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Sublethal Doses of Insecticides Affect the Fecundity and Fertility of the *Chrysodeixis includens*?

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Abstract

The objective was to determine lethal concentrations and to evaluate the effects of sublethal doses of insecticides on *Chrysodeixis includens* under laboratory conditions. The standard population of *C. includens* was kept on artificial diet under controlled conditions (25˚C ± 5˚C, 60% ± 10 RU, 12:12 h). The active principles used were *B. thuringiensis*, Indoxacarb and Teflubenzuron. The CL_{25} and LC_{50} obtained from dose response curve (estimated from eight concentrations of each active principle) were diluted in water and applied to the back of third instar caterpillars and compared to the control (water). The insecticides used showed lethal and sublethal effects in *C. includens*. *Bacillus thuringiensis* was 6.21 and 2.79 times more toxic to soybean looper when compared to the products Indoxacarb and Teflubenzuron, respectively. Insecticides applied to the caterpillars reduced survival and affected the biomass gain with increased larvae and pupae longevity, with a significant reduction in fertility (40%) and fecundity (94.97%) in adults. In the present study it was observed that both survival and larval biomass gain have a direct effect on fecundity and fertility, respectively. Therefore the active ingredients Indoxacarb, Teflubenzuron and *B. thuringiensis* can be used in rotation as a tool in the *C. includens*.

Keywords

Bacillus thuringiensis, Chemical Control, Indoxacarb, Soybean Looper, Teflubenzuron

1. Introduction

Crops such as soybeans and beans have suffered from constant population outbreaks of *Chrysodeixis includens* (Walker, 1857) (Lepidoptera: Noctuidae) [1] [2], known as soybean looper [3], with great defoliator potential [4] of polyphagous habit [5].

The use of chemicals and transgenic plants is a major control measure employed in the management of *C. includens* [1] [2]. Among the commonly used insecticides, there is an obstacle due to two aspects: resistant, and the lack of knowledge about the lethal and sublethal effects of insecticides on the pest [6] [7].

The toxicity of the insecticide reduces to the ability of a molecule to cause harmful damage to the insect. Since control failures during management [8] may result in exposure of the pest to sublethal doses [7]. In addition, insects that survive after toxic exposure to an insecticide may present changes in biological, reproductive, behavioral and physiological parameters [6] [9].

The main insecticides used in the management of lepidopteran pests are inhibitors of biosynthesis of chitin (Teflubenzuron); voltage-dependent sodium channel blockers (Indoxacarb); and the microbial disruptors of the mesenteric membrane (*Bacillus thuringiensis*) [10]. The sublethal effects of these insecticides are already documented in the literature, and the exposure of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to sublethal doses of the active ingredients *B. thuringiensis* and Indoxacarb resulted in an increase in larval longevity, reduction in biomass gain larvae and pupae in addition to affecting the fertility and fecundity of adults [7] [11].

Studies that seek to know the changes on the biology and reproduction of the pests in recurrence of control failures, are of great importance in the integrated pest management (IPM), to establish how and what characteristics of the insects are affected by the exposure of the caterpillar to an sublethal dose of the insecticide. The objective was to determine the lethal concentrations (CL_{25} and CL_{50}) and to evaluate the sublethal effects caused by the application of insecticide overdoses on *C. includens* under laboratory conditions.

2. Material and Methods

2.1. Creation Maintenance

The insects were maintained with artificial diet adapted from [12] wheat germ (237 g), beer yeast (152 g), ascorbic acid (15 g), sorbic acid (4.45 g), nipagin (9.45 g), agar (12.5 g) (propionic acid 41.8%, 4.2% phosphoric acid and 54% water). Caterpillars (<24 h old) were individualized and kept in plastic cups of 50 ml, capped, fed with artificial diet until reaching the pupal stage. The adults were transferred to PVC cages of 40 cm H × 30 cm Ø, internally covered with sheets of sulphite paper for oviposition. These were fed a honey-based solution (10%) and kept in controlled environment (25˚C ± 5˚C, 60% ± 10% RH and 12:12 h). The eggs were collected and stored in plastic cups of 50 ml with lid until the hatching of the caterpillars.

