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INFLUENCE OF SOME PLANT OILS ON SOME BIOLOGICAL ASPECTS OF THE COTTON LEAF WORM, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The effectiveness of some essential oils -menthol, lemon and their mixture was evaluated under laboratory conditions against egg masses, 2nd instars larvae and pupae of cotton leaf worm, *Spodoptera littoralis*..

The obtained results indicated that the effect of the oils on *S. littoralis* eggs can be summarized as delayed embryonic development and ovicidal activity. According to the hatchability % of the treated eggs, data showed that at the lowest concentration (5000 ppm), of the mixture (35.8 ± 6.1%) , lemon (41.7 ± 4.7 %) and menthol extracts (45.8 ± 7.8 %) caused a significant reduction in percent hatchability compared with control (90.3 ± 6.2 %). On the other hand ,mixture of menthol and lemon oils exhibited the highest toxic effect against egg stage followed by lemon and menthol extracts. However, the LC50 values were 456.09, 2130.13 and 3456.17 ppm, respectively.

The oils showed toxic and morphogenetic effects on *S. littoralis* 2nd instars larvae. Mixture of menthol and lemon was the most effective against 2nd instars larvae of *S.littoralis* followed by lemon and menthol alone. The LC_{50} values were 6813.79, 11410.92 and 11944.89 ppm, respectively. On the other hand, the extracted oils had been shown to cause some morphological changes in treated larvae.

In respect to pupal stage, menthol caused the highest toxic effect against *S.littoralis* pupae followed by the mixture and lemon extract .on the other hand, all extracted oils caused malformations in the emerged moths

Effect of LC_{50} of the plant extracts on 2nd instar larvae of *S. littoralis* was evaluated under semi-field conditions .Mixture was the most effective against *S.littoralis* larvae followed by lemon and menthol compounds. The LC_{50} values were 6813.8, 11410.9 and 11944.9ppm, respectively.

Keywords: *Spodoptera littoralis*, lemon oil, menthol oil, Malformation

INTRODUCTION

Among all cotton pests in Egypt, the cotton leaf worm, *Spodoptera littoralis* (Boisd.), is one of the most important pests. This pest has a very wide host range of at least 87 plant species over 40 plant families including many vegetable, fruit and ornamental crops. Thus, this pest seemed a destructive pest, not only to cotton, but also to other field crops (Reda *et al.,* 2013).it has at least 7 generations a year (Magd Eldin& El-gengaihi, 2000).

In Egypt, many problems have been uncounted as a result of the extensive use of synthetic chemical insecticides. Increasing problems concerning with the application of insecticides including insecticides resistance, residual contamination of human foods, mammalian toxicity and pollution of the environment (Abd El- Wahab, 2003).

Plant derived extracts and photochemical have long been a subject of research in an effort develop alternatives to conventional insecticides. It play an increasingly prominent role as alternatives pesticides due to the

increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma et al.,2006). Botanical extracts are important products for pest management (Farzana, 2012 ,Noureddin,et al 2012). The deleterious effects of plant extracts on insects can be manifested in several manners including insecticidal, repellence to pests, antifeedant effects and insect growth regulation (Wang, *et al.* 2009 and Noureddin,et al 2012).

Plant phenols represent a structurally diverse and widely distributed class of allelochemicals. The protection afforded by phenols against plant herbivores was primary factor in their selection during plant evolution (Kathirvelu Baskar 2012). A few phenolic compounds isolated from plants have been found to possess properties that alter insect behavior and physiology (Harwood et al, 1990, Michaelakis et al, 2011 and Karamaouna et al, 2013) . The characterization of the type of phenolics in response to herbivor is important to understand the interaction of plant and herbivore and is useful to develop new control strategies for pest control.

So, the aim of the present work aims to evaluate the influence of the phenolic compounds (menthol ;lemon and their mixture) on some biological aspects of *S. littoralis*, (Boisd) immature stages (eggs, 2nd instars larvae and pupae).

MATERIALS AND METHOD

Insect sources:

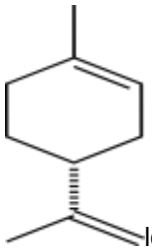
To have a culture of the cotton leaf worm, *Spodoptera littoralis* (Boisd.), freshly eggs masses were obtained from a susceptible laboratory strain maintained in the Integrated Pest Management (IPM) laboratory; Cotton leaf worm Department, Plant Protection Research Institute, Dokki, Egypt.

