

Toxicity of Chlorantraniliprole against Different Developmental Stages of the Cotton Leafworm under Laboratory Conditions

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ABSTRACT

Spodoptera littoralis management has been counted on the application of the synthetic chemical pesticides, but because of insecticides resistant problems in the cotton leafworm, the competence of these chemical have been reduced. So, new insecticides with different action could solve this problem. In this study, the activity of a new insecticide, chlorantraniliprole (Coragen® 20%SC), was evaluated against different stages of *S. littoralis*. For egg stage of *S. littoralis*, two different ages, < 24 h and 24-48 h were used and dipped in serial concentrations of chlorantraniliprole. The result presented that the young eggs were more sensitive than the old one with LC₅₀ value of 126.6 mg/l in case in the young eggs while it was 144.81 mg/l in the old one. In addition, the larvae of 2nd instar were exhibited a high susceptibility to chlorantraniliprole after 96 hrs. In contrast, when pupae were dipped in chlorantraniliprole different concentrations, the result showed no significant pupal mortality (20%) in the lower concentration (200-800 ppm), while the highest concentration (800 mg/l) showed 43.3% mortality. The letant effect of chlorantraniliprole at LC₂₅ (0.036 mg/l) on 2nd instar showed that the developmental time of larval and pupal stages has been prolonged and the percentage of hatchability was reduced as contrasted to untreated larvae. The present results propose that Coragen® could be a potentially effective and likely to be an alternative insecticide that can be used for *S. littoralis* management in Egypt.

keywords: Toxicity, *Spodoptera littoralis*, chlorantraniliprole, Coragen®

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd), is a polyphagous insect-pest in Egypt, and causes major damage not only to cotton plants but also for other crops and vegetables (Kandil *et al.*, 2003; Korrat *et al.*, 2012). The extensive application of chemical insecticides has been utilized for the control of this insect-pest, however, the steady of insecticides application led to the quick development of resistance to conventional insecticides (Ishaaya *et al.*, 1995). In addition, resistance of *S. littoralis* to some newer insecticides such as spinosad and abamectin has been documented (Gamal *et al.*, 2009). The resistance phenomena of insecticides has been recorded in 954 species of pests (Tabashnik *et al.*, 2014) including the cotton leafworm. To delay or prevent resistance development, there is a must for an alternative variety of insecticides with different action.

Diamid group of insecticides has been introduced for pest management as a new classe of insecticides with a low hazard on mammals (Lamb *et al.*, 2009). The anthranilic diamide, chlorantraniliprole, from DuPont, has an insecticidal activity on a wide range of pests including orders (Lepidoptera, Coleoptera, Diptera, Hemiptera and Isoptera) (Lahm *et al.*, 2005; Sattelle *et al.*, 2008; Lahm *et al.*, 2009; Lanka *et al.*, 2013). Chlorantraniliprole binds to the ryanodine receptor, which is a non-voltage-gated calcium channel that regulates the relief of calcium stores to the muscles. This reduces the regulation of muscle contraction

and leads to a unique symptoms including; feeding cessation, lethargy, paralysis, and death (Tohnishi *et al.*, 2005; Lahm *et al.*, 2007 and 2009; Cao *et al.*, 2010; Su *et al.*, 2012). In addition, no cross resistance has found with other insecticides, such as *Bacillus thuringiensis*, Cry toxins (Cao *et al.*, 2010) which could be used in the resistance management within integrated pest management (IPM) program.

Generally, insecticides consideration have been focused on the lethal activity on larval stage of insects, however, more studies on the sublatent effects of insecticides on pests on other stages are required. Many studies on the sublatent effects of insecticides have been confirmed on lepidopteran pests including; *Plutella xylostella* (Guo *et al.*, 2013), *Helicoverpa armigera* (Shen *et al.*, 2013), *S. littoralis* (El-Shiek, 2015), and *Mamestra brassicae* (Moustafa *et al.*, 2016).

