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Susceptibility of *Melipona scutellaris* Latreille (Hymenoptera: Apidae) to Biopesticides

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Abstract

Products based on entomopathogenic fungi are extensively used to control insect pests. These biopesticides can result in lethal or sublethal effects on non-target organisms. This study aimed to evaluate the survival of *Melipona scutellaris* Latreille workers exposed to commercial products based on *Beauveria bassiana* (IBCB 66), *Metarhizium anisopliae* (IBCB 425), and *Isaria fumosorosea* (ESALQ 1296). Fifty foragers were exposed to *B. bassiana* at 8.25×10^6 conidia/mL, *M. anisopliae* at 1.1×10^7 conidia/mL, and *I. fumosorosea* at 2.5×10^9 conidia/mL, using three routes of exposure (ingestion, contact with a surface, and topical application). Kaplan-Meier survival curves were estimated to determine the proportion of workers surviving after each application of the biopesticides. Workers exposed to *B. bassiana, M. anisopliae*, and *I. fumosorosea* presented survival rates of 74%, 34%, and 42%, respectively, after 168 hours of exposure by ingestion. For exposure via contact with a contaminated surface, survival rates were 64%, 70%, and 78%; for topical application, 68%, 66%, and 72% of the workers survived, respectively. The results highlight the variability in toxicity of biopesticides based on *M. anisopliae*, *B. bassiana*, and *I. fumosorosea* for *M. scutellaris* workers. Although all biopesticides resulted in mortality, bee survival rates varied depending on the exposure route. Understanding the effect of entomopathogenic fungi-based products on *M. scutellaris* may facilitate the development of strategies (such as timing and frequency of spraying) to reduce their impact on bees.

Introduction

Stingless bees (Apidae: Meliponini) stand out as pollinators in several crops (Heard, 1999; Sarto et al., 2005; Slaa et al., 2006; Giannini et al., 2020; Campbell et al., 2022; Roubik, 2023). *Melipona scutellaris* Latreille is a neotropical species, endemic to the vegetation of humid and sub-humid forests in Northeast Brazil (Alves et al., 2012; Camargo et al., 2023), that is important both for honey and pollen production (Oliveira et al., 2020; Nascimento et al., 2021).

The use of microbiological products has become an ally in pest control. Entomopathogenic fungi (EF) efficiently control insect vectors of diseases in several crops (Sharma & Sharma, 2021). However, fungi-based biopesticides can contaminate bees during foraging, ingestion of contaminated food, or through body contact or contact with contaminated surfaces, which results in the spread of conidia inside bee hives (Colombo et al., 2021). Like other pesticides, biopesticides can be pathogenic to pollinating insects such as bees (Potrich et al., 2018; Colombo et al., 2021). Their effects on non-target organisms as pollinators are best known on honeybees *Apis mellifera* L., *Apis cerana* Fabricius, and bumble bees (Hamiduzzaman et al., 2012; Ramanaidu & Cutler, 2012; Karise et al., 2015; Portilla et al., 2017; Challa et al., 2019; Colombo et al., 2021; Demirozer et al., 2022; Leite et al., 2022).

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There is very little knowledge about the effects of fungi-based pesticides on stingless bees. Some species assessed were *Melipona beecheii* Bennett (Toledo-Hernandez et al., 2016), *Melipona quadrifasciata* Lepeletier (Faita et al., 2023), *M. scutellaris* (Conceição et al., 2014), *Meliponula ferruginea* Lepeletier (Omuse et al., 2022a; 2022b), *Plebeia droryana* (Friese) (Faita et al., 2023), *Tetragonisca angustula* (Latreille) (Toledo-Hernandez et al., 2016; Almeida et al., 2022; Leite et al., 2022), *Scaptotrigona bipunctata* (Lepeletier) (Faita et al., 2023), *Scaptotrigona depilis* (Moure) (Leite et al., 2022), and *Scaptotrigona mexicana* Guérin-Meneville (Toledo-Hernandez et al., 2016).

Among the EF tested in bioassays with bees, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* are the most common. Toledo-Hernandez et al. (2016) investigated the susceptibility of the stingless bees *T. angustula*, *S. mexicana,* and *M. beecheii* to two *B. bassiana* isolates (Bea-TNK and BotaniGard), *M. anisopliae* (Meta-TNK and strain Ma-lu 01), and *I. fumosorosea* (strain Ifu-lu 01) via spraying, under laboratory conditions. Omuse et al. (2022a) evaluated the pathogenicity of five *M. anisopliae* isolates (ICIPE 7, ICIPE 20, ICIPE 62, ICIPE 69, and ICIPE 78) and one of *B. bassiana* (ICIPE 284) to *M. ferruginea* and *A. mellifera* workers, exposed via contact with contaminated surface. Leite et al. (2022) evaluated the effect of five concentrations of three commercial EF, *B. bassiana* PL63, *M. anisopliae* E9, and *Cordyceps fumosorosea* 1296, by topical contact and ingestion, on *S. depilis*, *T. angustula*, *A. mellifera*, and *Bombus terrestris* (L.), and Faita et al. (2023) assessed the effects of three commercial bioinsecticides formulated from *B. bassiana* (Boveril®WP), *M. anisopliae* (Metarril®WP), and *C. fumosorosea* (Octane®CS) on *M. quadrifasciata, P. droryana,* and *S. bipunctata* exposed by spraying. Additionally, recent studies have investigated the sublethal effects of EF on stingless bees. Omuse et al. (2022b) investigated the effect of *M. anisopliae* (ICIPE 69) on mortality, foraging behavior, and pollination success of *M. ferruginea* under semi-field conditions, and Almeida et al. (2022) assessed the effect of *B. bassiana* on *T. angustula* guard behavior under laboratory conditions.

