Applied Entomology and Zoology (2021) 56:115–124
https://doi.org/10.1007/s13355-020-00721-7

ORIGINAL RESEARCH PAPER

Toxicity and sublethal effects of chlorantraniliprole and indoxacarb on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

Moataz A. M. Moustafa[1] · Eman A. Fouad[2] · Yasmin Abdel-Mobdy[1] · Kamirán Áron Hamow[3] · Zsanett Mikó[4] · Béla Péter Molnár[3] · Adrien Fónagy[3]

Received: 22 April 2020 / Accepted: 17 December 2020 / Published online: 8 January 2021

Abstract

Chlorantraniliprole and indoxacarb insecticides exhibit good efficiency for control lepidopteran pests. The current study is a comprehensive analysis of the effect of lethal and sublethal concentrations of these insecticides on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) by using the leaf dipping technique. The LC_{50} values ranged from 0.06 to 1.07 mg/L, and 0.005 to 0.81 mg/L for chlorantraniliprole and indoxacarb, respectively. Our results showed that the treatment of the 2nd instar larvae with LC_{50} concentrations of these insecticides significantly increased the length of larval and pupal duration as well as pupal weight in most cases. While, no significant differences have been found in the percentage of hatchability except for LC_{50} equivalent of indoxacarb. Female behavior regarding calling activity decreased by 50–60% following exposure to the LC_{50} concentration of both insecticides. Gas chromatography analysis results showed that both insecticides lowered pheromone titer except at chlorantraniliprole LC_{50} equivalent for (*Z,E*)-9,12-tetradecadien-l-ol acetate, and indoxacarb LC_{10} equivalent for (Z)-9-tetradecenyl acetate. Additionally, the activity of mixed-function oxidases and glutathione S-transferase were elevated relative to control. The carboxylesterase activity significantly increased when assayed with both chlorantraniliprole concentrations and indoxacarb LC_{10} equivalent. These results indicate that chlorantraniliprole and indoxacarb could be effective for *S. littoralis* control.

Keywords Toxicity · Sublethal concentration · Chlorantraniliprole · Indoxacarb · *Spodoptera littoralis*

Introduction

Cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a destructive polyphagous insect pest of diverse field crops in different regions including; tropical and subtropical (Carter 1984). *S. littoralis* feeds on approximately 90 species of economic crops in 40 plant families (El-Sheikh et al. 2018). The regular use of chemical insecticides against *S. littoralis* resulted in the development of resistances to most of the traditional insecticides (Aydin and Gürkan 2006; Ishaaya et al. 1995) and some of the newer bioinsecticides such as spinosad and abamectin (Gamal et al. 2009). Therefore, there is an increasing need for alternative new classes of insecticides that may delay or prevent resistance development.

Diamide insecticides, such as chlorantraniliprole, for pest control, are one of the most promising new class of insecticides that have excellent efficacy and low hazard for mammals (Lahm et al. 2009). Chlorantraniliprole (Bentley et al. 2010), has an insecticidal effect on a wide range of

Supplementary Information The online version of this article (https://doi.org/10.1007/s13355-020-00721-7) contains supplementary material, which is available to authorized users.

✉ Moataz A. M. Moustafa
moataz.moustafa79@gmail.com

[1] Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza 12613, Egypt

[2] Bioassay Department, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Giza 12618, Egypt

[3] Zoology Department, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Hungary

[4] Lendület Evolutionary Group, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Hungary

lepidopteran pests (Hannig et al. 2009; Lahm et al. 2005) besides other orders including Coleoptera, and Diptera (Lanka et al. 2013; Sattelle et al. 2008). Chlorantraniliprole is classified by the insecticide resistance action committee as class 28 (IRAC 2019), which modulates functionality of the ryanodine receptor, that regulate the intracellular Ca^{2+} channels specialized for the release of Ca^{2+} into the muscles. Consequently, it has the potential to be one of the most successful agents in resistance management due to its mode of action (Guo et al. 2013).

Indoxacarb is another non-traditional insecticide that belongs to the oxadiazine insecticide group that is used against different species of insect pests in agricultural and urban environments (Gondhalekar et al. 2011; Harder et al. 1996; Wing et al. 2000). Indoxacarb is in class 22A (IRAC 2019) that effects by blocking the voltage-dependent Na^{+} channel and leading to paralysis of the insect. It is enzymatically bioactivated by insect esterases or amidases to a decarbomethoxylated metabolite, which is more effective than the parent compound, Indoxacarb (Wing et al. 1998; Zhao et al. 2005).

Successful pest control depends on the prolongation of the efficacy of insecticides. Therefore the assessment of the sublethal effects of an insecticide is important, and several studies on the sublethal effects of insecticides have been reported for a number of lepidopteran pests including *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Guo et al. 2013; Wang et al. 2011; Yin et al. 2008), *Helicoverpa armigera* (Hübner), *S. littoralis* and *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) (El-Sheikh 2015; Moustafa et al. 2016; Parsaeyan et al. 2013; Shen et al. 2013). The disturbance could reflect protective physiological responses such as the increment of cytochrome P450-dependent monooxygenases, carboxylesterases (CarE), and/or glutathione S-transferases (GST) that play important roles in insecticide metabolism (Yu 2004). The P450s and CarE catalyze phase I reactions by participating in the direct metabolism of insecticides, while the GSTs catalyze phase II reactions by increasing the molecule's hydrophilicity of compounds to be excreted by ABC transporters during phase III (Crava et al. 2016; Zhong et al. 2017). The insecticide resistance could be developed as a result of the induction of detoxification enzymes following insecticides exposure (He et al. 2019).

