



## RESEARCH ARTICLE - BEES

## Toxicity of Imidacloprid Against *Melipona scutellaris* (Latreille, 1811): Preliminary Risk Analysis

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### Abstract

The study aimed to evaluate the oral toxicity of the pesticide Imidacloprid Nortox 400 SC® to native bees (*Melipona scutellaris*, L) and assess the potential risk associated with crops treated with this product. Biotest was conducted using eight concentrations with triplicates. The lethal concentration (LC<sub>50</sub>) was determined through oral exposure, and the oral lethal dose (LD<sub>50</sub>) was estimated based on dietary consumption values. The hazard quotient (HQ) was then calculated using the LD<sub>50</sub> value. The LD<sub>50</sub> value for imidacloprid Nortox 400 SC® at 6h was 0.025 µg a.i. bee<sup>-1</sup>. Regarding the environmental risk to the species, the acute oral HQ exceeded the trigger value by 200 to 480 times across all evaluated crops. Additionally, the risk of product drift exceeded the trigger value within 50 meters of harvest. These results suggest that, during phase 1, it was not possible to dismiss the possibility of foliar spraying affecting *M. scutellaris* bees.

### Introduction

Pollination is an indispensable maintenance service for the terrestrial life cycle, as it preserves the genetic variability of ecosystems and their associated functions. Additionally, it ensures the production of plant-derived products, including fruits, seeds, and others, while fostering interactions with traditional knowledge, particularly indigenous peoples (IPBES, 2016; Costanza et al., 2017). Bees play a significant role in pollination as obligatory floral visitors, collecting pollen and nectar to sustain their biological cycles (Nogueira-Couto, 1994; Michener, 2000).

Among the bees currently under study are the stingless bees, characterized by their docility, ease of manipulation, and minimal resource requirements for successful reproduction. Geographically, they inhabit the pan and neotropical regions of the world (Nogueira-Neto, 1997). Brazil is recognized for having the highest biodiversity of stingless bees (Venturieri, 2008). Different genera of stingless bees contribute to pollinating numerous plant species, including economically important

crops and native species that play a crucial role in the regeneration of tropical forests (Kerr et al., 2001). For instance, in the Brazilian biomes of Caatinga and Pantanal, stingless bees participate in 30% of pollination interactions. Their contribution can reach up to 90% for regionally significant botanical species in the Atlantic and Amazon Forests (Kerr et al., 2001). Moreover, these bees directly benefit humans by pollinating various crops (Macieira & Proni, 2004).

A notable species within the stingless bee group is *Melipona scutellaris* (L), naturally found in the forested areas along the Bahia coast and the northeast region of Brazil (Nogueira-Neto, 1970). This species holds promise for rational reproduction and can contribute to agricultural pollination. It constructs its nests within hollow trunks of mature trees, featuring an ornamental entrance made of clay and vegetable resin known as geopropolis. The distinct shape and striated pattern of this entrance serve as identification factors for the species. (Nogueira-Neto, 1997). It exhibits a more selective floral preference than *Apis mellifera* (L), Brazil's polyhybrid species. It acts as a pollinator for crops such as avocado (*Persea*



*americana*), bell pepper (*Capsicum annuum*), and Surinam cherry (*Eugenia uniflora*). Additionally, it is attracted to the blossoms of clove basil, giant hyssop, Brazilian tulipwood, carnival cassia, rose cedar, cedar, red cedar, grey gum, lemon-scented gum, sensitive plant, pipe tree, yellow ipê, garden ipê, yellow bells, lead tree, uvaia-de-pera, uvaia-do-mato, among others (Aleixo et al., 2014).

Currently, there is a growing concern regarding the global decline of bee populations (Allen-Wardell et al., 1998; Westerkamp & Gottsberger, 2002; Kevan & Viana, 2003; Ellis et al., 2010; Lee et al., 2015; Corby-Harris et al., 2016; IPBES, 2016; Sánchez-Bayo & Wyckhuys, 2019). Various stressors have been identified as the cause of this phenomenon, particularly the use of pesticides in crops. Bees can be exposed to these pesticides when they land on flowers or other parts of plants that have been sprayed or through the consumption of contaminated nectar and pollen (Porrini et al., 2003).

