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Effects of chlorantraniliprole on development and reproduction of beet armyworm, *Spodoptera exigua* (Hübner)

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Abstract The lethal and sublethal effects of chlorantraniliprole against *Spodoptera exigua* (Hübner) were evaluated under laboratory conditions by oral exposure of neonate larvae to the compound. The 72 h LC_{50} value of this insecticide to *S. exigua* was found to be 12.747 µg l^{-1}. A progressive larval mortality of 24.32% for LC_{30} treatment and 42.61% for LC_{50} treatment was observed from 4th to 6th day after exposure, which resulted in the reduced pupation rates in exposure groups. The sublethal effects of this chemical were indicated by prolongation of larval period, the increase of pupal weight and decrease in hatch rate of egg. Chlorantraniliprole at LC_{30} and LC_{50} rate significantly delayed larval development; the developmental duration of surviving larvae was extended for 22.5 and 28.6%, respectively, compared with that of control group. LC_{30} treatment increased the mean weight of pupa and induced to the production of heavier pupa (>150 mg). In LC_{50} treatment, heavier pupa also showed up but the mean weight of pupa was not influenced. The egg hatch rate in LC_{50} group was significant lower than that in control and LC_{30} groups. No significant differences in pupal duration, emergence rate, sex ratio, egg number per female, and longevity of adults were observed among treatments. Chlorantraniliprole had exceptional activity against *S. exigua* according to concentration–response bioassay in laboratory, and the toxicities were primarily resulted from immediate lethality.

Keywords Chlorantraniliprole · Beet armyworm · *Spodoptera exigua* · Lethal effects · Sublethal effects

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Introduction

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a world widely distributed polyphagous pest of numerous cultivated crops (including vegetables, cotton, and ornamentals), and the most practical way to reduce beet armyworm population mainly depends on insecticide applications. Frequent application of insecticides had lead to resistance development of beet armyworm to conventional insecticides, and failure of chemical control was reported for this insect pest (Aldosari et al. 1996; Moulton et al. 2000, 2002; Liu et al. 2002a, b; Osoria et al. 2008). In this scenario, new insecticides with unique mode of action are required as an alternative in integrated management program of beet armyworm.

Chlorantraniliprole (Rynaxypyr™) is an anthranilic diamide, which belongs to insecticide resistance action committee (IRAC) mode of action class 28 (Cordova et al. 2006, Nauen 2006, Lahm et al. 2005, IRAC 2010), and has exceptional insecticidal activity on a range of Lepidopteran pests and other orders, such as Coleoptera, Diptera, Isoptera, and Hemiptera (Sattelle et al. 2008; Lahm et al. 2009). Chlorantraniliprole activates the unregulated release of internal calcium stores leading to Ca^{2+} depletion, feeding cessation, lethargy, muscle paralysis, and finally insect death (Lahm et al. 2007). It is characterized by its high levels of insecticidal activity and low toxicity to mammals attributed to a high selectivity for insect over mammalian ryanodine receptors. Additionally, because hasn't been found to exhibit cross-resistance with other commercial insecticides and low ecotoxicology, chlorantraniliprole is excellent selection for use in Integrated Pest Management (IPM) programs where commercial standards are no longer effective because of resistance (Lahm et al. 2009).

Potentially, all classes of insecticides may decrease the production of pest offspring through adverse lethal and sublethal effects, such as feeding behavior, larvae mortality, developmental time, pupal weight, sex ratio, time to adult emergence, fecundity, and development of the female ovipositor and egg hatch (Moreau and Bauce 2003; Seth et al. 2004; Galvan et al. 2005; Borchert et al. 2005; Eizaguirre et al. 2005; Abbott et al. 2008). Chlorantraniliprole has been shown to have ovicidal activity on eggs of *Lobesia botrana* (Denis & Schiffermüller) (Ioriatti et al. 2009), can disrupt the mating of codling moth (Lepidoptera: Tortricidae) (Knight and Flexner 2007), has suppressant activity against *Rhagoletis* fruit flies (Teixeira et al. 2009), and rapid feeding cessation faster than most recently developed insecticides (emamectin benzoate, indoxacarb, methoxyfenozide, and metaflumizone) (Hannig et al. 2009). To date, there were no published studies on sublethal effects of this insecticide on *S. exigua*. These effects may significantly influence exposed insect populations. The objectives of this study were to determine the lethal and sublethal effects of chlorantraniliprole on this insect pest.

