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## Ovicidal, Larvicidal and Biochemical Effects of Thyme, *Thymus vulgaris*, ON the Cotton Leafworm, *Spodoptera littoralis* (Boisd.)



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#### ABSTRACT



Ovicidal, larvicidal and biochemical effects of Thyme oil, Thymus vulgaris, and the carbamate insecticide, Methomyl, on the cotton leafworm, Spodoptera littoralis (boisd.) (Lepidoptera: Noctuidae) were studied. The calculated percentages of reduction in egg hatching were 32.5, and 57.1% for thyme oil tested at concentrations of 2 and 4% and 21.3, and 28.2% for Methomyl tested at concentrations of 2 and 4ppm, respectively. The calculated percentages of reduction were 100% for thyme oil and Methomyl tested at concentrations of 12% and 20ppm, respectively.Larval feeding assay showed that the calculated LC50 values for thyme oil were 0.701, 0.324 and 0.108% after 24, 48 and 72hrs, respectively. The calculated LC50 values for methomyl were 1.60, 0.918 and 0.51ppm after 24, 48 and 72hrs, respectively. Topical assay showed that the calculated LC50 values were 3.601, 3.601, and 2.089% after 24, 48, and 72hrs, respectively. Among methomyl, the calculated LC50 values were 2.12, 1.28 and 0.75ppm after 24, 48 and 72hrs, respectively. The in vitro inhibitory activity of Thyme oil and Methomyl on acetylcholinesterase (AChE) were also tested in larval head and midgut of S. littoralis in this study.Concentration of 6% of Thyme oil caused 68.6 and 68.7% inhibition of AChE in heads and midgut of the 4<sup>th</sup> larval instar of S. littoralis, respectively. While concentration of 20 ppm of methomyl caused 81.8 and 80.4% inhibition of AChE in heads and midgut of the 4<sup>th</sup> larval instar of S. littoralis, respectively. I<sub>50</sub> values for larval midgut AChE was about 1.3 and 1.52 folds more resistant to inhibition by thyme oil and methomyl, respectively, than those of larval head AChE.

#### Keywords: Essential oils, Thyme, acetylcholinesterase, Spodoptera littoralis.

#### INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is most important polyphagous pest, widely distributed all over the world. Larvae of this pest can feed on 90 economically important plant species belonging to 40 families and the rate of development has a strong nutritional component (Azab et al., 2001. Such pest is partly controlled by chemical insecticides but such insecticides now and since several years have come under increasing attack due to their hazards. There is therefore an urgent need to replace pesticides with alternative means of control that are less toxic and more environmentally friendly (Brown and Morra, 1997).

Essential oils (EOs) and their major components, mainly the monoterpenoids are potential source of ecologically safe botanical insecticides (Kalemba and Kunicka, 2003: Reichling *et al.*, 2009). The aromatic characteristics of essential oils provide various functions for the plants including (i) attracting or repelling insects, (ii) protecting themselves from heat or cold; and (iii) utilizing chemical constituents in the oil as defence materials (Koul et al., 2008). In nature, essential oils play major role in protection of plants as antibacterials, antivirals, antifungals (Kalemba and Kunicka, 2003: Reichling *et al.*, 2009). The insecticidal activity of essential

\* Corresponding author. E-mail address: drmelaw2000@yahoo.com DOI: 10.21608/jppp.2021.179474 oils has been reviewed (Regnault-Roger, 1997). Thyme volatile oil (*Thymus vulgaris* L., Lamiaceae) has an insecticidal action against several insects and mites of agricultural importance (Isman 2000; Kanat and Alma, 2004) and has many biological properties naturally (Matheen et al., 2021).

One of the most substantial issues in agriculture is the maxi-mization of the quality of agricultural products through the minimization of insect damage (Tharamak et al., 2020). The aim of this study was to evaluate the insecticidal activity of Thyme oil, *Thymus vulgaris*, and Methomyl on eggs and larvae of the cotton leafworm, *S. littoralis*. The study was conducted also to evaluate the biochemical effect of thyme oil on AChE in the head and midgut of the cotton leafworm, *S. littoralis*.

