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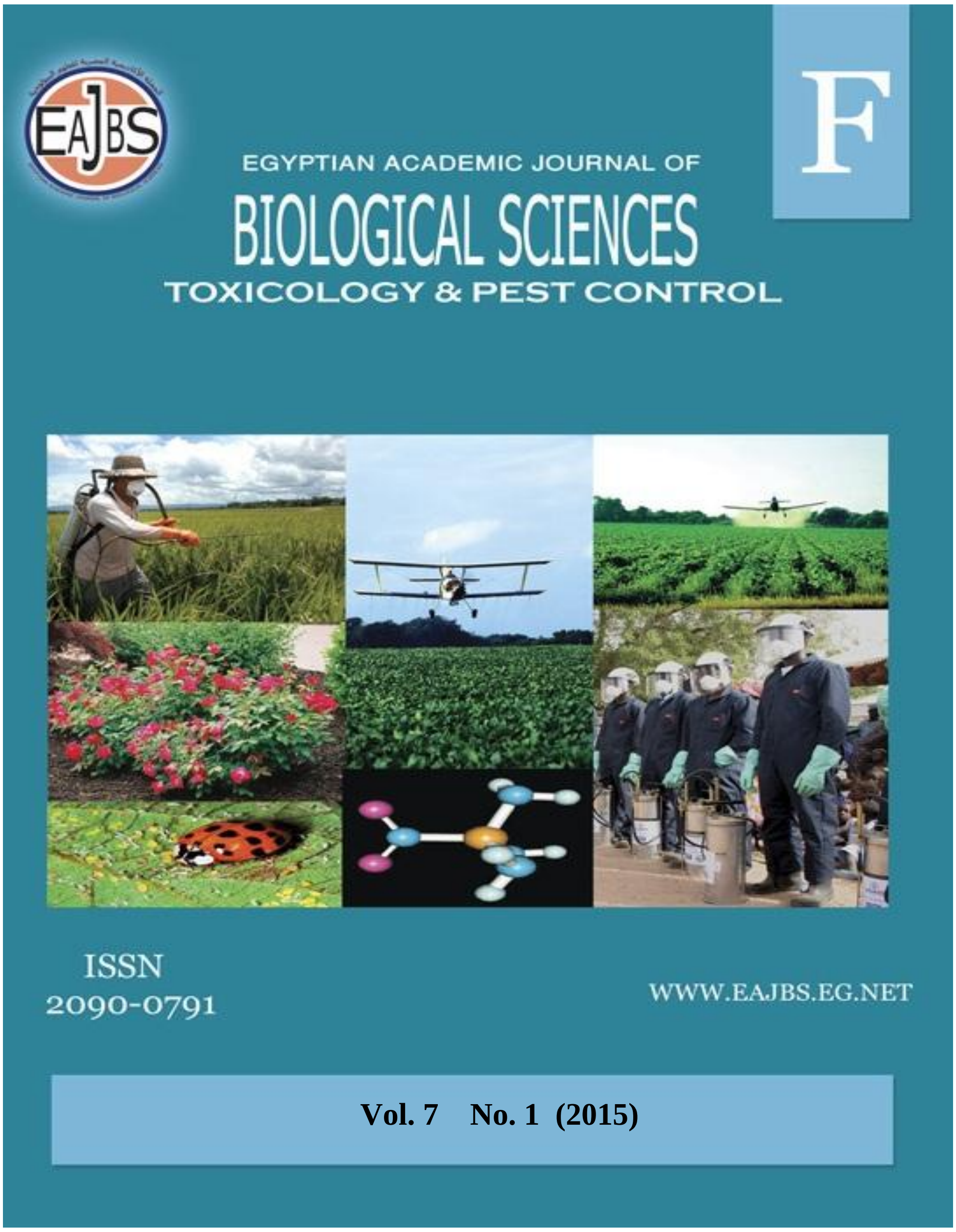