In Egypt, it is very important to investigate the sensitivity of the most dangerous pest on cotton plant to chlorantraniliprole. The objective of this study was to determine the toxic effect of chlorantraniliprole on the different developmental stages (eggs, larvae, and pupae) of *S. littoralis* and to evaluate the sublethal activity of this insecticide on developmental time and reproductive responses to detect its potential as a novel insecticide that can be used for *S. littoralis* control.

MATERIALS AND METHODS

Insect culture

A laboratory susceptible strain of the cotton leafworm, *S. littoralis* has been reared in the

laboratory under complete absence of insecticides as described by El-Defrawi *et al.* (1964). Larvae were reared on fresh castor bean leaves at 25 ± 1 °C, $75\pm 5\%$ RH. The pupae and adults were moved to another cages as planned for mating and egg deposition. Emerged moths were fed with a 10% sugar.

Chemical

The insecticide tested was chlorantraniliprole (Coragen® 20% SC, suspension concentrate, DuPont).

Bioassays of chlorantraniliprole on different stages of *S. littoralis*

Ovicidal activity

The activity of chlorantraniliprole was tested against a different egg age class of *S. littoralis* (<24 h and 24-48 h old) which were collected from females. Stock solution (200 g/L) of testing chlorantraniliprole was diluted with water to gain a serial concentrations of 5, 50, 100, and 150 mg/liter (ppm). The filter paper samples that contain egg batches were snip into small pieces and immersed for 5 second in each concentration. After drying at room temperature, the treated eggs were transferred individually to a clean jar and kept at 25 ± 1 °C, $70\pm 5\%$ RH. The mortality of treated eggs was calculated over 5 days post-treatment.

Sensitivity of 2nd instar larvae

The sensitivity of 2nd instar larvae of *S. littoralis* to chlorantraniliprole was investigated, by using the leaf dipping technique. Five serial concentrations of chlorantraniliprole; 0.0078, 0.0156, 0.0312, 0.0625, and 0.125 mg/liter (ppm), were prepared and used to deep the castor bean leaves in each concentration for 20 second. The treated leaves were allowed to dry, after which a pair of leaves was placed into a glass jar (0.5 l) with larvae (Hamada *et al.*, 2018). Untreated larvae (control larvae) were fed on leaves treated with water. Four replicates (25 larvae/replicate) for each concentration were allowed to feed on treating leaves for 24 hrs and then fresh untreated leaves were introduced to larvae. Mortality percentage was recorded after 96 hrs post-treatment to determine the lethal and sublethal concentrations.

Treatment of pupal stage

Four serial concentrations of chlorantraniliprole ranging from 200 to 800 mg/liter (ppm) were prepared to test their activity on the young pupae (24 h old) of *S. littoralis*. The pupae were immersed for 5 second in each concentration; three replicates (10 pupae/replicate) were used. Control pupae were handle with water. Mortality of pupae and adult emergence were recorded till a period of 10 days.

Sublethal effect of LC₂₅ of chlorantraniliprole on treated *S. littoralis* 2nd instar larvae

Effects on development of *S. littoralis*

LC₂₅ of chlorantraniliprole of a 96 hrs bioassay as sublethal concentration value was used to

determine its effects on developmental time of larval and pupal stages, pupation percentage and adult emergence. The larval duration from 2nd instar was recorded daily until the last instar larval stadium, then these larvae were transferred individually to a clean cup containing sawdust for pupation. Pupae were sexed, weighed individually, and kept with wet cotton to record the total pupal duration, pupation and emergence percentage.

Effects on fecundity and hatchability%

After emergence, groups of 5 females and 7 males were moved to glass jars (1 L), covered from inside with a white paper and covered with fine mesh close on the top and the adults were fed with 10% sugarsolution (Moustafa *et al.*, 2016). Three replicates (5 females +7 males / replicate) for each sublethal concentration of LC₂₅ or control were used. Deposited eggs were counted from day 2 to day 6, then kept for 3 to 5 days in clean Petri dish with a piece of wet cotton to hatch and recording the percentage of hatchability.