#### **MATERIALS AND METHODS**

#### Preparation of plant oil extract:

Fresh aerial parts (the top 25 cm) of *Thymus vulgaris* were collected from Borg El Arab, Alexandria-Egypt. Each sample (50 gm.) of *T. vulgaris* was subjected to hydro distillation separately for 3 hours, using a Clevenger-type apparatus, according to the European Pharmacopoeia (Council of Europe, 2007). The oil yield was estimated as a relative percentage to the weight (v/w)

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of plant materials. The collected oils were used in the present experiments.

#### Insects:

The larvae of *S. littoralis* were reared for several years under the laboratory of  $25C^{\circ}\pm 2.0$  and  $65\%\pm 5.0$  RH as susceptible strain. The larvae were fed on castor bean leaves, *Ricinus communis* L. (Fam. Euphorbiaceae). The resulted pupae were collected and sexed.

#### Egg Treatments:

Newly laid egg batches were dipped for 30 seconds in each concentration of 2, 4, 6, 8, 10, 12, and 14% from the crude extract of thyme oil, *Thymus vulgaris* in Aceton. Among Methomyl, concentrations of 2, 4, 8, 12, 16, and 20ppm were used. Ten batches were used for each concentration as well as for control (Aceton only). Treated egg batches were dried in air, placed individually in plastic cups and the cup were covered with tissue paper. The percentages of egg mortalities were calculated and corrected according to Abbott (1925). Obtained data were computerized according to Finney (1971) to calculate the LC<sub>50</sub> (the leathal concentration which cause 50% egg mortality).

#### The tasted compounds:

#### 1. Thyme oil, *Thymus vulgaris* (100%)

Chemical name: 1,3,3-Trimethyl-2-oxabicyclo{2.2.2}octane 2. Methomyl (90%)

**Chemical name:** (*E*,*Z*)-methyl *N*-{[(methylamino) carbonyl]oxy} ethanimidothioate

#### Larval feeding and treatment:

The 4th larval instar of S. littoralis was used in all larval treatments. Castor leaves were treated by dipping for 30 seconds in a series concentrations of each compound in Aceton (99%) (w/v) purchased from El Nasr Pharmaceuical Chemicals Co., and those used for control were dipped in Aceton (99%) only, and then dried on air. Experiments were carried out using 4 replicates for each concentration, each of which contained approximately 25 larvae fed on treated castor leaves in plastic plates (18cm\*18cm) and 12cm hight coverd with wight shefone and kept under the same laboratory rearing conditions of 25C°±2.0 and 65%±5.0 RH, for 24hr. Thereafter, treated larvae were fed on untreated castor leaves till the pupation. Larval mortality was recorded 24,48, and 72hrs after the beginning of the experiments according to each tasted compound, and till the pupation.

#### Larval feeding and treatment

The 4<sup>th</sup> instar larvae of *S. littoralis* were used in all larval treatments. Castor leaves were treated by dipping for 30 seconds in each concentration of of 2, 4, 6, 8, 10, 12, and 14% from the crude extract of thyme oil in Aceton (99%) (w/v), and those used for control were dipped in Aceton (99%) only, and then dried on air. Experiments were carried out using 4 replicates for each concentration, each of which contained approximately 25 larvae fed on treated castor leaves in plastic plates (18cm\*18cm) and 12cm hight coverd with white shefone and kept under the same laboratory rearing conditions of  $27\pm2.0$  °C and 65.0.  $\pm5.0\%$  R.H. for 24hr. Larval mortality percentages were recorded after 24, 48, and 72hrs. LC50 values were calculated according to Finney (1971).

