Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 14 (1)
January 2021
DOI: http://dx.doi.org/10.36560/14120211254
Article link
http://sea.ufr.edu.br/index.php?journal=SEA&page=article&op=view&path%5B%5D=1254&path%5B%5D=pdf
Included in DOAJ, AGRIS, Latindex, Journal TOCs, CORE, Discoursio Open Science, Science Gate, GFAR, CIARDRING, Academic Journals Database and NTHRYS Technologies, Portal de Periódicos CAPES, CrossRef, ICI Journals Master List.

ISSN 2316-9281

Toxicity and genetic analysis of *Scaptotrigona bipunctata* Lepeletier, 1836 contaminated with the pyrethroid cypermethrin

N. C. Pereira[1]*, T. O. Diniz[1], M. C. C. Ruvolo-Takasusuki[1]

[1] Universidade Estadual de Maringá – Departamento de Biotecnologia, Genética e Biologia Celular

*** Author for correspondence: naiaraclimas@gmail.com**

Abstract. Stingless bees are important pollinators for the native forest of tropical and subtropical regions, predominantly in Latin America. This group contains more than 300 species, many of them native from Brazil. Their colonies present various types of structures, formats and materials. *Scaptotrigona bipunctata* (Lepeletier, 1836) is a species of stingless bee that builds large colonies within tree trunks. They are ecologically important as pollinators, maintaining the ecological balance. However, studies indicate that the number of bees has been decreasing drastically over the years due to habitat destruction and intensive use of agrochemicals. High doses of insecticides can lead to the death of bees, but low concentrations may promote behavioral changes that affect the colonies and its services to ecosystem and agricultural crops. Around 40% of all insecticides applied in Brazil are toxic to bees. Cypermethrin insecticide belongs to the pyrethroid group and it is widely used in several crops, such as cotton, potato, coffee, maize and tomato. This study aimed to investigate the mortality rate, alterations in total protein and esterase expression in *S. bipunctata* after contamination by ingestion and contact with cypermethrin. At the end of the bioassays, there was found a low mortality rate, however, sublethal effects were expressive. Esterases showed different expression patterns after contamination, both by ingestion and contact and total protein analysis presented changes in expression, as well.
Keywords: stingless bee, esterase, protein, sublethal effects

Introduction

Bees have an important role in pollinating crops and native plants, increasing crops production, which is evaluated in $150 billion (Delaplane e Mayer, 2000). Agriculture dependent on pollination by insects grew 16.7% in developed countries and 9.4% in undeveloped countries between the years of 1961 and 2006 (Aizen et al. 2008).

Meliponines or stingless bees are social bees that have an atrophied sting, making it impossible to use (Lins et al. 2003). Stingless bees are among the most common pollinators in the tropical environment, being the dominant bees in certain regions, pollinating different types of crops (Macías-Macías et al. 2009).

Scaptotrigona bipunctata (Lepeletier, 1836) (Hymenoptera: Apidae: Meliponinae) is a species of stingless bee, which has a complex system of social interaction within colonies and with the environment. This species is found in several parts of Brazil and presents generalist behavior, collecting nectar from a vast number of plants (Pacheco et al. 2009).

In the last decade, Brazil expanded the use of agrochemicals by 190%, being number one in the world ranking since 2008 (Rigotto et al. 2014). Cypermethrin insecticide belongs to the pyrethroid group and represents more than 30% of the insecticides globally used (Clark et al. 2015; Sethi et al. 2015). In Brazil, this insecticide is used for cotton, rice, potato, coffee, citrus, beans, cassava, corn, soybean and tomato crops. Consequently, bees can easily be exposed to this type of insecticide.

Hence, the application of insecticides in agriculture has been widely employed, often without proper control. This fact has affected several aspects of the environment and its diversity, including pollinating insects, such as bees. A study conducted by Frazier et al. (2008) detected 17 types of pesticides in a sample of pollen from an Apis mellifera colony. Studies involving the use of insecticides and their effects on insect pollinators are important to understand their impact on biodiversity and agriculture (Brittain e Potts, 2011). Therefore, knowing how the insecticides works, especially in bees, is crucial to minimize their exposure to these pesticides, reducing their lethal and sublethal effects (Freitas e Pinheiro, 2012).

Tolerance to insecticides can be achieved in a variety of ways, such as mutations of target proteins or increasing the biodegradation of insecticides

(Terhzaz et al. 2015). Thus, biochemical mechanisms act in an attempt to eliminate or control the contamination process. However, there are few genetic and molecular studies regarding the mechanisms of insect defenses (You et al. 2013).

Detoxification enzymes, for instance, HPS90, HPS70 and P450, are known for their ability to metabolize insecticides in insects (Salinas and Wong, 1999; Li et al. 2007, Ding et al. 2013, Zimmer et al. 2014). These enzymes can hydrolyze the insecticide molecule and eliminate it from the body throughout metabolism (Hoy, 2003).

