Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-020-10191-7

RESEARCH ARTICLE

Effects of insecticides used in strawberries on stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae)

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Received: 8 May 2020 / Accepted: 20 July 2020

Abstract

The use of pesticides is considered one of the most important threats to pollinators, especially since they are widely used in agriculture for pest control. In the last years, several studies have reported severe secondary effects on various bee species, including exotic and native bees. In this study, lethal (mortality) and sublethal (locomotor activity) effects of insecticides and acaricides used in strawberries in Brazil (abamectin, novaluron, spinetoram, and thiamethoxam) were evaluated on the native stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi*. The results showed that the effects varied significantly according to the pesticide, type of exposure (oral or topical), and bee species. Through oral exposure, *M. quadrifasciata* was more susceptible to all insecticides except for abamectin, while in topical exposure, *T. fiebrigi* was more sensitive. Thiamethoxam followed by spinetoram and abamectin were the most lethal, regardless of species or exposure route; novaluron was not harmful at the highest tested dose. The locomotor activity of bees was altered in the presence of sublethal doses (LC_{10} and LC_{50}) of all insecticides. Spinetoram and abamectin can be as much as toxic as thiamethoxam against *M. quadrifasciata* and *T. fiebrigi* in laboratory experiments. These findings should be confirmed in field experiments to define possibilities to combine pest control and pollinator management. In crops like strawberries, the selectivity of native pollinators should be considered.

Keywords Acute toxicity · Behavior effects · Native bees · Pesticides · Selectivity · *Fragaria x ananassa* Duch

Introduction

Bees perform an important service as pollinators in a large number of agricultural interest crops as well as wild plants (Klein et al. 2007; Cresswell 2011). The honeybee *Apis mellifera* L., 1758, is the most widely used pollinator in commercial crops worldwide and the most often used as a model organism for nontarget toxicity studies (Minussi and Alves-dos-Santos 2007; Brittain and Potts 2011). However, in recent years, many beekeepers from different countries have been reporting unusual bee mortality resulting in high losses of honey beehives (Bortolotti et al. 2003). Therefore, the use of other bees, such as the stingless bees, has been encouraged, since these bees also contribute to pollination in agricultural crop areas (Slaa et al. 2006).

Among these are the *Melipona quadrifasciata* Lepeletier, 1836, known as "mandaçaia," with similar size of *Apis mellifera*, found in parts of Argentina, Brazil, and Paraguay, and *Tetragonisca fiebrigi* (Schwarz, 1938), known as "jataí," a small bee, found in parts of Argentina, Brazil, Paraguay, and Bolivia (Camargo and Pedro 2013). Several studies have shown that these bees play an important role in the pollination of crops in protected environments such tomatoes, eggplants, and strawberries (Free 1993; Del Sarto et al. 2005; Antunes et al. 2007; Nunes-Silva et al. 2013; Yankit et al. 2018) as well in open field to crops such as cotton and sesame among others (Stein et al. 2017), because it improves the production and the quality of fruits.

The strawberry is a plant cultivated and much appreciated worldwide (Witter et al. 2012). Although most commercial cultivars are hermaphrodite and self-pollinated, the lack of

Responsible Editor: Giovanni Benelli

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Published online: 24 July 2020

pollinators during the flowering of the crop associated with insufficient amounts of pollen results in deformations of the fruits and lower yields (Zebrowska 1998; Witter et al. 2014). The ease of management of *M. quadrifasciata* and *T. fiebrigi* combined with the absence of functional sting makes these species suitable for the pollination of strawberries in protected environments and with that they aid in the pollination deficit (Slaa et al. 2006). Also, because these bees are different in size, they may have a complementary effect on flower pollination, since medium to large bees fold at the top of the receptacle and pollinate the apical stigmas, while small bees circulate in the stamens and around of the receptacle, mainly to pollinate the basal stigmas (Chagnon et al. 1993; Malagodi-Braga and Kleinert 2007).

