Effects of some Insecticides on some Biological Parameters of Cotton Leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

Toxicity and sublethal effects of fipronil, chlorantraniliprole, emamectin benzoate and novaluron against the 2nd instar of *Spodoptera littoralis* larvae after 24 and 48 hrs were evaluated. The LC_5 values after 48 hrs of exposure were 0.062, 0.0008, 0.0001 and 0.048 mg L^{-1} for fipronil, chlorantraniliprole, emamectin benzoate and novaluron, respectively. While LC_{10} values after 48 hrs of exposure were 0.097, 0.0013, 0.0002 and 0.027 mg L^{-1}, respectively. Treated 2nd instar of *S. littoralis* larvae by LC_5 and LC_{10} values of fipronil, chlorantraniliprole, emamectin benzoate and novaluron showed significant ($P < 0.05$) reduction in larval body weights, larval duration, % pupation, pupal mean weight and % adult emergence rates. However, the average time to the pupation (pupal duration) for larvae treated did not change significantly in all treatments compared to control. Fecundity, fertility and adult longevity were strongly or softly reduced in all insecticide treatments compared to control. These results suggest that sublethal concentrations of the tested insecticides may reduce the population growth of *S. littoralis* by affecting the development and reproduction.

Keywords: *Spodoptera littoralis*, population growth, fipronil, chlorantraniliprole, emamectin benzoate, novaluron.

INTRODUCTION

Cotton leafworm, *Spodoptera littoralis* (Boisduval), (family: Noctuidae) is one of the most destructive agricultural lepidopterous pests of cotton and vegetable plants in Egypt through its different growth stages (Hatem *et al.*, 2009). The 1st, 2nd and 3rd generations of *Spodoptera* larvae reduces the yield by 50, 65 and 40%, respectively (El-Sherif *et al.*, 1991). The insect causes considerable damage by feeding on leaves, fruiting points, flower buds and, occasionally, also on bolls. So, it requires several insecticides and applications to control (Abou-Taleb; 2016).

The intensive use of broad-spectrum insecticides against *S. littoralis* has led to the development of resistance to many registered insecticides. To overcome problems associated with conventional insecticides, new insecticidal groups have been developed. Many of these compounds have greater selectivity to the target species, with likely less harmful (El-Sheikh, 2015).

Fipronil is a phenyl pyrazole insecticide commercially used since 1993 (Tingle *et al*., 2003). It is moderately hazardous pesticide and widely used to control veterinary, residential, and agricultural pests (Qureshi et al.; 2016). About 2000, 800 metric tons were produced worldwide (de Oliveira *et al.* 2011). In Australia, fipronil is registered for pest control in a broad range of crops, and is currently registered as a seed treatment for the control of phytophagous midge larvae in rice (Stevens *et al.* 2011). It is a nervous poison for insects by blocking GABA-gated and glutamate-gated chloride channels and subsequent death (Cole *et al.*, 1993 and Sefcikova *et al.* 2018). Fipronil exhibits high selectivity to insects which are resistant to cyclopentadiene, organic phosphorus, organic chlorine, pyrethroids, carbamate pesticides, and those which have no cross-resistance to existing pesticides (Tu *et al*. 2019).

Chlorantraniliprole is a new generation efficient anthranilic diamides insecticide developed by DuPont in 2000 (Sharma *et al.*, 2014 and Lahm *et al.*, 2007), with many advantages such as high efficiency, low toxicity, broad spectrum, long persistence and low residue (Ren *et al.*, 2008 and Lahm *et al.*, 2009). It has been reported to exhibit excellent efficacy against lepidopteran insects (Lavtizar *et al.*, 2015).

Emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopterans (Teran-Vargas *et al.*, 1997). Emamectin benzoate acts as a chloride channel activator, which decreases the excitability of neurons. Shortly after exposure, the insect larvae stop feeding, become irreversibly paralyzed, and die in 3-4 days (Grafton-Cardwell *et al.*, 2005).

Novaluron is a relatively new chitin synthesis inhibitor that inhibits the chitin formation on larvae of various insects (Lepidoptera, Coleoptera, Homoptera and Diptera). It has a potent insecticidal activity against several important foliage feeding insect pests (Cutler *et al*., 2005) with low toxicity to mammals, birds and earthworms.