Metabolic resistance to insecticides may occur due to increased expression or activity of enzymes that belong to three large families of detoxification-related genes: esterases, glutathione-S-transferase and enzymes from cytochrome P450 superfamily (Hemingway and Ranson, 2000, Hemingway et al. 2004, Li et al. 2007, Braga and Valle, 2007).

There is evidence that the esterase genes evolve rapidly and because of that, each insect species has a single set of detoxification genes, with only a few orthologues among the species (Montella et al. 2012).

Esterases isoenzymes are frequently related to resistance to several classes of insecticides, such as organophosphates, carbamates and pyrethroids. These isoenzymes act in gene amplification, upregulation, coding sequence mutations or a combination of these mechanisms to detoxify the insect organism (Li et al. 2007).

Finally, given the importance of stingless bees, both commercial and environmental, this study aims to analyze the effects of cypermethrin insecticide on *S. bipunctata*, regarding mortality, expression of esterases isoenzymes and peptides.

Methods

Biological material

S. bipunctata foragers were collected from six colonies in the meliponary from the Universidade Estadual de Maringá (UEM) (23°25'S and 51°57'O). Approximately 45 bees were collected from each beehive, totaling 270 bees for each test. Collections were made in clean plastic containers that were placed in the entrance of the colonies.

Thereafter, bees were transported to the animal genetic laboratory from UEM (-23.404024, -51.939619). After intoxication, bees were sacrificed and maintained in freezer at -20 °C.

Insecticide concentration

The commercial insecticide Cipermetrina Nortox® 250 EC was used in the bioassays. This insecticide presents a concentration of cypermethrin equal to 250 grams of active ingredient per liter (g a.i./L).

Previous bioassays were made to find sublethal concentrations to be used in this work. Therefore, we found five concentrations of cypermethrin for ingestion bioassays and five different concentrations for contact bioassays. This difference is because the concentration to kill 50% of bees (LC50) is higher for ingestion than for contact.

For ingestion the concentrations were: 0.25 µg a.i./mL, 0.5 µg a.i./mL, 1 µg a.i./mL, 2 µg a.i./mL, 4 µg a.i./mL and for contact the following concentrations were uses: 0.0625 mg a.i./mL, 0.125 mg a.i./mL, 0.25 mg a.i./mL, 0.5 mg a.i./mL, 1 mg a.i./mL.

Bioassays

Bioassays were performed in triplicate, containing 15 adult workers by concentration. Glass bottles measuring 18 cm in height and 13 cm in diameter, containing filter paper at the bottom, a water-soaked cotton swab and a container with food, were used.

For ingestion bioassay, the insecticide was placed into the food, which consisted of a liquid mixture of crystal sugar and water. To guarantee that the bees would feed efficiently small pieces of wood were placed into the food recipient to serve as support.

For the contact bioassay, 1 mL of the insecticide were added into the filter paper and left to dry. After dried glass bottles were assembled as described above.

Bioassays were stored in a BOD incubator, maintained in 28 ºC ± 2 °C and UR 70 ± 10%. Bees were maintained under these conditions for 72 hours and dead individuals were counted after 24, 48 and 72 hours of exposure.

Data analysis

The experimental delineation was completely randomized, and the data obtained were submitted to the nonparametric test Kruskal-Wallis, using the Action Software, considering that the data set was not normal according to the Shapiro-Wilk test and nonparametric tests are statistically more conservative and indicated in these cases. To calculate LC50 the results were submitted to Probit analysis, using the software IBM SPSS Statistics 22.

Esterases

For esterases extraction, samples from each surviving insect were macerated in 0.1% β-mercaptoethanol and 10% glycerol and centrifuged at 16 128 g for 10 min in a refrigerated centrifuge at 4 °C (Sigma 3K30). From the supernatant, an aliquot of 20 µL was applied to the gel. The vats of the electrophoresis system were filled with 0.1 M Tris-glycine buffer (pH 8.3) and electrophoresis was performed at 200 V for 5 h at 4 °C.

To identify the esterases, the gel was incubated for 30 min in 50 mL of 0.1 M sodium phosphate buffer, pH 6.2. After that, the gel was incubated for 20 min in the dark in 50 mL of 0.1 M sodium phosphate buffer (pH 6.2), n-propanol (5 mL), Fast Blue RR salt (0.06 g), and the substrates α-naphthyl acetate (0.04 g) and β-naphthyl acetate (0.04 g), previously diluted in 1 mL of acetone. The esterases were visualized on the gels as brown (α-esterases) or red (β-esterases) bands (Alfenas, 2006).

Proteins

For total protein extraction samples from each surviving insect were macerated in 0.1% β-

mercaptoethanol and 10% glycerol and centrifuged at 16,128 g for 10 min. After, 20 µL of the supernatant was added to a new microtube containing 40 µL of bromophenol blue. Samples were boiled at 100 °C for 3 min and 15 µL was applied to the SDS-PAGE gel.