Locating conspecific females for mating is a critical event in the life of adult moths. Most moth species produce in the female pheromone gland (PG) (Percy and Weatherston 1974) species-specific sex pheromones, composed of long-range aliphatic compounds (Ando et al. 2004). Release of sex pheromone blends correlates in time with high male responsiveness and locomotor activity (Raina et al. 1987). The circadian mating activity has been extensively studied by Silvegren et al. (2005) in *S. littoralis*. In *S. littoralis,* the highest pheromone titers are found in the PGs of 1–3 day (D) old females during the 2nd and 3rd hours of scotophase (Dunkelblum et al. 1987) with several C_{14} acetates identified in the PG extracts of *S. littoralis* (Nesbitt et al. 1973; Tamaki and Yushima 1974; The Pherobase). The Egyptian strain is characterized to include the major components (*Z*,*E*)-9,11-tetradecadienyl acetate [(*Z*,*E*) 9,11–14:Ac] and (*Z*,*E*)-9,12-tetradecadienyl acetate [(*Z*,*E*) 9,12–14:Ac] with 3 minor components: (*Z*)-9-tetradecenyl acetate (*Z*9–14:Ac), (*E*)-11-tetradecenyl acetate (*E*11–14:Ac) and (*Z*)-11-tetradecenyl acetate (*Z*11–14:Ac) (Campion et al. 1980; The Pherobase).

Sublethal doses/concentrations could result in the disorder of behavioral and physiological parameters of insects that survive after the initial insecticide exposure (Desneux et al. 2007). The current work provides information about the susceptibility of *S. littoralis* to chlorantraniliprole and indoxacarb and assesses their sublethal effects on insect development, various reproductive activity parameters (calling behavior, pheromone titer, fecundity and hatchability percentage) and critical detoxification enzyme activities such as mixed-function oxidases (MFOs), CarE and GST.

Materials and methods

Spodoptera littoralis culture

Spodoptera littoralis have been collected from the field at Giza governorate, Egypt. The colony is reared in the laboratory for more than 20 generations in the absence of insecticides as described by El-Defrawi et al. (1964). All stages of *S. littoralis* were maintained in a rearing room at 25 ± 1 °C, 75 ± 5% relative humidity under a reversed 16 h: 8 h (light: dark) regime, with lights-off at 8:00 a.m. and on at 4:00 p.m. Larvae were fed with fresh castor bean leaves (*Ricinus communis*; Malpighiales: Euphorbiaceae). Male and female pupae were separated to avoid mating. Emerged moths were supplied with a 10% sugar solution. For a limited number of experiments, assays were conducted separately in another room equipped with a dim bright red backlight, but under the same rearing conditions.

Insecticides and chemicals

Chlorantraniliprole (Coragen® 20%, suspension concentrate, DuPont, France), and Indoxacarb (Avaunt® 15%, emulsifiable concentration, DuPont) were used for the experiments. The pheromone standards, a blend comprising synthetic mixtures of neat compounds, were from Pherobank BV (The Netherlands). Fast blue salt, glutathione (GSH), p-nitroanisole (p-NA), 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma-Aldrich (Germany) and *n*-hexane

from Merck (Germany). Other substrates and reagent chemicals were purchased from Sorachim (Switzerland), and MP Biomedicals companies (India).

Bioassays

The toxicity of chlorantraniliprole and indoxacarb were tested using the leaf dipping technique on *S. littoralis* larvae comprising all 6 instars. The castor bean leaves were dipped for 20 s in five different concentrations ranging from 0.0078 to 4 mg/L of chlorantraniliprole and from 0.0019 to 4 mg/L of indoxacarb for each instar as indicated in the supplementary material. The treated leaves were allowed to dry, after which a pair of leaves were placed into a glass jar (0.5 L) with 25 larvae in 4 replicates. Control larvae were placed on untreated leaves. The larvae were allowed to feed for 24 h and then transferred onto untreated leaves. Mortality was recorded at 24 and 96 h to estimate the lethal and sublethal concentrations after 4 days post-treatment of each insecticide. The bioassay was repeated twice.

Sublethal effects of chlorantraniliprole and indoxacarb on *S. littoralis*: effects on insect development

Sublethal concentration values corresponding to the LC_{10} (0.01 and 0.001 mg/L) and LC_{50} (0.09 and 0.01 mg/L) of both chlorantraniliprole and indoxacarb were used to assess effects on the larval and pupal duration, pupation percentage, and emergence percentage. The larval duration was recorded daily until the last instar and then transferred individually to a clean cup for pupation. After 3 days, pupae were sexed, weighed, and kept separately to record the total pupal duration period, and emergence percentage. The following formula has been used:

Pupation percentage = Number of pupae/Total number of alive larvae after treatment ∗ 100

Emergence percentage = Number of moths/Total number of pupae ∗ 100.

