Original Article

Evaluation of the Pathogenicity of *Beauveria bassiana* Biopesticide against *Blattella germanica* (Blattaria: Ectobiidae)

Behzad Nourozi[1], Shahyad Azari-Hamidian[1, 2], Farzad Majidi-Shilsar[3], Mohammad Noori Sepehr[4], *Mohammad Naimi-Joubani[1, 4]

[1]Research Center of Health and Environment, School of Health, Guilan University of Medical Sciences, Rasht, Iran
[2]Department of Medical Parasitology, Mycology and Entomology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[3]Department of Plant Protection, Rice Research Institute, Rasht, Iran
[4]Department of Environmental Health, Research Center for Health, Safety and Environment, School of Health, Alborz University of Medical Sciences, Karaj, Iran

***Corresponding author:** Dr Mohammad Naimi-Joubani, E-mail: Naimijoubani@gums.ac.ir

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Abstract

Background: The German cockroach, *Blattella germanica* (Blattaria: Ectobiidae), is a worldwide urban pest. Due to limitations of conventional insecticides, alternative controls are needed. Entomopathogenic fungi-based biopesticides offer a promising solution. This study evaluated the pathogenicity of the indigenous *Beauveria bassiana* Rasht strain (Mcb_{18}) against the German cockroach.

Methods: A laboratory colony of *B. germanica* was maintained under controlled conditions. The toxicity of four spore concentrations (1.5×10^2, 1.5×10^4, 1.5×10^6, 1.5×10^8 conidia/ml) of *B. bassiana* was evaluated against adult cockroaches using the direct immersion bioassay. Daily mortality was recorded for 21 days using three replicates of 20 cockroaches each (n=60 per concentration). The lethal concentration (LC_{50} and LC_{90}) values were estimated using probit analysis.

Results: *Beauveria bassiana* caused dose-dependent mortality. The highest cumulative mortality (100%) was observed at 1.5×10^8 conidia/ml, while the lowest (15.79%) occurred at 1.5×10^2 conidia/ml. The LC_{50} and LC_{90} were estimated as 4.23×10^3 conidia/ml and 1.59×10^6 conidia/ml, respectively. The LT_{50} decreased with increasing concentration, reaching 8.475 days at 1.5×10^8 conidia/ml. Conidiation on cadavers increased with concentration but remained lower than mortality, indicating that death often occurred before external sporulation. One-way ANOVA revealed significant differences in mortality rates ($F=74.942$, $df=(3, 8)$, $p<0.001$). Post-hoc Tukey's HSD test showed that the two highest concentrations (1.5×10^6 and 1.5×10^8 conidia/ml) were not significantly different from each other ($p>0.05$), although both caused significantly higher mortality than lower concentrations ($p<0.001$).

Conclusion: Experimental findings confirmed *B. bassiana* as an effective entomopathogen against *B. germanica*, causing significant mortality through cuticular penetration and internal proliferation.

Keywords: Entomopathogenic fungi; Biological control; Integrated pest management; German cockroach

Introduction

The German cockroach, *Blattella germanica* (Blattaria: Ectobiidae), is one of the most notorious and widely distributed pests globally (1, 2). These cockroaches are vectors for a range of harmful pathogens and can carry significant loads of bacteria, viruses and parasites on their bodies or in their digestive systems due to their particular feeding habits and behavior (3). They are regularly found in human habitats, including apartments, homes and food-processing facilities, where they have access to a variety of food sources and thrive in warm, humid conditions (4). Conventional dependence on synthetic insecticides such as organophosphates, pyrethroids, carbamates and phenylpyrazole chemical family (e.g., fipronil) has been

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challenged by the development of resistance mechanisms, alongside growing public health and environmental concerns, which have required a shift toward sustainable alternatives. Significant resistance to several insecticides, such as DDT, Permethrin and Cypermethrin, has been demonstrated by German cockroaches. This resistance is primarily related to enzymatic and genetic factors, which have led to an essential need for alternative control strategies (5–7). As a result, biologically based insecticides, like entomopathogenic fungi, have drawn interest as environmentally friendly substitutes to chemical pesticides (8).