The only EF whose effects on the stingless bee *M. scutellaris* have been investigated was *B. bassiana*. Conceição et al. (2014) studied the susceptibility of newly emerged workers of *M. scutellaris* to a *B. bassiana* isolate (Biofungi 1). The bees were exposed to contact with contaminated surfaces and topical application (spraying) using four concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia/ml). Considering the ecological and economic importance of stingless bees and the lack of knowledge about the effects of fungi-based insecticides on these pollinators, we tested the hypotheses 1) the survival of *M. scutellaris* workers is affected by biopesticides based on *B. bassiana, M. anisopliae*, and *I. fumosorosea*; 2) the survival of *M. scutellaris* workers is affected differently depending on the route of exposure to the fungi-based bioinsecticides.

Material and Methods

Preparation of the workers

Brood combs containing *M. scutellaris* pupae about to emerge were taken from colonies housed in nest boxes INPA model (20 cm long x 20 cm wide x 8 cm high) in the meliponary of the Federal University of the Recôncavo of Bahia, municipality of Cruz das Almas, Bahia, Brazil (12º39'20" W; 39º07'23" S). In the laboratory, the brood combs were placed in plastic trays, covered with voile fabric, and kept in BOD at a 28 ºC temperature. The emerged adults were marked with water-based correction fluid and transferred to a nest box kept inside the laboratory, with an opening to the external area. When *M. scutellaris* workers reached 25 days old, they started foraging activity (personal observation; see also Giannini et al., 1997; Matheus et al., 2019). So, these marked foragers were collected at the nest entrance to carry out the bioassays.

To carry out the experiments, workers were kept in plastic cages (100 mm in diameter x 50 mm in height) with two lateral holes to fit two microtubes (one with water and the other with 50% sucrose solution) (Leite et al., 2018).

Bioassays

Workers were subjected to the EF at three routes of exposure:

1) Ingestion of contaminated food (IN) - In this bioassay, we prepared the dilution for each microbiological product following the manufacturers' recommendations for pest control. For B. bassiana, the solution was prepared using 1.5 g of the product + 200 ml of distilled water + 0.01% of Tween 80% + 200 g of sugar. For M. anisopliae, the solution was prepared using 0.1 g of the product + 200 ml of distilled water + 0.01% of Tween 80% + 200 g of sugar. For I. fumosorosea, the solution was prepared using 80 µl of the product + 20 ml of distilled water + 0.01% Tween 80% + 20 g of sugar. Then, we added 1 ml of contaminated food to the feeders. The uncontaminated 50% sucrose solution was used as a control treatment, a single control for three microbiological insecticides.

2) Contact with a contaminated surface (CS): In this bioassay, the recommended dilution for pest control was applied directly on Petri dishes through a spray tower, using the concentrations of 8.25×10^6 conidia/mL of *B. bassiana*; 1.1×10^7 conidia/mL of *M. anisopliae* and 2.5×10^9 conidia/mL of *I. fumosorosea*. The Petri dishes were dried at room temperature for two hours and placed at the bottom of the cages. Distilled water was used in the control treatment.

3) Topical application (TA): In this bioassay, fungal suspension was applied directly on the back of the workers by spraying through a spray tower. Workers were previously anesthetized at -4° C for two minutes. Spraying with distilled water was used in the control treatment.

Bioassays were performed using pesticides based on *B. bassiana* (IBCB 66) (8.25 x 10^6 conidia/mL), *M. anisopliae* (IBCB 425) (1.1 x 10^7 conidia/mL) and *I. fumorosorosea* (ESALQ 1296) (2.5 x 10^9 conidia/mL) (Table 1).

A completely randomized design (CRD) was used to evaluate workers' survival rate. The treatments comprised three biopesticides, three route of exposure (IN, CS, TA), and a control for each route of exposure. Each cage had ten bees and was considered a repetition, and we used five replicates to assess mortality caused by each entomopathogenic fungus in each route of exposure, totaling 50 bees. Bee mortality was recorded at 11 intervals after the exposure (0, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168h) (adapted from Potrich et al., 2018).

Statistical analysis

The data were analyzed using the survival package (Therneau, 2024) and ggplot2 (Wickham et al., 2016) in R software, version 4.3.3 (R Core Team, 2023). Survival analysis was conducted using the Kaplan-Meier method to estimate survival curves for bioinsecticide and control groups. The log-rank test was also performed to compare the survival curves among treatment groups. Log-rank tests were conducted for all possible combinations between treatment groups. The z-test for the difference in proportions with Bonferroni adjustment was employed to evaluate the proportion of surviving bees at the end of the experiment.

Results

The analysis of survival curves of workers subjected to ingestion of biopesticides revealed differences ($\chi^2 = 19.3$; $P < 0.0001$) in survival rate, according to the Cox-Mantel Log Rank test. There was a difference between *B. bassiana* and *M. anisopliae* ($\chi^2 = 13.4$; $P < 0.0001$), between *B. bassiana* and *I. fumosorosea* ($\chi^2 = 9.0$; $P = 0.003$), between *B. bassiana* and control ($\chi^2 = 5.8$; $P < 0.02$), between *M. anisopliae* and control ($\chi^2 = 13.4$; $P < 0.0001$), and between *I. fumosorosea* and control ($\chi^2 = 28.0$; $P < 0.0001$). However, there was no difference in survival rate between *M. anisopliae* and *I. fumosorosea* treatments ($\chi^2 = 0.3$; $P = 0.6$) for *M. scutellaris* (Figure 1).

Table 1. Biopesticides characteristics: active ingredient, biological measures prepared by the manufacturer to control insect pests, body of the fungal suspension in the Petri dish (113 cm^2).

Active ingredient	Class	Dilution	Suspension	Target organism	Contact/Topic
Beauveria bassiana-IBCB 66	Inseticide	750 g/100 L	1.5 g/200 mL	*Bemisia tabaci*	0.0845 mL/p-0.9 s
Metarhizium anisopliae- IBCB 425	Inseticide	50 g/100 L	0.1 g/200 mL	*Acrogonia gracilis*	0.113 mL/p-1.2 s
Isaria fumosorosea ESALQ 1296	Inseticide	400 mL/100 L	80 µL/20 mL	*Diaphorina citri*	0.113 mL/p-1.2 s

According to MAPA's (Ministry of Agriculture and Animal Breeding -Brazil) recommendation.

