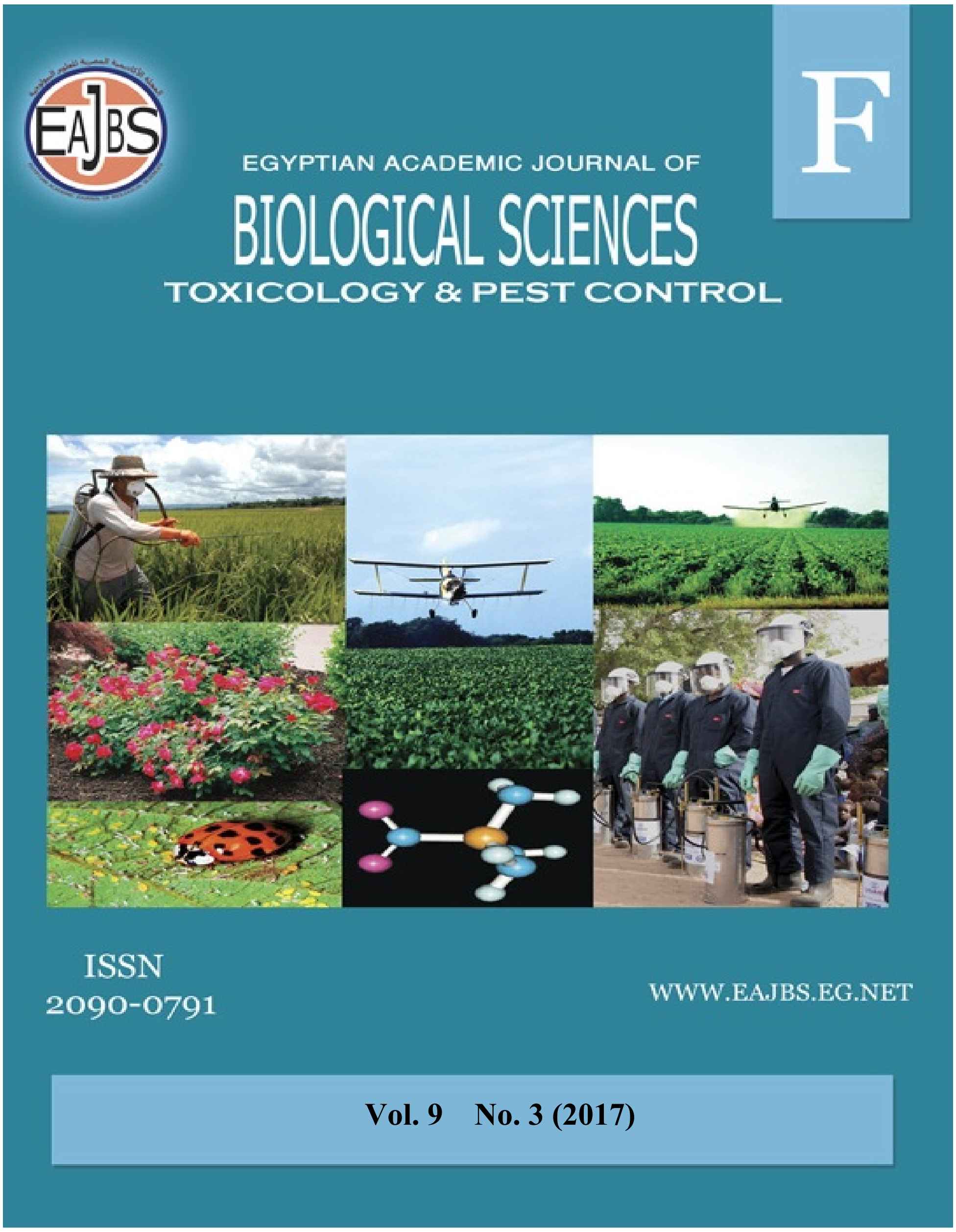

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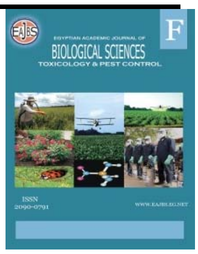

Biochemical Parameters, Toxicity, Development and Reproductive Effects of Two Novel Insecticides on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

The response of the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), to lethal, sublethal and biochemical parameters effects of clothianidin and metaflumizone were determined by a leaf dipping technique bioassays. Mortality of newly molted 4th instar larvae increased with the concentration resulting LC_{50} values of 70.24 ppm and 20.41 ppm, for clothiandin and metaflumizone respectively.