All stages of *S. littoralis* were reared and tested at 27±2° C and 65± 5 % R.H (EL-Defrawi *et al.,* 1964). Larvae were fed on fresh caster bean (*Ricinus communis* L) leaves until pupation. The full grown larvae were collected and placed in clean Jars with moist saw dust placed at the base to provide the pupation site. Adults were fed on 10% sugar solution offered in a piece of cotton tissue soaked in this solution., fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying.

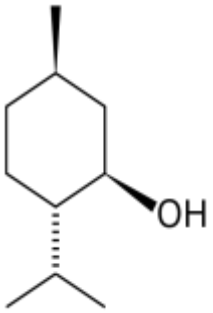
Plant materials and isolation of essential oils:

To have oil extraction from each host plant, fresh leaves of peppermint, *Mentha piperita* L. (Lamiales: Lamiaceae) as well as fresh peels of lemon fruits ,*Citrus limon* L. (Sapindales: Rutaceae) were washed and dried at room temperature.

Essenial oils (volatile oils) of lemon *Citrus limon* L. and peppermint leaves (*Mentha spesies*) were extracted by steam distillation apparatus in the laboratory at Plant Protection Institute, Mansoura, Egypt. The oil was separated dried over anhydrous sodium sulfate and stored in dark glass bottles at 4° C in refrigerator until used (Mohammed and Hany, 2013).the active ingredient of lemon oil and peppermint oil are lemonine and menthol .and the Chemical formula was:



lemonine formula (**OPENDER *et al.*, 2008**)



Menthol formula (**OPENDER *et al.*, 2008**)

Mixture of the two oils made by mix lemon oil and peppermint oil 1:1 proportion.

Crude oil was dissolved to give 200000 ppm stock solution of each extract. The stock solutions were prepared fresh by prior to doing the experiments, Tween-20 emulsifier was added at a concentration of 0.02% to the dilution before preparing the tested concentrations (5000 ,7500, 10000, 15ooo and 20000 ppm) for bioassays Mixture of lemon and peppermint oils(1:1 proportion) was used.

Laboratory bioassay:

i) Influence of tested oils on egg stage:

To study the effect of the previously extracted oils on *S. littoralis* egg stage, healthy tafla leaves with homogenous egg masses (100 eggs/ mass/ leaf) were collected from the laboratory colony. Leaves with egg masses on it were sprayed on each of different concentrations of the tested oils. Leaves sparyed with distilled water served as control. After spraying, the leaves were air - dried and placed at the bottom of glass Petri dishes (which was lined with a wet filter paper) until hatching. Three replicates were used for each concentration, .Microscopic examination was made after 3&4 days. In each treatment the number of egg hatches was calculated.

ii)Influence of tested oils on 2ndinstars larvae:

Fresh castor bean leaves homogenous in size were dipped for approximately 10 seconds into each of different concentrations (5000, 7500, 10000, 15ooo and 20000 ppm) of the tested oils. Leaves dipped in distilled

water served as control. After dipping, the leaves were air - dried and placed at the bottom of glass Petri dishes (which was lined with a wet filter paper). Ten 2nd instars larvae /replicate were allowed to feed on treated leaves .three replicates were done for each treatment. Larvae were allowed to feed on treated leaves for only 24 h, then, live were supplied with untreated castor bean leaves. The insect were observed and examined daily for 7 successive days after treatment. The toxicity data were recorded at one, three, five and seven days post treatment and LC_{50}values were calculated.

iii) Influence of tested oils on pupal stage:

The previously mentioned extracts wer bioassayed against the newly formed pupae by using sterilized Petri-dishes. Ten newly formed pupae were placed at the bottom of each Petri dish.Each concentration was sprayed directly on the pupae. Other Petri-dishes were treated with distilled water and used as controls. After spraying, the pupae were air - dried and kept in glass container until adult emergence .Three replicates were used for each concentration and maintained under laboratory conditions .Percentages of adult emergence were recorded.

The mortality percentage was estimated and corrected according to the Abbott's formula (1925). LC_{50} values were determined using probit analysis statistical method of Finney (1971)**.**

Equation: Sun, 1950 (to determine LC_{50} index**)**

$$\textbf{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Semi field bioassay:

To evaluate the semi -field efficiency of the extracted oils against cotton leaf worm, LC_{50} of each treatment was applied on pepper leaves (***Capsicum annuum*** l.) under field condition. .Treated pepper leaves were collected after zero time and transfer directly to the laboratory. Ten 2nd instars larvae /replicate were allowed to feed on treated leaves. Three replicates were done for each treatment. The mortality percent was calculated as previously mentioned.