Fecundity and fertility

After the 2nd instar larvae were treated with the LC_{10} and LC_{50} of both insecticides, the emerged adults were grouped as 5 females and 7 males (conferring to one replicate) as in an earlier similar study in *M. brassicae* (Moustafa et al. 2016). The groups were transferred into glass jars (1 L), placed underneath a white paper, and the jar covered with a fine mesh screen. Adults were fed as described above. Three replicates for each sublethal LC_{10} and LC_{50} concentrations were used. Egg batches were counted daily to day 6 (D6), and kept for D5 to record the hatchability percentage as follows:

Hatchability percentage = n. of hatching eggs/Total number of eggs.

Monitoring virgin female calling behavior

Calling behavior was recorded from D1 until D5 in surviving virgin female moths after sublethal (LC_{10} and LC_{50} values) insecticide exposure in second larval instar and controls according to Moustafa et al. (2016) with some modification. The observation was carried out in an experimental room equipped with a dim red light at 60 min intervals during scotophase, from 8:00 till 16:00. Data of 9 females (cumulated for 5 days), for each concentration was recorded. Each female was deemed calling or non-calling based on PG protrudence (calling = protruded PG; non-calling = a PG that was not visible).

Analysis of pheromone blends

Extraction of pheromone gland

For pheromone blend analysis, a pooled extract of 4 or 5 PGs was prepared. The glands were excised from D2 old virgin females between hours 2–3 of scotophase and extracted for an hour at room temperature in approximately 50 µL of *n*-hexane. The samples were transferred to conical glass inserts, then placed into 1.5 mL vials [suitable for gas-chromatography (GC) mass-spectrometry (MS) analysis], and a 500 ng/5µL internal standard (tridecyl acetate; 13:OAc) was added before sealing with a Teflon-lined screw cap. The vials were stored at − 30 °C until analysis.

GC-mass spectrometry analysis

Measurements were carried out on an Agilent (Santa Clara, California, USA) 6890 GC coupled to a 5973 MS system. The injector temperature was 220 °C, the injection volume was 1 µL in splitless mode, and the purge flow was 20 mL/min. Carrier gas of Helium 6.0 was used at the column flow rate of 1 mL/min in constant linear velocity mode. The separation was performed on an Agilent J&W VF WAXms (60 m × 0.25 mm × 0.25 µm) polar capillary column. The heat program for separation started with a 1 min 50 °C

hold, then increased by 20 °C/min to 90 °C, then increased by 10 °C/min to 190 °C and finally by 4 °C/min to 240 °C and held for 5 min. As a post-run function, the temperature was raised to 245 °C and held for 3 min before returning to starting conditions. For mass spectrometric detection, the source temperature was set to 230 °C while the quadrupole temperature was held at 150 °C. Positive electron ionization (EI+) was used with a standard electron energy level of 70 eV. The instrument was tuned using perfluorotributylamine according to the manufacturer's instructions. First, authentic standards were injected in scan mode to develop a Selected Ion Monitoring (SIM) method for quantitative mass spectrometric detection and to confirm compounds by their mass spectrum utilizing the NIST 17 mass spectral database. For quantitative measurements, the MS was operated in SIM mode at a cycle time of 20 Hz. The following ions were monitored, the first ion stated was the best unique ion for quantitation, the second was the qualitative ion for calculating ion ratios for unambiguous identification: for the internal standard (13:OAc) with a Retention Time (RT): at 16.97 min m/z 83, 69; for *Z*9–14:Ac (RT: 19.015 min) m/z 96, 86; for *E*11–14:Ac (RT: 19.05 min) for *Z*11–14:Ac (RT: 19.25 min) m/z 68, 82; for (*Z,E*) 9,12–14:Ac (RT: 20.19 min) and for (*Z,E*) 9,11–14:Ac (RT: 21.25 min) m/z 67, 79. Agilent Enhanced MSD ChemStation software was used to set the GC and MS parameters. For quantitative evaluation, Mass Hunter Workstation Quantitative Analysis B.09.00 software was used.

Activity of detoxifying enzymes

Sample preparations

At 4 days post-treatment of 2nd instar *S. littoralis* larvae were weighted and stored at -40 °C until biochemical analysis.

Mixed function oxidases (MFO) assay

The MFO activity was determined according to Hansen and Hodgson (1971). Treated and untreated larvae were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.8) then centrifuged at 15,000 *g* at 4 °C for 15 min. A hundred µL of 2 mM p-NA solution and 90 µL of the supernatant were added at 27 °C for 2 min, and then 10 µL of 9.6 mM NADPH were added. The optical density (OD) was recorded at 405 nm for 10 min by Vmax kinetic microplate reader (Molecular Devices).