Sublethal effects were studied by treatment of 4th instar larvae with a concentration equivalent to LC_{50}. The larval development time, from treatment until pupation, of the survivors were significantly increased in both insecticides, the pupation period shorted significantly for both sex but shorted insignificantly in male pupa treated with clothianidin, and the weighty of pupa was reduced significantly in both insecticides. However, no significant differences were found in the oviposition and oviposition days viability and percentage of egg viability, but significant differences were found in the preoviposition days when larvae treated with metaflumizone, fecundity percentage decreased in both insecticides and adult longevity was shorted in either both sex or both insecticides.

Biochemical analysis indicated that while treatment of 4th instar larvae with LC50 of both pesticides had no significant effect on acetylcholine esterase activity (AChE) in larval homogenate, it significantly decreased larval contents of total protein and total carbohydrates, reduced alkaline phosphatase (ALP), and esterase (EST) activities, and increased glutathione-s-transferase (GST) acitivity.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a key pest of cotton and other many crops in the Mediterranean area and Middle Eastern countries (Gómez and Rivero 1951; Campion *et al.* 1977; Nasr *et al.* 1984; Ahmad 1988; Domínguez 1993 and Belda *et al*., 1994). The fact that the insect infests more than 112 host plants belonging to 44 families (Moussa *et al.* 1960; Brown and Dewhurst 1975; Hatem *et al.* 2015 and Hatem *et al.* 2016) makes it a model of serious polyphagous pests. The control of this pest is focused to the searching of new insecticides with biological and ecological qualities (e.g. clothianidin and metaflumizone).

One of these strategies, the using of new pesticide group such as neonicotinoids like neonicotinoids are relatively new class of pesticides which belong to the fastest-growing class of pesticides in modern pest protection followed after the conventional pesticide group (Ahmed and Matsumura 2012).

Interestingly, neonicotinoids have a unique mode of action as compared to other classes of insecticides in that they act as the agonists to the nicotinic acetylcholine receptor (nAChRs) (Shao *et al*. 2013 and Sandor *et al*. 2015). Furthermore, their mammalian toxicities are generally low and also show low acute toxicities to birds, and fish, but displayed significant toxicity to bees (Ahmed 2011). Thus, the use of neonicotinoid pesticides is recommended to be increased in future to control the lepidopterous pests (Lorenzo and David 2015). Metaflumizone, was discovered by Nihon Nohyaku in the early 1990's and belongs to the new class of semicarbazone insecticides. Metaflumizone is derived from the pyrazoline chemistry and acts by the voltage-dependent sodium channels in insects binding. Metaflumizone produces a relaxed paralysis in a broad range of important pest insects (Lidia and Justin 2007and Kristopher *et al*. 2010).

The lethal effects are the most frequently evaluated parameters. Pesticides at sublethal concentrations have a strong impact on insects physiologically and behaviorally (Haynes 1988). Although sublethal effects on surviving insects should also be considered; for instance, insects not dying by product-insect interaction may have their physiological processes affected resulting in development and reproduction alterations (Desneux *et al*. 2007). Sublethal doses of insecticides may be potentially toxic to different instars and stages of insects through diverse effects such as interfering with the function of glutathione S-transferases (GST), carboxylesterase, and other metabolic enzymes, or changing the behavioural patterns associated with feeding, migration, reproduction, and/or the exchange of chemical information (Haynes 1988).

Furthermore, the extensive usage of these pesticides in cotton leafworm control has been lead to the emergence of pesticides resistance (Mahmoud *et al*. 2009 and Abdel Rahman *et al*. 2014). Thus, it is very considerable to use new effective countermeasure strategy in order to avoid the development of resistance particularly among major insect pests of great health concern. Kullik *et al*. (2011) reported that the clothianidin at a rate of 25 mg kernel-1 on Bt corn increased larval mortality and reduced larval weight of freshly molted third instar of black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). There is little information on lethal and sublethal effects of metaflumizone in *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae). Feng-Juan *et al*. (2012) showed that the pupation rate, emergence rate and total number of eggs laid by one female of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), in both generations were significantly lower in the LC15 or LC25 groups than in the control group. Zhang *et al*. (2012) reported that pupation rate, pupal period and pupal weight were significantly declined comparing with the control *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) third instar larvae were exposed to LC_{15} and LC_{25} of metaflumizone, the fecundity lagged behind control group. Han *et al*. (2012) found that the metaflumizone at the sublethal concentration (0.09 mg/L) tested decreased the pupation rate and pupal weight, prolonged the pupal duration and decreased the emergence rate, fecundity, oviposition duration and longevity of adults in the treated generation of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae).