RESULTS

(1) Laboratory Experiments:

Influence of tested oils on egg stage:

The effectiveness of the extracted oils and their mixture was evaluated under laboratory conditions. The obtained results are summarized in Tables (1 and 2) and illustrated in Figure (1).

The effect of the oils on *S. littoralis* eggs can be summarized as delayed embryonic development and ovicidal activity.

As shown in Table (1)**,** all extracted oils significantly inhibited the embryonic development of ***S.*** *littoralis* eggs, especially by using their mixture. However, the proportion of non hatching eggs was considerably high in

treated egg-mass in comparison with untreated one. On the other hand, at 15000 and 20000 ppm of the tested extracts, the percent of non hatching eggs was more than 90 % on third day of treatment in comparison with control (14.2 %). Also, it could be notice that the proportion of non hatching eggs increased as the oil concentration increased. So, it could be concluded that tested plant extracts had delayed effect on the embryonic development of *S. littoralis.*

According to the hatchability % of the treated eggs, data show that at the lowest concentration (5000 ppm), of the mixture (35.8 ± 6.1%) , lemon (41.7 ± 4.7 %) and menthol extracts (45.8 ± 7.8 %) caused a significant reduction in percent hatchability compared with control (90.3 ± 6.2 %).

Statistical analysis indicated that mixture of both lemon and menthol extracts had the highest efficiency against *S. littoralis* eggs, while lemone and menthol oils ranked second in the order of activity.

Table (1):Efficacy of some plant oils (menthol, lemon and their mixture) against the cotton leaf worm, *Spodoptera littoralis* Boisd.eggs under laboratory conditions (27±2 ◦C and 65±5% RH.) (L.S.D (5 %) =9.4).

Treatments	Conc. (ppm)	Non hatching proportion after treatments				Hatchability % ± SE
		One day	Three days	Five days	Seven days	
Menthol oil	5000	0	66.67	73.33	76.67	45.8 ± 7.8 b
	7500	0	86.67	70	70	43.3 ± 6.3 b
	15000	0	93.33	73.33	66.67	41.6 ± 9.9 b
	20000	0	96.66	83.33	93.33	31. 7 ± 5.6 bc
Lemon oil	5000	0	80	80	73.33	41.7 ± 4.7 b
	10000	0	88.33	93.33	68.33	37.5 ± 8.6 b
	15000	0	96.67	73.33	90	35.0 ± 7.2 bc
	20000	0	100	93.33	90	29.2 ± 5.3 bc
Mixture of menthol+ lemon oils	5000	0	86.67	80	90	35.8 ± 6.1 bc
	10000	0	96.67	86.67	83.33	33.3 ± 7.3 bc
	15000	0	100	83.33	93.33	30.8 ± 8.4 bc
	20000	0	100	91.67	95	28.3 ± 7.6 bc
Control (Distilled water)		0	14.2	9.7	9.7	91.6±6.2a

Data presented in Table (2) and Fig. (1), revealed that mixture of menthol and lemon oils had the highest toxicity against egg stage of *S.littoralis* followed by lemon and menthol extracts. The LC_{50} values were 456.09,2130.13 and 3456.17ppm, respectively. The toxicity index values were 100, 21.41 and 13.20 for mixture oils followed by lemon and menthol oils, respectively. The slope values indicated that, mixture of both oils had the lowest value was 0.34 followed by 0.51 and 0.51 for lemon oil and menthol oil, respectively. Also, LC_{90}/ LC_{50} values were 64o2.81, 325.75 and 344.72, respectively.

Table (2): Toxic effectof some plant oils (menthol, lemon and their mixture) against eggs of the cotton leaf worm, *Spodopter littoralis* Boisd.

