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Toxicity of insecticides on Neotropical stingless bees *Plebeia emerina* (Friese) and *Tetragonisca fiebrigi* (Schwarz) (Hymenoptera: Apidae: Meliponini)

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Abstract
Use of pesticides in agroecosystems is considered a major cause of bees diversity losses in the Neotropics, where *Plebeia emerina* (Friese) and *Tetragonisca fiebrigi* (Schwarz) (Hymenoptera: Apidae: Meliponini) are wild pollinators of native and crop plants. The aim of this study was to know the acute lethal toxicity of acetamiprid, malathion, phosmet and spinosad insecticides on *P. emerina* and *T. fiebrigi*. We obtained the mean concentration and mean lethal dose (LC_{50} and LD_{50}) and the mean survival of workers after oral and topical exposure to insecticides, respectively. The LC_{50} values (ng a.i./µl of diet) and the decreasing order of toxicity for *P. emerina* was spinosad (4.96) > malathion (18.75) > phosmet (97.33) > acetamiprid (4204.06), and for *T. fiebrigi* also was spinosad (5.65) > malathion (8.39) > phosmet (53.91) > acetamiprid (9841.32), when orally exposed. The LD_{50} values (ng a.i./bee) and the decreasing order of toxicity for *P. emerina* was spinosad (1.90) > malathion (10.90) > phosmet (19.54) > acetamiprid (6216.55) and for *T. fiebrigi* was malathion (29.29) ≥ spinosad (29.79) > phosmet (41.95) > acetamiprid (1421.23), when topically exposed. The mean survival (hours) of contaminated bees by malathion, phosmet, and spinosad, was 11.81, 7.20, and 12.32 for *P. emerina* and 8.55, 7.20, and 13.34 for *T. fiebrigi* when orally exposed; and was 4.87, 9.87 and 11.17 for *P. emerina*, and 4.87, 4.76, and 19.05 for *T. fiebrigi* when topically exposed. Malathion, phosmet, and spinosad were highly toxic, while acetamiprid was moderately toxic. Our results indicated that the insecticides tested, mainly malathion, phosmet, and spinosad may be harmful to *P. emerina* and *T. fiebrigi*, making it essential to propose measures to minimize their impact on wild pollinators.

Keywords Pesticides · Lethal effect · Acute toxicity · Native bees · Pollination

Introduction

Pollination is one of the first and most important steps in fruit production and for approximately 90% of the angiosperms (Ollerton et al. 2011) and to increases the fruit quality and, consequently, the economic value of agricultural production (Klatt et al. 2013; Garratt et al. 2014). This ecological service is performed mainly by bees (managed and wild species), which are recognized as the main and most efficient pollinators (Michener 2007; Potts et al. 2010).

Apis mellifera Linnaeus (Hymenoptera: Apidae), have been the most commonly used species in fruit pollination (Giannini et al. 2015a). However, with the decline of populations of this species, great concern has arisen about the stability of pollination services and its consequences on food production around the world (Potts et al. 2010; Pires et al. 2016).

Wild bees are also important to pollination services, due to the high species richness, variety of sizes and ecological habits, allowing pollination of a large number of plants (Imperatriz-Fonseca et al. 2006; Giannini et al. 2015b). Stingless bees, belonging to the Meliponini tribe, constitute

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the most diverse group of social bees and are pollinators of native plants grown in tropical and subtropical regions and are considered promising for use in fruit crops (Slaa et al. 2006; Michener 2007; Witter et al. 2014).

The genus *Plebeia* Schwarz, popularly known as "abelha mirim", is the second genus of Meliponini with the highest number of described species, constituting a wide group distributed in the Neotropical region. (Michener 2013). Due to the absence of functional sting, low aggressiveness and lower population size of the colonies, "abelhas mirins" are suitable for pollination of agricultural crops in populated areas or protected environments (Slaa et al. 2006; Venturieri et al. 2011). In this context, stands out *Plebeia emerina* (Friese) (Hymenoptera: Apidae: Meliponini) as a complementary agent in the fruit pollination, such as apple and strawberry (Orth 1984; Ortolan and Laroca 1996; Piovesan 2018).

Stingless bees could be rationally created and many species are promising in the honey production and marketing, as well as improving agricultural production due to the crop pollination services that can provide (Cortopassi-Laurino et al. 2006; Slaa et al. 2006). Honey of the Meliponini has greater ecological and commercial value when compared to the honey of *A. mellifera*, becoming an alternative to add economic value to Brazilian agro-ecosystems sustainably (Magalhães and Venturieri 2010).