Statistical analysis

Data were subjected to probit analysis using the EPA Probit analysis program used for calculating LC/EC values, version 1.5 to have the LC values as described by Finney (1971). Data analysis of all biological aspects, and reproductive activity were performed using one-way ANOVA (SAS, 2001) followed by Duncan's multiple range test.

RESULTS

Toxicity of chlorantraniliprole on different stages of *S. littoralis*

Ovicidal activity of chlorantraniliprole

Effect of chlorantraniliprole on two different egg ages of the cotton leafworm *S. littoralis*, was summarized in Table 1. The results showed that LC₅₀ value was 126.6 ppm in case of < 24 h old eggs, while it was 144.81 ppm in 24-48 h old eggs. The young eggs were more sensitive than the older one.

Sensitivity of 2nd instar larvae to chlorantraniliprole

Toxicity of chlorantraniliprole on 2nd instar larvae of *S. littoralis* after 96 hrs was presented in Table 2. The results exhibited that the lethal concentration of LC₉₀, and LC₅₀ values of chlorantraniliprole were 0.41 and 0.09 mg/liter (ppm), respectively, while the sublethal concentration of LC₂₅ was 0.036 ppm.

Effect of chlorantraniliprole on pupal stage

Result showed no harmful effects, when the young pupae (24 h old) of *S. littoralis* were dipped in 200 ppm of chlorantraniliprole (Figure 1). In contrast, high concentrations of chlorantraniliprole (800 mg/liter) caused significant pupal mortality (43.3%). Pupal mortality of the control was 10%.

Table 1: Ovicidal activity of chlorantraniliprole on *S. littoralis* eggs of different age.

Eggs age	LC ₅₀ (Confidence limits 95%)	LC ₉₀ (Confidence limits 95%)	Slope ± SE
<24 h old eggs	126.63 (99.20-174.50)	319.29 (211.57-1439.12)	3.19 ± 0.96
24-48 h old eggs	144.81 (113.73-242.88)	417.85 (246.90-3414.67)	2.78 ± 0.87

Table 2: Toxicity of chlorantraniliprole to 2nd instar larvae of *S. littoralis* after 4 days post-treatment.

	LC values (Confidence limits 95%)	Slope ± SE
LC ₂₅	0.036 (0.007-0.069)	
LC ₅₀	0.09 (0.075-0.114)	1.91 ± 0.190
LC ₉₀	0.41 (0.286-0.729)	

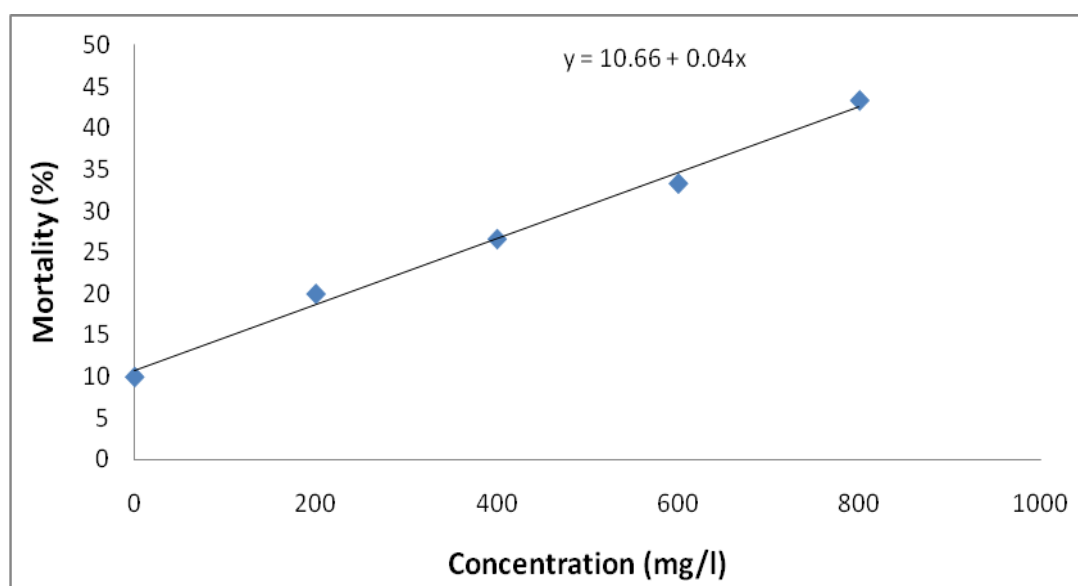


Figure 1: Regression analysis of mortality dependence on the concentrations of the chlorantraniliprole to young pupae (24 h old) of *S. littoralis*

