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Amazonian stingless bees: lethal concentration and mortality after exposure to insecticide in *Melipona interrupta* Latreire, 1811 (Hymenoptera: Apidae)

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Abstract. Neonicotinoid insecticides can cause a reduction in feeding rate, locomotion, and behavioral changes such as a reduction in flight speed and distance traveled by adult bees. Chronic exposure to sublethal concentrations can result in behavioral disorders and memory loss. This study investigated the effects of insecticides on Amazonian stingless bees, evaluated whether *Melipona interrupta* Latreire, 1811 (Hymenoptera: Apidae), is sensitive and does not reject food contaminated with Thiamethoxam, and compared the effects on native stingless bees from the Brazilian northern region to *Apis mellifera* L., 1758 (Hymenoptera: Apidae). Mortality was evaluated in these bees when exposed to Thiamethoxam (absolute standard AS and Actara 250 WG - commercial product) and Dimethoate AS as a positive control, thus verifying the lethal concentration 50 (LC50) for the species *M. interrupta*, popularly known as jupará. The mortality of forager workers exposed to active ingredient formulations indicated an LC50 of 24.77 ng/μL for Dimethoate, validating the tests, and 1.28 ng/μL for Thiamethoxam AS and 1.22 ng/μL for Actara 250 WG, values much lower than those found for *A. mellifera* bees, which were 4.28 ng i.a./μL of Thiamethoxam. Therefore, we concluded that formulations with the active ingredients Dimethoate and Thiamethoxam are highly toxic to Amazonian bees of the species *M. interrupta*.

Keywords: Environmental Impact; Insecticide; Mortality; Thiamethoxam; Toxicology.

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The Amazon rainforest plays a vital role in maintaining globally reaching ecosystem services. It contains over half of Earth's biodiversity and a third of the world's tropical forests (Castro & Andrade 2016; Prado 2021). Despite its singular importance, the agricultural frontier has been expanding over legal Amazonia with the cultivation of grains, especially soybeans, corn, and rice, as well as the intensification of livestock farming (IBGE 2022). To protect crops from potential pests, farmers resort to pesticides, whose prominence is directed towards soybean cultivation, responsible for 52% of pesticide sales in Brazil (Bombardi 2017).

In Amazonas, pesticide consumption has been growing at alarming levels since 2005 (Carneiro 2015). According to Inhuma *et al*. (2020), about 57% of pineapple producers in Novo Remanso (Itacoatiara/AM - Brazil) use insecticides to control pests, while 70% of farmers in the municipalities of Rio Preto da Eva/AM and Careiro da Várzea/AM confirm using pesticides in their crops (Fraxe *et al*. 2020). A study conducted with 27 pesticides by the Water Quality Surveillance Information System for Human Consumption (Sisagua) of the Ministry of Health detected the presence of all 27 tested pesticides in the waters that supply Manaus/AM between 2014 - 2017 (Aranha & Rocha 2019). According to Rosa-Fontana *et al*. (2020), water plays an important role when bees are foraging in agricultural areas treated with neonicotinoid insecticides, as it may allow for the dilution of high doses found in food and minimize mortality, considering that it is common to find use of extrapolated doses of insecticides in the field.

Among the various factors related to the causes of this loss, the main ones are deforestation (Brown & Oliveira 2014) and intensive use of insecticides in crops (Sanchez-Bayo & Goka 2014; Sanchez-Bayo *et al*. 2016). Research on the causes of bee disappearance, a phenomenon called Colony Collapse Disorder - CCD, has been carried out since the initial observation of this fact (in the years 2006/2007, according to Hood 2021), and among the groups of insecticides examined, neonicotinoids are prominent. This class of insecticides competes with acetylcholine for receptors that mediate nerve impulses in insects (Rigitano 2001; Gallo *et al*. 2002; Tomizawa & Cassida 2003, 2005), resulting in tremors, discoordination, and eventually, the collapse of the central nervous system and death of the bee (Faria 2009).

Neonicotinoid insecticides can cause a reduction in feeding and locomotion rate in *Bombus terrestris* Linnaeus, 1758 (Hymenoptera: Apidae) bees (Cresswell *et al*. 2012), behavioral changes such as a reduction in flight speed and distance traveled in adult bees of *Tetragonisca angustula* Latreille, 1811 (Hymenoptera: Apidae) (Jacob *et al*. 2019a), and changes in the average speed, distance traveled, duration, and frequency of rest and continuous mobility in *Scaptotrigona postica* (Latreille, 1807) (Hymenoptera: Apidae) (Jacob *et al*. 2019b). Effects from exposure to sublethal concentrations of these agents, such as behavior disorder and memory loss, have been observed (Goulson 2013; Arena & Sgolastra 2014). The routes of exposure to insecticides can vary as bees engage in external activities, visiting plants for

pollen collection, nectar, resins, and aromatic substances, as well as watercourses and soil for water, seeds, and mud collection.