"Jataí", *Tetragonisca* Moure, is very popular in meliponiculture and produces one of the most wanted kinds of honey of stingless bees (Oliveira et al. 2013). Bees of the Meliponini genus have commonly found in three Brazilian biomes (Pampa, Atlantic forest and Pantanal) (Camargo and Pedro 2013). The species *Tetragonisca fiebrigi* is found in Rio Grande Sul, Santa Catarina, and Paraná, and it is a floral visitor of native and crop plants, such as strawberry and canola, providing the establishment of consortium between the production of honey and fruits (Camargo and Pedro 2013; Vossler et al. 2014; Witter et al. 2014).

Several species of bees have been suffering from large population declines, which raises discussions about possible consequences in global agricultural practices and food production (Goulson et al. 2015). The unsustainable use of agro-ecosystems and excess of pesticides are considered the main causes of bees diversity losses (Wiest et al. 2011; Nicholls and Altieri 2013; Sanchez-Bayo and Goka 2014). As an example, the insecticides of organophosphates and neonicotinoids could be highlighted, which have a broad spectrum of action and high toxicity may be directly related to the pollinator populations decline (Becher et al. 2013; Godfray et al. 2014; Goulson et al. 2015). Among the new insecticides, an alternative to synthetic ones, the spinosad stands out, a product derived from aerobic fermentation of actinomycete *Saccharopolyspora spinosa* Mertz e Yao (Thompson et al. 2000; Urbaneja et al. 2009). This insecticide has low toxicity to mammals and fish, and higher selectivity to non-target insects (Thompson et al. 2000; Bailey et al. 2005). However, there is little information about the effects of these insecticides on wild pollinators such as stingless bees.

Bees are part of a very diverse group, divided into different taxonomic groups and have varying susceptibility to pesticides (Desneux et al. 2007). Currently, there are few available information on international protocols regarding toxicological experiments conducted with native species, therefore, it is necessary to study the toxicity of these insecticides formulations on Brazilian stingless bees. This study aimed to know the acute lethal toxicity of formulations of malathion and phosmet (Organophosphates), acetamiprid (Neonicotinoids) and spinosad (Spinosyns) on workers of stingless bees *P. emerina* and *T. fiebrigi*.

Material and methods

Insects

We used adult workers of *P. emerina* e *T. fiebrigi*, created in the meliponary of "Eliseu Maciel" Faculty of Agronomy, Federal University of Pelotas (UFPel) (register SisGen A7B64FD). For collection, we verified the health conditions and the physiological state of the bees, according to the guidelines for chemical tests on bees (OECD 1998a, b). We collect bees from three distinct non-parent hives, in sunny days with temperatures above 18 °C. *T. fiebrigi* workers were collected individually with the aid of collecting forceps, while *P. emerina* workers were collected in groups through an aspirator. We collected workers were located at the top of the hive.

The collected bees were placed in cages composed of transparent plastic pots of 250 mL capacity (internal diameter: 90 × 60 mm; external diameter: 105 × 65 mm), drilled at the top to ensure gas exchange. We lined the cage with filter paper on the floor and at the top, we coupled an Eppendorf® tube (1.5 mL) for food supply. After collection, we took the workers to the laboratory and kept them in air-conditioned rooms (temperature: 28 ± 1 °C; RH: 70 ± 2%; scotophase: 24 h). In order to minimize the stress caused by confinement, the workers remained in adaptation for 24 h and received sucrose solution (sucrose/water 1:1) *ad libitum* for food, before the beginning of tests.

Insecticides

Four commercial formulations of insecticides registered and used for pest management in Brazilian fruit crops were selected for conducting bioassays (Brasil 2018; PIC 2018; PIM 2018) (Online Resource 1).

Acute lethal toxicity bioassays

The toxicity of insecticides on stingless bees was based determination of the median lethal concentration (LC_{50}) and the median lethal dose (LD_{50}) representing, respectively, a concentration or dose capable of causing mortality of 50% of the experimental population. The susceptibility of workers to insecticides was evaluated using two exposure methods: oral and topical, for this, we used the adapted methodology from Felton et al. (1986), OECD (1998a, b) and Medrzycki et al. (2013).

The concentrations evaluated for each insecticide were determined based on the active ingredient concentration indicated on the formulations label. The tests were performed in two stages: (i) preliminary tests: the purpose of this test was to recognize the range of doses with response variation. We established a stock concentration (SC) of 1000 ng a.i./μL for malathion, phosmet and spinosad and 100,000.00 ng a.i./μL for acetamiprid. Through serial dilutions (1:10) of SC in distilled water, six concentrations were obtained in descending order for the recognition of the doses ranges that provided 0 to 100% mortality; (ii) definitive tests: after establishing the response range of preliminary tests, were established seven until nine doses in increasing concentrations of their active ingredients to be used in the bioassays.

