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Acute Oral Toxicity of Fipronil on the Stingless Bee *Frieseomelitta varia* (Lepeletier, 1836)

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Abstract

Stingless bees are essential pollinating insects, contributing to the maintenance of biodiversity and agricultural production. However, during their foraging activity, they encounter pesticides that can harm their populations. The present study aims to evaluate the acute oral lethal concentration (LC) of fipronil in the stingless bee species *Frieseomelitta varia*. The LC10, LC25, and LC50 were calculated for a 48-hour period, which were 61.2, 112.2, and 219.9 ng/mL, respectively, and for the 96-hour period, which were 15.07, 29.3, and 61.33 ng/mL, respectively. The study highlights the importance of the conscious use of pesticides, as damage to pollinating insect populations can be both economically and ecologically detrimental.

Introduction

Pollination by animals is responsible for enhancing the productivity of plant crops, making it more stable and optimized (Bishop et al., 2022). It is estimated that there is an annual gain in agricultural production of US$235 – 577 billion (Potts et al., 2016). The cultivation of pollination-dependent crops such as canola, soybean, and sunflower has been increasing over the decades, with up to 70% in developing countries (Aizen et al., 2008). This dependence is also evidenced by the fact that 87 of the top 124 crop plants used directly in human food are dependent on animal pollination (Klein et al., 2007). Bees are the main pollinating animals (Gallai et al., 2009; Winfree et al., 2008). There are 20,000 species of bees, of which a thousand species are considered social and half of them compose the stingless bee group, responsible for pollinating a large part of the tropical flora of their endemic locations (Imperatriz-Fonseca & Nunes-Silva, 2010; Vit et al., 2013). It is also assumed that these species have a better pollination capacity when compared to exotic bee species, as they evolved alongside the local flora (González- Acereto et al., 2006). Among the biological diversity of stingless bees, the species *Frieseomelitta varia* (Lepeletier, 1836), popularly known as "Marmelada amarela" (Yellow Marmalade), is distributed from Southwestern Mexico to Southeastern Brazil (Silveira et al., 2002; Teixeira et al., 2007). Their beehives have around 3,000 individuals and swarms of a few hundred (Oliveira et al., 2012). This species is an important generalist pollinator of Malpighiaceae, Proteaceae, Labiatae, Connaraceae, Caryocaraceae, and Araliaceae (Teixeira et al., 2007), Cecropiaceae, Sapotaceae, Myrtaceae, and Moraceae families (Marques-Souza, 2010).

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The foraging activity of bees in agricultural areas can expose them to pesticides, widely used in agriculture to prevent or eliminate pests or diseases and increase production (Fenik et al., 2011). However, it is important to note that these substances can cause significant harm to organisms that provide ecosystemic services, such as bees (Souza et al., 2024; Costa et al., 2024; Assis et al., 2022; Akinsanya et al., 2021; Fenik et al., 2011; Li, 2020). Pollen and nectar contaminated with pesticides, when brought to the hive, affect the entire colony (Blacquière et al., 2012; Kessler et al., 2015; Sgolastra et al., 2019; Tomasini et al., 2012), so the indiscriminate use of these products can result in ecological and financial losses, rather than the intended gains.

For a pesticide commercialization approval by the regulatory organs, it must undergo a series of tests that determine its degree of toxicity to pollinators (Abdullah et al., 2007). Studies that define the acute lethal concentration of pesticides on pollinators are important for setting hazard parameters for public policies and concentrations to be investigated in further studies (OECD, 1998; Jacob et al., 2013; Sanchez-Bayo & Goka, 2014). However, stingless bees are rarely used in toxicity assessments for the approval of new pesticides (Cham et al., 2019), which may lead to overgeneralizations from a few species and consequently erroneous conclusions (Thompson, 2016).

