



## Plant Protection and Pathology Research

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# THE EFFECT OF NON-TRADITIONAL COMPOUNDS ON SOME BIOLOGICAL ASPECTS OF THE COTTON LEAF WORM, *Spodoptera littoralis* UNDER LABORATORY CONDITIONS

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**ABSTRACT:** The effect of sublethal concentration of tested compounds on some biological aspects of the cotton leaf worm, *Spodoptera littoralis* under laboratory were evaluated. There were significant differences between tested compounds, Chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in the larval duration period of the second and fourth instar larvae of cotton leaf worm, *Spodoptera littoralis*. As for, larval mortality, chlorpyrifos was the most toxic compound on second and fourth instar larvae of *S. littoralis*, recording 53.33 and 50.0%, respectively. There were significant differences between tested compounds and control on pre-pupal period, Pupal Pupation% and Pupal weight (g) of second and fourth instar larvae of cotton leaf worm, *Spodoptera littoralis*. Chlorpyrifos recording the highest Pupal duration 15.00 and 12 day compared with other tested compounds and control on second and fourth instar larvae of cotton leaf worm, *S. littoralis*. There were significant differences between tested compounds and control on adult emergence (%), sex ratio and male longevity in second and fourth instar larvae of cotton leaf worm, *S. littoralis*. On the other hand, there were significant differences between tested compound and control except Alpha Cypermethrin and Orange oil in second instar larvae of *S. littoralis* and Chlorpyrifos and *B. thuringiensis* in fourth instar larvae of *S. littoralis*. As for number of eggs/female, fertility, hatchability (%) and deformation (%), Chlorpyrifos recorded the best results in these items.

**Key words:** Bioagents, biological control, bacteria, chemical insecticides.

## INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) is a major pest causing harmful and destructive effects to many economically agricultural and horticultural crops in Egypt. Larvae of *S. littoralis* are the principal damaging stage for cotton, vegetable and ornamentals vegetative and fruiting growth. Scientists conducted many researches to control insects by safe agents and microorganisms to avoid the harmful effects of chemical insecticides. Desuky *et al.* (2006) found that soybean extract recorded the highest reduction of the fourth instar larvae of the cotton leafworm, *Spodoptera littoralis* (44.86%) while NeemAzal extract recorded the highest larval mortality (80.0%) at

all concentrations compared with control. In pupal stage, NeemAzal recorded the lowest pupation (75.00%), as well as Biorepel and soybean decreased pupal weight for all tested extracts compared with control, moreover NemmAzal recorded the highest pupal mortality (25.00%). All tested agents reduced longevity fecundity and hatchability. Hussien *et al.* (2006) evaluated the toxicity of *B.t* toxin 3Aa, on *Spodoptera littoralis*. Moth in spite of similar food utilization and a relatively small difference in the body mass at pupation, female adults that developed from caterpillars fed on newleaf superior lay a mean of 309 eggs compared to a mean of 713 eggs deposited by females that developed from caterpillar fed on superior. Because of this difference and simultaneous

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reduction fertility (egg hatchability), a pair of adults that fed as larvae on New leaf superior produces only 148 karvae, whereas a pair of adults that fed as larvae on Superior produces 556 larvae. It is suggest that small amounts of cry 3Aa that accumulate in insect tissue and persist until the adult stage are responsible caused decline in reproduction.

**Pineda *et al.* (2006)** evaluated the toxicity of spinosad and methoxyfenozide against neonates and fourth instars of *S. littoralis* according to LC<sub>50</sub> values and found that no significant difference were observed between spinosad and methoxyfenozide after 48 hr., of treatment on neonate larvae. In addition, spinosad and methoxyfenozide significantly suppressed weight gain of neonates and 4<sup>th</sup> instars continuously fed with artificial diet containing the insecticides. **El-Morshedy *et al.* (2016)** studied the lethal and sublethal effects of chlorfluazuron, emamectin benzoate, pyrethrins and *Bacillus thuringiensis* sub sp. *kurstaki* and (chlorpyrifos), on the second instar larvae of a laboratory strain of cotton leaf worm, *Spodoptera littoralis*. Emamectin benzoate proved to be the most toxic compound among all the tested insecticides. Furthermore, some biological aspects were also investigated to show the latent effect of the tested compounds, such as: duration periods of larval and pupal stages, mortality (%) in pupal stage, weight of pupae, percentage of emergence, longevity, sex ratio and the hatchability. Data revealed that all compounds varied in their influences on biological aspects and could have relation with toxicity insecticides. **Kedr and El-Kawas (2013)** reported that the biological parameters of *Spodoptera littoralis* and *Tetranychus urticae* were affected due to *Corianderum sativum* essential oil treatment. Both larval and pupal duration were elongated compared to control, where the larval mortality of *S. littoralis* recorded 20 and 16%, respectively and reduced the total number of eggs/female. **Sharaby and El-Nojiban (2015a)** found that the sub lethal concentrations of oils (Garlic, Mint, Cumin, Caraway and Parsley) achieved remarkable significant increase in larva and pupa duration with retardation in their development and increase percentage of mortality of black cutworm, *Agrotis ipsilon*. **Sharaby and El-Nojiban (2015b)** evaluated the biological activity of essential oil of Sage plant leaves against second instar larvae of *Agrotis ipsilon*.

