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Impact of some Essential Plant Oils and Insect Growth Regulators on Immature Stages of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Egypt

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ABSTRACT

The cotton leafworm *Spodoptera littoralis* (Boisd) (Lepidoptera, Noctuidae) is consider a polyphagous pest infesting cotton and some important vegetables and field crops in Egypt. The latent effects (LC_{25}) of three insect growth regulators (IGRS); Runner (24% SC), Virtu (5% SC) and Roxy (10% EC) and three essential plant oils; coriander, basil and mustard oils were evaluated against fourth larval instar until adult emergence and oviposition under laboratory conditions. The influence of LC_{25} of tested materials on some biological aspects, nutrition indices for cotton leafworm were evaluated. Novaluron (roxy) showed a great ovicidal activity where recorded the lowest number of laid eggs (611 eggs) compared to control and coriander oil (1103.3 and 871 eggs, respectively). Also, novaluron was the most female oviposition deterrence (23%) and had the highest sterility (68.9%). On the other hand, there was a significant differences between novaluron and tested oils in sterility. Coriander oil recorded the highest feeding deterrence index (FDI) against fourth larval instar which statistically different with all tested treatment, while, tested IGRs had the lowest effect as a feeding deterrent for larvae varied between 7-9%. Relative growth Rate (RGR) had insignificantly varied between tested treatments. Larval duration was significantly elongated when larvae fed on treated castor bean leaves with coriander oil reach to 16.2 days compared to control (13.8 days). While IGRs treatments recorded that shorter larval duration rather than coriander oil, but significantly similar to control. Chromafenozide (virtu) pesticide had the highest larval mortality compared to control and essential plant oils treatments.

Keywords: *Spodoptera litterolis,* Insect Growth Regulators, Essential plant oils, mortality, survival, Oviposition deterrent, Feeding Deterrence Index

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a great polyphagous pest attacking wide range of economic crops such as cotton in Egypt. It has minimum 7-9 generations during the cotton season, additionally infesting more than 29 other crops and vegetables (El-din and El-Gengaihi, 2000). Cotton leafworm (CLW) larvae mainly feed on leaves and stems and can extremely postpone growth or decrease cotton production. Furthermore, during heavy infestations, CLW can also penetrate flowers and bolls, causing substantial loss up to 50% reduction in yield (Russell *et. al.,* 1993).

Based on the great interaction between plant and insects, the plant has improved the defense mechanism against herbivores and very well sources for toxic materials for insects (Pickett *et al.,* 2006). Essential plant oils produced from aromatic plants are introduced as a natural biopesticides among many different levels of natural materials (Isman and Grieneisen, 2014; Prakash *et al.,* 2014). Plant natural product like plant oils and plant derivatives could be an alternative agent for insect control, where they constitute rich sources of bioactive chemicals as well as, they are often biodegradable to non-toxic products. Furthermore, plant-derived materials have a great influence against pests, which appears resistant to insecticides (Arnason *et al.,* 1989; Kwon *et al.,* 1996 and Ahn *et al.,* 1997).

Insect growth regulators (IGRs) received a great attention as a hope for controlling insects in the future that showed diversely affect against *S. littoralis,* and caused large selectivity to beneficial insects (Raslan 2002). Using IGRs pesticides could result in growth reduction, moulting inhibition, anatomical abnormalities as well as mortality, in a wide range of insect species, most of them belonging to Order Lepidoptera, where its action depends on insect species and the applied concentration (Khedr *et al.,* 2005).

Ongoing research objective was evaluating the toxicity of plant oils and IGRs pesticides against CLW fourth larval instar and the influence of the latent effect of tested materials on some biological aspects of CLW fourth larval instar.

MATERIALS AND METHODS

1. Insect culture

Spodoptera littoralis were obtained as a pupae from Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt, then kept in the growth chamber at at 25 ± 2 ^{o}C and 65 ± 5% RH with a photoperiod of 16h:8h (light: dark) until adult emergence under Plant Protection Laboratory at Faculty of Agriculture, Minia University, El Minya, Egypt. The emerged adults were provided with Tafla branches (*Nerium oleander*) for oviposition and supplied with 10% (W/V) of honey solution. Tafla leaves with freshly deposited eggs were collected daily and placed in a glass jar provided with castor bean leaves.

2. Tested materials

1. Essential plant oils

The toxicity of three essential plant oils was evaluated against fourth larval instar in this experiment; Coriander oil (*Corianderum sativum* L.) (Fam: Apiaceae), Basil oil (*Ocimum basilicum* L.) (Fam: Lamiaceae) and Mustard oil (*Brassica alba*) (Fam: Brassicaceae). Essential plant oils were obtained as a (100% crude oils) from EL-Captain Company.

2. Insect growth regulators pesticides

1- Runner® 24% Suspension Concentrate (SC). Common name: Methoxyfenozide.

Chemical name: 3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide.

Action: Second generation ecdysone agonist. Causes cessation of feeding and premature lethal molt, agonist of 20-hydroxyecdysone a key hormone in the molting process.

2- Virtu® 5% Suspension Concentrate (SC). Common name:

Chromafenozide. Chemical name: 3,4-dihydro-5-methyl-2H-1-benzopyran – 6 - carboxylic acid 2 - (3,5-dimethylbenzoyl)- 2- (1,1-dimethylethyl) hydrazide.

Action: is a novel dibenzoylhydrazine and is categorized to be an insect hormone ecdysone (moulting hormone agonists).

3- Roxy 10% Emulsifiable concentrate (EC):

Common name: Novaluron. Chemical name:(±)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy) phenyl]-3-(2,6 difluorobenzoyl) urea.

Action: Chitin Synthesis Inhibitor.