#### Topical toxicity assay.

The insecticidal activity of monoterpenes against the 4<sup>th</sup> larval instar of *S. littoralis* was evaluated by direct topical application assay (Qi and Burkholder 1981; Broussalis et al. (1999). A series of concentrations of 2, 4, 6, 8, 10, 12, and 14% from the crude extract of thyme oil in Acetone (99%) (w/v) and one milliliter of each concentration was applied on the dorsal side of the thorax. For control the crude extract of thyme oil were dissolved in Aceton (99%) only. Three replicates ten larvae each were used for each concentration. Mortality percentages were recorded 24hr. post-treatment, and LC<sub>50</sub> values were obtained using the method of Finney (1971).

#### AChE Activity Assay:

*S. littoralis* larval heads (0.5 g) were separately homogenized in 5 ml of 0.1 M ice-cold phosphate buffer (pH 7.0) using a Teflon glass tissue homogenizer. Homogenates were centrifuged (5,000 rpm for 20 min at 0°C), and supernatants were used as the enzyme source for determination of AChE activity. Inhibition of AChE by Thyme oil and/or Methomyl test solutions was determined by the colorimetric method of Ellman *et al.* (1961) using ATChI as substrate. The two tested compounds were examined at six concentrations. Each test and control was corrected by blanks for nonenzymic hydrolysis. All the experiments were done in triplicate. *In vitro* inhibition percentages of AChE activity was calculated as follows:

AChE inhibition  $\% = (ODB - ODT)/ODB \times 100$ .

#### Where

## ODB is the optical density of blank enzyme and ODT is the optical density of treatment.

#### Statistical Analysis of Data:

Data of the different experiments were statistically analyzed as Completely Randomized Design (CRD) and comparison between means of treatments using "t" tast in groups as appropriate for each experiment, using one-way ANOVA followed by Tukey test for post hoc comparisons. A Student's t-test was used to compare each DC value with the respective control group.  $I_{50}$  values were calculated with their confidence intervals using the statistical software for correlated data developed by Throne *et al.* (1995).

#### **RESULTS AND DISCUSSION**

The current study was designed to compare the insecticidal activity of pure thyme oil at concentrations ranging from 2 to 14% as well as Methomyl at concentrations ranging from 2 to 20ppm against eggs and larvae of *S. littoralis.* The ovicidal and larvicidal activities of the two tested compounds and their possible mechanism of action were studied.

#### 1- Ovicidal activity of the tested compounds against Spodoptera littoralis eggs:

The ovicidal effect of tow compounds, Thyme oil and Methomyl against *S. littoralis* eggs was carried out in the present study.

#### (a) Effect of Thyme oil on egg hatching (%).

Table (1) showed the ovicidal effect of Thyme oil against eggs of *S. littoralis*. Data showed that there was a strong effect of thyme oil expressed as percentages of reduction in egg hatching. The calculated percentages of reduction were 32.5, and 57.1 for thyme oil tested at a concentration of 2 and 4%, respectively. While the

concentrations of 6, 8, and 10% caused percentages of reduction of 78.8, 79.7, and 84.8%, respectively. The calculated percentages of reduction were 100% for thyme oil tested at a concentration of 12 and 14%, respectively.

Thyme oil showed ovicidal activity more than 50% reduction in egg hatching at 4% concentration, whereas at 12% it achieved 100% reduction in egg hatching. However, the higher the concentration of thyme oil the higher the percentages of reduction in the egg hatching.

Table 1.Ovicidal effect of thyme oil, *T. vulgaris* against the eggs of *S. littoralis* 

Conc.	Egg hatching (%)	% of
(%)	Mean±SE*	R
2	52.00 <sup>b</sup> ±3.21	32.47
4	33.00 <sup>c</sup> ±4.51	57.14
6	16.33 <sup>d</sup> ±2.03	78.79
8	15.67 <sup>d</sup> ±1.45	79.65
10	11.67 <sup>d</sup> ±1.76	84.84
12	$0.00^{e}\pm0.00$	100
14	$0.00^{e}\pm0.00$	100
Control	77.00 <sup>a</sup> ±3.21	
LSD0.05	7.52	

\* No significant differences obtained for the same letters at 0.05 levels.

