EFFECTIVENESS OF LEMON AND SPEARMINT OIL EXTRACTS ON THE SPINY BOLLWORM, *EARIAS INSULANA* (BOISD.)

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Abstract

Laboratory experiments were conducted at Plant Protection Research Institute (Sharkia branch), Bollworms Research Department to evaluate the toxicity of two medicinal plant extracts i.e. lemon, C*itrus limon* (L.) and spearmint, *Mentha spicata* (Briq.) oils against the spiny bollworm, *Earias insulana* (Boisd.). The LC_{50}, LC_{90} for both extracts after 24 hours were determined. Moreover, the effects of lemon and spearmint oils on some biological and physiological aspects of the SBW were studied. The obtained results showed that C*. limon* and *M. spicata* oils had toxic effect against newly hatched larvae of *E. insulana*. The LC_{50}, LC_{90} values were 0.033, 0.2418 for C*. limon*, and 0.0007, 0.1597 ppm for the *M. spicata*, respectively. For lemon, the mortality percentage recorded 78.67, 71.33, 65.33 and 24.00 % for 1st instar larvae at concentration 0.150, 0.0750, 0.0375 and 0.0156 ppm. While mortality percentage for spearmint extract was 80.00, 77.42, 75.94 and 62.59 % at concentration of 0.0325, 0.0162, 0.0081 and 0.0040 ppm, respectively. Also, the spearmint oil was more toxic than Lemon oil. Otherwise, treatment with both oils caused pronounced short larval duration, pupal period, adult longevity, oviposion periods; larval and pupal weight, pupation and sex ratio percentages. Both oils significantly reduced adults fecundity and eggs hatchability percentage compared to control. On the other hand, adult moths fed on *C. limon* and *Mentha spicata* oils decreased oviposion periods compared to untreated adults. Additionally, it exhibited reduction in the numbers of laid eggs, while, in case of *M. spicata* the higher concentrations caused infertility for female moths compared to control. The results of the physiologic analysis showed a significant decrease in some biological characteristics of the spiny bollworm, especially the weight of the larvae and pupae, the number of eggs and the hatching rate. The results showed the changes in the activity of carbohydrate enzymes in the spiny bollworm larvae treated with *C. limon* and *M. spicata* was a significant increase in amylase and trehalase, aspirate amino transferase (AST) enzymes and total soluble protein contents in the spiny bollworm larvae treated with *C. limon* and *M. spicata* compared to control. While, the invertase, alanine amino transferase (ALT) and acetylcholinestriase (AChE) enzyme fluctuated from negative to positive activity up to the oil concentration compared to control. In conclusion, the lemon and spearmint oils could be tested in semi-field and field experiments to clarify its role in reduction infestation of SBW in cotton fields especially at the low infestation in the begging of cotton season and then incorporated into integrated control programs as one of vital factors for control the spiny bollworm.

Key words: Plant extracts, Spiny bollworm, SBW, *Earias insulana*, Lemon, C*itrus limon*, Spearmint, *Mentha spicata,* Toxicity, Biology and Biochemical.

INTRODUCTION

The spiny bollworm, *Earias insulana* (Boisd.) is one of the most important economic insect pests in Egypt and all over the world (Abdel-Salam, and Negm 2009). Villafañe *et al.* (2011) stated that *C. limon* oil influence on the *Spodoptera frugiperda* larvae, pupal mortalities and cause mortality and antifeedant action. On the other hand, the essential oils from *Mentha spicata* were carvone d-limonene and dihydrocarvone as major constituents out of the 21 components spearmint compounds contained more properties: antifungal, antiviral, antimicrobial, insecticidal, antioxidant, antiallergic, diuretic and stimulating (Ioan *et al.,* 2014). The activity of GOT and GPT enzymes of trehalase, invertaze and amylase activities and the total soluble protein in larvae of pink and spiny bollworms were studied after treatment with garlic extract. Previously, many researchers reported that achetylcholine (AChE) has a key role in neurotransmitter by hydrolyzing the neurotransmitter in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides Amer (2004).

Therefore, this study aimed to evaluate the toxicity of two natural plant extracts: *Citrus limon* and *Mentha spicata* against 1st instar larvae and adult stage of the spiny bollworm, *Earias insulana*. The study also, involved the effects of the previous compounds on some physiological aspects and biochemical parameters of the insect.

