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IMPACT OF RESIDUAL INSECTICIDES USED IN SUGAR CANE ON ADULTS OF *Cotesia flavipes* (HYMENOPTERA: BRACONIDAE)

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Abstract: The present study aimed to evaluate the selectivity of adults of the parasitoid Cotesia flavipes on insecticides used in sugarcane. The experiment was carried out in a greenhouse and in the Entomology laboratory of ILES/ULBRA in the municipality of Itumbiara-GO. The experiment was carried out in a completely randomized design with 7 treatments and 10 replications.The insecticides used were: Chlorantraniliprole, Chlorantraniliprole + lambda-cyhalothrin, Triflumuron, Bacillus thuringiensis, Lambda-cyhalothrin + thiamethoxam and Fipronil. For each insecticide, the commercial doses recommended for the control of Sugarcane Borer (Diatraea saccharalis) were used.. For installation of the experiment, pre-sprouted seedlings of sugarcane variety RB85 5156 were planted in 10 L polyethylene pots. After the application of insecticides, the sugarcane leaves were cut into pieces of 5 cm in length in order to occupy the internal area of the plate. In eachIn a petri dish, ten adults of C. flavipes were released, constituting an experimental plot. Petri dishes were kept at room temperature in the shade until the insect mortality was evaluated, which was carried out at 6, 12, 18 and 24 hours after exposure of natural enemies to insecticides.The insecticides tested significantly affected the mortality of C. flavipes on the day of spraying and at 5 and 10 days after spraying. The biological insecticide B. thuringiensis, when compared to the other insecticides evaluated, showed greater selectivity to the natural enemy C. flavipes (10 days) after spraying the products. However, the insecticides Chlorantraniliprole, Chlorantraniliprole + lambda-cyhalothrin, Triflumurom, Lambda-cyhalothrin + thiamethoxam and Fipronil caused 100% mortality on C. flavipes adults, being considered non-selective to the natural enemy. Thus, the data obtained emphasize the importance of determining the appropriate moment for the release of C. flavipes after the

use of insecticides in the field.

Keywords: *Saccharum officinarum*, MIP, Residual,Parasitoid, Cane Borer.

INTRODUCTION

Brazil is the world's largest producer of sugarcane (Saccharum officinarum) with a planted area of approximately 8,954 thousand ha and production of 655.16 million tons, thus making Brazil the world leader in sugar production and conquered the second position among the world's ethanol producers (CONAB, 2018). The cultivation of sugarcane has been increasing its importance in the Brazilian economy in recent decades, mainly due to the growing performance of sugarcane fields driven by new forms of management and technologies (DINARDO MIRANDA, 2008).

The sugarcane agroecosystem, despite being simplified, still harbors numerous species of insects, some of which vary according to the time of year and the region, which can cause economic losses. However, other species are beneficial and can play an important role in controlling pest species (MACEDO, 2006).

According to Broglio Micheletti et al., (2006) D. saccharalis is considered a key pest of the crop, causing losses of 1.5 t of cane per ha for every 1% of infestation. In the sugarcane crop, chemical control is the most used method, being inefficient due to the habit of D. saccharalis to remain, most of its development, inside the culms of the crop (PINTO, 2006).

Biological control has been shown to be efficient in the control of D. saccharalis due to the great diversity of parasitoid and predatory insects that act on the larval and egg stages of this lepidopteran pest (FIGUEIREDO et al., 2010). Among the natural enemies of the larval parasitoid D. saccharaliso, Cotesia flavipes, is the most used in Brazil, providing the field with a parasitism rate of up to 90% and can be raised relatively easily with low economic cost (DINARDO MIRANDA, 2008). different studies have shown that the association of the parasitoid with chemical control can affect its survival rates in the field due to the low selectivity of this natural enemy to the insecticides used to control the sugarcane borer (GUEDES et al. 2016;MATIOLI, 2019).

Thus, it is of great importance to carry out studies in order to demonstrate the effects of the different insecticides used in the sugarcane crop on the survival rates of the parasitoidC. flavipes in order to enable integrated pest management programs.

