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Survival of Indian Honey Bee *Apis Cerana indica* Fabricius exposed to Microbial Control Agents: Insights into Its Pathogenicity and Toxicity under Varied Exposure Methods

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Abstract

This study investigates the effects of commercially available entomopathogenic fungal formulations on the Indian honey bee *Apis cerana indica* Fabricius (Hymenoptera: Apidae). A 10-day bioassay conducted under laboratory conditions used four methods (topical, spray, leaf dip, feeding) to evaluate the pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae), *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Cordycipitaceae), *Metarhizium sp*. (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae), and *Lecanicillium sp*. (Zimmermann) Zare & Gams (Hypocreales: Cordycipitaceae) on *A. cerana indica* at 5×10^8 conidia/ml. After exposure to fungal formulations via different methods, we recorded conidial acquisition by bees ($4.36 \times 10^4 – 21.08 \times 10^4$ conidia/bee). The mortality rates were significantly lower in the topical (6.58%), leaf dip (10.17%), spray (9.92%), and feeding (9.17%) exposure methods, indicating their nontoxicity (< 25% mortality) according to IOBC classification. Among the fungal formulations tested, *Lecanicillium* (93.25%) recorded significantly higher survival rates. Mycosis percentage (3.66 – 15%) was observed in the 10-day-old moribund bees. The lethal time (LT) estimates, viz., LT_{10} and LT_{25}, were comparatively lower in the topical exposure method (LT_{10}: 13.4 – 16.08 days, LT_{25}: 17.08 – 19.68 days). However, the effects of entomopathogenic fungal formulations must be further confirmed under field conditions.

Introduction

In the recent era of sustainable agriculture, biocontrol agents have emerged as an alternative to pesticides in agricultural production (Chaudhary et al., 2024). Among these, the commercially available entomopathogens are considered economical, eco-friendly, effective, and safe to non-target organisms (Tomar & Yadav, 2024), playing a crucial role in integrated pest management (IPM) programs and contributing to sustainable agriculture. Due to their increased adoption and effectiveness, the entomopathogens market has expanded in Asia, Europe, and the United States (Leite et al., 2022) for field and horticultural crops.

Over 170 mycoinsecticides have been developed using entomopathogenic fungi, which are available in the market for use due to their broad host ranges and effectiveness against various agricultural pests. Fungi of the phylum Ascomycota, particularly of the genera such as *Beauveria*, *Metarhizium*, *Isaria*, *Lecanicillium*, and *Paecilomyces* (Mascarin & Jaronski, 2016), possess broad-spectrum insect pathogenic activity, infecting the insect species of Coleoptera, Lepidoptera, Diptera, and Hymenoptera (Castro et al., 2016).

As the application of entomopathogenic fungi has become more frequent and widespread, concerns have been raised regarding their non-target effects on beneficial arthropods, such as pollinators, predators, and parasitoids (Bamisile et al., 2021). Many crops, such as mango, guava, tomato, chilli, and cucumber, benefit from entomopathogenic fungi-based pest control and rely on pollination services by bees, including both *Apis* and non-*Apis* species (Klein et al., 2007). Under favorable environmental conditions and at standard application doses, entomopathogenic fungi may potentially infect non-target insects through contact, ingestion, or entry via natural body openings (Leite et al., 2022).

Although several bioassays have already been conducted to evaluate the effects of entomopathogenic fungi on various bee species, including *Melipona beecheii* (Toledo-Hernandez et al., 2016)*, M. scutellaris* (Vieira et al., 2024)*, M. ferruginea* (Omuse et al., 2022)*, Scaptotrigona depilis* (Toledo-Hernandez et al., 2016)*, S. mexicana* (Toledo-Hernandez et al., 2016)*, Tetragonisca angustula* (Toledo-Hernandez et al., 2016)*, Bombus terrestris* (Akkoç et al., 2019) and *Apis mellifera* (Potrich et al., 2018; Akkoç et al., 2019; Omuse et al., 2022), a significant knowledge gap remains concerning the effects of these factors on *A. cerana indica,* a vital native pollinator in South and Southeast Asian countries.