Materials and methods

Insects and insecticide

Spodoptera exigua was provided by Wuhan Kernel Biopesticide Company, Hubei, China, in May 2001. This population has been maintained in the laboratory without exposure to any insecticide. Newly laid eggs were sterilized with 5% formaldehyde to prevent viral pathogens, and larvae were reared with artificial diet (Jia et al. 2009). Adults were fed 10% sugar solution. All stages were kept in the same standard conditions of 27 ± 1°C, 60–70% RH, and 14:10 h light:dark photoperiod.

Chlorantraniliprole (20% SC, Rynaxypyr^{TM}, DuPont Crop Protection) was commercially available.

Bioassay

Bioassay was performed with neonate larvae (newly hatched larvae <12 h old) of *S. exigua* using artificial diet. Seven concentrations of chlorantraniliprole using twofold dilutions ranging from 1.25 to 80 μg/l were prepared with distilled water. After preparing the diet in the laboratory, a quantity of 5 mL of diluted chlorantraniliprole was mixed thoroughly with 45 mL of artificial diet in a 100-mL beaker during the normal course of cooling (54 ± 1°C), and then all the insecticide-treated as well as the water-treated control diet were placed into cylindrical identical tissue culture tubes (2 × 8 cm, each tube containing approximately 2.5 mL of diet). 20 tubes were prepared for each concentration; every tube was set of 5 neonate larvae which a total of 100 larvae were used for each concentration. All the tubes were closed with cotton pads and kept in an incubator (27 ± 1°C, 60–70% RH, and 14:10 h light:dark photoperiod). Mortalities were assessed after 72 h. Larvae not responding with head movements or peristaltic contractions when touched with art brush were scored as dead (Jia et al. 2009).

Observations on development and reproduction

Sets of 100–120 neonate larvae (newly hatched larvae <12 h old) were exposed to two concentrations (LC_{30} and LC_{50}) of chlorantraniliprole as well as to a water-treated control, and four replicates were performed for each concentration and control. After exposure for 72 h, the larvae were scored for mortality, and the surviving larvae were transferred individually to untreated artificial diet and reared until pupation (one larva per tube). Larvae mortality was checked every day until pupation. Pupae from each cohort were incubated in containers under the same standard conditions. Time until pupation, pupal weight and pupal sex were determined. The adults were previously sexed as pupae and maintained in separate plastic containers before emergence. One male and one female emerging from the plastic container were transferred to oviposition chamber (cylinder cup, 15 cm long, 8 cm diameter) and covered with mesh cloth. Sugar solution (10%) was provided as food, and changed for new cups and mesh clothes each day until the adult moths died. The oviposition, fertility (percentage of egg hatch), and longevity of moths were recorded.

Statistical analysis

Larval mortality was analyzed using POLO-Plus program (LeOra 2002) to estimate slope, sublethal concentration values (LC_{30} and LC_{50}), and their 95% confidence limits (CLs). Treatment means, standard deviations (SDs), and significant differences were analyzed using SAS (SAS 1999).

Results

The lethal effect of chlorantraniliprole on beet armyworm

The degree of mortality of larvae *S. exigua* increased with increasing concentration of chlorantraniliprole, the data on mortality indicated a good fit to the probit model (Fig. 1)