#### (b) Effect of Methomyl on egg hatching (%).

Table (2) showed the ovicidal effect of Methomyl against eggs of *S. littoralis*. Data showed that there was strong effect of Methomyl expressed as percentages of reduction in egg hatching. The calculated percentages of reduction were 21.3, and 28.2 for Methomyl tested at concentrations of 2 and 4%, respectively. While the concentrations of 8, 12, and 16ppm caused percentages of reduction of 40.2, 53.6, and 68.5%, respectively. The calculated of reduction was 100% for Methomyl tested at a concentration of 20ppm.

 Table 2. Ovicidal effect of Methomyl against the 4<sup>th</sup> larval instar of S. littoralis

Conc.	Egg hatching (%)	% of
(ppm)	Mean±SE	<b>R</b> *
2.0	63.20 <sup>b</sup> ±1.97	21.32
4.0	57.67°±1.20	28.21
8.0	48.07 <sup>d</sup> ±2.37	40.16
12.0	37.30 <sup>e</sup> ±1.23	53.57
16.0	25.27 <sup>f</sup> ±2.20	68.54
20.0	$0.00^{g}\pm 0.00$	100
Control	80.33 <sup>a</sup> ±0.94	
LSD0.05	4.89	

\*% of R: Reduction percentage = (Control-Treatment)/Control × 100.

## 2- Larvicidal activity of the tested compounds against the 4<sup>th</sup> larval instar of *Spodoptera littoralis*:

The larvicidal activity effect of the tow compounds, Thyme oil and Methomyl against the  $4^{th}$  larval instar of *S*. *littoralis* was carried out in the present study using feeding and contact assay (Tables 3 and 4).

#### a) Feeding toxicity

Table (3) showed the feeding toxicity of thyme oil against the  $4^{th}$  larval instar of *S. littoralis*, expressed as the LC50 values (the lethal concentration causing 50% mortality after 24 h).

Thyme oil was tested at concentrations of 2, 4, 8, 12, and 14% of the extract. The larval mortality was observed after 24 h of exposure. The calculated LC50 values were 0.701, 0.324, and 0.108 % after 24, 48, and

72hrs, respectively. The result revealed that Thyme oil promising as larvicidal activity against *S. littoralis*.

 Table 3. LC50 values of thyme oil against the 4<sup>th</sup> larval instar of S. littoralis.

Treatment	Harm	LC50*	Confidence	e Limits	Slope   SE	X <sup>2</sup>			
Treatment	nour	(%)	Lower	Upper	Slope±SE	Λ-			
	24	0.701	0.366	1.121	0.911±0.135	5.544			
Feeding	48	0.324	0.144	0.551	0.972±0.157	2.064			
-	72	0.108	0.017	0.258	0.737±0.159	4.840			
*The lethal o	*The lethal concentration causing 50% mortality								

Table (4) showed the feeding toxicity of Methomyl against the  $4^{th}$  larval instar of *S. littoralis*, expressed as the LC50 values.

Methomyl was tested at concentrations of 2, 4, 8, 12, 16 and 20% of the extract. The larval mortality was observed after 24 h of exposure. The calculated LC50 values were 1.6, 0.918 and 0.51 ppm after 24, 48 and 72hrs, respectively. The result revealed that the Methomyl promising as larvicidal activity against *S. littoralis*.

 Table 4. LC<sub>50</sub> values of Methomyl against the 4<sup>th</sup> larval instar of S. littoralis.

Treatment	Uoun	ur <u>LC50 Confidence Limit</u> (ppm) Lower Uppe		e Limits	Slope±SE*	<b>X</b> <sup>2</sup>
meannent	nour	(ppm)	Lower	Upper	Slope±SE*	Λ
	24	1.600	1.28	1.970	1.308±0.134	6.980
Feeding	48	0.918	0.551	1.144	$1.375\pm0.140$	4.240
Ū.	72	0.510	0.329	0.692	1.207±0.154	5.676
*Slop of concentration-mortality regression line±standard error.						