However, in these crops, the application of pesticides is a common practice in the management of pest arthropods (Bernardi et al. 2015). Abamectin, thiamethoxam, spinetoram, and novaluron are used to control mites, aphids, thrips, and caterpillars, respectively, during strawberry crop production in Brazil (Agrofit 2019). Abamectin belongs to chemical group to avermectinas and acts as GABA agonist (gamma-aminobutyric acid). Thiamethoxam is an insecticide from the group of neonicotinoids that act as nicotinic acetylcholine receptor agonists (nAChR) in post-synaptic neurons of the central nervous system, competing for the action site of the neurotransmitter acetylcholine (Irac 2020). Spinetoram belongs to spinosyns chemical group and which acts as modulators of acetylcholine receptors. Some studies highlight that insecticides in this group have low residual toxicity, in relation to other insecticides and greater selectivity to beneficial insects (Williams et al. 2003; Ruiz et al. 2008). Novaluron is an insecticide of chemical group of benzoylureas that works by inhibiting chitin biosynthesis. The first three insecticides act in synaptic transmission, while the last act in the change of instars of insects (Irac 2020). Thus, during the pollination activity, stingless bees can also be exposed directly or indirectly to these products (Talebi et al. 2008; Mullin et al. 2010), directly through the contact of the body of the insect with chemical molecules suspended in the air and indirectly through the ingestion of pesticide residues present in pollen, nectar, or water (Girolami et al. 2009; Johnson et al. 2010). Several studies have demonstrated the occurrence of serious lethal and sublethal effects in these native bees species exposed to insecticides (Tomé et al. 2015; Pitts-Singer and Barbour 2016; Dorneles et al. 2017; Prado-Silveira et al. 2018; Brito et al. 2020; Padilha et al. 2020); however, little information on most of the species is available.

Therefore, due to the importance of pollination for strawberry production, it is necessary to know the toxicity of pesticides on these bees. The objectives of this study were to establish the lethal dose and lethal concentration (LD_{50} and LC_{50}) as well as to evaluate the sublethal effects (locomotor activity) of insecticides used in strawberry on species of the native bees *M. quadrifasciata* and *T. fiebrigi*.

Material and methods

Bee collection

Foragers bees of *M. quadrifasciata* and *T. fiebrigi* were collected from three different colonies in the Meliponary at Federal University of Pelotas (UFPel), (Capão do Leão, RS, Brazil) and maintained in disposable plastic cages of 250 mL for all time of assay. The bee collecting was made in sunny days with temperatures above 18 °C. After the collection, the bees were transported to the laboratory (28 °C ± 1 °C temperature, 70% ±5% relative humidity, and scotophase of 24 h). In order to minimize the stress caused by confinement, the workers remained in adaptation for 24 h and received sucrose solution (50% v/v) ad libitum in an Eppendorf® tube (1.5 mL) for food supply, before the beginning of tests.

Toxicity bioassays

The tested insecticides were the following: abamectin (Vertimec® 18 EC 1.8% a.i.), thiamethoxam (Actara® 250 WG 25.0% a.i.), spinetoram (Delegate® 25.0% a.i.), and novaluron (Rimon Supra® 10.0% a.i.) all registered for the strawberry crop in Brazil. The concentrations evaluated for each insecticide were determined based on the active ingredient concentration described on the formulations label. Susceptibility of *M. quadrifasciata* and *T. fiebrigi* was assessed through oral and topical exposure. The experiments were conducted in two steps using a combined methodology adapted from Felton et al. (1986), OECD (1998a, b), and Medrzycki et al. (2013).

1) *Preliminary tests*: serial dilutions were performed (1:10) with insecticide stock concentration (1000 ng a.i./μL) in distilled water. Six concentrations were obtained in descending order for the recognition of the doses ranges that provided 0 to 100% mortality.
2) *Final tests*: after establishing the response range of preliminary tests, were established six until eight doses in increasing concentrations of their active ingredients to be used in the bioassays.

Six replicates with ten adult bees from different colonies were used for each treatment. The bioassays were conducted under a randomized design. Bioassays with mortality higher than 10% in the control treatment were not considered for analysis.

Determination of lethal oral concentration (LC_{50})

The commercial formulations of selected insecticides were diluted in sucrose solution (50% v/v). To stimulate consumption, the insects were starved for 2 h before the experiments.

Each group of bees was fed with 1 mL of insecticide solution for 6 h. Subsequently, the insecticide solution was replaced by sucrose solution ad libitum. The concentrations of thiamethoxam, spinetoram, abamectin, and novaluron ranged from 0.1 to 100 ng a.i./μL diet, 0.1 to 50 ng a.i./μL diet, 0.1 to 100 ng a.i./μL diet, and 1.0 to 50,000 ng a.i./μL diet to *T. fiebrigi* and from 0.01 to 1.0 ng a.i./μL diet, 0.1 to 10 ng a.i./μL diet, 2.0 to 50 ng a.i./μL diet, and 1.0 to 50,000 ng a.i./μL diet to *M. quadrifasciata*, respectively. The bees from the control group were fed with sucrose solution only. Dead insects, as well as abnormal symptoms, were recorded during 48 h after the initial exposition.