A. cerana indica is an indigenous species of the Indian subcontinent that plays a vital role both in honey production and crop pollination. Unlike the exotic *Apis mellifera*, which has dominated beekeeping in northern India, *A. cerana indica* remains more prominent in southern states where it is better adapted to local ecological conditions and coevolved with native flora (Kamaraj & Rasappan, 2024). Colonies of this species are smaller in size yet highly resilient, and their survival is strongly linked to efficient foraging behavior. Most floral visits occur in the early morning when lower temperatures and higher humidity are favorable, while elevated temperatures above 30 °C and rainfall reduce activity (Kamaraj & Rasappan, 2024). The pollination efficiency index of the species was exceptionally high (747,035.5), and colonies placed in the crop ecosystem showed substantial growth, including a 67.85% increase in sealed honey area and a 15.07% rise in adult population (Murali et al., 2021).

Management practices such as migratory beekeeping have proven particularly effective with *A. cerana indica*, ensuring colony survival during the dearth period and improving productivity. By relocating colonies to landscapes with sequentially blooming resources such as teak, eucalyptus, cashew, moringa, and sesame, beekeepers can secure four to five harvests annually, making the system more profitable than stationary beekeeping (Sharma et al., 2013; Martinez-Lopez et al., 2022; Kamaraj & Rasappan, 2024). The economic significance of this species extends far beyond honey yields; its pollination services are crucial in preventing yield losses in cross-pollinated crops, which in India are estimated at ₹10,000–55,000 per hectare when pollinators are scarce (Mohapatra et al., 2019).

Therefore, the current study aims to assess the direct effects of four of the most commercialised entomopathogenic fungi, such as *B. bassiana, B. brongniartii, Metarhizium,* and *Lecanicillium,* on *A. cerana indica* by topical, spray, leaf dip, and oral exposure methods (Ausique et al., 2017; Castro et al., 2016). It is hypothesized that while these fungi act against target pests (Table 1), they may also have sublethal or lethal effects on *A. cerana indica* under different exposure modes.

Materials and Methods

The study was conducted in accordance with guidelines from the Organization for Economic Cooperation and Development (OECD) for testing the acute contact toxicity of honey bees (Organization for Economic Cooperation and Development [OECD], 1998).

Collection of bees

The Indian honey bee colonies were maintained in Newton hives for the study in the Insectary (11.0167° N, 76.9292° E), Department of Agricultural Entomology, Tamil Nadu Agricultural University, from October to December 2024. Colony strength metrics were assessed in relation to the health of naturally mated colonies (Medrzycki et al., 2013). Before the bioassay, the colonies were visually examined to determine the pest- and disease-free status of the brood and colony members. The foragers returning to the hive and bees present in the super chamber were collected between 0800-1100 hours and immobilized by refrigerating them at 4 ºC for a few seconds and transferred to a wooden box (25 ± 2 ºC,

Table 1. Overview of entomopathogenic fungal isolates that were assessed for pathogenicity to *Apis cerana indica* Fabricius.

S. No.	Treatment	Trade Name, Formulation and Concentration (Conidia/ml)	Target Pest	Geographical Origin
1	*Beauveria bassiana*	Bannari Larval Hunter (Dust, 5 ×10^8)	Fall Army Worm, European Corn Borer, Codling moth	Asia
2	*Beauveria brongniartii*	Bannari Larval Hunter (Dust, 5 ×10^8)	*Melolontha, Epilachna*	France
3	*Metarhizium sp.*	Bannari Grub Hunter (Dust, 5 × 10^8)	Grubs, Termite, Locust	India
4	*Lecanicillium sp.*	Bannari Pest Hunter (Dust, 5 × 10^8)	Whitefly, Aphid	Asia

Data on target pest were given in Zimmermann (2007); Saharwat et al., (2021); Liu et al., (2023).

65 ± 5% relative humidity, 0:24 Light: Dark photoperiod) with sugar syrup (1:1 w/v cane sugar: water) ad libitum for 2 days. The adequately fed, healthy, disease-free bees were visually selected for the bioassay.