2.2. Dose Response Curve

A preliminary test of the toxicity of 13 insecticides on *C. includens* was carried out, using the 50%, 100% and 150% fractions of the recommended dose, the experiment was conducted in a Double Factorial (Product x Dose).

The active principles selected were *B. thuringiensis*, Indoxacarb and Teflubenzuron. To estimate the dose response curve, the dose recommended for the management of *C. includens* in the soybean crop was fractionated in eight concentrations (0%, 5%, 15%, 25%, 50%, 75%, 100% and 125%) (**Table 1**).

The bioassay was performed and conducted according to the adapted methodology of [13], using 3rd instar caterpillars of *C. includens*. After assembly of the experiment the caterpillars were kept under controlled conditions, previously described. The experiment was conducted in DIC with three replicates (15 caterpillars/replicate). Mortality was assessed every 24 hours for a period of 96 hours. The caterpillars, which after physical stimulation with the tweezers in the prothorax region showed no movement, were considered dead. Lethal concentrations (CL_{25} and CL_{50}) were estimated using the Probit routine with the statistical program SAS Version University Edition [14].

2.3. Sublethal Effects

The concentrations (CL_{25} and CL_{50}) obtained in the previous bioassay were used to characterize the sublethal effects of insecticides on *C. includens* and compared to a control (water). The bioassay was performed and conducted according to the methodology described by [13].

The larval survival was evaluated daily until the caterpillars reached the pupal stage. The obtained pupae were weighted at 24 hours, separated by sex and transferred to Petri dishes lined internally with filter paper, and evaluated daily until the adult phase, to evaluate longevity, emergence, fertility and fecundity of adults.

The values for the number of caterpillars that became pupae and the number of pupae that hatched were used to estimate the survival of larvae and pupae,

Table 1. Insecticides and doses used to determine the dose response curve in *Chrysodeixis includens*.

Treatments	Concentration of the active ingredient (μg ia. mL^{-1})						
	5%	15%	25%	50%	75%	100%	125%
Indoxacarb	3.75	11.25	18.75	37.5	56.25	75	93.75
Bacillus thuringiensis	1.176	3.528	5.88	11.76	17.64	23.52	29.4
Teflubenzuron	1.125	3.375	5.625	11.25	16.875	22.5	28.125

i.a. Active ingredient.

respectively. Pupae sexing was performed according to the adapted methodology of [15]. To determine the sex ratio, the number of females was divided by total pupae (number of pupae). The bioassay referring to the sublethal effects of insecticides on larvae and pupae of *C. includens* were conducted in completely randomized design (CRD) with four replicates (20 caterpillars/replicate).

After sexing, individuals up to two days old were grouped in pairs and fed with 10% honey solution, provided in 50 mL cups with cotton, kept in PVC cages (15 cm high/10 cm Ø) covered with A4 paper and sealed at the lower end with cardboard and top with elasticated "voile" type fabric. The cages were randomly distributed on shelves maintained in a controlled environment (25˚C ± 5˚C, 60% ± 10 RU and 12:12 h), egg counting was performed every 48 hours using a stereomicroscope, until the end of period of oviposition. The mean number of eggs per female was defined as fecundity and fertility was calculated from the viability of 100 eggs from each couple. Evaluations related to oviposition were conducted in CRD using 6 replicates of each concentration (one pair/replicate).

The sublethal effects evaluated were survival (larvae and pupae), biomass and longevity of larvae and pupae; sexual reason; fecundity and fertility.