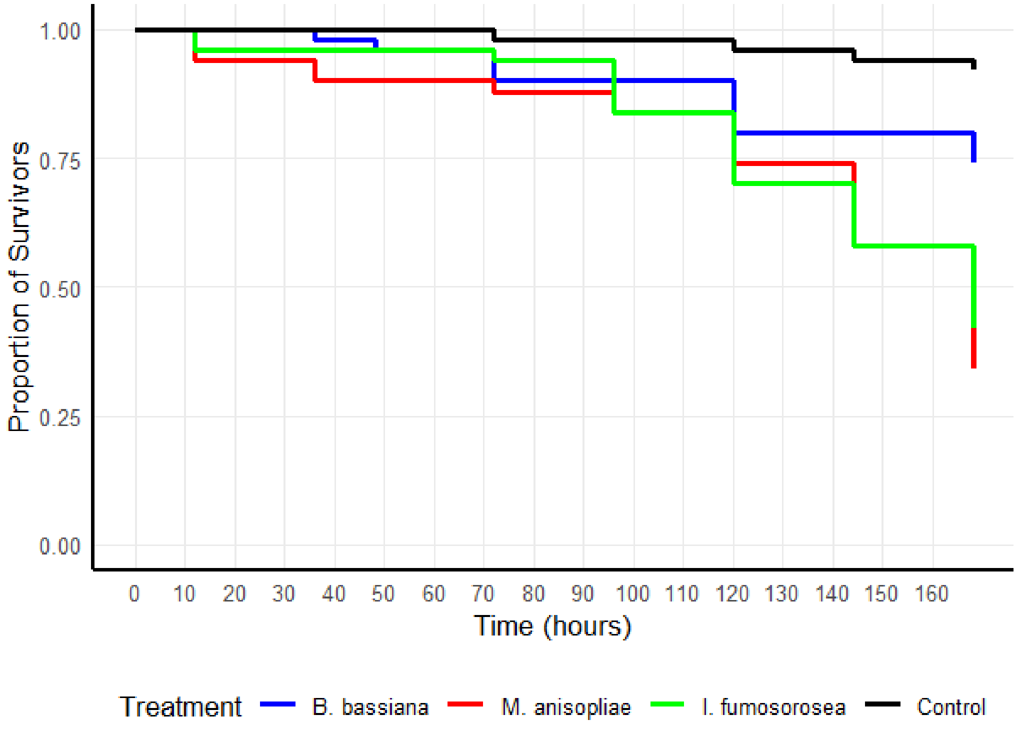

Fig 1. Survival curves of *Melipona scutellaris* workers plotted from the time of exposure via ingestion until death. The curves indicate the median and the 95% percentile, respectively.

The survival curves of bees exposed to contact with a surface contaminated with biopesticides showed differences ($\chi^2 = 18.7$; $P < 0.0001$) in survival rate, according to the Cox-Mantel Log Rank test. There was a difference in survival rate between *B. bassiana* and control ($\chi^2 = 18.6$; $P < 0.0001$), between *M. anisopliae* and control ($\chi^2 = 14.1$; $P < 0.0001$), between *I. fumosorosea* and control ($\chi^2 = 9.3$; $P = 0.002$). However, there was no difference for the biopesticides *B. bassiana* and *M. anisopliae* ($\chi^2 = 0.9$; $P = 0.3$), between *B. bassiana* and *I. fumosorosea* ($\chi^2 = 2.6$; $P = 0.1$), and between *M. anisopliae* and *I. fumosorosea* ($\chi^2 = 0.6$; $P = 0.4$) for *M. scutellaris* bees (Figure 2).

The survival curves of bees subjected to topical application of biopesticides showed differences ($\chi^2 = 19.3$; $P < 0.0001$) in survival rate, according to the Cox-Mantel Log Rank test. There was a difference in survival rate between *B. bassiana* and control ($\chi^2 = 18.9$; $P < 0.0001$), between *M. anisopliae* and control ($\chi^2 = 20.3$; $P < 0.0001$), and between *I. fumosorosea* and control ($\chi^2 = 16.2$; $P < 0.0001$). However, there was no difference between *B. bassiana* and *M. anisopliae* ($\chi^2 = 0$; $P = 0.9$), between *B. bassiana* and *I. fumosorosea* ($\chi^2 = 0.1$; $P = 0.8$), and between *M. anisopliae* and *I. fumosorosea* ($\chi^2 = 0.2$; $P = 0.7$) in the survival rate of *M. scutellaris* (Figure 3).

