Original Research Articles

Toxicity of insecticides in *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), a new strawberry pest in Brazil <mark>under laboratory conditions</mark>

ABSTRACT

The strawberry caterpillar, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) was recently found in Brazil causing significant losses for this crop. Because it is a recent pest in the country, still, there is no record of insecticides for its control. Thus, this work evaluated the toxicity of different commercial insecticides on *D. fovealis* second instar caterpillars. The insecticides tested were: acetamiprid, cyromazine, thiamethoxam + lambda-cyhalothrin, milbemectin, chlorantraniliprole, lambda-cyhalothrin, thiamethoxam, fenpropathrin, alpha-cypermethrin, chlorfenapyr, indoxacarb, and lambda-cyhalothrin + chlorantraniliprole. The application was carried out by spraying the caterpillars in a Potter tower. Insecticides indoxacarb, lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr provided mortality data of *D. fovealis* caterpillars from 70.9 to 100%, and could be considered as promising for the management of this pest, once duly registered with the Ministry of Agriculture, Livestock and Supply (MAPA).

Keywords: Fragaria x ananassa, strawberry caterpillar, chemical control

1. INTRODUCTION

Duponchelia fovealis Zeller (Lepidoptera: Crambidae) is a microlepidopteran originating in the Mediterranean region and the Canary Islands, which is currently distributed throughout much of Europe, Asia, India, Africa, Canada and the United States [1,2]. *D. fovealis* is a polyphagous pest with a high number of host species (cereals, vegetables, medicinal and ornamental plants) [1,3–5]. In Brazil, the occurrence was reported in 2008 [1,2,5–7]. In the country, the pest was established in strawberry (<mark>*Fragaria x ananassa*</mark>) crops in the metropolitan regions of Curitiba - PR and Mountainous state of Espírito Santo, where it has caused losses of up to 100% in production [2,8,9]. For this reason, the pest was known as a strawberry caterpillar or crown caterpillar [1,5,9].

Duponchelia fovealis caterpillars pierce the crown region and fruit insertion, weakening the circulation of nutrients [7]. The attack also occurs in the vegetative phase and the defoliation damages the photosynthetic process and, consequently, reduces the production, which in severe attacks can lead the plants to death [1,2]. <mark>The main losses are due to the impossibility of commercialization, since the fruits are not adequate for consumption [5,6,9].</mark>

The management of *D. fovealis* in strawberry plantations has been hampered by the fact that it is an exotic pest and there is still no registration of products for control by the Ministry of

Livestock and Supply (MAPA) [10]. However, some insecticides such as lambda-cyhalothrin, deltamethrin and chlorfenapyr are being used in emergencies in Portugal and Brazil [11].

The control of *D. fovealis* by application of insecticides with contact action is difficult because the caterpillars remain in the basal part of the plant, where they are protected from spraying by the leaves of the strawberry plants [12,13]. Another characteristic that causes difficulty in the control is the habit of this species not to feed during the last larval instar; so insecticides with action by ingestion do not have a lethal effect in this stage [4]. Due to these difficulties and scarcity of control methods, it is necessary to evaluate the potential of synthetic insecticides in the control of this pest. Thus, the objective of this work was to evaluate the toxicity of different commercial insecticides to *D. fovealis* caterpillars.

2. MATERIAL AND METHODS

2.1 Rearing of *Duponchelia fovealis*

Catterpilar of *Duponchelia fovealis* were collected in the state of Espírito Santo from areas with strawberry crops (Latitude: 20 ° 07'50 "S, Longitude: 40 ° 58'15"W), were sent the of Entomology sector of the Nucleus of Scientific and Technological Development in Phytosanitary Management of Pests and Diseases (Nudemafi) of the Center of Agrarian Sciences and Engineering of the Federal University of Espírito Santo (CCAE-UFES), Alegre, Espírito Santo, Brazil. They were kept in a controlled environment at 25 ± 1°C, 70 ± 10% relative humidity (RH) and 14 hours of photophase (14L: 10D). Rearing of the juvenile and adult insects followed the methodology described by Pirovani et al.[8].

