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Determination of acute lethal doses (LD_{50} and LC_{50}) of imidacloprid for the native bee *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae)

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Abstract

The bee species *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) is native to Brazil and, stingless. In Brazil, stingless bees are responsible for 40% to 90% of tree species pollination, depending on the considered ecosystem. However, their survival has been threatened since the country has been standing out as a big consumer of pesticides. Many of the pesticides used are considered toxic to bees, including imidacloprid. Although the bees are not the target of these substances, they are highly vulnerable to contamination. Thereby, the objective of this study was to establish the mean lethal dose (LD_{50}) and the mean lethal concentration (LC_{50}) of imidacloprid for the *M. scutellaris*. In order to carry out this experiment, bees were collected and the test was performed according to OECD's protocol (1998a, 1998b), developed for *A. mellifera*. For the determination of LD_{50} and LC_{50}, data was analyzed through the Probit method. The topical LD_{50} established in this study was 2.41 ng/bee for 24 hours and 1.29 ng/bee for 48 hours. The oral LC_{50} was 2.01 ng i.a./µL for 24 hours and 0.81 ng i.a./µL for 48 hours. Thus, it is important to establish management methods which take this higher susceptibility into account to protect native species.

Introduction

Bees belong to the class Insecta, order Hymenoptera, as well as wasps and ants. These insects are the most important ones for biodiversity conservation since they have the biggest number of pollinators which use pollen and nectar as food and energy sources (Nogueira-Neto, 1997). During the collection process of these resources on flowers, they transfer pollen grains from the male reproductive organs to the female ones, ensuring pollination success.

In Brazil, the Apidae family is the biggest one, with solitary and social bee species. Among representatives of this family we find bees from the *Melipona* Illiger, 1806 genus (which belongs to the Meliponini tribe), with more than 50 species. In this genus, the stingless and eusocial bee, *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae), also commonly known as "uruçu" or "uruçu do nordeste", stands out (Nogueira-Neto, 1997; Kerr et al., 2001; Imperatriz-Fonseca & Santos, 2014). This species is endemic among Brazilian Northeastern region, inhabiting hot and humid forest regions of the Bahia Coast and Chapada Diamantina, and it is also adapted to the ecological and climatic conditions of the state of São Paulo (Nogueira-Neto, 1997). In the Atlantic Rainforest this bee is found only in places which present a low level of disturbance, fact that can be considered as an indication of environmental quality (Ramalho & Batista, 2005).

In addition to the pollination services, *Melipona* provides products and by-products with high economic value, such as: honey, pollen, and propolis. However, the importance of these bees goes far beyond the economic benefits, they also help in the rebuilding process of tropical forests and in the conservation of the existing ones, being able to act as bioindicators of environmental quality and having a key role in the ecosystem processes in which they are involved (Ballivián, 2008).

Unfortunately, little is known about Brazilian bees and, for this reason, there are difficulties in establishing conservation initiatives for this insect. At the same time there is also a reduction of food sources and nesting areas, an intensive occupation of lands and, most importantly, an excessive and/or incorrect use of pesticides, contributing to the reduction of populations (Kerr et al., 2001).

Neonicotinoids acts on post-synaptic nicotinic acetylcholine receptors in the central nervous system. After binding, nerve impulses are discharged at first, followed by failure of the neuron to propagate any signal. Acetylcholinesterases are not able to break down the neonicotinoid. This binding process is irreversible. The symptoms resulting from intoxication are tremors, seizures and death (National Pesticide Information Center [NPIC], 2010).

Although bees are not the target of these toxic compounds, they may do their foraging in contaminated fields, being highly vulnerable to contamination (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA], 2012). Thereby, the objective of this study was to establish the mean lethal dose (LD_{50}) and the mean lethal concentration (LC_{50}) of imidacloprid for the *M. scutellaris*.