3. Bioassays for tested materials and LC_{50} calculation

The toxic effect of tested materials was evaluated against larval stage, where newly fourth larval instar were fed on castor bean leaves dipped for 60 seconds in different five concentrations of essential plant oils and IGRS pesticides (500, 1000, 2000, 3000 and 4000 ppm) and (50, 100, 200, 300 and 400 ppm) respectively. Four replicates per concentration were assayed to calculate the LC_{50}. For the controls, leaves were dipped in Tween-20 (0.5%). The feeding period on treated leaves was for 4 days. Mortality was daily recorded up to four days post treatment. Mortality in treated treatments was corrected with the corresponding mortality in the untreated check and subjected to Finney probit analysis (Finney, 1971). Toxicity of tested materials on eggs and fourth larval instar was compared based on the LC50 and LC90 values.

4. Latent effect of LC_{25} of tested materials on:

1. Some biological aspects of cotton leafworm

Newly molted fourth larval instar were placed individually in plastic vial provided with a disc of fresh castor bean leaves and covered with a pored lid for refreshing the air. Two groups of 100 larvae, each was set up to conduct this study. One group was served as a control, receiving untreated leaves. Another group was received tested materials treated castor bean leaves with a concentration corresponding to the 96 hrs (100 larvae for each tested materials). LC_{25} that established from the previous study. Each group was divided to ten replicates of ten individual larvae each, which used to follow the daily effects on food consumption, larval weight, larval mortality and finally on larval duration from fourth instar until pupation. Every day, surviving larvae were individually weighed and food consumption was calculated. The Date of pupation was recorded in each vial and pupae were daily observed for recording the date of emergence and the % of emerged female adults.

2. Nutritional indices assay

Feeding deterrence index (FDI) were studies for tested materials using leaf disc no choice bioassay method. Fresh castor leaf disc were dipped for 60 seconds in LC_{25} of each tested material, then the leaf disc was kept in individual petri dish (9 cm diameter). In each petri dish a single pre starved CLW fourth instar larva was introduced. The larva was allowed to feed on treated discs for 24 h and changed every day with untreated leaf disc for 10 days, whereas control leaf disc were dipped in water only. Leaf discs were weighted in the beginning of the experiment and the consumed area of leaf disc was calculated by reweighting the left leaf disc. Ten replicates for each tested materials were studied for 10 days and the feeding deterrence index was calculated based on the formula of (Huang *et al.,* 2000). Also, the relative growth rate was determined, where the fourth larval instar were weighted before introducing them to the treated leaf disc with tested materials. Then the larvae reweighted after treatment every day till 10 days and ten replicates were evaluated for each pesticide. The nutritional indices were calculated for larvae according to Huang et al. (2000) formula:

Relative Growth Rate (RGR) and Feeding Deterrence Index (FDI)

$$= \frac{\text{Food consumed in control (mg) - Food consumed in treatment (mg)}}{\text{Food consumed in control (mg)}} \text{ X } 100$$

Feeding Deterrence Index (FDI)

$$= \frac{\text{(Weight of live insects after experiment (mg) - Weight of live insects before experiment (mg)}}{\text{Weight of live insects before experiment (mg) x day (feeding period)}} \text{X } 100$$

Relative Growth Rate (RGR)

3.Oviposition deterrent activity and sterility

For treated and control treatments, 10 pairs of treated adults were coupled in a jar provided with Tafla branch and a piece of cotton pad soaked in 10% sugar solution and jars were covered with muslin cloth tied with a rubber band. The jars were observed daily for collecting Tafla leaves having egg masses and placed after counting in Petri dishes. For each replicate, numbers of eggs laid and numbers of un-hatched eggs were counted in control and treatment. Mean number of eggs laid in control and treatment and % hatchability were used to calculate the percentage oviposition deterrent activity (Arivoli and Tennyson, 2013) and the overall latent effect on reproduction was expressed as percent sterility (Toppozada *et al.,* 1966) among control and treated treatments.

$$= \frac{\text{Number of eggs laid in control – Number eggs laid in treated}}{\text{Number of eggs laid in control + Number eggs laid in treated}} \text{ X } 100$$

Percent oviposition deterrent activity:

$$= \frac{\text{No. of deposited eggs in treatment x \%hatchability in treatment}}{\text{No. of deposited eggs in control + \%hatchability in control}} \text{ X } 100$$

Percent Sterility

Data analysis

Data was analyzed using SAS software (SAS Inst. Inc, 2016). Biological aspects, ovicidal activity and nutritional indices parameters were subjected to an analysis of variance using the SAS procedure GLIMMIX. Predicted treatment means were calculated using the (LSMEANS statement. Tukey's studentized range test was used for testing the null hypothesis of no mean difference between a pair of treatment, in an effort to control Type I Error.

Mean response and 95% confidence interval for essential plant oil treatments, and IGR pesticide treatments were calculated using the ESTIMATE statement. Null hypothesis of no difference between these two treatment groups were analyzed through a contrast using the ESTIMATE and a 95% confidence interval for their mean difference calculated. Level of significance was set to 0.05 (α=0.05).

Daily larvae mortality counts from day 1 to day 10 was analyzed using the SAS procedure LIFETEST. Life Test Mortality curves were calculated for each treatment and Wilcoxon test, with Sidak adjustment for multiple comparison to control Type I Error, was used for pairwise comparison of treatment curve. Average life test curves were

calculated for the three treatment groups: Control, Oil based and PGRP. Wilcoson test with Sidak correction was used to pairwise compare treatment groups. Level of significance was set to 0.05 (α=0.05).

RESULTS

1. **Toxicity of tested essential plant oils and IGRs pesticides on *S. littoralis* fourth larval instar:**

CLW fourth larval instar were fed on castor bean leaves treated with different concentrations of tested materials. LC_{90}, LC_{50} and LC_{25} values of each tested material predicated by Probit analysis for fourth larval instar after 72 hr of treatment (Table 1). According to LC_{25}, LC_{50} and LC_{90} values, coriander oil showed that the highest LC50 and LC_{25} values (955 ppm and 354.8 ppm, respectively) compared to other tested materials. Otherwise, runner pesticide had the lowest LC_{50} and LC_{25} values (24 ppm and 15.5 ppm, respectively). In general, data showed that essential plant oils needed higher concentration for LC_{25}, LC_{50} and LC_{90} than pesticides and this is an evidence that the pesticides have a severe effect on this pest.