Determination of lethal topical dose (LD_{50})

The commercial formulations of selected insecticides were diluted in distilled water and acetone (50% v/v) with a range of concentrations. Prior topical application, all the bees (including control group) were anesthetized with CO_2 for 10 s. Using a micro applicator (Burkard Scientific, UK) a drop with 0.5 μL (*T. fiebrigi*) or 1.0 μL (*M. quadrifasciata*) was deposited on the pronotum of each bee. The concentrations of thiamethoxam, spinetoram, and abamectin ranged from 0.5 to 50 ng a.i./bee, 0.5 to 50 ng a.i./bee, and 0.25 to 125 ng a.i./bee to *T. fiebrigi* and from 0.1 to 100 ng a.i./bee, 1.0 to 500 ng a.i./bee, and 1.0 to 1000 ng a.i./bee to *M. quadrifasciata*, respectively. Control bees received a drop of distilled water and acetone (50% v/v). Bees were fed with a sucrose solution (50%) ad libitum. Mortality and abnormal behavior were recorded 48 h after initial exposure.

Behavioral bioassays

Locomotor activity

Bees were exposed to sublethal concentrations LC_{10} and LC_{50}. The treatments were conducted as described previously to determine the oral LC_{50}. The insects were individually released at one end of a silicone tube with a total length of 60 cm (Fig. 1). A fluorescent lamp was used at the opposite end of the tube to stimulate the bees. The time that each bee spent to walk a distance of 50 cm toward the light source was recorded. Based on untreated/control bees, the maximum walking period was about 1 min. Subsequently, the average speed for each bee was calculated. The bioassays using 30 bees per treatment were performed under 28 ± 1 °C, at 4 and 24 h after initial exposure.

Statistical analyses

Statistical analyses to determine the LC_{50} and LD_{50} values were performed with the "four-parameter log-logistic function" of the "drc" package (Analysis of Dose-Response Curves using the statistical software R®) (Ritz and Streibig 2005). Toxicity was assessed by comparing the LC_{50} and LD_{50} values between the insecticides for each species of bee and also comparing these values among the species. In both cases, the values of the LC_{50} and LD_{50} confidence intervals were used, being significantly different when no overlap occurred in the confidence intervals, at 95% probability. For locomotor activity, analysis of variance was performed using the Kruskal-Wallis test, and when statistics obtained a significant p value (< 0.05), the Dunn test was applied to 95% probability.

Fig. 1 Experimental setup. A silicone tube (50 cm) coupled to an apparatus consisting of a wooden plate (angle 0°)

Results

Toxicity bioassays

Acute oral toxicity

The calculated values to LC_{50} (48 h) of thiamethoxam, spinetoram, and abamectin to *T. fiebrigi* were 2.05 ng a.i./μL, 2.72 ng a.i./μL, and 3.53 ng a.i./μL diet, respectively (Table 1). In the bioassays performed with *M. quadrifasciata*, the LC_{50} (48 h) of thiamethoxam, spinetoram and abamectin were 0.18 ng a.i./μL, 2.45 ng a.i./μL, and 8.81 ng a.i./μL diet, respectively (Table 1). Thiamethoxam presented a higher lethal effect, with an LC_{50} value considered extremely low. When comparing the toxicity of the insecticides among the species in this route of exposure, *M. quadrifasciata* presented greater susceptibility to insecticides than *T. fiebrigi* (except for abamectin). The overlapping in the confidence interval of LC_{50} for spinetoram indicated that the two species did not differ in susceptibility to this

Table 1 Relative toxicity of orally exposed insecticides to *T. fiebrigi* and *M. quadrifasciata*

Insecticide	Specie	Slope (± SE)	LC_{50} (95% FL) (ng a.i./μL)	*t*	*p* value
Thiamethoxam	*T. fiebrigi*	1.0047 (± 0.11)	2.05 (1.5217–2.5858)	7.73	< 0.0001
	M. quadrifasciata	1.7364 (± 0.32)	0.18 (0.1453–0.2311)	8.84	< 0.0001
Spinetoram	*T. fiebrigi*	2.4631 (± 0.38)	2.72 (2.2599–3.1792)	11.92	< 0.0001
	M. quadrifasciata	1.5342 (± 0.30)	2.45 (1.6001–3.3013)	5.80	< 0.0001
Abamectin	*T. fiebrigi*	2.7584 (± 0.44)	3.53 (3.0996–3.9732)	16.22	< 0.0001
	M. quadrifasciata	9.5683 (± 1.96)	8.81 (8.4575–9.1725)	49.69	< 0.0001

insecticide. For both species, the most toxic insecticides when ingested were thiamethoxam, spinetoram, and abamectin, respectively.

Novaluron caused a low percentage of mortality for adults of *T. fiebrigi* (10%) and *M. quadrifasciata* (16%) at the maximum tested concentration (50,000 ng a.i./μL diet) and not possible to construct the dose-response curve.