2.2 Insecticides used

The insecticides and dosages, in g active ingredient (a.i.)/L, tested were: acetamiprid (Saurus[®], Iharabras S.A. Chemical Industries) – 0.08; cyromazine (Trigard[®] 750 WP, Syngenta Proteção de Cultivos Ltda) – 0.1125; methoxyfenozide (Intrepid[®] 240 SC, Dow Agrosciences Industries Ltda) – 0.12; thiamethoxam + lambda-cyhalothrin (Platinum Neo[®], Syngenta Proteção de Cultivos Ltda) – 0.10575 + 0.0795; milbemectin (Milbeknock[®], Iharabras S.A. Chemical Industries) - 0,02; chlorantraniliprole (Premio[®], Du Pont of Brazil S.A) – 0.0368; lambda-cyhalothrin (Karate Zeon[®] 50 CS, Syngenta Proteção de Cultivos Ltda) – 0.04; deltamethrin (Decis[®] 25 EC, Bayer CropScience Ltda) – 0.0125; thiamethoxam (Actara[®] 250 WG, Syngenta Proteção de Cultivos Ltda) – 0.75; fenpropathrin (Danimen[®] 300 EC, Sumitomo Chemical of Brazil Representations Ltda) – 0.195; alpha-cypermethrin (Fastac® 100, Basf S.A) – 0.01; chlorfenapyr (Pirate[®], Basf S.A) – 0.24; indoxacarb (Rumo[®] WG, Du Pont of Brazil S.A) – 0.048 and lambda-cyhalothrin + chlorantraniliprole (Ampligo[®], Syngenta Proteção de Cultivos Ltda) – 0.025 + 0.05. The highest doses recorded in the package leaflets of each product were used and as a control treatment distilled water was used.

2.3 Toxicity Bioassay

For the bioassay, foliar discs of strawberry of the variety "Tudla" (ø = 4 cm) were sterilized in 5% (v / v) sodium hypochlorite solution and washed immediately under running water. For the removal of excess water, the leaf discs were kept on paper towel, then transferred to glass Petri dishes (9.5 x 1.5 cm), lined with filter paper. After this procedure, five second instar caterpillars were transferred to the leaf discs and in the sequence were sprayed on Potter's Tower[®]. The second instar larvae of *D. fovealis* were selected for use in the bioassay due to the fact that only from this instar it is possible to manage without causing mortality in the manipulation. Moreover, it is from this stage the larvae begin to cause injury

to plants. For the calculation of the dilutions the application volume of 300 L/ha was adopted. The equipment was set at a pressure of 15 lb/in², using 18 mL of each treatment, measured with 2 mL automatic pipette. The upper part of the Petri dish (6 mL), the abaxial face (6 mL) and the adaxial leaf discs (6 mL) were sprayed. After drying, the glass Petri dishes were closed and transferred to an air-conditioned room set at a temperature of 25 ± 1ºC, relative humidity of 70 ± 10% and photophase of 12h (12L: 12D).

The bioassay was installed in a completely randomized design with 14 treatments (insecticides + control) with five replicates (25 caterpillars / replicate) for each treatment. The evaluation of larval mortality was performed after 24, 48, 72 and 96 h after spraying. The accumulated mortality data in 96h were corrected by Abbott's formula [14], transformed into $\sqrt{x+1}$ and submitted to analysis of variance [15]. Mortality averages were grouped by the Scott-knott test ($P ≤ .05$) using the software R version 3.3.3 [16] using the ExpDes.pt package [17]. The Henderson and Tilton formula [18] was used to calculate control efficiency.

2.4 Concentration-Mortality Curves of Insecticides

From the results of the previous experiment, the concentration-mortality curves of caterpillars were established for insecticides that caused mortality higher than 70% of caterpillars. In this way, the insecticides chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole were selected (Table 1). The spraying and evaluation procedure were the same as in the previous bioassay. The concentrations used were based on preliminary tests aiming at a mortality between 0 and 100%, being equidistantly spaced by means of logarithmic transformation [15]. Based on previous results, insecticides (concentration range, mg a.i./L) were selected for this test: chlorfenapyr (25.0-250.0); indoxacarb (0.025-1.0) and; lambda-cyhalothrin + chlorantraniliprole (10.0-2,250.0).