For each treatment (insecticide concentration or dose) three replicates were used, each replicate was composed of ten adult workers, and the experiment was repeated twice, totaling 60 bees per treatment. The cages were kept in air-conditioned (temperature: 28 ± 1 °C; RH: 70 ± 2%; scotophase: 24 h). The mortality was observed 48 h after contamination, to determine the LC_{50} and LD_{50}.

Oral toxicity

The different concentrations of insecticides were offered to workers through diet (sucrose solution) into Eppendorf® tubes (1.50 mL). These minimum and maximum concentrations (in ng a.i./μL diet) were used in the tests with *P. emerina*: acetamiprid (Mospilan®) 625.00 to 50,000.00; malathion (Malathion® 1000 EC) 1.00 to 100.00; phosmet (Imidan® 500 WP) 60.00 to 200.00 and spinosad (Tracer®) 0.10 to 100.00. These minimum and maximum concentrations (in ng a.i./μL diet) were used in the tests with *T. fiebrigi*: acetamiprid (Mospilan®) 500.00 to 50,000.00; malathion (Malathion® 1000 EC) 1.00 a 200.00; phosmet (Imidan® 500 WP) 10.00 to 500.00 and spinosad (Tracer®) 2.00 to 100.00.

To induce food consumption, the workers were prevented from food for 2 h before the start of experiments. After the fasting period, each group of bees received 1.00 mL of contaminated feed, while the control received 1.00 mL of insecticide-free feed. Six hours after the contaminated food supply, all feeders were replaced by a new one containing only sucrose solution. The amount of food consumed by workers was obtained by weighing the feeder before and after exposure.

Topical toxicity

To obtain topical toxicity, the stock concentration of each insecticide was diluted, in decreasing concentrations, into a solution of acetone (water/acetone, 1:1). These minimum and maximum doses (in ng a.i./bee) were used in the tests with *P. emerina*: acetamiprid (Mospilan®) 625.00 to 50,000.00; malathion (Malathion® 1000 EC) 0.25 to 250.00; phosmet (Imidan® 500 WP) 2.50 to 100.00 and spinosad (Tracer®) 0.25 to 25.00. These minimum and maximum doses (in ng a.i./bee) were used in the tests with *T. fiebrigi*: acetamiprid (Mospilan®) 156.20 to 25,000.00; malathion (Malathion® 1000 EC) 5.00 to 500.00; phosmet (Imidan® 500 WP) 10.00 to 100.00 and spinosad (Tracer®) 2.50 to 250.00. For the control group, only the solvent (acetone solution) was used.

Before topical application, workers were anesthetized with CO_2 for ten seconds. The exposure of workers to insecticides occurred individually, by applying 0.50 μL of each dose in the insect's pronotum with the aid of the Burkard® hand micro applicator (Burkard Scientific, Rickmansworth, England).

Survival time

The insecticides were used at 1000 ng/μL of diet, for the oral bioassays, and 1000 ng/bee for the topical bioassays. Exposure of insecticides to bees and experimental design were performed as described above for oral and topical toxicity bioassays. Mortality assessments were performed 0.50; 1.00; 2.00; 3.00; 6.00; 12.00; 24.00 and 48.00 h after exposure to insecticides.

Statistical analysis

The normality and homoscedasticity of mortality data and food consumption data were verified through the Shapiro-Wilk and Bartlett test, respectively. In order to compare if there was a difference in the consumption between contaminated food and uncontaminated food, during oral exposure, ANOVA and Tukey *post hoc* was made ($P < 0.05$), using R® (R Development Core Team 2015).

The mortality of the control group did not exceed 10% and the 95% confidence interval was respected. The LC_{50} and LD_{50} values, 95% confidence interval and chi-square values were determined using the log-logistic function of "drc" – Analysis of Dose-Response Curves, compiled by

R® (R Development Core Team 2015) (Ritz and Streibig 2005).

After obtaining the LC_{50} and LD_{50}, we evaluated the toxicity of the insecticides in two aspects: i) comparing the LC_{50} or LD_{50} values of each insecticide among the stingless bees species e; ii) comparing the LC_{50} or LD_{50} values among the insecticides for each stingless bees species. For this, LC_{50} and LD_{50} values and confidence intervals were used, the values were considered significantly different when there was no overlap of these ranges, at 95% probability.

The Kaplan–Meier estimates (Log-Rank method) were used to evaluate the stingless bees survival (hours) and the survival curves were compared by Holm-Sidak test ($P < 0.05$) using SigmaPlot 12.3 software (Systat Software, San Jose, CA, USA).

Results

Our acute lethal toxicity tests performed with the commercial formulations of acetamiprid, malathion, phosmet, and spinosad showed different levels of toxicity for *P. emerina* and *T. fiebrigi*, depending on the mode of exposure (Tables 1 and 2).