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Toxicity and Biochemical Effects of Four Plant Essential Oils Against Cotton Leafworm, *Spodoptera littoralis* (Boisd)

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ABSTRACT

This study was undertaken to investigate the toxicity of four natural source essential oils (*Trigonella foenum graecu, Sesamum indica, Eucalyptus camaldulensis* and *Nigella sativa*) on 4th larval instar of *Spodoptera littoralis*. Biochemical changes in larvae induced by treatment with LC_{50} of tested essential oils were also studied. Bioassay technique was performed using thin film to estimate LC_{25}, LC_{50} and LC_{90} values. Results showed that, *T. foenum graecu* was the most effective oil followed by *S. indica, E. camaldulensis* and finally *N. sativa*. As regards to biochemical changes induced by treatment with LC_{50} of each oil. Remarkable reduction in acetyl cholinesterase (AChE) enzyme was recorded in *T. foenum graecu* and *N. Sativa* treatments. However *E. camaldulensis* and *S. indica* induced significant elevation in glutamate oxaloacetate transaminase (GOT) enzyme, while glutamic pyruvic transaminase (GPT) reported significant elevation in all treatments. Remarkable reduction in total protein content was observed in *N.sativa* treatment. Whereas, treatment with *T. foenum graecum* oil induced significant increase in total protein content.

In conclusion, results indicated that the plant essential oils had insecticidal effect and also showed adverse effect on the tested biochemical parameters, suggesting the possibility of using them as an alternative to conventional insecticides for cotton leafworm control.

INTRODUCTION

Chemical control methods using insecticides had been favored so far because of their speedy action and easy application. However, the public concern over harmful effect of chemical insecticides on the environment and human health has enhanced the search for safer and environmentally friendly control alternatives where plant oils seem to be relevant and had great promise as an alternative to the conventional pesticides.

Terpenes and terpenoids are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with diverse functions (Bakkali *et al*., 2008). Insecticidal properties of numerous essential oils and some monoterpenes have been extensively studied against various insects (Lee *et al*., 2003, Abdel Aziz, *et al*., 2007, Bashir *et al*., 2013. and Hany, 2013). Eugenol, citronellal and thymol are reported as toxic to *Spodoptera litura* and *Musca domestica* (Lee *et al*., 1997: Hummel runner & Isman, 2001).

Estragole is an example of a toxic fumigant compound in the essential oils from coriander (*Coriandrum sativum*), caraway (*Carum carvi*) and basil (*Ocimum basilicum*) that is active against insect pests (Lopez *et al*., 2008).

Compounds extracted from plants, or the derivatives of such compounds may affect insect physiology in various ways (Shekari *et al.,* 2008).

AChE is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter, acetylcholine, in the nervous system of various organisms (Wang *et al*. 2004).

The possible sites of action of essential oil toxicity are acetylcholinesterase and the octopamingeric system in insects (Kostyukovsky *et al*. 2002; Evans 1981).

Transamination has been demonstrated in a number of insect tissues, particularly that concerning glutamate, aspartate and alanine (Gilmour, 1961). The glutamic oxaloacetate transaminase (GOT) and glutamic pyruvic transaminase (GPT) are key enzymes in the formation of non-essential amino acids, in metabolism of nitrogen waste, gluconeogenesis and correlated with protein anabolism and catabolism (Mordue and Golworthy, 1973). Moreover, transaminases, especially GPT, act as a catalytic agent in carbohydrates metabolism (Katumuma *et al*., 1968).

The present study was conducted to investigate the toxicity level and biochemical effects of four plant essential oils against 4^{th} larval instar of *Spodoptera littoralis.*

MATERIALS AND METHODS

Test insects:

Fourth instar larvae of *S. littoralis* were obtained from a continuous stock susceptible colony maintained in the Central Agricultural Pesticides Laboratory (CAPL), Dokki, Giza, Egypt. Larvae were reared throughout the experiments as described by (El-Defrawi *et al.*, 1964) under laboratory conditions (25±2°C and 65±5% R/H.).