2.4. Statistical Analyses

Pearson correlation coefficients were estimated between the evaluated characters (sublethal effects). From the correlations estimated the trail coefficient, this study deals with the decomposition of the correlation between the basic variable (fecundity and fertility) and the explanatory ones (survival, biomass and longevity of larvae and pupae, sexual ratio). All the mathematical description of the track coefficient estimation is found [16], all statistical procedures were performed based on the genetic-statistical procedure of the Genes software [17]. The design used in all bioassays was completely randomized design since the obtained data were submitted to analysis of variance (ANOVA), by the test f at 5% of probability. Significant results were compared by the Tukey Means Differentiation Test with program R Version 3.2.1 [18].

3. Results

Bacillus thuringiensis was considered to be the most toxic product to *C. includens* because it presented the lowest value for LC50, being 6.21 and 2.79 times lower than the Indoxacarb and Teflubenzuron products, respectively (**Table 2**). By means of the obtained results the three active principles can be used in rotation in the *C. includens.*

The results concerning the effect of the insecticides on the development and reproduction of *C. includens* are described in **Table 3**. The three active principles evaluated reduced the biomass gain in the larvae. The changes on the biomass and the longevity of the pupae were significant, however they were not affected drastically the development of the insect. According to the results obtained for the characteristics of adults of *C. includens*, it was observed that the

Table 2. Lethal concentration (CL_{25} and CL_{50}) of insecticides tested on *Chrysodeixis includens* after 96 hours of exposure.

Treatments	Answer Dose μg ia. mL^{-1}		Inclination ± DP	X^2	F**
	CL_{25} (IC 95%)	CL_{50} (IC 95%)			
Bacillus thuringiensis	1.5 (1.23 - 1.75)	2.45 (2.13 - 2.79)	2.554 ± 3.80	15.09	0.96
Indoxacarb	11.10 (4.91 - 15.69)	15.21 (10.21 - 27.19)	1.598 ± 8.25	249.14	0.006
Teflubenzuron	3.28 (1.69 - 4.80)	6.84 (4.62 - 8.74)	1.501 ± 2.72	20.08	0.389

DP: Standard deviation; CL: Lethal concentration; CI: Confidence interval; X2: Chi-square; C.V.: Coefficient of variation **($p \leq 0.01$). ia—active ingredient.

Table 3. Biological and reproductive parameters of *C. includens* after application to CL_{25} and LC_{50} of different active principles.

Parameter[1]	Dose	*Bacillus thuringiensis*	Indoxacarb	Teflubenzuron	Witness	F	C.V. (%)
Larval Biomass (mg)	CL_{25}	43.09 ± 2.57 b	61.25 ± 3.85 ab	40.51 ± 1.21 b	74.00 ± 3.42 a	5.77**	24.08
	CL_{50}	48.57 ± 1.09 b	50.95 ± 1.29 b	35.60 ± 1.97 b	74.00 ± 3.42 a	11.02**	18.41
Larval Longevity (Days)	CL_{25}	21.18 ± 0.43 b	20.31 ± 0.08 b	22.26 ± 0.42 a	20.72 ± 0.09 b	12.26**	2.32
	CL_{50}	21.28 ± 0.21 ab	20.47 ± 0.13 b	21.38 ± 0.18 a	20.72 ± 0.09 b	7.92**	1.35
Pupae Biomass (mg)	CL_{25}	242.79 ± 1.42 a	250.32 ± 2.79 a	231.01 ± 5.91 b	245.15 ± 1.38 a	18.32**	1.60
	CL_{50}	235.5 ± 5.83 b	243.06 ± 2.79 ab	231.73 ± 1.84 b	245.15 ± 2.15 a	3.85**	3.85
Longevity of Pupae (Days)	CL_{25}	7.44 ± 0.15 b	6.38 ± 0.08 c	7.86 ± 0.06 a	7.26 ± 0.05 b	48.33**	2.45
	CL_{50}	7.67 ± 0.24 a	6.48 ± 0.09 b	7.79 ± 0.30 a	7.26 ± 0.05a b	8.28**	5.44
Sexual Reason	CL_{25}	0.61 ± 0.06 a	0.44 ± 0.01 b	0.63 ± 0.04 a	0.39 ± 0.05 b	12.60**	14.30
	CL_{50}	0.48 ± 0.11	0.383 ± 0.04	0.45 ± 0.06	0.39 ± 0.05	2.38[N.S.]	25.22
Fecundity of Adults	CL_{25}	41.00 ± 1.06 d	107.16 ± 4.83 b	78.50 ± 1.60 c	405.00 ± 5.77 a	1703**	5.99
	CL_{50}	114.16 ± 3.51 c	132.83 ± 2.35 b	20.33 ± 1.02 d	405.00 ± 5.77 a	2001*	5.29
Fertility (%)	CL_{25}	58.33 ± 2.47 b	70.00 ± 6.95 b	87.50 ± 3.81 a	99.16 ± 0.83 a	17.9**	13.05
	CL_{50}	60.00 ± 3.41 b	60.83 ± 3.00 b	60.83 ± 3.74 b	99.16 ± 0.83 a	39.35**	10.58