Fungal Material

The commercially available entomopathogenic fungi *B. bassiana* (Bannari Larval Hunter®), *B. brongniartii* (Banari Larval Hunter®), *Metarhizium* (Bannari Grub Hunter®), and *Lecanicillium* (Bannari Pest Hunter®) were obtained from the Agri Natural Fertiliser Unit of Bannari Amman Sugars Limited, Tamil Nadu, India. Hundred µL in each of these formulation suspensions was cultured individually on potato dextrose agar to determine their concentrations. Each fungus was scraped using a glass rod and rinsed in glass tubes containing distilled water and 0.05% Tween 80. A homogenous conidial suspension was obtained by vortexing the glass tubes, and the suspension was serially diluted to determine the concentration. Two hundred µL was pipetted out from the lowest dilution, adjusted to 0 in the Neubauer hemocytometer, and conidia were counted. While counting the conidia under the phase contrast microscope (Primostar 3, Carl Zeiss, Suzhou, China) (×40X magnification), it was considered viable if its germination tube was two times longer than its width (Goettel & Inglis,1997). Furthermore, an even concentration of 5×10^8 was ensured in all plates before exposing the bees to fungal cultures during the bioassay.

Fungal Exposure Methods

The assay was conducted using four exposure methods (topical, feeding, spray, and leaf dip), with five treatments comprising 5×10^8 conidia concentration each of *B. bassiana, B. brongniartii, Metarhizium, Lecanicillium,* and control (water with 0.05% Triton X-100) and three replications. Five µg of formulated microbial product dust were applied to the pronotum of the test worker bees in the topical application method and held for 20 seconds to spread. Bees were given a fungal formulation mixed sugar solution (1:1 w/v) *ad libitum* as part of the feeding method. Ten mg dust formulation was evenly spread on a Whatman™ Grade 1 qualitative filter paper dipped in sugar solution (1:1 w/v) and used for the spray exposure. The sprayed filter paper was air dried for 10 minutes before introduction into the cage. Cotton (MCU 5 variety) leaves were used in the leaf dip method, and protocols were followed similar to those used in the spray exposure method. The exposure to entomopathogenic fungi was controlled in topical application, while in the other treatments, the bees might not receive equal amounts of inoculum. Hence, it was assumed that the amount of inoculum exposure was proportional to the body size of the bees. Sterile 0.05% Triton X-100 was used as a control in the topical, spray, and leaf dip methods, while plain sugar solution was used as a control in the feeding method. Bees visit flowers and leaves of plants sprayed with entomopathogenic fungi, and the conidia become attached to the bees' legs (Butt & Goettel, 2000). Hence, spray and leaf dip methods were used to inoculate the fungal entomopathogen on the exposed bees.

Testing Cages

A total of 60 cylindrical plastic cages (4 cm in height and 10 cm in diameter) were designed, with the bottom (8 cm in diameter) and two sides (1.5 cm²) covered by wire mesh for ventilation. Ten bees per replication were released using a soft tweezer, and a sugar solution (1:1 w/v) was provided in glass vials (5 × 10 × 2 mm) to each cage individually. The bioassay was maintained for 10 days, and daily mortality was recorded.

Conidial Acquisition by Fungal-Exposed Bees

To assess conidial acquisition by bees, a 0.05% Triton X-100 solution was prepared, and bees were immersed in the solution for a minute. Then, the exposed bees were ground using a sterile pestle and mortar with 1 ml of distilled water and centrifuged at 1000 rpm for 5 minutes. The supernatant with dislodged conidia was carefully collected without disturbing the sedimented body segments and enumerated using an improved Neubauer hemocytometer. Then, the conidial concentration was calculated using the following formula (Omuse et al., 2022):

$$\text{Conidia (cells per ml)} = \frac{\text{Average conidia per square} * \text{Dilution factor}}{\text{Volume of square (ml)}}$$

Fungal Conidiogenesis

The moribund bees in all methods and treatments were collected, surface sterilized with 70% ethanol for 3 minutes and 3% sodium hypochlorite for 1 minute, and kept in a Petri dish (90 mm) with moistened Whatman™ Grade 1 qualitative filter paper in controlled conditions (25 ± 2 °C, 0:24 L:D) to confirm the fungal conidiogenesis. Mycosis percent was observed for 2-7 days to determine the development of white-colored conidial outgrowth from the exposed bees, and its identity was compared with the mother cultures. Conidial identity was further confirmed by preparing slides from the mycelial outgrowth.