Honey samples (198) from all continents except Antarctica were analyzed by Mitchell *et al*. (2017), who found the presence of five commonly used neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam) in these locations. The authors found that 75% of all honey samples contained quantifiable levels of at least one neonicotinoid, and 45% of samples were contaminated with two to five neonicotinoids, indicating that a cocktail of neonicotinoids is being applied in high concentrations worldwide. This situation reveals a concerning issue that directly affects the health of the environment, as these products target pest insects but also end up eliminating beneficial insects for crops such as bees (Gallo *et al*. 2002; Desneux *et al*. 2007).

Brazil has 1,961 described species of bees (Ascher & Pickering 2020), of which 259 species are stingless bees (Nogueira 2023). Brazilian native social stingless bees belong to the Meliponini tribe, whose singular characteristic is the atrophied sting. Therefore, meliponine are commonly called stingless bees or indigenous bees (Nogueira-Neto 1997; Lopes *et al*. 2005; Rodrigues 2006), with emphasis on the genus *Melipona* Illiger, 1806 (Hymenoptera: Apidae), which groups 74 species, of which 40 occur in Brazilian territory. In the state of Amazonas, there are 20 species of *Melipona* (Pedro 2014). One of the most commonly cultivated in Meliponiculture is *Melipona* (*Melikerria*) *interrupta* Latreille, 1811 (Hymenoptera: Apidae) (Carvalho-Zilse & Nunes-Silva 2012), a species popularly known as jupará or black jandaíra from the Amazon, present in Brazil (Amazonas, Pará, Amapá), Guyana, French Guiana, and Suriname (Camargo & Pedro 2013; Pedro 2014).

Given the lack of information on species from the Amazon biome, which has very different characteristics from the country's major agricultural centers, located in the southeast and south, and also distinct from the northeastern region of Brazil, we aimed to estimate the lethal concentration, by ingestion, of the insecticides Thiamethoxam (Absolute Standard - AS and Actara 250 WG - commercial product) and Dimethoate AS a positive control for adult workers of the Amazonian autochthonous species *M. interrupta*, as well as quantify the consumption of food contaminated with each of the insecticides.

MATERIAL AND METHODS

The experiments conducted here were based on the international protocols for acute toxicity testing of pesticides from the Organization for Economic Cooperation and Development (OECD 1998a, 1998b), which uses the species *Apis mellifera* L., 1758 (Hymenoptera: Apidae) as the model organism. These protocols were adapted for the species *M.* (*M.*) *interrupta*.

To determine the lethal concentration 50 (LC50) in *M. interrupta*, foraging workers were collected from healthy colonies with a known history, housed in standardized INPA model boxes, maintained in the commercial meliponaries Urutau, owned by Mr. Hélio Vilas Boas - Iranduba-AM (-3.21661111, -60.12811111); private "Mundo das Abelhas" meliponary, owned by Josimar Rodrigues Marinho - Iranduba-AM (-3.27772222, -60.18900000); and at the Scientific Meliponary of the Grupo de Pesquisa em Abelhas (GPA) - INPA in Manaus - AM (-3.09727778, -59.98511111).

A total of 930 bees from 46 colonies were collected in three periods (310 individuals in each repetition carried out in June/2020, December/2020, and April/2021) to compose the sample sets used in each experimental repetition. Adult workers were collected at the entrance of the colonies using a plastic pot. The first 10 bees to leave the colony and enter the pot were collected, forming groups of 10 bees per pot (plastic pot with a diameter of 11 x 9 x 7 cm at the top, height, and bottom diameter, with a volume of 500 mL). For each experimental repetition, new bees were collected using the same method, so that there were three experimental repetitions for each insecticide, with each repetition formed by individuals from different hives.

Three cuts of 1 cm² were made in the lid of each pot to create an opening with a flap (a window-like structure that opens and closes to allow the introduction or removal of bees). A circular hole of 0.5 mm was also made for the feeder attachment. The feeder consisted of a 2 mL Eppendorf® microtube with lateral microholes made with a 25 x 0.7 mm hypodermic disposable needle - Black Cannon, to allow feeding of the bees.