**Significant to 1 of probability by the test f. N.S. Not significant by test f. 1 Medias followed by the same letter on the line do not differ by test Tukey ($p \leq 0.05$).

CL_{25} of the active ingredients *B. thuringiensis* and Teflubenzuron increased in 56.86% and 60.61%, respectively, the sexual ratio in relation to the control. The CL_{25} of the active ingredient *B. thuringiensis* reduced the adult fecundity by 87.87% (355.87 eggs), while the Teflubenzuron LC_{50} decreased by 94.97% (384.62 eggs) (**Table 3**). The three active principles reduced fertility by approximately 40% for LC_{50}, with the highest percentages for *B. thuringiensis* and Indoxacarb (**Table 3**).

The survival of *C. includens* was affected by insecticides, and the results obtained were statistically significant (**Table 4**), within each LC at each stage of development evaluated. The CL_{25} of *B. thuringiensis* and Teflubenzuron caused a reduction in larval survival of 28.75% and 275%, respectively, and in total survival 57.50% and 66.25%, respectively (**Figure 1**).

Table 4. Coefficients of the Pearson correlation between the biological parameters of *C. includens* after application to CL_{25} and LC_{50} of different active principles.

	SL	BL	LL	BP	LP	SP	RS	ST	FT
BL	0.70**								
LV	−0.47*	−0.49**							
BP	0.48*	0.40*	−0.51**						
LP	−0.49**	−0.29$^{N.S.}$	0.73**	−0.41*					
SP	0.35*	0.49**	−0.52**	0.23$^{N.S.}$	−0.19$^{N.S..}$				
RS	−0.22$^{N.S.}$	−0.32$^{N.S.}$	0.21$^{N.S.}$	0.08$^{N.S.}$	0.15$^{N.S.}$	−0.21$^{N.S.}$			
ST	0.83**	0.75*	−0.56*	0.41*	−0.37$^{N.S.}$	0.78**	−0.29$^{N.S.}$		
FT	0.50**	0.52**	0.12$^{N.S.}$	0.05$^{N.S.}$	0.15$^{N.S.}$	0.43**	−0.03$^{N.S.}$	0.62**	
FC	0.53**	0.73**	−0.29$^{N.S.}$	0.25$^{N.S.}$	−0.18$^{N.S.}$	0.48*	−0.31$^{N.S.}$	0.66**	0.63**

SL—Larval Survival; BL—Larval biomass; LG—Larval Longevity; BP—Biomass of the pupa; Longevity of the pupa; SP—Survival of the pupa; RS—Sexual Reason; ST—Total Survival; FT-Fertility; Total FC—Fecundity. Ns, *, ** not significant, significant at 5% and 1% respectively by the test t.