Sublethal effects may be as important as lethal effects in crop protection programs as a result of feeding suppression, delayed development, and reduced reproductive potential of survivos. Thus, sublethal effects of clothianidin and metaflumizone may be great importance in regulating population of a target species, particulary in polivoltine species.

The objectives of this study were to determine: (1) lethal effects of clothianidin and metaflumizone on *S. littoralis* larvae. (2) sublethal effects of both insecticides on development and reproduction, and (3) sublethal effects of clothianidin and metaflumizone as a biological control agent on some biochemical parameters such as total protein total carbohydrates and enzyme activity in 4th larval instars of *S. littoralis*.

MATERIALS AND METHODS

Insects:

A laboratory colony of *S. littoralis* continuously was reared away from insecticidial contamination in Plant Protection Research Institute. Rearing of insects were conducted following the technique described by (El- Defrawi *et al*. 1964). Larvae were fed on fresh castor bean (*Ricinus communis* L.) leaves until pupation. Moths were fed on 10% sugar solution soaked a piece of cotton tissue. Each jar was provided with branches of tafla, *Nerium oleander* L. as an oviposition site. The insects were maintained at 25±2 °C, 65±5% RH and a photoperiod of 16:8 h (L:D).

Bioinsecticides:

- Clothianidin 48% SC wt/vol SC (Supertox-1): was obtained from Jiangsu Flag chemical industry Co., China.

- Metaflumizone 24% SC wt/vol SC (Alverde): was obtained from BASF, France.

Bioinsecticidal effects:

Different concentrations of clothianidin (20, 40, 60, 80 and 100 ppm for *S. littoralis* and metaflumizone (5, 10, 20, 40 and 80 ppm were assayed against *S. littoralis* 4th larvae by using leaf dipping technique. Fresh castor bean leaves were dipped in each concentration for 10 second. Each concentration was replicated three times and each replicate contained 30 larvae. Control larvae were fed on castor bean leaves immersed in distilled water. Mortality was recorded every 24 hrs after treatment.

Sublethal effects on reproductive:

Newly molted 4th instar of *S. littoralis* larvae was treated with LC_{50}'s for clothianidin 70.24 ppm and metaflumizone 20.41 ppm. Larvae fed on treated leaves during 24-48 hrs. Control larvae were fed on caster bean leaves immersed in distilled water. Males from the nighty after emergence were paired with 1 or 2-day-old virgin females in jars (6 cm in diameter and 12 cm high) which were supplied with leaves of *N. oleander* as an oviposition site, one pair per jar and fed on 15% honey solution and maintained in the containers until they died. Egg production was recorded daily and eggs were allowed to hatch. All bioassays were conducted at 25±2 ºC. Mortality was recorded every 24hrs.

Biochemical parameters:

Sample preparation for biochemical analysis:

The 4th instar larvae of *S. littoralis* were immobilized by chilling on ice for 15 minutes. The larvae were cleaned with distilled water, weighed and homogenized in cold distilled water. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants obtained after centrifugation were used for determination of total protein, total carbohydrates and enzyme activity assays.

Total carbohydrates assay:

Total carbohydrates were extracted, prepared for assay and estimated in samples by the phenol-sulphuric acid reaction according to procedure of Dubois *et al*. (1956) and Crompton and Birt (1967). Total carbohydrate is expressed as mg glucose/gm larval fresh weight.

Total protein assay:

Total protein concentration was determined by using Coomassie Brilliant blue G-250 reagent and bovine albumin as a standard according to the method of Bradford (1976). Total protein is expressed as mg/g larval fresh weight.

Enzyme activity assays:

Alpha esterases (α-EST) and beta esterases (β-EST) were determined according to Van Asperen (1962) using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively. The activity of the EST was presented as ug α-nphthol/min /g.b.wt.

Acetylcholinesterase (AchE) activity was measured according to the method described by Simpson *et al*. (1964) using acetylcholine bromide (AchBr) as substrate and expressed as ug AchBr/min/g.b.wt.

Alkaline phosphatase (ALP) activity was measured according to the method described by Powell and Smith (1954) using p-nitrophenyl phosphate (p-NP) as substrate. the enzyme activity is expressed by unit (U), where 1 unit represents amount of enzyme that hydrolyze 1.0 u mole of p-nitophenyl phosphate per minute at 37°C, at pH 10.4.

Glutathione S-transferase (GST) activity was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate based on the methods of Habig *et al*. (1974). The activity of the GST was presented as m.mole sub.conj/min/g.b.wt.