Treatments	Conc.	Corrected non hatching%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Menthol oil	5000	54.17	3456.17	1191400.86	0.51±0.27	13.20	344.72	0.861	0.528
	7500	56.67							
	15000	58.44							
	20000	68.33							
Lemon oil	5000	58.33	2130.13	693951.29	0.51±0.28	21.41	325.75	0.949	0.837
	10000	62.5							
	15000	65							
	20000	70.83							
Mixture of menthol+ lemon oils	5000	64.17	456.09	2920255.45	0.34±0.29	100	6402.81	0.977	0.969
	10000	66.67							
	15000	69.17							
	20000	71.67							

R: Regression **P: Propability**

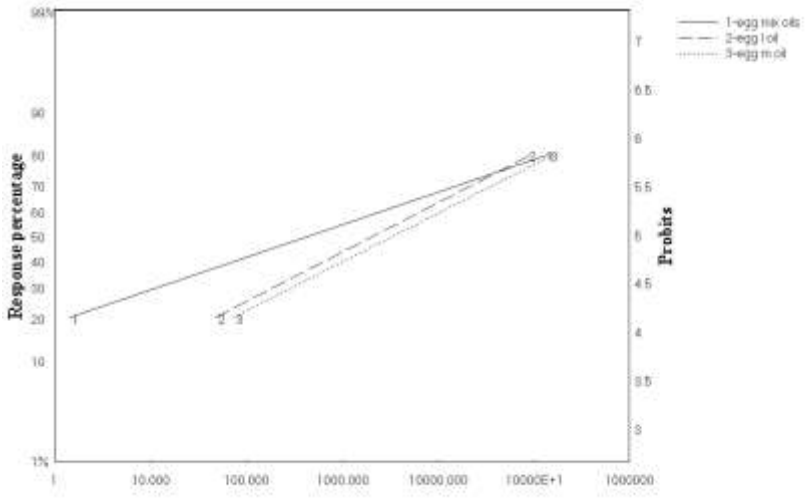


Fig. (1): LC-P lines for some plant oils against eggs of cotton leaf worm, *S. litoralis*

Influence of tested oils on 2nd instar larvae of *S.littoralis:*

The obtained data are summarized and illustrated in Table (3 and 4) and Figure (2).

The effect of the oils on *S. littoralis* 2nd instars larvae can be summarized as follows: 1) toxic effect, and 2) morphogenetic effect. The effect of the extracts on *S.littoralis* larvae revealed that mixture oils recorded relatively higher mortality percentage on *S.littoralis* larvae than Lemon oil and menthol oils (Table, 3). At higher concentration 20000 ppm mixture oils caused 86.7± 19.4 % mortality of treated *S.littoralis* larvae followed by menthol (86.7± 13.3) and lemon (80.0± 17.5 %).

Table (3): Corrected mortality % of 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* treated with some plant oils under laboratory conditions 27±2 ◦C and 65±5% RH.(L.S.D. == 15.4)

No.	Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
			One day	Three days	Five days	Seven days	
1	Menthol oil	5000	------	------	------	6.67	6.7± 2.8 a
		7500	------	6.67	6.67	-----	13.4± 5.9 a
		15000	------	40	13.33	13.33	66.6± 10.8 c
		20000	------	6.67	40	40	86.7± 13.3 c
2	Lemon oil	5000	-----	6.67	-----	------	6.7± 4.8 a
		10000	6.67	-----	26.67	20	53.4± 15.8 b
		15000	6.67	6.67	6.67	40	60.1± 11.6 bc
		20000	6.67	-----	20	53.33	80.0± 17.5 c
3	Mixture of menthol+ lemon oils	5000	6.67	13.333	-----	20	40.0± 12.8 b
		10000	----	6.67	33.33	20	60.0± 16.6 bc
		15000	----	13.33	46.67	20	80.0± 22.8 c
		20000	----	20	40	26.67	86.7± 19.4 c
4	Control (Distilled water	----	0	0	0	0	0

As shown in Table (4), mixture of menthol and lemon oils exhibited the highest toxicity against 2nd instar larvae of *S.littoralis* followed by lemon and menthol extracts. The LC_{50} values were 6813.79,11410.92 and 11944.89 ppm, respectively. The toxicity index values were 100, 59.71 and 57.04 for mixture oils followed by lemon and menthol oils, respectively. The slope values indicated that, mixture oils had the lowest value was 2.29 followed by 3.53 and 4.6 for lemon oil and menthol oil, respectively. Also, LC_{90}/ LC_{50} values were 3.64, 2.30 and 1.90 for mixture of oils, lemon and menthol oil, respectively.