Experimental plant essential oils:

The four plant essential oils used in this study (*Trigonella foenum graecum Sesamum indica*, *Eucalyptus camaldulensis* and *Nigella sativa*) were purchased from El-captain Company (CAP. PHARM., Cairo), Egypt.

Method of application:

Thin film technique was used as a method of application in this study (Asher and Mirion, 1981), where the tested concentrations were applied through acetone to the surface of 9 cm in petri-dish. One ml of each concentration of the tested oils was spread on the inner surface of a petri-dish, by moving the dish gently in circles. Petri-dish used as control was treated with 1 ml of acetone only. The solvent was evaporated under room conditions leaving a thin film of oil on the surface of petri-dish. Ten newly moulted 4^{th} larval instar of *S. littoralis* were exposed to the tested oils for 6 hrs in each petri-dish, then transferred to clean glass containers and fed on fresh castor bean leaves. Five replicates of each concentration and the control were made. The mortality percentages were recorded after 24 hr from treatment and corrected as compared to control larvae according to Abbott formula (Abbott, 1925). The LC_{25}, LC_{50} and LC_{90} values for each oil were calculated according to Finney (1971).

To evaluate the toxicity index (TI) of the tested oils, the following equation (Sun, 1950) was applied:

$$Toxicity\ index = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the compound used}} \times 100$$

To investigate the biochemical effects of the tested oils, newly moulted 4th larval instars were treated with LC_{50} of tested essential oils as described before to determine their potential effects on the activities of GOT, GPT, AChE and total protein content as well.

Sample preparation for biochemical assays:

The larvae were collected after treatment and starved for about 4 h before being homogenized in (1/5 w/v) homogenization buffer (pH 7.8) which prepared by dissolved 15 ml of glycerol into 75 ml distilled water and added 606 mg Tris, 292 mg EDTA and 5 mg phenyl thiourea. The pH was adjusted to 7.8, then final volume was completed to 100 ml. Homogenates were centrifuged at 10000 rpm for 15 min at 4°C and resulting supernatants were held on ice and used to determine the activity of enzymes and total protein content (Mohamady, 2005).

Biochemical assays:

GOT and GPT activities were determined in homogenate of larvae according to Harold (1975). AChE activity was determined according to Ellman *et al*. (1961). The total protein content in the samples was determined according to Lowry *et al*. (1951).

Statistical Analysis:

The data obtained from the present study was analysed using one-way ANOVA at ($P < 0.05$). The data was expressed as mean ± SE. Probitanalysis was performed for calculating LC_{25}, LC_{50}, and LC_{90} according to Finney (1971).

RESULTS

Data in Table (1) represented the LC_{25}, LC_{50} and LC_{90} values of tested plant essential oils against 4th larval instar of *S. littoalis*.

Results showed that, the LC_{50} values were 8.69, 10.24, 11.79 and 24.46 ppm for *T. foenum gaecum*, *S. indeca*, *E. camaldulensis* and *N. sativa*, respectively.

Based on LC_{50} values and toxicity index in Table (1) data revealed that, the *T. foenum gaecum* was the most effective oil against 4th larval instar of *S. littoalis*, where (TI =100%) followed by *S. indeca* (TI =84.86 %) and *E. camaldulensis* (TI = 73.71%), while *N. sativa* showed the lowest toxic effect (TI = 35.53%).

Table 1: Toxicity values of tested plant essential oils against 4th larval instar of *S. littoralis*.

Treatments	Toxicity parameters				
	LC_{25}	LC_{50}	LC_{90}	Slope	Toxicity index
Trigonella foenum graecum	3.42	8.69	51.00	1.66	100
Sesamum indica	4.52	10.24	38.00	2.22	84.86
Eucalyptus camaldulensis	6.00	11.79	42.55	2.3	73.71
Nigella sativa	16.53	24.46	51.15	3.9	35.53

Certain biochemical parameters were investigated in homogenate of 4th larval instar of *S. littoralis* treated with LC_{50} of tested plant essential oils (Table 2).