MATERIALS AND METHODS

1. The plant extracts:

A. Lemon, C*itrus limon* (L.) oil (Order: Sapindales, Fam.: Rutaceae)

A commercial formulation form was purchased from EL-Captain Company, Egypt.

The amount for lemon oil, D-limonene (64%), 2-cyclohexen-1-ol (6.21%) and β-Pinene (3.822%) (Njoku and Evbuomwan 2014).

B. The leaves of Spearmint, *Mentha spicata* subsp. *condensate* (Briq.) (Order: Lamiales) Fam.: Lamiaceae) that collected from Sharkia Governorate, Egypt were air dried at room temperature. Essential oil was extracted from the leaves of *M. spicata*. The spearmint leaves extract was prepared in study as follows:

The essential oil was extracted by steam distillation for 4-6 h, using a Clevenger-type apparatus (Clevenger, 1928) where 300 gm of plant materials in 300 ml of water subjected to hydro distillation (Marcus and Lichtenstein, 1979). The oil

was separated, dried over anhydrous sodium sulfate $Na_2 SO_4$ and stored in dark glass bottles at 4 ºC in the refrigerator until used.

The essential oil from *M. spicata* contains carvone (73.29 %), d-limonene (7.59 %) and dihydrocarvone (3.83 %) as major constituents out of the 21 components. The essential oil from *M. cardiaca* contains carvone (60.9 %) and limonene (21.58 %) as major constituents out of the 35 components (Chowdhury *et al.,* 2007).

2. Insect Rearing Technique: To evaluate the effects of *C. limon* and *M. spicata* oils on the *E. insulana* larvae (SBW). A batch of SBW larvae was obtained from Bollworms Research Department, Plant Protection Research Institute, Sharkiya Branch, (ARC). This strain was collected from cotton field and reared in an incubator at 26 ±1˚C and 80 ± 5 % R.H. on a modified artificial diet as described previously by **(**Amer, 2015) for many generations free from any insecticide contamination.

3. Plant extracts processing: To evaluate the toxic effect of the two tested plant extracts against newly hatched larvae of *E. insulana*, serial aqueous dilutions of *C. limone & M. spicata* were prepared (0.150, 0.0750, 0.0375 and 0.0156 ppm) & (0.0325, 0.0162, 0.0081 and 0.0040 ppm) using distilled water and add some drops of Triton B as an emulsifier, respectively.

4. Bioassay tests:

4. a. The procedure: Ten grams of artificial diet was putted in a Petri-dish (9 cm in diameter) and one ml of the tested concentration was distributed on the surface of the diet and left until dryness. Each concentration was replicated three times. Twenty five of newly hatched larvae of SBW of each replicate were transferred to the surface of treated diet using a soft brush and kept at 26 ±1ºC and 80 ± 5 % R. H. at an electrical incubator. Similar number of larvae was transferred into untreated diet. Larvae were allowed to feed on the tested diets for 24 hours. Percentages of mortalities were recorded and corrected according to Abbott's formula (1925) as follows: $\%\text{Corrected mortality} = \frac{\text{T - C}}{\text{100 - C}} \times 100$

Where: T: %mortality in treatment

C: %mortality in check

The LC_{50}, LC_{90} and the slope values were determined according to (Finney, 1971).

After 24 hours from treatment any alive larvae were transferred individually to glass tubes (2.0 X 7.0 cm) containing 3.0 gm of control diet. Tubes were plugged with absorbent cotton and incubated at aforementioned conditions for ten days. The tubes were inspected daily and larval mortalities were recorded. Larval mortality percentage, larval and pupal duration, larval and pupal weight, pupation percentage, sex ratio

percentages were recorded. Newly emerged moths of SBW were sexed and paired into glass Jars (250 ml in size) 3pairs/ glass Jars for mating. The upper and lower surfaces of each were covered with muslin cloth held in position by rubber bands. Three replicates/ conc. / extract were used. The emerged moths were fed on 10 % sucrose solution, as well as, control by providing each cage with soaked piece of cotton wool. (Each Jar was inspected daily to record the number of deposited eggs/ female till death, the oviposition periods, longevity of males and females, the number of deposited eggs / female and hatchability percentages.