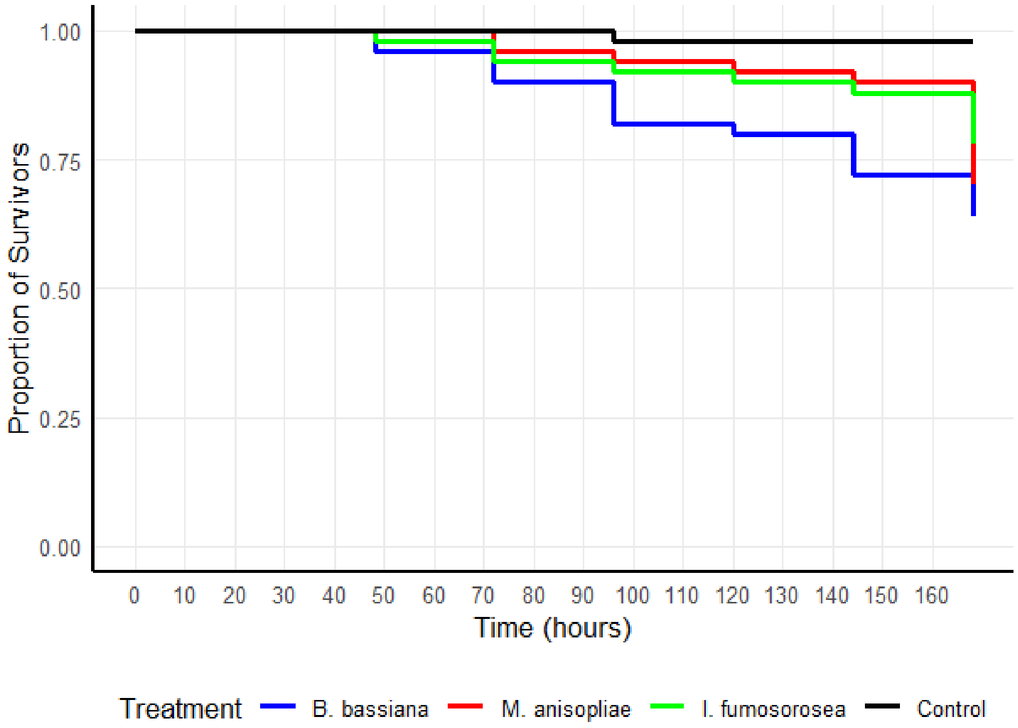

Fig 2. Survival curves of *Melipona scutellaris* workers plotted from the time of exposure via contact with a contaminated surface until death. The curves indicate the median and the 95% percentile, respectively.

All the biopesticides evaluated in the study caused mortality in *M. scutellaris* workers. The biopesticide based on *M. anisopliae* was quite toxic to *M. scutellaris* via ingestion, resulting in only 34% survival at the end of the experiment. However, this survival rate did not differ ($P > 0.05$) from biopesticide *I. fumosorosea*, which showed 42% survival. *B. bassiana* showed less toxicity than the other biopesticides ($P < 0.05$), with a bee survival rate of 74% at the end of the experiment. Notably, the control group exhibited higher survival ($P < 0.05$), with 92% survival after 168 hours. Workers exposed for 168 hours to contact with a surface contaminated with products based on *B. bassiana, M. anisopliae,* and *I. fumosorosea* did not differ from each other ($P > 0.05$) and exhibited survival rates of 64%, 70%, and 78%, respectively. The control group showed 98% survival of workers and differed ($P < 0.05$) from the other treatments. Workers exposed to the treatment of topical application with biopesticides based on *M. anisopliae*, *B. bassiana*, and I. *fumosorosea* did not differ from each other ($P > 0.05$). At the end of the experiment, they exhibited survival rates of 66%, 68%, and 72%, respectively. No mortality was observed in the control group exposed via topical application (Table 2).

Discussion

Route of exposure by ingestion

The three entomopathogenic fungi affected the survival of *M. scutellaris* workers exposed to the tested isolates by ingesting a contaminated diet. On this route of exposure, *M. anisopliae* and *I. fumosorosea* had a high impact on *M. scutellaris* workers and caused mortality similar to each other

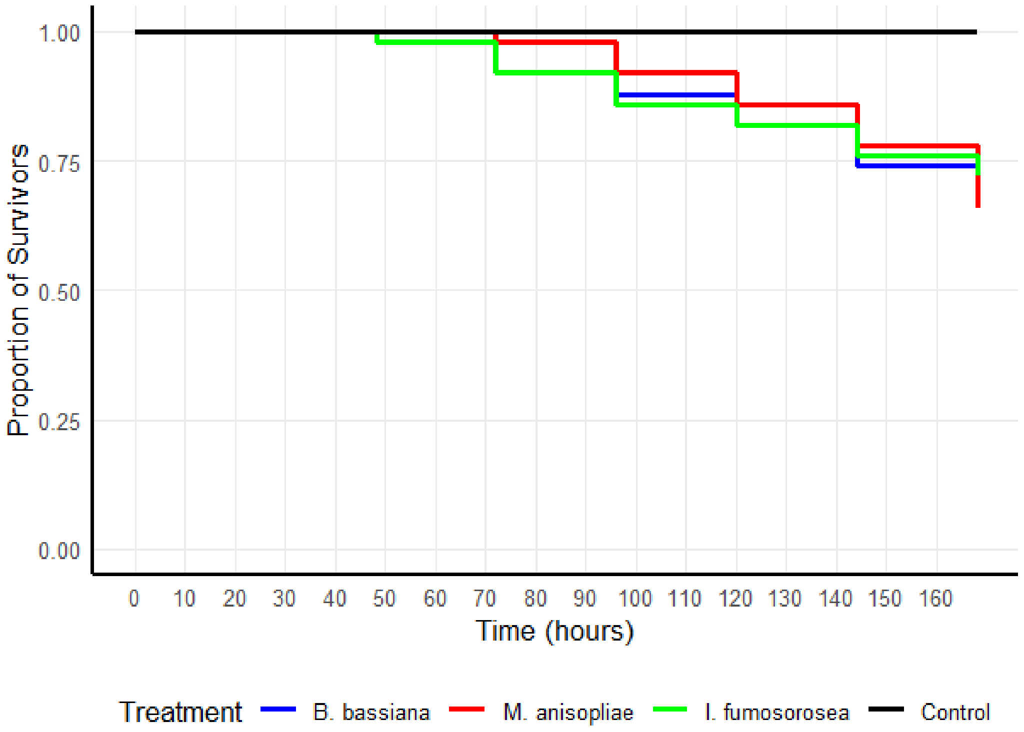

Fig 3. Survival curves of *Melipona scutellaris* workers plotted from exposure time via topical application until death. The curves indicate the median and the 95% percentile, respectively.

(66% and 58%, respectively) and significantly higher than that recorded by oral exposure to *B. bassiana* (26%). These results for *M. scutellaris* are similar to those found by Colombo et al. (2021), who also observed that *M. anisopliae* caused a significantly higher reduction in the survival of Africanized *A. mellifera* than the biopesticide *B. bassiana* when workers were orally exposed.