All materials used in the experiment were previously washed in running water with neutral liquid soap, rinsed, dried with a paper towel, and sterilized under UV light in a QUIMIS mm-80 Manometer laminar flow chamber for one hours. The pots were prepared in advance, with feeders attached containing 1 mL of sucrose syrup (in a ratio of 100 g of sugar diluted in 60 mL of water), to transport the bees from the collection sites (bee hives) to the Laboratório de Genética de abelhas no Grupo de Pesquisas em Abelhas (LGA/GPA), so that the bees could feed during transport. In the laboratory, the pots were incubated in a BOD MIR - 254 - PA oven at 30 ºC and relative humidity around 85% ± 5% (maintained with 1.5 L of water in a standard metallic form of 42 x 30 x 10 cm inside the oven). The first 24 h in the BOD oven were used for the adult workers' acclimation, using the feeder with 1 mL of syrup (*ad libitum*) for the first 18 h and fasting for the last six hours before starting the tests with contaminated food. For fasting, the feeders were removed, and the bees were left without access to food. This acclimation was performed to avoid stress and stimulate the bees' food consumption.

Preparing stock solutions and serial dilutions to estimate the lethal concentration (LC50) of each insecticide. Three insecticide formulations were used, two pure formulations being Dimethoate absolute standard (AS) as a positive control (standard reference in toxicity tests) and Thiamethoxam AS, and one commercial formulation of Thiamethoxam - Actara 250 WG (Actara 25%, dispersible granules). The products, originally synthesized in the absolute standard formulation of the active ingredient Dimethoate (100 mg, powder) and Thiamethoxam (100 µg/mL diluted in methanol), were obtained from the company Interprise Analytical Instruments LTDA. The Actara commercial formulation was obtained as a courtesy from Embrapa Amazônia Ocidental. To obtain the stock solutions from the original presentations of the company, the following preparations were made.

Preparation of homogeneous stock solutions:

a) Dimethoate AS (powder): 10 mg of pure Dimethoate was diluted in 10 mL of distilled water to obtain a concentration of 1,000 ng/µL;

b) Thiamethoxam AS (100µg/mL, liquid): 100 µL of pure Thiamethoxam was diluted in 10 mL of sucrose syrup to obtain a solution of 1,000 ng/µL; and,

c) Thiamethoxam in Actara 250 WG formulation (250 g/kg, Batch No. 0027-19-4950, powder): 10 mg of Actara 250 WG was diluted in 10 mL of distilled water to obtain a solution of 1,000 ng/µL.

From these stock solutions at a concentration of 1,000 ng/µL, serial dilutions were made for tests to establish LC50 at 10 ratios: tests to establish LC50 at the following ratios: For

Dimethoate: 1/1 (one part of the active ingredient to one part of sucrose), 1/10, 1/50, 1/100, 1/200, 1/400, 1/600, 1/700, 1/800 and 1/1,000, and for Thiamethoxam and Actara 250 WG it was: 1/1, 1/10, 1/50, 1/100, 1/1,000, 1/2,000, 1/3,000, 1/4,000, 1/5,000, 1/7,500, 1/9,000. The tests were divided into three repetitions (three collections and their respective bioassays in each collection). A survival test was performed to verify the concentrations in the range of the survival curve.

Survival curve determination from serial dilutions of mother solutions. Bioassays were performed with the 10 serial dilutions from the mother solutions, in addition to the control group with sucrose. 0.5 mL of contaminated food was offered in each feeder (Eppendorf® microtube with holes), testing one insecticide per pot (with 10 bees each) in addition to the control pot with sucrose. The bees consumed the food *ad libitum* for 24 h. Before and after 24 h of exposure to the insecticides, the feeders were weighed with the food for consumption recording of each insecticide. After exposure, the number of dead individuals was counted in each pot, recording the mortality per dose of each insecticide.

This made it possible to verify the survival curve profile according to the analyzed serial dilution proportions for each insecticide. Applying the Survival Test, with the aid of the R software, the survival curve was obtained for each insecticide, and then, to proceed with the search for the lethal concentration (LC50) of the insecticides, 10 sub-dilutions were made within the mortality limits.

Determination of the lethal concentration (LC50) with sublethal doses of Dimethoate (AS) and Thiamethoxam (AS). Based on the survival curves obtained for each insecticide, sub-dilutions were made from the range of serial dilution proportions of the mother solutions of each insecticide, generating 10 sublethal concentrations to be tested, in addition to the control group with sucrose, to estimate the lethal concentration (LC50) of each insecticide.

The 10 sublethal concentrations established for Dimethoate and Thiamethoxam were:

1) Dimethoate (10 homogenized sub-dilutions): 500 ng/μL; 100; 50; 33.3; 25; 20; 16.6; 14.28; 12.5; 10 ng/μL.