Analysis of data:

Median lethal concentrations (LC50,s) were determined by linear regression analysis and a test was made for parallelism according to the relative potency estimation method (Finney 1971) using the microcomputer program POLO-PC (Russell *et al*. 1977). The larval and pupal development, preoviposition, oviposition period, total fecundity, egg viability and adult longevity data were analysed by ANOVA and comparison of means by the least significant difference test (LSD) were calculated. The assays for total protein, total carbohydrates and enzyme activity were performed in 3 replicates, and the data were subjected to one way ANOVA. The means were separated by Tukey Multiple Range Test for significance ($p<0.05$) using costat software for windows (Costat 2007).

RESULTS

Bioinsecticidal effects:

The mortality percentage increased with the concentration of Clothianidin and Metaflumizone. The larvae died from 1 to 2 days after treatment, for both insecticides (Table 1) but the most of them died in the same instar of treatment for both insecticides.

Table 1: Mortality of *Spodoptera littoralis* larvae treated in fourth instar with clothianidin and metaflumizone.

Treatment	Concentration (ppm)	Nº	Mortality	
			nº	%
Clothianidin	0	90	0	0
	20	90	10	11.11
	40	90	20	22.22
	60	90	44	48.89
	80	90	52	57.79
	100	90	55	61.11
Metaflumizone	0	90	0	0
	5	90	30	27.78
	10	90	44	48.89
	20	90	46	51.11
	40	90	49	54.44
	80	90	60	66.67

Nº=Treated larvae number. nº=Died larvae number.

Analysis by probit regression line revealed the following equations and LC_{50}'s (with 95% confidence limits): y=2.36X+4.13; χ^2=3.35 (3df) and 70.42 ppm (62.16±80.30) for Clothianidin; y=0.71X+6.65; χ^2=3.94 (3df) and 20.41 ppm (13.56±30.96) for Metaflumizone. The adjustment was acceptable using the test χ^2.

Sublethal effects on reproductive:

Development period of the male and female larvae treated with Clothianidin (8.56 and 7.62 days respectivily) and Metaflumizone (12.19 and 12.00 days respectivily) were prolonged significantly for male and femal (F=369; df=2; p=0.0000 and F=416; df=2; p=0.0000) respectivily (Table 2). Development period of the pupae treated with each insecticide was shorter than the control pupae, while males tended to develop slower than females too (Table 3). Pupal development of females was significantly (F=32.5; df=2; p=0.0000 and F=242; df=2; p=0.0000) decreased but the male pupae were not affected by the treatments. Pupal weights differed significantly depending on the insecticide on which the larvae were treated and differed significantly between females and males when they treated with both insecticide (F=12.70; df=2; p=0.0000 and F=47.60; df=2; p=0.0000) (Table 4). The female pupae were generally heavier than their male, and heavier in control than treatment.

Table 2: Effects of clothianidin and metaflumizone on larval development of *Spodoptera littoralis*.

Concentration (ppm)	Sex	Nº	Treatment	Time (days)		
				Mean	Range	S.E.
0.0	♀	34	Control	7.29 a	7-9	0.14
70.24		29	Clothianidin	7.62 a	7-8	0. 15
20.41		43	Metaflumizone	12.00 b	11-14	0.12
0.0	♂	36	Control	8.11 a	7-9	0.12
70.24		34	Clothianidin	8.56 b	7-9	0.12
20.41		42	Metaflumizone	12.19 c	11-13	0.11

Means followed by the same letter are not significantly different (LSD, p = 0.05). S.E. = Standard error.

Table 3: Effects of clothianidin and metaflumizone on pupal development of *Spodoptera littoralis*.

Concentration (ppm)	Sex	Nº	Treatment	Time (days)		
				Mean	Range	S.E.
0.0	♀	34	Control	9.50 a	9-11	0.13
70.24		29	Clothianidin	9.03 b	8-10	0.14
20.41		43	Metaflumizone	8.14 c	7-9	0.11
0.0	♂	36	Control	11.47 a	10-12	0.11
70.24		34	Clothianidin	11.26 a	10-13	0.11
20.41		42	Metaflumizone	8.59 b	8-10	0.10

Means followed by the same letter are not significantly different (LSD, p=0.05). S.E=Standard error.

Table 4: Effects of clothianidin and metaflumizone on pupal wieghty of *Spodoptera littoralis*.