#### b) Topical toxicity

Table (5) showed the topical toxicity of thyme oil against the  $4^{th}$  larval instar of *S. littoralis*, expressed as the LC50 values. The calculated LC50 values were 3.6, 3.6, and 2.1% after 24, 48, and 72, respectively.

 Table 5. Acute toxicity of the Thyme oil to the 4<sup>th</sup> larval instar S. littoralis by topical application.

Treatment	House	LC50	Confidenc	e Limits	Slope±SE	<b>v</b> 2*			
Treatment	Hour	(%)	Confidenc Lower	Upper	Slope=SL	$\Lambda^{-}$			
	24	3.601	1.305	4.907	2.24±0.675	1.282			
Topical	48	3.601	1.305	4.907	2.24±0.675	1.282			
-	72	2.089	0.534	4.175	1.574±0.694	1.181			
*Chi cauaro v	Chi sayara valua								

\*Chi square value.

Table (6) showed the topical toxicity of Methomyl against the  $4^{th}$  larval instar of *S. littoralis*, expressed as the LC50 values. The calculated LC50 values were 2.12, 1.28 and 0.75ppm after 24, 48 and 72hrs, respectively.

 Table 6. Acute toxicity of Methomyl to the 4<sup>th</sup> larval instar S. littoralis by topical application.

Treatment	Hour	LC50	Confidence	e Limits	Slope±SE	<b>X</b> <sup>2</sup>
Heatment	nour	(ppm)	Lower	Upper	Slopeist	
	24	2.120	1.35	2.590	1.370±0.675	5.049
Topical	48	1.280	0.550	2.190	$1.416\pm0.675$	9.392
-	72	0.750	0.290	1.130	$1.575\pm0.694$	9.693

3- Effects of Thyme Oil on head and midgut acetylcholinesterase in the 4<sup>th</sup> larval instar of *S. littoralis*.

The effect of Thyme oil on head and midgut acetylcholinesterase in the  $4^{th}$  larval instar of *S. littoralis* was also studied in the present work. Table (7) showed the concentration-response plots for AChE inhibition in heads of the  $4^{th}$  larval instar of *S. littoralis* with Thyme oil. The percentage of inhibition of AChE activity in head

homogenates of the  $4^{th}$  larval instar of *S. littoralis* using different concentrations of the Thyme oil was increased with increasing the concentration. Their inhibitory abilities have been compared with methomyl, a prototypical naturally occurring AChE inhibitor. Concentration of 6ppm of Thyme oil caused 68.6 and 68.7% inhibition of AChE in heads and midgut of the  $4^{th}$  larval instar of *S. littoralis*, respectively.

 Table 7. Larval head and midgut acetylcholinesterase activity and inhibition in the 4<sup>th</sup> larval instar of S. littoralis using defferent concentrations of Thyme oil

111	yme on.					
Conc.	Acetylcholinesterase Inhibition, %					
(%)	Head	Midgut				
0.0	0.00 <sup>e</sup> ±0.00	$0.00^{g}\pm0.00$				
0.5	11.93 <sup>d</sup> ±2.52	16.53 <sup>f</sup> ±2.83				
1.5	39.53°±1.93	20.83°±0.96				
2.5	41.83°±1.94	37.67 <sup>d</sup> ±0.92				
4.0	52.70 <sup>b</sup> ±2.93	44.60°±0.10				
5.0	57.47 <sup>b</sup> ±0.71	54.50 <sup>b</sup> ±1.06				
6.0	68.60 <sup>a</sup> ±0.79	68.70 <sup>a</sup> ±0.66				
LSD0.05	5.56	3.87				

The activity of each sample was expressed in terms of  $I_{50}$  (the concentration required to inhibit the acetylcholinesterase level by 50%), which was calculated from log dose curves (Table 8).  $I_{50}$  values were 3.17 and 4.01% for larval head and midgut AChE.  $I_{50}$  values for larval midgut AChE was about 1.3 fold more resistant to inhibition by Thyme oil than those of larval head AChE.

