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Toxicity of Imidacloprid to the Stingless Bee *Scaptotrigona postica* Latreille, 1807 (Hymenoptera: Apidae)

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Abstract The stingless bee *Scaptotrigona postica* is an important pollinator of native and cultivated plants in Brazil. Among the factors affecting the survival of these insects is the indiscriminate use of insecticides, including the neonicotinoid imidacloprid. This work determined the toxicity of imidacloprid as the topical median lethal dose (LD_{50}) and the oral median lethal concentration (LC_{50}) as tools for assessing the effects of this insecticide. The 24 and 48 h LD_{50} values were 25.2 and 24.5 ng of active ingredient (a.i.)/bee, respectively. The 24 and 48 h LC_{50} values were 42.5 and 14.3 ng a.i./μL of diet, respectively. Ours results show the hazard of imidacloprid and the vulnerability of stingless bees to it, providing relevant toxicological data that can used in mitigation programs to ensure the conservation of this species.

Keywords Acute toxicity · Neonicotinoid · Stingless bee

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Bees are major pollinators of wild and cultivated plants (Castro et al. 2006). The interaction between bees and plants ensures the development of bee colonies, because pollen and nectar are the primary protein and carbohydrates sources, as well as the success of plants due to cross-breeding and the resultant increases in genetic variability and fruit and seed production (Kearns and Inouye 1997; Brittain and Potts 2011).

According to Kerr et al. (1999, 2001), stingless bees (Hymenoptera: Apidae, Meliponini) are responsible for 30 % of the pollination of the Brazilian Caatinga and Pantanal ecosystems, and up to 90 % of the pollination of some plant species of the Atlantic Forest and Amazon. In agricultural areas, these bees are important for pollination of crops such as watermelon (*Citrulus lanatus* L.), onion (*Allium cepa* L.) and sunflower (*Helianthus annuus* L.) (Macieira and Proni 2004).

However, even with a richness of stingless bees in Brazil that can exceed 500 species (Castro et al. 2006), only the honey bee (*Apis mellifera* L., 1758; Hymenoptera: Apidae) is considered as a model organism in toxicological studies. The reason for this choice is mainly due to the ability of the honey bee to adapt to different habitats, facility in the management/maintenance of colonies and the value-added in products such as honey, propolis and other (Kevan et al. 2007; Klein et al. 2007). Despite the importance of these insects to agriculture and maintenance of different ecosystems, studies report that human activities such as deforestation, fragmentation of forest areas and indiscriminate use of insecticides are among the main factors in the disappearance of both honey bees and stingless bees (Kerr et al. 2001; Alix and Vergnet 2007; Ellis et al. 2010).

Commercially launched in 1991 and used in approximately 140 different crops (Elbert et al. 2008),

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imidacloprid is a neurotoxic insecticide that acts in the synapses of the central nervous system of insects as an agonist of acetylcholine, competing with this neurotransmitter by nicotinic receptors. Unlike what occurs with acetylcholine, the imidacloprid isn't metabolized by the enzyme acetylcholinesterase and the persistent binding of this pesticide with the nicotinic receptors promotes continuous transmission of nerve impulse (Nauen et al. 2001; Faria 2009). Moreover, due to the properties of imidacloprid as a systemic insecticide and the long-term presence of residuals in the environment, this insecticide can be taken up and translocated in plants, resulting in its occurrence in pollen and nectar (Goulson 2013).

Data on the risks of stingless bee exposure to imidacloprid are scarce, but several studies show its toxicity to honey bees. According to Schmuck et al. (2001), the LD_{50} (48 h) in the honey bee could reach up to 40.90 and 242.60 ng/bee for both oral and topical treatments, respectively. In another study, Nauen et al. (2001) reported values of 81 and 102 ng/honey bee for oral and topical treatments, respectively, after 48 h for the same bee species. In addition, studies by Medrzycki et al. (2003), Yang et al. (2008) and (Schneider 2012) showed that sublethal doses of imidacloprid can induce behavioral changes in honey bee like learning ability, orientation, foraging and brood care.

Little is known about the tolerance of stingless bees following exposure to pesticides. This study aimed to evaluate the acute toxicity of imidacloprid for the species *Scaptotrigona postica* by topical (LD_{50}) and oral (LC_{50}) treatments.

Materials and Methods

The workers of stingless bee *S. postica* were collected at the meliponary from the Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Rio Claro, São Paulo, Brazil. To obtain newly-emerged bees, combs with pupae were collected from three different colonies, immediately placed in plastic cages (250 mL), and maintained under darkness in a BOD chamber at 28 ± 2°C, with relative humidity of 70 ± 10 %, simulating the conditions of the colony.

