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Toxicity of anthranilamides used in cucurbit cultivation on *Apis mellifera*

Ewerton Marinho da Costa[1]*, Carlos Henrique Peixoto de Barros[1], Kaique Oliveira Silva[1], Allysson Jonhnny Torres Mendonça[1], Tiago Augusto Lima Cardoso[1], Carlos Eduardo Souza Bezerra[2], Elton Lucio Araujo[3]

[1]Universidade Federal de Campina Grande, Campina Grande-PB, Brazil.
[2]Centro Universitário de Várzea Grande, Várzea Grande-MT, Brazil.
[3]Universidade Federal Rural do Semi Árido, Mossoró-RN, Brazil.
*Corresponding author, e-mail: ewerton.marinho@professor.ufcg.edu.br

Abstract

This study aimed to evaluate the toxicity of insecticides belonging to the chemical group Anthranilamide on *Apis mellifera*. Toxicity was evaluated in the two experiments, which corresponded to two exposure modes: direct spraying of the products on the bees and ingestion of a contaminated diet. The two bioassays were performed in a completely randomized design composed of eight treatments (absolute control – distilled water; positive control: Thiamethoxam at 0.03 and 0.3 g.a.i. L^{-1}; Chlorantraniliprole: 0.015 g.a.i. L^{-1}; Cyantraniliprole: 0.05 and 0.1 g.a.i. L^{-1}; Chlorantraniliprole + Abamectin: 0.027+0.0108 g.a.i. L^{-1} and 0.045+0.018 g.a.i. L^{-1}), with 10 replications each. Via exposure through direct spraying, the insecticide Chlorantraniliprole + Abamectin, regardless of the dose used, caused 100% bee mortality, showing a TL_{50} of 10.05h and 8.36h for the minimum and maximum doses, respectively. The insecticide Cyantraniliprole caused the death of 33.1% and 44.9% of the bees, respectively at the minimum and maximum doses, with a TL_{50} of 87.79 h for the two doses. The insecticide Chlorantraniliprole showed the lowest mortality rate, 24.7%, with a TL_{50} of 118.88h. Via ingestion of a contaminated diet, the insecticide Chlorantraniliprole + Abamectin caused 100% mortality and showed a TL_{50} of 27.83h and 24.01h at the minimum and maximum doses, respectively. The insecticide Cyantraniliprole caused the death of 67.6% and 68.1% of bees at the minimum and maximum doses, respectively, with a TL_{50} of 58.63 h for the two doses. Chlorantraniliprole shows the lowest mortality rate, 48.1%, and a TL_{50} of 79.68h.

Keywords: conservation, honey bee, insecticides, mortality, pollinator

Introduction

In commercial cucurbit growing areas, e.g., melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*), the use of bee colonies with *Apis mellifera* (Hymenoptera: Apidae) for pollination and chemical pest control are indispensable practices to ensure fruit production on a commercial scale (Klein et Al., 2007; Guimarães et al., 2008; Bomfim et al., 2013; Kiill et al., 2015.).

However, it is known that the abusive use of pesticides is one of the most impacting factors for pollinator decline in agricultural areas (Godfray et al., 2014; Christen & Fent, 2017), being associated with the causes of the Colony Collapse Disorder – CCD (van Engelsdorp et al., 2009) and the loss of bee colonies worldwide (Castilhos et al., 2019). The impact of the contact between bees and pesticides can occur at the individual level, especially on workers during foraging, or affect the entire colony (Barganska et al., 2016; Cham et al., 2017).

Given this problem, it is necessary to understand the impacts on bees of pesticides used in agricultural areas (Pinheiro & Freitas 2010; Pires et al., 2016). In the chemical group of the anthranilamides, it has been reported that Chlorantraniliprole and Cyantraniliprole show low toxicity on bees (Dinter et al., 2009; Larson et al., 2013). In Brazil, only the effect of Chlorantraniliprole was evaluated on *A. mellifera* at the dose registered for use in melon (via ingestion and contact with the product's residues on leaves), in a study in which the authors observed low mortality and reductions flight capacity of bees (Gomes et al., 2020). Furthermore, Chlorantraniliprole has been reported to cause alterations in genes that regulate the immune system of *A. mellifera* (Christen & Fent, 2017).

However, despite the existing contributions, it is necessary to expand and generate new information about the effect of anthranilamides on *A. mellifera*, especially at the different doses registered for use in

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cucurbit growing areas. Therefore, this study aimed to evaluate the toxicity of the insecticides of the chemical group Anthranilamide on *A. mellifera* via spraying and the supply of a contaminated diet, under laboratory conditions.