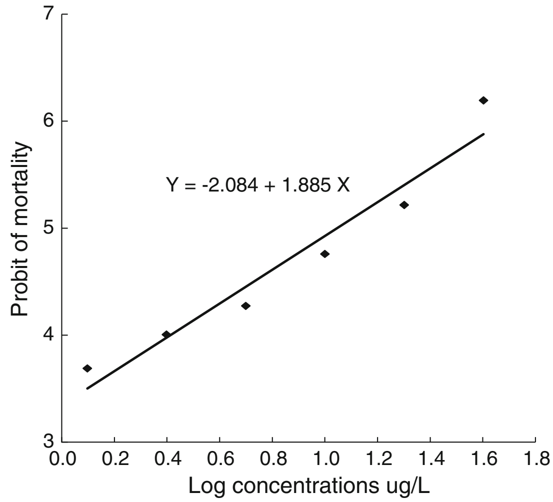

Fig. 1 Toxicity response of neonate larvae of *S. exigua* to chlorantraniliprole

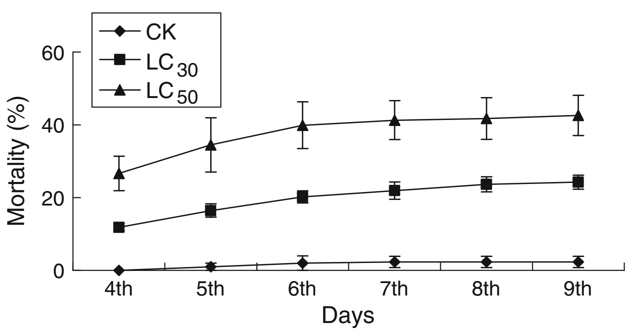

Fig. 2 Mortality of surviving larvae after transferring to diet without chlorantraniliprole

($P > 0.05$; $\chi^2 = 7.444$, df = 4). The estimated 72 h LC_{30} and LC_{50} values are 6.718 μg/l (1.082–11.425) and 12.747 μg/l (4.987–19.240), respectively. In order to evaluate the sublethal effects, neonate larvae were orally exposed to chlorantraniliprole, the 72 h mortality of larvae were 1.90 ± 0.96, 27.68 ± 1.41, and $49.71 \pm 3.01\%$ for 0, 6.7, and 12.7 μg/l of chlorantraniliprole, respectively. The surviving larvae were then transferred to diet without insecticide, the daily mortality were scored. A progressive larval mortality of 24.32% for LC_{30} treatment and 42.61% for LC_{50} treatment was observed from 4th to 6th day (Fig. 2) and resulted in significantly lower pupation rate for LC_{30} and LC_{50} concentrate exposure (74.75 and 54.68%, respectively) compared with control (96.33%) (Table 1).

Chlorantraniprole effects on development

Beet armyworm larvae challenged by chlorantraniprole take more days to finish larvae development, the development duration of larvae survived from LC_{30} and LC_{50} rate exposure had been extended by 22.5 and 28.6%, respectively, compared with non-chlorantraniliprole exposure (Table 1). The post-exposure effects on development time were carried over to pupa stage, the pupal durations were prolonged slightly, but there were no significant difference between chlorantraniliprole exposure and control. No significant difference of male and female pupa duration between exposed and non-exposed groups was observed. There were decreased female percentages in chlorantraniliprole treatments compared with control, but the differences were not statistically significant. No significant difference in emergence rate of moth was observed (Table 1).

Obviously lower concentrate of chlorantraniliprole (LC_{30}) increased the pupal weight (Table 1). The mean weight of pupae from larvae in LC_{30} group was significantly greater (125.01 mg) than those from control (111.94 mg). The distribution pattern of pupal weight was greatly changed by chlorantraniliprole challenge (Fig. 3), and the pupae with heavier weight increased after exposure to this insecticide. The peak value of pupal weight distribution was 100–110 mg (27.4%), 120–130 mg (31.3%), and 110–120 mg (36.4%) for control, LC_{30}, and LC_{50} group, respectively. There were no individual pupae weighted over 150 mg from control and no individual pupa less than 80 mg from exposed groups. Chlorantraniliprole exposure induced the occurrence of heavier pupae over 150 mg.

Chlorantraniprole effects on moth longevity and reproduction

The adult longevity of male *S. exigua* was shorter than that of female, 6.35 and 6.82 day for male and female in control group, respectively (Table 2). The adult longevity were extended slightly for male and female in two exposure groups except the male adult in LC_{30} group, but no significant difference among the three groups were found. Egg number and hatching percentage were investigated to evaluate the effect on reproduction of chlorantraniliprole. There were not statistically significant differences among treatments in the number of eggs laid per female. Exposing neonate larvae to 0, LC_{30}, and LC_{50} concentrations of chlorantraniliprole resulted in 863.64, 798.30, and 767.70 eggs per female, respectively. But high concentration (LC_{50}) reduced the hatch rate of eggs significantly. No significant difference was observed in hatch rate between control and LC_{30} concentration treatment (Table 2).