Among commonly used pesticides, fipronil is a formicide and termicide from the pyrasol chemical group (Simon-Delso et al., 2015), which acts on GABA (γ-aminobutyric acid) receptor-regulated channels, altering their function and causing extreme excitation of the nervous system, resulting in death (Wang et al., 2016). It is well established that sublethal concentrations of fipronil are detrimental to the memory, vision, neural capacity, and circadian clock control of bees (Bernadou et al., 2009; Roat et al., 2014; Astolfi et al., 2025). Due to its toxicity to bees, a recent decision in Brazil has prohibited the aerial application of fipronil (IBAMA, 2023). The countries of the European Union have even more restrictive rules for fipronil use (PE, 2023). Other countries, such as China and the United States, have also banned the use of fipronil within their territories (Gonçalves et al., 2022).

Studies on acute oral toxicity can help explain how, in the long term, concentrations of the pesticide may damage the hives and agricultural production (Sherman & Visscher, 2002). Thus, the present study aims to assess the acute oral lethal concentration of fipronil in the stingless bee species *F. varia*.

Materials and Methods

Toxicity tests were conducted using the Fipronil Sulfone Pestanal (Sigma-Aldrich). We diluted the pesticide to 1mg/mL in Acetone. We collected forager bees of *F. varia* from four hives maintained at the meliponary of the Institute of Biological Sciences, Federal University of Goiás, in the city of Goiânia-GO, Brazil (16º 36' S, 49º 15' W). To assess the acute oral toxicity of fipronil, the OECD protocol 213 (OECD, 1998) was used with adaptations. We collected 240 bees, which were assigned to six treatments (40 bees per treatment). Within each treatment, four replicates of ten bees were established. To ensure independence of the four replicates, each group of ten bees came from a different hive. We kept the bees in 250 mL plastic jars, in a BOD incubator (Biological Oxygen Demand) at 28 ºC and relative humidity of 70%.

We subjected the bees from all experimental groups to a 2-hour fasting period to homogenize their intestinal content. After the fasting period, we fed them with a diet of 50% sucrose syrup. We fed bees from treatments F1, F2, F3, F4, and F5 syrup supplemented with increasing dosages of fipronil (15, 30, 60, 120, 240 ng/mL, respectively), and a dilution vehicle (acetone, maximum concentration: 0.024%). We fed the Control group sucrose syrup supplemented with 0.024% of acetone. We offered contaminated food for four hours. After this period, we removed the contaminated food and replaced it with 50% sucrose syrup without contaminants. Food consumption was measured every 24 hours over a 96-hour period (24, 48, 72, and 96 hours) by weighing the Eppendorf tubes with correction for evaporation. The number of living bees was also recorded every 24 hours; however, for analysis and reporting, we used only the 48- and 96-hour time points. We chose these intervals because most studies with other Hymenoptera species calculate the LC50 over 48 hours, and the 96-hour interval is considered the limit of acute tests for bees (OECD, 1998).

To calculate the results for the LC50 calculation, we used the "Ecotoxicology" package from the "R" program, extracting the values of the concentrations capable of killing 10% (LC10), 25% (LC25), and 50% (LC50) of the bees after 48 and 96 hours.

Results and Discussion

It was possible to calculate the fipronil LC10, 25, and 50% after 48 and 96 hours of exposure of adults of *F. varia*. For 48 hours after the exposition period, the values of LC 10, 25, and 50 are 61.2, 112.2, and 219.9 ng/ml, respectively (Table 1).

For 96 hours after the exposition period, the values of LC 10, 25, and 50 are 15.1, 29.3, and 61.3 ng/ml, respectively (Table 1). The increase of fipronil concentration in food results in increasingly higher mortality rates in both observation periods (Figs 1 and 2).

Table 1. Results for lethal concentrations of fipronil (LC) 10, 25, and 50% for 48 and 96 hours for *F. varia*.