As new types of insecticides currently generated more attention regarding *Spodoptera* pest control due to

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concerns about environmental risks and resistance for conventional insecticides. Therefore, the main objective of this study is to investigate the sublethal effects of fipronil, chlorantraniliprole, emamectin benzoate and novaluron on life parameters (longevity, fecundity, and fertility) and progeny (pupal formation and adult emergence), of *S. littoralis*.

MATERIALS AND METHODS

Insects

A susceptible strain of the *S. littoralis* has been reared for many years in the Plant Protection Research Institute, Giza, Egypt. Larvae were fed on castor bean leaves under controlled laboratory conditions (25 ± 2 °C, RH 65%) for several years avoiding exposure to any pesticides according to the method of Eldefrawi *et al.*, (1964).

Tested insecticides

Fipronil (Rado-X 80%, WG), chlorantraniliprole (Coragen 20%, SC), emamectin benzoate (Albin- X 50%, WG) and novaluron (Roxy 10% EC), were obtained from Jiangsu Tuoqiu Agrochemical Co., DU PONT DU NEMOURS Co., Shandong Sino-Agri United Biotechnology LTD. and United Phosphorus Ltd., respectively.

Toxicity of sublethal concentrations of tested insecticides against *S. littoralis*

A leaf dip bioassay method (Eldefrawi *et al.*, 1964) was used. Homogenous castor bean leaf pieces were dipped in six concentrations of each tested insecticide (prepared in water) for 10 sec., and dried at room temperature. Treated castor bean leaf pieces were introduced to ten 2nd instar larvae (5 ± 0.3 mg mg/larva), which had been starved for two hrs. The cups were covered with lids and maintained at 25 ± 2 °C. Each concentration was replicated five times. After 24 hrs., fresh untreated castor bean leaf pieces were added to each cup. Mortality was recorded after 24 and 48 hrs, corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). The median lethal concentrations, confidence limits and the slope were calculated. LC_5 and LC_{10} after 24 hrs insecticide exposure followed by other 24 hrs without insecticides were selected as sublethal concentrations for the subsequent experiments.

Latent effects of sublethal concentrations of tested insecticides against *S. littoralis*

Castor bean leaves were dipped in the determined LC_5 and LC_{10} of the tested insecticides. Four hundred 2nd instar larvae (5 ± 0.3 mg / larva) in 4 replicates was used for each treatment and provided with treated leaves. After 24 hrs, surviving larvae were transferred to jars containing fresh untreated leaves and observed daily for pupation and emergence. Larval, pupal and adult durations were determined. Larval and pupal weights and percentages of adult emergence were recorded. Resulted adults were placed in plastic cups provided with a folded sheet paper as oviposition site. Two adult males were kept with one adult female to maximize the probability of successful mating. The sublethal effects of tested insecticides on fecundity (total number of eggs/female) and fertility (hatchability percentages of eggs) were determined.

Statistical Analysis:

The data was analyzed using CoStat Statistical software,1998, according to statistical procedure of analysis of variance (ANOVA), and in case of significant differences, (L.S.D) at 5% level of probability.

RESULTS AND DISCUSSION

Toxicity of fipronil, chlorantraniliprole, emamectin benzoate and novaluron against 2nd instar of *S. littoralis* larvae after 24 and 48 hrs are presented in Table (1). The LC_{50} values after 24 hrs for fipronil, chlorantraniliprole, emamectin benzoate and novaluron were 0.711, 0.06, 0.017 and 5.439 mg L^{-1}, respectively, and 0.458, 0.009, 0.0014 and 0.357 mg L^{-1}, respectively, after 48 hrs. The LC_5 and LC_{10} values after 48 hrs of exposure were 0.062 and 0.097 mg L^{-1} for fipronil, 0.0008 and 0.00013 mg L^{-1} for chlorantraniliprole, 0.0001 and 0.0002 mg L^{-1} for emamectin benzoate, and 0.027 and 0.048 mg L^{-1} for novaluron.

Sublethal effects of the tested insecticides on some biological aspects of *S. littoralis*:

Effects of sublethal concentrations of the tested insecticides on some biological parameters of *S. littoralis* are presented in Tables (2, 3 and 4). The average weight of treated larvae was decreased significantly compared to control during the observation period (Table 2). It is clear that, the higher concentration of all tested insecticides (LC_{10}) was more potent to reduce the larval weight compared with the LC_5, and chlorantraniliprole and novaluron were the most potent.