As demonstrated in Table (2) the activity of acetylcholinesterase (AChE) was reduced after treatment of larvae with all tested oils.

Treatment with *T. foenum graecum* and *N. sativa* significantly reduced

(AChE) activity to (-20.87% and -19.99 %, respectively), while there is no significant change with *E. camaldulensis* and *S. indica* as compared to control.

Data assorted in Table (2) declared enhancing effect in the activity of GOT after treatment of larvae with tested plant essential oils. One exception was exhibited. This exceptional was the remarkable reduction in enzyme activity in the homogenate of larvae (Change %: - 5.76) after treatment with *N. sativa* oil.

Table 2: Activity levels of some biochemical parameters in larvae homogenate of S. *littoralis* after treatment by LC_{50} of tested plant essential oils.

Treatments	AChE Activity (U/ml)	% Change	GOT Activity (U/ml)	% Change	GPT Activity (U/ml)	% Change	Total protein content (mg/bw)	% Change
Control	287.6 ±7.57	-	27.45 ± 0.80	-	14.15 ±0.08	-	5.83 ±0.30	-
Trigonella foenum graecum	239.81* ± 9.40	-19.99	29.33 ns ± 0.17	6.85	25.62* ±0.28	81.06	6.87 ns ±0.34	17.84
Eucalyptus camaldulensis	281.52ns ± 7.82	-6.08	42.61* ±0.23	55.23	41.52* ±0.30	193.43	5.39 ns ±0.41	-7.55
Nigella sativa	237.21* ± 5.21	-20.87	25.87 ns ±0.16	- 5.76	22.17* ±0.51	56.67	4.57* ±0.29191	-21.61
Sesamum indica	277.61 ns ± 6.77	-0.867	30.57* ±0.24	11.37	56.74* ±1.54	300.99	5.19 ns ±0.08	-10.98

Results were expressed as mean **±SE.** *significance difference versus control at P<0.05; nsnonsignificance difference versus control at P<0.05.

$$\% \, Change = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$$

The highest inducing effect was exhibited in the activity of GOT enzyme after treatment of larvae with *E. camaldulensis* oil (Change %:55.23) in comparison with control. On the other hand, the least inducing effect was detected in homogenate of larvae treated with *T. foenum graecum* (Change %:6.85) as compared to control.

Significant elevation in GPT activity was recorded in homogenate of larvae treated with LC_{50} of all tested plant essential oils as shown in Table (2). The strongest inducing activity was detected in homogenate of larvae treated with *S. indica* (Change %:300.99). In respect to the other three plant essential oils, treatment with *E. camaldulensis* significantly increased GPT activity to193.43% followed by *T. foenum graecum* (Change %:81.06) and finally *N. sativa* (Change %:56.67) as compared to control.

No significant changes in the total protein content in larvae homogenate after treatment with *E. camaldulensis* and *S. indica* (Change %:-7.55 and -10.98, respectively). However treatment with *N. sativa* induced significant decrease in total protein content with Change % -21.61 as compared to control. On the other hand the protein content was increased significantly (Change %:17.84) from control when larvae treated with *T. foenum graecum* oil.

DISCUSSION

Many researchers have reported on the effectiveness of plant essential oils against insects, especially stored-products insects. In our study, we are concentrated on *S. littoralis*.