Material and Methods

Bee Collection

Foragers bees of *M. scutellaris* were collected in the apiary of the Biosciences Institute at São Paulo State University "Júlio de Mesquita Filho", Rio Claro campus. For all the performed tests, 30 bees were used with three repetitions of 10 bees, being each one from three different colonies. The chosen colonies were free from diseases and presented a queen in good health. The tests were performed at the Center for the Study of Social Insects in the Bioscience Institute at UNESP, Rio Claro.

Determination of Mean Lethal Topical Dose

The procedures for the determination of the topical LD_{50} were based on the OECD (1998a) developed for *A. mellifera* (the temperature was changed to 28 °C). The imidacloprid pesticide (degree of purity 95%) was initially diluted in acetone and by means of successive dilutions were prepared the desired doses for application (2; 4; 8; 16; 32; and 64 ng a.i./µL to establish LD_{50} for 24 hours and 0.3; 0.6; 1.2; 2.4; 4.8; and 9.6 ng a.i./µL to establish LD_{50} for 48 hours).

The collected bees were transferred to plastic containers with volume of 250 ml with perforations on the lid for air circulation. In each container there was a plastic microvial with food (50% sucrose solution) ad libitum. Bees in the experimental group received a topical application of 1 µL of one respective the solution containing the tested substance in the dorsal area of the thorax. Bees in the control group received only 1 µL of acetone. The bees were held in climatic room at 28 ± 1°C, the relative humidity of 70 ± 5% and darkness.

Determination of Mean Lethal Oral Concentration

The procedures for the determination of the oral LC_{50} were based on the OECD (1998b) developed for *A. mellifera* (the temperature was changed to 28 °C).

To obtain the desired concentrations of imidacloprid, from the stock solution were prepared some others by dilutions on cascade using as solvent a mixture of 85% water + 15% acetone. The concentrations used for the bee's contamination were 0.3; 0.6; 1.2; 2.4; 4.8; and 9.6 ng/µL. For the control group, food was supplied without imidacloprid. The tests were conducted in BOD chamber with a temperature of 28 ºC ± 1.0 and with 70% ± 5 of relative humidity.

Statistical Analysis

Mortality data obtained from the assays were subjected to statistical analysis using the Probit method (Finney, 1952) using the R® software. LD_{50} and LC_{50} values were determined, as well as their respective 95% confidence intervals values.

Results and Discussion

The values for the topical LD_{50} of the imidacloprid obtained for *M. scutellaris* were: 2.41 and 1.29 ng a.i./bee (Table 1 and Figs 1 and 2), for 24 and 48 hours, respectively. The oral LC_{50} obtained were of 2.01 and 0.81 ng a.i./µL (Table 1 and Figs 3 and 4), for 24 and 48 hours, respectively.

Table 1. Acute toxicity values of imidacloprid for *Melipona scutellaris*.

Exposure mode	Time (hours)	LD_{50}	LC_{50}	C.I.$_{95\%}$	χ^2	D.F.
Topic ng a.i./ bee	24	2.41	–	1.63 – 3.27	0.753	4
	48	1.29	–	0.813 – 1.903	2.642	4
Ingestion ng a.i./ diet µL	24	–	2.01	1.551 – 2.618	2.534	4
	48	–	0.81	0.264 – 1.538	4.001	4

(LD50) mean lethal dose; (LC50) mean lethal concentration; (C.I.95%) confidence interval 95%; (χ2) chi-square, and (D.F.) degree of freedom.

According to Johansen and Mayer classification (1990), which consider insecticides with a LD_{50} < 2.000 ng/bee as highly toxic to bees, imidacloprid is considered highly toxic for *M. scutellaris*. For *A. mellifera* there are several reports for the topical LD_{50} of imidacloprid, among them: 17.9

ng a.i./bee (24 hours) (Iwasa et al., 2004); 24 ng a.i./bee (24 and 48 hours) (Suchail et al., 2003); 42 – 104[1] ng a.i./bee (48 hours) (Schmuck et al., 2003); 49 – 102[2] ng a.i./bee (48 hours) (Nauen et al., 2001). The values of LC_{50} for *A. mellifera* are: 81 ng a.i./µL diet (48 hours) (Nauen et al., 2001) and 40.9 ng a.i./µL diet (48 hours) (Schmuck et al., 2001). These values show that the *M. scutellaris* bees are more sensitive to imidacloprid than *A. mellifera.*