Results showed that 75% larval mortality after 8 days of treatment, then all larvae died at the tenth day, as well as at the sub lethal concentration of the oil reduced egg deposition by 67.4 and 69.4% in egg hatchability and caused above 31.2% sterility among the resulting females, and increased deformities (%) and prolongation of larval period. Pupation percentage decreased with 58% in moths. **Abdel-Aziz *et al.* (2013)** studied the latent effects thyme, bitter and neem oils on certain biological parameters of *Spodoptera littoralis* larvae, and reported all oils caused deformations with various degrees for larvae, pupae and adults. This work aimed to study the effect of Chlorpyrifos, Alpha cypermethrin, Spinosad, *B. thuringiensis* and orange oil on some biological aspects of larvae of cotton leafworm, *Spodoptera littoralis*

## MATERIALS AND METHODS

The present study was carried out in the Cotton Leafworm Research Department, Plant Protection Research institute, Sharkia branch and the Apiary and laboratories of Plant Protection Dept., Fac., Agric. Zagazig Univ. to study at the level of LC<sub>50</sub>. This work aimed to study the effect of Chlorpyrifos, Alpha cypermethrin, Spinosad, *B. thuringiensis* and orange oil on some biological aspects of larvae of cotton leafworm, *Spodoptera littoralis*.

### Tested Compounds

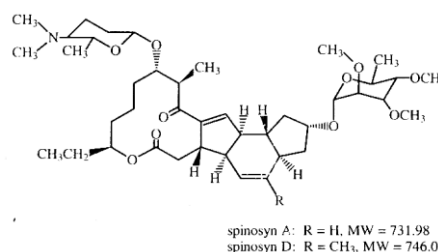
#### *Bacillus thuringiensis*

Trade Name: Dipel 2x (6.4% W.P).

الشركة المنتجة: فالينت بيوسينس- USA

#### Spinosad

Trade Name: Tracer (24% SC)



الشركة المنتجة: داواجر و سنسز

#### Orange oil

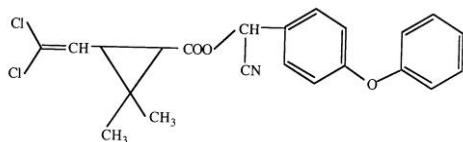
Trade Name: PREV- AM 6(% SL)

الشركة المنتجة: ORO- AGRI, USA

### Alpha-cypermethrin

Trade Name: (Icta Alpha) (10% EC)

IUPAC Name: (RS)-ch-cyano-3-phenoxybenzyl (1RS, 3RS, 1RS, 3SR)-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate.

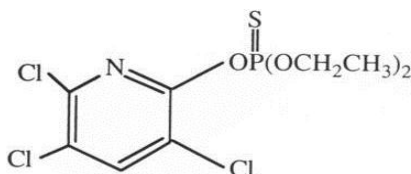


الشركة المنتجة: باريجات انداستريز - بي في تي المحدودة - الهند

### Chlorpyrifos

Trade Name: Robest (48% EC)

IUPAC Name: O, O, diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate.



الشركة المنتجة: باجير اكيمايز انداستريز المحدودة - الهند

### Rearing Technique

The culture of Egyptian cotton leafworm *S. littoralis* was initiated from egg masses collected from cotton field located in Sharkia Governorate. The egg masses were placed in glass jars covered with muslin cloth and fastened with rubber band under laboratory conditions of  $25 \pm 2^\circ\text{C}$ ,  $70 \pm 5$  RH% till hatching. Daily fresh cotton leaves were provided to the larvae. The accumulated feces were cleaned out daily. After pupation, pupae were collected and placed in clean jars until the emergence of adults. Newly emerged moths were sexed and kept in mating cages, each jar containing 10 males and 10 females. Saturated 15% sugar solution cotton wool were placed in each jar and changed daily.