The vats of the electrophoresis system were filled with 0.1 M Tris-glycine buffer (pH 8.3) and 10% SDS. Electrophoresis was performed at 90 V, for 6h at room temperature.

The gel was then incubated in 50 mL of 50% ethyl alcohol, 12% acetic acid and 0.0075% formaldehyde for 12 h. After, the gel was washed for 20 min in 1:1 ethanol and distilled water.

The gel was then treated for one minute in 0.02% thiosulfate solution and washed three times with distilled water, then incubated in 0.2% AgNO3 and 0.0075% formaldehyde for 20 min. Finally, the gel was revealed in 6% NaHCO3, 2% thiosulphate and 0.005% formaldehyde until the bands appear in about 5 minutes.

Results and discussion

To analyze the mortality, dead individuals were counted after 24, 48 and 72 hours after insecticide contamination. The mortality rates did not differ statically after 48 hours of intoxication by ingestion (48 hours: Kruskal-Wallis Chi-Square (5) = 0.37, $p > 0.05$). On the other hand, after 24 and 72 hours, there was a significant difference in mortality (24 hours: Kruskal-Wallis Chi-Square (5) = 12.09, $p < 0.05$; 72 hours: Kruskal-Wallis Chi-Square (5) = 12.04, $p < 0.05$). The multiple comparison tables are shown below (table 1 and 2).

Table 1 The sum of ranks of the Kruskal-Wallis test for mortality of *Scaptotrigona bipunctata* intoxicated by ingestion of Cypermethrin after 24 hours of exposure with the groups resulting from the pairwise comparison. Concentrations with statistical difference do not share the same group

Concentration	Rank	Groups		
0.25 µg a.i./mL	17	A		
1 µg a.i./mL	10.66		B	
0.5 µg a.i./mL	9.66		B	C
4 µg a.i./mL	9.66		B	C
2 µg a.i./mL	5			C
Controle	5			C

Table 2 The sum of ranks of the Kruskal-Wallis test for mortality of *Scaptotrigona bipunctata* intoxicated by ingestion of Cypermethrin after 72 hours of exposure with the groups resulting from the pairwise comparison. Concentrations with statistical difference do not share the same group

Concentration	Rank	Groups		
Controle	15.83	A		
2 µg a.i./mL	13	A	B	
4 µg a.i./mL	8.66		B	C
0.25 µg a.i./mL	6.5			C
0.5 µg a.i./mL	6.5			C
1 µg a.i./mL	6.5			C

After 24 and 72 hours of intoxication by contact, mortality did not differ statically (24 hours: Kruskal-Wallis Chi-Square (5) = 6.75, $p > 0.05$; 72 hours: Kruskal-Wallis Chi-Square (5) = 0.60, $p > 0.05$). Otherwise, after 48 hours of intoxication, there was a significant difference in mortality (48 hours: Kruskal-Wallis Chi-Square (5) = 11.75, $p < 0.05$). Table 3 shows the multiple comparison tests after 48 hours of intoxication by contact.

Table 3 The sum of ranks of the Kruskal-Wallis test for mortality of *Scaptotrigona bipunctata* intoxicated by contact with Cypermethrin after 48 hours of exposure with the groups resulting from the pairwise comparison. Concentrations with statistical difference do not share the same group

Concentration	Rank	Groups		
0.5 mg a.i/mL	16	A		
1 mg a.i/mL	12.16	A	B	
0.0625 mg a.i/mL	9.33		B	C
0.125 mg a.i/mL	6.5			C
0.25 mg a.i/mL	6.5			C
Controle	6.5			C

The estimated LC50 by ingestion after 72 hours of exposure was 27.224 µg a.i/mL and for intoxication by contact was 3.663 mg a.i/mL.

The relative esterase activity after 24 hours of exposure by ingestion (Fig 1A) showed a slight inhibition in the EST-1 and EST-3 at the concentration of 4 µg a.i./mL when compared to the control group. After 72 hours (Fig 1B) at the concentration of 0.25 µg a.i./mL, all esterase activity decreased when compared to the control group. The activity of EST-3 was inhibited after 24 hours of exposure at the concentration of 4 µg a.i./mL, but it increased again after 72 hours, showing normal activity (Table 5).

The relative activity of EST-3 presented an increase after 24 hours of exposure by contact (Fig 1C) at the concentrations of 0.125 mg a.i./mL and 0.25 mg a.i./mL and its activity decreased at the concentrations of 0.5 mg a.i./mL and 1 mg a.i./mL.

Therefore, after 72 hours of exposure by contact (Fig 1D) EST-3 increased its activity at the concentration of 0.0625 mg a.i./mL, when compared with the control group. Elseways, in the concentrations of 0.125 mg a.i./mL and 0.5 mg a.i./mL EST-3 decreased its activity when compared to the control group (Table 6).