4. b. Moths feeding test: This experiment was directed to evaluate the effect of the three concentrations of *Citrus limon* oil (0.15, 0.075 and 0.0375 ppm) and (0.0325, 0.0162 and 0.0081 ppm*)* concentrations of *Mentha spicata* oil on *E. insulana* (SBW) moths. Pairs of emerged adult moths', were placed in glass chimney for mating. The upper and lower surfaces of each were covered with muslin cloth held in position by rubber bands. Moths were fed by providing each cage with soaked piece of cotton wool in 10 % sugar solution only as control. The cages were kept under the previous condition. Treatment the alive and dead moths were recorded after 24 hrs. The cages were inspected daily until moth death. Pre-oviposition, oviposition and post-oviposition periods, female & male longevity, number of deposited eggs/female and hatchability percentages were recorded.

5. Biochemical effects of C. *limon* and *M. Spicata* on larvae of spiny bollworm:

The present experiment was designed to study the changes in the activities of the major biochemical components, which are necessary for organism development, and perform its vital activities: carbohydrate hydrolyzing enzymes, total soluble protein and transaminas enzymes (AST& ALT) and acetylcholine eateries (AchE) in the supernatant of the homogenate of the spiny bollworm larvae as affected by different concentrations of *C. limon* and *M. Spicata* oils (0.150 and 0.0750 ppm & 0.0325 and 0.0162 ppm, respectively) as compared to untreated larvae (control).

5. 1. The preparation of samples: Newly hatched larvae were allowed to feed on artificial diet treated with (0.150 and 0.0750 ppm & 0.0325 and 0.0162 ppm, of *C. limon* and *M. Spicata* oils, respectively for 24 hours and then transferred to feed on untreated diet. Seven days after treatment. Twenty larvae from each treatment and control were placed in clean Jars and left four hours to starve. The starved larvae were homogenized in distilled water (1 larvae: 1 ml) using a Teflon homogenizer surrounded with jacket of crushed ice for three minutes. The homogenate larvae were centrifuged at 3500 R.P.M. for 10 minutes at 5^{o}C. The supernatant was immediately

assayed to determine amylase, invertase, trehalase, (AST) & (ALT) enzymes, total soluble protein and acetylcholinesterase (AchE) activities.

a. Carbohydrate hydrolyzing enzymes: The methods used to determine the activities of amylase, invertase and trehalase enzymes according to (Ishaaya and Swiriski, 1976).

The activities of AST and ALT enzymes were determined calorimetrically according to the method of (Reiteman and Frankle 1957).

b. Determination of total soluble protein: Colorimetric determination of total soluble protein in total homogenate of larvae of spiny bollworm was carried out as described by (Gornall *et al.,* 1949).

c. Enzymes measurements:

Acetylcholinesterase (AchE) activity: Acetylcholinesterase was measured according to the method described by (Simpson *et al*., 1964).

6-Statistical analysis: The obtained data were statistically analyzed. Toxicological data were statistically calculated through a Proban program, software computer program (Jedrychowski, 1991). The proper "F" and LSD value was calculated as described by (Fisher, 1944) using Costat program software computer**.**

RESULTS

1. Toxicity of the two oils:

Results in Table (1) showed that the LC_{50} and LC_{90} values were 0.033 and 0.2418 ppm for 1st instar larvae of (SBW) treated with *C. limon* oil, while it gave 0.0007 and 0.1597 ppm for *M. Spicata* oil, respectively. The 1st instar larvae of SBW was highly susceptible for *M. spicata* than *C. limon* oils. (Amani *et al*., 2015) proved that *Ocimum basilieum* (L.) had toxic effect against newly hatched larvae of *E. insulana*. The LC_{50} and LC_{90} values of basil oil against SBW larvae were 41.34 and 178.76 ppm**.**

Table 1. Toxicity of *C. limon* and *M. spicata* oils against *E. insulana* 1st instar larvae insect

Plant oils	LC_{50}	LC_{90}	Slope
Citrus limon	0.033	0.2418	1.4811
Mentha spicata	0.0007	0.1597	0.5485

2. Effect of *C. limon* and *M. spicata* on some biological aspects of the 1st instar larvae of spiny bollworm:

Larval mortality percentage: Data in Table (2) indicated highly significant differences between larval moralities of SBW treated with different concentrations of

C. limon and *M. spicata* concentrations compared with untreated check. Increased concentrations from each oils increased progressively larval mortality percentage.