Statistical Analysis

Data were analyzed using R software version 4.4.2 (R Core Team, 2020). Everyday mortality was subjected to Abbott's correction to adjust the treatment mortality according to control mortality (Abbott, 1925). Kaplan-Meier Survival estimator curves (Kaplan & Meier, 1958) were generated using the survival (Therneau, 2024) and survminer

(Kassambara & Kosinski, 2023) packages with cage members as a random factor. The lsmeans package (Lenth, 2016) was used to separate means with Bonferroni-adjusted p-values. The LT_{10} and LT_{25} regression slopes, along with 95% fiducial limits (FL), were estimated using adjusted mortality in the ecotox package (Hlina et al., 2021), based on probit regression (Finney, 1971). The overlapping 95% FL ($\alpha = 0.05$) estimates were used to assess the difference in LT. A multicomp package (Hothorn et al., 2008) with Tukey's adjusted p-values was used to separate means of mycosis percent, and a generalised mixed effect model with log distribution using the glmer function from the lme4 package (Bates et al., 2015) was used in estimation, where cage members acted as a random factor. The lmer function with the lme4 package was used to analyze conidial acquisition data after log transformation, implementing a linear mixed-effect model (Bates et al., 2015). Filter paper spraying acted as a random factor, and mean values were separated with Tukey's adjusted p-values.

Results

Survival of A. cerana indica on post-exposure to Entomopathogenic fungi

Post-exposure survival of *A. cerana indica* exposed to entomopathogenic fungi under different methods is summarized in Fig 1. Significant differences were observed in the survival of bees across various exposure methods and treatments. The survival rate in topical methods (93.73%) was higher than in leaf dip (90.53%), spray (90.4%), and feeding (90.3%) exposure methods, which were comparable to one another. The interaction between exposure methods and treatment was nonsignificant. A significantly lower mortality and higher survival rate of bees were recorded in the treatment with the commercial formulation of *Lecanicillium*, with corrected mortality ranging from 2.66% to 7.33% in the 10-day bioassay across all exposure methods. However, among the fungal treatments, there was no significant difference in the survival of bees in the feeding exposure method. A lower survival rate was recorded in *B. brongniartii* (88.7%) under topical exposure. In the spray method, a statistically on par level of low survival rate was recorded for all the agents used, *B. brongniartii* (88.3%), *B. bassiana* (86.3%), and *Metarhizium* (85%). In the leaf dip exposure method, the level of lower survival rates was recorded at par for *Metarhizium* (86%) and *B. bassiana* (86.7%).

Time Response Mortality

Regression slopes, lethal time (LT_{10}, LT_{25}), and the fiducial limits on the effects of the entomopathogenic fungi against *A. cerana indica* under different exposures of entomopathogenic fungi are presented in Table 2.