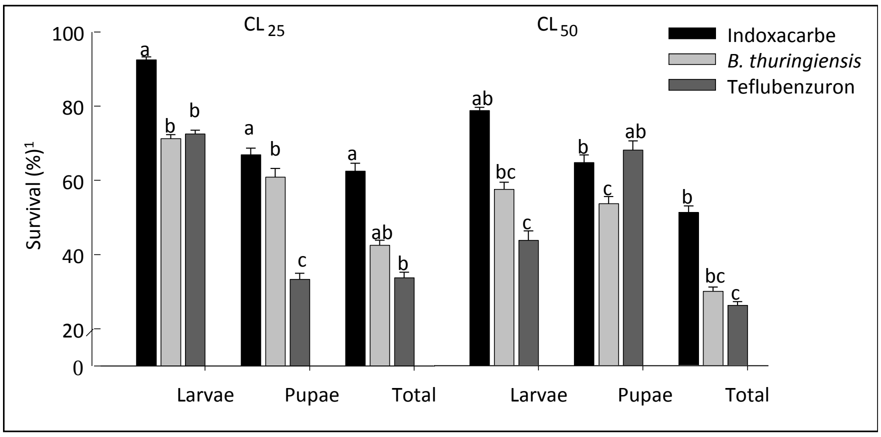

Figure 1. Survival (±EPM) of *C. includens* total and larvae and pupa stages after toxic exposure to different insecticides for CL_{25} and CL_{50}.

The Teflubenzuron LC_{50} decreased by 56.25% and 73.75%, larval and the total survival, respectively, whereas in the same dose *B. thuringiensis* reduced the survival of *C. includens* pupae by 56.35% (**Figure 1**).

According to the Pearson correlation coefficients established among the biological parameters evaluated (**Table 4**), it was observed that the larval biomass presented a negative correlation with larval and pupal longevity and positive survival (larval, pupal and total), fecundity and fertility.

According to the coefficients estimated by the trail analysis (**Table 5**), it can be established that fecundity has a positive, strong and direct influence in relation to total survival, with the same effect in relation to fertility in contrast to biomass larval. The fecundity is affected indirectly by the parameters biomass, survival and longevity of larval and pupae; as well as the parameters longevity of larvae and pupae in conjunction with total survival indirectly affect the fertility of adults (**Table 5**).

Table 5. Estimates of the direct and indirect effects of the variables survival, biomass, longevity and sexual reason on the correlations with the main variables fecundity and fertility of *C. includens* after application to CL_{25} and LC_{50} of different active principles.

Variable	Association Ways	Track Coefficients with Collinearity		Variable	Association Ways	Track Coefficients with Collinearity	
		Fecundity				Fecundity	Fertility
Larval Survival	DE	−0.613	0.520	Pupae Longevity	DE	0.040	−0.043
	DI - LB	0.200	−0.367		DI - LS	0.302	−0.256
	DI - LL	−0.086	0.220		DI - LB	−0.113	0.598
	DI - PB	0.009	−0.046		DI - LL	0.140	−0.124
	DI - PL	−0.020	0.021		DI - PB	−0.008	0.042
	DI - OS	−0.099	0.141		DI - PS	0.088	−0.125
	DI - SR	0.010	−0.033		DI - SR	−0.011	0.037
	DI - TS	1.210	0.014		DI - TS	−0.609	−0.007
Larval Biomass	DE	0.318	0.821	Pupae Survival	DE	−0.443	0.628
	DI - LS	−0.386	−0.233		DI - LS	−0.137	0.117
	DI - LL	−0.093	−0.170		DI - LB	0.131	−0.399
	DI - PB	0.008	0.051		DI - LL	−0.093	0.144
	DI - PL	−0.014	−0.031		DI - PB	0.003	−0.017
	DI - PS	−0.182	−0.305		DI - PL	−0.008	0.009
	DI - SR	0.018	0.028		DI - SR	0.012	−0.041
	DI - TS	1.048	−0.010		DI - TS	1.137	0.013
Larval Longevity	DE	0.192	0.349	Sexual Reason	DE	−0.057	0.193
	DI - LS	0.274	0.327		DI - LS	0.103	−0.088
	DI - LB	−0.155	−0.399		DI - LB	−0.098	0.119
	DI - PB	−0.010	−0.043		DI - LL	0.028	−0.107
	DI - PL	0.029	0.015		DI - PB	0.002	−0.012
	DI - PS	0.215	0.259		DI - PL	0.008	−0.008
	DI - SR	−0.008	−0.059		DI - PS	0.094	−0.134
	DI - TS	−0.831	0.012		DI - TS	−0.417	−0.005
Pupae Biomass	DE	0.019	−0.097	Total Survival	DE	1.520	0.018
	DI - LS	−0.288	0.244		DI - LS	−0.488	0.414
	DI - LB	0.140	−0.435		DI - LB	0.219	−0.449
	DI - LL	−0.102	0.153		DI - LL	−0.105	0.240
	DI - PL	−0.017	0.019		DI - PB	0.007	−0.037
	DI - PS	−0.078	0.110		DI - PL	−0.016	0.017
	DI - SR	−0.007	0.023		DI - PS	−0.331	0.470
	DI - TS	0.578	0.007		DI - SR	0.016	−0.053