Therefore, this study aimed to evaluate the selectivity of adults of C. flavipes on the various insecticides used in the cultivation of sugarcane.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse and at the Agricultural Entomology Laboratory of ILES/ULBRA located in the municipality of Itumbiara-GO, with an average altitude of 491m defined by the geographic coordinates of latitude: 18º 40' 97" South and longitude: 49º 19' 19" West. The climate in the region is AW type, characteristic of humid tropical climates, with two well-defined seasons, dry in winter and wet in summer, with average annual precipitation between 1200 and 1800 mm. The relief of the municipality of Itumbiara presents wavy parts and mountainous parts, but mostly flat. Average temperatures range from a minimum of 19°C to a maximum of 39°C.

The Pre-Sprouted Seedlings (MPB) were supplied by STA Techcana, located in the municipality of Itumbiara in Goiás on BR-153, Km 493.5. The variety of sugarcane used in the test was (RB85 5156) they were kept in a greenhouse and watered daily until they reached the age for transplanting. Upon reaching 20 days after sowing, the seedlings were transplanted into 10 L black plastic pots. In each pot, 3 seedlings were deposited, containing a mixture of soil and substrate in a 2:1 ratio. One week later, NPK fertilization (20-0-20) was performed, 2 grams per pot, applying water immediately and repeating 30 days later.

Cane seedlings with the same phenological age (60 days) and with seven perfectly developed leaves were used to conduct the assay. (2009), being supplied in cups containing cocoons from which the parasitoid adults emerged.

The insecticides used to assess the effects of mortality on the parasitoid C. flavipes were: Chlorantraniliprole, Chlorantraniliprole + lambda-cyhalothrin, Triflumuron, Bacillus thuringiensis, Lambda-cyhalothrin + thiamethoxam and Fipronil. The commercial doses recommended for the control of Sugarcane Borer (Diatraea saccharalis) were used. The list of treatments studied and the respective commercial registration dose for controlling the sugarcane borer are shown in Table 1.

The pre-sprouted seedlings (MPB) were sprayed with insecticides using a 20-liter backpack pump with a spray volume of 134.2 liters per hectare, using a TT 11002 fan nozzle and working with constant pressure. The control treatment did not receive any type of exposure to insecticides.

For the test of selectivity of insecticides to C.flavipes, a completely randomized design with 7 treatments and 10 replications was used. Mortality bioassays were performed with adults of C. flavipes mounted in a petri dish (90 x 15mm). For the installation of the experiment, leaves of sugarcane plants in the vegetative phase were collected. The leaves were cut into 5 cm long pieces.in order to occupy the internal area of the plate, allowing the insect constant contact with the leaf. Next, the sugarcane leaves were sprayed according to the treatments described in Table 1. After spraying, the leaves were dried in the shade for one hour.

In eachIn a petri dish, ten adults of C. flavipes were released, constituting an experimental plot. The petri dishes were then kept at room temperature in the shade until the insect mortality was evaluated, which was carried out at 6, 12, 18 and 24 hours after the exposure of natural enemies to insecticides. For the feeding of the parasitoids, cotton wool soaked in a solution of honey (20%) was used, being deposited on the lower sides of the petri dishes, which were covered with white organza and secured with rubber gums.

The sprayed plants were kept in a covered shed in the absence of rain and irrigated daily by drip. The evaluation of C.flavipes mortality occurred on the day of spraying the insecticide on the plants, being the count performed at 0, 6, 12, 18 and 24 hours after releasing the adults in petri dishes and at 5 and 10 days after spraying the plants. plants, in the same time interval, in order to determine the residual of insecticides on the mortality of C. flavipes exposed for different periods to the tested insecticides.

Parasitoids that did not show movement of their appendages and those that were unable to return to their normal position and walk when touched by a brush were considered dead. To determine the selectivity of the insecticides used in the control of D.saccharalis, the methodology ofHassan (1988), which subdivides the different insecticides into classes according to the observed parasitoid mortality rates, as follows: Class I - Harmless (selective) less than 50% adult mortality; Class II - Little harmful, 50 to 79% mortality; Class III - Moderately harmful with 80 to 99% mortality and Class IV - Harmful above 99% mortality of parasitoids.

For statistical analysisdata were submitted to analysis of variance (ANOVA) and treatment means were compared at 5% significance levels ($p<0.05$), by Tukey's test, using the MiniTab 18 statistical program.

RESULTS AND DISCUSSION

The insecticides tested to control the sugarcane borer significantly affected the mortality of adults of Cotesia flavipes on the day of spraying and at 5 and 10 days after spraying by the Anova test at 5% probability (Table 2, 3 and 4).