2) Thiamethoxam (10 homogenized sub-dilutions): 10 ng/μL; 5; 4; 3.33; 2; 1.66; 1.25; 1; 0.5; 0.33 ng/μL.

Then, 0.5 mL of contaminated food was offered in each feeder (Eppendorf® microtube with holes), with one insecticide tested per pot (with 10 bees each) in addition to the control pot with sucrose. The bees consumed the food *ad libitum* for 24 h. Before and after 24 h of exposure to the insecticides, the feeders with food were weighed to record the consumption of each insecticide. After exposure, the number of dead individuals was counted in each pot per dose per insecticide. A total of 330 individuals were used, 300 individuals for each active ingredient, per concentration, and 30 individuals for the control group fed only with sucrose.

Determination of lethal concentration (LC50) with sublethal doses of Actara 250 WG (Thiamethoxam in commercial formulation). The commercial product used in this test was Actara - 250 WG (Syngenta Crop Protection Ltd., Paulínia, SP, Brazil, Lot No. 0027-19-4950) whose formulation is composed of 25% of the active ingredient (a.i.) Thiamethoxam and 75% of inert ingredients. Similarly, to the other insecticides, nine sub-dilutions were made from the dilution proportions indicated by the Survival Test, homogenized, at the following concentrations: 10 ng/μL; 6.66; 5; 2.5; 2; 1.42; 1.25; 1; 0.66 ng/μL and a control group containing only sucrose syrup. 0.5 mL of contaminated food was offered in each feeder (Eppendorf® microtube with holes), with one insecticide tested per pot (with 10 bees each) in addition to the control pot with sucrose. The bees consumed the food *ad libitum* for 24 hours. Before and after 24 h of exposure to the insecticides, the feeders with food were weighed to record the consumption of each insecticide. After exposure, the number of dead individuals in each exposure pot for each of the insecticides was counted. For this experiment, 300 adult workers were used, with 30 individuals per concentration.

Statistical analysis. The data regarding bee mortality at different lethal and sublethal doses were subjected to dose-response statistical analysis using the four-parameter log-logistic function of the "drc" package, Analysis of Dose-Response Curves, version 3.0.1 (Ritz & Streibig 2005). Model validation was performed in the R program for all analyses, and the data were run on the statistical software R version 4.1.0 (R Development Core Team 2021). The dataset underwent a model validation using the "Modelfit" in the R program to verify if the data obtained for the analysis were suitable for the used model. For the analysis of food consumption by insecticide concentration, a Shapiro-Wilk test was initially performed to verify data normality. For non-parametric data (which did not follow a normal distribution), a Kruskal-Wallis test and Dunn's Test were applied for non-parametric data.

RESULTS

Mortality of forager workers of *M.* (*M.*) *interrupta*, was recorded for all active ingredient formulations, Dimethoate and Thiamethoxam (absolute standard AS and Actara 250 WG).

Lethal concentration 50 for Dimethoate. The synthetic product Dimethoate AS (positive control) showed high mortality rates (above 60%) for concentrations above 20 ng/μL (10 concentrations were tested ranging from 500 to 10 ng/μL). Only concentrations lower than 16.6 ng/μL had mortality rates below 50% of the group, and the lethal concentration 50 (LC50) of Dimethoate AS estimated for *M. interrupta* was 24.77 ng/μL ($p < 3.883e-11$) (Figure 1A). There was a significant difference in mortality between concentrations (Kruskal-Wallis chi-squared = 26.856, df = 10, p-value = 0.002744). Differences were observed at concentrations lower than 16.66, with concentrations above 20 ng showing a significant difference in mortality. The data recorded regarding the consumption of food contaminated with Dimethoate did not follow a normal distribution, and thus, a Kruskal-Wallis test was applied (chi-squared = 7.7891, df = 10, p-value = 0.6494). Based on analyses, it was found that there was no significant difference between the consumption of food contaminated with Dimethoate (regardless of concentration) compared to the control group (Figure 2).

Lethal concentration 50 (LC50) for Thiamethoxam. The survival test applied to estimate the LC50 with the synthesized product Thiamethoxam absolute standard (AS) showed a high mortality rate for most of the concentrations used in the experiment, with mortality being recorded at all tested concentrations (10 concentrations ranging from 10 to 0.33 ng/μL). Only concentrations below 0.5 ng/μL had mortality below 50% of the group. The estimated LC50 of Thiamethoxam (AS) for *M. interrupta* was 1.28 ng/μL ($p = 3.026e^{-05}$) (Figure 1B). There was a significant difference in mortality only at the concentration of 4 ng compared to the control group ($p<0.005$). The data on food consumption contaminated with Thiamethoxam during the experiment did not follow a normal distribution. Kruskal-Wallis chi-squared = 15.874, df = 10, p-value = 0.1033 was applied, and it was found with Dunn's test (similar to Tukey's test for non-parametric data) that there was no significant difference ($p>0.05$) in the consumption of food contaminated with Thiamethoxam between the concentrations used in the LC50 tests compared

to the control group with sucrose (Figure 2).