Table 1. Toxicity of the tested insecticides against 2nd instar of *S. littoralis* larvae after 24 and 48 hrs

Insecticide	Time after exposure (hrs)	LC_{50} (mg L^{-1}) (95% CL)	LC_5 (mg L^{-1}) (95% CL)	LC_{10} (mg L^{-1}) (95% CL)	Slope ± SE
Fipronil	24	0.711 0.509-0.953	0.016 0.002-0.046	0.038 0.008-0.086	1.01±0.17
	48	0.458 0.247-0.683	0.062 0.002-0.057	0.097 0.006-0.094	1.89±0.24
Chlorantraniliprole	24	0.06 0.046-0.079	0.005 0.003-0.007	0.008 0.005-0.012	1.49±0.14
	48	0.009 0.007-0.011	0.0008 0.0004-0.0012	0.0013 0.0008-0.0019	1.53±0.14
Emamectin benzoate	24	0.017 0.014-0.022	0.0023 0.0013-0.0035	0.0036 0.0023-0.0051	1.88±0.18
	48	0.0014 0.0012-0.0017	0.0001 0.00007-0.0002	0.0002 0.0001-0.0003	1.65±0.19
Novaluron	24	5.439 2.878-11.653	0.319 0.039-0.445	0.596 0.107-0.853	1.34±0.12
	48	0.357 0.276-0.450	0.027 0.013-0.045	0.048 0.026-0.074	1.47±0.14

Table 2. Effect of the tested insecticides on the larval weight, larval duration and pupation of 2nd instar larvae of *S. littoralis*

Insecticide	Conc. (mg L^{-1})	Mean weight (mg/larva) after treatment ± SE			Mean larval duration ± SE	Mean Pupation (%) ± SE
		4 days	8 days	12 days		
Control		61.4±3.2	323.3±5.6	812.7±8.5	14.5±1.0a	97.5±1.1a
Fipronil	0.062	54.2±2.1	264.4±3.3	575.4±6.8	18.0±1.5b	81.6±3.2b
	0.097	50.4±1.9	244.6±3.1	565.4±11.7	19.0±1.5b	78.4±2.5b
Chlorantraniliprole	0.0008	45.3±1.5	211.1±3.1	441.8±4.8	19.5±1.4b	66.6±1.8c
	0.0013	42.3±1.4	199.5±3.3	389.1±7.5	20.4±1.2b	64.4±2.4cd
Emamectin benzoate	0.0001	49.4±1.3	225.4±2.1	465.4±7.5	16.2±1.1ab	52.2±2.1d
	0.0002	48.2±1.5	222.9±2.4	411.4±9.8	17.4±0.9b	50.4±1.1d
Novaluron	0.027	44.2±1.8	198.2±2.2	399.4±10.5	22.1±1.1c	68.8±2.2c
	0.048	42.4±1.7	190.4±3.2	410.5±12.7	23.2±2.1c	56.1±1.5d

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

The average time to the pupation for larvae treated with the LC_5 and LC_{10} of fipronil, chlorantraniliprole, emamectin benzoate and novaluron were significantly longer than those in the control treatment. These times were 18.0 and 19.0 days for fipronil, 19.5 and 20.4 days for chlorantraniliprole, 16.2 and 17.4 days for emamectin benzoate and 22.1 and 23.2 days for novaluron at LC_5 and LC_{10}, respectively, where it was 14.5 days for control. Sublethal concentrations of the tested insecticides had a considerable effect on pupation. The LC_5 treatments of fipronil, chlorantraniliprole, emamectin benzoate and novaluron caused lower pupation percentage (81.6, 66.6, 52.2 and 68.8%, respectively) than the control treatment (97.5%). Also, significant decrease in pupation percentages (78.4, 64.4, 50.4 and 56.1) were achieved with the LC_{10} of fipronil, chlorantraniliprole, emamectin benzoate and novaluron, respectively. The present results are in agreement with Zhang *et al*. (2013), who reported that, chlorantraniliprole at sublethal concentrations significantly reduced the larval body mass of *Helicoverpa armigera*. Also, Nawaz *et al*. (2017) observed that chlorantraniliprole was toxic to *Harmonia axiyridis*, and increased the length of the pre-adult developmental time. On the other hand, the sublethal concentrations of emamectin benzoate against *S. littoralis* larvae were significantly reduced the time taken for 50% death, and larval weight, and disrupted larval development, and stopped insects from feeding after exposure (El-Sheikh, 2015). In addition, the present data are similar to those Moustafa *et al.* (2016); Abou-Taleb (2016) and Khan *et al.* (2018).