Table 1. Comparative toxicity of different essential plant oils and IGRs pesticides against *S. littoralis* fourth larval instar

Tested materials	Treatment	Equation	LC_{90} (ppm)	LC_{50}(ppm)	LC_{25} (ppm)
Essential plant oils	Coriander	Y= 1.5823x + 0.2915	6025.59	954.99	354.81
	Basil	Y= 1.3513x + 1.1544	6165.95	707.95	223.87
	Mustard	Y= 1.2638x + 1.296	8709.64	851.14	245.47
IGRs pesticides	Virtu 5% SC	Y= 1.7036x + 2.205	245.47	54.95	17.37
	Roxy 10% EC	Y= 1.2564x + 2.6961	707.95	67.61	19.49
	Runner 24% SC	Y= 1.383x + 2.6756	407.38	23.99	15.48

2. Effect of sublethal concentration of tested materials on some biological aspects of CLW

Fourth larval instar were fed on treated castor bean leaves with tested essential plant oils and IGRS pesticides at the concentration corresponding to LC_{25}. Results showed that there is a significant differences in larval duration between treatments (ANOVA, DF=28, F=23.17, P<0.0001). Larval duration was significantly elongated when larvae fed on treated castor bean leaves with coriander oil reach to 16.2 days and other leaves treated with basil oil (15.7 days) compared to control treatment (13.8 days) and all other treatment (Tukey, DF=28, P<0.0001) except basil oil, where larval duration reach to 15.7 days (Tukey, DF=28, P>0.05) (Table 2). Otherwise, larval duration for larvae which fed on castor beans leaves treated with pesticides showed similar larval duration as a control treatment ranged between 13.4-14 days (Tukey, DF=28 , P>0.05).

Mustard oil treatment had statistically similar larval duration for basil oil, Chromafenozide pesticide and Methoxyfenozide pesticide (Tukey, DF=28, P>0.05), but shorter larval duration (14.9 days) than coriander oil treatment and longer larval duration than novaluron pesticide and control treatments (Tukey, DF=28, P<0.0001).

Pupation percentage was significantly varied between treatments (ANOVA, DF=28, F=24.74, P<0.0001). Pupated larvae was reached to 92% in control treatment, which not significantly different with pupated larvae from coriander oil and mustard oil treatments (84% and 87%, respectively) (Tukey, DF=28, P>0.05), but significantly different with all other treatments (Tukey, DF=28, P<0.0001). Pesticides treatments showed the lowest percentage of pupated larvae especially for Methoxyfenozide pesticide (59%) compared to control and essential plant oils treatments (Tukey, DF=28, P<0.0001), while statistically similar percentages for pupated larvae were recorded for tested pesticides ranged between 59% to 69% (Tukey, DF=28, P>0.05). Coriander oil and mustard oil treatment had almost similar percentage for pupated larvae, but both of them are significantly different with tested pesticides.

On the other hand, 78% of larvae pupated in the basil oil treatment, which significantly different with Chromafenozide and Methoxyfenozide pesticides treatments (Tukey, DF=28, P<0.0001), but not significant with novaluron pesticide treatment (Tukey, DF=28, P>0.05).

Pupal duration was significantly different between treatments (ANOVA, DF=63, F=24.42, P<0.0001). There was a highly significant differences between coriander oil and all other treatment (Tukey, DF=63, P<0.0001) except basil oil (Tukey, DF=63, P>0.05). Results showed that pupal duration for larvae which fed on coriander oil treatment was elongated and reached to 12.4 days compared to control treatment (10.6 days). Basil oil showed almost similar pupal duration to coriander oil and mustard oil reached to 12 days (Tukey, DF=63, P>0.05), but significantly different with all other treatments (Tukey, DF=63, P<0.0001). Pupal duration for larvae fed on mustard oil did not significantly different with Chromafenozide treatment (Tukey, DF=63, P>0.05), while pupal duration was significantly higher than novaluron and Methoxyfenozide treatments (Tukey, DF=63, P<0.0001). Tested pesticides showed almost similar pupal duration like control treatment did not exceed 10.7 days (Tukey, DF=63, P>0.05).

The percentage of females were not significantly different between treatments (ANOVA, DF=63, F=1.95, P>0.05), where the percentage of females were ranged between 50-51.2% for all treatments.

Emergence related to the number of treated larvae was highly significant different between treatments (ANOVA, DF=63, F=36.9, P <0.0001). The highest percentage for emerged larvae was recorded for control treatment (92%) which significantly higher than all tested pesticides (Tukey, DF=63, P <0.0001), where % emerged larvae for tested pesticides ranged between 59-70%. Otherwise, Coriander and mustard oils not significantly different with control treatment or between each other (Tukey, DF=63, P >0.05), whereas %emerged larvae were 84% and 87%, respectively. On the other hand, Methoxyfenozide pesticide showed the lowest percentage for emerged larvae (59%), which significantly lower than

all treatments (Tukey, DF=63, P <0.0001), except Chromafenozide pesticide (63%) (Tukey, DF=63, P >0.05). Chromafenozide pesticide had significantly lower emerged larvae than the three tested oils, while novaluron pesticide was significantly lower than coriander and mustard oils, but not significantly different with basil oil.

Results for emergence related to the number of formed pupae showed that, there is a significant differences between treatments (ANOVA, DF=63, F=31.17, P<0.0001).