Lethal concentration 50 for Actara 250 WG. For the commercial product Actara 250 WG, the result showed mortality at concentrations higher than 1 ng/µL (9 concentrations were tested between 10 to 0.66 ng/µL). In 7 out of 10 tested concentrations, mortality above 50% was observed (concentrations greater than 1.25 ng/µL). The estimated LC50 of Actara 250 WG for *M. interrupta* was 1.22 ng/µL (p=1.496e^{-09}) (Figure 1C). The food consumption during the experiment did not follow a normal distribution. Kruskal-Wallis chi-squared = 18.422, df = 16, p-value = 0.3, and it was found that there was no significant difference in the consumption of food contaminated with Actara 250 WG at the concentrations used in the LC50 tests (Figure 2).

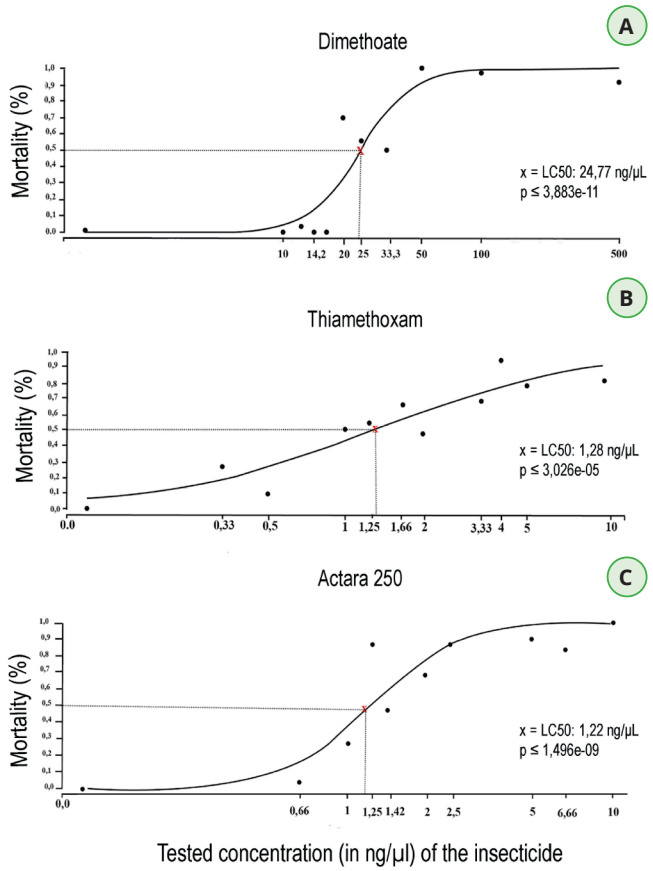

Figure 1. Mortality curve (% index) of *Melipona interrupta* workers after feeding on different concentrations of insecticides. A: Dimethoate, B: Thiamethoxam, C: Actara 250 WG, dots represent the mean mortality by concentration; "x" indicates the lethal concentration 50 (LC50) of the insecticide in nanograms (ng/µL).

DISCUSSION

The data from this study were validated by the results of tests with the positive control Dimethoate AS, which is a reference for toxicity standards and indicated the sensitivity of *M. interrupta* bees to its active ingredient. The CL50 for Dimethoate recorded here was the lowest among *Melipona* bees compared to available data in the literature for *Melipona quadrifasciata* Lepeletier, 1836 (Hymenoptera: Apidae) (Piovesan *et al*. 2020) and *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) (Brigante *et al*. 2021) (Table 1). It was also lower than for non-Melipona bees, such as *Scaptotrigona postica* (Latreille, 1807) (Hymenoptera: Apidae) (Jacob *et al*. 2019b). However, it is worth noting that Dimethoate is a chemical that belongs to the group of organophosphates, a class of insecticides considered one of the most toxic. This insecticide has been widely used in several studies as a standard for validating toxicity tests conducted in the laboratory for bees (Gough *et al*. 1994; Aupinel *et al*. 2007).