Discussion

Chlorantraniliprole, with a novel mode of action, potently activate ryanodine receptor, releasing stored calcium from the sarcoendoplasmic reticulum causing impaired

Table 1 Effects of chlorantraniliprole on development of *S. exigua*

Treatment	Larvae duration (±SD) (days)	Pupation rate (±SD) (%)	Pupal weight (±SD) (mg)	Pupal duration		Emergence rate (±SD) (%)	Female percentage (%)
				Female (±SD) (days)	Male (±SD) (days)		
Control	9.53 ± 0.61a	96.33 ± 1.53a	111.94 ± 13.42a	5.34 ± 0.50a	5.37 ± 0.52a	97.57 ± 0.38a	53.93
LC_{30}	11.67 ± 1.09b	74.75 ± 2.85b	125.01 ± 15.70b	5.74 ± 0.55a	5.81 ± 0.54a	97.65 ± 0.47a	48.59
LC_{50}	12.26 ± 1.09c	54.68 ± 6.47c	114.59 ± 14.37a	5.63 ± 0.57a	5.74 ± 0.59a	89.45 ± 3.21a	47.24

[a] Means within a column followed by different letters are significantly different ($P < 0.05$)

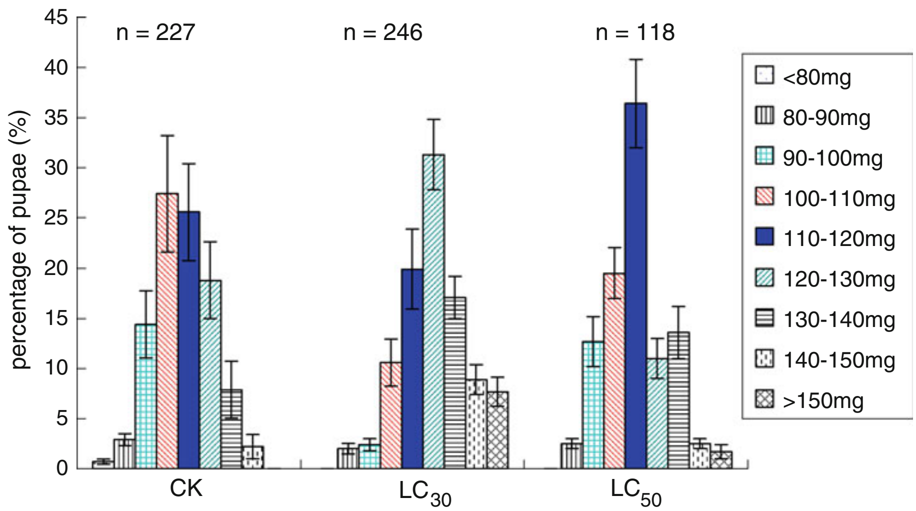

Fig. 3 Frequency distribution of pupal weight

Table 2 Effects of chlorantraniliprole on moth longevity and reproduction of *S. exigua*

Treatment	Number of moth pair	Adult longevity (days)		Number of eggs laid per female (±SD)	Percentage of egg hatch (%) (±SD)
		Male (±SD)	Female (±SD)		
Control	53	6.35 ± 1.63a	6.82 ± 1.55b	863.64 ± 223.23a	55.24 ± 11.78a
LC_{30}	47	6.16 ± 1.10a	7.31 ± 1.36b	798.30 ± 205.58a	51.95 ± 12.60ab
LC_{50}	31	6.59 ± 1.50a	7.22 ± 1.43b	767.70 ± 142.44a	47.79 ± 10.77b

[a] Means within a column followed by different letters are significantly different ($P < 0.05$)

regulation of muscle contraction (Cordova et al. 2006). The data obtained in this study showed that chlorantraniliprole was a highly toxic insecticide against *S. exigue*, the 72 h LC_{50} values to neonate larvae was as low as 12.747 μg/l. This result was in accord with that of Lahm et al. (2007) who had reported high toxicity of this chemical to several lepidopteran pests, such as *Plutella xylostella*, *Spodoptera frugiperda*, and *Heliothis virescens*.