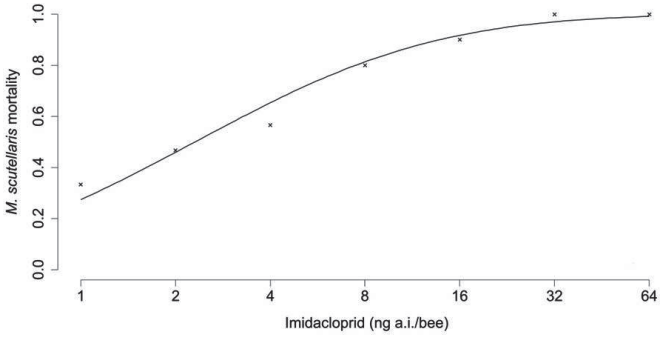

Fig 1. Mortality of *Melipona scutellaris* (24 hours) after the intoxication with imidacloprid by contact.

Our results corroborate with the work of Soares et al. (2015), which determined the topical LD_{50} and oral LC_{50} of imidacloprid for native bee S*captotrigona postica* Latreille, 1807 (Hymenoptera: Apidae). The obtained values were: topical LD_{50} of 25.20 (24 hours) and 24.46 ng a.i./bee (48 hours) and oral LC_{50} of 42.5 (24 hours) and 14.3 ng a.i./µL diet (48 hours), indicating that this species is also more susceptible to the neonicotinoid pesticide than *A. mellifera*.

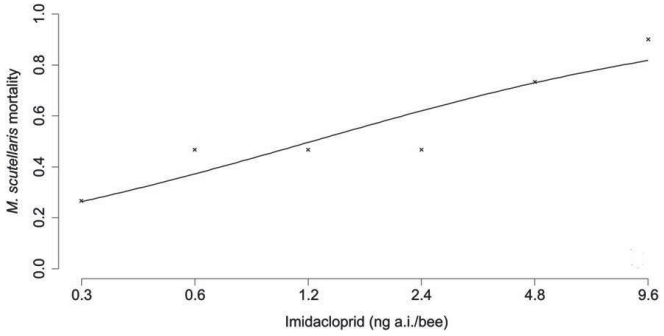

Fig 2. Mortality of *Melipona scutellaris* (48 hours) after the intoxication with imidacloprid by contact.

Comparing values of LD_{50} and LC_{50} (48 hours) of imidacloprid for *S. postica* and *M. scutellaris*, it was noted that this bee is 19 times more sensitive when compared to the other bee.

The fact that *M. scutellaris* bee is more sensitive than *A. mellifera* bee was also proved by Lourenço et al. (2012a, 2012b) who did a toxicity study of fipronil with native bee species and verified that the pesticide is highly toxic, showing a topical LD_{50} (48 hours) of 0.41 ng a.i./bee and oral LC_{50} (48 hours) of 0.011 ng a.i./ µL diet. Jacob et al. (2013) also demonstrated the higher sensitivity of native bee *S. postica* to fipronil. In this study, the topical LD_{50} (24 hours) determined was 0.54 ng a.i./bee and the oral LC_{50} (24 hours) was 0.24 ng a.i./µL diet. For *A. mellifera* the topical LD_{50} of fipronil is 4 ng a.i./bee and the oral LC_{50} is 1.27 ng/µL diet (Tingle et al., 2003; Decourtye et al., 2005).