This study demonstrated that *M.* (*M.*) *interrupta* bees ingest food contaminated with Dimethoate and Thiamethoxam insecticides, regardless of the concentration. Bee mortality was recorded at any of the concentrations of insecticides used, and the majority of concentrations showed a high mortality rate. These findings suggest the high sensitivity of Amazonian bees, as well as low resistance to lower doses of insecticides.

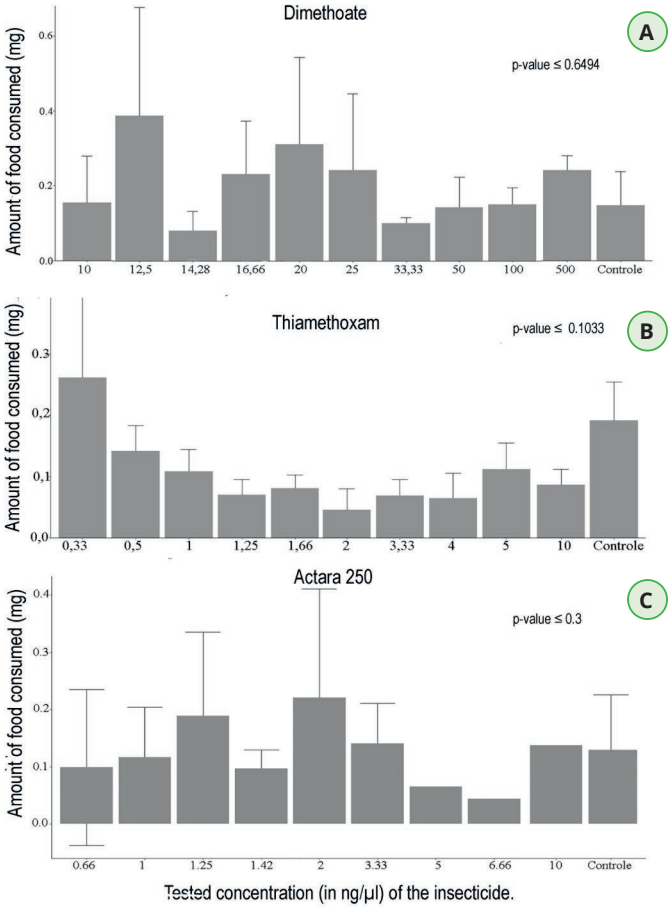

Figure 2. Profile of food consumed (expressed in mg) by *Melipona interrupta* workers after feeding on different concentrations of insecticides. A: Dimethoate, B: Thiamethoxam, C: Actara 250 WG.

Despite the high mortality, no significant difference existed between the mean consumption of contaminated food, regardless of the insecticide and concentration tested. Therefore, *M. interrupta* workers did not reject any of the insecticide-contaminated foods, but rather accepted and consumed them, leaving them exposed and vulnerable to their effects.

Considering the three formulations of insecticides tested on adult *M. interrupta*, Actara 250 WG showed the highest toxicity with an LC50 of 1.22 ng/µL, followed by Thiamethoxam with 1.28 ng/µL and Dimethoate with 24.77 ng/µL. The LC50 of Actara 250 WG estimated for *M. interrupta* is also lower compared to that of *Tetragonisca angustula* (Latreille, 1811) (Hymenoptera: Apidae) at 6.664 ng/µL, *Scaptotrigona xanthotricha* Moure, 1950 (Hymenoptera: Apidae) at 0.1848 ng/µL (Quiroga-Murcia *et al*. 2017), and *Scaptotrigona bipunctata* (Lepeletier, 1836) (Hymenoptera: Apidae) 2 µg/L (Moreira *et al*. 2018). Such comparisons point to the consideration of the geographical location factor of the *M. interrupta* species in the Amazon, a region with low agricultural speculation. Therefore, it is likely that this species has not been exposed to intense contact with insecticides compared to other bee species studied in the southeast and southern regions of Brazil, known for their agricultural development. It is known that contact with high concentrations can lead to the death of foraging bees. In sublethal doses, it can lead to the colony's

Table 1. Data on toxicity tests of the insecticides Dimethoate AS, Thiamethoxam AS and Actara 250 WG on bees and plants.