Fresh leaves of *Nerium oleander* were introduced daily into breeding cages as an oviposition site. Laid egg masses were collected daily and transferred into the rearing jars, each jar containing five egg masses.

### Effect of Tested Compounds against *Spodoptera littoralis*

The toxicity of *Bacillus thuringiensis* at the concentrations of 500, 250, 125, 62.5 and 31.25  $10^5$  CFU/ml, while spinosad at 1000, 500, 250,

125 and 62.5 ppm and orange oil at 1000, 500, 250, 125 and 62.5 ppm, and Alpha-cypermethrin and chlorpyrifos at concentrations of 500, 250, 125, 62.5 and 31.25 ppm to determine the  $LC_{50}$  for each.

The tested compounds were evaluated against the first day of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* by leaf dipping technique.

Series concentrations at 3 replicates were prepared for each tested compounds, fresh castor bean leaves dipped into these solutions for 10-30 seconds and air dried at room temperature. The larvae were left to feed on the treated leaves for 48 hrs.

Ten larvae of 2<sup>nd</sup> instar larvae of *S. littoralis* were introduced into 1 L glass jars and were offered for 48 hr., and replaced by untreated leaves. Mortality of larvae was daily observed and calculated till the end of study.

The effect of tested compounds was evaluated on the duration post treatment, pupation%, pupal duration, weight of pupae and adult emergence%.

Mal formation of *S. littoralis* emergence of moths calculated according to Redfera *et al.* (1970) and Staal (1972).

### Statistical Analysis

Collected data were subjected to statistical analysis of variance (ANOVA) at 5% probability, and the measurements were separated using Duncan's Multiple Range Test (SAS, 1990) (DMRT) through CoStat software program (Version 6.400). CoStat version 6.400 Copyright © 1998-2008 Cohort Software. 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

## RESULTS AND DISCUSSION

### Effect of Chlorpyrifos, Alpha Cypermethrin, Spinosad, *B. thuringiensis*, Orange Oil on some Biological Aspects of 2<sup>nd</sup> Larvae of Cotton Leafworm, *Spodoptera littoralis*.

**Effect of sublethal concentration of tested compounds on larval duration period, larval mortality %, pupation%, pre-pupal period, pupal duration (days) and pupal weight (g)**

The obtained data in Table 1 indicated that there was significant difference between chlorpyrifos and control treatment, while there

**Table 1. Biological aspects of *S. littoralis* for 2<sup>nd</sup> instar larvae treated with sublethal concentration (LC<sub>50</sub>) for tested compounds**

Compound	LC <sub>50</sub> ppm	Larval duration (days)	Larval Mortality (%)	Pre pupal period (days)	Pupation (%)	Pupal duration (days)	Pupal weight (g)
Chlorpyrifos	1.14	12.00±	53.33±	1.50±	46.67±	15.00±	0.2900±
		1.00 <sup>a</sup>	5.77 <sup>a</sup>	0.50 <sup>ab</sup>	4.23 <sup>a</sup>	1.00 <sup>a</sup>	0.0100 <sup>bc</sup>
Alpha cypermethrin	35.286	11.00±	50.00±	1.20±	50.00±	13.50±	0.2960±
		1.00 <sup>ab</sup>	5.77 <sup>a</sup>	0.20 <sup>ab</sup>	4.23 <sup>a</sup>	1.00 <sup>ab</sup>	0.0100 <sup>bc</sup>
Spinosad	548.497	10.50±	46.67±	1.60±	53.33±	12.00±	0.2740±
		1.00 <sup>ab</sup>	5.77 <sup>a</sup>	0.30 <sup>a</sup>	4.23 <sup>a</sup>	1.00 <sup>bc</sup>	0.0085 <sup>cd</sup>
<i>B. thuringiensis</i>	152.4	10.10±	50.00±	1.31±	50.00±	11.00±	0.2986±
		1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.30 <sup>ab</sup>	4.23 <sup>a</sup>	1.00 <sup>cd</sup>	0.0015 <sup>b</sup>
Orange oil	97.453	10.53±	46.67±	1.45±	53.33±	13.00±	0.2650±
		1.00 <sup>ab</sup>	5.77 <sup>a</sup>	0.00 <sup>ab</sup>	4.23 <sup>a</sup>	1.00 <sup>b</sup>	0.0200 <sup>d</sup>
Control	-	10.00±	0.00±	1.06±	100±	10.00±	0.3550±
		0.001 <sup>b</sup>	0.00 <sup>b</sup>	0.03 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>d</sup>	0.0200 <sup>a</sup>
LSD 5%	-	1.62	8.39	0.49	2.33	1.62	0.0238

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean ± standard deviation (SD).

were no significant differences among alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control treatments in the larval duration period of the 2<sup>th</sup> instar larvae of cotton leaf worm, *S. littoralis*.