Carboxylesterase (CarE) assays

The activity of CarE (including; α- and ß- esterase) was determined according to Van Asperen (1962) modified by Cao et al. (2008). Larvae were homogenized in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 12,000 *g* on 4 °C for 15 min. A 50 µL aliquot of the supernatant was incubated with 50 µL of (30 mM) alpha (α) or beta (ß)—naphthyl acetate at 30 °C for 15 min to evaluate α- and ß- esterase activities, respectively. The reaction was stopped by adding 50 µL of stop solution 2 Fast Blue RR (1%): sodium dodecyl sulphate (5%). The color change was measured at 600 nm for hydrolysis of α-naphthyl acetate and at 550 nm of hydrolysis of ß-naphthyl acetate by V-530 UV/Vis Spectrophotometer (JASCO Corporation). Bradford Coomassie brilliant blue assay and α- and ß- naphthyl acetate standard curves were used to calculate the mean levels of enzyme activity.

Measurement of glutathione S-transferase (GST) activity

GST activity was determined as described by Habing et al. (1974). The larvae were homogenized in 0.1 M phosphate buffer (pH 6.5) and centrifuged at 12,000 *g* on 4 °C for 15 min. The reaction solution contained 100 µL enzyme stock solution, 10 µL 30 mM CDNB, and 10 µL 50 mM GSH, which was measured at 430 nm on 25 °C for 3 min by V-530 UV/Vis spectrophotometer.

Statistical analysis

Probit analysis (EPA Probit analysis program, version 1.5) was used to estimate the lethal and sublethal values (LC_{10} and LC_{50}) of chlorantraniliprole and indoxacarb on different instar *S. littoralis* larvae at 4 days post-exposure. Further data analyses were performed using one-way ANOVA (SAS 2001) followed by Tukey's Honestly Significant Different.

Results

Lethal effects of chlorantraniliprole and indoxacarb on different larval instars

The results of feeding the various *S. littoralis* larval instars on castor bean leaves treated with different concentrations of chlorantraniliprole or indoxacarb are presented in Tables 1 and 2, respectively. The chlorantraniliprole LC_{10}, and LC_{50} values ranged from 0.014 to 0.323, and 0.06 to 1.07 mg/L, respectively for the 1st to 6th instars, while the LC_{90} values were 0.34 to 3.54 mg/L (Table 1). In contrast, the LC_{10}, and LC_{50} values of indoxacarb were between 0.001 to 0.055, and 0.005 to 0.81 mg/L, respectively for the 1st to 6th instars, while the LC_{90} values were from 0.021 to 11.87 mg/L (Table 2).

Table 1 Susceptibility of laboratory-reared *S. littoralis* larvae to chlorantraniliprole

Larval instar	LC_{10} (mg/L)[a] (95% Confidence limit)	LC_{50} (mg/L)[b] (95% Confidence limit)	LC_{90} (mg/L)[c] (95% Confidence limit)	Slope ± SE
1st	0.014 (0.004–0.023)	0.06 (0.044–0.145)	0.34 (0.157–2.981)	1.82 ± 0.284
2nd	0.019 (0.015–0.024)	0.09 (0.075–0.114)	0.41 (0.286–0.729)	1.91 ± 0.190
3rd	0.024 (0.01–0.10)	0.20 (0.001–0.479)	1.67 (0.646–2.429)	1.39 ± 0.404
4th	0.058 (0.039–0.078)	0.23 (0.195–0.270)	0.93 (0.765–1.214)	2.12 ± 0.187
5th	0.175 (0.70–0.288)	0.76 (0.554–0.970)	3.37(2.445–5.883)	1.99 ± 0.418
6th	0.323 (0.152–0.483)	1.07 (0.809–1.360)	3.54 (2.529–6.499)	2.46 ± 0.319

First to sixth instar larvae were treated with five different concentrations (ranging from 0.0078 to 4 mg/L) of chlorantraniliprole by the leaf dipping technique. After 24 h they were fed with untreated fresh castor leaves. Larvae were monitored throughout development. The test was performed in four replicates ($n = 25$). For concentrations see Supplementary material

[a]LC_{10}: concentration causing 10% mortality

[b]LC_{50}: concentration causing 50% mortality

[c]LC_{90}: concentration causing 90% mortality

Table 2 Susceptibility of laboratory-reared *S. littoralis* larvae to indoxacarb

Larval instar	LC_{10} (mg/L)[a] (95% Confidence limit)	LC_{50} (mg/L)[b] (95% Confidence limit)	LC_{90} (mg/L)[c] (95% Confidence limit)	Slope ± SE
1st	0.001 (0.001–0.002)	0.005 (0.004–0.006)	0.021 (0.014–0.039)	2.00 ± 0.303
2nd	0.001 (0.001–0.002)	0.01 (0.008–0.020)	0.17 (0.084–1.260)	1.15 ± 0.278
3rd	0.003 (0.001–0.010)	0.03 (0.017–0.057)	0.44 (0.240–2.140)	1.20 ± 0.286
4th	0.016 (0.002–0.040)	0.13 (0.062–0.188)	1.04 (0.660–2.820)	1.41 ± 0.304
5th	0.041 (0.021–0.063)	0.31 (0.251–0.380)	2.43 (1.735–3.952)	1.44 ± 0.150
6th	0.055 (0.011–0.124)	0.81 (0.547–1.145)	11.87 (5.779–50.448)	1.09 ± 0.199

First to sixth instar larvae were treated with five different concentrations (ranging from 0.0019 to 4 mg/L) of indoxacarb. Further conditions as in Table 1

[a]LC_{10}: concentration causing 10% mortality

[b]LC_{50}: concentration causing 50% mortality

[c]LC_{90}: concentration causing 90% mortality

Sublethal effects of chlorantraniliprole and indoxacarb on development

Both tested insecticides significantly increased the larval and pupal duration (Table 3). Both insecticides decreased pupation rate at the concentration equivalent to LC_{50}, while pupal weight significantly increased after the larvae were treated with the LC_{10} and LC_{50} of chlorantraniliprole and indoxacarb LC_{50} value. In contrast, there were no significant effects on sex ratio and emergence rate between the treated larvae and untreated larvae (Table 3).