In the field of research, many scientists are studying the effects of neonicotinoid pesticides on bees. This group acts as an agonist for acetylcholine, and when it binds to nicotinic acetylcholine receptors, it is not degraded by acetylcholinesterase, resulting in hyperexcitability and death of the insect (Casida & Durkin, 2013). Exposure to these substances has been observed to cause early cell death in the brain region (Tavares et al., 2015), morphological changes in brain structures with increased cell elimination in the ventricular region (Oliveira et al., 2014), as well as morphophysiological changes at the enzymatic level, affecting cellular detoxification enzymes and activating Heat Shock Proteins (HSPs), known to be involved in response to cellular stress (Silva-Zacarin et al., 2006). Other sublethal effects observed from exposure to neonicotinoids in bees include changes in insect behavior, which can compromise colony functioning (Lambin et al., 2001; El Hassani et al., 2008; Teeters et al., 2012), a reduction in resistance to pathogens (Pettis et al., 2012), and diminished olfactory abilities (Tan et al., 2014).

Given that neonicotinoids are widely used in various crops, it is crucial to investigate their effects on *M. scutellaris* species. Therefore, the aim of this study is to determine the oral sensitivity of this species to the commercial product Imidacloprid Nortox 400 SC® and outline the potential risk scenario. This includes considering that bees can be exposed to the pesticide in the crop and adjacent vegetation, which can be affected by spray plume drift.

## Materials and Methods

### Organisms

Adult forager bees of the *M. scutellaris* species were used in this study. The bees were sourced from 14 sister hives in the Experimental Meliponary of the Center for Water Resources and Environmental Studies (CRHEA, in Portuguese, EESC, USP) in Itirapina (SP), Brazil. The hives

were situated in a natural environment, and only healthy bees without any signs of disease or pests were selected. The bees also exhibited good queen posture, as specified in OECD/OECD Protocol No. 213 (Organization for Economic Cooperation and Development [OECD], 1998).

### Pesticide

The pesticide used in this study was Imidacloprid Nortox 400 SC®, which contains imidacloprid as the active ingredient. The product is registered with the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) under registration number 11012. It is authorized for use in controlling pests in various crops.

### Experimental Design

#### Determination of Acute Oral Toxicity

The acute oral toxicity test followed the general guidelines outlined in OECD/OECD Protocol No. 213 (OECD, 1998). Each biological test involved testing eight concentrations of Nortox 400 SC® imidacloprid. The concentrations tested were determined based on preliminary bioassays, which resulted in mortality rates ranging from 10% to 90% in the sample. These data were used to establish the final doses, following a geometric series with a variable factor between 1.8 and 2.5. The tested concentrations were 0.00075, 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, and 0.05  $\mu\text{g a.i. } \mu\text{L}^{-1}$ . The product was directly diluted in a 50% sucrose solution. Each sample was prepared in triplicate, with ten bees in each, and offered 1 mL of the sucrose solution (50%) (OECD, 1998). The oral test results, given as the 50% lethal concentration ( $\text{LC}_{50}$ ), were converted into a 50% lethal dose ( $\text{LD}_{50}$ ) based on the average daily consumption of sucrose solution under laboratory conditions, estimated from the volumes of daily dietary consumption offered to the bees.

#### Preliminary Estimate of Environmental Risk

For the preliminary Phase 1 environmental risk assessment, the terrestrial application method, specifically the foliar application of imidacloprid, was considered. The methodology adopted for the Environmental Risk Assessment (ARA, in Portuguese) of pesticides to bees, as defined in the Ibama Normative Instruction 02/2017 (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis [IBAMA], 2017) and systematized in the Pesticide Risk Assessment Guidelines for Bees (IBAMA, 2020), was applied. The ARA aimed to determine the Hazard Quotient (HQ) value of the product for bees. The HQ was calculated by establishing the relationship between the Estimated Environmental Concentration (CAE, in Portuguese) of the active ingredient in relevant matrices for bees (nectar and pollen) and the endpoint ( $\text{LD}_{50}$ ) of acute oral toxicity of imidacloprid on *M. scutellaris*. The CAE and HQ were calculated using the BeeREX spreadsheet (Environmental Protection Agency [EPA], 2021a), and the HQ value was compared to the acute risk threshold of 0.4.