As observed in *M. scutellaris*, Leite et al. (2022) recorded high mortality in the stingless bees *S. depilis* (64%) and *T. angustula* (58%), and *B. terrestris* (64%) exposed orally to *M. anisopliae* E9. Potrich et al. (2018) and Colombo et al. (2021) also reported a high impact on honey bee survival when exposed by ingestion to *M. anisopliae* E9 (Potrich et al., 2018) and IBCB 425 (Colombo et al., 2021) isolates.

The *I. fumosorosea*-based product also caused high mortality (58%) in *M. scutellaris* exposed orally. This result agrees with those reported for other bee species. Leite et al. (2022) recorded high mortality caused by *C. fumosorosea* in the stingless bees *S. depilis* (66%) and *T. angustula* (70%), as well as in *B. terrestris* (92%) and *A. mellifera* (58%).

Table 2. Proportion of workers survival after 168 hours of ingestion, contact, and topical application with biopesticides and control.

Treatment	Pesticide	Survival after 168 hours (n = 50)		95% C.I.
		Estimative*	Stt. err	
Ingestion	*B. bassiana*	0.74a	0.0620	(0.63; 0.87)
	M. anisoplae	0.34b	0.670	(0.23; 0.50)
	I. fumosorosea	0.42b	0.0698	(0.30; 0.58)
	Control	0.92c	0.0384	(0.85; 1.00
Contact	*B. bassiana*	0.64a	0.0679	(0.52; 0.79)
	M. anisoplae	0.70a	0.0648	(0.58; 0.84)
	I. fumosorosea	0.78a	0.0586	(0.67; 0.90)
	Control	0.98b	0.0198	(0.94; 1.00)
Topical application	*B. bassiana*	0.68a	0.0660	(0.56; 0.82)
	M. anisoplae	0.66a	0.0670	(0.54; 0.81)
	I. fumosorosea	0.72a	0.0635	(0.61; 0.86)
	Control	1.00b	-	-

Proportions followed by different lowercase letters differ among the microbiological pesticides within each exposure route by the Bonferroni test.

The bioinsecticide based on *B. bassiana* also significantly reduced the survival of *M. scutellaris* via ingestion, but it caused a lower percentage of mortality (26%) than the other two biopesticides tested. An effect on reducing the longevity of Africanized *A. mellifera* fed with sweet paste contaminated with *B. bassiana* was found by Potrich et al. (2018) and Colombo et al. (2021), who recorded approximately 25% and 15% mortality, respectively, after six days (144h). Leite et al. (2022) found much higher mortality in *A. mellifera* (50%) as well as in *S. depilis* (80%), *T. angustula* (58%), and *B. terrestris* (66%) orally exposed to *B. bassiana.* The responses of bee species to exposure to *B. bassiana* via ingestion are pretty variable, and our results suggest that, under laboratory conditions, *M. scutellaris* is less sensitive to oral exposure to *B. bassiana* than the other stingless bees assessed.

Route of exposure by contact with a contaminated surface

The three EF tested affected the survival of *M. scutellaris* workers exposed via contact with a contaminated surface, causing significantly higher mortality than in the control, and there were no differences between the biopesticides. Our bioassay of exposure of *M. scutellaris* to *B. bassiana* (IBCB 66) at 8.25 x 10^6 conidia/ml via contact with a contaminated surface resulted in 36% worker mortality, similar to that previously observed by Conceição et al (2014) who reported around 24% mortality in *M. scutellaris* after 168h of contact with a surface contaminated with suspension containing a *B. bassiana* isolate (Biofungi 1) at 1 x 10^8 conidia/ml.

The responses of different bee species to contamination via residual contact with *B. bassiana* are heterogeneous, but there are few studies on stingless bees (Conceição et al., 2014). This EF caused 100% mortality in Africanized *A. mellifera* workers exposed via contact with smooth surface and soy leaves six days after the exposure (Potrich et al., 2018). Colombo et al. (2021) recorded around 40% mortality in Africanized *A. mellifera* exposed via contact with eucalyptus leaves contaminated with *B. bassiana*, although this reduction in survival did not differ from mortality in the control treatment. The bumble bee *B. terrestris* also suffered a high impact on survival (>60%) after exposure through contact with a surface contaminated with *B. bassiana* (Demirozer et al., 2022).

Exposure to *M. scutellaris* via contact with a surface contaminated with *M. anisopliae* also resulted in moderate mortality (30%). In contrast, Omuse et al. (2022a) found that none of the five isolates of *M. anisopliae* tested significantly affected the African stingless bee *M. ferruginea* survival, whose mortality ranged from 10.9 to 19.1%. These authors reported statistically significant mortality in African *A. mellifera* maintained in Kenya, ranging from 15.3-17.4%, exposed via contact with a contaminated surface with three *M. anisopliae* isolates (ICIPE 7, ICIPE 20, ICIPE 69). At the same time, there was no significant reduction in the survival of the honey bee in treatments with ICIPE 62 and ICIPE 78 isolates. In contrast, the survival of Africanized *A. mellifera* from Brazil was severely affected after workers were exposed to surfaces contaminated with *M. anisopliae* E9 (Potrich et al., 2018) and IBCB 425 isolates (Colombo et al., 2021), with approximately 90% mortality on the 6th day of exposure. Colombo et al. (2021) highlighted that discrepancies in the results of different studies may be related to using different isolates of an entomopathogenic fungus in bioassays, of different concentrations and different bees.

As in the treatments with *B. bassiana* and *M. anisopliae*, exposure of *M. scutellaris* via contact with a surface contaminated with *I. fumosorosea* 1296 isolate (2.5 x 10^9 conidia/ml) had an impact on the survival of *M. scutellaris*, resulting in 22% mortality by the 7th day. The effects of this EF on the survival of stingless bees exposed via contact with contaminated surfaces are still poorly investigated. Our results for *M. scutellaris* agree with those recently observed in *B. terrestris*, when exposed to a surface contaminated with the commercial biopesticide Priority (1.5% *I. fumosorosea*) reached 38% mortality (Demirozer et al., 2022).