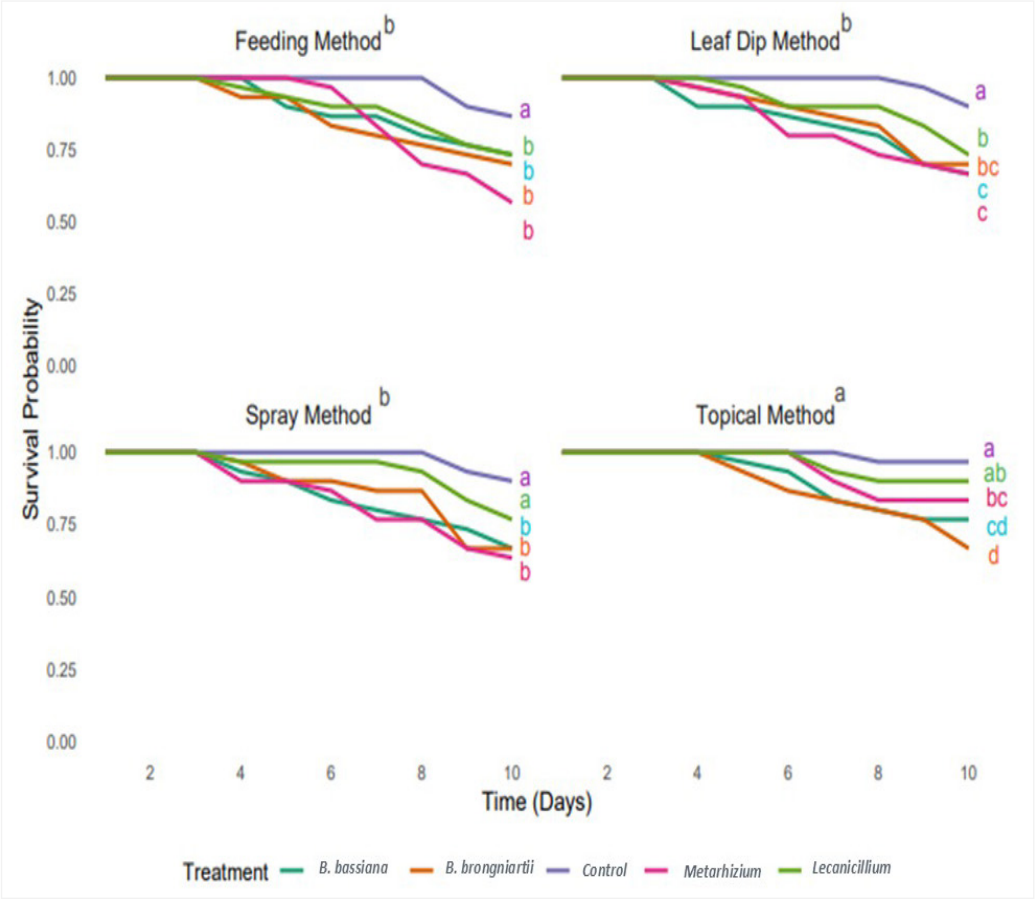

Fig 1. Kaplan Meier Survival curve for *Apis cerana indica* Fabricius exposed to 5×10^8 conidia/ml of commercial entomopathogenic fungal formulations.

Table 2. Lethal Time (LT_{10}, LT_{25}) in days of Entomopathogenic fungi (1×10^8 conidia/ml) under different exposure method against *Apis cerana indica* Fabricius.

Treatment	Feeding Method			Leaf Dip Method		
	Slope ± SE	LT_{10} (+95% FL)	LT_{25} (+95% FL)	Slope ± SE	LT_{10} (+95% FL)	LT_{25} (+95% FL)
Beauveria bassiana	0.115 ± 0.49	15.60 (7.15, 24.05)	20.88 (12.42, 29.33)	0.111 ± 0.46	15.46 (7.23, 23.68)	20.89 (12.67, 29.11)
Beauveria brongniartii	0.118 ± 0.44	14.51 (7.07, 21.95)	19.66 (12.21, 27.10)	0.082 ± 0.47	19.42 (8.15, 30.69)	26.79 (15.52, 38.06)
Metarhizium sp.	0.100 ± 0.49	17.19 (7.51, 26.87)	23.22 (13.54, 32.90)	0.129 ± 0.48	14.14 (6.87, 21.41)	18.83 (11.56, 26.10)
Lecanicillium sp.	0.101 ± 0.49	17.19 (7.51, 26.87)	23.22 (13.54, 32.90)	0.164 ± 0.74	14.50 (5.60, 23.39)	18.20 (9.31, 27.09)
Treatment	**Spray Method**			**Topical Method**		
	Slope ± SE	LT_{10} (+95% FL)	LT_{25} (+95% FL)	Slope ± SE	LT_{10} (+95% FL)	LT_{25} (+95% FL)
Beauveria bassiana	0.109 ± 0.45	14.51 (7.07, 21.95)	19.66 (12.21, 27.10)	0.162 ± 0.636	13.77 (6.1, 21.44)	17.51 (9.83, 25.18)
Beauveria brongniartii	0.111 ± 0.47	15.58 (7.26, 23.89)	21.05 (12.73, 29.37)	0.158 ± 0.567	13.40 (6.36, 20.44)	17.25 (10.21, 24.28)
Metarhizium sp.	0.126 ± 0.47	14.34 (6.95, 21.75)	19.17 (11.77, 26.58)	0.193 ± 0.915	13.94 (4.64, 23.22)	17.08 (7.78, 26.38)
Lecanicillium sp.	0.107 ± 0.51	16.59 (7.33, 25.86)	22.26 (12.99, 31.53)	0.168 ± 1.11	16.08 (3.13, 29.04)	19.68 (6.73, 32.64)

LT_{10} or LT_{25} followed by the same letters do not differ significantly at $\alpha = 0.05$, according to the degree of overlap in the 95% FL. N = 10 bees per treatment and replications = 3. [a]Probit regression model ± standard error (SE). [b]FL = fiducial limits.