	48 hours	96 hours
LC10	61.2 ng/mL	15.1 ng/mL
LC25	112.2 ng/mL	29.3 ng/mL
LC50	219.9 ng/mL	61.3 ng/mL

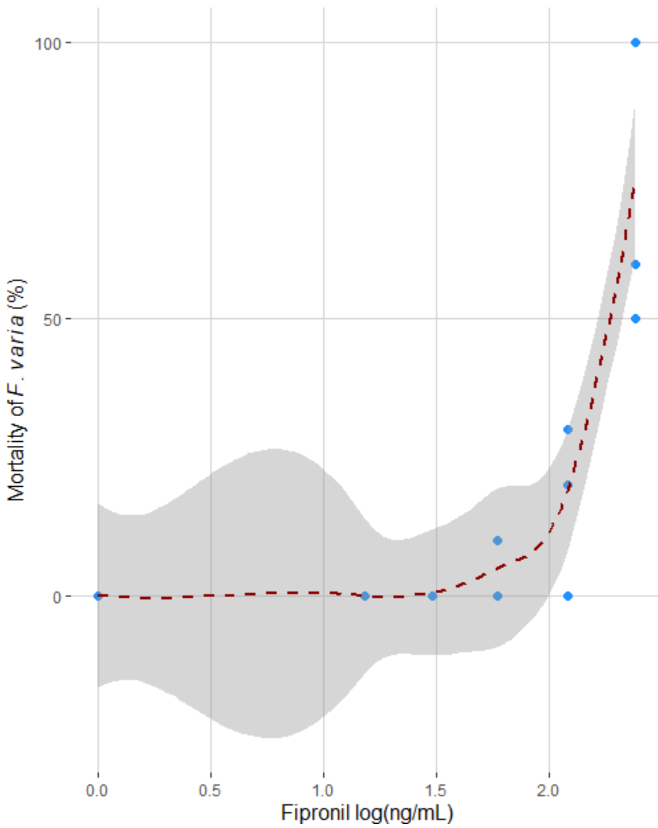

Fig 1. Graphical representation of 48h acute oral toxicity for adult individuals of the species *F. varia*, concentration given in logarithm.

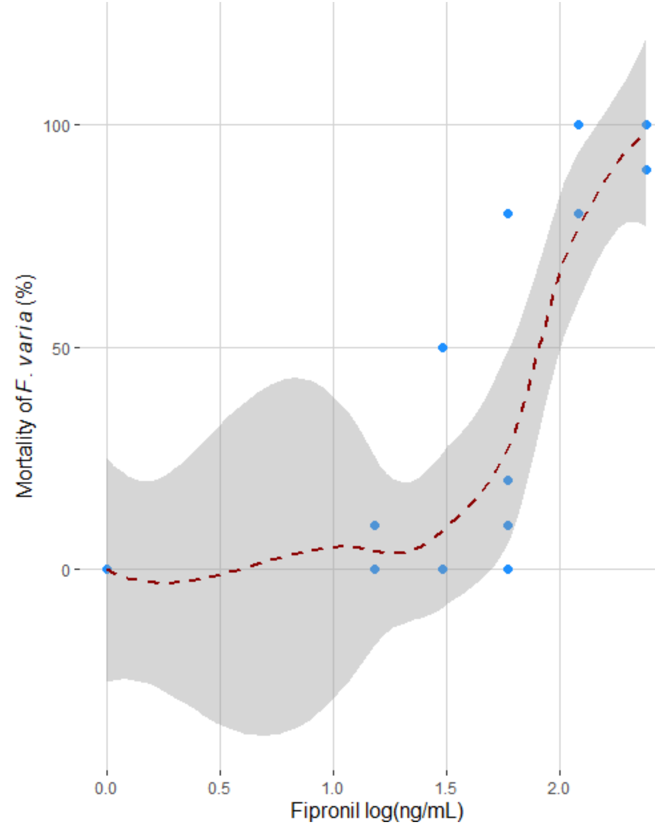

Fig 2. Graphical representation of 96h acute oral toxicity for adult individuals of the species *F. varia*, concentration given in logarithm.