The LC_{50} values for acetamiprid, malathion, and phosmet after oral exposure were significantly different among *P. emerina* and *T. fiebrigi*, however, the LC_{50} values for spinosad did not differ among stingless bees species (Table 1). The LC_{50} values ranged from 4.96 to 4204.06 ng a.i./µl diet for *P. emerina* and from 5.65 to 9841.32 ng a.i./µl diet for *T. fiebrigi*, and the decreasing order of toxicity for both species was: spinosad > malathion > phosmet > acetamiprid. Significant differences in LC_{50} values were evidenced by means non-overlapping of 95% confidence intervals (Table 1).

Both stingless bees showed no difference in the consumption of food with insecticide compared to its control groups, demonstrated by Tukey's test: *P. emerina* (acetamiprid: $F = 0.04$, $P = 0.99$, $df = 7$; malathion: $F = 0.18$, $P = 0.99$, $df = 8$; phosmet: $F = 1.52$, $P = 0.18$, $df = 8$; spinosad: $F = 0.44$, $P = 0.89$, $df = 8$); *T. fiebrigi* (acetamiprid: $F = 0.13$, $P = 0.99$, $df = 7$; malathion: $F = 0.32$, $P = 0.94$, $df = 7$; phosmet: $F = 0.97$, $P = 0.46$, $df = 6$; spinosad: $F = 0.09$, $P = 0.99$, $df = 7$).

The LD_{50} values for acetamiprid, malathion, phosmet, and spinosad after topical exposure were significantly different among *P. emerina* and *T. fiebrigi* (Table 2). The LD_{50} values ranged from 1.90 to 6216.55 ng a.i./bee for *P. emerina* and from 29.29 to 1421.23 ng a.i./bee for *T. fiebrigi*. The decreasing order of toxicity to *P. emerina* was spinosad > malathion > phosmet > acetamiprid; however, for *T. fiebrigi* the decreasing order of toxicity was malathion ≥ spinosad > phosmet > acetamiprid (LD_{50} values with overlapping 95% confidence intervals were classified as having the same toxicity level) (Table 2).

The survival of *P. emerina* and *T. fiebrigi* workers was significantly reduced after oral exposure (Log-Rank = 320.84; $df = 4$; $P < 0.001$); (Log-Rank = 217.67; $df = 4$; $P < 0.001$) and topical exposure (Log-Rank = 274.88; $df = 4$; $P < 0.001$); (Log-Rank = 246.10; $df = 4$; $P = <0.001$), respectively.

P. emerina workers showed rapid mortality after oral exposure to phosmet (mean lethal time (LT_{50} [±SE] = 7.20 ± 1.64 h) and did not differ from malathion (LT_{50} [±SE] = 11.81 ± 2.08 h). Acetamiprid did not reduce bee survival and was not significantly different from control (Fig. 1a).

Phosmet and malathion also provided rapid mortality to *T. fiebrigi* workers (LT_{50} [±SE] = 7.20 ± 1.58 h) and (LT_{50} [±SE] = 8.55 ± 1.65 h), did not differ significantly from each other. However, both insecticides caused a greater reduction in bee survival when compared to spinosad (LT_{50} [±SE] = 13.34 ± 1.89 h) and acetamiprid (LT_{50} [±SE] = 28.88 ± 2.66 h). All insecticide treatments differed from the control group, which showed no reduction in survival during the test period (Fig. 1b).

After the topical exposure of malathion, *P. emerina* workers had a mean survival (±SE) of 4.87 (±0.71) hours, differing from the other treatments. Spinosad and phosmet also reduced bee survival (LT_{50} [±SE] = 11.17 ± 1.18 h; LT_{50} [±SE] = 9.86 ± 1.18 h, respectively) differing significantly from acetamiprid (LT_{50} [±SE] = 42.21 ± 1.91 h). All insecticide treatments differed from the control group, which showed no reduction in survival during the test period (Fig. 2a).

Malathion and phosmet, topically exposed, provided rapid mortality to *T. fiebrigi* workers (LT_{50} [±SE] = 4.87 ± 0.71 h) and (LT_{50} [±SE] = 4.76 ± 0.89 h), respectively and did not differ significantly. *T. fiebrigi* workers had mean survival (±SE) of 19.05 (±1.76) hours after exposure to spinosad and 42.21 (±1.91) hours, after acetamiprid exposure. Acetamiprid showed a lower reduction in survival when compared to the other treatments. All treatments with insecticides differed from the control group, which also did not show a reduction in survival during the test period (Fig. 2b).