The LT_{10} and LT_{25} of exposed bees were shorter with the topical application method than with other exposure methods. In the topical application method, the LT_{10} (16.08 days) and LT_{25} (19.68 days) were higher in *Lecanicillium,* indicating its lesser toxicity to *A. cerana indica*. In the spray method, LT_{10} and LT_{25} were low in *Metarhizium* (14.34, 19.17) and *B. bassiana* (14.51, 19.66). *B. brongniartii* recorded a maximum of 19.42 and 26.79 days to kill 10% and 25% of the treated bees in the leaf dip exposure method. *Metarhizium* (17.19, 23.22) and *Lecanicillium* (17.19, 23.22) expressed similar LT_{10} and LT_{25} values in the feeding exposure method. However, the differences in LT_{10} or LT_{25} values among the exposure methods were statistically nonsignificant.

Mycosis percent under post-entomopathogenic fungi

Mycosis percent was comparatively lower in the topical (7.58%) than in spray (11.58%), leaf dip (11.5%), and feeding (11.5%) exposure methods (Fig 2). Bees were free from mycosis in the control. A lower mycosis percent was recorded under different exposure methods for *Lecanicillium* (3.67 – 9.67%). A higher mycosis percentage was recorded for *B. brongniartii* (11.3%) on topical exposure, and for *B. bassiana* (13.3%) and *Metarhizium* (14%) under the leaf dip exposure method. Under the spray exposure, the mycosis percentage was not significantly different in *B. bassiana* (13.7%), *B. brongniartii* (11.7%), and *Metarhizium* (15%) treatments. The mycosis percentage on moribund *A. cerana indica* is depicted in Fig 3.

Conidia acquisition by A. cerana indica post-exposure to entomopathogenic fungi

Conidial acquisition by *A. cerana indica* under different treatments and exposure methods is depicted in Table 3. Bees bodily acquired the conidia of all entomopathogenic fungi under different exposure methods, with significant differences in acquisition. The conidia/bee recorded were significantly fewer in *Lecanicillium* (4.36-7.83 × 10^4) under different exposure methods. However, it was significantly higher *Metarhizium* in the leaf dip (19.12 × 10^4) and spray (21.08 × 10^4) methods, while *B. brongniartii* in topical (14.14 × 10^4) and feeding (18.75 × 10^4) methods.

Table 3. Conidial acquisition by bees under different exposure methods to Entomopathogenic fungi (1×10^4 conidia/bee).

Treatment	Topical Method	Leaf Dip Method	Spray Method	Feeding Method
Beauveria bassiana	11.97 ± 0.75[b]	13.89 ± 0.61[b]	12.85 ± 0.78[b]	7.94 ± 0.39[c]
Beauveria brongniatii	14.14 ± 0.71[a]	9.46 ± 0.41[c]	10.47 ± 0.31[c]	18.75 ± 1.14[a]
Metarhizium sp.	8.61 ± 0.52[c]	19.12 ± 0.83[a]	21.08 ± 0.92[a]	12.81 ± 0.38[b]
Lecanicillium sp.	7.83 ± 0.23[c]	6.52 ± 0.51[d]	7.69 ± 0.33[d]	4.36 ± 0.19[d]

Mean ± Standard Error with the same letter followed by does not have any significant difference at α = 0.05 based on the Tukey test.