Fecundity and fertility

Both insecticides showed no significant differences in the percentage of hatchability at all concentrations tested (LC_{10} and LC_{50} equivalent) except for LC_{50} equivalent of indoxacarb compared to the control (Table 4). In contrast, there was no significant difference in the number of eggs laid by one female (fecundity) between treated and untreated larvae (Table 4).

Calling behavior

Calling activity was the most intense between the 2nd (09:00) and 4th (11:00) hours of scotophase. Female calling behavior after treating 2nd instar larvae of *S. littoralis* with both insecticides were (24.42 ± 4.1%) and (31.08 ± 8.1%) for chlorantraniliprole LC_{10}, (19.98 ± 2.2%) and (22.20 ± 7.8%) for chlorantraniliprole LC_{50}, (19.98 ± 2.2%) and (31.08 ± 6.4%) for indoxacarb LC_{10}, while (13.32 ± 2.2%) and (17.76 ± 4.4%) for indoxacarb LC_{50} at the 2nd and 4th hours of scotophase respectively, and clearly decreased towards the end of scotophase (Fig. 1). Overall, female calling behavior following exposure to the LC_{50} concentration of both insecticides compared to controls significantly decreased (50–60%) (Fig. 1).

Table 3 Effects of chlorantraniliprole and indoxacarb on development of *S. littoralis* from 2nd instar larvae to emergence

Treatments	Mean ± SE							
	Larval duration (days)*	Pupation rate (%)	Pupal duration (days)**	Pupal weight (g)		Sex ratio		Emergence %
				Female	Male	Female	Male	
Control	16.70 ± 0.55^{d}	97.29 ± 3.48^{a}	8.43 ± 0.47^{b}	0.296 ± 0.004^{b}	0.274 ± 0.002^{b}	47.89 ± 6.68^{a}	52.10 ± 6.68^{a}	96.23 ± 3.77^{a}
Chlorantraniliprole								
LC_{10}	17.99 ± 1.27^{c}	98.26 ± 1.71^{a}	8.59 ± 0.84^{ab}	0.323 ± 0.05^{a}	0.294 ± 0.04^{a}	55.19 ± 1.77^{a}	44.80 ± 1.78^{a}	98.62 ± 1.62^{a}
LC_{50}	18.20 ± 1.51^{c}	95.76 ± 2.88^{cb}	8.78 ± 0.87^{a}	0.318 ± 0.05^{a}	0.290 ± 0.05^{a}	50.64 ± 5.26^{a}	49.32 ± 5.27^{a}	95.63 ± 3.14^{a}
Indoxacarb								
LC_{10}	18.79 ± 0.08^{b}	93.20 ± 0.97^{ab}	10.37 ± 0.08^{a}	0.296 ± 0.004^{b}	0.269 ± 0.003^{b}	46.52 ± 6.22^{a}	53.47 ± 6.22^{a}	95.72 ± 1.84^{a}
LC_{50}	19.6 ± 0.11^{a}	92.74 ± 0.72^{b}	10.38 ± 0.90^{a}	0.354 ± 0.006^{a}	0.317 ± 0.005^{a}	48.68 ± 2.42^{a}	51.31 ± 2.42^{a}	95.97 ± 0.80^{a}

Second instar larvae were treated with different (LC_{10} and LC_{50}) sublethal concentrations of chlorantraniliprole and indoxacarb. After 24 h they were fed with untreated fresh castor leaves ($n = 25$ in four replicates)

Values marked with the same letters are not significantly different ($p > 0.05$: Tukey's Honestly Significant Different) between control and each treatment of both chlorantraniliprole and indoxacarb

*Number of days from 2nd instar larvae till pupation

**Number of days from the pupation till the emergence

Table 4 Mean fecundity and hatchability percentage (± SE) of *S. littoralis* females

Treatments	Fecundity*	Hatchability %**
Control	573.73 ± 28.92^{a}	92.95 ± 2.17^{a}
Chlorantraniliprole		
LC_{10}	577.44 ± 61.62^{a}	75.13 ± 7.06^{ab}
LC_{50}	511.13 ± 86.07^{a}	67.76 ± 5.93^{ab}
Indoxacarb		
LC_{10}	533.09 ± 104.34^{a}	67.78 ± 6.66^{ab}
LC_{50}	352.15 ± 8.98^{a}	62.73 ± 7.48^{b}

Second instar larvae were treated with different sublethal concentrations (LC_{10} and LC_{50}) of chlorantraniliprole and indoxacarb. After 24 h they were fed with untreated fresh castor leaves. Three replicates (5 females + 7 males) were assayed at each concentration