Additionally, the AgDrift® tool, provided by the EPA (2021b), was used to predict the risk of imidacloprid drift outside the crop.

To ensure that the exposure hypothesis was not rejected, only those crops permitted for the use of Imidacloprid Nortox in Brazil were selected for the risk study. Among these crops, those known to be attractive to bees, such as tomato, cotton, potato, citrus, and beans, were chosen. The package insert for these plants recommends using boom tractors or knapsack sprayers with cone jet nozzles for application (Agência de Defesa Agropecuária do Paraná [ADAPAR], 2022). The control pressure of the nozzles must be adjusted to produce droplet sizes between 110 and 250 microns in diameter, as specified in the ASAE S572 technical standard (American Society of Agricultural Engineering [ASAE], 2004).

#### Statistical Analysis

The toxicity data were subjected to analysis of variance (ANOVA) at a significance level of 5% or higher. The toxicity endpoint (LC<sub>50</sub>) was determined using a logistic distribution and non-linear regression analysis. The statistical software used for the analysis was SigmaPlot application, version 11.0, provided by Systat Software, Inc., San Jose, California, USA (www.systatsoftware.com).

## Results and Discussion

### Toxicity

The acute oral toxicity bioassay was conducted to assess the toxicity of imidacloprid. The results in Table 1 indicate an LC<sub>50</sub> value of 0.0039 (0.0017 – 0.0061) µg a.i. µL<sup>-1</sup> of dietary exposure and an LD<sub>50</sub> value of 0.025 (0.011 – 0.039) µg a.i. bee<sup>-1</sup>. Due to the widespread use of *A. mellifera* in neonicotinoid research, there is limited information available on the acute sensitivity of native species to imidacloprid. Soares et al. (2015) conducted a study on *Scaptotrigona postica* (L) and found that the species exhibited varying resistance to imidacloprid depending on the route of exposure, with greater sensitivity than *M. scutellaris*.

Costa et al. (2015) reported LD<sub>50</sub> values of 2.01 ng a.i. µL<sup>-1</sup> at 24 hours and 0.81 ng a.i. µL<sup>-1</sup> at 48 hours for *M. scutellaris*, which are 1.24 and 30 times lower than the values obtained in our research. In a study by Jacob et al. (2019), *A. mellifera* was found to have 3.91 times higher oral susceptibility to imidacloprid compared to *S. postica*, suggesting that this insecticide is highly lethal to both species compared to other pesticides. These authors reported similar lethal concentrations for *M. scutellaris* and *A. mellifera*, while *S. postica* exhibited greater resistance to imidacloprid than *M. scutellaris*.

**Table 1.** Relative toxicity of imidacloprid for *M. scutellaris*.

Pesticide	Exposure time (h)	Angular coefficient	LC <sub>50</sub> µg a.i. µL <sup>-1</sup> *	LD <sub>50</sub> µg a.i. bee <sup>-1</sup> **
Imidacloprid Nortox 400 SC®	6	11.3 (10.1 – 12.5)	0.0039 ± 0.0022	0.025 ± 0.014

\*LC<sub>50</sub> determined from ANOVA and non-linear regression analysis, with a 95% confidence interval.

\*\*LD<sub>50</sub> estimated from sucrose consumption with a 95% confidence interval at a significance level greater than 5%.

Schmuck et al. (2001) reported variations in the oral toxicity of imidacloprid to *A. mellifera*, with LD<sub>50</sub> values ranging from 0.0037 to 0.04 µg bee<sup>-1</sup>. Similarly, Schmuck et al. (2003) obtained LD<sub>50</sub> values ranging from 0.041 to 0.081 µg bee<sup>-1</sup>. Despite such variations in sensitivity, it can be concluded that *M. scutellaris* is more susceptible to imidacloprid than *A. mellifera*. Araújo et al. (2018) evaluated the effects of different insecticides on *A. mellifera* through topical exposure and found that imidacloprid was the second most lethal pesticide, with poisoning symptoms observed within the first few hours such as paralysis, disorientation, and eventual death. According to the categorization by Felton et al. (1986), imidacloprid was highly toxic to *M. scutellaris* (LD<sub>50</sub> < 1.0 µg a.i. bee<sup>-1</sup>).