As for mortality % of larvae, the results in Table 1 indicated that chlorpyrifos, alpha cypermethrine and *B. thuringiensis* were the most toxic compounds on 2<sup>nd</sup> instar larvae of cotton leaf worm, *S. littoralis*, recording 53.33, 50.0 and 50.0% compared with other compounds which recorded 46.67%.

The data on the effect of tested compounds on pre-pupal period of 2<sup>nd</sup> instar larvae of *S. littoralis*, Table 1 indicated that there was significant differences between spinosad and control treatments recording pre-pupal period 1.60 day compared to control 1.06 day, while there were no significant differences between the rest compounds and control in this direction.

With respect to pupation%, the data in Table 1 indicated that there were significant differences between tested compounds and control on 2<sup>th</sup> instar larvae of *S. littoralis*, where the pupation% were 46.67, 50.00, 53.33, 50.00, 53.33 and 100% for chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control, respectively.

As for pupal duration, the data in Table 1 indicated that there were no significant differences between all tested compounds and control except *B. thuringiensis*, recording 15.0, 13.5, 12.0, 11.0, 13.0 and 10.0 day for Chlorpyrifos, alpha cypermethrin, spinosad and orange oil and control, respectively. It was obvious that chlorpyrifos recording the highest pupal duration 15.0 day compared with other tested compounds and control.

The data in Table 1 show the effect on pupal weight (g). The obtained data indicated that there were significant differences between tested compounds and control, recording 0.29, 0.296, 0.274, 0.2986, 0.265 and 0.355 g for

chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control, respectively.

#### **Effect of sublethal concentration of tested compounds on adult emergence, sex ratio, and longevity**

The obtained data in Table 2 indicated that there were no significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in adult emergence (%) of the second instar larvae of cotton leaf worm, *S. littoralis*.

As for deformation percentage, data in Table 2 recorded that *B. thuringiensis* and orange oil recorded the highest deformation percentage 9 and 7% compared with other tested compounds and control.

As for sex ratio, the obtained results in Table 2 indicated that there were no significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in female (%) and male (%).

The effect of tested compounds on longevity periods for male and female of the cotton leaf worm, *S. littoralis* Table 2 revealed that there were no significant differences between all tested compounds and control in male longevity period except orange oil, recording 8 day as longevity period.

As for the longevity period for female in pre oviposition, oviposition and post oviposition period, the obtained data show that there were significant differences in pre-oviposition period between alpha cypermethrin, *B. thuringiensis*, chlorpyrifos and control recording 1.7, 1.8, 2.0, 2.0, 1.8 and 1.0 day, respectively. Also, there were significant differences in oviposition period between chlorpyrifos and alpha cypermethrin, comparing to other treatments and control. There were significant differences in post oviposition period between spinosad and control compare to other treatments and control.

As for the effect of tested compounds on longevity periods for female of *S. littoralis* the data in Table 2 show that there were significant differences between *B. thuringiensis*, Orange oil, Control and other three treatments:

Chlorpyrifos, Alpha cypermethrin, Spinosad recording 7.80, 7.90 and 7.0 day, respectively.

#### **Effect of sublethal concentration of tested compounds on fertility and hatchability**

The obtained data in Table 3 indicated that there were significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in the number of eggs/female of the second instar larvae of *S. littoralis*, where, orange oil recorded 1360 eggs/female as the highest, on the other side, chlorpyrifos were the least one recording 905.67 eggs/female comparing with control 1800.0 eggs/female.

As for fertility, the results in Table (3) indicated that there were significant differences among tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* & orange oil and control in the fertility of the 2<sup>nd</sup> instar larvae of cotton leaf worm, *S. littoralis*, where, orange oil recorded 1244 eggs/female as the highest one, on the other side, chlorpyrifos were the least one recording 408 eggs/female, comparing with control 1745.0 eggs/female.

With respect to hatchability %, data in Table 3 indicated that there were significant differences among tested compounds and control on 2<sup>nd</sup> instar larvae of *S. littoralis*, except orange oil which recorded 94.49%. It was obvious that chlorpyrifos recorded the lowest hatchability 75.00% compared to control recording 98.05%.

#### **Effect of sublethal concentration of tested compounds on larval period, larval mortality (%), Pupation (%), pre-pupal period, pupal duration and pupal weight**

The obtained data in Table 4 indicated that there were no significant differences between tested compounds alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in the duration period of the 4<sup>th</sup> instar larvae of cotton leaf worm, *S. littoralis* except chlorpyrifos, recording 10 day duration period.