Discussion

According to our results, it was observed that the recommended concentrations for formulations of malathion, phosmet, and spinosad, for use in Brazilian citrus, apple and peach orchards, are considerably higher than the LC_{50} values determined in this study. The recommended

Table 1 Acute lethal toxicity of formulations of acetamiprid, malathion, phosmet and spinosad (LC_{50} ng a.i./μL) orally exposed to stingless bees *Plebeia emerina* and *Tetragonisca fiebrigi*

Insecticide	Species[a]	*n*	Slope ± SE	LC_{50}[b] (95% CI)*	χ^2	*P* value
Acetamiprid	*P.e.*	480	2.13 ± 0.20	4204.06 (3752.51–4655.60)	18.7	<0.0001
	T.f.	480	0.97 ± 0.12	9841.32 (5959.94–13722.71)	5.1	<0.0001
Malathion	*P.e.*	540	1.93 ± 0.24	18.75 (16.36–21.13)	15.7	<0.0001
	T.f.	480	4.96 ± 0.84	8.39 (7.63–9.16)	22.1	<0.0001
Phosmet	*P.e.*	540	2.78 ± 0.86	97.33 (95.12–99.53)	8.6	<0.0001
	T.f.	420	2.76 ± 0.74	53.91 (42.56–65.25)	9.6	<0.0001
Spinosad	*P.e.*	540	2.16 ± 0.35	4.96 (4.16–5.75)	12.5	<0.0001
	T.f.	480	2.31 ± 0.51	5.65 (4.56–6.73)	10.5	<0.0001

[a]*P.e. Plebeia emerina*; *T.f. Tetragonisca fiebrigi*

[b]Lethal concentration 50: insecticide concentration that causes 50% mortality of the population (ng a.i./μL diet)

The asterisk indicates that values whose confidence intervals (95% CI) do not overlap are considered significantly different

Table 2 Acute lethal toxicity of formulations of acetamiprid, malathion, phosmet and spinosad (LD_{50} ng a.i./bee) topically exposed to stingless bees *Plebeia emerina* and *Tetragonisca fiebrigi*

Insecticide	Species[a]	*n*	Slope ± SE	LD_{50}[b] (95% CI)*	χ^2	*P* value
Acetamiprid	*P.e.*	540	1.09 ± 0.11	6216.55 (4664.52–7768.57)	8.0	<0.0001
	T.f.	540	1.02 ± 0.12	1421.23 (966.95–1875.51)	6.2	<0.0001
Malathion	*P.e.*	540	1.18 ± 0.19	10.90 (7.96–13.83)	7.3	<0.0001
	T.f.	540	1.72 ± 0.20	29.29 (24.84–33.73)	13.2	<0.0001
Phosmet	*P.e.*	480	1.59 ± 0.22	19.54 (14.81–24.24)	8.3	<0.0001
	T.f.	480	2.70 ± 0.29	41.95 (37.96–45.95)	21.1	<0.0001
Spinosad	*P.e.*	540	1.67 ± 0.24	1.90 (1.58–2.22)	11.8	<0.0001
	T.f.	480	1.38 ± 0.13	29.79 (24.96–34.63)	12.4	<0.0001

[a]*P.e. Plebeia emerina*; *T.f. Tetragonisca fiebrigi*

[b]Lethal dose 50: insecticide doses that causes 50% mortality of the population (ng a.i./bee)

The asterisk indicates that values whose confidence intervals (95% CI) do not overlap are considered significantly different

concentrations for malathion (Malathion® 1000 EC), phosmet (Imidan®) and spinosad (Tracer®) are 53.33–106.66; 2.56–10.27; and 12.09–14.51 times the LC_{50} value for *P. emerina*; 119.18–238.37; 4.0–18.53; and 10.61–12.74 times the LC_{50} value for *T. fiebrigi*, respectively. However, the recommended concentration for acetamiprid (Mospilan®) is 52.55 times lower than the LC_{50} for *P. emerina* and 123.01 times lower than the LC_{50} for *T. fiebrigi*.

According to LD_{50} values obtained through the topical exposure tests, malathion, phosmet, and spinosad were classified highly toxic to both stingless bees (LD_{50} < 1000 ng a.i./bee). However, acetamiprid was classified moderately toxic (LD_{50} 1000–10000 ng a.i./bee) (adapted from Felton et al. 1986).

The insecticides application is a widely used tool in pest control, being crucial for food production (Aktar et al. 2009; Schreinemachers and Tipraqsa 2012; Guedes et al. 2016). The insecticides formulations tested in this study: acetamiprid, malathion, phosmet, and spinosad are molecules used in formulations for control of fruit crops pests, such as Tephritidae, Tortricidae and Hemiptera. These insecticides are commonly used via the aerial application, spraying in total area, or smaller volumes with directed applications, such as toxic baits (Brasil 2018; PIC 2018; PIM 2018).