Route of exposure by topical application

The topical application (TA) of all bioinsecticides caused significant mortality in *M. scutellaris,* and the three EF had a similar impact on worker survival. The bioinsecticide *M. anisopliae* (IBCB 425; 1.1 x 10^7 conidia/mL), when sprayed directly on *M. scutellaris,* resulted in 34% mortality. Other neotropical stingless bee species of several genera (see Toledo-Hernandez et al., 2016; Leite et al., 2022; Faita et al., 2023), and *A. mellifera* (Espinoza-Ortiz et al., 2011; Potrich et al., 2018; Colombo et al., 2021) also showed high susceptibility to different *M. anisopliae* isolates when topically applied.

Similar to what we found for *M. scutellaris*, Toledo-Hernandez et al. (2016) also observed significant mortality in *M. beecheii* (52%; 40%), as well as in *T. angustula* (95%; 60%) and *S. mexicana* (38%; 30%) topically exposed to two *M. anisopliae* isolates (strain Ma-lu 01 and Meta-TNK) at 1 x 10^9 conidia/ml. These authors reported that among three EF tested (*M. anisopliae, B. bassiana,* and *I. fumosorosea*) using this route of exposure, *M. anisopliae* isolates caused the highest mortalities in these three stingless bees. Stingless bees topically exposed to a commercial bioinsecticide Metarril®WP formulated from *M. anisopliae* at 10^7 conidia/ml showed a wide variation in mortality, with *M. quadrifasciata* being the most affected among the three species assessed (61% mortality) (Faita et al., 2023). Likewise, Leite et al. (2022) found that *M. anisopliae* E9 isolate, when topically applied, significantly affected the survival of *T. angustula*, causing the death of 50% of workers, and the survival of *S. depilis* was affected with two of the five concentrations tested, resulting in a mean mortality of 38%.

The *B. bassiana* IBCB 66 isolate at 8.25 x 10^6 conidia/ml caused a significant reduction in survival when sprayed directly on *M. scutellaris* workers (32% mortality). This finding agrees with the previous study by Conceição et al. (2014), which highlighted that *B. bassiana,* the Biofungi 1 isolate, was virulent to *M. scutellaris*, as it caused the death of 40% of workers sprayed with a solution at 1 x 10^8 conidia/ml within 168h (7 days). Assessing the mortality caused by *B. bassiana* solutions at different concentrations, they observed that the corrected mortality in 10 days post-exposure was 56% in the treatment using the lowest concentration (10^5 conidia/ml) and reached 85% at 10^8 conidia/ml.

As observed for *M. scutellaris*, other stingless bee species, as well as *A. mellifera* and *B. terrestris,* also had survival affected by topical exposure to *B. bassiana* (Mommaerts et al., 2009; Potrich et al., 2018; Colombo et al., 2021; Leite et al., 2022; Demirozer et al., 2022; Faita et al., 2023). Mortality caused by two *B. bassiana* isolates in another species of the genus *Melipona* (*M. beecheii*) was 28% (Bea-TNK) and 30% (Botanigard - GHA) at 10^9 conidia/ ml (Toledo-Hernández et al., 2016), similar to that we found for *M. scutellaris.* These authors found mortality between 20-30% in *S. mexicana* and 4-20% in *T. angustula*, and they concluded that these isolates are not detrimental to these bee species, as well as *M. beecheii*, as the mortality caused was <30%. In contrast, another species of *Melipona* assessed (*M. quadrifasciata*), suffered a high impact (66.3% mortality) when topically exposed to the commercial bioinsecticide Boveril®WP formulated from *B. bassiana*. This bioinsecticide also affected the stingless bees *P. droryana* and *S. bipunctata*, causing mortality of 43.3 and 42.6%, respectively, and it was considered slightly harmful to these species and moderately harmful to *M. quadrifasciata* (Faita et al., 2023). Leite et al. (2022) found high mortality in *S. depilis* (76%), as well as in *T. angustula* (48%) exposed to *B. bassiana* PL63 isolate. In contrast, Omuse et al. (2022a) reported that the survival of the African stingless bee *M. ferruginea*, as well as the honey bee *A. mellifera* was not significantly affected by topical application of the *B. bassiana* ICIPE 284 isolate, as also observed by some studies on *A. mellifera* (but see Potrich et al., 2018; Colombo et al., 2021; Leite et al., 2022).

The *I. fumosorosea* 1296 isolate at 2.5 x 10^9 conidia/ml caused significant mortality in *M. scutellaris* workers (28%) when topically applied. These results agree with those obtained by Leite et al. (2022) in bioassays with the stingless bees *S. depilis* and *T. angustula* (mean mortality 48% each) and *A. mellifera* (30%) after exposure to the same isolate (1296), and by Demirozer et al. (2022) in *B. terrestris* (34% mortality) topically exposed to the commercial biopesticide Priority (1.5% *I. fumosorosea*, strain PFs-1; 1 x 10^8 CFU/ml). Faita et al. (2023) found a much higher mortality rate in *M. quadrifasciata* (95%), *P. droryana* (80%), and *S. bipunctata* (74.7%) topically exposed to Octane®CS, a commercial bioinsecticide formulated from *C. fumosorosea.* Faita et al. (2023) highlighted that bioinsecticides formulated as concentrated solutions, such as Octane®CS, are more harmful to bees than wettable powder (WP) bioinsecticides. In contrast, Toledo-Hernandez et al. (2016) found little impact of *I. fumosorosea* (strain Ifu-lu 01 at 10^9 conidia/ml) on the mortality of three other stingless bee species, *M. beecheii*, *T. angustula* (<10% each), and *S. mexicana* (< 20%).