The procedures for determining the lethal dose (LD_{50}) were based on the OECD protocol (1998a) for *A. mellifera*. A stock solution of imidacloprid (Sigma-Aldrich, São Paolo, BR) was prepared at 1,000 ng a.i./μL acetone. Subsequently, dilutions were made covering a range of 150 to 0.01 ng a.i./μL.

For application of 1 μL of respective solution on the pronoto region, we used a microsyringe coupled with a repetitive dispenser. For each dose tested, 60 individuals were divided into three replicates of 20 bees each, from three different colonies. A control group received only acetone. After 5 min of the insecticide application, the bees were placed in plastic cages (250 mL), lined with filter paper and maintained under darkness in a BOD chamber as described above. The water, offered in soaked cotton, and the food, made with honey + water (1:1) enriched with 30 % of sucrose and 4 % of gelatin, were placed *ad libitum* on each cage. After 24 and 48 h, the number of dead bees was recorded and the values subjected to statistical analysis. In addition, any atypical behavior of the bees was recorded.

The lethal concentration (LC_{50}) of imidacloprid was determined according to the methodology proposed by the OECD (1998b) with some modifications. To prepare the contaminated diets, a stock solution of imidacloprid in acetone was prepared at 1,000 ng a.i./μL, and a cascade of dilutions with food was prepared to produce seven dietary treatments from 1 to 120 ng a.i./μl of diet. For the control group, food was supplied without imidacloprid. As described above, a group of 60 bees per concentration (three replicates of 20 bees), were placed in plastic cages lined with filter paper, with contaminated food and water-soaked cotton. Feeding was *ad libitum*. Before starting the assay, bees were not fed for 2 h.

Mortality data obtained from the assays were subjected to statistical analysis using the software R® (R Core Team 2014) and the package drc (Ritz and Streibig 2005). With the log-logistic model fitted, both LD_{50} and LC_{50} values were determined, as well as their respective 95 % confidence intervals and Chi square values.

Results and Discussion

Topical treatments with imidacloprid resulted in LD_{50} values of 25.2 and 24.5 ng a.i./bee at 24 and 48 h following application, respectively (Table 1; Figs. 1, 2). Dietary LC_{50} values were 42.5 and 14.3 ng a.i./μl of diet after 24 and 48 h of exposure, respectively (Table 1; Figs. 3, 4). These results showed only a minor variation between 24 and 48 h when imidacloprid was administered topically, but a greater than three-fold difference when administered in the diet.

A comparison of toxicity data from our study with literature values for honey bees shows a greater sensitivity of the stingless bee to this neonicotinoid. Our 48 h LD_{50} value for *S. postica* was 24.5 ng a.i./bee. Considerably higher LD_{50} values of 102 ng/bee (Nauen et al. 2001) and 243 ng/bee (Schmuck et al. 2001) have been reported for *A. mellifera*. Similarly, our 48 h dietary LC_{50} value of 14.3 ng a.i./μL of diet may be compared to values of 81 and 41 ng/μL of diet in the honey bee, as reported by Nauen et al.

Table 1 Summary of the acute toxicity tests of imidacloprid against *S. postica*

Exposure route	Time[a]	LD_{50}^{b}	LC_{50}^{c}	$CI_{95\ \%}^{d}$	χ^{2e}	DF[f]
Topical (ng a.i./bee)	24	25.2	–	20.13–30.28	10.964	16
	48	24.5	–	13.71–35.22	20.729	15
Ingestion (ng a.i./μL diet)	24	–	42.5	36.91–48.09	22.047	29
	48	–	14.3	11.87–16.68	36.734	28

[a] Time in hours after the administration of imidacloprid; [b] lethal dose 50 %; [c] lethal concentration 50 %; [d] confidence interval at 95 %; [e] Chi-square from model; [f] degrees of freedom