The insecticides of Organophosphorus chemical group, malathion, and phosmet, are known examples of compounds that are among the most common insecticides used in agriculture and are lethal to honeybees and native bees (McBride 2011; Stanley et al. 2015; Dorneles et al. 2017). Studies conducted by Stevenson (1978) and Rinkevich et al. (2015) have demonstrated that malathion is highly toxic to *A. mellifera* with LD_{50} values of 1,10 ng a.i./mg of bee, and 0,27 μg a.i./bee, respectively. Likewise, the topical LD_{50} calculated for phosmet to *A. mellifera* is 1.13 μg a.i./bee (Sylvia 2010).

The high acute mortality reached by insecticides formulations in stingless bees is a consequence of insecticide interaction with its primary site of action in these species after exposure to lethal doses. Organophosphates, for example, are neurotoxic compounds that inhibit

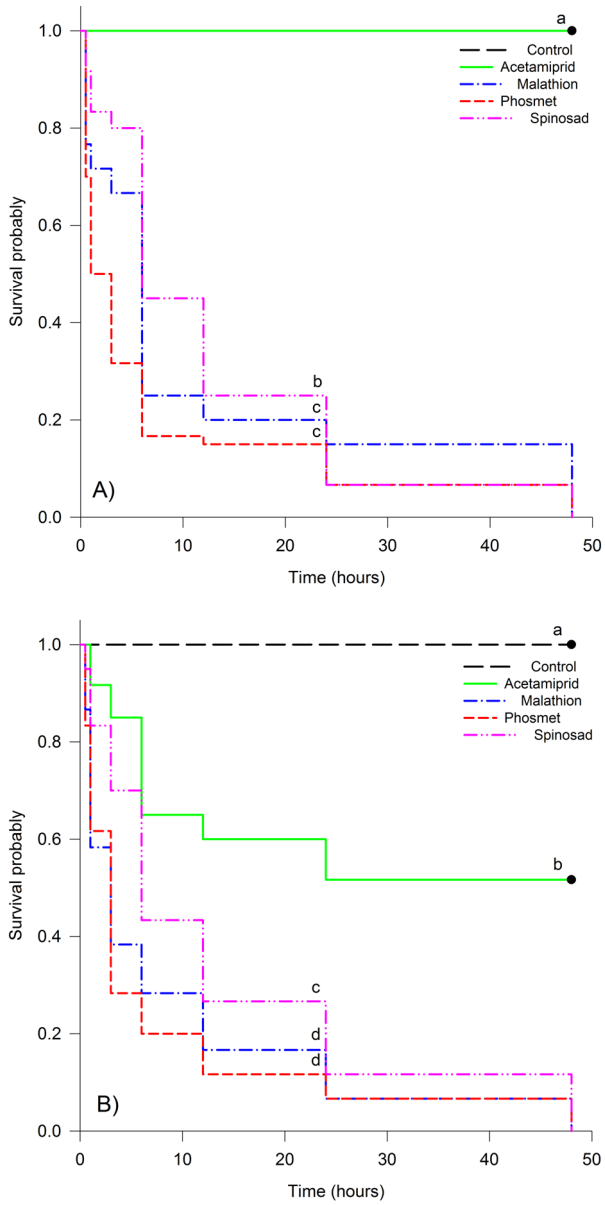

Fig. 1 Survival curves for *Plebeia emerina* (**a**) and *Tetragonisca fiebrigi* (**b**) orally exposed to 1000 ng/μL diet of formulations of acetamiprid, malathion, phosmet, and spinosad. The mean survival time followed by the same lowercase letter did not differ significantly using the Holm-Sidak test ($P < 0.05$)