DE—Direct Effect; DI—Indirect Effect; LS—Larval Survival; LB—Larval Biomass; LL—Larval Longevity; PB—Pupae Biomass; PL—Pupae Longevity; PS—Pupae Survival; SR—Sexual Reason; TS—Total Survival.

4. Discussion

The use of active principles with different modes of action is suggested as a tool of phytosanitary protection [7], in order to avoid the occurrence of problems related to resistance of insect pests [8]. Based on this premise the present study had the purpose of evaluating the toxicity of different insecticides on the soybean looper (*C. includens*), being found that the active principles *B. thuringiensis*, Indoxacarb and Teflubenzuron can be used in the management of *C. includens*, because the values obtained for LC_{50} were similar to those reported in the literature for other pest lepidoptera, such as *H. armigera* [7] [11] [19]. Since studies referring to the baseline characterization of susceptibility of soybean looper to insecticides are scarce in the literature [20].

Among the evaluated products, the active ingredient *B. thuringiensis* was considered the most toxic to *C. includens* because it presented the lowest value of LC_{50}. This is an important finding because the entomopathogenic bacterium, *B. thuringiensis*, is a biological control agent that allows the control of a pest range, with great efficiency and with less damage to the environment [21].

During the phytosanitary management of the crop, application failures may occur [8], resulting in the exposure of the pest to insecticide sublethal doses, according to results found, where changes in the biology and reproduction of *C. includens* were observed through the toxic action of sublethal doses of insecticides *B. thuringiensis*, Indoxacarb and Teflubenzuron. This is because insect exposure to a sublethal concentration of insecticide may not lead to target pest death, but may affect biochemical reactions and physiological processes at secondary sites of action [7]. It is of fundamental importance to perform studies aimed at characterizing the sublethal effects of different insecticides on the soybean looper, since they provide prior knowledge to be increased within the IPM, especially when faults occur during the application [22] [23].

It was found that *C. includens* exposure to *B. thuringiensis*, Indoxacarb and Teflubenzuron active substances affect longevity as well as reduce the biomass gain of larvae and pupae, besides affecting the fecundity and fertility of adults [7] [22] [24].

Teflubenzuron caused the highest percentages of reduction in larval biomass gain. For Teflubenzuron is an inhibitor of the biosynthesis of chitin type 0, which affects the development and metamorphosis of the insect [10], which can promote the loss of hemolymph and body fluids during ecdysis, of larval biomass [22]. This is an important finding within IPM because smaller caterpillars cause minor damage [7].