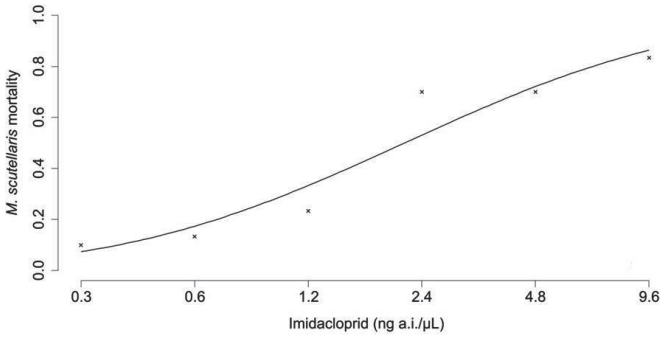

Fig 3. Mortality of *Melipona scutellaris* (24 hours) after the intoxication with imidacloprid by ingestion.

When we compare the LC_{50} and LD_{50} found in this study with values obtained by other studies presented here, it is possible to infer that *M. scutellaris* species is more sensitive to the fipronil than to imidacloprid.

Others studies that compare the tolerance between stingless and Africanized honey bee species showed that the former are usually more sensitive to pesticides (Moraes et al., 2000; Valdovinos-Núñez et al., 2009; Del Sarto et al., 2014).

The toxicity of neonicotinoid pesticides for bees can be classified in two groups based on the presence of nitro or cyan grouping. The pesticides with nitro grouping are the most toxic ones, such as imidacloprid, because the presence of this functional group grants to the pesticide great affinity with the nicotinic receptor of acethylcholine and, therefore, its high toxicity (Tomizawa & Casida, 2003).

The bees exposed to imidacloprid, either topically or orally, presented signs of paralysis, tremors and some of them were even dead, which are common symptoms of intoxication by neonicotinoid pesticides observed by Suchail et al. (2001), since the target organ of this substance is the nervous system.

[1] This range is because one of the objects of study Schmuck et al. (2003) it was to verify if there was difference between the sensitivity of bees from different European apiaries to imidacloprid. The toxicity tests were carried out in different European laboratories. The calculated LD_{50} values did not indicate significant differences in sensitivity between honeybees of different apiaries.

[2] This range is because one of the objects of study Nauen et al. (2001) it was to verify if there was difference between the sensitivity of bees from different European apiaries to imidacloprid. The toxicity tests were carried out in different European laboratories. The calculated LD_{50} values did not indicate significant differences in sensitivity between honeybees of different apiaries.

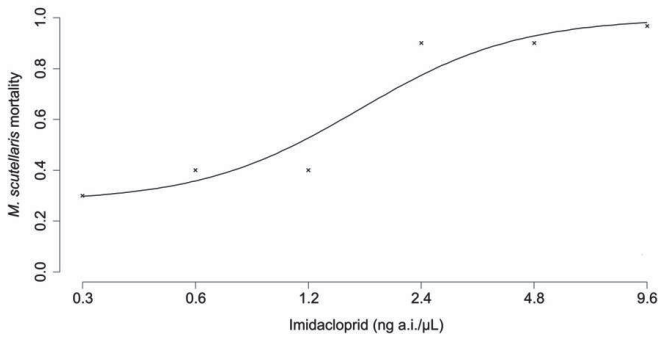

Fig 4. Mortality of *Melipona scutellaris* (48 hours) after the intoxication with imidacloprid by ingestion.

A study performed by Suchail et al. (2003) indicated that the high oral toxicity of imidacloprid for *A. mellifera* might be caused by the fact that this molecule is rapidly metabolized in olefin and 5-hydroxyimidacloprid. Such metabolites are toxic with acute exposure, highly suggesting that 5-hydroxyimidacloprid and/or olefin contribute for an increased action of imidacloprid in bees.

Depending on the cultivation and application method, imidacloprid can show an application concentration of 70 µg/mL, exceeding in 54 times the value capable of causing bee mortality (LD_{50} for 48 hours). Therefore, the use of imidacloprid should be avoided during the bloom period (Suchail et al., 2000).

In conclusion, our study showed that *M. scutellaris* is highly sensitive to the action of the insecticide imidacloprid after topical and oral intoxication. Because of this and of the economic and ecological importance, native species of stingless bees should be more studied, especially in relation to pesticide impact.

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