Entomopathogenic fungi are organisms that cause disease in insects; their main manner of action involves adhesion to the insect cuticle, enzymatic cuticle degradation, penetration and internal proliferation leading to host death. This infection process differs from neurotoxic chemical insecticides, which reduce the possibility of cross-resistance. Among entomopathogenic fungi, *Beauveria bassiana* is widely studied and applied. *Beauveria bassiana* infects its insect hosts by penetrating the exoskeleton and proliferating inside the body; the fungus produces cuticle-degrading enzymes and toxins that contribute to mortality (9–12). Laboratory bioassays have demonstrated that virulent strains of *B. bassiana* can induce high mortality (70–90%) in *B. germanica* populations within 7–14 days under controlled conditions (13). This fungus has also been reported as effective against agricultural pests (14). Although promising efficacy, the practical application of *B. bassiana* faces challenges related to mass production, formulation stability and environmental sensitivity of conidia. Recent advances, including solid-state fermentation on agricultural residues (such as rice husk) and formulation with carriers or oils to improve shelf-life and field persistence, have reduced cost and increased the feasibility of commercial use (15, 16). Still, some challenges remain; consequently, localized screening for indigenous strains is essential for the optimal control of specific insect populations depending on the localized conditions.

Insect pathogens such as *Metarhizium*, *Paecilomyces*, *Verticillium*, *Aspergillus* and *Beauveria* have a long history in biological control of various pests (17–20). First identified by Agostino Bassi in 1834 as the causative agent of silkworm disease (13), *Beauveria bassiana* has subsequently been developed for use against a wide range of insect pests across multiple orders, including Blattodea, Coleoptera, Diptera and Lepidoptera (21–24).

The reasons for considering *B. bassiana* as a biopesticide candidate are based on its ability to infect the host both by cuticular contact as well as ingestion, its broad host range, limited effect on many non-target organisms and the feasibility of mass production as commercial formulations. However, experimental designs must carefully account for formulation effects (for example, Tween 80 or carrier oil effects) and control-mortality and must report spore viability and storage conditions to ensure reproducibility. While *B. bassiana* is well-recognized as an entomopathogenic fungus with potential for cockroach control, its virulence is highly strain and host-specific. Quantitative data on lethal concentration (LC_{50}, LC_{90}), lethal time (LT_{50}) and sporulation potential, parameters essential for developing effective, locally-adapted biocontrol strategies, remain scarce for indigenous Iranian strains against *B. germanica*. To address this gap, the present study aimed to quantitatively evaluate the virulence of a locally isolated *B. bassiana* Rasht strain (Mcb_{18}) as a biopesticide against *B. germanica* by determining its LC_{50}, LC_{90}, LT_{50} and conidiation capacity on cadavers under controlled laboratory conditions.

Materials and Methods

Insect colony

The German cockroach, *B. germanica*, was obtained from the Medical Entomology Department of Tehran University of Medical Sciences. The colonies were kept in the insectary of the School of Health, Guilan University of

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Medical Sciences (Rasht), inside plastic containers (1.3×0.5×0.6 m). Environmental enrichment, including pleated cardboard cylinders (25×40 cm) and egg cartons, was used to create sheltered microhabitats known to improve cockroach survival and reproduction while reducing stress (25). The colonies were kept under controlled conditions of 27±2°C, 70±5% relative humidity and a 12:12 h light: dark photoperiod, simulating natural habitat conditions (26). A diet of bread and dried fruit, along with water, was provided to maintain the colony (27). The described rearing conditions were selected to maintain a stable, low-stress environment and minimize external influences on physiological and behavioral traits. In order to ensure that baseline susceptibility to *B. bassiana* was not affected by prior environmental or chemical exposures, the colony originated from a controlled laboratory strain with no history of pesticide exposure. For experiments, adult males and females at a 1:1 ratio were selected using CO_2 anesthesia to reduce mobility and transferred to test containers.