Control treatment showed significantly the highest percentage of emerged pupae (100%) compared to tested pesticides (Tukey, DF=63, P <0.0001), while there were no significant differences between control and tested oils (Tukey, DF=63, P >0.05), where the percentage of emerged pupae ranged between 91-94%. Methoxyfenozide pesticide had the lowest percentage of emerged pupae at 56% which significantly lower than all treatments (Tukey, DF=63, P <0.0001). Similar results were recorded for novaluron pesticide, where 61% of pupae were emerged which significantly different with all other treatments. Otherwise, Chromafenozide pesticide showed significantly less percentage of emerged pupae (82%) than mustard oil (94%), while no significant differences were found between Chromafenozide pesticide and coriander and basil oils.

3. Feeding Deterrence Index (FDI):

The mean feeding deterrence index for tested materials was calculated against *S. littoralis* fourth larval instar after feeding them on treated castor leaves (Figure 1). Results showed that there was a highly significant differences between treatments (ANOVA, DF=54, F=74.08, P<0.0001). Coriander oil was the highest feeding deterrent for fourth larval instar (25.6 as an average for 10 days after the treatment) which statistically different with all other treatments (Tukey, DF=54, P<0.0001). While, tested pesticides had the lowest effect as a feeding deterrent for larvae varied between (7-9 %). No significant differences were found between tested pesticides, also there is no differences between mustard and basil oils (Tukey, DF=54, P>0.05). Similarly, no differences were found between novaluron and Chromafenozide pesticides and mustard and basil oils (Tukey, DF=54, P>0.05).

Table 2. Means of some biological aspects for CLW fourth larval instar corresponding to LC_{25} of tested essential plant oils and IGRS pesticides

Biological aspects	Control	Tested essential plant oils			Tested IGRs pesticides		
		Coriander	Basil	Mustard	Virtu	Roxy	Runner
Larval duration	13.8 d	16.2 a	15.7 ab	14.9 bc	13.9 cd	13.4 d	14 cd
% Pupation	92 a	84 ab	78 bc	87 ab	63 d	69 cd	59 d
Pupal duration	10.2 d	12.4 a	12.0 ab	11.5 bc	10.7 cd	10.3 d	10.3 d
% Emergence*	100 a	92 ab	91 ab	94 a	82 b	61 c	56 d
%Emergence**	92 a	84 ab	78 bc	87 a	63 de	70 cd	59 e
% Female	50.1 a	51.0 a	50.6 a	50.6 a	51.2 a	51.4 a	52.1 a

Values within a row followed by the same letter are not significantly different via multiple comparison with Tukey (α=0.05).
****%Emergence related to number of treated larvae**
*** %Emergence related to number of formed pupae**

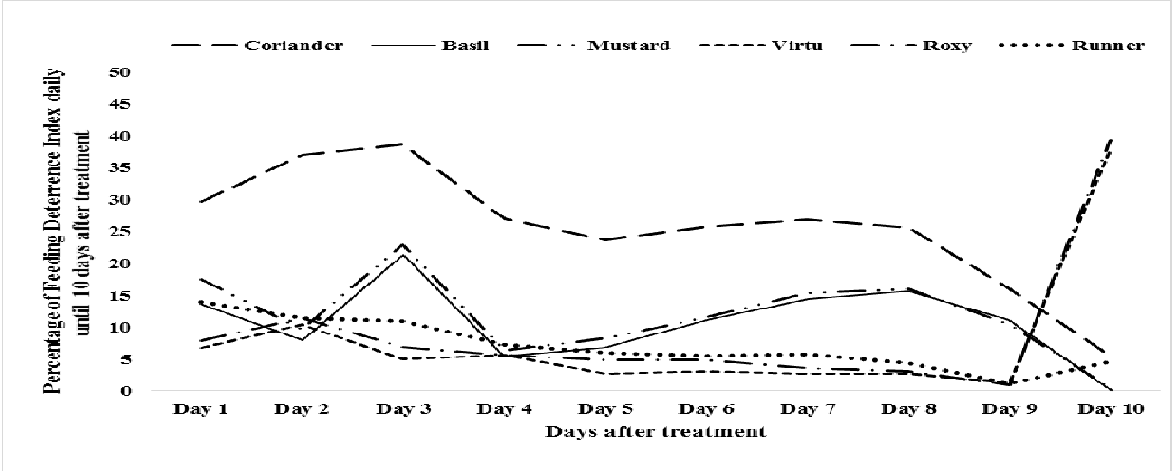

Fig. 1. Percentage of feeding deterrence index (FDI) of tested essential plant oils and IGRs pesticides against CLW fourth larval instar daily until 10 days after treatment

4. Relative Growth Rate (RGR):

Fourth larval instar which fed on untreated and treated leaf disks were weighted every day after treatment until tenth day (Table 3). The relative growth rate was calculated every day after treatment and determined after 10 days of treatment. Results showed that the growth rate for larvae after 10 days of treatment not varied between tested treatments. Also, statistically, no significant differences were found between treatments (ANOVA, DF=63, F=0.7, P>0.05), where relative growth rate for fourth larval instar ranged between 1.90-2.32 for all treatments.

5. Total mortality for CLW fourth larval instar after 10 days of treatment:

The effect of LC_{25} for each essential plant oil and IGRS pesticide was evaluated on CLW fourth larval instar. The accumulative number of dead larvae were recorded

daily for 10 days after treatment (Figure 2). Results showed that the total mortality of larvae after 10 days of treatment is significantly different between all treatments (control, essential plant oils and IGRS pesticides) (DF= 6, F= 27.9, P<0.0001). Chromafenozide pesticide showed the highest toxic effect and recorded significantly the highest number of total mortality for larvae at day tenth (37% dead larvae) with control and essential plant oils (Tukey, DF=63, P<0.0001). Whereas, no significant differences were found between chromafenozide and other IGRS pesticides (methoxyfenozide and novaluron) (Tukey, DF=63, P>0.05). However, the lowest total mortality appeared in control (8% dead larvae), which was significantly different with IGRS pesticides (Tukey, DF=63, P<0.0001) and basil essential plant oil (Tukey, DF=63, P=0.003). Otherwise, control treatment was not significantly different than coriander and mustard essential plant oils (Tukey, DF=63, P>0.05). On other hand, basil essential plant oils was higher and significant different than mustard (Tukey, DF=63, P=0.02), but not significant with coriander (Tukey, DF=63, P>0.05). Also, no significant differences were found between mustard essential plant oil and coriander (10% and 15% dead larvae, respectively) (Tukey, DF=63, P>0.05).