The accumulated caterpillar mortality data were submitted to probit analysis [15,19], using the ecotoxicology package [20]. The toxicity ratios (TR) were obtained by means of the quotients between the LC_{50} of the insecticide that showed the lowest toxicity and the LC_{50}s of the other treatments [15]. The LC_{dq} values obtained for insecticides were compared based on the confidence intervals at 95% probability, as proposed by Carvalho et al. [15].

3. RESULTS

3.1 Toxicity of insecticides

The corrected mortality of *D. fovealis* varied between the insecticides studied (F = 5.404, df = 14, 32, $P < .001$) (Table 1). The insecticides, lambda-cyhalothrin, milbemectin, cyromazine, thiamethoxam, methoxyfenozide and deltamethrin did not cause mortality and therefore were not considered in the analysis (Table 1). Among the other insecticide mortality ranged from 17 to 100%. The insecticides chlorfenapir, indoxacarb and lambda-cyhalothrin + chlorantraniliprole were induced higher mortality, being statistically similar among each other and significantly different with other insecticides tested. Thus, these insecticides were the most efficient in controlling *D. fovealis* (Table 1).

The insecticides alpha-cypermethrin, chlorantraniliprole, acetamiprid, thiamethoxam + lambda-cyhalothrin and fenpropatrin caused mortality of less than 41%.

Table 1. Corrected mortality[1] of 2nd instar caterpillars of *Duponchelia fovealis* treated with different insecticides and control efficiency. Temp .: 25 ± 1 ° C, RH 70 ± 10% and 12h of photophase

Active ingredient	Corrected Mortality [2,3]	EC[4]
Alfacipermethrin	40.6 ± 6.46 b	40.0
Indoxacarbe	70.9 ± 12.47 a	73.3
Chlorantraniliprole	34.6 ± 12.36 b	35.0
Acetamiprid	24.5 ± 10.75 b	33.3
Lambda-cyhalothrin + Chlorantraniliprole	80.0 ± 12.93 a	80.0
Thiamethoxam + Lambda-cyhalothrin	24.5 ± 10.75 b	33.3
Phenpropathic	16.9 ± 6.23 b	20.0
Chlorfenapyr	100.0 ± 0.00 a	100.0
Lambda-cyhalothrin*	0.0 ± 0.00	-
Milbemectin*	0.0 ± 0.00	-
Cyromazine*	0.0 ± 0.00	-
Thiamethoxam*	0.0 ± 0.00	-
Methoxyfenozide*	0.0 ± 0.00	-
Deltamethrin*	0.0 ± 0.00	-
F	5.404	
P	<.001	

[1] Mortality corrected by Abbott's formula (1925);
[2] Means followed by the same letter in the column do not differ from each other by the Scott-Knott test (*P* ≤ .05).
[3] Data transformed by $\sqrt{x + 1}$.
[4] Efficiency of control [Henderson and Tilton equation (1955)].
* Products that did not cause mortality were omitted and did not participate in the analysis.

3.2 Concentration-Mortality Curves of Insecticides

The mortality data of *D. fovealis* treated with different concentrations of the three insecticides selected were adjusted to the Probit model (*P* > .05). The LC_{50} values for chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole were 64, 0.08 and 30 (mg a.i./L), respectively (Figure 1, Table 2). Based on these results, the lowest LC_{50} was observed for indoxacarb, followed by lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr. The toxicity ratio of indoxacarb is 800 times more toxic than chlorfenapyr.