Abelha	Median Lethal Concentration (LC50)			
	Thiamethoxam	Actara 250 WG	Dimethoate	Reference
Apis mellifera	4,28 ng i.a./µL			Oliveira *et al*. 2014
	5.0 ng / bee			Godfray *et al*. 2014
Melipona interrupta	1,28 ng/µL	1,22 ng/µL	24,77 ng/µL	The present article
Melipona quadrifasciata	0,18 ng/µL			Piovesan *et al*. 2020
Melipona scutellaris	0,053 ng/µL			Miotelo *et al*. 2021
			43 µg i.a./bee	Brigante *et al*. 2021
	2,01 ng/mL^{-1}			Costa *et al*. 2015
Scaptotrigona bipunctata		0,002 ng/µL^{-1}		Moreira *et al*. 2018
Scaptotrigona postica			67,52 ng/µL	Jacob *et al*. 2019b
Scaptotrigona xanthotricha		0,1848 ng/µL^{-1}		Quiroga-Murcia *et al*. 2017
Tetragonisca angustula		0,28 ng i.a./ µL	7,81 ng i.a./ µL	Jacob *et al*. 2019a
		6,664 ng/µL^{-1}		Quiroga-Murcia *et al*. 2017
Tetragonisca fiebrigi	2,05 ng/µL			Piovesan *et al*. 2020
Root collar of coffe		1,0 µg/g (after 30 days of application)		Torres *et al*. 2010
Coffee leaves		0,8 µg/g (after 30 days of application)		Torres *et al*. 2010
Apis mellifera honey (melon pollination)		32,3 µg/kg		Pacífico da Silva *et al*. 2015

extermination in a few days because foragers can bring contaminated nectar and pollen into the colony and thus cause the death of other individuals. According to Torres *et al*. (2010), a dosage of 0.8 µg/g of Thiamethoxam was found in the first sample collected in coffee plant leaves, and 0.15 µg/g was collected in the coffee fruit peel 30 days after application. Souza *et al*. (2006) found 1.0 µg/g after 30 days of application via spray in the plant's collar region, between the roots and the stem. Such records lead to the possible deduction of the accumulation of these agents in nectar and pollen, resources widely collected by stingless bees in coffee (A.b.e.l.h.a. 2015).

In the present study, the mortality rate of *M. interrupta* workers exposed to feed containing the insecticide Actara 250 WG was higher compared to the mortality of bees exposed to feed contaminated with the synthesized product Thiamethoxam PA. One possible explanation for this is that Actara 250 WG is composed of 75% of ingredients considered inert and only 25% of the active ingredient Thiamethoxam, thus the inert ingredients may be influencing the efficiency of Thiamethoxam. However, there are few scientific studies on the real action of commercial formulations, with most of them focusing on the active principle of pesticides, and few studies analyzing the inert ingredients that make up commercial pesticides. According to manufacturers, such inert ingredients are biologically harmless, but they are used for many reasons, including to make a pesticide easier to apply or to improve its effectiveness. Queiroz *et al*. (2008) state that adjuvants or additives are inert substances that are part of some pesticides and are designed to facilitate penetration and overcome target organism barriers, modifying the activity of the applied products and promoting greater efficiency in application. Therefore, caution should be taken in the evaluations of toxicological risks of pesticides that only address active ingredients without their adjuvants, avoiding neglecting important toxicity results for non-target organisms, especially bees (Mullin *et al*. 2016). Here, the observed result of higher mortality of *M. interrupta* bees consuming Actara 250 WG compared to pure Thiamethoxam demonstrates that the commercial product has greater efficiency in its intoxication process, facilitating the absorption of the insecticide and enabling a lower dose of the active principle to achieve mortality close to that obtained with the absolute standard (pure Thiamethoxam).

Data related to toxicity tests using Thiamethoxam on stingless bees are still scarce. The information available for this group of bees is restricted to a few species such as *M. scutellaris* (Miotelo *et al*. 2021; Brigante *et al*. 2021), *S. bipunctata* (Moreira *et al*. 2018), *T. angustula* (Jacob *et al*. 2019a), *M. quadrifasciata* and *Trigona fiebrigi* (Schwarz, 1938) (Piovesan *et al*. 2020). When it comes to Amazonian stingless bee species, the data from this study are unprecedented as there are no bibliographic records of research on the toxicity of chemical agents related to effects of insecticides with Amazonian meliponinios, most likely due to these species occurring in regions that are not extensively agricultural in tropical climate countries, and it is known that the main toxicity studies have been conducted in temperate climate countries (Moraes *et al*. 2000).

Pacífico da Silva *et al*. (2015) have been warning since 2015 that bees foraging in areas treated with the pesticides analyzed here are likely to be collecting floral resources with neonicotinoid residues and, in this case, leading to indirect contamination of the colony. These authors, by chemically analyzing the honey from colonies of *A. mellifera* used for melon pollination, detected maximum concentrations of the insecticide Thiamethoxam, at levels of 19.1 µg.kg^{-1}, in approximately 1/3 of the analyzed colonies. Such a situation can be as harmful as acute exposure that occurs over a shorter period and involves higher toxic concentrations because the effect of sublethal doses of neonicotinoids can negatively affect both social interactions and overall orientation of bees (Bortolotti *et al*. 2003; Henry *et al*. 2012) as well as cause damage to the physiology of queens, leading to a reduction in egg-laying and their subsequent replacement (Sandrock *et al*. 2014; Williams *et al*. 2015).