Table 8. I<sub>50</sub> values for larval head and midgut AChE after treatment with deferent concentrations of thyme oil.

No.	Treatment	т	Confider	inder	Folda	Slone	
190.	Treatment	150	Confider Lower	Upper	maex	roius	Slope
1	Head	3.17	2.65	3.85	100	1	1.36
2	Midgut	4.01	2.76	7.98	79.00	1.27	1.33

4- Effects of Methomyl on acetylcholinesterase in the 4<sup>th</sup> larval instar of Spodoptera littoralis.

The effect of methomyl on head and midgut acetylcholinesterase in the  $4^{th}$  larval instar of *S. littoralis* was also studied in the present work. Table (9) showed the concentration-response plots for AChE inhibition in heads of the  $4^{th}$  larval instar of *S. littoralis* with Methomyl, the prototypical naturally occurring AChE inhibitor.

# Table 9. Larval head and midgut acetylcholinesterase activity and inhibition in the 4<sup>th</sup> larval instar of S. littoralis using different concentrations of Methomyl.

Conc.	Acetylcholinesterase Inhibition, %				
(ppm)	Head	Midgut			
0.0	$00.00^{f} \pm 0.00$	$00.00^{g}\pm0.00$			
0.5	38.07 <sup>e</sup> ±1.88	26.30 <sup>f</sup> ±1.59			
1.5	46.17 <sup>d</sup> ±0.58	34.90 <sup>e</sup> ±2.70			
2.5	49.67 <sup>d</sup> ±0.88	$46.17^{d} \pm 1.75$			
4.0	58.33°±1.23	56.97°±0.39			
5.0	71.47 <sup>b</sup> ±1.36	66.07 <sup>b</sup> ±2.02			
6.0	81.77 <sup>a</sup> ±2.92	80.37 <sup>a</sup> ±0.27			
LSD0.05	4.67	4.75			

The percentage of inhibition of AChE activity in head homogenates of the  $4^{th}$  larval instar of *S. littoralis* 

using different concentrations of the methomyl was increased with increasing the concentration of methomyl. The plots indicate that methomyl exhibited the strongest AChE inhibitory effect over the range of concentrations. Concentration of 20ppm of methomyl caused 81.8 and 80.4% inhibition of AChE in heads and midgut of the  $4^{th}$  larval instar of S. littoralis, respectively.

 $I_{50}$  values were 0.27 and 0.41 ppm for larval head and midgut AChE.  $I_{50}$  values for larval midgut AChE was about 1.52 fold more resistant to inhibition by thyme oil than those of larval head AChE (Table 10). However, the AChE inhibitory potential decreased in the following order: methomyl > thyme oil.

 Table 10. I<sub>50</sub> values for larval head and midgut AChE after treatment with deferent concentrations of Methomyl.

Na	Transformert	т (	Confiden	ce limits		Folds Slope	
No.	Ireatment	150	Lower	Upper	index		
1	Head	0.27	0.19	0.36	100.00	1.00 0.93	
2	Midgut	0.41	0.33	0.51	66.26	1.51 1.19	

#### Discussion

The aim of this study was to evaluate the insecticidal activity of Thyme oil, *Thymus* vulgaris, and Methomyl on eggs and larvae of the cotton leafworm, S. littoralis. According to Regnault-Roger et al. (2012) the major plant families from which EOs are extracted include Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae. However, Thyme, *T. vulgaris*, used in the present work belonging to the Lamiaceae family, is a well-known spice plant possessing excellent medicinal properties (Mandal and DebMandal, 2016). have repellent, insecticidal, and growth-reducing effects on a variety of insects. They have been used effectively to control preharvest and postharvest phytophagous insects and as insect repellents for biting flies and for home and garden insects.