As shown in Table (3), all treatments significantly suppressed the pupal weight compared to control treatment. The average of pupae weights were 305.4, 232.4, 289.4 and 270.4mg / pupa in the LC_5 of fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments, respectively, compared to 411.5 mg / pupa in the control treatment. While the weight averages were 299.5, 211.1, 266.7 and 231.4mg / pupa in the LC_{10} of fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments, respectively. However, pupal duration did not significantly change in all treatments compared to control. Reduction in the adult emergence rates were significantly achieved by all treatments. Adult emergence were 78.4, 69.1, 80.0 and 65.3% for fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments (LC_5), respectively, compared to 99.0% in the control treatment. On the other hand, the percentages were 79.0, 65.4, 78.4 and 57.4% respectively, for the LC_{10} of fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments.

Table (4) represents the effect of insecticides treatments on the adult fecundity, fertility and longevity. It is clear that the LC_{10} of the tested compounds were reduced the adult fecundity (number of eggs laid) to 543.0, 336.5, 390.0 and 288.8/female for fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments, respectively compared to 859.0/female in control treatment. Also, the LC_5 significantly decreased the adult fecundity (567.5, 382.0, 418.0 and 308.5/female), respectively. Fertility (percentages of egg hatch) was significantly decreased under all insecticides treatments, at LC_{10} , 79.0, 65.0, 78.0 and 54.0% for fipronil, chlorantraniliprole, emamectin benzoate and novaluron treatments, respectively, compared to 98.0% in control. On the other hand, the treatments had low different effect on adult longevity compared to control.

Table 3.Effect of the tested insecticides on the pupal weight, pupal duration and %adult emergence of 2nd instar larvae of *S. littoralis*

Insecticide	Conc. (mg L^{-1})	Pupal weight (mg/pupa) ± SE	Pupal duration (days) ± SE	%Adult emergence ± SE
Control		411.5±4.2a	8.0±0.6a	99.0±1.0a
Fipronil	0.062	305.4±4.1b	7.5±0.4a	78.4±2.2b
	0.097	299.5±3.1b	7.4±0.4a	79.0±2.4b
Chlorantraniliprole	0.0008	232.4±3.4d	7.1±0.7a	69.1±2.7c
	0.0013	211.1±4.5e	7.2±0.8a	65.4±2.2c
Emamectin benzoate	0.0001	289.4±4.5b	7.5±0.4a	80.0±1.0b
	0.0002	266.7±5.3c	7.3±0.6a	78.4±2.1b
Novaluron	0.027	270.4±6.1c	7.8±0.6a	65.3±2.4c
	0.048	231.4±5.7d	7.7±0.7a	57.4±2.2d

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Table 4. Effect of the tested insecticides on adult fecundity, fertility and longevity of 2nd instar larvae of *S. littoralis*

Insecticide	Conc. (mg L^{-1})	Fecundity (No. eggs laid/female) ± SE	fertility (% egg hatch)	Adult longevity (days) ± SE
Control		859.0±55.5a	98.0±2.0a	6.0±0.5a
Fipronil	0.062	567.5±45.5b	81.8±1.8b	5.6±0.3ab
	0.097	543.0±34.5b	79.0±2.2b	5.3±0.4ab
Chlorantraniliprole	0.0008	382.0±22.4c	68.5±3.2c	5.1±0.2ab
	0.0013	336.5±32.4c	65.0±2.5c	5.0±0.4bc
Emamectin benzoate	0.0001	418.0±41.1c	77.8±3.3b	5.5±0.6ab
	0.0002	390.0±45.0c	78.0±2.0b	5.4±0.3ab
Novaluron	0.027	308.5±26.4d	55.0±4.5d	5.0±0.6ab
	0.048	288.8±42.8d	54.0±6.7d	5.2±0.2bc