Fungal preparations

All chemicals used in this study were of analytical grade and all solutions were prepared using deionized water made by a hydroservice reverse osmosis/ion-exchange system. To ensure complete sterility during preparation and incubation, all glassware and instruments were sterilized before use either by autoclaving at 121 °C for 15 min or by UV irradiation. In addition, all solutions, including deionized water, were sterilized and all procedures, such as inoculation and incubation, were carried out under aseptic conditions in a laminar flow hood to avoid any possible contamination.

The *B. bassiana* (Mcb_{18}) strain used in the present study was provided by the Pest Management Department of the Iran Rice Research Institute (IRRI). It had been initially isolated from a cadaver of *Chilo suppressalis* (Lepidoptera: Crambidae). The conidia were first grown in Sabouraud Dextrose Broth (SDB) at 25±1 °C for 48 h. The autoclaved medium was then poured into aseptically sterilized Petri dishes (10×15 cm), with approximately 15 ml of molten Sabouraud Dextrose Agar (SDA).

A conidial suspension was then prepared by harvesting spores into 30 ml of sterile 0.1% Tween 80 solution in a 50 ml Falcon tube, followed by homogenization for 3 min using a vortex mixer. Subsequently, 0.1 ml of this suspension was inoculated onto the center of each SDA plate. The plates were maintained at 25± 1 °C under a 12:12 h light/dark photoperiod for 14 days. At the end of the incubation period, conidia were collected from the surface of the plates using a sterile spatula. Harvested conidia were suspended in sterile water containing 0.02% Tween 80, filtered through a double layer of sterile cheesecloth, centrifuged at 4000×g for 15 min at 4 °C and again suspended in the same solvent. The final spore concentration was counted using a hemocytometer. Before conducting the bioassays, single-spore germination assays were performed to evaluate conidial viability. Individual conidia were transferred onto SDA plates and allowed to germinate at 25±1 °C for 24 h. Conidial viability was confirmed by plating 100 µl of each suspension onto SDA and incubating the plates at 25±1 °C. Germination rates were assessed microscopically and only conidial suspensions with viability above 95% were used for subsequent experiments. This procedure confirmed that metabolically active conidia with high viability were used in this study (28, 29).

Bioassay test protocol

Four spore concentrations (1.5×10^2, 1.5×10^4, 1.5×10^6 and 1.5×10^8 conidia/ml) were prepared by serial dilution of the stock suspension. Sterile distilled water containing 0.02% Tween 80 served as the control treatment. To evaluate pathogenicity, a direct immersion bioassay was conducted, with parallel control groups included to account for background mortality.

Direct immersion bioassay

To perform the direct immersion bioassay, aqueous conidial suspensions were prepared

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at the four specified concentrations via serial dilution. Groups of 20 adult cockroaches were immersed individually in each suspension for 5 seconds, with three replicates per concentration. Each replicate consisted of 20 adult cockroaches and three replicates were performed for each concentration (total n=60 per concentration, with an overall sample size of N=300 including the control group). A control group was immersed in 0.02% Tween 80 solution, which has previously been documented as an inert medium with no significant effects on cockroach survival or behavior (30). This confirmed that any observed mortality was attributable to fungal infection rather than the carrier solution.

After immersing and treating each group in their respective methods, cockroaches from each treatment and control group were transferred to separate plastic containers supplied with food and water. All containers were maintained at 27±2 °C, 70±5% relative humidity and a 12: 12 h light/dark photoperiod for 21 days. Environmental conditions were carefully monitored during the course of the experiment to avoid deviations that may have impacted fungal pathogenicity or survival of the cockroaches. Containers were inspected daily and dead cockroaches were removed and recorded. Any cockroach that developed white muscardine growth on the body surface was classified as having mortality due to *B. bassiana* infection (17).