Acute topical toxicity

Topical treatments in *T. fiebrigi* with thiamethoxam, spinetoram, and abamectin resulted in LD_{50} values of 5.50 ng a.i./bee, 5.79 ng a.i./bee, and 8.07 ng a.i./bee, respectively (Table 2). The values for the topical LD_{50} of the thiamethoxam, spinetoram, and abamectin for *M. quadrifasciata* were as the following: 9.06 ng a.i./bee, 26.27 ng a.i./bee, and 237.74 ng a.i./bee, respectively (Table 2). In this route of exposure, *T. fiebrigi* was the specie most susceptible to the insecticides studied, according to the values of the confidence intervals of each LD_{50}. For the two species, the most toxic insecticides were thiamethoxam, spinetoram, and abamectin, respectively.

Behavioral bioassays

Locomotor activity

Exposure to insecticides caused changes in the locomotor activity of the bees, varying according to species, concentration, insecticide, and period evaluated. Thiamethoxam significantly reduced the average speed of *T. fiebrigi* ($X^2 = 52.48$; df = 2; $p < 0.0001$) and *M. quadrifasciata* ($X^2 = 30.76$; df = 2; p < 0.0001) 4 h after the oral exposure. However, after 24 h, no statistically significant difference was observed, either for *T. fiebrigi* ($X^2 = 0.09$; df = 2; $p = 0.9538$) as for *M. quadrifasciata* ($X^2 = 5.18$; df = 2; $p = 0.07$). When comparing the average speed of *T. fiebrigi* bees from the control treatment with the speed of bees exposed to LC_{10} and LC_{50} of thiamethoxam after 4 h, a statistically significant difference was observed, since the control group walked the established distance with a higher velocity than the others (2.20 cm/s) (Fig. 2a). Bees of the LC_{10}, although presenting a lower average speed in relation to the control (1.85 cm/s), were less impaired than those exposed to LC_{50}, which presented reduced locomotor activity (0.52 cm/s). *M. quadrifasciata* presented similar behavior, and after 4 h, the average speed of bees exposed to LC_{10} (2.19 cm/s) and LC_{50} (1.85 cm/s) differed statistically from the control group (4.18 cm/s), but there was no difference between bees of LC_{10} and LC_{50}. No significant difference was observed at 24 h, of the control group (3.59 cm/s) (Fig. 2b).

Bees exposed to LC_{50} and LC_{10} of spinetoram showed a significant difference in average speed values compared with control group bees at 4 h (*T. fiebrigi*: $X^2 = 10.57$; df = 2; $p = 0.005$; *M. quadrifasciata*: $X^2 = 28.75$; df = 2; $p < 0.0001$) and 24 h after (*T. fiebrigi*: $X^2 = 28.01$; df = 2; $p < 0.0001$; *M. quadrifasciata*: $X^2 = 25.35$; df = 2; $p < 0.0001$). Bees from the control treatment walked the established distance with higher speed (*T. fiebrigi*: 2.13 cm/s (4 horas) and 2.01 cm/s (24 horas); *M. quadrifasciata*: 2.52 cm/s (4 horas) and 3.45 cm/s (24 horas)) than bees submitted to feeding with the insecticide, which presented minimum average speed of 1.27 cm/s (*T. fiebrigi*) and 1.24 cm/s (*M. quadrifasciata*) after 24 h exposed to LC_{50} (Fig. 3 a and b).

Table 2 Relative toxicity of topically exposed insecticides to *T. fiebrigi* and *M. quadrifasciata*

Insecticide	Specie	Slope (± SE)	LD_{50} (95% FL) (ng a.i./bee)	*t*	*p* value
Thiamethoxam	*T. fiebrigi*	2.9547 (± 0.65)	5.50 (4.8082–6.2068)	15.87	< 0.0001
	M. quadrifasciata	3.7765 (± 1.32)	9.06 (8.1888–9.9387)	20.88	< 0.0001
Spinetoram	*T. fiebrigi*	1.0743 (± 0.14)	5.79 (3.7172–7.8718)	5.62	< 0.0001
	M. quadrifasciata	2.0000 (± 0.59)	26.27 (18.2274–34.3170)	6.58	< 0.0001
Abamectin	*T. fiebrigi*	0.9860 (± 0.15)	8.07 (4.4483–11.6968)	4.48	< 0.0001
	M. quadrifasciata	1.3457 (± 0.21)	237.74 (152.9804–322.4947)	5.65	< 0.0001

