu (1990) for the larvae of mosquito *C. pipiens* after treatment with IGR diflubenzuron. These results also coincided with other reports of plant extract treatments of insects. For example, Senthil-Nathan (2006) showed that treatment of rice plants with *Melia azedarach* Juss (Meliaceae) extracts decreased the activity level of ALP in *Cnaphalcrocis medinalis* (Guenee). Previously published research reported that feeding *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) on *Ricinus communis* L. treated with azadirachtin decreases the amount of this enzyme in the midgut (Senthil-Nathan and Kalaivani, 2005). Glutathione S-transferases (GST) are mainly cytosolic enzymes that catalyze the conjugation of electrophile molecules with reduced glutathione (GSH), potentially toxic substances become more water soluble and generally less toxic (Grant and Matsumura, 1989). Application of other xenobiotics such as plant defense allelochemicals against phytophagous insects may induce GST activity (Vanhaelen *et al*., 2001). In this study, activity level of GST at 24 h after treatment decreased significantly compared with control. This result indicates its inhibition by essential oil more significantly after 24 h. These findings are in agreement with Nasr *et al*. (2017) who found significant decrease in GST activity in larvae of *Plutella xylostella* treated

with sub lethal concentration of *O. vulgare* extract. On the other hand, these findings were contradicting to Khosravi *et al*., (2011) who reported that GST and general esterase activity increased in the larvae of *G. pylolais* treated with *A. annua* methanolic extract. Also, Vanhaelen *et al*. (2001) showed that Brassicaceae secondary metabolites induced GST activity in *Myzus persicae* and several lepidopterous species such as *Heliothis virescens* Fabricius, *Trichoplusia ni* Hubner and *Anticarsia gemmatalis* Hubner.

Table (3): Non-specific esterases (α- and β- esterases) activity in 4th instar larvae of *Spodoptera littoralis* treated with $LC_{50}$ of tested essential oils

| Essential oils | Non-specific esterases activity (µg α-/β-naphthol/min./g.b.wt) (Mean ±S.E.) | |
|---|---|---|
| | α-esterase | B-esterase |
| Garlic | $145.6 \pm 1.8^{bc}$ | $116.3 \pm 1.0^{b}$ |
| Peppermint | $139.0 \pm 1.2^{c}$ | $121.1 \pm 1.5^{b}$ |
| Eucalyptus | $150.0 \pm 1.4^{b}$ | $116.8 \pm 1.7^{b}$ |
| Lavender | $143.4 \pm 5.7^{bc}$ | $114.6 \pm 1.4^{b}$ |
| Control | $200.0 \pm 9.0^{a}$ | $133.0 \pm 9.84^{a}$ |

Means followed by the same letters are insignificantly different at $P < 0.05$

**Table (4): Acid and Alkaline Phosphatases (ACP and ALP) activity in 4th instar larvae of *Spodoptera littoralis* treated with $LC_{50}$ of tested essential oils**

| Essential oils | Activity ($U \times 10^3$ ml/gm per body weight) (Mean ±S.E.) | |
|---|---|---|
| | Acid Phosphatase | Alkaline Phosphatase |
| Garlic | $210.7 \pm 0.30^{ab}$ | $328.0 \pm 2.00^{d}$ |
| Peppermint | $199.9 \pm 0.45^{c}$ | $394.0 \pm 1.51^{b}$ |
| Eucalyptus | $187.6 \pm 0.55^{d}$ | $406.7 \pm 1.07^{a}$ |
| Lavender | $200.3 \pm 0.67^{bc}$ | $312.5 \pm 1.40^{e}$ |
| Control | $215.0 \pm 3.75^{a}$ | $341.0 \pm 6.90^{c}$ |

-Means followed by the same letters are insignificantly different, P≥0.05.

**Table (5): Mixed Function Oxidase (MFO) and Gluthion S-Transferase (GST) activity in 4th instar larvae of *Spodoptera littoralis* treated with $LC_{50}$ of tested essential oils**

| Essential oils | Enzyme Activity (Mean ±S.E.) | |
|---|---|---|
| | MFO (nm/min/mg protein) | GST (µmol/min/mg protein) |
| Garlic | $0.027 \pm 0.08^{b}$ | $0.030 \pm 0.05^{e}$ |
| Peppermint | $0.030 \pm 0.06^{a}$ | $0.053 \pm 0.14^{b}$ |
| Eucalyptus | $0.025 \pm 0.05^{c}$ | $0.033 \pm 0.09^{d}$ |
| Lavender | $0.024 \pm 0.05^{c}$ | $0.045 \pm 0.07^{c}$ |
| Control | $0.024 \pm 0.00^{c}$ | $0.075 \pm 0.003^{a}$ |

-Means followed by the same letters are insignificantly different, P≥0.05.

**Conclusion:**

The use of plant essential oils has considered as an important alternative for pest control because of their environmental and mammals safety properties. From results of the present study, could be concluded that the tested oils (Garlic, Peppermint, Eucalyptus and Lavender) possess toxic effect against 2nd and 4th instar larvae of *S. littoralis*. In addition, all the tested oils exhibited antifeedant effects. Because the induction and inhibition of detoxification metabolic system plays an important role in insect's detoxification mechanism, inhibition of enzyme activities in treated 4th instar larvae indicate that some extracts may not be detoxified by these enzymes. And so, tested oils which exhibited inhibitory effects on the esterase, phosphatase, and GST activities may be potential agents for controlling *S. littoralis*, especially as a part of Integrated Pest Management. However, further investigation should be carried out in future to ascertain the active ingredient (s) in the tested oils responsible for the inhibition of these detoxifying enzymes.

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## ARABIC SUMMERY

# التغيرات الأنزيمية والأثر السام لبعض الزيوت النباتية العطرية على دودة ورق القطن

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أجريت الدراسة الحالية بهدف تقييم التأثير الابادي والبيو كيميائي لبعض الزيوت النباتية العطرية على يرقات دودة ورق القطن. تم اختبار تأثير أربعة زيوت عطرية تجارية وهي زيت الثوم، زيت النعناع، زرت الكافور، وزيت اللافندر على يرقات العمرين الثاني والرابع. وقد أظهرت النتائج فاعلية جميع الزيوت المختبرة على كلا العمرين، وخاصة؛ زيت الثوم حيث كانت له أقل قيمة للتركيز القاتل للنصف لكل من يرقات العمرين الثاني والرابع. كما أظهرت النتائج أن معاملة يرقات العمر الرابع بالتركيز القاتل للنصف أدت إلى اختلال فى نشاط الإنزيمات المقدرة مقارنة باليرقات الغير معاملة. وفي الختام، فقد أثبتت الزيوت المختبرة أن لها تأثيرات سمية، ومثبطة للشهية، ومثبطة لنشاط الإنزيمات. لذلك، يمكن تطبيق الزيوت المختبرة بأمان في برنامج الإدارة المتكاملة للآفات لدودة ورق القطن.