The selectivity data for C. flavipes to the tested insecticides, collected on the day of spraying treatments and evaluated at 6, 12, 18 and 24 hours after the release of adults on the leaves submitted to spraying are shown in (Table 2) below with the respective mean tests and represented in (Figure 1). The chemical insecticide Chlorantraniliprole in the commercial dosage registered in the control of Diatraea saccharalis in sugarcane (60 g/ha), showed greater selectivity to the parasitoid C. flavipes, with six hours of exposure to the treated leaves, different from the control as indicated in (Table 3). However, after 12 hours of exposure to the treated leaves, the mortality rate of C. flavipes was 100% of the individuals tested.

As for the insecticides Chlorantraniliprole + lambda-cyhalothrin;*Bacillus thuringiensis*; Lambda-cyhalothrin + thiamethoxam; Fipronil; in their respective commercial dosages of registry caused the mortality of 100% of the adults of C. flavipes, already with six hours of exposition of the insects being considered harmful according to classification of (HASSAN, 1988).According to Mena (2010), some tests carried out evaluating the residual of insecticides in adults of the parasitoid, it was found that the insecticide Lambda-cyhalothrin was highly toxic to C. flavipes at all concentrations tested. However, the spraying of Triflumuron showed low toxicity, even when the parasitoids were exposed soon after spraying the insecticides. However, corroborating the results obtained by Malvina (2017), where the toxicity of different insecticides was observed in the laboratory with immersion of sugarcane leaves for 5 seconds, showing that 24h after immersion of leaves some insecticides such as Triflumuron, Lambda-cyhalothrin + thiamethoxam and Fipronil had 100% mortality in adults of C. flavipes. As in the experiment carried out by Malvina (2017).

The selectivity data for Cotesia flavipes to the tested insecticides, collected on the fifth day after spraying the treatments and evaluated at 6, 12, 18 and 24 hours after the release of C. flavipes adults on the leaves subjected to spraying are shown in (Table 4) below with the respective average tests and represented in (Figure 2) below.

At 5 days after spraying and after 6 hours of exposure of the adults of Cotesia flavipes to the treated leaves, there was an increase in the selectivity of the insecticide.*Bacillus thuringiensis*which caused mortality of 57.23% of adults of C. flavipes, however, when reaching 12 hours of insect exposure, the observed mortality was 87.19% of the population of C. flavipes (Figure 2). However,Hassan (1988) states that studies involving the effect of biological insecticides based on Bacillus thurigiensis on adults of C. flavipes have not yet been investigated in the national and international literature. Prutz and Dettner (2004), working with the larval stage of the parasitoid, observed high mortality rates of C. flavipes when submitted to a diet with the insecticide Dipel® under laboratory conditions.

Other insecticides Chlorantraniliprole, Chlorantraniliprole + lambda-cyhalothrin; Triflumuron; Lambda-cyhalothrin + thiamethoxam and Fipronil; not showing a reduction in the mortality of the parasitoid C. flavipes, presenting a mortality of 100%

of the adults of C. flavipes (Figure 1) being considered harmful according to the classification of (HASSAN, 1988).

In the reassessment of the experiment at 10 days after spraying the products, greater selectivity was observed in the treatment sprayed with*Bacillus thuringiensis*with 75.91% mortality. Çcorroborating with the experiment carried out by Malvina (2017), at the same time,Triflumuroma showed 89.34% (Figure 3), thus demonstrating that they have been showing a decrease in their residual after (10 days) of spraying them. Malvina (2017), states that when adults of Cotesia flavipes were placed in contact with sugarcane leaves of sugar, one day after immersion under the evaluated insecticides, high mortality rates were observed.

100.00% mortality was observed in treatments based on Triflumurom, Fipronil andLambda-cyhalothrin + thiamethoxam24 hours after the release of C. flavipes adults. Thus, the current experiment has shown significant differences 10 days after spraying, with insecticides *Bacillus thuringiensis* and Chlorantraniliprole showing a reduction in its residual as shown in (Figure 3).