Table (4): Toxic effect of some plant oils against 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis.*

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Menthol oil	5000	6.67	11944.89	22716.50	4.6± 0.95	57.04	1.90	0.990	0.783
	7500	13.33							
	15000	66.67							
	20000	86.67							
Lemon oil	5000	6.67	11410.92	26309.88	3.53±0.89	59.71	2.30	0.974	0.529
	10000	53.33							
	15000	60.00							
	20000	80.00							
Mixture of menthol+ lemon oils	5000	40.00	6813.79	24782.15	2.29±0.78	100	3.64	0.989	0.898
	10000	60.00							
	15000	80.00							
	20000	86.67							

R: Regression P: Propability

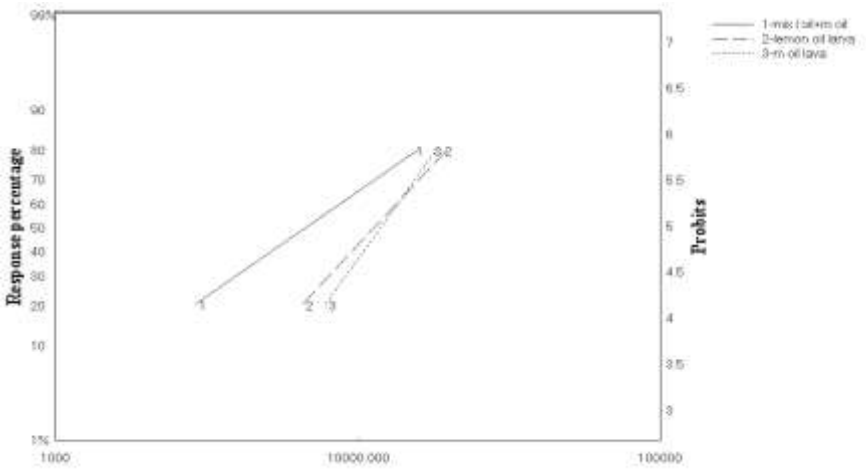


Fig. (2): LC-P lines for some plant oils against 2nd instar larvae of cotton leaf worm, *S. litoralis*

Treated 2nd instar larvae of cotton leaf worm, *S. littoralis* with each of menthol and lemon oils or their mixture caused malformation in treated larvae as shown in Fig. (3).However, the extracted oils had been shown to cause some morphological changes in treated larvae.

Fig.(3): Malformation of *S. littoralis* larvae treated with mixture of lemon and menthol oils (a), lemon oil (b) and menthol oil (c).

Influence of tested oils on *S.littoralis* pupae:

Data shown in (Table 5) indicated that, the mean proportion of non emerged pupae increased with increasing concentration of the extracted oils.

The toxic effect of the tested oil extracts on *S. littoralis* pupae revealed that all oil treatments significantly recorded high mortality percentages of *S. littoralis* pupae, especially with the mixure of menthol and lemon extract.

At the concentration of 20000 ppm, the mixture, lemon and menthol caused 86.7 ± 10.7,84.4 ± 17.3 and 82.2 ± 16.6 %mortality, respectively.

Table (5): Non emergence proportion of *Spodopteralittoralis* pupae treated with some plant oils under laboratory conditions 27±2 ºC and 65±5% RH (L.S.D. 5% = 12.4).

No.	Treatments	Conc. (ppm)	Non emergence after treatments %			Mean of Non emergence %
			Three days	Five days	Seven days	
1	Menthol oil	5000	86.67	60	60	68.89± 15.2 c
		7500	86.67	66.67	60	71.11± 14.3 c
		15000	93.33	73.33	73.33	80± 13.7 c
		20000	93.33	80	73.33	82.22± 16.6 cd
2	Lemon oil	5000	26.67	60	73.33	53.33± 13.9 b
		10000	86.68	46.67	93.33	75.56± 12.6 c
		15000	100	66.67	66.67	77.78± 15.6 c
		20000	100	80	73.33	84.44± 17.3 cd
3	Mixture of menthol+ lemon oils	5000	53.33	80	80	71.11± 12.6 c
		10000	60	80	86.67	75.56± 17.5 c
		15000	100	66.67	80	82.22± 16.4 cd
		20000	100	86.67	73.33	86.67± 10.7 cd
4	Control (Distilled water					

Control adult emergence after seven days 100%

As shown in Table (6), menthol was the most effective against *S.littoralis* pupa followed by mixture and lemon extract. The LC_{50} values were 1206.94, 1300.82 and 4135.59 ppm, respectively. Also, The toxicity index values were 100, 92.8 and 29.18 for menthol, mixture oils and lemon oils, respectively.