Sublethal effect of chlorantraniliprole on the development of *S. littoralis*

The determined LC₂₅ value of chlorantraniliprole after 4 days post-treatment on early 2nd instar larvae of *S. littoralis* was used to investigate its effects on developmental time of *S. littoralis* (Table 3). The result showed that there was a significant increase of the larval and pupal developmental time of the treated 2nd instar larvae comparing with control. In contrast, there were no significant differences exhibited on pupation %, female pupal weight, sex ratio and emergence % between the treated larvae with chlorantraniliprole and untreated larvae (Table 3).

Sublethal effect of chlorantraniliprole on fecundity and fertility of *S. littoralis*

Chlorantraniliprole was significantly decreased the percentage of hatchability of those adults resulted from 2nd instar larvae treated with LC₂₅ of chlorantraniliprole as compared with the control (Table 4). The hatchability% between the mean numbers of deposited egg/female was 77.12 while in

the control was 92.95. In contrast, there were no significant differences on the number of laid eggs by one female between treated and untreated larvae as shown in Table (4).

DISCUSSION

In order to avoid the insecticide resistance, novel products with new action are needed. Chlorantraniliprole is a new compound which could be used in pest control within the Integrated Pest Management (IPM) programs. Studying the sensitivity of insects to chlorantraniliprole before use, will aid in realization of resistance management strategies before control failures occur (Lai *et al.*, 2011).

The ovicidal activity of chlorantraniliprole on *S. littoralis* was observed in both egg ages (<24 h and 24-48 h old), however the recently laid eggs (< 24 h) was more sensitive to chlorantraniliprole than older laid eggs (24-48 h).

Table 3: Latent effects of sublethal concentration (LC₂₅) of chlorantraniliprole on development of *S. littoralis* from 2nd instar larvae to emergence.

Treatments	^a Larval duration (days)	Pupation %	^b Pupal duration (days)		Pupal weight (mg)		Sex ratio		Emergence %
			Female	Male	Female	Male	Female (%)	Male (%)	
Control	16.70 ^b ±0.98	97.29 ^a ±3.48	8.43 ^c ±0.82	0.296 ^b ±0.05	0.274 ^b ±0.04	47.88 ^a ±6.68	52.10 ^a ±6.68	96.23 ^a ±3.77	
LC ₂₅	18.15 ^a ±1.71	94.71 ^a ±4.80	9.08 ^a ±1.28	0.287 ^b ±0.06	0.281 ^{ab} ±0.05	51.83 ^a ±13.82	48.15 ^a ±13.82	95.99 ^a ±4.51	

Values marked with the same letters are not significantly different (P > 0.05; Duncan's multiple range test).

a = number of days from 2nd instar larvae till pupation

b= number of days from the pupation till the emergence

Table 4: Mean Fecundity and Hatchability % (\pm SD) of *S. littoralis* female after expose of 2nd instar larvae to sublethal concentration (LC₂₅) of chlorantraniliprole

Treatments	^a Fecundity	^b Hatchability %
Control	573.73 ^a \pm 50.09	92.95 ^a \pm 3.77
LC ₂₅	556.08 ^a \pm 144.26	77.12 ^{ab} \pm 14.65

Values marked with the same letters are not significantly different ($P > 0.05$: Duncan's multiple range test)

^aFecundity was estimated by counting the eggs from the first day till the sixth day (total number of eggs laid by one female).