The opposite result was observed with the insecticide Chlorantraniliprole, which had its selectivity index reduced at 0 (32.43%), 5 (89.67%) and 10 days (95.71%) after spraying the insecticide. The period of biological activity of an insecticide, also called the residual period, is the period between spraying the product and the period that shows the last signs of lethal effect of the insecticide. This period can vary with numerous factors, such as rainfall, temperature, humidity, crop stage, insect stage, chemical characteristics of the molecule, interaction with the plant, among others. Larger doses can increase the residual period, which consequently increases the interval between applications (PEZZINI et al., 2015). In the case of the experiment where the sugarcane plants were kept in a covered environment after spraying the insecticides, the absence of precipitation contributed to the prolongation of the residual of the insecticides. The data of the present study related to the selectivity of the Altacor insecticide; corroborate those obtained by Pezzini et al., (2015) working with Chlorantraniliprole in soybean at a dose of 45 ml/ha, also found high biological activity on Helicoverpa armigera even 10 days after spraying the insecticide, surpassing the efficiency obtained at 0 days after the spray. Similarly, Sial and Brunner (2010), working with Chlorantraniliprole, found that a minimum time of spraying the insecticide on the plant is necessary to increase the mortality of Choristoneura rosaceana (Harris) (Lepidoptera:

This way, the data obtained emphasize the importance of the producer to evaluate within the IPM of the sugarcane borer (Diatraea saccharalis) the appropriate time after chemical control to adopt biological control, that is, to release the parasitoideC. flavipes. This care is usually neglected by rural producers who associate the two controls simultaneously. In order to achieve these results, a minimum period of 10 days must be used between chemical control and the release of parasitoids. The present study demonstrates the need to carry out new studies with evaluations greater than 10 days after spraying in order to determine the residual of the products under conditions protected from precipitation,

Treatment	Active ingredient	Commercial Dose	Insecticide Amount per liter of syrup
T1	Witness	-	-
T2	Chloranthraniliprole	60 g/ha	0.45 g
T3	Chlorantraniliprole + lambda-cyhalothrin	200 ml/ha	1.49 ml
T4	triflumuron	80 ml/ha	2.98 ml
T5	*Bacillus thuringiensis*	1 kg/ha	7.4 g
T6	Lambda-cyhalothrin + thiamethoxam	200 ml/ha	1.49 ml
T7	fipronil	500 g/ha	1.86 g

Table 1- List of treatments studied withrespective doses for the selectivity test of theCotesia flavipes (Cameron, 1981) (Hymenoptera: Braconidae) on sugarcane.

PV	GL	medium square			
		6 hours	12 hours	18 hours	24 hours
Treatment	6	16080.07**	14285.71*	14285.71*	14285.71*
Mistake	63	44.60	0.00	0.00	0.00
Average		71.76	85.71	85.71	85.71
CV%		52.86	41.12	41.12	41.12

** Significant at the 1% probability level, by the F test.

* Significant at the 5% probability level, by the F test.

Table 2- Analysis of variance for the parasitoid mortality parameterCotesia flavipes (Cameron, 1891) (Hymenoptera: Braconidae) at 6, 12, 18 and 24 hours after release of adults in petri dishes containing leaves freshly sprayed with different insecticides recommended for controlling the sugarcane borer (Itumbiara, GO, 2018)).

PV	GL	medium square			
		6 hours	12 hours	18 hours	24 hours
Treatment	6	13806.95**	13910.17*	14285.71*	14285.71*
Mistake	63	73.15	26.83	0.00	0.00
Average		76.95	83.88	85.71	85.71
CV%		46.27	41.88	41.12	41.12

** Significant at the 1% probability level, by the F test.

* Significant at the 5% probability level, by the F test.

Table 3- Analysis of variance for the parasitoid mortality parameterCotesia flavipes (Cameron, 1891) (Hymenoptera: Braconidae) at 6, 12, 18 and 24 hours after releasing the adults in petri dishes containing sprayed leaves 5 days after spraying with different insecticides recommended for the control of the sugarcane borer (Itumbiara) , GO, 2018).

PV	GL	medium square			
		6 hours	12 hours	18 hours	24 hours
Treatment	6	13251.75**	13251.75**	13740.77**	14285.7
Mistake	63	23.82	23.82	15.49	0
Average		80.14	80.14	81.66	85.71
CV%		42.76	42.76	42.58	41.12

** Significant at the 1% probability level, by the F test.

* Significant at the 5% probability level, by the F test.