Statistical analysis

For data analysis of the bioassay experiment, Daily mortality records for each concentration were documented during the 21-day observation period. Mortality rates were first corrected using Abbott's formula to eliminate background mortality, with control mortality remaining below 5%, confirming all experimental replicates for statistical analyses (31). Dose–mortality relationships were analyzed using probit regression with statistical software (SPSS Ver. 19), generating LC_{50} and LC_{90} values with their corresponding 95% confidence intervals. To estimate the time-dependent virulence of the fungal pathogen, LT_{50} values (median lethal time) were also calculated using probit-time analysis. Statistically different speed of kill by LT_{50} was shown by non-overlapping 95% confidence intervals (CIs). In addition to probit analysis, one-way ANOVA was performed to compare Abbot corrected mortality rates of the four different tested concentrations. The assumptions of homogeneity of variance were checked before conducting the ANOVA and then any significant differences ($p<0.05$) were further examined using Tukey's HSD post hoc test (32, 33).

Results

Beauveria bassiana (Mcb_{18}) conidial suspensions caused dose-dependent mortality in *B. germanica* compared with the control group. Cumulative mortality was recorded daily and observations continued for up to 21 days post-exposure. The cumulative mortality trends across different concentrations are illustrated in Fig. 1. The highest cumulative mortality was observed at the concentration of 1.5×10^8 conidia/ml, whereas the lowest mortality occurred at 1.5×10^2 conidia/ml (Table 1).

Mortality in the control group remained below 5% during the experiment, indicating stable and suitable bioassay conditions. To standardize mortality estimates and eliminate the effects of unintended factors, mortality rates in all treated groups were corrected using Abbott's formula (34). The corrected mortality data (Mean±SD) are presented in Table 1.

As shown in Table 1, the Abbott-corrected mortality rate showed an increasing trend with an escalation in conidial concentrations from 15.79% at 1.5×10^2 conidia/ml to complete mortality (100%) at 1.5×10^8 conidia/ml. Conidiation on cockroach cadavers showed an identical trend with an increase in conidial concentrations; however, conidiation percentages were regularly lower than mortality rates, demonstrating that death occurred before external sporulation in a proportion of individuals. The highest conidiation rate (32.46%) was recorded at 1.5×10^8 conidia/ml.

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The LC_{50} and LC_{90} of *B. bassiana* against *B. germanica* were estimated at 4.23×10^3 and 1.59×10^6 conidia/ml, respectively (Table 2). The probit model showed an acceptable goodness of fit (χ^2=2.701, df=3, p=0.259), supporting the reliability of the concentration–mortality relationship within the tested dose range.

The median lethal time (LT_{50}) values decreased consistently with increasing conidial concentration (Table 3). LT_{50} declined from 19.379 days at 1.5×10^2 conidia/ml to 8.475 days at 1.5×10^8 conidia/ml. Therefore, the data demonstrate a clear time-mortality relationship, with higher conidial concentrations leading to faster death. The concentration of 1.5×10^8 conidia/ml yielded the highest mortality with the narrowest confidence intervals, indicating a more precise estimation compared to other concentrations. One-way ANOVA revealed a significant effect of conidial concentration on Abbott-corrected mortality (F=74.942, p< 0.001), indicating statistically significant differences among treatments (Table 4). Post-hoc pairwise comparisons using Tukey's HSD test revealed three statistically homogeneous subsets (Fig. 2; Table 4). The lowest concentration (1.5×10^2 conidia/ml) formed subset a, with a mean corrected mortality of 15.79±5.26%, which was significantly lower than all other treatment groups (p< 0.05). The intermediate concentration (1.5×10^4 conidia/ml) formed subset b, with a mean mortality of 59.6±12.15%, showing a statistically significant increase compared to the lowest concentration. The two highest concentrations (1.5×10^6 and 1.5×10^8 conidia/ml) were grouped in subset c, with mean mortality rates of 82.46±6.08% and 100.00±0.00%, respectively. No statistically significant difference was observed between these two concentrations (p> 0.05), although both caused significantly higher mortality than the lower concentrations (p< 0.001).