Table 3. Mean of Relative Growth Rate (RGR) for CLW fourth larval instar when fed on treated castor bean leaves after 10 days of treatment

Days after treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	*Day 10
Control	0.14	0.27	0.26	0.34	1.12	0.71	1.20	1.45	1.25	1.45a
Coriander	0.03	0.06	0.20	0.23	0.29	0.77	0.97	1.95	1.52	2.57a
Basil	0.02	0.13	0.45	0.46	0.57	1.00	1.32	1.15	1.72	2.81a
Mustard	0.12	0.12	0.16	0.46	0.56	0.99	1.30	2.42	1.72	1.85a
Virtu	0.05	0.27	0.26	0.33	0.71	0.70	1.20	1.45	1.25	1.97a
Roxy	0.12	0.26	0.25	0.33	1.11	0.70	1.82	1.44	1.24	2.99a
Runner	0.11	0.24	0.24	0.32	0.70	0.70	1.80	1.42	1.23	2.82a

Relative Growth Rate was calculated at day 10 at the end of the experiment
***Values within a column followed by the same letter are not significantly different via multiple comparison with Tukey (α=0.05).**

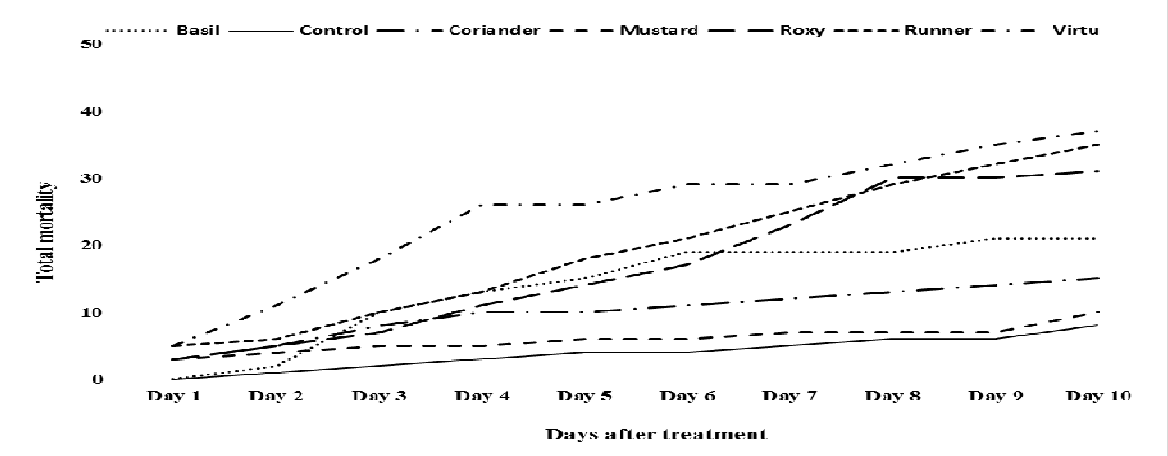

Fig. 2. Total mortality of CLW fourth larval instar after 10 days of treatment by essential oils and IGRs pesticides

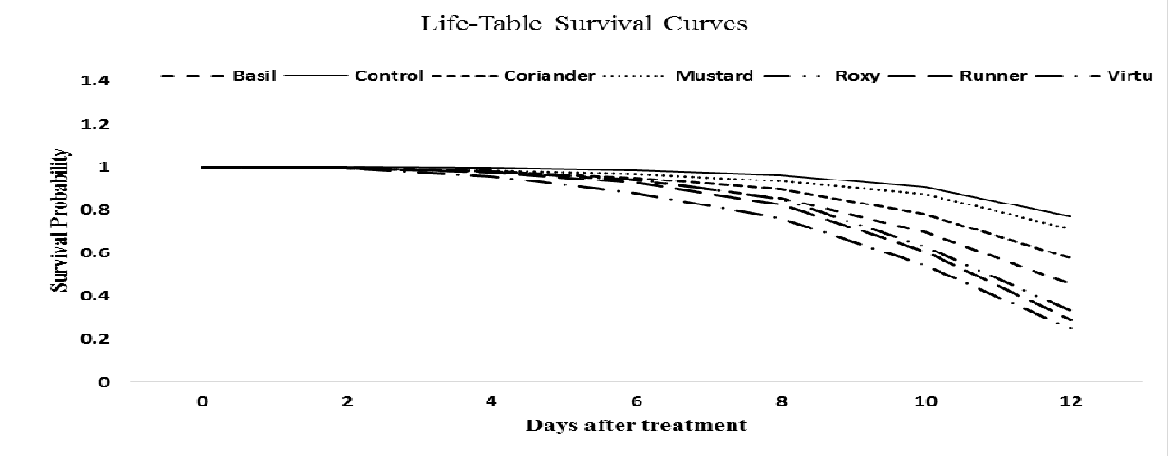

Fig. 3a. Total survival curve of CLW fourth larval instar by treatments (control, tested essential oils and IGRS pesticides) (Life Test Curves)

Based on recording mortality for larvae, the survival of larvae also were estimated (Figure 3a). Results showed that survived larvae were significantly different between treatments (DF= 6, F= 27.9, P<0.0001). Data showed that the highest survival was recorded on control treatment (92% survived larvae) and significantly different with tested IGRS pesticides and basil essential plant oils (Tukey, DF=63, P<.0001). While, control treatment was not significant different with other essential plant oils (coriander and mustard) (Tukey, DF=63, P>0.05). Larvae survival was significantly higher in mustard (90% survived larvae) than basil essential plant oil (79% survived larvae) (Tukey, DF=63, P=0.02), but no differences were found between coriander oil and basil oil and mustard oil (Tukey, DF=63, P>0.05). As expected that the lowest larvae survival was occurred on chromafenozide pesticide (63% survived larvae, which showed significantly different with all tested essential plant oils. Otherwise, larvae survival at chromafenozide pesticide was not significantly different with other tested pesticides (65% and 69% survived larvae for methoxyfenozide and novaluron, respectively) (Tukey, DF=63, P>0.05).