^bHatchability is calculated by counting of the emerged larvae from collected eggs batch

Generally, no information is available about the ovicidal effect of chlorantraniliprole on *S. littoralis*, however, several studies have been confirmed the ovicidal activity of different insecticides on *S. littoralis* and *Heliothis virescens* including; spinosad and methoxyfenozide (Bret *et al.*, 1997; Pineda *et al.*, 2004). For larval stage of *S. littoralis*, the presented results specified that the 2nd instar larvae was susceptible to chlorantraniliprole with LC₅₀ value of 0.23 mg/l. Recently, the field strains of *Spodoptera litura* were found to be more susceptible to chlorantraniliprole contrasted with the laboratory strain (Su *et al.*, 2012). Herein, (Lai *et al.*, 2011) mentioned that the laboratory strain of *Spodoptera exigua* was more sensitive to chlorantraniliprole with LC₅₀ 0.014 mg/L than field strains with LC₅₀ ranged value from 0.039 to 0.240 mg/L. In addition, the chlorantraniliprole showed a little effect on the pupal stage of *S. littoralis*, at the highest concentration 800 mg/L by dipped treatment, which possibly due to low penetration of this compound.

Surviving of exposed larvae to sublethal concentration (LC₂₅) of the chlorantraniliprole exhibited a significant reduction in development rate of 2nd instar larvae till emergence. In some other studies it was established that sublethal concentrations affected *P. xylostella* and inhibited insect development (Guo *et al.*, 2013). Moreover, a reduction in the percentage of hatchability was set in *S. littoralis* following treated the 2nd instar larvae with LC₂₅ value of the chlorantraniliprole. These findings could be related to some physiological aspects (Yin *et al.*, 2008).

The present results suggested that, the commercialization of chlorantraniliprole provided a useful tool for the cotton leafworm *S. littoralis* management.

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سمية الكلورنترانيلبيرول ضد الأطوار المختلفة لدودة ورق القطن تحت الظروف المعملية

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الملخص العربي

اعتمدت إدارة مكافحة حشرة دودة ورق القطن على استخدام المبيدات الكيميائية التقليدية وقد أدى الاستخدام المتكرر لهذه المبيدات الي ظهور مشكلة ظاهرة مقاومة دودة ورق القطن لهذه المبيدات الأمر الذي أدى الي خفض كفاءة وفاعلية هذه المبيدات الكيميائية. ويعتبر استخدام المبيدات الجديدة والتي لها طرق فعل حديثة من الحلول التي قد تؤدي لحل هذه المشكلة. وتتناول الدراسة تقييم فعالية مبيد كلورنترانيلبيرول (كوراجين 20%) ضد الأطوار المختلفة لدودة ورق القطن. ففي طور البيضة فقد تم معاملة فئتين عمريتين مختلفتين هما: 24 ساعة، ومن 24-48 ساعة بطريقة العمر في مجموعة من التركيزات المختلفة من مبيد كلورنترانيلبيرول، وقد أظهرت النتائج أن البيض الأصغر عمرا (24 ساعة) كان أكثر حساسية من الأكبر عمرا (24-48 ساعة) بقيمة LC_{50} 126.6 مجم / لتر (جزء في المليون) في حالة البيض (24 ساعة) في حين كان 144.81 مجم / لتر في البيض (24-48 ساعة). بالإضافة الي ذلك فإن يرقات العمر اليرقي الثاني قد أظهرت درجة من الحساسية العالية تجاه مبيد كلورنترانيلبيرول بعد 96 ساعة. في حين أظهرت النتائج قلة السمية عند عمر عذاري دودة ورق القطن في مجموعة من التركيزات المختلفة المجهزة (200-800 جزء في المليون) من مبيد كلورنترانيلبيرول. وقد وجد أن معاملة يرقات العمر اليرقي الثاني بالتركيز التحت مميت (LC_{25}) لمبيد كلورنترانيلبيرول، قد تسبب في أطاله فترة عمر الطور اليرقي وطور العذراء كما أدى الي خفض نسبة الفقس مقارنا باليرقات غير المعاملة. الأمر الذي يشير إلى أن مبيد كلورنترانيلبيرول (كوراجين 20%) من الممكن استخدامه بكفاءة في مكافحة حشرة دودة ورق القطن في مصر.