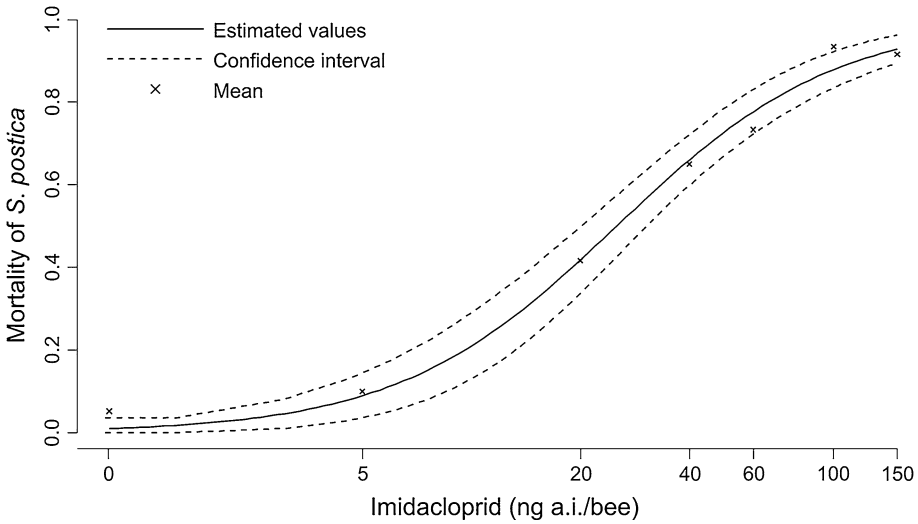

Fig. 1 Mortality of *S. postica* at 24 h following topical application of imidacloprid for LD_{50} determination

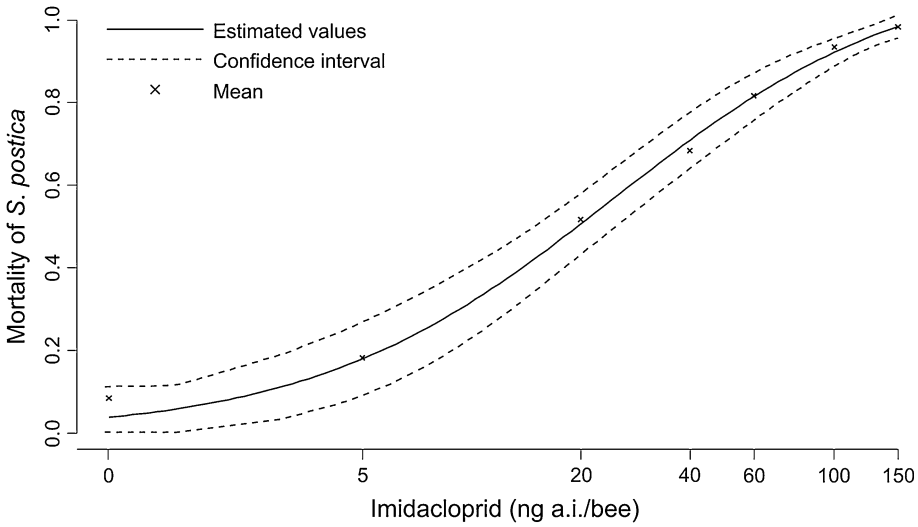

Fig. 2 Mortality of *S. postica* at 48 h following topical application of imidacloprid for LD_{50} determination

2001 and Schmuck et al. 2001, respectively. These data corroborate with studies that used several species of wild bees in evaluations of the toxicity of cyhalothrin (Mayer et al. 1998), malathion (Moraes et al. 2000) and fipronil (Lourenço et al. 2012; Jacob et al. 2013), where the toxicity of these insecticides was higher to wild bees than to *A. mellifera*.

In addition, studies from our laboratory with dimethoate, insecticide used as a standard in toxicity tests (OECD 1998a, 1998b), showed that the LD50 value for *S. postica* (0.087 μg a.i./bee) was lower than for Africanized (0.223 μg a.i./bee) (Roessink et al. 2011) and European (0.10–0.30 μg a.i./bee) *A. mellifera* (OECD 1998a). This is an indication that the bees present in Brazil have a higher

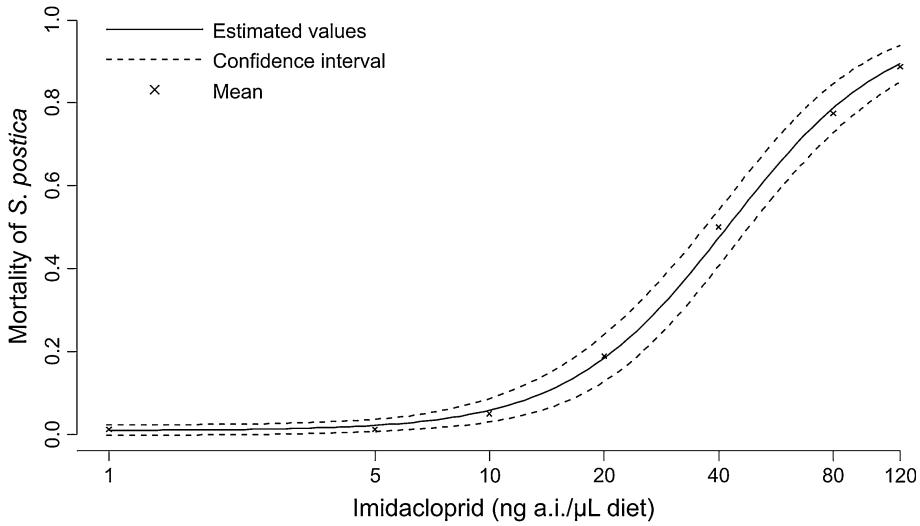

Fig. 3 Mortality of *S. postica* after 24 h of dietary exposure to imidacloprid for LC_{50} determination