Life test mortality curves were calculated for each treatment, and the analyses showed that total larval survival is significantly higher different between control treatment and all other treatments including IGRS pesticides and essential plant oils except mustard oil (Multiple Comparisons for the Wilcoxon Test, P<0.0001) (P>0.05) (Figure 3b), respectively. Mustard oil showed significantly higher survival than basil oil (P=0.0005), otherwise, no significant differences were found between coriander oil and basil oil and mustard oil (P>0.05). The lowest survival was significantly appeared on chromafenozide pesticide compared to all tested treatments (P<0.0001). On the other hand, no significant differences were found between basil oil and novaluron and methoxyfenozide pesticides, also there was no significant differences between novaluron and methoxyfenozide pesticides (P>0.05).

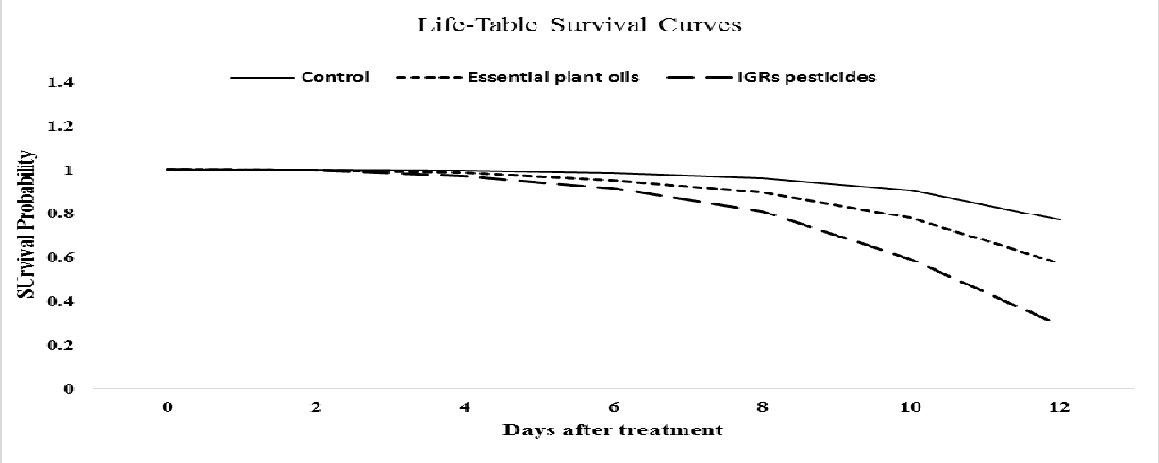

Fig. 3b. Total Survival curve of CLW fourth larval instar by tested group (control, tested essential oils and IGRS pesticides) (Life Test Curves).

Average life test curves were calculated for the three treatment groups (control, essential plant oils and IGRS pesticides). Results showed that, there was a significant differences between IGRS pesticides and control and essential plant oils treatments (P<0.0001), where the lowest larval survival was recorded at IGRS pesticides treatment. While, there was no significant differences between control and essential plant oil treatments (P>0.05).

6. Ovicidal activity of tested essential plant oils and IGRS pesticides on :

a. Laid egg and % egg hatchability:

The latent effect of LC_{25} of tested essential plant oils and IGRS pesticides was evaluated against CLW females adults, where the number of laid eggs were recorded in all treatments and percentage of hatchability was calculated (Table 4). The number of eggs was significantly different between treatments (ANOVA, DF=21, F=4.95, P=0.002). Results showed that the control had the highest number of laid eggs compared to other treatments (1103.25 eggs), but not significantly different with essential plant oils treatments and Chromafenozide pesticide (ANOVA, DF=21, P>0.05). Control treatment was significantly different with Methoxyfenozide and novaluron pesticides (ANOVA, DF=21, P<0.01). Novaluron pesticide appeared to have the most effect on laid eggs, where showed the lowest number of laid eggs (611 eggs) but not significantly different from other IGRS pesticides, basil and mustard oils (ANOVA, DF=21, P>0.05). Otherwise, novaluron pesticide showed significant different with coriander essential plant oils (ANOVA, DF=21, P<0.01). No significant differences were found between tested essential plant oils (ANOVA, DF=21, P>0.05).