As for larval mortality (%), results in Table 4 indicated that chlorpyrifos, spinosad and *B. thuringiensis* were the most toxic compounds on 4<sup>th</sup> instar larvae of cotton leaf worm, *S. littoralis*, recording 50% compared with other compounds which recorded 46.67% larval mortality.

**Table 2. Biological aspects of *S. littoralis* larvae after treating 2<sup>nd</sup> instar with the tested insecticides (LC<sub>50</sub>) under laboratory conditions**

Compound	LC <sub>50</sub> ppm	Adult Emergence (%)	Defor- mation %	Sex ratio		Longe- vity ♂	Longevity ♀			longevity ♀
				Female (%)	Male (%)		Pre- ovi.	Ovi.	Post- ovi.	
Chlorpyrifos	1.14	80.00±	2.00±	50.00±	50.00±	9.00±	1.70±	2.17±	2.67±	6.54±
		14.43 <sup>a</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>a</sup>	0.11 <sup>a</sup>	0.50 <sup>b</sup>	0.29 <sup>b</sup>	0.11 <sup>b</sup>
Alpha cypermethrin	35.286	80.00±	3.00±	50.00±	50.00±	9.00±	1.80±	2.50±	2.50±	6.80±
		13.23 <sup>a</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.97 <sup>a</sup>	0.15 <sup>a</sup>	0.29 <sup>b</sup>	0.50 <sup>b</sup>	0.15 <sup>b</sup>
Spinosad	548.497	85.00±	1.00±	50.00±	50.00±	8.50±	2.00±	3.00±	3.11±	6.11±
		11.55 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.90 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.29 <sup>a</sup>	0.29 <sup>b</sup>
<i>B. thuringiensis</i>	152.4	93.33±	9.00±	50.00±	50.00±	8.30±	2.00±	3.00±	2.80±	7.80±
		11.55 <sup>a</sup>	7.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.90 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.29 <sup>b</sup>	0.29 <sup>a</sup>
Orange oil	97.453	93.33±	7.17±	50.00±	50.00±	8.00±	1.80±	3.60±	2.50±	7.90±
		11.55 <sup>a</sup>	6.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	0.50 <sup>a</sup>	0.30 <sup>a</sup>	0.50 <sup>b</sup>	0.15 <sup>a</sup>
Control	-	100.00±	0.00±	50.00±	50.00±	8.00±	1.00±	3.00±	3.00±	7.00±
		0.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.05 <sup>b</sup>	0.50 <sup>b</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.00 <sup>a</sup>
LSD 5%	-	20.32	0.75	0.00	0.00	0.55	0.43	0.54	0.36	0.79

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean ± standard deviation (SD).

**Table 3. Biological aspects of *S. littoralis* larvae after treating 2<sup>nd</sup> instar with the tested insecticides (LC<sub>50</sub>) under laboratory conditions**

Compound ppm	LC <sub>50</sub> ppm	No. of eggs/female	Fertility	Hatchability (%)
Chlorpyrifos	1.14	905.67±	408.00±	75.00±
		70.00 <sup>c</sup>	31.51 <sup>c</sup>	2.19 <sup>c</sup>
Alpha cypermethrin	35.286	970.00±	455.33±	77.00±
		37.64 <sup>c</sup>	17.79 <sup>e</sup>	2.22 <sup>c</sup>
Spinosad	548.497	1042.33±	740.00±	87.00±
		96.00 <sup>c</sup>	67.51 <sup>d</sup>	2.76 <sup>b</sup>
<i>B. thuringiensis</i>	152.4	1219.00±	1072.33±	90.90±
		154.04 <sup>b</sup>	135.54 <sup>c</sup>	1.24 <sup>ab</sup>
Orange oil	97.453	1360.00±	1244.00±	94.49±
		47.00 <sup>b</sup>	43.00 <sup>b</sup>	1.80 <sup>a</sup>
Control	-	1800.00±	1745.00±	98.05±
		96.00 <sup>a</sup>	93.50 <sup>a</sup>	1.88 <sup>a</sup>
LSD 5%	-	163.51	135.54	9.56

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean ± standard deviation (SD).