Concluding, the relative activity of EST-3 at the concentration of 0.125 mg a.i./mL increased after 24 hours of exposure by contact and decreased after 72 hours. On the other hand, at the concentration of 0.5 mg a.i./mL the activity of EST-3 was normal after 24 hours but decreased its activity after 72 hours.

The SDS-PAGE gel after 24 hours of exposure by ingestion showed that the majority of proteins increased their expression at the concentrations of 0.5 µg a.i./mL, 1 µg a.i./mL and 2 µg a.i./mL. On the other hand, the expression at the concentration of 4 µg a.i./mL decreased (Fig 2A).

The electrophoretic pattern after 72 hours of exposure by ingestion showed that the regions of approximately 80, 35, 26 and 27 kDa had a progressive decrease of expression from the lowest to the highest concentrations tested (Fig 2B).

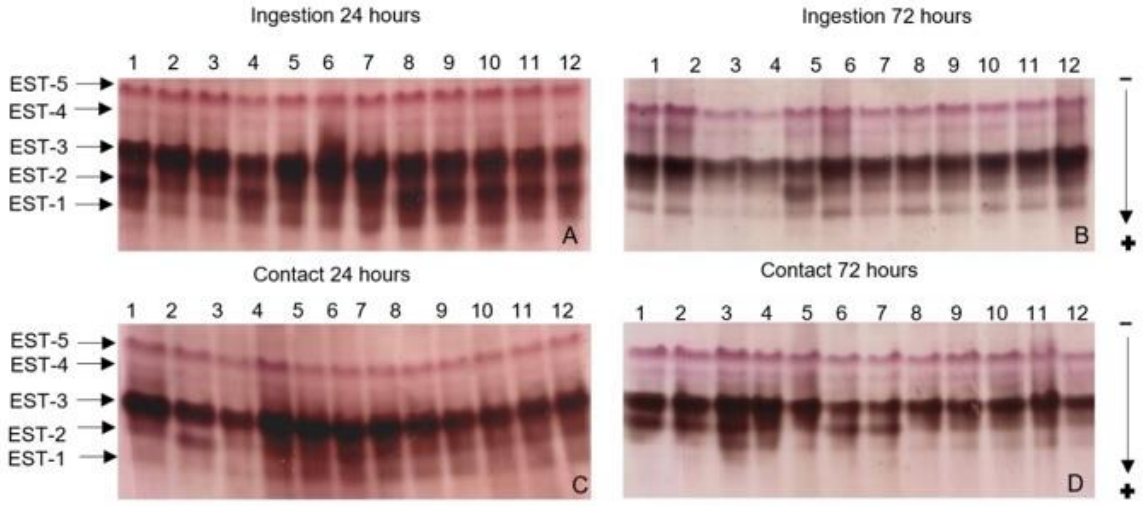

Figure 1 Electrophoretic profile of polyacrylamide gel esterases. (A) After 24 hours of contamination with cypermethrin by ingestion. 1-2: control; 3-4: 0.25 µg a.i./mL; 5-6: 0.5 µg a.i./mL; 7-8: 1 µg a.i./mL; 9-10: 2 µg a.i./mL; 11-12: 4 µg a.i./mL. (B) After 72 hours of contamination with cypermethrin by ingestion. 1-2: control; 3-4: 0.25 µg a.i./mL; 5-6: 0.5 µg a.i./mL; 7-8: 1 µg a.i./mL; 9-10: 2 µg a.i./mL; 11-12: 4 µg a.i./mL. (C) After 24 hours of contamination with cypermethrin by contact. 1-2: control; 3-4: 0.0625 mg a.i./mL; 5-6: 0.125 mg a.i./mL; 7-8: 0.25 mg a.i./mL; 9-10: 0.5 mg a.i./mL; 11-12: 1 mg a.i./mL. (D) After 72 hours of contamination with cypermethrin by contact. 1-2: control; 3-4: 0.0625 mg a.i./mL; 5-6: 0.125 mg a.i./mL; 7-8: 0.25 mg a.i./mL; 9-10: 0.5 mg a.i./mL; 11-12: 1 mg a.i./mL.