Material and Methods

The study was conducted at the Entomology Laboratory (room climatized at 25 ± 2°C, 50 ± 10% RH, and a photoperiod of 12 h) of the Center of Sciences and Agri-food technology (CCTA) of the Federal University of Campina Grande (UFCG), Pombal – PB. The bees used in the experiment were obtained from three colonies belonging to the apiary of CCTA/UFCG.

The anthranilamides evaluated were Chlorantraniliprole, Cyantraniliprole, and Chlorantraniliprole + Abamectin (insecticide composed of two chemical groups: Anthranilamide and Avermectin). All insecticides were evaluated at the minimum and maximum doses recommended by the manufacturers for use in melon and watermelon, except for Chlorantraniliprole, which has a single registered dose. The insecticide Thiamethoxam was used as a positive control at the minimum and maximum doses registered for pest control in melon (**Table 1**).

Insecticide toxicity on the bees was evaluated in two different experiments, which corresponded to two types of exposure: direct spraying of the products on the bees and ingestion of a contaminated diet. The two experiments were performed according to the methodology used by Costa et al. (2014), in which manual sprayers were used to spray the solution on the bees and the artificial diet. It should be noted that the bees selected to ingest the contaminated diet were in a previous fasting period for two hours before the beginning of the experiment.

Plastic containers (15cm X 15cm) with the end partially covered by an anti-aphid screen and the sides containing small openings (±0.1 cm) were used in the two bioassays as arenas. All arenas received a sugar paste (artificial bee diet) inside a plastic container (28 mm) and a cotton wad soaked in distilled water (the wad was re-moistened every hour). For bee handling during the bioassays, the insects were previously anesthetized by cold (+ 4°C for 90 seconds).

The two bioassays were performed in a completely randomized design composed of eight treatments (absolute control – distilled water; positive control: Thiamethoxam at 0.03 and 0.3 g.a.i L^{-1}; Chlorantraniliprole: 0.015 g.a.i L^{-1}; Cyantraniliprole: 0.05 and 0.1 g.a.i L^{-1}; Chlorantraniliprole+ Abamectin: 0.027+0.0108 g.a.i L^{-1} and 0.045+0.018 g.a.i L^{-1}) with ten replications, and each experimental unit consisted of 10 adult bees. Each bioassay was repeated twice.

After the application of treatments, mortality and motor disorders (prostration, tremors, paralysis and reduced of feeding) of the bees were evaluated in 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60 and 72 h after the beginning of exposure to insecticides. Were considered dead the bees that did not respond to mechanical stimuli (touching with a fine brush).

The mortality means were corrected by Abbott's equation (1925), after which the non-parametric test of Kruskal & Wallis (1952) was applied at 5% significance, followed by Wilcoxon's test. The bee survival data were analyzed using the 'survival' package (Therneau & Lumley 2010) of the R software and subjected to a Weibull distribution analysis. Treatments with similar effects with regard to toxicity and mortality speed were clustered through contrasts. The median lethal time (TL_{50}) was also calculated for each group formed. All analyses were performed through the statistical software R (R CORE TEAM 2020).

Results and Discussion

Toxicity of anthranilamides via direct spraying on *A. mellifera*.

The insecticide Chlorantraniliprole + Abamectin, regardless of the dose used, was extremely toxic to *A. mellifera*, causing 100% mortality and a TL_{50} of 10.05h and 8.36h for the minimum and maximum doses, respectively,

Table 1. Insecticides and respective doses (minimum and maximum) evaluated for toxicity to *Apis mellifera* via direct spraying and ingestion of contaminated diet, Pombal – PB, 2022.

Active ingredient	Chemical group	Dose	Target pest
Thiamethoxam	Neonicotinoid	60 and 600 g/ha*(0.03 and 0.3 g.a.i L^{-1})	*B. tabaci* biotype B *Aphis gossypii*
Chlorantraniliprole	Anthranilamide	7.5 mL/100 L(0.015 g.a.i L^{-1})	*Diaphania nitidalis*
Chlorantraniliprole +Abamectin	Anthranilamide + Avermectin	300 and 500 mL/ha* (0.027+0.0108 g.a.i L^{-1} and 0.045+0.018 g.a.i L^{-1})	*Liriomyza huidobrensis* *Diaphania nitidalis*
Cyantraniliprole	Anthranilamide	250 to 500 mL/ha* (0.05 and 0.1 g.a.i L^{-1})	*B. tabaci* biotype B *Liriomyza sativae* *Diaphania nitidalis*

*A mean application volume of 500 liters/ha was considered for the dilutions. At the laboratory, 1 L of each mixture was prepared for each insecticide by maintaining the referred proportion.

being as lethal as the positive control (Thiamethoxam). The insecticide Cyantraniliprole caused the death of 33.1% and 44.9% of the bees at the minimum and maximum doses, respectively, with a TL_{50} of 87.79 h for the two doses. In turn, the insecticide Chlorantraniliprole showed the lowest mortality rate, with 24.7% and a TL_{50} of 118.88h (**Table 2 and Figure 1**).