**Table 4. Biological aspects of *S. littoralis* larvae after treating 4<sup>th</sup> instar with the tested insecticides (LC<sub>50</sub>) under laboratory conditions**

Compound	LC <sub>50</sub> ppm	Larval duration (day)	Larval mortality (%)	Pre pupal period (day)	Pupation (%)	Pupal duration (day)	Pupal weight (g)
Chlorpyrifos	1.239	10.00±	50.00±	1.50±	50.00±	12.00±	0.2933±
		1.00 <sup>a</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>b</sup>	1.00 <sup>a</sup>	0.0100 <sup>b</sup>
Alpha cypermethrin	48.948	9.50±	46.67±	1.45±	53.33±	11.00±	0.2960±
		1.00 <sup>ab</sup>	5.77 <sup>a</sup>	0.51 <sup>ab</sup>	5.55 <sup>b</sup>	1.00 <sup>ab</sup>	0.0090 <sup>b</sup>
Spinosad	633.853	9.00±	50.00±	1.75±	50.00±	10.30±	0.2830±
		1.00 <sup>ab</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.00 <sup>b</sup>	0.70 <sup>bc</sup>	0.0130 <sup>b</sup>
<i>B. thuringiensis</i>	234.6	8.50±	50.00±	1.50±	50.00±	9.50±	0.2990±
		1.00 <sup>ab</sup>	0.00 <sup>a</sup>	0.50 <sup>ab</sup>	0.00 <sup>b</sup>	0.10 <sup>bc</sup>	0.0100 <sup>b</sup>
Orange oil	131.924	8.85±	46.67±	1.30±	53.33±	10.80±	0.2820±
		0.90 <sup>ab</sup>	5.77 <sup>a</sup>	0.30 <sup>ab</sup>	5.55 <sup>b</sup>	0.90 <sup>ab</sup>	0.0080 <sup>b</sup>
Control	-	8.00±	0.00±	1.00±	100.00±	9.00±	0.3880±
		0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>c</sup>	0.0090 <sup>a</sup>
LSD 5%	-	1.59	5.93	0.69	12.36	1.506	0.0177

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean  $\pm$  standard deviation (SD).

The data on the effect of tested compounds on pre-pupal period of 4<sup>th</sup> instar larvae of cotton leaf worm, *S. littoralis* Table 4, indicated that there were no significant differences between tested compounds and control, except spinosad exhibited significant differences recording pre-pupal period 1.75 day compared with control which recorded 1.00 day.

With respect to pupation (%), data in Table 4 indicated that there were no significant differences between tested compounds and control on 4<sup>th</sup> instar larvae of *S. littoralis*, where the pupation (%) were 50, 53.33, 50, 50, 53.33 and 100% for chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control, respectively.

#### **Effect of Chlorpyrifos, Alpha Cypermethrin, Spinosad, *B. thuringiensis*, Orange Oil on Some Biological Aspects 4<sup>th</sup> Cotton Leaf Larvae**

As for pupal duration, data in Table 4 indicated that there were significant differences between chlorpyrifos, alpha cypermethrin,

orange oil and control, recording, 12.0, 11.0, 10.8 and 9.0 day, respectively.

Regarding to pupal weight, data in Table 4 recorded that there were no significant differences between tested compounds and control, recording 0.2933, 0.296, 0.283, 0.299, 0.282 and 0.388 g for chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control, respectively.

#### **Effect of sublethal concentration of tested compounds on adult emergence, sex ratio, and longevity**

The obtained data in Table 5 indicated that there were no significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in adult emergence % of the 4<sup>th</sup> instar larvae of *S. littoralis*.

As for deformation percentage, the data in Table 5 indicated that there were significant differences between *B. thuringiensis* and orange oil treatments and other treatments and control. It was obvious that *B. thuringiensis* and orange

**Table 5. Biological aspects of *S. littoralis* larvae after treating 4<sup>th</sup> instar with the tested insecticides (LC<sub>50</sub>) under laboratory conditions**