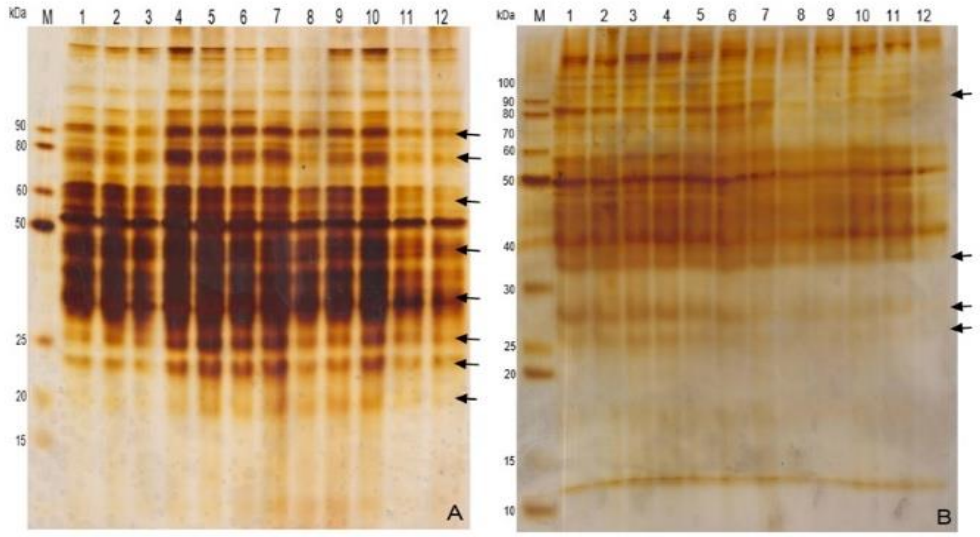

Figure 2 Total protein SDS-PAGE electrophoresis after contamination with cypermethrin by ingestion. (A) After 24 hours of contamination. (B) After 72 hours of contamination.1-2: control; 3-4: 0.25 µg a.i./mL; 5-6: 0.5 µg a.i./mL; 7-8: 1 µg a.i./mL; 9-10: 2 µg a.i./mL; 11-12: 4 µg a.i./mL.

Within 24 hours of exposure by contact with Cypermethrin, there was an increase in the protein expressions at the region of approximately 80 and 70 kDa at the concentration of 0.5 mg a.i./mL and 1 mg a.i./mL. Moreover, at the regions of approximately 26 and 27 kDa, there was a progressive decrease in the expression from the lowest to the highest concentrations (Fig 3A).

Furthermore, the results after 72 hours of exposure by contact showed that there was an increase of expression at the regions of approximately 41, 26 and 27 kDa at the concentrations of 0.5 a.i./mL and 1 mg a.i./mL when compared to the control group. Also, it can be observed a significant increase in the expression at approximately 16 kDa at the concentration of 1 mg a.i./mL, when compared to the individuals that were not intoxicated with the insecticide (Fig 3B).

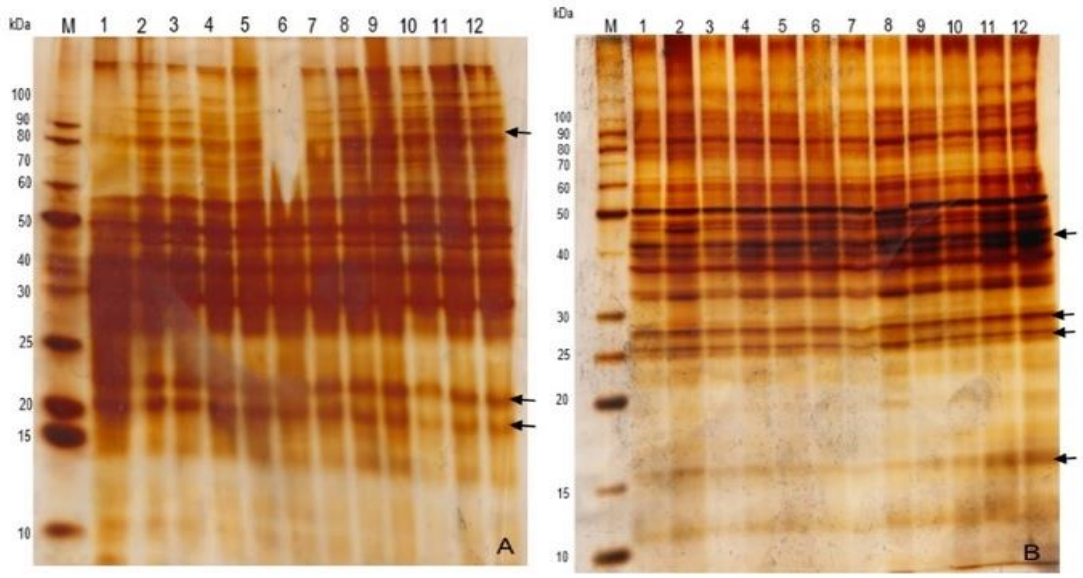

Figure 3 Total protein SDS-PAGE electrophoresis after contamination with cypermethrin by contact. (A) After 24 hours of contamination. (B) After 72 hours of contamination. 1-2: control; 3-4: 0.0625 mg a.i./mL; 5-6: 0.125 mg a.i./mL; 7-8: 0.25 mg a.i./mL; 9-10: 0.5 mg a.i./mL; 11-12: 1 mg a.i./mL.

It is important to emphasize that the amount of studies with *S. bipunctata* is scarce, mainly in relation to their interaction with insecticides. Therefore, it is possible to correlate the data obtained in this work with studies that used cypermethrin insecticide in different species.