The lower accumulation of biomass results in an increase in the larval longevity of *C. includens*. [7] in his work with the exposure of *H. armigera* to CL30 of Hexaflumuron (analogous to Teflubenzuron) incorporated into the diet, observed a considerable reduction in larval biomass gain with an increase in larval longevity (five days), as observed in the present study. As the increase in larval longevity gives rise to natural biological control, as the period of exposure of the pest to natural enemies increases [25]. It was found a low larval biomass with an increase in larval and/or pupal longevity, which delays the emergence of the adult, besides reducing the fecundity and fertility [7] [13].

The active principles *B. thuringiensis* and Teflubenzuron increased the sexual ratio of adults, being an unfavorable point in pest management, since a larger number of females result in a high number of offspring, increasing the pest damage potential. However, low fecundity and fertility rates were obtained, these results reinforce the importance of conducting experiments related to the complete characterization of the insect-pest biology.

There was a considerable reduction in the fertility and fecundity of adults through the toxic action of the products, a significant result for the context of pest management, did not occur adult death, but there was a decrease in population density. These results corroborate with those found in the literature, where applications of insecticides made in immature stages caused changes in the frequency of copula, female fertility, in the production of sperm, which results in a reduction in the proliferation (fecundity and fertility) of the insect-plague [26] [27]. The active principle Teflubenzuron caused the highest percentages of reduction of survival of the soybean looper. These results are consistent with the literature, since the same active principle incorporated in the diet reduced the survival of *H. armigera* by 60% [28]. A significant reduction in pupal survival was observed, resulting in a lower emergence of adults, where the active principles *B. thuringiensis* and Teflubenzuron had the highest percentages of reduction. For the insect, pupae and pupa-adult transformation phases, the low concentrations of *B. thuringiensis* are the most sensitive [29]. Results lower than those obtained were the sublethal concentrations—CL_{25} and CL_{50}—of *B. thuringiensis*, with 40.20% and 30.5%, respectively, of the reduction in the emergence of adults [11]. Reducing survival through the use of insecticide sublethal doses is important in integrated pest management, as the pest population may remain below the control level when the insect is exposed at doses below the recommended dose.

The reduction in the survival of *C. includens*, reconciled with the increase in the time of development, the reduction in the fertility and fecundity of the adults; may increase population doubling time and subsequently lead to lower population growth [6]. These findings are important because the insect-pest population may require a longer period of time to reach the level of control.

A positive correlation was observed between the reduction in the larval biomass gain of the insects with a reduction in the fertility and fecundity of the adults. Because caterpillars weakened from a nutritional point of view, they originate smaller adults with lower fecundity [7]. When analyzing the results obtained it was determined that reductions in larval biomass gain can lead to changes in the cycle and reproduction of *C. includens*. And according to the track analysis it can be established that both the reduction in survival and the gain of larval biomass have a direct impact on the fecundity and fertility of adults. The larval biomass gain, longevity of larvae and pupae indirectly affect fertility and fecundity. It can be established that the reproductive parameters are affected negatively, when the caterpillars suffer reductions in the gain of larval biomass. It is necessary to carry out studies that seek to establish how insecticides sublethal doses affect the nutrition, fertility and fecundity of *C. includens* adults.

In summary, a drastic reduction in survival was observed, as well as in the fertility and fecundity of adults, resulting in a smaller number of fertile adults. These findings are consistent with the existing premises where applications of insecticide sublethal doses in immature stages of development affect the development.

5. Conclusions

The active ingredient *B. thuringiensis* presents higher toxicity to *C. includens* when compared to Indoxacarb and Teflubenzuron products.

The active principles evaluated affect the survival, biology, fecundity and fertility of *C. includens*.

The survival and the gain of larval biomass have a strong direct effect on fecundity and fertility, respectively.

Indoxacarb, Teflubenzuron and *B. thuringiensis* can be used in rotation as a tool in the management of *C. includens.*

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