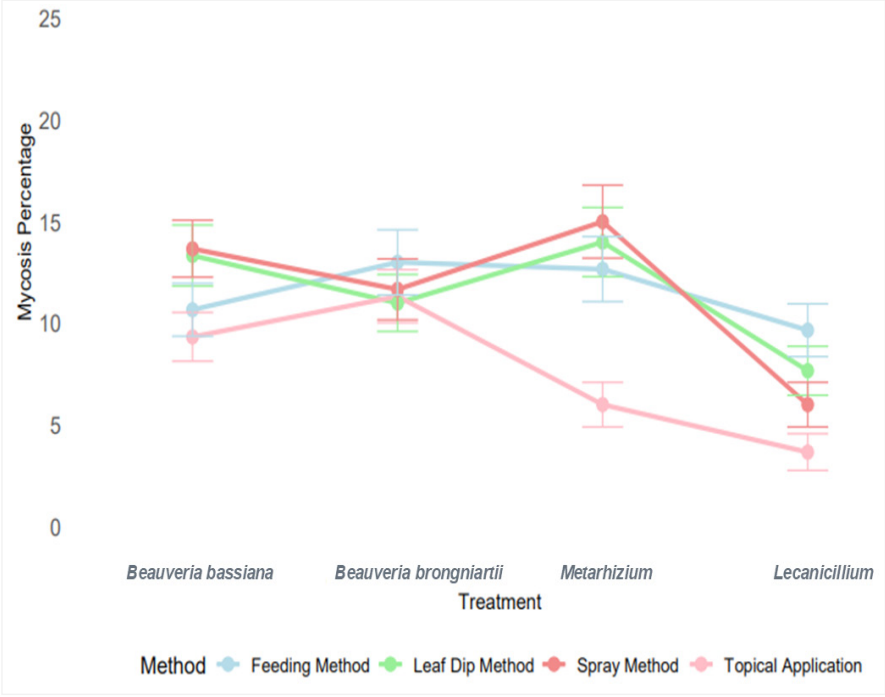

Fig 2. Mycosis percent in fungal-exposed bees after 10 days of exposure to 5×10^8 conidia/ml of entomopathogenic fungi. Error bars indicate the percentage error values.

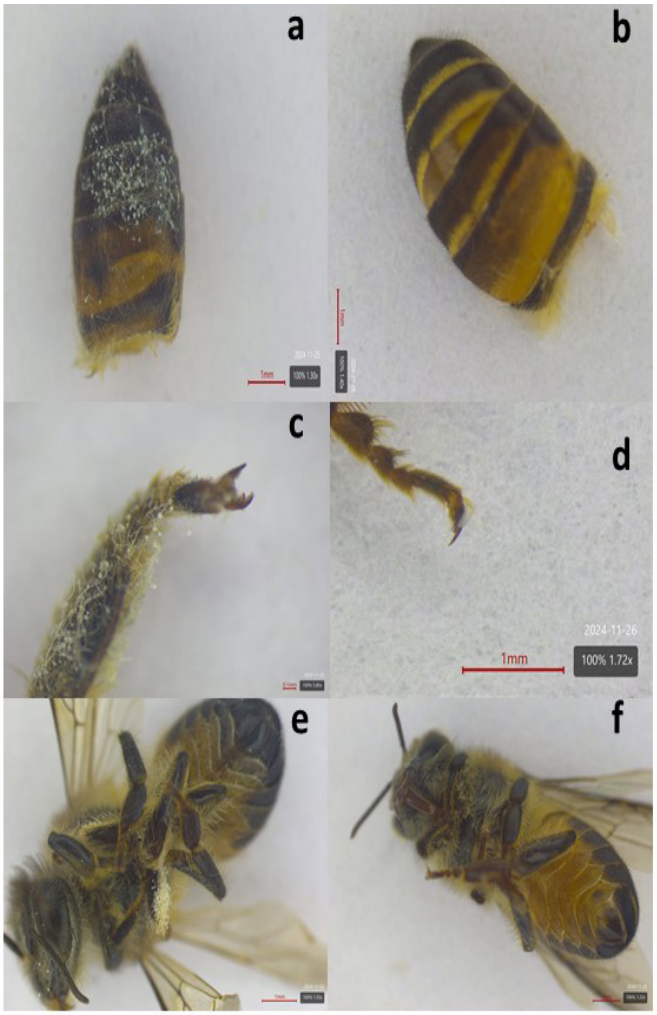

Fig 3. Morphological comparison observed in 10-day-old moribund bees. (a, b) Abdomen; (c, d) Legs; (e, f) Whole body; Treated bees are shown in (a, c, e); control bees in (b, d, f).

Discussion

In this study, *A. cerana indica* acquired 10^4 conidia/bee when exposed to 5×10^8 conidia/ml of all the commercial entomopathogenic fungi tested. According to the OECD guidelines, this concentration is considered safe for the exposed organisms in the short term (Organization for Economic Cooperation and Development [OECD], 1998).