Lambda-cyhalothrin + Chlorantraniliprole

$M = \phi\left(3.6128 + 0.9322 \cdot \log_{10}(x)\right)$

$\chi^2 = 5.84,\ p = 0.559$

$LC_{90} = 2.86$ [729 mg/L]

$LC_{50} = 1.49$ [30.7 mg/L]

Chlorfenapyr

$M = \phi\left(0.8207 + 2.3126 \cdot \log_{10}(x)\right)$

$\chi^2 = 8.74,\ p = 0.8801$

$LC_{90} = 2.36$ [229 mg/L]

$LC_{50} = 1.81$ [64 mg/L]

Indoxacarbe

$LC_{90} = -0.16$ [0.69 mg/L]

$LC_{50} = -1.09$ [0.08 mg/L]

$M = \phi\left(6.4989 + 1.3995 \cdot \log_{10}(x)\right)$

$\chi^2 = 2.06,\ p = 0.159$

- Observed values
- Estimated values
- CI95

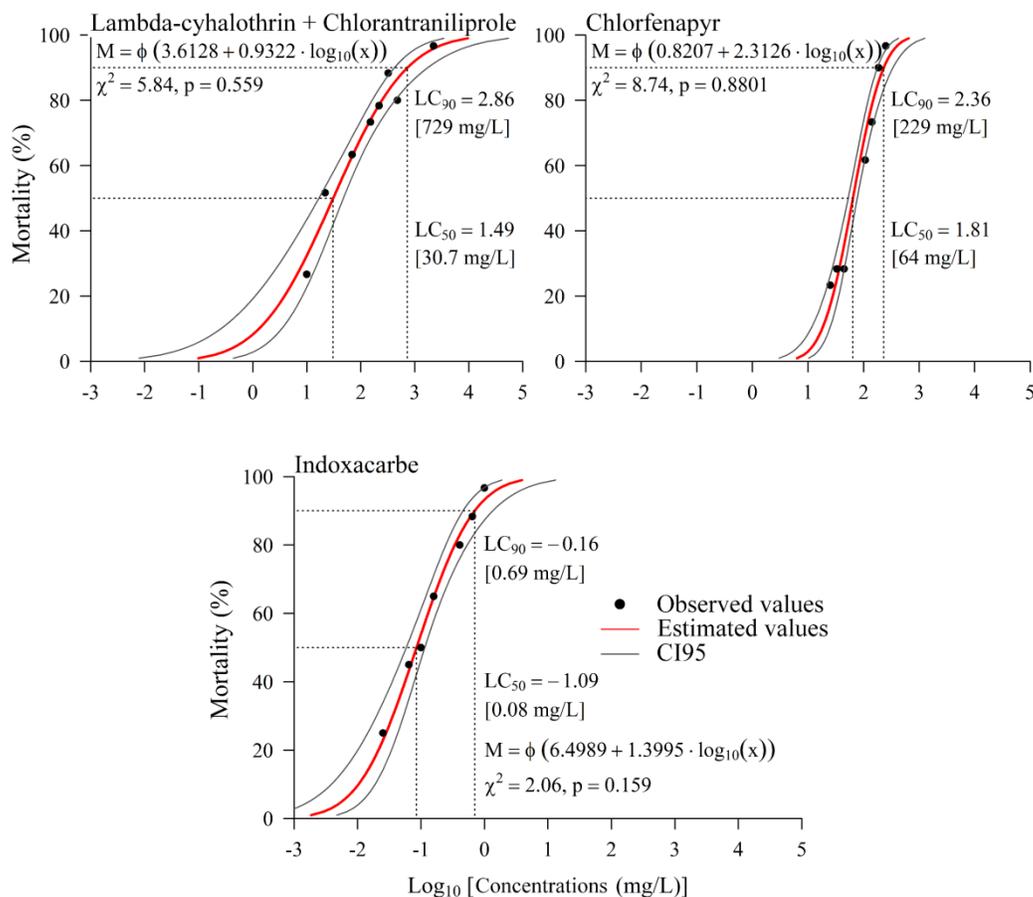Mortality (%) — $\log_{10}$ [Concentrations (mg/L)]

Figure 1. Concentration-mortality curves of chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole on second instar caterpillars of *Duponchelia fovealis*. Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase.