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$

In the current study, the fecundity and fertility of *S. littoralis* were adversely affected when the 2nd instar larvae were treated with two sublethal concentrations (LC_5 and LC_{10}) of these compounds. The present results are parallel with the results of (Abd El-kader *et al*., 1994; Shaaban and Mourad, 1994; Shaurub *et al*., 1999 and Cao *et al*. 2010) who observed a reduced fecundity and fertility of several lepidopteran pests after exposure to conventional or nonconventional insecticides, either through topical application or by ingestion. Also, results are in agreement with EL-Tahawe.*et al.* (2018) who reported that fipronil had highly toxic effect against newly hatched larvae of pink bollworm followed by 2nd and 4th instar larvae of *S. littoralis*. The latent effects of the pyrazole compounds on the two insects were presented in increasing the duration of larval and pupal stages. Also significant decrease in larval and pupal weight of the two insects, male and female longevities and reduced the fecundity were observed. Zhang *et al*. (2013), reported that, chlorantraniliprole at sublethal concentrations significantly reduced the adult longevity and egg hatching rate of *Helicoverpa armigera*. Also, the pupation and copulation rate in the parental generation and the pupal mass in the offspring also strongly decreased. In addition, Nawaz *et al.* (2017), reported that, chlorantraniliprole was increased the length of the pre-adult developmental time while decreased adult longevity and fecundity of *H. axiyridis*. Lefebvre *et al*., (2011) measured the effects of, chlorantraniliprole and novaluron on eggs, larvae, adults, and female fecundity of *Galendromus occidentalis*. They found that, novaluron slightly affected fecundity for only the first 24 hrs. Chlorantraniliprole appeared to have no adverse effects on fecundity throughout the observation. Khan *et al*. (2018) determined the lethal and sublethal effects of emamectin benzoate on the rove beetle, *Paederus fuscipes*, and found that, the LC_{30} reduced the pre-oviposition period, the fecundity and the body weight of adults emerged from treated larvae. In the sublethal experiment with adults, the fecundity and the feeding potential were significantly reduced. Moustafa *et al*. (2016) reported that, the use of insecticides may result in multiple sublethal effects on insect pests with detrimental impacts on some physiological or behavioral process in the surviving insects, sublethal concentrations of emamectin benzoate were affected larval development time, pupation, pupal duration and weight, emergence percentages, and reproductive activity of *Mamestra brassicae*.

In conclusion, the present study suggests the using of sublethal concentration of either fipronil, chlorantraniliprole, emamectin benzoate and novaluron reduce the population growth of *S. littoralis* by affecting on the development and reproduction.

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الملخص العربي

تأثير بعض المبيدات على بعض العوامل البيولوجية لدودة ورق القطن

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تم تقدير سمية وتأثير تركيزات منخفضة للفبرونيل، الكلورانترانيليبرول، إيمامكتين بنزوات والنوفاليورون على العمر اليرقي الثاني لدودة ورق القطن. أوضحت النتائج أن التركيزات المسببة لموت ٥% من اليرقات المعاملة بعد ٤٨ ساعة هي ٠,٠٦٢، ٠,٠٠٠٨، ٠,٠٠٠١، ٠,٠٢٧ مجم/لتر للفبرونيل، الكلورانترانيليبرول، إيمامكتين بنزوات والنوفاليورون، على التوالي، بينما كانت التركيزات المسببة لموت ١٠% من اليرقات المعاملة بعد ٤٨ ساعة هي ٠,٠٩٧، ٠,٠٠١٣، ٠,٠٠٠٢، ٠,٠٤٨ مجم/لتر، على التوالي. أظهرت يرقات العمر الثاني المعاملة بالتركيزات اللازمة لموت ٥ و١٠% انخفاض معنوي فى متوسط وزن اليرقة، طول فترة العمر اليرقي ونسبة التعذير، كما أظهرت انخفاضا معنويا فى متوسط وزن العذارى ونسبة تحول العذارى إلى حشرات كاملة، بينما لم تؤثر تلك المعاملات على فترة التعذير. كما أوضحت النتائج أن معدل التبويض لكل أنثى وخصوبة البيض قد انخفضت فى المعاملات بصوره معنوية مقارنة باليرقات الغير معاملة (كنترول)، كما أن فترة التبويض أوعمر الحشرة الكاملة انخفض فى المعاملات بصوره معنوية مقارنة بالكنترول. من هذه النتائج يتضح أن التركيزات المنخفضة من المبيدات السابقة تؤثر سلبيا على تعداد الحشرات الناتجة من دودة ورق وذلك كنتيجة للتأثير على تطور الحشرة وتكاثرها.