Concentration (ppm)	Sex	Nº	Treatment	weighty (grams)		
				Mean	Range	S.E.
0.0	♀	34	Control	0.26 a	0.2-0.36	0.005
70.24		29	Clothianidin	0.24 b	0.21-0.29	0.006
20.41		43	Metaflumizone	0.23 c	0.20-0.33	0.005
0.0	♂	36	Control	0.26 a	0.20-0.31	0.005
70.24		34	Clothianidin	0.20 b	0.16-0.25	0.005
20.41		42	Metaflumizone	0.20 b	0.16-0.25	0.005

Means followed by the same letter are not significantly different (LSD, p=0.05). S.E.=Standard error

Most of females were mated and most of them were fecund and fertil, independently of treatment (Table 5). Mean number of eggs laid per female surviving to Clothianidin and Metaflumizone were decreased in all mating combinations respect to the control combination, but this difference were statistically significant (p=0.0000). The mean percentage of viability per female for both insecticide bioassays decreased in most of the combinations related to the control, but the differences were not statistically significant (p=0.5378) (Table 6). Mean number of laid eggs and laid eggs viable days did not differ significanlty among treated and control (p=0.2244 and p=0.2041). Mean days of preovipostion were longer in larvae treated with Metaflumeziron than either Clothianidian or control with significant differences (p=0.0000) (Table 7). The mean of adults longevity for males and females were shorted significantly in treated larvae than control (p=0.0000 and p=0.0000) (Table 8).

Table 5: Response of *Spodoptera littoralis* females came from larvae treated with clothianidin and metaflumizone according to different mating combinations.

Combination	Nº	Nº females	
		Eggs laid	Eggs viable laid
♀ $_{control}$ x ♂ $_{control}$	15	10	10
♀ $_c$ x ♂ $_c$	16	12	12
♀ $_m$ x ♂ $_m$	15	13	13

Nº = Mated female number. c = clothianidin treatment. m = metaflumizone treatment.

Table 6: Reproductive potential of *Spodoptera littoralis* larvae treated with clothianidin and metaflumizone according to different mating combinations.

Combination	Nº	Fecundity		% Viability	
		Mean	Range	Mean	Range
♀ $_{control}$ x ♂ $_{control}$	10	1485.0 a	1075-2050	76.03 a	56-100
♀ $_c$ x ♂ $_c$	12	721.2 b	200-1550	73.32 a	42.86-100
♀ $_m$ x ♂ $_m$	13	655.8 b	125-1350	64.97 a	20-97.41

Means followed by the same letter are not significantly different (LSD, p= 0.05). Nº = Mated female number.
c = clothianidin treatment. m = metaflumizone treatment.

Table 7: Laid periods of *Spodoptera littoralis* females came from larvae treated with clothianidin and metaflumizone according to different mating combinations.

Combination	N_1	Nº Preoviposition days		Nº Oviposition days		N_2	Nº Oviposition days viability	
		Mean	Range	Mean	Range		Mean	Range
♀ $_{control}$ x ♂ $_{control}$	10	3.10 a	1-5	3.8 a	2-5	10	3.4 a	1-5
♀ $_c$ x ♂ $_c$	12	2.00 a	1-3	3.0 a	1-5	12	2.5 a	1-4
♀ $_m$ x ♂ $_m$	13	3.23 b	2-4	3.1 a	1-5	13	2.92 a	1-5

Means followed by the same letter are not significantly different (LSD, p= 0.05). N1 = Fecundity female number.
N2 = Fertility female number. c= clothianidin treatment. m= metaflumizone treatment

Table 8: Adults longevity of *Spodoptera littoralis* came from larvae treated with clothianidin and metaflumizone according to different mating combinations.

Combination	Nº	Males		Females	
		Mean	Range	Mean	Range
♀ $_{control}$ x ♂ $_{control}$	10	11.2 a	6-14	9.5 a	6-11
♀ $_c$ x ♂ $_c$	12	5.7 b	4-11	5.8 b	4-9
♀ $_m$ x ♂ $_m$	13	7.1 b	5-10	6.9 b	5-9

Means followed by the same letter are not significantly different (LSD, p= 0.05). Nº = Mated female number.
c = clothianidin treatment. m = metaflumizone treatment.

(Table 9) shows the results of biochemical analysis of *S. littoralis* 4th instar larvae treated with LC_{50} of Clothianidin and Metaflumizone compared with untreated control larvae. Treatment with Clothianidin and Metaflumizone resulted in slight reduction of larval total protein contents (32.1±0.8 and 34.2±0.7 mg /g.b.wt, respectively) compared with the untreated control (40.9±1.4 mg /g.b.wt). Total carbohydrates also significantly decreased from 11.8±0.9 mg /g.b.wt for untreated larvae to 9.5±0.4 and 6.1±0.5 mg /g.b.wt for larvae treated with Clothianidin and Metaflumizone, respectively. The activity of α-EST was significantly lower in Clothianidin treated larvae than control (249.0±8.0 Vs 326.3±15.5 respectively) while both insecticides causes significant reduction in β-EST and ALP activities.