Table 2. Response of the concentration-mortality curves of chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole on second instar caterpillars of *Duponchelia fovealis*. Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase

Active ingredient	n^1	LC_{50} (CI a 95%)[2] * (mg a.i./L)		TR_{50}[3]
Chlorfenapyr	420	64 (52-77)	c	-
Lambda-cyhalothrin + chlorantraniliprole	480	30.7 (16.7-47.5)	b	2.13
Indoxacarbe	420	0.08 (0.07-0.1)	a	800

[1] Number of insects used in the test;

[2] Confidence interval of the LC_{50} at 95% probability;

[3] Toxicity ratio;

* LC_{50}s accompanied by the same letter do not differ from each other based on the confidence interval at 95% probability.

4. DISCUSSION

The absence or reduced of mortality of *D. fovealis* by the insecticides lambda-cyhalothrin, milbemectin, cyromazine, thiamethoxam, methoxyfenozide and deltamethrin, alpha-cypermethrin, chlorantraniliprole, acetamiprid, thiamethoxam + lambda-cyhalothrin and fenpropatrin may be related the low concentrations tested, or this species may have some degree of resistance/tolerance to the active ingredients. Information on insecticide toxicity bioassays for *D. fovealis*, as well as insect resistance to active ingredients, have not been reported in the literature, possibly because this pest was not considered a problem for crops in other countries.

Among the insecticides tested, chlorfenapyr, which caused higher mortality in *D. fovealis* caterpillars, is a de-coupling of oxidative phosphorylation through the interruption of the H^+ proton gradient, acting on the respiratory metabolism of the insect [21]. This insecticide presented the highest slope of the concentration-mortality curve among the three products (Figure 1). High line slope values indicate that small variations in insecticide concentration promote greater responses in target pest mortality. Thus, small amount of the active principle is effective in the mortality of this pest species in less time of exposure as it occurs with *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) after 24h exposure [22].

The insecticide indoxacarb is used in the control of different Lepidoptera species; for example, damage by *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) has an 80% reduction effectiveness [23]. In addition, indoxacarb has low toxicity to non-target organisms [24], which makes this insecticide an option to be used in association with other management tactics, such as biological control.

Indoxacarb acts after bioactivation in the activity of enzymes present in the insect and that result in the formation of the metabolite JT333 (N-decarbomethoxyllated) that causes the blockade of the sodium channels. The metabolism of indoxacarb in JT333 causes small seizures that paralyze the insects, followed by death [25]. Activities such as feeding and oviposition are interrupted rapidly after exposure to the product, whereas death may take 4 to 72 hours [21], which can be proven in the present study with *D. fovealis*.

The insecticide based on lambda-cyhalothrin + chlorantraniliprole is available as a ready-to-use mixture with activity at two sites of action. The active principle lambda-cyhalothrin belongs to the chemical group of pyrethroids and they are known as modulators of the sodium channels of the nerve cells of the peripheral nervous system, prolonging the influx and reducing the threshold of the action potential of the membrane, causing immediate paralysis and mortality, which is called a knock-down effect [25].

On the other hand, chlorantraniliprole is an insecticide modulator of rianodine receptors, which regulate the release of calcium in the sarcoplasmic reticulum of muscle cells, thus affecting the muscular contraction of insects [21,26]. These insecticides require a longer time to cause insects to die [27]. However, the association between lambda-cyhalothrin and chlorantraniliprole, in the insecticide tested, promoted mortality similar to those of indoxacarb and chlorfenapyr at the same time exposure period.

Although the LC_{50} of lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr in *D. fovealis* is superior, when compared to that of indoxacarb, these insecticides are promising for the integrated management of the pest, since they can be used in rotation of mechanisms of action, thus minimizing the risk of selection of insecticide-resistant individuals.

5. CONCLUSION

Of the insecticides tested, indoxacarb, lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr were the most effective for the control of *D. fovealis* under laboratory conditions. The indoxacarb was the most lethal for *D. fovealis* and could be considered for semi-field and field bioassays for pest control.

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