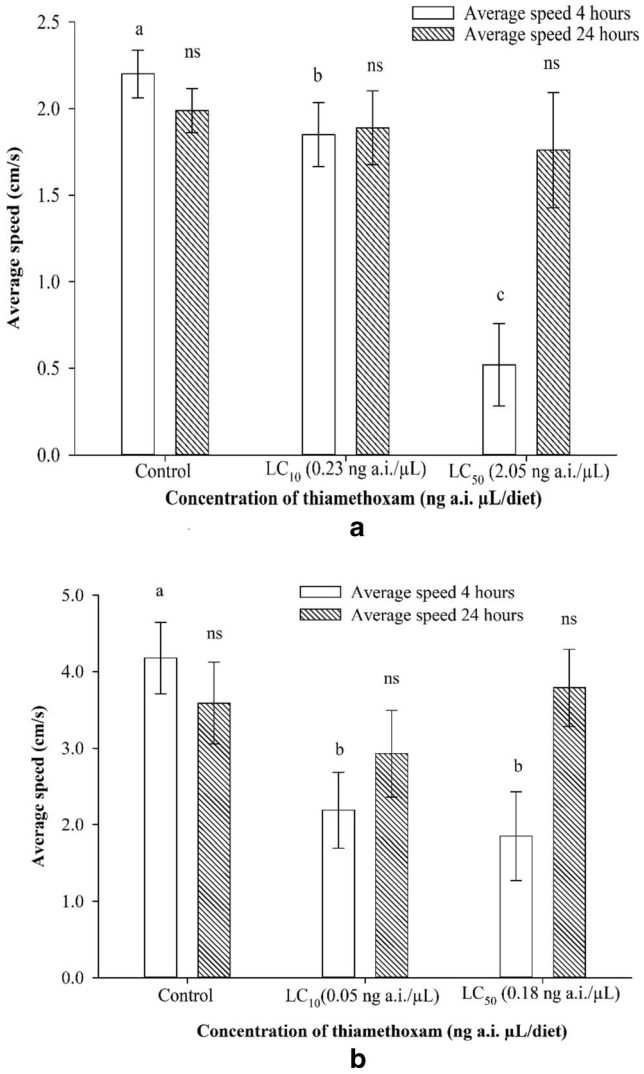

Fig. 2 Locomotor activity of adult *T. fiebrigi* (**a**) and *M. quadrifasciata* (**b**) 4 and 24 h after oral exposure to thiamethoxam. * Averages followed by the same letter do not differ statistically from one another by the Dunn test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 h after oral exposure to insecticide; dashed bars indicate the average speed (cm/s) 24 h after exposure of the bees to the insecticide

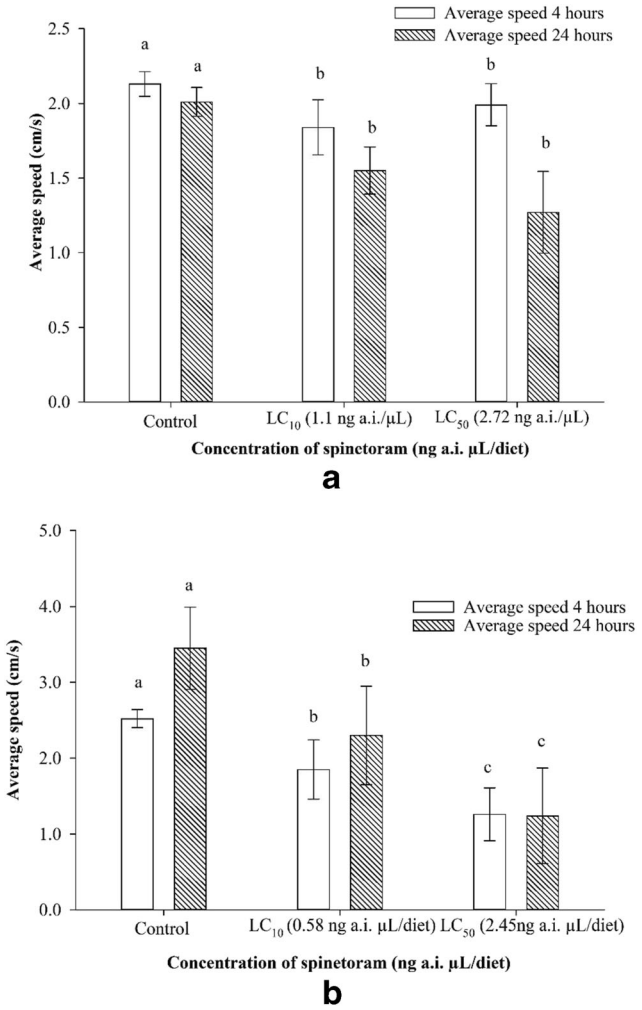

Fig. 3 Locomotor activity of adult *T. fiebrigi* (**a**) and *M. quadrifasciata* (**b**) 4 and 24 h after oral exposure to spinetoram. * Averages followed by the same letter do not differ statistically from one another by the Dunn test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 h after oral exposure to insecticide; dashed bars indicate the average speed (cm/s) 24 h after exposure of the bees to the insecticide