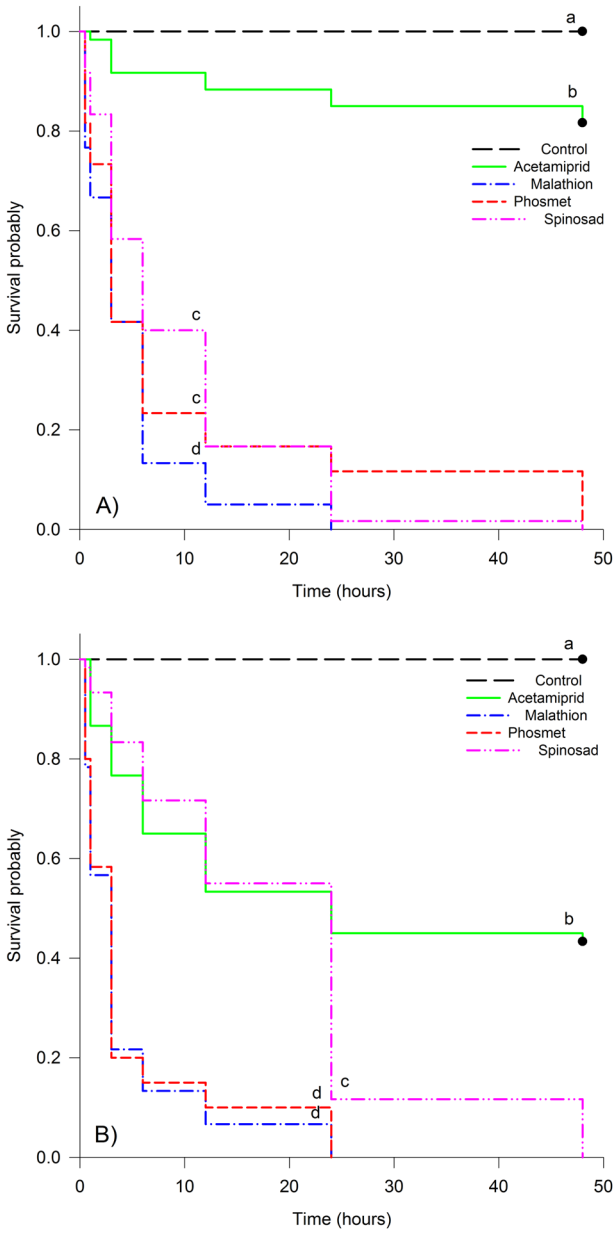

Fig. 2 Survival curves for *Plebeia emerina* (**a**) and *Tetragonisca fiebrigi* (**b**) topically exposed to 1000 ng/bee of formulations acetamiprid, malathion, phosmet, and spinosad. The mean survival time followed by the same lowercase letter did not differ significantly using the Holm-Sidak test ($P < 0.05$)

acetylcholinesterase (AChE), which is responsible for the hydrolysis of acetylcholine (ACh) in the synaptic regions of cholinergic nerve endings. Inhibition of AChE results in the accumulation of ACh and excessive stimulation of cholinergic receptors (Fukuto 1990; Casida and Durkin 2013).

Our results showed that spinosad was more toxic than malathion, phosmet, and acetamiprid for *P. emerina* and *T. fiebrigi*, orally-exposed. When spinosad was topically-exposed, it remained the most toxic for *P. emerina* and equaled to malathion for *T. fiebrigi*. However, in another study, spinosad was considered safe for several non-target arthropods (Sarfraz et al. 2005). Spinosad is a bioinsecticide made from a mixture of the macrolide molecules, spinosyn A and spinosyn D, produced by the actinomycete bacterium *S. spinosa* Mertz e Yao (Sparks et al. 2001).

In acute tests by topical and oral exposure for *A. mellifera*, spinosad was classified as highly toxic to workers, with LC_{50} values 0.058 μg a.i./bee and 0.053 μg a.i./bee, respectively (Miles 2003). Spinosad was also considered highly toxic to *Melipona quadrifasciata* Lepeletier workers (LD_{50} 12.07 ng a.i./bee), presenting a higher lethal effect

than imidacloprid (Tomé et al. 2015). A similar result was observed for spinetoram, also from Spinosyns chemical group, this insecticide was highly toxic to *T. fiebrigi* and *M. quadrifasciata* (Piovesan 2018).

These results demonstrate that the low adverse effects of the Spinosyns chemical group on non-target insects could be overestimated. The high toxicity of this chemical group may be related to the neurotoxic action of these insecticides (Jeschke et al. 2011). Spinosad act through allosteric modulators of nicotinic acetylcholine receptors (nAChRs) at their cholinergic synapses (Kirst 2010), and this could be the reason for high stingless bees mortality.

Acetamiprid was the insecticide that showed the lowest toxicity for *P. emerina* and *T. fiebrigi* adult workers, in the oral and topical bioassays. Acetamiprid also showed low toxicity for *Apis cerana japônica* (Radoszkowski) with LD_{50} values of 0.278 µg a.i./bee (Yasuda et al. 2017) and for *A. mellifera* (LD_{50} 8.09 µg a.i./bee) (Decourtye and Devillers 2010). Neonicotinoids acting as nAChR agonists while mimicking the activity of ACh excitatory neurotransmitter (Salgado and Saar 2004; Casida and Durkin 2013).

Some insecticides of Neonicotinoid chemical group, such as acetamiprid and thiacloprid, have relatively low toxicity to bees because they have cyan substitutions in the molecule chemical composition (Stanley et al. 2015). Iwasa et al. (2004) reported that Neonicotinoids containing nitro groups exhibited higher toxicity in *A. mellifera* than those containing cyan substitutions in the molecule chemical composition. The lower toxicity of Neonicotinoids with the cyan group could be attributed to their rapid biotransformation, because it may be metabolized generating harmless degradation compounds, while the other Neonicotinoids produce toxic metabolites to bees (Iwasa et al. 2004; Brunet et al. 2005).