It is clear from the obtained results that, *T. foenum graecu* was the most toxic compound and has low LC_{50} value followed by *S. indica*, *E. camaldulensis* and finally *N.sativa* which has high value of LC_{50}. These results are in agreement with the findings of Pavela (2004) and Krishnappa *et al.* (2010) who reported that some medicinal plants essential oils are larvicidal to the larval instar of *S. littoralis*. Hazrat and Soaib (2012) showed that those essential oils from *C. sativum* are effective against Mosquito larvae. Rana *and Rana* (2012) found that EO from *F. vulgare* kills 100% of *Culex quinquefasciatus* (Linnaeus) larvae at 250 ppm after 40 min. Similar toxic effects also were observed for essential and edible oils including sesame oil in the control of the pulse beetle *C. chinensis* (Ali *et al.*, 1983, Khalequzzaman *et al*., 2007and Kumar *et al.*, 2008), larvae of *S. littoralis* (Mesbah *et al*., 2006) and *C. maculatus* (Abd El-Razik and Zayed, 2014). Likewise Kanat and Alma (2003) and Sampson *et al*., (2005) found insecticidal effects of essential oils from *Eucalyptus camaldulensis* against the larvae of pine processionary moth, *T. pityocampa* and adult turnip aphids, *Lipaphis pseudobrassicae*. Also Chaubey (2008) evaluated the toxic effects of seven different essential oils against *Callosobruchus chinensis* and found that *Nigella sativa* was the most effective at all stages.

On the other hand Omara *et al*. (2014) reported that clove and sesame oils can be used as repellent botanical insecticides, against *P. Americana*. Ebadollahi (2011) studied the antifeedant activity of essential oils from *Eucalyptus globulus* Labill and *Lavandula stoechas* L. on *Tribolium castaneum* Herbst

Marei *et al.* (2009) showed that sesame oil has a latent effect on larvae up to certain limit while pupal mortality was affected with jojoba and sesame oil extracts at 3% concentration, being 50% and 80% respectively.

Essential oils or some of their substances, respectively, may exhibit mutual synergistic effects. For example the susceptibility of *S. littoralis* larvae to cyhalothrin increased when treated after treatment with LC_{50} of essential oils, (Ismail and Shaker 2014).Visetson *et al*. (2003) found that sesame oil showed good synergism with cypermethrin.

Also the obtained results agree with Souguir *et al.* (2013) who recorded that the essential oils of *S. officinalis* leaves, *C. sativum* seeds, *D. carota* flowers, and *F. vulgare* seeds, may be serving as a lepidopteran agricultural pest control of *S. littoralis*.

In the present study change in the level of some biochemical paramatersin larvae homogenate of *S. littoralis* may be due to physiological alterations which are induced by compounds found in tested plant essential oils.

Regarding the mode of action of essential oils against insect pests, little information is available but treatment with various essential oils or their constituents cause symptoms that suggest a neurotoxic mode of action (Kostyukovsky *et al*., 2002; Priestley *et al*., 2003; Lu and Wu, 2010). In the present study, activity of AChE decreased in all treatments in comparison to control. These findings are coincide with that reported by Chaubey (2011) who found that, fumigation of *S. oryzae* adults with sublethal concentrations of *C. cyminum* and *P. nigrum* essential oils inhibited AChE activity. Previous researchers have reported the competitive inhibition of AChE activity by monoterpenes and monoterpenoids. Most of the essential oil components like cuminaldehyde, limonene, α – pinene and β – phellendrene inhibiting AChE activity (Lee *et al*., 2001, Abdelgaleil *et al.*, 2009 and Zapata & Snagghe, 2010). Several essential oils from aromatic plants, monoterpenes, and natural products act as AChE inhibitors (Shaaya and Rafaeli 2007; Ló opez *et al.*, 2010).

On the other hand the obtained results indicated that, treatment with all plant essential oils induced an elevation of GOT and GPT activities except *N. sativa* induced a reduction in GOT activity.

Early studies were revealed that, the GPT activity was disrupted in *S. gregaria* by Neemazal (a neem preparation) and *N. sativa* extracts (Hamadah, 2009) as well by *F. bruguieri* extracts (Tanani *et al.,* 2009). A considerable inducing effect on GOT and GPT activities in haemolymph of nymphs and adults of *S. gregaria* after treatment with *P. granatum* peel extracts, was recorded by Ghoneim *et al*. (2014) .