The impact of pyrethroid insecticides in *Apis mellifera* are well known. Honeybees suffer directly and indirectly damages due to the action of these insecticides used in crop fields (Atkins e Kellum, 1985). Besides causing mortality of honeybees, they compromise foraging activity, decrease queen fecundity, alter motor coordination, cause muscle tremors and compromise learning behaviours (Decourtye et al. 2004; Freitas e Pinheiro, 2010; Dai et al. 2010).

Cypermethrin has been shown to be a repellent in *A. mellifera* at concentrations ranging from 5 to 25 g/ha (Cox and Wilson, 1984). Tests designed to simulate long exposure to this insecticide demonstrated that doses of 12.5 µg/L cause serious damages to *A. mellifera* colonies (Bendahou et al. 1999). Thus, it can be observed that small doses of cypermethrin can affect the colonies development in a short and long term.

Motricity changes were observed in *S. bipuncta*, such as tremors and difficulty to fly after 24 hours of intoxication, both in ingestion and contact intoxication bioassays. Considering that, field studies will be necessary to investigate the impact of this insecticide on *S. bipunctata* colonies.

Feo et al. (2010) showed that the half-life of cypermethrin in water exposed to the sun is 50 days. Delabie et al. (1985) found an LC_{50} of 0.025 µg per honeybee for cypermethrin contamination. Given that, it was found in this work that *S. bipunctata* are more tolerant to cypermethrin than *A. mellifera*.

In *Aedes aegypti* it was found an LC_{50} of 0.01022 mg/L for contamination with cypermethrin (Calderón-Arguedas and Troyo, 2016). Moreover, the stingless bee *S. bipunctata* is less tolerant to this pyrethroid when compared to *A. aegypti*.

Thus, it can be concluded that this species shows a certain degree of tolerance to cypermethrin regarding mortality, but the effects of sublethal doses on the individuals and consequently on the dynamics of the colony are more relevant.

Aldridge (1953) initially classified the esterases in two classes, A and B, according to their interaction with organophosphates, where esterase A hydrolyzes this compound, whereas esterase B is inhibited by it. From then on, methods that are more refined subdivided the esterases into four classes: (1) arylesterases, (2) acetylesterases, (3) carboxylesterases and (4) cholinesterases (Augustinsson, 1968; Heymann, 1980).

According to the inhibition pattern on *S. bipunctata* EST-1 and EST-2 were classified as cholinesterase I, EST-3 could not be classified and EST-4 and EST-5 were classified as cholinesterase II (Moreira et al., 2018).

The cholinesterases are enzymes that act in a range of important areas such as neurobiology, toxicology and pharmacology, acting as dietary detoxification, hormone and pheromone degradation, and neurodevelopment. These proteins hydrolyze carboxylic esters to alcohols and acids (Claudianos et al. 2006; Miao et al. 2010).

These enzymes are responsible for the metabolism of a broad range of substrates, including insecticides. There is evidence that the acquisition of insecticide resistance can arise either by mutations or by amplification of genes in this group. Such phenomena have been observed in many insect species and there might be common mechanisms for

the acquisition of insecticide resistance in these species based on their cholinesterases (Tsubota and Shiotsuki, 2010).

Thus, esterase isoenzymes are part of the detoxification mechanism of the insects. Therefore, it can be inferred that these isoenzymes may act as detoxifiers in *S. bipunctata* after contamination with insecticides at sublethal concentrations.

It is worth noting that resistance is a hereditary characteristic and the *S. bipunctata* bees analyzed in this study were workers, thus they do not have reproductive capacity. Therefore, no resistance was observed, but tolerance to sublethal concentrations of cypermethrin due to the low mortality rate detected after 72 hours of intoxication.

However, this pyrethroid caused changes in the relative activity of esterase that act on the intermediate metabolism of these bees. Consequently, environmental contamination and intoxication of animals with insecticides alters the intermediate metabolism of individuals of different species, so it is important to study and understand these effects in various organisms.

The analysis of proteins is an important approach that can reveal differences in genes expression. Therefore, identifying regions that increased or decreased expression after contamination of bees with insecticides can indicate putative genes related to tolerance to these agrochemicals.

It can be observed that the difference of protein expression after contamination with cypermethrin was more significant than the ones detected in esterases. This response of the bees to cypermethrin may be because the analyzed isoenzymes probably showed an immediate response to the intoxication (24 hours) and afterwards (72 hours) other enzymes were mobilized to act in the detoxification mechanism.

This may be due to the functionality of esterases that play a role in secondary metabolism, including detoxification. Thus, it can be inferred that the enzymes that act most actively in cypermethrin detoxification belong to different classes, other than esterases, for instance, glutathione S-transferases (GST) and cytochrome P450 (P450s) (Claudianos et al. 2006).