Larval duration: Results indicated that no significant differences was found between larval duration of SBW treated with different concentrations of the two oils compared to untreated check (Table 2).

Larval weight: Table (2) show that no significant differences were found between the tested concentrations of the oils as compared with the control. The lowest tested concentration of *C. limon* and *M. spicata* oils caused a decreases in larval weight as compared to the control.

Pupal period: The results in Table (2) showed that all concentrations of *C. limon* led to shorten the SBW pupal period significant compared with the untreated check.

Pupal weight: Also, *C. limon* and *M. spicata* oils caused no significant & significant effect on pupal weight of SBW by raising concentrations as compared with control (Table 2).

Pupation percentage: Results in Table (2) indicate that pupation percentages of SBW decreased with increasing of *C. limon* and *M. spicata* oils as compared with control.

Adult stage: *C. limon* and *M. spicata* plant extracts caused significant effect in sex ratio of SBW with the two tested oils, respectively, compared with control. In case of pre - and oviposition periods the results in Table (3) showed that the tested concentrations indicated no significant effect and significant effect of post- oviposition periods compared to untreated check. Also, results showed that *C. limon* and *M. spicata* plant extracts shorten adult longevity than control. The tested oils significantly reduce numbers of laid eggs and the hatchability percentages compared with check. The rate of hatchability % was increased with concentration decreased in each oil and

Table 2. Effect of different concentrations of *C. limon* and *M. spicata* oils on immature stages of *E. insulana* under laboratory conditions

Plant oils	Concentrations ppm	Larval Mortality %	Larval duration (days)	Larval Weight (g)	Pupal Period (days)	Pupal Weight (g)	Pupation %
Citrus limon	0.150	78.67^{a}	12.25	0.0432	5.16^{b}	0.0324	21.33^{e}
	0.0750	71.33^{a}	13.75	0.0461	5.18^{b}	0.0306	29.33^{d}
	0.0375	65.33^{b}	14.42	0.0483	7.75^{ab}	0.0315	34.67^{c}
	0.0156	24.00^{c}	14.00	0.0588	8.82^{ab}	0.0426	76.00^{b}
	Control	0.00^{d}	16.14	0.0685	11.21^{a}	0.0504	97.33^{a}
	P	0.000 ***	0.3866 N.S.	0.3776 N.S.	0.0159*	0.5470 N.S.	0.000 ***
	$LSD_{0.05}$	5.10			4.43		4.23
Mentha spicata	0.0325	80.00^{a}	13.30	0.0432	7.25^{b}	0.030^{b}	18.66^{d}
	0.0162	77.42^{a}	13.67	0.0556	6.33^{b}	0.0435^{ab}	22.67^{b}
	0.0081	75.94^{a}	13.75	0.0562	7.38^{b}	0.0427^{ab}	28.00^{c}
	0.0040	62.59^{b}	14.20	0.0610	8.05^{b}	0.0536^{ab}	37.33^{b}
	Control	0.00^{c}	16.45	0.0718	10.89^{a}	0.0640^{a}	98.67^{a}
	P	0.000 ***	0.5849 N.S.	0.5527 N.S.	0.1341*	0.2667*	0.000 ***
	$LSD_{0.05}$	6.30			3.06	0.0325	4.07

*=Significant **= Highly significant

Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately).

Table 3. Effect of *C. limon* and *M. spicata* oils on mature stages of the spiny bollworm.