Table (6): Toxic effect of some plant oils against cotton leaf worm, *Spodoptera littoralis* pupae and laboratory conditions.

Treatments	Conc.	Corrected non emergence %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Menthol oil	5000	68.89	1206.94	60217.22	0.75± 0.29	100	49.89	0.926	0.990
	7500	71.11							
	15000	80							
	20000	82.22							
Lemon oil	5000	53.33	4135.59	29895.50	1.49±0.30	29.18	7.23	0.977	0.572
	10000	75.56							
	15000	77.78							
	20000	84.44							
Mixture of menthol+ lemon oils	5000	71.11	1300.82	37295.36	0.88± 0.31	92.8	28.67	0.961	0.707
	10000	75.56							
	15000	82.22							
	20000	86.67							

R: Regression P: Propability

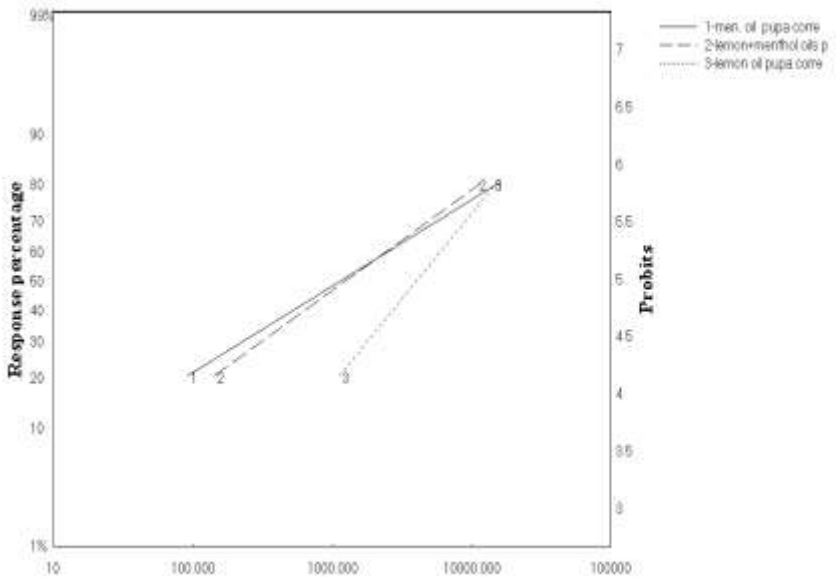


Fig.(4):LC-P lines for some plant oils against pupae of cotton leaf worm, *S. litoralis.*

S. littoralis pupae treated with the extracted oils (menthol, lemon and their mixture) caused malformation in the emerged moths as shown in Fig. (5). However, the extracted oils had been shown to cause some morphological changes in treated larvae.

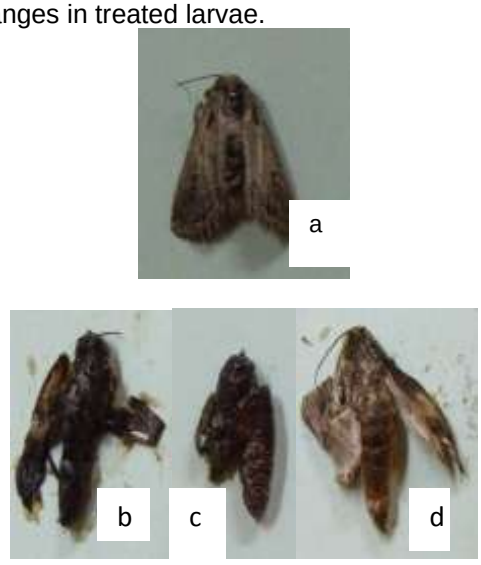


Fig.(5): Malformation of cotton leaf worm adults due to treatment with mixture of lemon and menthol oils (b), lemon oil (c) and menthol oil (d) and the control (a).

(2) Semi-field bioassay

Effect of LC_{50} of each extract on 2nd instar larvae of *S. littoralis* was evaluated under semi-field conditions.

The obtained results indicated that ,mixure oils were the most effective against *S.littoralis* larvae followed by lemon and menthol compounds (Table, 7). TheLC_{50} values were 6813.8, 11410.9 and 11944.9 ppm, respectively.

Table (7): Effiency of LC_{50} of plant extracts on the larvae of *S. littoralis* which fed on treated pepper leaves in semi- field conditions.