Our results showed that all microbiological products tested caused a reduction in *M. scutellaris* workers' survival, regardless of the route of exposure. *M. anisopliae* was more toxic to workers when fed a contaminated diet, followed by *B. bassiana* and *I. fumosorosea.*

As highlighted by other authors, our study also revealed differences in the responses of bee species exposed to different EFs and routes of exposure. The differences found between studies are influenced by several factors, among them, bee species assessed, different EF isolates tested, variations in solution concentrations, variations in the age of workers, route of exposure used, and period of application of the product (Mommaerts et al., 2009; Ramanaidu & Cutler, 2012; Colombo et al., 2021; Leite et al., 2022). It is essential to carry out selectivity tests regarding natural enemies and pollinating insects, including stingless bees, due to their importance in ecosystems in producing honey, pollen, and crop pollination.

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Authors' Contribution

JBV: conceptualization, investigation, resources, writing-original draft.
CMLA: conceptualization, writing-original draft, writing-review, and editing.
EDC: formal analysis, designing the figures, writing-review and editing.
MF: conceptualization, methodology, writing-original draft.
CADB: conceptualization, methodology, writing-original draft.
CALC: conceptualization, methodology, writing-original draft, writing-review and editing, funding acquisition.

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References

Almeida, F.C.R., Magalhães, D., Favaris, A.P., Rodríguez, J., Azevedo, K.E.X., Bento, J.M.S. & Alves, D.A (2022). Side effects of a fungal biopesticide without guarding behavior of

stingless bees. Chemosphere, 287: 132147.
https://doi.org/10.1016/j.chemosphere.2021.132147

Alves, R.M., Carvalho, C.A., Souza, B.A., & Santos, W.S. (2012). Areas of natural occurrence of *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) in the state of Bahia, Brazil. Anais da Academia Brasileira de Ciências, 84: 679-688.

Camargo, J.M.F., Pedro, S.R.M & Melo, G.A.R. (2023). Meliponini Lepeletier, 1836. In Moure, J. S., Urban, D. & Melo, G. A. R. (Orgs). Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version. Available at https://www.moure.cria.org.br/catalogue. Accessed Apr/22/2024

Campbell, A.J., Lichtenberg, E.M., Carvalheiro, L.G., Menezes, C., Borges, R.C., Coelho, B.W.T., Freitas, M.A.B., Giannini, T.C., Leão, K.L., Oliveira, F.F., Silva, T.S.F. & Maués, M.M. (2022). High bee functional diversity buffers crop pollination services against Amazon deforestation. Agriculture, Ecosystems & Environment, 326: 107777.
https://doi.org/10.1016/j.agee.2021.107777

Challa, G.K., Firake, D.M. & Behere, G.T. (2019). Bio-pesticide applications may impair the pollination services and survival of foragers of honey bee, *Apis cerana* Fabricius in oilseed brassica. Environmental Pollution, 249: 598-609.
https://doi.org/10.1016/j.envpol.2019.03.048.

Colombo, F.C., Maciel, R.M., Abati, R., Raulino-Domanski, F., Longhi, S.J., Costa-Maia, F.M., & Potrich, M. (2021). Do *Beauveria bassiana* and *Metarhizium anisopliae* affect worker survival and the production of Africanized *Apis mellifera* queens? Journal of Apicultural Research, 60: 260-269.
https://doi.org/10.1080/00218839.2020.1828237

Conceição, J.P., Lyra, N.C.M., Sodré, G.S., Carvalho, C.A.L., Souza, A.V., Ribeiro, G.S., & Campos, P.R. (2014). Susceptibility of *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) worker bees to *Beauveria bassiana* (Bals.) Vuill. Sociobiology, 61: 184-188.
https://doi.org/10.13102/sociobiology. v61i2.184-188

Demirozer, O., Uzun, A. & Gosterit, A. (2022). Lethal and sublethal effects of different biopesticides on *Bombus terrestris* (Hymenoptera: Apidae). Apidologie, 53: 24.
https://doi.org/10.1007/s13592-02200933-6

Espinoza-Ortiz, G.E., Lara, R.J., Otero, C.G., Alatorre, R.R. & Valdez, C.J. (2011). Susceptibilidade de larvas, pupas y abejas adultas a aislamientos de *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Sorokin) y *Paecilomyces fumosoroseus* (Wize). Interciencia, 36: 148-152.

Faita, M.R., Pereira, E. & Poltronieri, A.S. (2023). Effect of entomopathogenic fungus-formulated bioinsecticides on stingless bees (Hymenoptera: Apidae: Meliponini) in the laboratory. Journal of Apicultural Research, 62: 1-8.
https://doi.org/10.1080/00218839.2023.2230717

Giannini, K.M. (1997). Labor Division in *Melipona compressipes fasciculata* Smith (Hymenoptera: Apidae: Meliponinae). Anais da Sociedade Entomológica do Brasil, 26: 153-162

Giannini, T.C., Alves, D.A., Alves, R., Cordeiro, G.D,. Campbell, A.J., Awade, M., Bento, J.M.J., Saraiva, A.M. & Imperatriz-Fonseca, V.L. (2020). Unveiling the contribution of bee pollinators to Brazilian crops with implications for bee management. Apidologie, 51: 406-421.
https://doi.org/10.1007/s13592-019-00727-3

Hamiduzzaman, M.M., Sinia, A., Guzman-Novoa, E., & Goodwin, P.H. (2012). Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, *Varroa destructor*, and their effect on the immune response of honey bees (*Apis mellifera* L.). Journal of Invertebrate Pathology, 111: 237-243.
https://doi.org/10.1016/j.jip.2012.09.001

Heard, T.A. (1999). The role of stingless bees in crop pollination. Review of Entomology, 44: 183-206.
https://doi.org/10.1146/annurev.ento.44.1.183.