Plant oils	Concentrations ppm	Sex ratio %		Oviposition period/female(days)			Adult longevity(days)		Female fecundity/	Hatchability %
		Female ♀	Male ♂	Pre-oviposition Period	Oviposition period	Post-oviposition period	Female ♀	Male ♂		
Citrus Limon	0.150	50.00^{ab}	50.00^{bc}	2.00	5.00	1.50^{b}	8.50^{b}	11.50	9.75^{a}	13.02^{d}
	0.0750	53.38^{a}	46.62^{bc}	2.50	5.50	1.70 b	9.70^{b}	12.50	44.50^{c}	64.55^{ab}
	0.0375	44.44^{bc}	48.89^{ab}	3.25	6.00	2.00^{b}	11.25^{b}	12.25	84.50^{b}	70.20^{c}
	0.0156	42.30^{c}	57.70^{a}	3.30	6.38	2.25^{b}	11.93^{b}	10.75	92.75^{b}	79.00^{ab}
	Control	46.37^{bc}	53.63^{ab}	4.12	6.25	6.21^{a}	16.58^{a}	12.11	23.00^{a}	94.00^{a}
	P	0.0129*	0.0129*	0.2965 N.S.	0.6636 N.S.	0.0053*	0.0015*	0.850N.S.	0.000 ***	0.0009 ***
	$LSD_{0.05}$	5.92	5.92			2.30	3.82		13.40	28.98
Mentha spicata	0.0325	66.67^{a}	33.33^{b}	2.50	8.00	2.00^{b}	12.50	11.00	64.00^{d}	12.81^{c}
	0.0162	54.55^{bc}	45.45^{b}	3.00	7.50	2.75^{ab}	13.25	11.50	70.75^{d}	44.30^{b}
	0.0081	56.25^{bc}	43.75^{b}	4.34	8.22	3.00^{ab}	14.56	12.00	108.72^{c}	79.24^{a}
	0.0040	40.00^{c}	60.00^{a}	4.50	8.00	3.50^{ab}	16.00	13.75	114.67^{c}	81.02^{a}
	Control	57.78^{ab}	42.22^{b}	4.63	7.60	4.37^{a}	16.60	14.25	245.00^{a}	92.00^{a}
	P	0.0173 ***	0.0138 ***	0.3779 N.S.	0.9686 N.S.	0.0890*	0.8068 N.S.	0.4953 N.S.	0.000 ****	0.000 ***
	$LSD_{0.05}$	16.27	13.02			1.79			23.01	16.12

NS=Non-significant *=Significant **= Highly significant

Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately).

3. Moths feeding test: The tested concentrations showed no significant & significant effect on pre-oviposion period for *C. limon* and *M. spicata* oils compared to

control. The tested concentrations of *C. limon* and *M. spicata* indicated significant effect on oviposition and post oviposition periods compared with check. In addition, results show that increasing the concentration of the tested oils induced a reduction in numbers of laid eggs i.e. decreasing the concentration increasing laid eggs and % hatchability compared with control. In case of *C. limon* oil, the lowest rate of hatchability achieved 50.69 % in the highest concentration compared with 95.00 % in untreated moth Table (4). While, in case of *M. spicata*, infertile moths appeared in the highest concentration with no laying eggs compared with control Table (4).

Table 4. Effect of *Citrus limon* and *Mentha spicata* oils on *Earias insulana* moths.

Plant oils	Concentrations Ppm	Oviposion periods/ days			Adult longevity / days		Female fecundity	Hatchability %
		Pre	Ovi	Post	Female ♀	Male♂		
C. limon	0.150	3.00	8.00^{b}	2.00^{a}	12.00	14.30	73.94^{c}	50.69^{d}
	0.0750	3.52	9.08^{ab}	3.75^{ab}	16.35	15.18	111.50^{b}	58.84^{cd}
	0.0375	4.00	10.50^{a}	5.50^{a}	20.00	15.30	128.00^{b}	72.30^{bc}
	Control	4.50	6.83^{b}	4.90^{a}	20.00	15.18	202.66^{a}	95.00^{a}
	P	0.7691 N.S.	0.1590*	0.0340*	0.2908 N.S.	0.9441 N.S.	0.000 ***	0.001 **
	$LSD_{0.05}$		3.39	2.30			16.59	16.79
M. spicata	0.0325	0.00^{b}	0.00^{b}	0.00^{b}	3.25^{c}	4.50^{c}	0.00^{c}	0.00^{b}
	0.0162	0.00^{b}	0.00^{b}	0.00^{b}	4.50^{c}	8.00^{bc}	0.00^{c}	0.00^{b}
	0.0081	4.00^{a}	8.50^{a}	3.75^{a}	13.30^{b}	11.75^{ab}	43.63^{b}	82.11^{a}
	Control	5.50^{a}	7.50^{a}	5.20^{a}	18.20^{a}	15.70^{a}	220.00^{a}	93.60^{a}
	P	0.005 ***	0.000**	0.000 ***	0.000* **	0.0024 ***	0.000 ***	0.000 ***
	$LSD_{0.05}$	2.11	2.11	2.10	3.77	4.51	13.31	13.30

NS=non-significant *=significant ** highly significant

Within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately).