Egg hatchability was recorded for each treatment and results showed that statistically, there a significant differences between treatments (ANOVA, DF=21, F=14.17, P<0.0001). Highest percentage of hatchability was found on control treatment (94%) which was significantly different with tested pesticides (Tukey, DF=21, P<0.0001), but not significantly different with tested essential plant oils (Tukey, DF=21, P>0.05). No differences were found between tested oils, where percentage of hatchability ranged between 82%-85%. Meanwhile, the lowest percentage of hatchability was detected on novaluron pesticide (50.5%), which significantly different with tested oils (Tukey, DF=21, P<0.0001), but not significantly different with other tested pesticides (Tukey, DF=21, P>0.05).

b. %Oviposition deterrent activity:

The effect of LC_{25} for tested materials as a oviposition deterrent activity was determined. Significant differences were found between treatments (ANOVA, DF=21, F=3.04, P=0.02). It's obvious that novaluron had the most effect against females as an oviposition deterrent activity (28.3%), which significantly different with control (Tukey, DF=21, P=0.02). On the other hand, no significant differences were found between control and all other treatments. Also, no differences were found between tested oils and tested pesticides (Tukey, DF=21, P>0.05).

c.%Sterility:

Percentage of sterility for tested materials based on number of eggs and percentage of hatchability was also evaluated and results showed that there is a significant differences between treatments (ANOVA, DF=21, F=17.69, P<0.0001). The highest sterility was found on novaluron (68.9%), which significantly different with control and tested oils (Tukey, DF=21, P<0.0001). Otherwise, there is no differences were found between novaluron and other tested pesticides (Tukey, DF=21, P>0.05). No sterility was recorded on control, while low sterility was found on tested oils ranged between 26-31%.

Table 4. Ovicidal activity of some essential plant oils and IGRS pesticides against CLW*

Tested materials		Measured parameters			
		Eggs#/ female	% Hatchability	% Oviposition deterrent activity	% Sterility
Essential plant oils	Coriander	871.25ab	84.70a	11.86ab	28.78c
	Basil	909.25abc	81.95ab	9.35ab	26.61cd
	Mustard	850.75abc	82.75ab	12.45ab	30.83bc
	Virtu	698.50abc	62.03bc	21.89ab	57.13ab
IGRS pesticides	Roxy	611.25c	50.55c	28.28a	68.86a
	Runner	692.25bc	53.15c	22.59ab	64.78a
Control		1103.25a	94.18a	0.0b	0.0d

***Values within a column followed by the same letter are not significantly different via multiple comparison with Tukey (Alpha=0.05).**

DISCUSSION

Currently, the larvicides used for cotton leaf worms control are neurotoxic products. Beyond the pollution for the environment, these insecticides present a toxic effect to other class of animals like birds, fish, bees and mammals (Quarles, 2001 and Rose, 2001). The interest then has been focused on the development of new products that could be good alternatives for pest control. During the last decays a new class of pesticides, acting via the development process on the insect, known the insect growth regulators (IGRs) (Khan and Qamar, 2012 and Smagghe and Degheele, 1994). It was suggested as an alternative to classical synthetic chemical pesticides because of do not present any effect on the environment and non targer animals. The IGRs were developed in order to inhibit or to disrupt the insect growth, the molt and or the metamorphosis (Salem *et al.,* 1997 and Dhadialla *et al.,* 1998).

On the other hand, essential plant oils from plants could also be an alternative source of *S. littoralis* fourth larval instar control. The reason for this is because plant oils are a source of bioactive compounds that are safe for human health and the environment.

The present study was conducted to evaluate the toxicity of some essential plant oils and some IGRs on performance and some biological aspect of CLW. Our results showed that coriander oil have the highest feeding deterrence against CLW fourth larval instar, this may be happened because more than 80% of coriander oil consisted of various terpenes and lacked any identifiable esters, where Khedr and El-Kawas (2013) reported that the major compounds of the coriander oils were Linalool 64.10%, α-pinene 11.96% and β-pinene 4.84%. Also, basil oil and mustard oil showed a fewer feeding deterrence rather than coriander, where researcher mentioned that two major active components of basil oil were identified as: Linalool (33.9 %) and Eugenol (8.31 %) (El-Mesallamy *et al.,* 2015), while allyl isothiocyanate being the main component (71.06%) of mustard oil (Peng *et al.,* 2014). Pavela (2004) reported that some medicinal plants essential oils are larvicidal to the third instar larvae of *S. littoralis*.

In addition, coriander oil elongated the larval duration about 3 days more control followed by mustard oil and basil oil (about1day and 2 days, respectively). Similar results for pupal duration, where essential plant oils elongated the pupal duration 1-2.5 days more than control. This may happened because larvae need food to and energy to molt to the next larval stage or restore food for pupa stage, and in case of coriander oil which showed a feeding deterrent to larval stage, this is may be influence her molt.

Basil oil had affected the percentage of emerged larvae, where fewer larvae were emerged in basil oil compared to control, otherwise no difference were found between basil oil and novaluron, but significantly higher than chromafenozide and methoxyfenozide. Coats *et al.,* (1991) suggested that basil oil mode of action related to neurotoxic, linalool acts on nervous system which effect on ion transport and release of acetylcholine esterase. Enan, (2005) mentioned that, eugenol mimicked octopamine in increasing intracellular calcium level in cloned cells from the brain of *Periplaneta americana* and *Drosophila melanogaster* and cellular changed by eugenol responsible for its insecticidal property (Price and Berry, 2006). Researchers have confirmed the antimicrobial, insecticidal and antifungal properties of the essential oil of basil (Bagamboula *et al.,* 2004; Sacchetti *et al.,* 2004).

Otherwise, tested IGRs did not influenced larval and pupal durations, but have a great effect on the percentage of pupation, emerged larvae and emerged pupae. This could be refer to the natural of IGRs which were developed in order to inhibit or to disrupt the insect growth, the molt and or the metamorphosis (Salem *et al.,* 1997 and Dhadialla *et al.,* 1998). These results could be explained by the accumulation and persistence of ecdysone agonists in the larval tissue until

the pupal molt, at which point the agonist kills the insect (Zarate *et al.,* 2011). In addition, Sundaram *et al.,* (2002) reported the presence of an ecdysone receptor complex in the lepidopteran pupae, also, he mentioned that larval stage is susceptible to ecdysone agonists.