Compound	LC <sub>50</sub> ppm	Adult Emergence (%)	Deform- ation (%)	Sex ratio		Longevit y ♂	Longevity ♀			Longevit y ♀
				Female (%)	Male (%)		Pre- ovi.	Ovi.	Post- ovi.	
Chlorpyrifos	1.239	85.00±	2.00±	50.00±	50.00±	9.88±	1.70±	2.50±	3.00±	7.20±
		13.23 <sup>a</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.80 <sup>a</sup>	0.29 <sup>b</sup>	0.35 <sup>b</sup>	0.50 <sup>a</sup>	0.06 <sup>b</sup>
Alpha cypermethrin	48.948	93.33±	3.00±	50.00±	50.00±	9.67±	1.80±	2.33±	3.00±	7.13±
		11.55 <sup>a</sup>	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>a</sup>	0.25 <sup>b</sup>	0.58 <sup>b</sup>	0.50 <sup>a</sup>	0.68 <sup>b</sup>
Spinosad	633.893	91.67±	1.00±	50.00±	50.00±	9.50±	2.10±	2.18±	3.00±	7.28±
		14.43 <sup>a</sup>	0.50 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.90 <sup>a</sup>	0.53 <sup>a</sup>	0.50 <sup>b</sup>	0.58 <sup>a</sup>	0.83 <sup>b</sup>
<i>B. thuringiensis</i>	234.6	93.33±	9.00±	50.00±	50.00±	9.85±	2.22±	2.50±	3.00±	7.72±
		11.55 <sup>a</sup>	7.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.85 <sup>a</sup>	0.40 <sup>a</sup>	0.50 <sup>b</sup>	0.58 <sup>a</sup>	0.36 <sup>a</sup>
Orange oil	131.924	91.67±	7.17±	50.00±	50.00±	8.55±	1.80±	3.00±	3.50±	7.80±
		14.43 <sup>a</sup>	6.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.50 <sup>a</sup>	0.87 <sup>a</sup>
Control	-	100.00±	0.00±	50.00±	50.00±	8.00±	1.00±	3.00±	3.00±	7.00±
		0.00 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	0.50 <sup>c</sup>	0.76 <sup>a</sup>	0.50 <sup>a</sup>	0.87 <sup>b</sup>
LSD 5%	-	21.2778	0.75	0.00	0.00	1.18	0.58	0.9376	0.33	0.69

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean  $\pm$  standard deviation (SD).

oil recorded the highest deformation percentage 9.00 and 7.00% compared with other tested compounds and control.

As for sex ratio, the obtained results in Table 5 indicated that there were no significant differences between tested compounds and control in female and male % of the 4<sup>th</sup> instar larvae of *S. littoralis*.

The effect of tested compounds on longevity periods of male and female of *S. littoralis* were determined (Table 5). Data found that there were no significant differences between all tested compounds and control in male longevity period.

As for the longevity period for female, the obtained data in Table 5 show that there were significant differences in pre oviposition period between Chlorpyrifos, Alpha Cypermethrin, *B. thuringiensis*, and control recording 1.7, 1.8, 2.1, 2.22, 1.8 and 1.0 day, respectively. Also, there were significant differences in oviposition period between orange oil and control and chlorpyrifos, alpha, cypermethrin, *B. thuringiensis* recording, 3.0 and 3.0 day.

As for female longevity periods, there were significant differences between *B. thuringiensis* and orange oil and other treatments recording 7.72 and 7.80 day, respectively, while it was 7.2, 7.13, 7.28, and 7.0 for the treatments of Chlorpyrifos, Alpha Cypermethrin, Spinosad and control, respectively.

#### Effect of sublethal concentration tested compounds on fertility, hatchability, and deformation

The obtained data in Table 6 indicated that there were significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in the number of eggs/female of the 4<sup>th</sup> instar larvae of *S. littoralis*, where, orange oil recorded 1680 eggs/ female as the highest, on the other side, chlorpyrifos were the least one recording 935 eggs/female.

Regarding to fertility results in Table 6 revealed that there were significant differences between tested compounds, chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil and control in the fertility where,



**Table 6. Biological aspects of *S. littoralis* after treating 4<sup>th</sup> instar with the tested insecticides (LC<sub>50</sub>) under laboratory conditions**

Compound	LC <sub>50</sub> ppm	No. of eggs/female	Fertility	Hatchability (%)
Chlorpyrifos	1.239	935.00±	447.00±	75.00±
		63.84 <sup>c</sup>	74.91 <sup>d</sup>	2.50 <sup>c</sup>
Alpha cypermethrin	48.948	980.67±	512.00±	77.00±
		17.62 <sup>c</sup>	94.40 <sup>d</sup>	2.86 <sup>c</sup>
Spinosad	633.853	1123.00±	730.00±	87.00±
		80.99 <sup>d</sup>	68.51 <sup>c</sup>	2.93 <sup>b</sup>
<i>B. thuringiensis</i>	234.6	1551.67±	843.33±	90.90±
		81.68 <sup>c</sup>	71.06 <sup>bc</sup>	1.33 <sup>b</sup>
Orange oil	131.924	1680.00±	912.00±	94.67±
		78.73 <sup>b</sup>	90.02 <sup>b</sup>	1.83 <sup>a</sup>
Control	-	1919.67±	1840.00±	98.55±
		37.90 <sup>a</sup>	52.12 <sup>a</sup>	1.93 <sup>a</sup>
LSD 5%	-	115.41	136.03	5.68

Means with the same letter in each column are not significant different ( $p < 0.05$ ).