Table 9: Amounts of total protein, total carbohydrates and the activities of selected enzymes in *S. littoralis* 4th instar larvae treated with LC_{50} of metaflumizone and clothianidin compared with untreated control larvae (mean ± SD).

	Control	Clothianidin	Metaflumizone
Total protein (*mg /g.b.wt*)	40.9±1.4 a	32.1±0.8 b	34.2±0.7b
Total carbohydrates (*mg /g.b.wt*)	11.8±0.9 a	9.5±0.4 b	6.1±0.5b
α-EST *(ug α-nphthol/min /g.b.wt)*	326.3±15.5 a	249.0±8.0 b	327.7±5.5a
β-EST *(ug β-nphthol/min /g.b.wt)*	163.0±9.8 a	116.7±4.2 b	143±6.1c
AchE *(ug AchBr/min/g.b.wt)*	251.3±8.1 a	238.3±7.6 a	267.7±8.9a
ALP *($U \times 10^3$ /g.b.wt)*	433±27.4 a	278.7±6.8 b	271.7±7.4b
GST *(m mole sub.conj/min/g.b.wt)*	25.3±1.7 a	30.4±0.6 b	31.2±1.1b

Means in the same raw with different letters are significantly different (LSD, $p < 0.05$).

The GST activity was obviously higher in Clothianidin and Metaflumizone treated larvae than untreated control larvae (30.4±0.6, 31.2±1.1 and 25.3±1.7 respectively). Table 9 also shows that AChE activity does not change significantly in larvae exposed to LC_{50} of Both insecticides compared with control

DISCUSSION

Bioinsecticidal effects:

The laboratory colony of *S. littoralis* tested was susceptible to both insecticides. Metaflumizone and Clothianidian were present a high toxicity for some lepidopteran species like *Agrotis segetum* (Denis & Schiffermuller) (Lepidoptera: Noctuidae) was evaluated through leaf dipping method of bioassay against third and sixth instar larvae of *A. segetum*. The Clothianidin was more toxic for the third instar larvae and the LD50 was 1.6 ng a.i./larva but for sixth instar larvae LD_{50} was 3.5 ng a.i./larva (Nikhil and Verma 2015). The LC_{50} value obtained in *S. litttoralis* was similar to the LC_{50} reported by Tofangsazi *et al*. (2015) in the larvae of *Herpetogramma phaeopteralis* (Guenée 1854) (Lepidoptera: Crambidae) (46.6 ppm), treated with clothianidin by topical application of insecticides against medium-sized (third and fourth instar) *H. phaeopteralis*.

Khakame *et al*. (2013) evaluated the toxicity to Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to third instar larvae using the leaf dipping bioassays. The concentrations were calculated in milligrams per liter (ppm). The result showed that Metaflumizone toxic to *P. xylostella*. The populations of *P. xylostella* from different 14 geographical areas in China displayed a narrow variation in LC50 ranged from 1.34 to 8.16 ppm. Metaflumizone could provide an effective alternative insecticide for Diamondback moth management. Tian *et al*. (2014) reported that the

Metaflumizone was toxic to beet armyworm, Spodoptera exigua. Emmanouil *et al*. (2013) were used in a leaf-dipping bioassay for second-instar larvae of Tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) from Greece. All assays were performed in a 32- cell replication dish (RT32W; Bioserve, USA, www.insectrearing.com). The LC_{50} ranged from 31.8 to 159.5 mgL-1, to the second instar *T. absoluta*. The LC_{50} values for Metaflumizone were different in the Ankara-Beypazarı 52.7 ppm and Antalya-Serik 21.02 ppm population of *T. absoluta* L2 larvae. The experiment was conducted using a leaf-dipping bioassay method with L2 larvae. This indicates that the susceptibility levels of these populations to this insecticide are different. However it is difficult to decide using only two populations without a susceptible population (Karaagac 2012).