Table 2. Mortality (%) of *Apis mellifera* after direct insecticide spraying on the bees.

Treatments	% of mortality	
	Dose 1 (Minimum)	Dose 2 (Maximum)
Absolute control	0.0a	
Positive control (Thiamethoxam)	100d	100d
Chlorantraniliprole	24.7b	
Cyantraniliprole	33.1bc	44.9c
Chlorantraniliprole + Abamectin	100d	100d

Chlorantraniliprole + Abamectin reduced bee mobility, followed by prostration and death. Cyantraniliprole and Chlorantraniliprole did not cause apparent changes in the behavior of bees that survived the long evaluation period.

Toxicity of anthranilamides on A. mellifera via ingestion of a contaminated diet.

The insecticide Chlorantraniliprole + Abamectin, regardless of the dose used, was extremely toxic to *A. mellifera* via diet, causing 100% mortality and showing a TL_{50} of 27.83h and 24.01h for the minimum and maximum doses, respectively, values statistically equal to the insecticide Thiamethoxam. Cyantraniliprole caused the death of 67.6% and 68.1% of the bees at the minimum and maximum doses, respectively, with a TL_{50} of 58.63 h for the two doses. In turn, the insecticide Chlorantraniliprole showed the lowest mortality rate: 48.1%, with a TL_{50} of 79.68h (**Table 3 and Figure 2**).

After ingestion of the diet contaminated with Chlorantraniliprole + Abamectin, there was a perceptible reduction in bee mobility, evolving over time to paralysis and prostration, followed by death. The bees showed reduced movements after ingesting the insecticides

Table 3. Mortality (%) of *Apis mellifera* after ingestion of a diet contaminated with insecticides.

Treatment	% Mortality	
	Dose 1 (Minimum)	Dose 2 (Maximum)
Absolute control	0.0a	
Positive control (Thiamethoxam)	100d	100d
Chlorantraniliprole	48.1b	
Cyantraniliprole	67.6bc	68.1c
Chlorantraniliprole + Abamectin	100d	100d

Cyantraniliprole and Chlorantraniliprole over the evaluation period.

Toxicity of anthranilamides via direct spraying on ***A. mellifera.***

At the minimum and maximum doses evaluated, the insecticide Chlorantraniliprole + Abamectin reduced bee mobility, with subsequent prostration followed by death, thus being extremely toxic to *A. mellifera* and as lethal as the neonicotinoid Thiamethoxam, an insecticide that has already been reported in various studies as lethal to bees and with a strong effect on the central nervous system of insects (Laurino et al., 2011; Costa et al., 2014; Araújo et al., 2017). However, when applied in isolation, the anthranilamide Chlorantraniliprole resulted in the lowest mortality rate (24.7%) among the insecticides evaluated, thus being less toxic for bees than the positive control and showing a TL_{50} higher than 100 hours. Therefore, the presence of Abamectin can be pointed out as the cause of the high mortality observed for the insecticide formulated with Chlorantraniliprole + Abamectin when sprayed on the bees since the referred active ingredient had already been mentioned as highly toxic to *A. mellifera* in several studies (Carvalho et al., 2009; Costa et al., 2014). Furthermore, Chlorantraniliprole has shown to be little toxic to other bees, e.g., *Bombus terrestris* and *B. impatiens* (Dinter et al., 2009; Larson et al., 2013). However, despite causing low mortality in adults of *A. mellifera*, there are already records that Chlorantraniliprole has caused changes in genes that regulate the immune system (Christen & Fent, 2017) and compromised the flight capacity of this bee (Gomes et al., 2020), factors that can ultimately compromise the whole colony.

The insecticide Cyantraniliprole, despite causing higher mortality than Chlorantraniliprole, was significantly less harmful than Thiamethoxam when sprayed on *A. mellifera*. Dinter & Samel (2014) reported that que Cyantraniliprole offered low risks to *A. mellifera* through direct contact, which was also observed in the present study. With that, the results obtained indicate that direct bee contact with the insecticides Chlorantraniliprole and Cyantraniliprole, at the doses recommended for pest control in cucurbits, is little harmful to the survival of adult workers of *A. mellifera* under laboratory conditions, not causing adverse effects on the behavior of the bees that survived spraying.