The high toxicity of neonicotinoids was also reported by Oliveira *et al*. (2014) for *A. mellifera* with an estimated lethal concentration (LC50) of the analytical standard Thiamethoxam (92.5% purity) equal to 4.28 ng of active ingredient/µL (i.a./

µL). Results reported by Miotelo *et al*. (2021) showed that the stingless bee *M. scutellaris* is more sensitive to Thiamethoxam than *A. mellifera*, with an LC50 of 0.0543 ng of active ingredient (ai)/µL compared to 0.227 ng a.i./µL for *A. mellifera*. The sensitivity of different bee species to insecticides depends on several factors, such as body size, age, floral specialization, flight period, sociability, and nest behavior (Brittain & Potts 2011). The high toxicity of this insecticide was also demonstrated for the species *T. angustula* as workers of this species, when exposed to feed containing four different neonicotinoid insecticides, showed greater sensitivity to Thiamethoxam (Actara 250 WG), with an LC50 of 0.28 ng a.i./µL and 7.81 ng a.i./µL for Dimethoate, compared to the other insecticides Acetamiprid, Thiacloprid, and Imidacloprid. The difference in acute toxicity levels of the neonicotinoid group is related to the time of metabolism of the active ingredients, where acetamiprid and thiacloprid are metabolized more quickly than Thiamethoxam and imidacloprid (Jacob 2019a). A similar result was found by Piovesan *et al*. (2020) in the species *M. quadrifasciata* and *T. fiebrigi*, with an LC50 of 0.18 ng/µL and 2.05 ng/µL, respectively, for Thiamethoxam when workers were exposed orally or topically to the insecticides Thiamethoxam, Spinetoram, and Abamectin. These authors found that Thiamethoxam was the insecticide that was most harmful to both species, and the different susceptibility observed may have been due to specific characteristics of the insecticide and bee species. Life history traits, body weight, detoxification capacity, and cuticle structure can alter the toxicity level (Hardstone & Scott 2010; Brittain & Potts 2011).

Ludicke & Nieh (2020) observed that neonicotinoid pesticides, especially Thiamethoxam, used in a wide variety of crops in the United States, affected the visual learning of *A. mellifera* bees and that the behavior of the bees was altered, presenting falls, tremors, and abnormal and rapid movements that were significantly increased according to the doses of Thiamethoxam tested, of 0.8 ng/bee and 1.34 ng/bee. According to Jiang *et al*. (2018), when measuring the levels of Thiamethoxam residues in pollen and nectar from cotton crops, residual amounts were found in 90% of the pollen samples and more than 60% of the nectar samples, with levels ranging from non-detectable to 14,521 $ng.g^{-1}$ in pollen and from non-detectable to 4,285 $ng.g^{-1}$ in nectar.

The data obtained here from the LC50 tests for Dimethoate, Thiamethoxam, and Actara 250 WG allow us to create a database, adding information to those already available for other native species (Table 1). This will allow us to verify the sensitivity curve of stingless bees to insecticides and to develop a comparison tool with other native stingless bee species. In this way, it will be possible to infer which species (*M. interrupta* or others) would be better bioindicators of environmental contamination by insecticides in the different Brazilian biomes. This database would support the availability of this information to support regulatory decisions in risk assessments for the conservation of bee biodiversity and the essential pollination services they provide. Finally, an environmental and food safety measure would be to restrict the use of these high-risk products and to seek new, less harmful, or more selective alternatives, *i.e.*, products that would kill pests while preserving beneficial arthropods, and to invest more in research on new, more selective active ingredients or to find biological methods for pest control.

Formulations containing the active ingredients Dimethoate and Thiamethoxam are highly toxic to the Amazonian stingless bees of the species *M.* (*M.*) *interrupta*, which accept and consume contaminated food regardless of the formulations and concentrations of the insecticides. Worrisomely, it was found that the commercial presentation of Thiamethoxam in the Actara 250 WG formulation is more efficient in poisoning the bees than its pure formulation. Such a result highlights the importance of conducting toxicity tests with commercial products to verify the sensitivity of native bees to such compounds.

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AUTHORS CONTRIBUTION

DDC: initial and final writing and statistical analysis, JCDS - Revision and final writing of the article. GACZ - Revision and final writing of the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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