Sublethal effects on reproductive:

Our results showed that the development period of the male and female larvae treated with Clothianidin and Metaflumizone were prolonged significantly. Pupation period of *S. littoralis* males was about two days longer than females, either treated or nontreated larvae, as have been previously reported by Vargas Osuna (1985). For this reason the study of pupation period was made considering the males and females separately. As general the development period of the pupae treated with each insecticide was shorter than the control pupae, while males tended to develop slower than females too. Pupal development of females was significantly decreased but the male pupae were not affected by the treatments. Pupal weights differed significantly depending on the insecticide on which the larvae were treated and differed significantly between females and males when they treated with both insecticide. The female pupae were generally heavier than their male, and heavier in control than treatment. Kullik *et al*. (2011) reported that the Clothianidin at a rate of 25 mg kernel-1 on Bt corn increased larval mortality and reduced larval weight of freshly molted third instar of Black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidopter: Noctudiae). There are little information on lethal and sublethal effects of Metaflumizone in *S. littoralis*. (Feng-Juan *et al*. 2012) showed that the pupation rate and emergence rate of *Spodoptera exigua* (Hübner) (Lepidopter: Noctudiae) in both generations were significantly lower in the LC_{15} or LC_{25} groups than in the control group. Zhang *et al*. (2012) reported that pupation rate, pupal period and pupal weight were significantly declined comparing with the control *P. xylostella* third instar larvae were exposed to LC_{15} and LC_{25} of Metaflumizone. Han *et al*. (2012) found that the Metaflumizone at the sublethal concentration (0.09 mg/L) tested decreased the pupation rate and pupal weight, prolonged the pupal duration and decreased the emergence rate, in the treated generation of *P. xylostella*.

The reproduction of *S. littoralis* has been affected with some of the treatments studied but in different form according to the insecticide. Mean number of eggs laid per female surviving to Clothianidin and Metaflumizone were decreased in all mating combinations respect to the control combination but this difference was statistically significant. The mean percentage of viability per female for both insecticide bioassays decreased in most of the combinations related to the control but the differences were not statistically significant. Mean number of laid eggs and laid eggs viable days did not differ significanlty among treated and control. Mean days of preovipostion were longer in larvae treated with Metaflumeziron than either Clothianidian or control with significant differences. Adults longevity of *S. littoralis* was not affected when treated with the treated insecticides. The mean of adults longevity for males and females were shorted significantly in treated larvae than control.

Feng-Juan *et al*. (2012) showed that the emergence rate and total number of eggs laid by one female of *S. exigua* (Hübner) in both generations were significantly lower in the LC_{15} or LC_{25} groups than in the control group. Zhang *et al*. (2012) reported that the fecundity of *P. xylostella* third instar larvae were exposed to LC_{15} and LC_{25} of Metaflumizone lagged behind control group. Han *et al*. (2012) found that the Metaflumizone at the sublethal concentration (0.09 mg/L) tested decreased the emergence rate, fecundity, oviposition duration and longevity of adults in the treated generation of *P. xylostella*.

Metaflumizone is a voltage-dependent sodium channel blockers that selectively target voltage-gated sodium channels in the slow inactivated state by binding at or near the local an aesthetic receptor within the sodium channel pore (Stein and Soderlund 2012 and Tian *et al*. 2014). Clothianidin is new insecticide similar to imidacloprid, and other neonicotinoids acting selectively on insect nicotinic acetylcholine receptors (nAChRs) as an activator of post-synaptic acetylcholine receptors but do not inhibit acetylcholine esterase (AChE) (Thany 2009). The two compounds are without ester bond in their chemical structure, which means that hydrolysis by esterases might not the major mechanism for their detoxification by insects. This explain why treatment of *S. littoralis* larvae with LC_{50} of the two insecticides did not affect the activity of AChE compared with untreated control as it act on ester bond. However alpha and beta esterase also act on ester bond and the significant decrease in alpha and beta esterase activities in larvae treated with both insecticides may be due to their inhibition by sequestration. Esterases have been reported to function primarily as sequestration proteins in several insect species including *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)*, Culex quinquefasciatus* (Say) (Diptera: Culicidae)*, Culex pipiens* (L.) (Diptera: Culicidae) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Lee and Clark 1998 and Tian *et al*. 2014). Sayyed and Wright (2006) confirmed that resistance to indoxacarb, another voltage dependent sodium channel blockers in a field population of diamondback moth was esterase associated. Similarly Smirle *et al*. (2010) and Jemec *et al*. (2007) reported significant decrease in the activity of esterase in *Aphis pomi* (de Geer, 1773) (Hemiptera: Aphididae), *Aphis spiraecola* (Patch.) (Hemiptera: Aphididae) and *Daphnia magna* (Straus 1820) (Cladocera: Daphniidae) exposed to LC_{50} of imidacloprid, another neonicotinoids similar to Clothianidin. Our results also showed significant decrease in alkaline phosphatase activity due to treatment with both insecticides. The decrease in level of alkaline phosphatase activity in response to treatments indicates insecticide's harmful effect on insect's digestive system and development as ALP; play an important physiological role in digestive system (Bharat *et al*. 2017). On the other hand *S. littoralis* larvae treated with LC_{50} of both Clothianidin and Metaflumizone were found to possess significantly higher GST activity compared to the untreated control. These results reflect the important role of GST in the detoxification of Clothianidin and Metaflumizone. In accordance with our results Yalcin *et al*. (2015) confirmed an increased enzyme activity of *T. absoluta* due to Metaflumizone treatment. Also GST activity was noticeably increased during exposure of *Helix aspersa* (Müller 1774) (Stylommatophora: Helicidae) (Radwan and Mohamed 2013) and zebrafish (Ge *et al*. 2015) to the neonicotinoid imidacloprid . Results of this study also showed that treatment of *S. littoralis* larvae with Clothianidin and Metaflumizone affected not only the investigated enzymes, but also significantly reduced larval contents of total carbohydrate and proteins, which reflects a generally impaired physiological state of an organism. The decreased amounts of total carbohydrates and proteins confers to the antifeedant nature of the investigated compounds where the larvae unable to metabolise the diet provided for its survival, growth, and reproduction (Jemec *et al*. 2007 and Bharat *et al*. 2017).