Results showed that novaluron possess an ovicidal action on CLW than control. Where, the highest percentage of oviposition deterrent activity was recorded for novaluron, but not significantly different with other tested IGRs and essential plant oils. Furthermore, novaluron had a high percentage of sterility of 68.86% followed by methoxyfenozide and chromafenozide which higher than plant oils which ranged between 26-31%, which appears that IGRs pesticides have a multiple effect than plant oils. Moreover, egg hatchability was statistically similar for control and plant oils, otherwise the least egg hatchability was recorded for IGRs pesticides. Furthermore, the lowest number of laid eggs were recorded in novaluron followed by methoxyfenozide. Novaluron is consider a chitin synthesis inhibitors influence insect's ability to produce new exoskeletons when molting. Additionally, its impact on the larval stages by inhibiting or blocking the synthesis of chitin which represent 30-60% of the insect exoskeleton structure. Also, result in increasing egg mortality. Chitin synthesis inhibitors include conventional benzoylureas, triazine / pyrimidine derivatives, and buprofezin (Perveen, 2012).

No specific studies have tested the mode of action of novaluron, but focused on applying the general mechanisms and effects with benzoylphenyl ureas. These compounds do not easily inhibit chitin synthesis in cell free systems, but they block the chitin biosynthetic pathway in intact larvae (Oberlander and Silhacek 1998). Although a precise biochemical explanation of the insecticidal activity of benzoylphenyl ureas has been elusive, the most likely hypothesis is that they interrupt *in vivo* synthesis and/ or transport of specific proteins required for assemblage of polymeric chitin (Oberlander and Silhacek 1998). In general, only larvae are affected and all effects, including complete molt inhibition, partial molt inhibition, malformed pupae and failure to feed are due to malformation of the cuticle (Retnakaran and Wright, 1987).

Methoxyfenozide also had a great effect on biological aspects of *S. littoralis*, where recorded the lowest percentage of pupation, lowest number of emerged larvae and emerged pupae (<60%). In other studies, both measured reproductive parameters (fecundity and fertility) were negatively affected in *S. littoralis* when fourth larval instar were exposed to methoxyfenozide (Adel and Shenal 2000). Carpenter and Chandler 1994 elucidated that there is a decrease in either the percentage of the eggs hatching in *H. zea*, or the number of eggs laid by the females in *P. idaeusalis* (Biddinger et al 2006).

Larval mortality was significantly higher on chromafenozide after 10 days of treatment compared to essential plant oils, while there was no significant different between chromafenozide and novaluron and methoxyfenozide. These results could be explained by the accumulation and persistence of ecdysone agonists in the larval tissue until the pupal molt, at which point the agonist kills the insect (Zarate *et al.,* 2011).

Pineda et al 2007 mentioned that treating early instars of cotton leafworm with methoxyfenozide leading to consequent larval mortality. This effect may be due to some combination of a high metabolic stability of the compounds within the larval body tissue and the larval food, and the compounds' high affinities for the target sites. In addition, continuous treating with these compounds for long period may cause sufficient accumulation in the larval tissue and result in induce a lethal molting cycle (Trisyono and Chippendale 1997, 1998).

CONCLUSION

The present study elucidated that the essential oils could be an alternative source of *S. littoralis* fourth larval instar control. Where plant oils are a source of bioactive compounds that are safe for human health and the environment. In addition, using IGRs pesticides had a great effect in controlling fourth larval instar. Mixing both essential plant oils and IGRs could be very effective in suppression of cotton leafworm in the integrated pest management (IPM) programs, further studied will be useful in this concern.

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تأثير بعض الزيوت النباتية الاساسية ومنظمات النمو علي الأطوار الغير كاملة لفراشة دودة ورق القطن في مصر

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تعتبر دودة ورق القطن فة متعددة العوائل تهاجم القطن والعديد من الخضروات والمحاصيل الهامة في مصر. تم تقييم التأثير الكامن لثلاثة مبيدات من منظمات النمو: رنر (٢٤%)، فيرتو (٥%) و روكسي (١٠%) وثلاثة من الزيوت النباتية الاساسية: زيت الكزبرة، زيت الريحان وزيت الخردل ضد الطور اليرقي الرابع وتأثيرها علي الحشرات الكاملة ووضعها للبيض وذلك تحت الظروف المعملية. تم اختبار تأثير التركيز القاتل ل ٢٥% للمواد المختبرة علي بعض النواحي البيولوجية و المؤشرات الغذائية لدودة ورق القطن. اظهر المبيد روكسي نشاط كبيركمانع لوضع البيض حيث سجل اقل عدد للبيض الذي تم وضعه بواسطة الاناث (٦١١ بيضة) مقارنة بالكنترول وزيت الكزبرة (١١٠٣.٣ و ٨٧١ بيضة علي التوالي). أيضا اظهر المبيد روكسي من اكثر المواد المختبرة مانع لوضع البيض بنسبة (٢٣%) وايضا سجلت اعلي نسبة للعقم بنسبة (٦٨.٩%). من ناحية اخري، كانت هناك اختلافات معنوية بين المبيد روكسي والزيوت النباتية المختبرة في العقم. اظهر زيت الكزبرة اعلي تأثير كمؤشرمانع للتغذية ضد العمر اليرقي الرابع والتي احصائيا تختلف معنويا عن بقية المواد المختبرة، بينما اظهرت مبيدات منظمات النمو اقل تأثير كمانع للتغذية تراوح ما بين ٧-٩%. كما لم يختلف معدل النمو النسبي معنويا بين المواد المختبرة. قد امتد العمر اليرقي الرابع ووصل الي ١٦.٢ يوم عند تغذية يرقات العمر اليرقي الرابع علي اوراق الخروع المعاملة بزيت الكزبرة مقارنة بالكنترول الذي استغرق ١٣.٨ يوم. بينما اظهر مبيدات منظمات النمو عمر يرقي اقصر مقارنة بزيت الكزبرة ولكنها معنويا متشابهه مع الكنترول. اوضح مبيد فيرتى اعلي نسبة موت للعمر اليرقي الرابع مقارنة بمعاملات الكنترول والزيوت النباتية.