4. Biochemical effect of *C. limon* and *M. spicata* oils on the 1st instar larvae of spiny bollworm: Data in Table (5) refer to the changes in the activity of carbohydrate hydrolyzing enzymes of *E. insulana* at seven days old larvae. The activity of amylase, trehalase, AST enzymes and total soluble protein were highly positively increased than control but invertase, ALT negatively activity appeared in most concentrations except the low one (7.50%) in *C. limon*. The actylecholinesterase (AChE) showed positively activity in high concentrations of both oils and vise versa (negatively activity) in low concentration of each oil than control (Table 5).

Table 5. Changes in activities of some enzymes, total soluble protein and acetylcolin esterase in the supernatant of the homogenated of spiny bollworm larvae as affected by C. *limon* and *M. spicata* oils

Plant oils	Concentrations ppm	Carbohydrate hydrolyzing enzymes mg carbohydrates/g b.wt						Transaminase enzymes				Total Soluble Protein mg protein /g b.wt.	% Increase or decrease than control	AChE	% Increase or decrease than control
		Amylase	% Increase or decrease than control	Invertase	% Increase or decrease than control	Trehalase	% Increase or decrease than control	AST	% Increase or decrease than control	ALT	% Increase or decrease than control				
Citrus limon	0.150	124.03a	+276.53	52.14c	-34.69	46.44a	+124.02	5.78c	+16.77	64.42a	+43.35	16.49a	+102.37	606.448a	+132.84
	0.0750	37.33d	+13.33	149.84a	+87.67	23.25c	+12.16	31.44a	+535.15	30.52c	-32.09	10.083b	+23.67	186.178e	-28.52
Mentha spicata	0.0325	46.93c	+42.47	51.44c	-35.57	46.93a	+126.39	34.03a	+587.47	64.06a	+42.55	14.601ab	+79.09	482.044b	+85.08
	0.0162	65.26b	+98.12	23.66d	-70.37	37.24b	+79.64	13.10b	+164.65	11.44d	-74.54	11.67ab	+43.14	192.283d	-26.17
Control		32.94d		79.84b		20.73c		4.95c		44.94b		8.153b		260.456c	
F. test		**		**		**		**		**		*		**	
$LSD_{0.05}$		6.91		6.50		7.91		4.49		6.59		6.57		5.57	

** Highly significant within the same column and source data followed by the same letter are not significantly different (P>0.05; LSD mean separately).

% Increase or decrease than control = treated - control ÷ control ×100.

AST= Aspartate aminotransferase ALT= Alanine aminotransferase AchE= Acetylcolinesterase

DISCUSSION

The effect of lemon, C*itrus limon* (L.) and spearmint, *Mentha spicata* (Briq.) oils on some biological aspects of the spiny bollworm, *Earias insulana* (Boisd.). The results indicated that both oils proved toxic effects against *E. insulana*. *M. spicata* was more toxic than *C. limon* oil. Larval mortality percentages were increased as concentration increased after ten days from treatment compared with the untreated check. Both extracts caused pronounced short larval duration, pupal period, pupation percentages, adult longevity, oviposion periods; larval and pupal weight, and sex ratio and reduce the fecundity and hatchability percentages. Adult moth fed on *C. limon* and *M. spicata* oils decreased oviposion periods, adult longevity and the numbers of laid eggs compared to untreated check. While, in case of *M. spicata* the highest concentration showed infertile moths with no laying eggs compared with control. Also, it was found that the two oils reduced the hatchability percentages compared with untreated moths. The results showed that the change in the activity of carbohydrate enzymes in the spiny bollworm larvae treated with *C. limon* and *M. spicata* induced a significant increase in amylase and trehalase, AST enzymes and total soluble protein contents. The invertase, ALT and acetylcholinestriase (AChE) enzyme were fluctuated from negative to positive activity up to the oil concentration compared to control.

The present results are in agreement with (Villafañe *et al*., 2011) who found that the essential oils of *C. aurantium* and *C. limon* were more effective on *Spodoptera frugiperda*. (Amani *et al*., 2015) stated that basil oil *Ocimum basilieum* (L.) significantly prolonged larval and pupal duration and decreased pupation and adult emergence percentages of *E. insulana*. (Amer, 2004) reported that decrease in the activity of GOT and GPT enzymes and increasing of trehalase, invertase and amylase activities as well as in the total soluble protein of pink and spiny bollworms larvae after treatment with garlic extract.