The insecticides formulations tested in this study did not present the same mortality rate for *P. emerina* e *T. fiebrigi* at the same concentration (1000 ng/µL). Organophosphates showed a fast reduction in stingless bees survival, usually during the first day assessments. Spinosad, although it has high toxicity, took longer to kill 50% of the tested population. Acetamiprid, in turn, did not significantly reduce bees' survival and, in general, took more than 31 h to kill half the population. This difference may be due to the low toxicity of acetamiprid for the stingless bees tested in this study.

There were differences in susceptibility among bee species tested in this study according to exposure methodology and insecticide. *T. fiebrigi* was more susceptible than *P. emerina*, to malathion and phosmet, but it was less susceptible to acetamiprid when oral-exposed. On the other hand, *T. fiebrigi* was less susceptible than *P. emerina* to malathion, phosmet, and spinosad, when topically-exposed. This difference in response among different bee species to insecticide exposure was also observed in other studies, and the results indicated that *A. mellifera* was more tolerant to insecticides than stingless bees species (Desneux et al. 2007; Nocelli et al. 2011; Arena and Sgolastra 2014).

The differential susceptibility observed in toxicity studies may have resulted from the specific characteristics of insecticides and bee species. The thickness and chemical composition of the cuticle, which is genetically determined and varies among species, could facilitate the insecticide penetration, causing higher toxicity according to bee species (Abdalla et al. 2003; Bacci et al. 2006; Blomquist and Bagnères 2010; Leonhardt et al. 2015). Other factors, intrinsic to each species, which can change the toxicity level are age, body weight and detoxification capacity (Oliveira et al. 2002; Hardstone and Scott 2010; Brittain and Potts 2011).

The lipophilic character of insecticide, when associated with lipid composition of the bees cuticle, can also be a determining factor for the higher spinosad toxicity, topically-exposed, compared with malathion for *P. emerina*. Lipophilic compounds have a higher affinity for being readily absorbed cuticle, reaching their target of action quickly (Milhome et al. 2009; Leite et al. 2012). This hypothesis is based on the low water solubility of spinosad (89.5 mg/L at 20 °C) compared with malathion (145 mg/L at 25 °C).

The oral susceptibility difference among the evaluated species could be related to its detoxification capacity. When the input pathway is through ingestion of contaminated pollen and nectar, the toxicity of the products may be reduced, due to the action of several detoxifying enzymes present in the digestive system of bees (Berenbaum and Johnson 2015).

In this study, we used commercial formulations of insecticides and two exposure routes: oral and topical, to evaluate acute toxicity on stingless bees. The use of this methodology is justified because the bees can get in contact with these products at the time of foraging in treated areas (Fletcher and Barnett 2003). The absorption of insecticides can occur through the ingestion of pollen and nectar with insecticides residues or topically exposed when chemicals suspended in the air come into contact with the body of bee (Johnson et al. 2010; Mullin et al. 2010; Dively and Kamel 2012).

In addition, unlike *A. mellifera*, native bees cannot be temporarily displaced during insecticides spraying, because the nests of these species are found in the trunks of the native trees present in the inside or edges of the orchards. Floral specialization, shorter nesting period and limited foraging range are other factors that may make stingless bees more susceptible to insecticides compared to honeybees (Thompson and Hunt 1999; Brittain and Potts 2011).

The choice of using commercial formulation instead of using the technical grade active ingredient (99.9% purity) may substantially alter the toxicity rating of many insecticides, due to the toxicity of the chemical itself, the

concentration of formulated insecticides and the potential interaction among the active ingredient and materials formulation. However, formulated insecticides are the only option for farmers to protect their crops when chemical control becomes necessary. Therefore, to know the toxicity of formulated insecticides is important because it includes the total toxicity of the factors mentioned above and potential additive and synergistic interactions (Zhu et al. 2015).

The data obtained in this study provide essential information to guide chemicals selection in the management of agricultural pests in order to minimize the risk for native bees. The mean lethal dose (LD_{50}) and the mean lethal concentration (LC_{50}) are parameters commonly used to measure the toxicity of a substance. In addition, our results confirm the importance of considering other bee species in risk assessments, not just using *A. mellifera* as a reference (Decourtye et al. 2013).

Despite the high acute toxicity of spinosad, phosmet and malathion on *P. emerina* and *T. fiebrigi*, demonstrated in the present study, acute lethality tests, made in the laboratory may be considered simplistic indicators of environmental impact. Because insecticides, especially Neonicotinoids, may have sub-lethal effects on bees (Desneux et al. 2007; Carvalho et al. 2009; Laycock et al. 2012; Henry et al. 2012). Thus, more studies evaluating sublethal effects, in addition to semi-field and field experiments are necessary to investigate the impacts of these products under more realistic conditions, aiming at preserving the action of pollinators at the pest control moment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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