Further studies will therefore aim to investigate the histology involved (e.g., midgut, ovary, testes,..etc) in these effects on development and reproduction of the surviving insects. These results were valuable for the practical use of the Clothianidin and Metaflumizone in Integrated Pest Management (IPM) programmes.

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ARABIC SUMMERY

دراسات بيوكيميائية وسمية وتطوريه و تكاثرية لاثنين من المبيدات الحشرية الجديدة على دودة ورق القطن الكبرى

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تم دراسة إستجابة دودة ورق القطن الكبرى للتركيزات القاتلة وللتركيز تحت المميت لكل من مبيد الكلوثيانيدين و مبيد الميتافلوميزون بالمعاملة ليرقات العمر الرابع عن طريق الغمر لورق الخروع. لوحظ أنة قد إزدادت نسبة الموت بزيادة التركيز لكلا المبيدين وقد أظهرت النتائج أن الجرعة النصفية القاتلة LC50 ليرقات العمر الرابع هى ٧٠,٢٤ و٢٠,٤١ جزء في المليون لمبيد الكلوثياندين ومبيد الميتافلوميزون على التوالي. وتمت دراسة التأثيرات تحت المميتة عن طريق معاملة يرقات العمر الرابع بالتركيز النصفى القاتل لكلا المبيدين. وقد أظهرت النتائج زيادة فترة تطور اليرقات بصورة معنوية مقارنة بالكنترول لكلا المبيدين، و هى الفترة من المعاملة بالمبيد و حتى التعذير، أيضا لوحظ إنخفاض فترة طور العذراء بشكل ملحوظ لكلا الجنسين ولكن بصورة أقل و بشكل ملحوظ في عذراء الذكور المعاملة بالكلوثيانيدين، وإنخفض وزن العذراء بشكل كبير لكلا المبيدين مقارنة بالكنترول. ومع ذلك لم يتم ملاحظة أى فروق معنوية في فترة وضع البيض بينما فى فترة وضع البيض المخصب تم ملاحظة فروق معنوية في فترة ما قبل وضع البيض عند معاملة اليرقات مع ميتافلوميزون، وإنخفضت نسبة الخصوبة لكلا المبيدين مقارنة بالكنترول وطول فترة حياة الفراش لكلا الجنسين لكلا المبيدين. وقد أظهر التحليل البيوكيميائي أن معاملة يرقات العمر الرابع بالجرعة النصفية القاتلة LC_{50} لكلا المبيدين لم يكن لهما تأثير معنوي على نشاط إنزيم أستيل كولين إستريز في تجانس اليرقات، فقد إنخفضت معنويا محتويات اليرقات من البروتين الكلي، وإنخفاض الألكلاين فوسفاتيز، وبيتا إستريز، وزيادة نشاط جلوتاثيون-s-ترانسفيرز. وبالإضافة إلى ذلك، أثرمبيد ميتافلوميزون بشكل ملحوظ على محتوى اليرقات من الكربوهيدرات الكلية، في حين أن مبيد كلوثيانيدين أدى إلى إنخفاض كبير في نشاط إنزيم ألفا إستيريز.

كلمات المفتاحية: تركيز تحت مميت ،الحيوية ، خصوبة البيض، طول عمر الفراش، القياسات البيوكيميائية.