The survival rate was significantly reduced in the leaf dip (88.5%), spray (88.42%), and feeding (88.5%) methods compared to the topical (92.41%) method of exposure. The high survival rate in the topical method may be attributed to the grooming behavior of honey bees (Erler et al., 2022).

Our results share several similarities with those of Omuse et al. (2022), who found that exposing *Apis mellifera* and *Melipona ferruginea* to 10^8 conidia/ml of fungal test isolates caused less than 25% mortality and was non-toxic based on IOBC classification. The mycosis percentage was lower in *A. mellifera* (18%) and *M. ferruginea* (11.7%). LT_{10} and LT_{25} recorded were in the range of 7.4 and 13.8 days, and 8.7 and 19.9 days, respectively. However, studies reported that the bee population was reduced regardless of the method of exposure to the fungi (Leite et al., 2022). Laboratory exposure to the recommended concentration of entomopathogenic fungi, such as *B. bassiana, Cordyceps fumosorosea*, and *Metarhizium anisopliae*, can potentially reduce bee survival (Leite et al., 2022). Furthermore, they also found that the mycosis percentage was low in topical exposure, followed by oral exposure, which agrees with the present study.

The survival of honey bees was reduced by exposure to *Metarhizium* (10^9 conidia/ml) and *Bacillus thuringiensis* (3×10^8 spores/ml) in a spray compared to leaf dip, feeding, and

topical application methods (Potrich et al., 2018). Likewise, *B. bassiana* treatment resulted in reduced survival across all exposure methods. However, these agents did not produce morphometric changes in the midgut. They also reported that 50% of the honey bee workers survived till 89.1 hours.

In contrast, the *B. bassiana* strain ATCC 74040 (107 conidia/mL) was found to be safe for non-target insects, such as *Chrysoperla lucasina* (Morda et al., 2024). In the laboratory bioassay, less than 30% mortality was reported in stingless bee species, *Tetragonisca angustula, Scaptotrigona mexicana*, and *Melipona beechei* exposed to *Isaria fumosorosea* (Ifu-lu-01) and *B. bassiana.* However, *T. angustula* (94.2%) and *M. beecheii* (53%) showed higher mortality when exposed to *M. anisopliae* (Toledo-Hernandez et al., 2016).

Additionally, at 168 hours post-treatment, the survival rates of *M. scutellaris* on exposure by ingestion, contact with contaminated surfaces, and topical application were 74%, 64% and 68% for *B. bassiana*, 34%, 70% and 66% for *M. anisopliae* and 42%, 78% and 72% for *I. fumosorosea*, respectively (Vieira et al., 2024).

The method of fungal exposure influences the behavioral response in bees (Akkoç et al., 2019). Specifically, the feeding exposure affected the locomotion of honey bees, while the spray exposure caused minimal mortality among the exposed bees. However, entomopathogens did not affect the movement behavior of bumble bees. These findings highlight the species-specific and method-dependent effects of fungal biocontrol agents on pollinators.

Conclusion

Our 10-day laboratory bioassay study with *A. cerana indica* exposed to 5×10^8 conidia/ml of the commercial formulations of entomopathogenic fungi resulted in no apparent effects classified as non-toxic according to the IOBC classification. Thus, these commercial formulations can be safely used on pollinator-dependent crop plants. The highest mortality observed in this laboratory study was only 13.3%. However, mortality may be lowered under field conditions due to environmental variables (Abbaszadeh et al., 2011), hive temperature (32-33 °C) (Jarimi et al., 2020), and bee grooming behavior (Erler et al., 2022). Although non-toxic effects are perceived, in field conditions, it is advisable to limit the timing of applying entomopathogens on crop plants during periods of peak bee foraging.

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Authors' Contribution

VD: Investigation, data curation, writing-original draft, writing-review & editing.
MRS: Conceptualization, methodology, writing-review & editing, supervision.
VRS: Resources, project administration, funding acquisition.
MM: Writing-review & editing, validation, formal analysis.
KA, EK, and KR: Validation, formal analysis, visualization.
RP and MI: Software, writing-review & editing, validation, formal analysis.

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