The post-exposure effects of chlorantraniliprole on *S. exigua* larvae were indicated by progressive larvae mortality until pupation (Fig. 2), extended larvae duration, increased pupa weight (Table 1) and decreased egg hatch rate (Table 2). In this study, neonate larvae were exposed to chlorantraniliprole for 72 h and surviving larvae were then transferred to vials with diet without insecticide. On diet without insecticide larvae from exposure groups incurred progressive larval mortality (mainly from 4th to 6th day). After 6th day, the survival rates of larvae were stable in exposure groups. This means the check time for toxicity bioassay of chlorantraniliprole on *S. exigua* larvae can be postponed to 6th day.

Chlorantraniliprole may delay larval development of surviving larvae at LC_{30} and LC_{50} rates significantly. Observation on behavior of beet armyworm larvae showed chlorantraniliprole may decrease the mobility of the larvae, however, the most of the larvae exposed to lower concentrate of insecticide may recovered once removed from exposure, the prolongation of larval duration perhaps due to starvation caused by feeding cessation (Hannig et al. 2009). Bt treatment may induce feeding inhibition of spruce budworm and increased larval development time by 14%. Appearance of supernumerary instars (6th-instar) larvae caused by insecticide could be another reason for prolonged larval duration (Moreau and Bauce 2003).

There were many reports on pupal weight reduction of insects after exposed to sublethal insecticides (Stapel et al. 1998; Seth et al. 2004; Pineda et al. 2007; Liu et al. 2008; Rodríguez Enríquez et al. 2010). Interestingly, in chlorantraniliprole exposed groups, the pupal weight was increased compared with control group, especially in LC_{30} group. Analysis on frequency distribution of pupal weight of *S. exigua* disclosed that chlorantraniliprole treatment induced the appearance of pupae with heavy weight and increased the percentage of heavy individual pupae. There may be two reasons for this phenomenon. Firstly, Chlorantraniliprole killed the weaker individuals; the survivors in exposure groups were strong and robust one. In control group, there were individual pupae weighed less than 80 mg (0.7%), pupae less than 80 mg disappeared in exposure groups. The pupae weighed over 150 mg showed up in exposure groups (7.7% in LC_{30} group and 1.7% in LC_{50} group); however, there were no pupae heavier than 150 mg in control. Secondly, chlorantraniliprole perhaps induced the generation of the supernumerary instars larvae of *S. exigua*. There are normally five instars and sometimes six instars in larval period, the percentage of 6th-instars of *S. exigua* may be increased under stress (Chen et al. 2008). Spruce budworms are able to recover from exposure to *Bt* variety kurstaki without suffering reduction in pupal weight, in the terms of pupal weight the ability of spruce budworm to compensate for sublethal *Bt* effects was directly related to whether it produced supernumerary instars (Moreau and Bauce 2003). The increase in pupal weight in chlorantraniliprole groups may be related to the generation of supernumerary instars, but this assumption needs experimental verification by measuring head capsule of *S. exigua* larvae.

Previous studies showed chlorantraniliprole had significant disruption effect on mating behavior of *Cydia pomonella* (Knight and Flexner 2007) and significant ovicidal activity on *Lobensia botrana* (Ioriatti et al. 2009), but in this study no significant effect on reproduction of *S. exigua* was observed in lower concentrate treatment which was similar with the result on fruit fly (Teixeira et al. 2009), only slight reduction in egg hatch percentage was observed in high concentrate treatment. In this study, results were obtained by exposing neonate larvae to chlorantraniliprole, from exposure to adult stage there was more than 15 days time interval, perhaps only weak effects were carried over to adult stage. In order to assess the sublethal effects of chlorantraniliprole on reproduction of *S. exigua* thoroughly, further assay by exposing old larvae, pupa, or adult to this chemical should conducted.

In conclusion, this study indicated that chlorantraniliprole had high toxicity on neonate larvae of *S. exigua*, the toxicities resulted mainly from immediate lethality. The sublethal effects on *S. exigua* were not obvious and were primarily indicated by prolongation of larval stage and by the increase of pupal weight. Chlorantraniliprole represents a novel mode of insecticide action, have a most favorable toxicological and ecotoxicological profiles, these profile make this chemical a useful tool in IPM of *S. exigua*, however, the resistance risk of beet armyworm on this insecticide should not be overlook.

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