NSNot Significant by F Test

Table 4- Analysis of variance for the parasitoid mortality parameterCotesia flavipes (Cameron, 1891) (Hymenoptera: Braconidae) at 6, 12, 18 and 24 hours after releasing the adults in petri dishes containing sprayed leaves 10 days after spraying with different insecticides recommended for controlling the sugarcane borer.

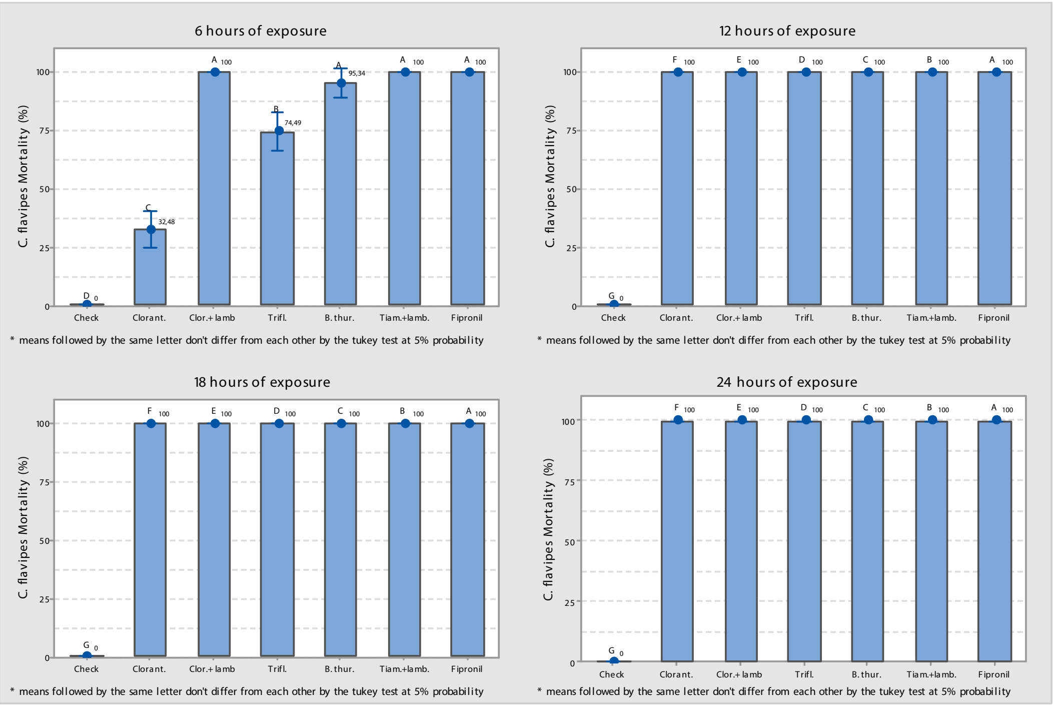


Figure 1- Mortality of the parasitoidCotesia flavipes (Cameron, 1891) (Hymenoptera: Braconidae) at 6, 12, 18 and 24 hours after releasing the adults into petri dishes containing leaves recently sprayed with different insecticides recommended for controlling the sugarcane borer.(Where: Clorant. = Chlorantraniliprole; Chlor.+ lamb. = Chloranthraniliprole + lambda-cyhalothrin; Trifl. = Triflumuron; B. thur. = Bacillus thuringiensis, Tiam.+lamb. = Thiamethoxam + Lambda-Cyalothrin).