The effect of sublethal doses of EOs from 13 aromatic plant species including *T. vulgaris* on the development and fertility of *S. littoralis* was studied by Pavela (2013). Thyme oil and Methomyl showed ovicidal and larvicidal activity against *S. littoralis* However, based on the results of Pavela (2013) and our results, *T. vulgaris* can be recommended for further development of botanical insecticides designed to protect plants against the important polyphagous pest *S. littoralis*. Pavela (2010) found acute toxicity on *S. littoralis* larvae for 6 monoterpenes which often represent the majority components of many essential oils obtained from aromatic plants of the Lamiaceae family. Their mutual synergistic relationship was found for fumigant as well as topical application.

The *in vitro* inhibitory activity exerted by the main constituents of essential oil obtained from the aromatic plant thyme, *T. vulgaris*, and Methomyl on AChE was also studied in the present work. The total essential oil was tested for AChE inhibition. Thyme oil exhibited the strongest AChE inhibitory effect over the range of concentrations. The AChE inhibitory potential decreased in the following order: methomyl > thyme oil. It is interesting that the head and midgut AChE inhibitory effect exerted by methomyl was 12 and 10 times stronger than that exerted by Thyme oil, respectively, although the inhibitory abilities of methomyl, a prototypical naturally

occurring AChE inhibitor, is recommended. Dewer et al. (2016) highlighted that sublethal doses of both pesticides did not induce a change in acetylcholinesterase activity in head of exposed larvae of *S. littoralis*.

Jukic et al. (2007) examine *in vitro* the inhibitory activity exerted by the main constituents of essential oil obtained from the aromatic plant *T. vulgaris* on acetylcholinesterase (AChE). The total essential oil and selected compounds, specifically linalool and thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone, were tested for AChE inhibition. Thymohydroquinone exhibited the strongest AChE inhibitory effect over the range of concentrations. The AChE inhibitory potential decreased in the following order: thymohydroquinone > carvacrol > thymoquinone > essential oil > thymol > linalool. The AChE inhibitory effect exerted by carvacrol was 10 times stronger than that exerted by its isomer thymol, although thymol and carvacrol have a very similar structure.

Compounds of the EOs exert their activities on insects through neurotoxic effects involving several mechanisms, notably through GABA, octopamine synapses, and the inhibition of acetylcholinesterase. (Regnault-Roger et al.,2012).

The biochemical effects of thyme oil on insect enzyme observed in the present study can be compared to effects observed in other work of Abdel-Aziz *et al.* (2013) who indicated that highly significant stimulation in chitinase and  $\alpha$ - &  $\beta$  -esterases activity was recorded with all treatments and the most effective one caused by thyme oil followed by bitter. Highly significant inhibition in protease activity and AChE was attained by (bitter & thyme) and (neem & thyme) oils, respectively. While high significant stimulation was recorded in protease and nonsignificant stimulation of AChE was noticed by neem and bitter, respectively (Abdel-Aziz *et al.*, 2013).

However, treatment with S. litura larvae did not produce any inhibitory effects of AChE, which may have been because AChE is not the main site of the action of thymyl cinnamate and thus is not necessarily related to insect mortality levels. A similar result was obtained wherein AChE was not affected by the treatment of terpinen-4-ol and 1,8-cineole against stored-product pests (Greenberg-Levy et al., 1993). Furthermore, fumigant toxicity tests with monoterpenes against Sitophilus oryzae adults did not produce a direct correlation between the toxicity of menthone from Mentha arvensis and AChE inhibition (Lee et al., 2001). It could be concluded that thyme oil may be used as an effective agent against the agricultural pest Spodoptera littoralis and also alternative for synthetic chemicals for sustainability of environment. Further suggest the value of exploring other Egyptian plants in search for new, environmentally acceptable pest control agents for S. littoralis.