When the species were exposed to abamectin, there was a significant difference between the groups for the two evaluated periods (4 h = *T. fiebrigi*: $X^2 = 34.27$; df = 2; $p < 0.0001$; *M. quadrifasciata*: $X^2 = 9.52$; df = 2; $p = 0.008$; 24 h = *T. fiebrigi*: $X^2 = 12.03$; df = 2; $p = 0.002$; *M. quadrifasciata*: $X^2 = 16.36$; df = 2; $p = 0.0002$). The average speed of *T. fiebrigi* bees belonging to the control after 4 h (1.54 cm/s) and 24 h (1.66 cm/s) was statistically higher than the speed of bees exposed to LC_{10} and LC_{50} (Fig. 4a). For *M. quadrifasciata*, at 4 h, the average speed of the control bees (3.49 cm/s) did not differ statistically from the speed of bees exposed to feeding with LC_{50} (3.13 cm/s), while both differed and were higher than those under LC_{10} (2.32 cm/s). On the other hand, after 24 h of feeding, both the average speed of bees exposed to LC_{50} (2.73 cm/s) and LC_{10} (1.95 cm/s) differed from the control (3.51 cm/s) showing lower average speeds. At this time, it was also observed that the bees exposed to LC_{50} presented higher average speed compared with LC_{10} bees (Fig. 4b).

Discussion

The results have shown that the toxicity of the insecticides and the acaricide abamectin vary according to the route of exposure and specie. Furthermore, it was observed that the recommended concentrations for formulations of thiamethoxam, spinetoram, and abamectin, for use in Brazilian strawberries, are considerably higher than the LC_{50} values determined in this study. For thiamethoxam, insecticide that was most toxic to both species and routes of exposure, the recommended concentration is 25.0 ng a.i./µL water (10 g/100 L water) which represents about

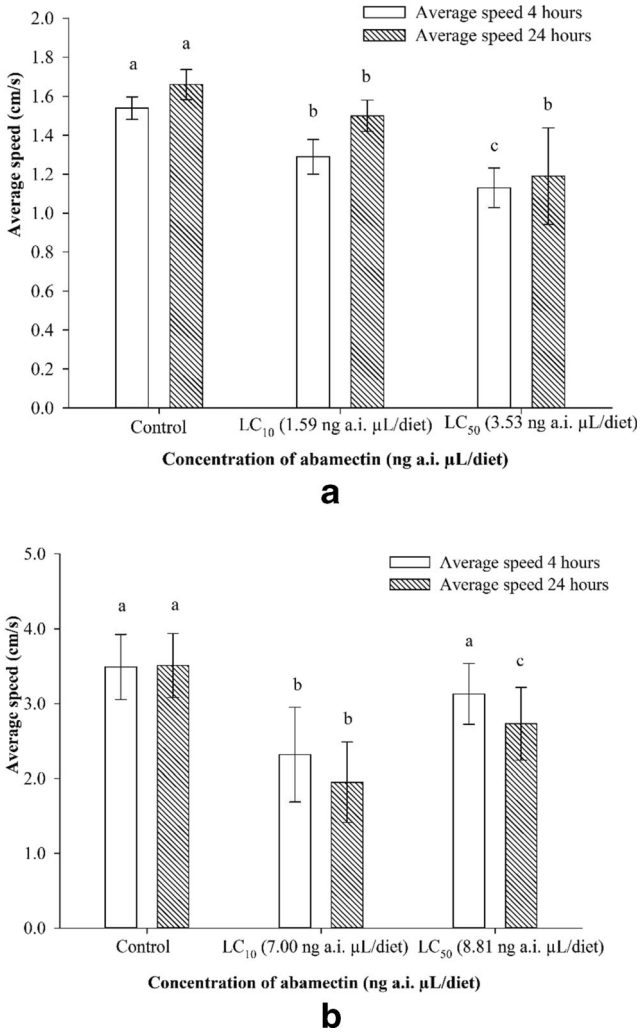

Fig. 4 Locomotor activity of adult *T. fiebrigi* (**a**) and *M. quadrifasciata* (**b**) 4 and 24 h after oral exposure to abamectin. * Averages followed by the same letter do not differ statistically from one another by the Dunn test at 5% probability. White bars indicate the average speed (cm/s) of bees 4 h after oral exposure to insecticide; dashed bars indicate the average speed (cm/s) 24 h after exposure of the bees to the insecticide