Karise, R., Muljar, R., Smagghe, G., Kaart, T., Kuusik, A., Dreyersdorff, G., Williams, I.H., & Mand, M. (2016). Sublethal effects of kaolin and the biopesticides Prestop-Mix and BotaniGard on metabolic rate, water loss and longevity in bumble bees (Bombus terrestris). Journal of Pest Science, 89: 171-178. https://doi.org/10.1007/s10340-015-0649-z

Leite, D.T., Sampaio, R.B., Santos, C.O., Santos, J.N., Chambó, E.D., De Carvalho, C.A.L., & Silva Sodré, G. (2018). Toxicity of fenpyroximate, difenoconazole and mineral oil on *Apis mellifera* L. Sociobiology, 65: 737-743.
https://doi.org/10.13102/sociobiology. v65i4.3416

Leite, M.O.G, Alves, D.A., Lecocq, A., Malaquias, J.B., Delalibera Jr., I., & Jensen, A.B. (2022). Laboratory risk assessment of three entomopathogenic fungi used for pest control toward social bee pollinators. Microorganisms, 10: 1800.
https://doi.org/10.3390/microorganisms10091800

Mateus, S., Ferreira-Caliman, M.J., Menezes, C., Grüter, C. (2019). Beyond temporal-polyethism: division of labor in the eusocial bee *Melipona marginata*. Insectes Sociaux.
https://doi.org/10.1007/s00040-019-00691-2

Mommaerts V, Sterk G, Hoffmann L, Smagghea G (2009). A laboratory evaluation to determine the compatibility of microbiological control agents with the pollinator *Bombus terres*tris. Pest Management Science 65: 949-955.
https://doi.org/10.1002/ps.1778

Nascimento, A.S., Silva, F.L., Silva, S.M.P.C., Sodré, G.S., Carvalho, C.A.L. (2021). Physicochemical Quality and Bioactive Compounds of the Honey of *Melipona scutellaris* Produced in an Urban-Industrial Region. Revista Virtual de Química, p. 1268.
https://doi.org/10.21577/1984-6835.20210071

Oliveira, D.J., Santos, D.R., Andrade, B.R., Nascimento, A.S., Silva, M.O., Merces, C.C., Lucas, C.I.S., Silva, S.M.P.C., Carvalho, P.D., Silva, F.L., Estevinho, L.; Carvalho, C.A.L. (2020). Botanical origin, microbiological quality, and physicochemical composition of the *Melipona scutellaris* pot-pollen (samburá) from Bahia (Brazil) Region. Journal of Apicultural Research, 59: 1-13. https://doi.org/10.1080/00218839.2020.1797271

Omuse, E.R., Niassy, S., Wagacha, J.M., Ong'amo, G.O., Michael, H., Lattorff, H. M.G., Kiatoko, N., Mohamed, S.A., Subramanian, S., Akutse, K.S., Dubois, T. (2022a). Susceptibility of the Western Honey Bee *Apis mellifera* and the African Stingless Bee *Meliponula ferruginea* (Hymenoptera: Apidae) to the Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Journal of Economic Entomology, 115:46-65. https://doi.org/10.1093/jee/toab211

Omuse, E.R., Niassy, S., Kiatoko, N., Lattorff, H. M.G., Wagacha, J.M. & Dubois, T. (2022b). A fungal-based pesticide does not harm pollination service provided by the African stingless bee *Meliponula ferruginea* on cucumber (*Cucumis sativus*). Apidologie, 53: 28. https://doi.org/10.1007/s13592-022-00938-1

Portilla, M., Luttrell, R., Snodgrass, G., Zhu, Y.C. & Riddick, E. (2017). Lethality of the entomogenous fungus *Beauveria bassiana* strain NI8 on *Lygus lineolaris* (Hemiptera: Miridae) and its possible impact on beneficial arthropods. Journal of Entomological Science, 52: 352-369. https://doi.org/10.18474/JES17-15.1

Potrich, M.; Silva, R.T, Maia, F.M., Lozano, E.R., Rossi, R.M. & Colombo, F.C., Gouvea, A. (2018). Effect of entomopathogens on africanized *Apis mellifera* L. (Hymenoptera: Apidae). Revista Brasileira de Entomologia, 62: 23-28. https://doi.org/10.1016/j.rbe.2017.12.002.

R Core Team. (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Ramanaidu K. & Cutler GC (2012). Diferent toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana, Bacillus subtilis* and spirotetramat. Pest Management Science 69: 949-954. https://doi.org/10.1002/ps.3456

Roubik, D.W. (2023). Stingless Bee (Apidae: Apinae: Meliponini) Ecology. Annual Review of Entomology, 68: 231-56. https://doi.org/10.1146/annurev-ento-120120- 103938

Sarto, D.E.L., Peruquetti, M.C.L. & Campos, R.C.L. (2005). Evaluation of the neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes. Journal of Economic Entomology, 98: 260-266. https://doi.org/10.1093/jee/98.2.260

Slaa, E.J., Chaves, L.A.S., Malagodi–Bragac, K.S., Hofsteded, F.E. (2006). Stingless bees in applied pollination: practice and perspectives. Apidologie, 37: 293-315. https://doi.org/10.1051/apido:20060 22

Sharma, R. & Sharma, P. (2021). Fungal entomopathogens: a systematic review Egyptian Journal of Biological Pest Control, 31: 57. https://doi.org/10.1186/s41938-021-00404-7

Therneau T. (2024). A package for survival analysis in R. R package version 3.5-8. URL: https://cran.r-project.org/package=survival.

Toledo–Hernandez, R.A., Ruiz–Toledo, J., Toledo, J., Sanchez, D. (2016). Effect of three entomopathogenic fungi on three species of stingless bees (Hymenoptera: Apidae) under laboratory conditions. Journal of Economic Entomology 109: 1015-1019. https://doi.org/10. 1093/ jee/ tow064.

Wickham, H. (2016). ggplot 2: elegant graphics for data analysis. New York: Springer.