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التأثيرات الإبادية لكل من زيت نبات الزعتر والميثوميل على البيض واليرقات وعلى بعض النظم البيوكيميائية لحشرة دودة القطن الكبرى (Lepidoptera: Noctuidae) (Spodoptera littoralis (Boisd.) محمد عبد القادر منصور العو<sup>1</sup>، خليل عبد الله أحمد دراز<sup>1</sup>، المعتز بالله على النجار<sup>2</sup> وفارس حسين عبد السلام<sup>2</sup> <sup>1</sup> قسم وقاية النبات - كلية الزراعة - جامعة دمنهور <sup>2</sup> قسم العقاقير الطبية - كلية الصيدلة - جامعة دمنهور

فى الدراسة الحالية تم دراسة التأثيرات الإبادية لكل من زيت نبات الزعتر ومبيد ميثوميل على البيض واليرقات وعلى بعض النظم البيوكيميائية الحيوية لحشرة دودة القطن الكبرى (Lepidoptera: Noctuidae) (.Spodoptera littoralis (Boisd). أظهرت النتائج وجود تأثير قوي لمستخلص الزعتر و 2.82 و 2.82% والميثوميل معبراً عنه كنسب مئوية من النقص في فقس البيض. كانت النسب المئوية المحسوبة للإختزال 3.25 و 7.71% لمستخلص الزعتر و 2.15 و 2.82% الميثوميل معبراً عنه كنسب مئوية من النقص في فقس البيض. كانت النسب المئوية المحسوبة للإختزال 3.25 و 7.71% لمستخلص الزعتر و 2.15 و 2.82% الميثوميل المختبرين بتركيزات 2% و 4 جزء في المليون، على الترتيب. كانت النسب المئوية المحسوبة للإختزال 3.25 و 7.71% لمستخلص الزعتر وتم إختبار مبيد ميثوميل المختبرين بتركيزات 21% و 4 جزء في المليون، على الترتيب. أظهر اختبار التغذية على ورق معامل بزيت الزعتر أن قيم التركيز المميت لنصف عدد اليرقات المعاملة بمستخلص الزعتر (100% لمستخلص الزعتر وتم و 2.15% لمستخلص الزعتر وتم و 2.15% لميثوميل المعنون على المريزين تركيزات 21% و 4.50 و 7.01% بعد 24 و 48 و 72 ساعة، على ورق معامل بزيت الزعتر أن قيم التركيز المميت لنصف عدد اليرقات المعاملة بمستخلص الزعتر (100% بعد 24 و 48 و 72 ساعة على التوالي. أظهرت المعاملة السطحية الليرقات بالمركين أن قيم 2.50 و 7.01% بعد 24 و 48 و 72 ساعة، على ورق معامل بزيت الزعتر والمركيز أن قيم 1.50% و 2.50% و 7.50% و 2.50% و 7.50% بعد 24 و 48 و 72 ساعة، على الترتيب. وفي حالة مبيد ميثوميل كانت قيم الركيز أن قيم 2.50% و 7.50% و 2.50% و 7.50% و 2.50% و 7.50% بعد 24 و 48 و 72 ساعة، على الترتيب. وفي حالة مبيد ميثوميل كانت قيم الدوجي المركيز أن قيم 2.50% ور 2.50% من زيت الزميز والمركيز أن قيم 2.50% من زيت الزميز والميوميل كانت قيم الميثون بين ي و 2.5% من الإلى واليريزين الم واليرقات المنوب من الميثوميل معن الميوميل مي والميون من الميون من الإلى والمعي المترزين المحسوبة 2.50% من زيت الزميز ولاح 2.50% من الإلى والمعي الوليزيم أستنب لل ورق ورق ورق و 2.50% ورو و 75% معام المرون الم والم من زيت الزميز ولي تركيز مي 2.50% من زيت الزميز ولوي وي 2.50% من الإلى والميون من الميثون من الميون من الميون من الميثوميل في تنبيل ورام والمعي المون من الميون من المنوم مان وليني كلائ