Data expressed as mean  $\pm$  standard deviation (SD).

orange oil recorded 912.0 eggs/female as the highest, on the other side, Chlorpyrifos were the least one 447.0 eggs/female.

With respect to hatchability (%), the data in Table 6 indicated that there were significant differences between tested compounds and control in this direction. It was obvious that chlorpyrifos recorded the lowest hatchability percentage 75% while orange oil recorded the highest one 94.67% compared with other tested compounds, while it was 98.55% in control.

Generally, nearly all tested compounds exhibited toxic effect on some biological aspects such as, pupal weight (g), pupal duration (day), longevity of male and female, number of eggs/female, fertility, hatchability (%) and Deformation (%) of cotton leaf worm, *S. littoralis*. Chlorpyrifos were the most toxic compound in 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of cotton leaf worm, *S. littoralis*.

The obtained data were in agreement with those conducted by **Ismail and Morshedy (2009)** who evaluated some environmental safe chemicals against *Spodoptera littoralis*, also

**Ismail and Shaker (2014)** who studied the efficacy of some essential oil against immature stages of *Spodoptera littoralis*. Moreover, **Sharaby and El-Nojiban (2015 a)** who found that the sub lethal concentrations of Garlic, Mint, Cumin, Caraway and Parsley oils achieved remarkable significant increase in larval and pupa duration with retardation in their development and increase percentage of mortality of black cutworm *Agrotis ipsilon*. **El-Morshedy et al. (2016)** found that emamectin benzoate proved to be the most toxic compound among all the tested insecticides chlorfluazuron, emamectin benzoate, pyrethrins and *Bacillus thuringiensis* sub sp. *kurstaki* and chlorpyrifos, on the second instar larvae of cotton leaf worm, *S. littoralis*. Some biological aspects also investigated to show the latent effect of the tested compounds, such as: duration periods of larval and pupal stages, mortality (%) in pupal stage, weight of pupae., percentage of emergence, longevity of adult male and female, sex ratio and the hatchability revealed that all compounds varied in their influences on biological aspects and these could have relation

with toxicity insecticides against *S. littoralis* larvae. In addition, the obtained results are confirmed with those of **Abd El-Aziz et al. (2011)**, **Hendawi et al. (2017)** and **Abd El-Samei et al. (2019)**.

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## تأثير بعض المبيدات الحشرية الغير تقليدية على بعض الصفات البيولوجية لدودة ورق القطن تحت الظروف المعملية

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تم تقييم تأثير التركيزات المثبطة للمركبات Chlorpyrifos, alpha cypermethrin, spinosad, *B. thuringiensis* and orange oil على بعض الجوانب البيولوجية للطور اليرقى الثانى والرابع لدودة ورق القطن *Spodoptera littoralis* تحت ظروف المعمل، أظهرت النتائج انه توجد فروق معنوية بين المركبات المختبرة، الكلوربيريفوس، ألفا سايبيرمثرين، سبينوساد، باسيلس ثورينجينسيس وزيت البرتقال والكنترول في فترة الطور اليرقى لكل من العمر الثانى والرابع لدودة ورق القطن. أما بالنسبة لموت اليرقات، فقد كان الكلوربيريفوس هو المركب الأكثر سمية ليرقات الطورين الثانى والرابع من *S. littoralis* مسجلين 53.33 و 50.0% على التوالي، توجد فروق معنوية بين المركبات المختبرة والكنترول في فترة ما قبل العذارى ونسبة تشرنق العذراء ووزن العذراء ليرقات العمر الثانى والرابع لدودة ورق القطن. سجل الكلوربيريفوس أعلى مدة للعذارى 15.00 و 12 يوماً مقارنة بالمركبات الأخرى المختبرة والكنترول على يرقات الطورين الثانى والرابع لدودة ورق القطن *S. littoralis*، أظهرت نتائج التحليل الاحصائي أنه توجد فروق معنوية بين المركبات المختبرة والكنترول في النسبة المئوية لخروج الفراشات الكاملة ونسبة الجنس وعمر الذكور ليرقات العمر الثانى والرابع من دودة ورق القطن. من ناحية أخرى، توجد فروق إحصائية معنوية بين المركبات المختبرة والكنترول باستثناء زيت البرتقال و Alpha Cypermethrin في يرقات الطور الثانى من *S. littoralis* والخصوبة، ونسبة الفقس والتشوه، فقد سجل الكلوربيريفوس أفضل النتائج في كل هذه العناصر بالمقارنة بباقي المواد المختبرة.

الكلمات الإسترشادية: المواد الحيوية، المكافحة البيولوجية، البكتيريا، المبيدات الحشرية الكيميائية.

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