Conclusion

The mortality rates of bees after contamination with cypermethrin, both by ingestion and contact, at the concentrations tested in this study was not high. However, the effects observed on its behavior after 24 hours of contamination, such as tremors and difficulty in flight, were significant.

The expression of the esterases presented discrete differences between the treatments. We can see greater differences between treatments in the analysis of total proteins. Thus, we can conclude that other families of enzymes probably act more actively in the detoxification of the cypermethrin insecticide in *S. bipunctata* and the action of esterases is an initial and immediate response, and later other enzymes and/or proteins perform the detoxification of the organism.

Acknowledgements

Research supported by CAPES, CNPq, Fundação Araucária and Secretaria de Estado da Ciência, Tecnologia e Ensino Superior - FUNDO PARANÁ. We gratefully acknowledge all the staff members of Laboratório de Genética Animal for their assistance.

References

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2008) Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Current Biology 18:1572-1575. https://doi.org/10.1016/j.cub.2008.08.066

Aldridge WN (1953) Serum esterases. I. Two types of esterase (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate and a method for their determination. Biochemical Journal 53:110-117

Alfenas AC (2006) Eletroforese e marcadores bioquímicos em plantas e microrganismos. UFV, Viçosa

Atkins EL, Kellum D (1985) Comparative morphogenesis and toxicity studies on the effect of pesticides on honeybee brood. Journal of Apicultural Research 24:245-255. https://doi.org/10.1080/00218839.1986.11100725

Bendahou N, Fleche C, Bounias M (1999) Biological and Biochemical Effects of Chronic Exposure to Very Low Levels of Dietary Cypermethrin (Cymbush) on Honeybee Colonies (Hymenoptera: Apidae). Ecotoxicology and Environmental Safety 44:147-153. https://doi.org/10.1006/eesa.1999.1812

Braga Ia, Valle D (2007) *Aedes aegypti*: inseticidas, mecanismos de reação e resistência. Epidemiologia e serviços em saúde pública. Revista do Sistema Único de Saúde, 16:279-293. http://dx.doi.org/10.5123/S1679-49742007000400006

Brittain C, Potts SG (2011) The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. Basic and Applied Ecology 12: 321-331. https://doi.org/10.1016/j.baae.2010.12.004

Calderón-Arguedas Ó, Troyo A (2016) Evaluación de la resistencia a insecticidas en cepas de *Aedes aegypti* (Diptera: Culicidae) de la Región Caribe de Costa Rica. Revista Cubana de Medicina Tropical 68:95-104

Clark SL, Ogle RS, Gantner A, Hall Jr LW, Mitchell G, Giddings J, Mccoole M, Dobbs M, Henry K, Valenti T (2015). Comparative sensitivity of field and laboratory populations of *Hyalella azteca* to the pyrethroid insecticides bifenthrin and cypermethrin.

Environmental Toxicology 34:2250-2262. https://doi.org/10.1002/etc.2907

Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, Berenbaum MR, Feyereisen R, Oakeshott JG (2006) A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology 15:615-636. https://doi.org/10.1111/j.1365-2583.2006.00672.x

Claudianos C, Ranson H, Johnson RM, Biswas S, Shuler MA, Berenbaum MR, Feyereisen R, Oskeshott JG (2006) A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology 15:615-636. https://doi.org/10.1111/j.1365-2583.2006.00672.x

Cox RL, Wilson TW (1984) Effect of permethrin on the behaviour of individually targeted honeybees, (*Apis mellifera*). Environmental Entomology 2:375-378

Dai P, Wang Q, Sun J (2010) Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development on the honeybee *Apis mellifera* ligustica. Environmental Toxicology and Chemistry 29:644-649. https://doi.org/10.1002/etc.67

Decourtye A, Devillersb J, Cluzeauc S, Charretona M, Pham-Delègue M (2004) Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicology and Environmental Safety 57:410-419. https://doi.org/10.1016/j.ecoenv.2003.08.001

Delabie J, Bos C, Frota C, Masson C (1985) Toxic and repelente effects of Cypermethrin on the honeubee: laboratory, glasshouse and field experiments. Pesticide Science 16:409-415. https://doi.org/10.1002/ps.2780160417

Delaplane KS, Mayer DR, Mayer DF (2000) Crop Pollination by Bees. CABI Publishing, London

Ding Z, Wen Y, Yang B, Zhang Y, Liu S, Han Z (2013) Biochemical mechanisms of imidacloprid resistance in *Nilaparvata lugens*: over-expression of cytochrome P450 CYP6AY1. Insect Biochemistry and Molecular Biology 43:1021-1027. https://doi.org/10.1016/j.ibmb.2013.08.005

Feo ML, Ginebreda A, Eljarrat E, Barcelo D (2010) Presence of pyrethroid pesticides in water and sediments of Ebro River Delta. Journal of Hydrology 393:156-162. https://doi.org/10.1016/j.jhydrol.2010.08.012