Values marked with the same letters are not significantly different ($p > 0.05$: Tukey's Honestly Significant Different) between control and each treatment of both chlorantraniliprole and indoxacarb

*Fecundity was estimated by counting the eggs from the first day till the sixth day (total number of eggs laid by one female)

**Hatchability is calculated by counting the emerged larvae from collected eggs batches

Pheromone production

Based on the described GC–MS methodology we determined the changes of five pheromone components including the two most decisive ones as (*Z,E*)9,12–14:Ac and (*Z,E*)9,11–14:Ac. On Table 5, the five different blend component amounts (in ng/PG) are listed according to their retention times. Treatments did not result in significant differences in the amount of pheromone components in comparison to controls, except in the case of chlorantraniliprole LC_{50}, for a major component (*Z,E*)9,12–14:Ac and indoxacarb LC_{10} equivalent Z9–14:Ac, which is a minor component.

Detoxification enzyme activities

The activity of MFO was much higher (15-fold) at a sublethal LC_{10} concentration of indoxacarb compared with that of control, but was only threefold higher at the LC_{50} (Table 6). In contrast, MFO activities increased to 3.9-fold at LC_{10} and 4.3-fold at LC_{50} of the chlorantraniliprole. The higher α-esterase activity was found in all treatments except for the indoxacarb LC_{50} value (Table 6). In addition, the chlorantraniliprole LC_{50} and indoxacarb LC_{10} concentrations significantly increased the ß-esterase activity (Table 6). Likewise, GST activity was elevated at chlorantraniliprole LC_{50} and indoxacarb LC_{10} concentrations (Table 6).

Discussion

Chlorantraniliprole and indoxacarb are promising alternative compounds that could be effectively used in crop protection. Understanding the effects of any pesticide is important to implement appropriate resistance management strategies or to reduce the pesticide treatment thresholds before control failures occur (Liu et al. 2011). This study aimed to advance our knowledge of the insecticidal activity and the latent effects of chlorantraniliprole and indoxacarb against *S. littoralis.*

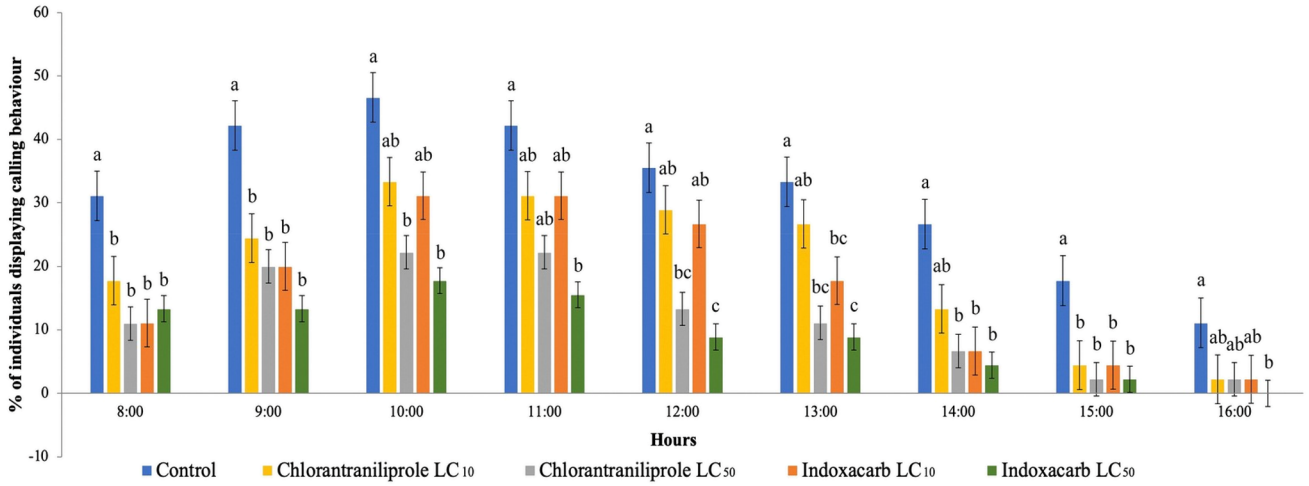

Fig. 1 Calling behavior of adult *S. littoralis* females. Percentage (charts represent means ± SE; $n = 9$ recorded from D1 till D5) of *S. littoralis* females exhibiting calling behavior in scotophase (8 h, from 8:00 till 16:00.). Females were derived from 2nd instar larvae fed with leaves treated with sublethal concentration LC_{10} and LC_{50} equivalent of chlorantraniliprole or indoxacarb. Controls are larvae fed with untreated leaves. One-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test was performed among the control and each treatment of both insecticides at each time point. In each time point, values marked with the same letters are not significantly different ($p > 0.05$: Tukey's HSD post hoc test)