#### Preliminary Risk Analysis

Preliminary risk analysis helps assess the probability of negative effects on bees resulting from pesticide exposure

in crops and surrounding areas affected by product drift. In this analysis, the risk scenario for *M. scutellaris* exposed to imidacloprid in various crops (cotton, potato, citrus, bean, soybean, and tomato) was determined using Hazard Quotient values in Phase 1. Conservative risk estimates were based on data from application rates specified on the label and toxicity endpoints at the individual level rather than the colony level. The hazard quotient values for in-crop and off-crop bees are summarized in Table 2.

#### Field Risk Assessment

Among the crops and their respective maximum application rates in the field, the hazard quotient acute oral values were significantly higher than the trigger value (200 to 480 times higher), indicating that the risk hypothesis in Phase 1 cannot be ruled out for all crops treated with imidacloprid foliar spray and for *M. scutellaris*. The highest HQ values were observed for cotton (194.62) and citrus (164.85) crops.

**Table 2.** Imidacloprid oral acute HQ values in- and off-crop for *M. scutellaris*.

Crop	Application rate (kg a.i./ha)	Field	Off-crop	
		Acute oral HQ	Buffer Zone (m)	Drift HQ
Cotton	0.17	194.62	50	2.96
Potato	0.072	82.43	50	1.28
Citrus	0.144	164.85	50	2.44
Bean	0.1056	120.89	50	1.80
Soybean	0.12	137.38	50	2.06
Tomato	0.096	109.9	50	1.67

Fonte: Author (2023).

### Risk Assessment Off-crop

The droplet size becomes crucial in estimating the risk of product drift in Phase 1. Fine to medium droplet sizes were considered as specified in the product insert (110 to 250  $\mu\text{m}$ ). It should be noted that fine droplets provide good surface coverage and uniformity of the pesticide mixture, resulting in more deposition on the target, which directly affects effective pest control (Nuyttens et al., 2007; Cunha & Pereira, 2009; Nuyttens et al., 2009). However, fine droplets have a higher potential for drift.

In the worst-case scenario, the application bar height was 1.27 meters from the ground. Additionally, the oral toxicity parameter for *M. scutellaris* was considered. The results indicated that, for all crops, the minimum distance (50 meters) required to implement mitigating measures and minimize the threat to bees in the surrounding environment was insufficient to rule out the risk of drift, with HQ values at 50 meters ranging from 3.2 to 7.4 times higher than the trigger value. When the risk of drift is identified within 50 meters, more stringent mitigating actions must be implemented to reduce the HQ value to the trigger level.

Considering that native bees, including *M. scutellaris*, are more sensitive to imidacloprid than *A. mellifera* (de Assis et al., 2022), the use of imidacloprid at the recommended doses for these crops poses a risk to *M. scutellaris*, contributing to population decline and a subsequent reduction in the services they provide. It is important to validate standardized methodologies for other environmental realities and focus on native bees for pesticide risk assessment in the Neotropical region, as they are more commonly found in these areas and are more likely to be exposed compared to Apis bees (Tomé et al., 2017).

### Conclusions

Imidacloprid was found to be ten times more toxic to *M. scutellaris*, possibly due to other ingredients present in the syrup. The preliminary risk analysis revealed significantly

higher risk ratios for imidacloprid, indicating that further investigation is needed to understand the behavior of this pesticide in different exposure scenarios. This research provided important data on the sensitivity and risk to native stingless bees, particularly *M. scutellaris*, a promising species for agricultural pollination in Brazil.

Further researches should address gaps in our knowledge of *M. scutellaris*, such as its biology, adaptability, flowering preferences, and the need to design risk scenarios for crops that can benefit from their pollination. Emphasizing the importance of expanding toxicity studies to include native bees, as it is evident that these species exhibit different sensitivities compared to Africanized exotic species. Ecotoxicological and environmental risk researches focused on native stingless bees provide valuable information on their sensitivities to pesticides, enabling the design of more realistic hazard scenarios in the environment and formulating public policies to preserve their essential pollination services in ecosystems.

### Declaration of conflict of interest

The authors confirm that there is no conflict of interest.

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### Authors' Contributions

JOC: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review & editing, Visualization.

JB: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Review & editing.

EMV: Conceptualization, Supervision.

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