12 and 140 times the oral LC_{50} value for *T. fiebrigi* and *M. quadrifasciata*, respectively. The high toxicity of neonicotinoids to bees has been reported in several studies (Biddinger et al. 2013; Soares et al. 2015; Jiménez and Cure 2016; Jacob et al. 2019). *Melipona scutellaris* exposed by feeding with imidacloprid presented oral LC_{50} of 0.81 ng a.i./µL diet (Costa et al. 2015). For *A. mellifera*, independent of the route of exposure, thiamethoxam was extremely toxic (Costa et al. 2014). The LC_{50} oral for this specie was 0.12 ng a.i./µL diet (48 h) (Laurino et al. 2011), which corroborates the results observed in this study. Neonicotinoids cause alterations in the nerve impulses of insects since they interfere in the nicotinic acetylcholine receptors (nAChR) (Brown et al. 2006; Tan et al. 2007). The linkage of the insecticide molecules with nAChR is irreversible, resulting in paralysis and death of insects (Jeschke et al. 2011). This scenario becomes even more worrying, since neonicotinoid insecticides, once absorbed by the plant, can translocate up to the water of guttation, nectar, and pollen of the crops, which in the flowering stage attract several bees (Krupke et al. 2012). In cucurbit crops, the foliar application and drip irrigation of thiamethoxam during flowering resulted in high average levels of residues in pollen (122 ng/g) and nectar (17.6 ng/g) (Dively and Kamel 2012). According to Dively and Kamel (2012), environmental conditions have a significant effect on overall residue levels, and therefore, further studies are needed to determine the real dose that bees may be exposed in the pollen and/or nectar of the crops.

In the case of spinetoram, the recommended concentration is 50.0 ng a.i./µL water (20 g/100 L water) which represents about 19 and 20 times the oral LC_{50} value observed for *T. fiebrigi* and *M. quadrifasciata*, respectively. Despite the biological origin of the insecticides of the spinosyns group, they also act in synaptic transmission which may explain the high mortality. These results demonstrate that the lower adverse effects of this group on beneficial insects may be overestimated, and it is, therefore, important to emphasize the importance of non-exemption of new bioinsecticidal molecules from the risk assessment analysis for bees. A similar result was observed with spinosad (also belonging to the spinosyns) that presented a higher lethal effect than the imidacloprid insecticide for *M. quadrifasciata* (Tomé et al. 2015). Spinosad and spinetoram were found to be dangerous for *Megachile rotundata* in contact with adults, causing changes also in the immature stages of this species (Gradish et al. 2012).

T. fiebrigi was more susceptible to abamectin than *M. quadrifasciata* in the two exposure routes evaluated. The recommended concentration of this product is 13.5 ng a.i./µL water (75 mL/100 L water) which represents about 14 and 1.5 times the oral LC_{50} value for *T. fiebrigi* and *M. quadrifasciata*, respectively. Del Sarto et al. (2014) observed that *A. mellifera* also presented lower tolerance of this acaricide than *M. quadrifasciata*. The oral administration of abamectin significantly reduced the survival of *A. mellifera*, with a lethal time about three times less than deltamethrin (Aljedani 2017). Ingestion of abamectin may cause changes in bees midgut cells, leading to severe digestive disturbances (Aljedani 2017).

The low toxicity of novaluron to adult bees can be explained by the fact that growth-regulating insecticides act especially on the immature stage of insects, interfering in the process of ecdysis (Mommaerts et al. 2006). The maximum tested concentration, which caused inexpressive mortality, was about 1400 times the recommended concentration for the field 36.0 ng a.i./µL water (60 mL/100 L water). Due to these factors, such an insecticide was not used in sublethal tests. Similar results to adult bees have been reported in several studies (Malone et al. 2007; Scott-Dupree et al. 2009). For adults of *Bombus terrestris*, this group of insecticides also did not provoke acute toxicity; however, for the immature stages, harmful effects were observed, including egg mortality and larval deformation (Mommaerts et al. 2006). Studies

exploring the effects of these insecticides on the early stages of development of *M. quadrifasciata* and *T. fiebrigi* are required.

In topical application, *M. quadrifasciata* and *T. fiebrigi* presented high susceptibility to thiamethoxam, being more sensitive when compared with *A. mellifera* (29.90 ng a.i./bee) (Iwasa et al. 2004). The high lethality of this insecticide was also observed for *Nannotrigona perilampoides* (LD_{50} = 4.0 ng a.i./bee) (Valdovinos-Núñez et al. 2009), which corroborates the lethal dose obtained for *T. fiebrigi* (5.50 ng a.i./bee). Despite the lack of studies, Dorneles et al. (2017) also reported high sensitivity of *T. fiebrigi* to the topical application of chlorpyrifos (organophosphate). The greater susceptibility of this species compared with *M. quadrifasciata* may be related to the structure of the cuticle, which possibly facilitates the absorption of insecticides. According to Bacci et al. (2007), the penetration rate is related to the composition and chemical thickness of the insect cuticle and the physic-chemical characteristics of the compounds.