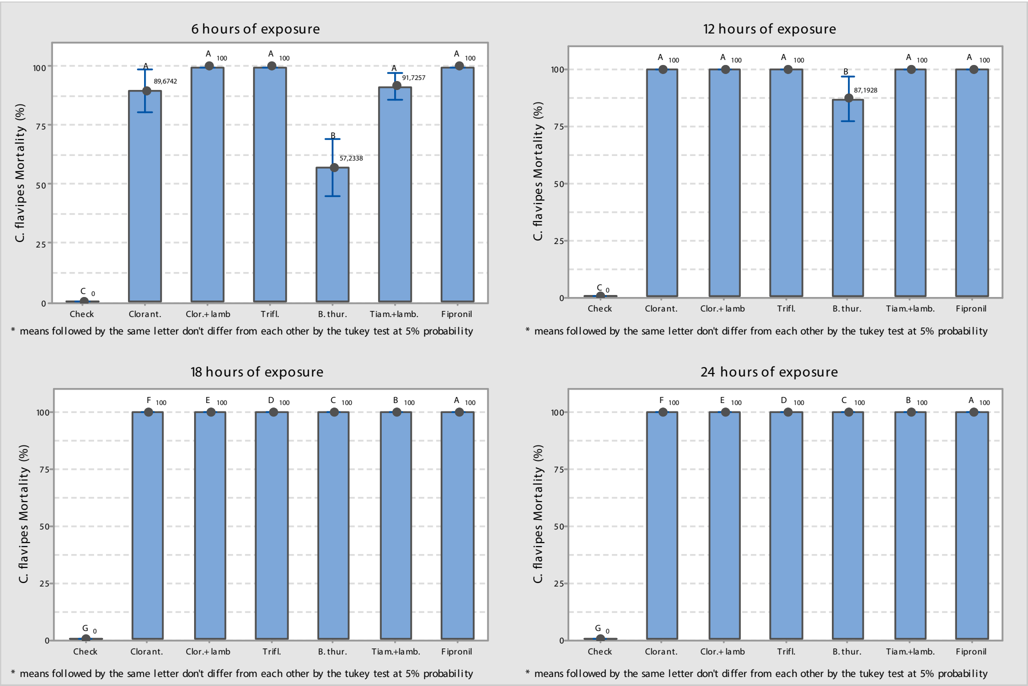
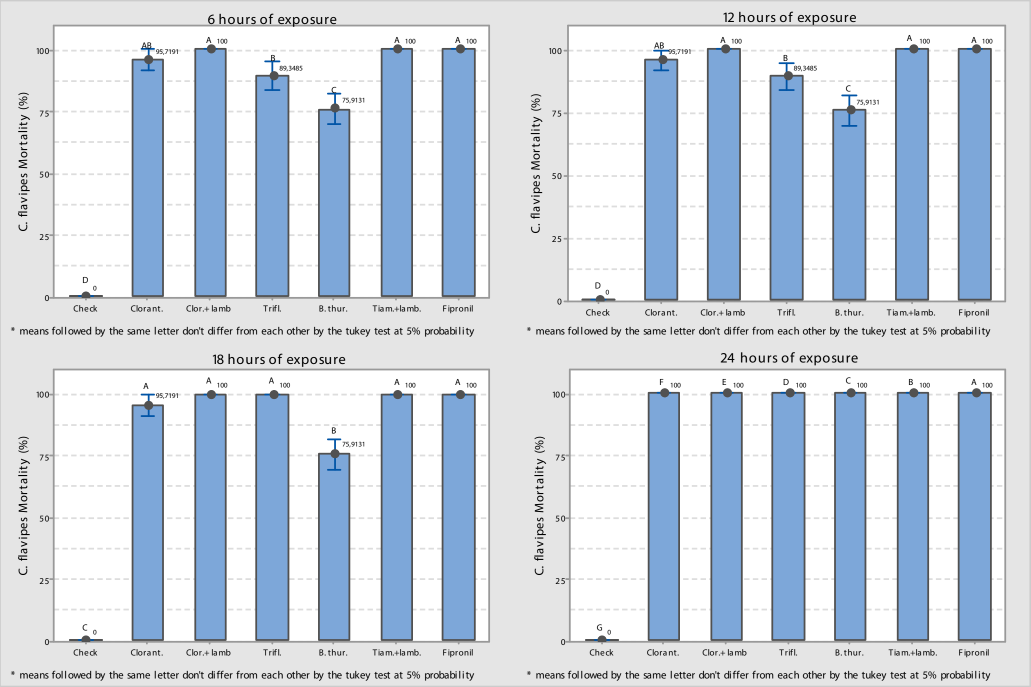


Figure 2- Mortality of the parasitoid Cotesia flavipes (Cameron, 1891) (Hymenoptera: Braconidae) at 6, 12, 18 and 24 hours after releasing the adults in petri dishes containing leaves sprayed for 5 days with recommended insecticides for controlling the sugarcane borer (Where: Chlorant. = Chlorantraniliprole; Chlor.+ lamb. = Chlorantraniliprole + lambda-cyhalothrin; Trifl. = Triflumuron; B. thur. = Bacillus thuringiensis, Tiam.+lamb. = Thiamethoxam + Lambda-Cyalothrin).



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