It is of interest to mention that GOT and GPT serve as a strategic link between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari *et al*., 2005). Accordingly, the disturbance in GOT and GPT levels will be closely related to metabolism of proteins and amino acids. Thus it will disrupt many physiological functions and ultimately lead to death, in other way control the pest (Ezz and Fahmy, 2009).

Khatter and Abuldahb (2010) revealed that, the botanicals and mineral oil inhibited the anabolism of the treated insects. The metabolic activity is mostly of catabolic pattern.

Also total protein content decreased after treatment with tested plant essential oils except *T. foenum graecu* increased the level of protein content.

Medhini *et al*. (2012) studied that the highest reduction in protein content of the larvae of *Spodoptera litura* when treated with *Calendula olficinalis* extracts. The level of protein content in the body of larva is dependent upon the rate of synthesis, the breakdown of proteins and even water movement between tissues. Moreover, Krishnaveni *et al.* (2013) revealed that, treatment of the larvae of *Spodoptera litura* with Pongam oil and neem oil decreased the total protein content and this reduction may be due to increased breakdown of proteins to detoxify the active principles present in the pongam oil.

CONCLUSION

The results indicated that tested plant essential oils (*T. foenum graecu, S. indica, E. camaldulensis* and *N. sativa*) gave toxicity and biochemical effects against 4th larval instar of *S. littoralis*. Based on these results we recommended the use of plant essential oils in control of *S. littoralis* as alternative to chemicals insecticides. Because of these oils come from natural resources, safe, cheap and efficient and they will help to decrease the negative effects of synthetic chemicals such as residues in products, insect resistance and environmental pollution.

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ARABIC SUMMERY

التأثيرات السامة والبيوكيميائية لأربعة من الزيوت النباتية ضد دودة ورق القطن

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أجريت هذه الدراسة لتقييم التأثير السام لأربعة من الزيوت النباتية (الحلبة ، السمسم ، الكافور، حبة البركة) على يرقات العمر الرابع لدودة ورق القطن. وكذلك تمت دراسة التغيرات البيوكيميائية في اليرقات الناتجة عن المعاملة بالتركيز النصف مميت للزيوت المختبرة.

وتم عمل الاختبار الحيوي عن طريق الطبقة الرقيقة لحساب قيم LC_{90}, LC_{50}, LC_{25}. وقد أظهرت النتائج أن زيت الحلبة كان أكثر الزيوت تأثيرا يليه زيت السمسم ثم زيت الكافور وأخيرا زيت حبة البركة. وفيما يتعلق بالتغيرات البيوكيميائية الناتجة عن المعاملة بالتركيز النصف مميت لكل زيت. فقد سجلت النتائج إنخفاضا ملحوظا في نشاط أنزيم (AChE) نتيجة المعاملة بزيت الحلبة وزيت حبة البركة. ولكن أدت المعاملة بزيت الكافور وزيت السمسم إلى زيادة معنوية في نشاط أنزيم **(GOT)**، بينما سجلت النتائج زيادة معنوية في نشاط انزيم (GPT) في كل المعاملات. وقد لوحظ انخفاضا ملحوظا في محتوي البروتين الكلي نتيجة المعاملة بزيت حبة البركة ،في حين أدت المعاملة بزيت الحلبة إلى زيادة معنوية في محتوى البروتين الكلي.

وأخيرا تشير النتائج إلى أن الزيوت النباتية المختبرة لها تأثيرات سامة وأيضا أظهرت تأثير سلبي على القياسات البيوكيميائية المختبرة ، مما يوحي إلى إمكانية استخدامها كبديل للمبيدات الحشرية التقليدية لمكافحة دودة ورق القطن.