The routes of exposure studied in this work sought to simulate the possible forms of contamination of bees with insecticides in the field. In general, the species *M. quadrifasciata* and *T. fiebrigi* showed greater susceptibility to the insecticide thiamethoxam followed by spinetoram and abamectin in the two routes evaluated. The differential susceptibility observed may have occurred due to specific characteristics of insecticides and bee species. Life story traits, body weight, detoxification capacity, and cuticle structure are factors that may change the level of toxicity (Oliveira et al. 2002; Hardstone and Scott 2010; Brittain and Potts 2011). The lower molecular weight of thiamethoxam (291.71), followed by spinetoram (754.00) and abamectin (873.10) (Yu 2008), may have influenced the different degrees of toxicity of insecticides. According to Stock and Holloway (1993), substances with smaller molecular weights have greater penetration capacity in the cuticle of the insects.

The locomotor activity of bees was altered in the presence of sublethal doses of insecticides. Sublethal responses should be considered since the lethality is only a simplistic indicator of environmental impact (Tomé et al. 2015). Thiamethoxam decreased the locomotor activity of *T. fiebrigi* and *M. quadrifasciata* shortly after exposure (4 h). Although the short-term response to neurotoxic insecticides usually occurs through hyperexcitation, in this study, the initial decrease in activity may have occurred due to the severe symptoms observed, including spasms and disorientation, which made it difficult to move. The symptoms of prostration and motor disturbance caused by thiamethoxam on honey bees are due to the effect of the compound on the synapses of the insect central nervous system (Kagabu 1997). After 24 h, bees of the species *M. quadrifasciata* exposed to LC_{50} presented average speed superior to the bees belonging to LC_{10} and control. This species exhibited similar behavior when exposed to imidacloprid (Tomé et al. 2015). Contradictory results were observed for *A. mellifera*, which, after 24–48 h after application of thiamethoxam, showed lower mobility and flight activity (Charreton et al. 2015; Tosi et al. 2017). El Hassani et al. (2008) did not observe changes in the locomotor activity of bees exposed to thiamethoxam.

Different behavioral responses may have occurred due to exposure time, bee species, and doses used in each study. Lambin et al. (2001) point out that sublethal effects are highly dependent on the dose of the insecticide. Moreover, several nicotinic receptor subtypes are involved in complex behaviors and memory processes and may be differentially altered by sublethal doses of neonicotinoids (Gauthier 2010). In *A. mellifera*, exposure to sublethal doses of thiamethoxam (10 ppb) increased the expression of two subunits nAChRs, nAChRα9, and nAChRβ2, indicating a compensatory reaction to the functional loss of nAChRs due to the neonicotinoid (Christen et al. 2016; Shi et al. 2017). This insecticide seems to induce changes in the regulation of the gene responsible for memory formation in *A. mellifera* (NMDA receptor 1 (NR1)) (Shi et al. 2017), which may explain in part the adverse behavioral effects observed.

Spinetoram and abamectin also interfered in the locomotor activity of both species. Disorientation was the typical symptom of the first. Spinosad, although it did not alter the locomotion, affected the flight activity of *M. quadrifasciata* (Tomé et al. 2015). The sublethal toxicity of spinosyns has been reported as negative in bumblebees (Morandin et al. 2005).

Tremors and spasms, followed by disorientation and paralysis, were caused when insects were exposed to abamectin. Avermectins, as well as neonicotinoids and spinosyns, act in the transmission of the nerve impulse; however, the first group acts like agonist of the gamma-aminobutyric acid (GABA), causing immobilization and paralysis of the insects, due to neuromuscular action (Sánchez-Bayo 2011). This fact may explain the locomotor difficulty observed in bees exposed to abamectin. These effects may cause impacts on the survival of all colony, which require active and healthy bees for feeding, cleaning, cell building, and various tasks (Winston 1987).

In conclusion, the insecticides thiamethoxam, spinetoram, and abamectin presented high lethality for *M. quadrifasciata* and *T. fiebrigi* under the conditions (oral and topical) evaluated. The recommended concentrations for use in Brazilian strawberries are considerably higher than the LC_{50} values here determined. Novaluron was not harmful at the highest tested dose. The sublethal test suggests that abamectin and spinetoram can be as toxic as thiamethoxam neonicotinoid toward native bees and therefore implies that the molecules to be used would need to be carefully selected. These results confirm the importance of considering other species of bees in the risk assessments, not only using *A. mellifera* as reference (Decourtye et al. 2013). Further studies evaluating sublethal effects in semi-field and field experiments are necessary to investigate the impacts of these products under more realistic conditions and the possibility of integrated pest and pollinator management.

Funding information To Coordination of Improvement of Higher Education Personnel (CAPES, Finance Code 001) for granting scholarships.

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