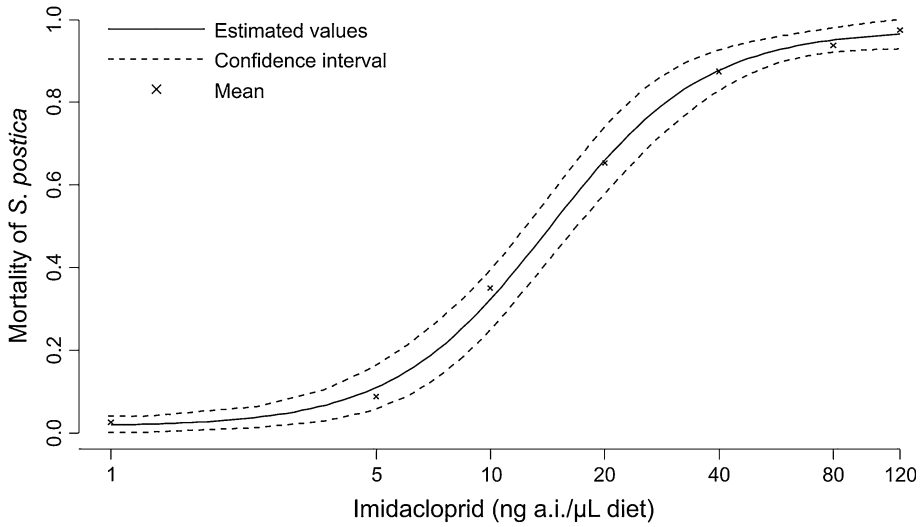

Fig. 4 Mortality of *S. postica* after 48 h of dietary exposure to imidacloprid for LC_{50} determination

sensitivity to insecticides than species identified as surrogates in toxicological tests, showing the importance of properly assessing the impact of these molecules for species that are found in our forests and farmland to ensure their conservation.

The route of intoxication is an important factor for risk assessment, as it may determine the time for an insecticide to reach its target site. It was observed that a topical exposure (24 or 48 h) to imidacloprid was more toxic to *S. postica* than a 24 h dietary exposure. This may have been due to the fact that imidacloprid is a neurotoxic insecticide with action by contact, and that it readily penetrated the integument of the insect when diluted in acetone. When exposure was by ingestion, the insecticide present in the midgut likely encountered a variety of enzymes involved in the metabolism of neonicotinoids (e.g. cytochrome P450, aldehyde oxidase and gluthatione S-transferase) (Casida 2011). However, the greater toxicity observed at 48 h after the ingestion of imidacloprid may be linked with its activation during metabolism. This hypothesis was discussed by Suchail et al. (2003) considering the mortality kinetics and the neurotoxicity symptoms. For them, the mortality of *A. mellifera* was due to increases of 5-hydroxyimidacloprid and olefin (metabolites) in comparison with parent imidacloprid that decreased with time, and was related to the early neurotoxicity symptoms.

The symptoms resulting from poisoning by imidacloprid were similar for the topical and oral treatments, including paralysis, tremors, prostration and death, but it was observed that the symptoms of intoxication appeared earlier in bees exposed by topical application. Medrzycki et al. (2003) and Faria (2009) showed that the decreased mobility and the shivering of wings and body were common symptoms of poisoning by neonicotinoid insecticides in *A. mellifera*,

resulting in the disappearance of symptoms or in death of the individuals depending on the dose administered.

In conclusion, our study showed the vulnerability of stingless bees to the action of the insecticide imidacloprid after topical and oral intoxication. Moreover, the use of bee species other than *A. mellifera* must be encouraged as models in toxicological studies, as well as in programs whose goals are to assess the effects of anthropogenic activities in the environment. This statement should be considered in view of the fact that single species are unable to represent the array of behavioral, morphological and physiological attributes of the natural community (Decourtye et al. 2004). Toxicological data reported here are important for the development of new research on levels of imidacloprid that cause sublethal effects, and as a contribution toward policies with a goal of reducing the hazard of pesticides to bees (van der Valk et al. 2013).

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