Treatments	LC_{50} Conc. (ppm)	Mortality after treatments % (days)					Total Mortality %
		1	3	5	7	14	
Menthol oil	11944.9	--------	--------	6.67	6.67	20	33.34
Lemon oil	11410.9	--------	-------	6.67	6.67	20	33.34
Mixture of menthol+ lemon oils	6813.8	--------	6.67	6.67	13.33	20	46.67

DISCUSSION

Essential oils – inhibit embryonic development of *S. littoralis*

In the present study, oil extracts inhibit the embryonic development of *S. littoralis* eggs and treated eggs failed to hatch. Also, phenolic compounds significantly inhibited the embryonic development of the lepidopteron species, *Earias vittella* (Fab.) (Rao *et al,* 2005). It is possible that essential oils interact with a component of the membranes of the eggs (Opender and. Dhaliwal ,2001).

Essential oils – control metamorphosis in *S. litoralis*.

The tested oils had significant inhibitory effects on the larval development of *S. littoralis* .A similar response to crude oil extracts has been recorded against *S. littoralis* (Mohammed and Hany, 2013) as well as several lepidopteran species (Abd. El-Aziz and E.zz EL –Din 2007 & Hung et al., 2004). Hung et al (2007) demonstrated that the failure of *S. littoralis* larvae to grow was attributed to phenolic compounds in the essential oils.

The LC_{50} values revealed that mixture of both methanol and limonene extracts was the most toxic to *S. littoralis* larvae. According to Sintim(2009) methanol extract exhibited high toxicity to *S. littoralis* larvae and the toxicity of the extract was attributed to the presense of phenolic compounds. Methanol and limonene (Opender and. Dhaliwal ,2001) caused a significant reduction in relative growth rate of lepidopteron larvae .Roth et. al (1997) illustrated that a reduction in relative growth rate due to both reduced efficiency of conversion of digested food and reduced relative consumption rate and caused a significant increase in mortality.

The present investigation obviously indicated that the tested oils exhibited deleterious effect on the pupae of *S. littoralis*. Also, phenol extracts caused reduction in moth performance of *S.littoralis* (Prakash and Ghosal, 1979).

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تأثير بعض الزيوت النباتية على بعض الخصائص البيولوجية لدودة ورق القطن

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تم دراسة تأثير كلا من زيت النعناع وزيت الليمون وخليطهما (بنسبة 1 : 1)والتى تم استخلاصهما فى معمل معهد وقاية النبات بالمنصورة – محافظة الدقهلية على كلا من كتل البيض والعمر اليرقى الثانى والعذراء لدودة ورق القطن .

ولقد اوضحت الدراسة مايلى:-

عند معاملة بيض دودة ورق القطن: كان للذيوت المستخدمة تأثير مثبط للنمو الجنينى وتأثير مباشر بموت البيض خاصة عند المعاملة بمخلوط زيت النعناع وزيت الليمون. فعند استخدام التركيز المنخض (5000 جزء فى المليون) كان هناك انخفاض معنوى فى نسبة الفقس حيث بلغت 35.8 و 41.7 و 45.8 عند المعاملة بالمخلوط و ذيت الليمون وذيت النعناع على التوالىمقارنة بالكنترول91,6%.وقد بلغت الجرعة اللاذمة لقتل 50 % 456,09 و 2130,13 و 3456,17 جزء فى المليون على التوالى.

أبدت المستخلصات تأثير مثبط للتطور فى اليرقات المعاملة مع ظهور تشوهات فى شكل اليرقات ا بالمقارنة بالكنترول , هذا بالاضافة الى التأثير القاتل. وقد بلغت الجرعة اللاذمة لقتل 50 % 6813,79 و 11410,93 و 11944,89 جزء فى المليون على التوالى.

بالنسبة لمعاملة طور العذراء: كان المينثول الاكثر سمية على طور العزراء يلية المخلوط ثم الليمينول- كما تم حدوث تشوهات فى الفراشات الناتجة من العزارى المعاملة

4- كما تم تقيم تأثير التركيز نصف المميت لكل مستخلص فى تجربة حقلية – معملية.وقد أظهرت النتائج ان خليط كلا الزيتين كان له تأثيرا أعلى من كل زيت على حدة والتركيز النصف مميت كان ,68138، 11410,9، 11944,9 جزء فى المليون لكل من خليط الزيتين، زيت الليمون وزيت النعناع، على التوالى.

قام بتحكيم البحث

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