Table 5 Pheromone production in *S. littoralis* females

Treatments	Mean titer (ng)/female (PG) ± SE (CV%)				
	Z9–14:Ac	*E11–14:Ac*	*Z11–14:Ac*	*(Z,E) 9,12–14:Ac*	*(Z,E) 9,11–14:Ac*
Control	1.75 ± 0.04^{a} (0.022)	1.10 ± 0.04^{a} (0.036)	0.57 ± 0.03^{a} (0.052)	4.27 ± 0.15^{a} (0.035)	3.14 ± 0.08^{a} (0.025)
Chlorantraniliprole					
LC_{10}	1.57 ± 0.08^{a} (0.05)	1.03 ± 0.21^{a} (0.199)	0.51 ± 0.09^{a} (0.169)	3.96 ± 0.92^{ab} (0.298)	3.26 ± 0.29^{a} (0.089)
LC_{50}	1.42 ± 0.37^{ab} (0.264)	0.78 ± 0.19^{a} (0.243)	0.40 ± 0.10^{a} (0.250)	1.42 ± 0.64^{b} (0.450)	2.60 ± 0.52^{a} (0.20)
Indoxacarb					
LC_{10}	1.18 ± 0.07^{b} (0.035)	0.72 ± 0.09^{a} (0.123)	0.32 ± 0.05^{a} (0.128)	2.74 ± 0.84^{ab} (0.306)	2.30 ± 0.26^{a} (0.102)
LC_{50}	1.61 ± 0.16^{ab} (0.106)	0.84 ± 0.07^{a} (0.083)	0.44 ± 0.04^{a} (0.088)	2.64 ± 0.61^{ab} (0.286)	2.57 ± 0.29^{a} (0.113)

Mean pheromone blend component titers ng/female ± SE (CV%, SE/Mean, $n = 4$–5 pheromone glands /sample in three replicates) of 2-day-old *S. littoralis* females (at the 2nd–3rd hour of scotophase) treated as 2nd instar larvae with LC_{10} and LC_{50} values of chlorantraniliprole or indoxacarb. After 24 h they were fed with untreated fresh castor leaves

Values marked with the same letters are not significantly different ($p > 0.05$: Tukey's Honestly Significant Different) between control and each treatment of both chlorantraniliprole and indoxacarb

CV % = SE/Mean

Our results indicate that the susceptibility of *S. littoralis* to chlorantraniliprole and indoxacarb decreased with larval age; 6th instar larvae had much higher tolerance levels compared to 1st and 2nd instars (Tables 1, 2). The susceptibility of an organism to a particular chemical is influenced by several factors including size, nutrition and physiological status (Liu and Trumble 2005; Stark and Rangus 1994; Yin et al. 2008). The sensitivities of early and late instar as 6th instar larvae tolerance were significantly greater than that of 1st instars (~ 283.3-fold for chlorantraniliprole and 162-fold for indoxacarb). Similarly, Gamil et al. (2011) found that the 2nd instar larvae of *S. littoralis* were more susceptible than 4th instar to indoxacarb. *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) laboratory strain was found to be more susceptible to chlorantraniliprole ($LC_{50} = 0.014$ mg/L) than 18 different field strains in China (Lai and Su 2011). A laboratory strain of *H. armigera* was likewise more tolerant to indoxacarb ($LC_{50} = 0.147$ µg/mL) than chlorantraniliprole ($LC_{50} = 0.0147$ µg/mL) (Bird 2015). Recently, Cui et al. (2018) reported an LC_{50} value of 5.93 mg/L for indoxacarb in 3rd instar *H. armigera* larvae, which is remarkably high.

Table 6 Detoxification enzyme activities in *S. littoralis* larvae

Treatments	MFO (mOD/min/mg protein)	Carboxylesterase (mol/min/mg protein)		GST (mmol/min/mg protein)
		α-esterase	β-esterase	
Control	0.36 ± 0.02^{d}	0.0026 ± 0.00025^{c}	0.202 ± 0.017^{c}	1.78 ± 0.04^{cd}
Chlorantraniliprole				
LC_{10}	1.39 ± 0.04^{b}	0.0045 ± 0.00017^{b}	0.242 ± 0.017^{c}	1.59 ± 0.08^{d}
LC_{50}	1.53 ± 0.86^{b}	0.0072 ± 0.00020^{a}	0.488 ± 0.006^{a}	3.75 ± 0.16^{b}
Indoxacarb				
LC_{10}	5.72 ± 0.13^{a}	0.0037 ± 0.00019^{b}	0.329 ± 0.003^{b}	4.85 ± 0.05^{a}
LC_{50}	0.94 ± 0.05^{c}	0.0014 ± 0.00023^{d}	0.206 ± 0.027^{c}	2.10 ± 0.17^{c}

Mixed Function Oxidases (MFO), Carboxylesterase (α- and β- esterase) and Glutation S-transferase (GST) activities of *S. littoralis* following treatment as 2nd instar larvae with sublethal (LC_{10} and LC_{50}) concentrations of chlorantraniliprole and indoxacarb. After 24 h they were fed with untreated fresh castor leaves. Samples were taken four days post-treatment. For each enzyme assay five replicates/concentration were used

Enzyme activity is showed as mean ± SE and means followed by different letters are significantly different by Tukey's honestly significant different ($p < 0.05$) between control and each treatment of both chlorantraniliprole and indoxacarb