Toxicity of anthranilamide on A. mellifera via ingestion of a contaminated diet.

The insecticide Chlorantraniliprole + Abamectin

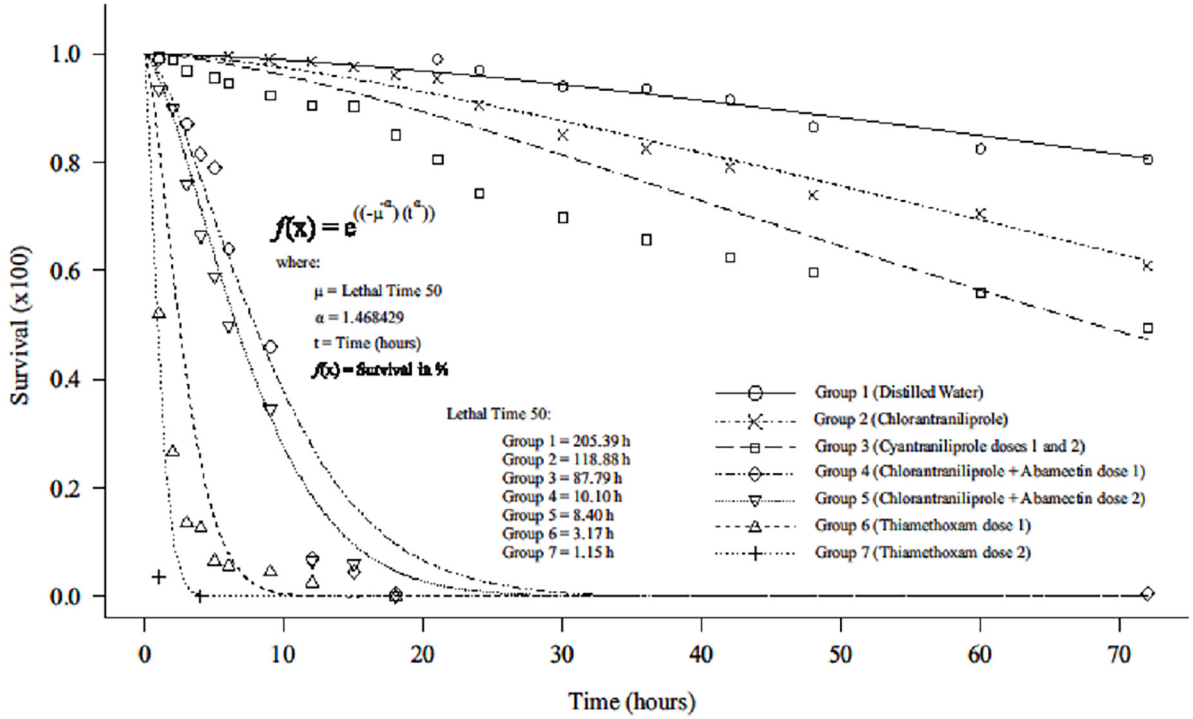

Figure 1. Survival (%) and median lethal time (TL_{50} in hours) of *Apis mellifera* after ingestion of a diet contaminated with insecticides.