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فعالية مستخلصات زيت الليمون والفلية على دودة اللوز الشوكية

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أجريت التجارب المعملية في معهد بحوث وقاية النباتات (فرع الشرقية) قسم بحوث ديدان اللوز لتقييم تأثير زيت الليمون والفليه على بعض الجوانب البيولوجية لدودة اللوز الشوكية. أشارت النتائج إلى أن زيت الليمون والفليه كان لهما تأثير سام ضد دودة اللوز الشوكية. قيمة LC_{50} ٠,٠٣٣ و LC_{90} ٠,٢٤١٨ جزء في المليون لزيت الليمون، ٠,٠٠٠٧ و٠,١٥٩٧ جزء في المليون لزيت الفليه بعد مرور ٢٤ ساعة من معاملة الفقس الحديث . من هذه النتائج وجد ان زيت الفليه كان أكثر سمية من زيت الليمون . بالنسبة لزيت الليمون ، سجلت النسبة المئوية للموت ٧٨,٦٧، ٧١,٣٣، ٦٥,٣٣ و٢٤,٠٠% للعمر اليرقي الأول عند التركيزات ٠,١٥ ، ٠,٠٧٥٠، ٠,٠٣٧٥، و٠,٠١٥٦ جزء في المليون . بينما نسبة الموت لمستخلص الفليه كانت ٨٠,٠٠ ، ٧٧,٤٢، ٧٥,٩٤ و ٦٢,٥٩٪ عند التركيزات ٠,٠٣٢٥ ، ٠,٠١٦٢ ، ٠,٠٠٨١ و ٠,٠٠٤٠ جزء في المليون ، على التوالي.أيضا وجد أن زيت الفلية اكثر سميه من زيت الليمون . على الجانب الآخر سببت المعاملة بكلا من الزيوت قصر في مدة الطور اليرقي، فترة التعذير، وفترة حياة الحشرات الكامله وفترات وضع البيض ووزن الطور اليرقي والعذري والنسبة الجنسية (للفراشات الناتجة من معاملة الفقس الحديث). كما خفض كلا الزيتين نسبة خصوبة الحشرات الكاملة ونسبة الفقس إلى حد كبير مقارنة بالكنترول. وبتغذية الفراشات على زيت الليمون والفليه خفضت فترات وضع البيض مقارنة بالكنترول . أيضا وجدت فروق غير معنوية ومعنوية بين تركيزات الزيوت المختبرة لفترة حياة الحشرات الكامله على التوالي .زيت الليمون خفض عدد البيض الموضوع مقارنة مع الكنترول .بينما في حالة زيت الفلية وجد أن أعلى التركيزات سببت عقم لإناث الفراشات مع عدم وضع بيض مقارنة مع الكنترول. وقد خفضت الزيوت نسب الفقس مقارنة مع الكنترول .كما أوضحت نتائج التحليل الفسيولوجى إنخفاضا" معنويا" في بعض الصفات البيولوجيه لدودة اللوز الشوكيه خاصة وزن الطور اليرقي وعدد البيض الموضوع ونسبه الفقس . وأظهرت النتائج أن التغير في نشاط إنزيمات الكربوهيدرات الأميليز والتريهالاس، في يرقات دودة اللوز الشوكية المعاملة بزيت الليمون والفليه كانت معنويا في AST والمحتوى الكلى للبروتين في حين أن إنزيم إنفرتاز، AST وأسيتيلكولينستريبس (AchE) أظهر انخفاض أو زيادة تدريجية للزيوت المختبره مقارنة بالكنترول. حدث زيادة معنوية في إنزيم الأميليز وتريهالاس،في حين أن إنزيم إنفرتاز، AST والمحتوى الكلى للبروتين أظهر تذبذب بين النقص والزيادة التدريجية في إنزيمات انفرتيز ،ALT و أسيتيلكولينستريس (AChE) مقارنة بالكنترول. ويمكن إدراج زيت الليمون والفلية ضمن برامج المكافحة المتكاملة كأحد عوامل المكافحة الحيوية الآمنة لمكافحة دوده اللوز الشوكية بعد إجراء المزيد من الدراسات الحقلية لتأكيد دورهما في خفض الإصابة بدودة اللوز الشوكية .