Comparing the lethal concentration values obtained for *F. varia* with those of other stingless bees, such as *Melipona scutellaris*, Schrottky 1902, and *Scaptotrigona postica* Latreille 1807 (Table 2) (Jacob et al., 2013; Lourenço et al., 2012), it is observed that *F. varia* seems to be more resistant to fipronil compared to other Meliponini species. However, the honey bee *Apis mellifera* is much more resistant to fipronil than the stingless bees already studied (Table 2) (Roat et al., 2013), reinforcing the need for further studies investigating native bees rather than generalizing results from *A. mellifera*. Studies involving pollen analyses from various locations around the world (USA, France, Spain, Poland) observed the maximum concentration of fipronil detected of 29 ng/g (Sanchez-Bayo & Goka, 2014). The maximum concentration of fipronil found in the environment poses a considerable danger to the population of pollinators such as *F. varia,* given that the LC25 calculated in the present study is 29.3 ng/ml. This is particularly concerning for other stingless bees, such as *M. scutellaris,* which is more sensitive to fipronil (Table 2) (Lourenço et al., 2012).

Even though fipronil is a widely used insecticide for controlling ants, those species generally displayed greater resistance to it (Hayasaka et al., 2015; Xiong et al., 2019) when compared to non-target insects (bees) (Table 2). It is known that resistance to pesticides in insects can be associated with genes from the P450 superfamily. Those genes operate in the

Table 2. Comparison between the lethal concentrations of fipronil for stingless bees (*Melipona scutellaris; Scaptotrigona postica*), the main commercial bee (*Apis mellifera*), and ants targeted by the pesticide used (*Solenopsis invicta*; *Linepithema humile*; *Camponotus bishamon*; *Lasius japonicas*).

Species	**Lethal concentration (LC)**	**Reference**
Melipona scutellaris (stingless bee)	LC50 (48 hrs) 11 ng/mL	(Lourenço et al., 2012)
Scaptotrigona postica (stingless bee)	LC50 (24 hrs) 240 ng/mL	(Jacob et al., 2013)
Apis mellifera (honeybee)	LC50 (24 hrs) 1270 ng/mL	(Roat et al., 2013)
Solenopsis invicta (ant)	LC50 (48 hrs) 2510 ng/mL	(Xiong et al., 2019)
Linepithema humile (ant)	LC50 (48 hrs) 271 ng/mL	(Hayasaka et al., 2015)
Camponotus bishamon (ant)	LC50 (48 hrs) 767 ng/mL	(Hayasaka et al., 2015)
Lasius japonicas (ant)	LC50 (48 hrs) 1944 ng/mL	(Hayasaka et al., 2015)

metabolism of xenobiotics, mutagens, hormones, fatty acids, and steroids (Liu et al., 2011; Pavek & Dvorak, 2008). It has been observed that some of these genes become overexpressed in cases of contact with pesticides, such as in *Solenopsis invicta* (Zhang et al., 2016). Genome sequencing of different bee species reveals that they have a lower diversity of P450 genes compared to other insects. While beetle and mosquito species have over 100 P450 genes, bee species already studied have fewer than 50 genes (Claudianos et al., 2006; Darragh et al., 2021). The lower diversity of P450 genes may explain why bees are more susceptible to pesticides than other insects.

Conclusions

In this study, the insecticide fipronil was used to determine the LC10, LC25, and LC50 for the stingless bee *F. varia*. This species appears to be more resistant to fipronil than other Meliponini species, but less resistant than the honey bee, *A. mellifera*. According to the literature, environmental concentrations of fipronil exceed the 96-hour LC25 for *F. varia*, indicating a potential hazard to this species. The target taxa for this insecticide (ants) are reported to be far more resistant to fipronil than stingless bees, implying that necessary applications may pose disproportionate risks to non-target insects. The importance of this study lies in quantifying species-specific mortality thresholds to inform safer levels of pesticide use; moreover, the use of this pesticide threatens key pollinators, potentially causing environmental and economic losses given the essential role of pollination in plant reproduction.

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Authors' Contribution

MMG: Formal analysis, writing-original draft
DMS: Project administration, funding acquisition, writing-review & editing
PVAB: Conceptualization, methodology, funding acquisition, writing-review &editing

Data availability statement

All data generated from this study will be made available to interested parties upon request through contact with the corresponding author.

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