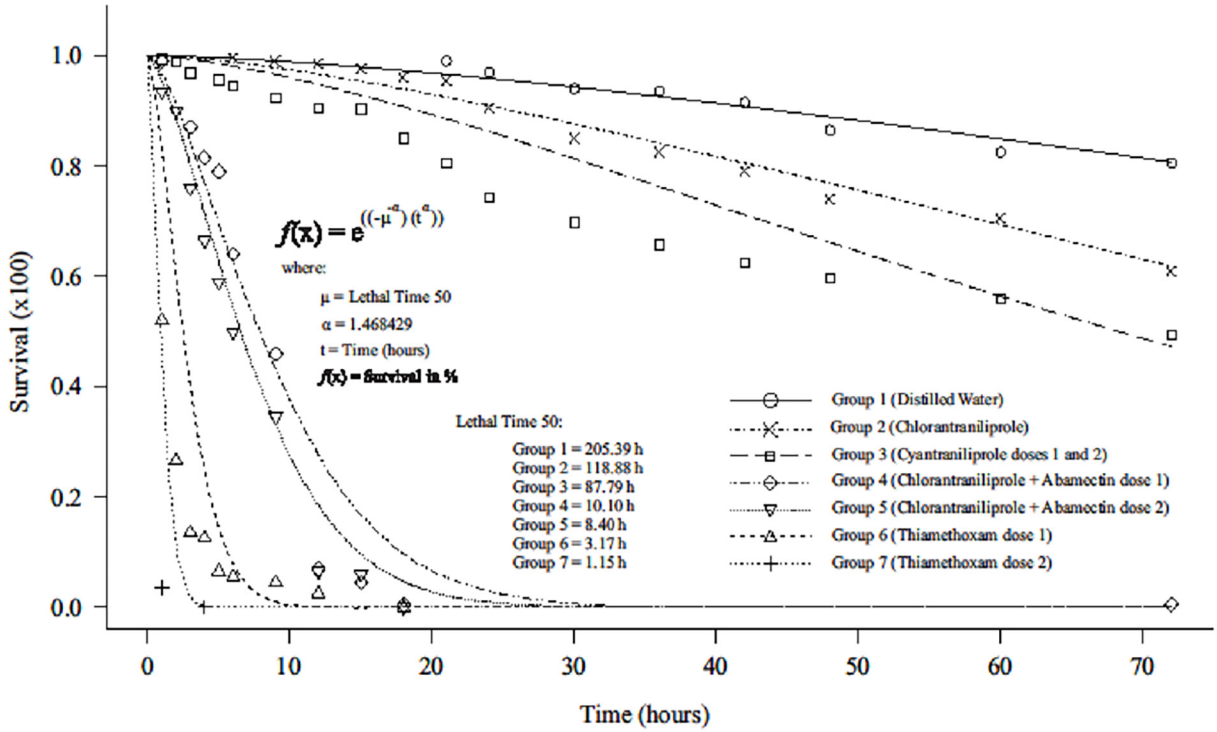

Figure 2. Survival (%) and median lethal time (TL_{50} in hours) of *Apis mellifera* after ingestion of a diet contaminated with insecticides.

was also extremely harmful to bees via ingestion, with Abamectin being once more the likely responsible for the high mortality observed since it has been reported as highly toxic on *A. mellifera* when ingested (Costa et al., 2014). Whereas Chlorantraniliprole, at the dose recommended for melon, caused low mortality in an oral test (Gomes et al., 2020). However, with the median lethal time of 27.83h for the minimum dose and 24.01h for the maximum dose, Chlorantraniliprole + Abamectin took longer to cause death than the positive control, the insecticide Thiamethoxam, i.e., the insecticides performed differently with regard to the speed of mortality.

The insecticides Chlorantraniliprole and Cyantraniliprole, even with mortality rates below

Thiamethoxam, caused more mortality compared to the exposure via direct spraying, in addition to reducing bee mobility soon after the ingestion of the contaminated diet. This mobility reduction occurs because this chemical group acts mainly by ingestion and acts in the ryanodine receptor, compromising muscle activity and causing, for example, reduced locomotor activity and paralysis (Cordova et al. 2006). Gomes et al. (2020) and Williams et. al. (2020) also reported low bee mortality caused by Chlorantraniliprole after the ingestion of a contaminated artificial diet. Nevertheless, Gomes et al. (2020) reported that Chlorantraniliprole significantly affected the flight activity of bees. In another study, Kadala et al. (2019) evaluated the effects of sublethal doses of Chlorantraniliprole through topical application to the thorax of *A. mellifera* and verified adverse effects on locomotion, stressing that the locomotor deficits lasted for a long period, which is extremely harmful to the insect. The reduction in locomotor activity compromises the natural behavior and the performance of bees in daily activities, e.g., foraging, directly affecting the stocking of food (Desneux et al.,2007; Sandrock et al., 2014). In the long term, the adverse effects on the locomotor activity of bees can cause the colony to collapse (Stanley et al., 2015).

The results obtained in the present study indicate that the anthranilamides evaluated, except for the insecticide mixed with Abamectin, are little lethal to *A. mellifera*, especially through exposure via direct spraying. However, it should be noted that, when ingested, despite causing less mortality than Thiamethoxam (positive control), Chlorantraniliprole and Cyantraniliprole caused higher mortality rates than those observed in the direct exposure treatment, in addition to reductions in the locomotor activity of bees, which requires attention since, under field conditions, these effects can ultimately compromise the colony. It should be noted that the results obtained expand the current information related to the toxicity of anthranilamides to *A. mellifera* and can subsidize new research, especially with other dosages registered for use in Brazil, different types of exposure, evaluations on the flight capacity, and the impact of contact under field conditions.

Conclusions

The insecticide Chlorantraniliprole + Abamectin was extremely toxic to *A. mellifera*, regardless of the dose and type of exposure evaluated.

The insecticides Chlorantraniliprole and Cyantraniliprole were little toxic to *A. mellifera* via direct spraying.

The insecticides Chlorantraniliprole and Cyantraniliprole caused higher mortality rates for *A. mellifera* via ingestion of a contaminated diet.

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