Frazier M, Mullin C, Frazier J, Ashcraft S (2008) What have pesticides got to do with it? American Bee Journal 148:521-523

Freitas BM, Pinheiro JN (2010) Efeitos sub-letais dos pesticidas agrícolas e seus impactos no manejo de polinizadores dos agroecossistemas brasileiros. Oecologia Australis 14:282-298

Freitas BM, Pinheiro JN (2012) Polinizadores e Pesticidas: Princípios de Manejo para os Agroecossistemas Brasileiros. MMA, Brasília

Hemingway J, Hawkes NJ, Mccarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology, 34:653-665. https://doi.org/10.1016/j.ibmb.2004.03.018

Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annual Review of Entomology 45:371-391. https://doi.org/10.1146/annurev.ento.45.1.371

Hoy M (2003) Insect molecular genetics. Academic Press, London

Li X, Schuler MA, Berenbaum MR (2007) Molecular Mechanisms of Metabolic Resistance to Synthetic and Natural Xenobiotics. Annual Review of Entomology 52:231-253. https://doi.org/10.1146/annurev.ento.51.110104.151104

Lins ACS, Silva TMS, Câmara CA, Silva SEM, Freitas BM (2003) Flavonóides isolados do pólen coletado pela abelha *Scaptotrigona bipunctata* (canudo). Revista Brasileira de Farmacognosia 13:40-41. http://dx.doi.org/10.1590/S0102-695X2003000400013

Macías-Macías O, Chuc J, Ancona-Xiu P, Caunich O, Quezada-Eúan JJG (2009) Contribuition of native bees and Africanized honey bees (Hymenoptera: Apoidea) to solenaceae crop pollination in tropical México. Journal of Applied Entomology 133:456-465. https://doi.org/10.1111/j.1439-0418.2009.01399.x

Miao Y, He N, Jun-Jie Z (2010) History and New Developments of Assays for Cholinesterase Activity and Inhibition. Chemical Reviews 110:5216-5234. https://doi.org/10.1021/cr900214c

Montella IR, Schama R, Valle D (2012) The classification of esterases: an important gene family involved in insecticide resistance - A Review. Memórias do Instituto Oswaldo Cruz 107:437-449. http://dx.doi.org/10.1590/S0074-02762012000400001

Moreira, DR, Gigliolli, AAS, Falco, JRP, Julio, AHF, Volnistem, EA, Chagas F, Toledo, VAA, Ruvolo-Takasusuki, MCC (2018) Toxicity and effects of the neonicotinoid thiamethoxam on *Scaptotrigona bipunctata* lepeletier, 1836 (Hymenoptera: Apidae). Environmental Toxicology https://doi.org/10.1002/tox.22533

Pacheco WF, Alves JE, Ribeiro MF, Freitas BM (2008) Comportamento alimentar de *Scaptotrigona bipunctata* (Hymenoptera, Apidae, Meliponini) em três Municípios do Estado do Ceará. Disponível em: https://ainfo.cnptia.embrapa.br/digital/bitstream/CPATSA-2009-09/40387/1/OPB2266.pdf. Accessed 12 September 2018

Rigotto RM, Vasconcelos DP, Rocha MM (2014) Uso de agrotóxicos no Brasil e problemas para a saúde pública. Cadernos de Saúde Pública 30:1-3. http://dx.doi.org/10.1590/0102-311XPE020714

Salinas AE, Wong MG (1999) Glutathione S-transferases – a review. Current Medicinal Chemistry 6:279-309

Sethi BK, Pradhan S, Behera N, Sahoo SL (2015) Effect of cypermethrin, a pyrethroid insecticide on dynamics of soil microflora. Journal of Applied Biology and Biotechnology 3:019-025. https://10.7324/JABB.2015.3504

Takuya T, Takahiro S (2010) Genomic analysis of carboxyl/cholinesterase genes in the silkworm *Bombyx mori*. BMC Genomics 11:377. https://doi.org/10.1186/1471-2164-11-377

Terhzaz S, Cabrero P, Brinzer RA, Halberg KA, Dow JAT, Davies SA (2015) A novel role of *Drosophila* cytochrome *P450-4e3* in permethrin insecticide tolerance. Insect Biochemistry and Molecular Biology 67:38-46. https://doi.org/10.1016/j.ibmb.2015.06.002

You M, Yue Z, He W et al (2013) A heterozygous moth genome provides insights into herbivory and detoxification. Nature Genetics 45:220-225. https://doi.org/10.1038/ng.2524

Zimmer CT, Bass C, Williamson MS, Kaussmann M, Woelfel K, Gutbrod O, Nauen R (2014) Molecular and functional characterization of CYP6BQ23, a cytochrome P450 conferring resistance to pyrethroids in European populations of pollen beetle, *Meligethes aeneus*. Insect Biochemistry and Molecular Biology 45:18-29. https://doi.org/10.1016/j.ibmb.2013.11.008