It is a common phenomenon that insects are exposed to sublethal concentrations of insecticides because of their degradation after initial application in crops. So, when larvae are exposed to sublethal concentrations of chlorantraniliprole and indoxacarb it models such circumstances, and well demonstrated that developmental rates had significantly decreased and prolonged the larval and pupal stages (Table 3). These results are in agreement with El-Dewy (2017) who found that both insecticides significantly increased the larval duration after 4th instar larvae of *S. littoralis* were treated with LC_{25} value. Also, both insecticides have been found to inhibit *P. xylostella* development (Guo et al. 2013; Wang et al. 2011). These findings on life span/length and rate of development that occur after larval insecticide exposures are consistent with Yin et al. (2008), and Liu and Trumble (2005) in both spices of *P. xylostella* and *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) respectively. No significant differences in the eggs that hatched were found following the exposure of 2nd instar larvae to LC_{10} and LC_{50} values of either chlorantraniliprole or indoxacarb. This is in accordance with the study of Mahmoudvand et al. (2011) who proved that indoxacarb when tested individually on *P. xylostella* didn't significantly increase egg mortality.

Female adult calling behavior in non-treated control was similar to that described earlier (Dunkelblum et al. 1987; Silvegren et al. 2005). As shown in Fig. 1, intensive calling behavior occurs between the 2nd and 4th hours in scotophase, but then gradually drops to around 10% at the end of scotophase. A similar drastic drop was observed *M. brassicae* following treatment with sublethal doses of spinosad or emamectin benzoate (Moustafa et al. 2016). For *P. xylostella* females, 3rd instar larvae treated with a sublethal dose of indoxacarb resulted in robust calling behavior during the initial scotophase, but decreased with following scotophases (Wang et al. 2011).

Sex pheromone production is tightly coordinated with physiological events that are under hormonal and neuronal control. For moths, pheromone biosynthesis is typically regulated by a neuropeptide, pheromone biosynthesis activating neuropeptide (PBAN) (Bloch et al. 2013; Hull and Fónagy 2019). Unlike most Noctuids, pheromone biosynthesis peaks in *S. littoralis* during the 2nd to 3rd hours of scotophase, which correlates with their calling activity (Silvegren et al. 2005) (Fig. 1). In earlier studies, *S. littoralis* pheromone biosynthesis and production were reported as ng pheromone/PG and the measured amount at peak production of the main component, (*Z,E*)9,11–14:Ac, was around 7–8 ng/PG (Dunkelblum et al. 1987; Marco et al. 1996;). In our study, we obtained 3.26 ± 0.29 ng/PG of (*Z,E*)9,11–14:Ac and 3.96 ± 0.92 ng/PG of (*Z,E*)9,12–14:Ac (Table 5), respectively, with LC_{10} equivalent chlorantraniliprole due to using a very sensitive heat program for the developed SIM method, which two components when summed are comparable to that previously reported (Dunkelblum et al. 1987; Marco et al. 1996;). The results regarding pheromone production in *M. brassicae* had significant differences also in comparison to controls when 2nd instar larvae were treated with different sublethal concentrations of spinosad or emamectin benzoate (Moustafa et al. 2016).

Sublethal concentrations of insecticides could prompt detoxification enzymes such as GSTs that are responsible for insecticide resistance. Increased MFO and GST activities were detected in both insecticide treatments (Table 6). These results indicated that MFO and GST are closely related to chlorantraniliprole and indoxacarb detoxification enzymes system. However, no significant increase in alpha-esterase activity when exposed to indoxacarb LC_{50}. This finding may

be related to the activation of indoxacarb converting it into a decarbomethoxylated metabolite as well demonstrated in *Periplaneta americana* (Linnaeus) (Blattodea: Blattidae) (Gondhalekar et al. 2016; Zhao et al. 2005). In contrast, GST activity was increased 24 h post-treatment in 3rd instar *H. armigera* larvae exposed to LC_{30} value of indoxacarb and hexaflumuron (Vojoudi et al. 2017), whereas CarE and GST activities were reduced after 3 days post-treatment. Sial et al. (2011) proposed that the chlorantraniliprole resistance strain of *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) had higher CarE activity after 9 generations of selection. In contrast, the activity of MFO enzymes in 9 resistant field populations of *S. litura* was significantly higher compared with the susceptible strain, while only 2 populations had higher activities of CarE and GST (Su et al. 2012).

Conclusion

Both chlorantraniliprole and indoxacarb showed high toxicity against *S. littoralis* larvae. The lethal and sublethal exposures to these insecticides significantly affected the larval and pupal developmental period. Additionally, among the detoxification enzymes, MFO, and GST activities increased. However, insecticide resistance development could reduce the efficiency of the two insecticides. Consequently, resistance monitoring should be conducted to generate the information needed for establishing sustainable and effective management strategies for *S. littoralis* that utilize either chlorantraniliprole or indoxacarb.

Acknowledgements This research was funded by the Science & Technology Development Fund (STDF), Egypt (Project ID; 33353). Author P. B. M. is thankful for the János Bolyai Grant fellowship. We would like to express our special thanks to Dr. Ibrahim S. Ahmed (Faculty of Agriculture, Cairo University, Egypt), Dr. József Fodor (Plant Protection Institute of CAR, Budapest, Hungary) and Dr. J. Joe Hull (USDA, ARS, Maricopa AZ, U.S.A.) for improving the manuscript considerably, including English grammar and styles.

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