Table 1. Percentage of conidiation and Abbott-corrected mortality (Mean±SD) of *Blattella germanica* at day 21 exposed to different concentrations of the *Beauveria bassiana* Rasht strain (Mcb_{18}) spores

Concentration (conidia/ml)	Corrected Mortality Rate (Abbot)	Conidiation (%)
1.5×10^2	15.79±5.26	4.23
1.5×10^4	59.65±12.15	9.53
1.5×10^6	82.46±6.08	13.68
1.5×10^8	100.00±0.00	32.46

Table 2. Lethal concentration (LC_{50} and LC_{90}) values of the *Beauveria bassiana* Rasht strain (Mcb_{18}) against *Blattella germanica* based on probit analysis

Parameter	Value	95% confidence limits (Lower)	95% confidence limits (Upper)
Probit equation	Y=0.498x−1.806		
Chi-square (goodness-of-fit)	2.701(p=0.259)		
LC_{50}	4.23×10^3 conidia/ml	1.49×10^3	1.07×10^4
LC_{90}	1.59×10^6 conidia/ml	4.58×10^5	9.40×10^6

Table 3. Lethal time values of the *Beauveria bassiana* Rasht strain (Mcb_{18}) spores against *Blattella germanica* at different concentrations

Concentration (conidia/ml)	LT_{50}(day)	95% confidence limits (lower)	95% confidence limits (upper)	Chi-square
1.5×10^2	19.379	18.125	21.409	16.206
1.5×10^4	15.150	14.372	16.151	23.562
1.5×10^6	11.700	10.943	12.499	67.778
1.5×10^8	8.475	8.253	8.697	2.316

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Table 4. One-way ANOVA of Abbott-corrected mortality of *Blattella germanica* exposed to different concentrations of *Beauveria bassiana* Rasht strain (Mcb_{18})

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11936.750	3	3978.917	74.942	< 0.001
Within Groups	424.746	8	53.093		
Total	12361.496	11			

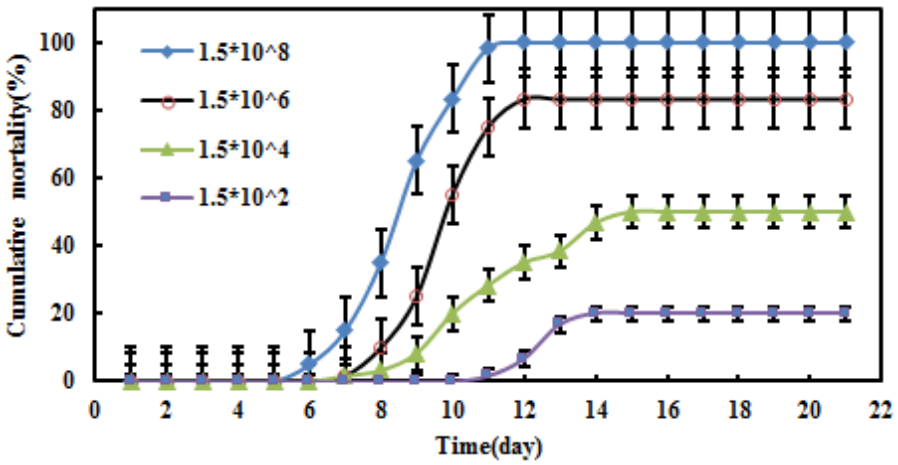

Fig. 1. Cumulative mortality rate of *Blattella germanica* exposed to different concentrations of the *Beauveria bassiana* Rasht strain (Mcb_{18}) spores. Mortality was recorded daily for up to 21 days. Results are expressed as means±SD of three independent experiments

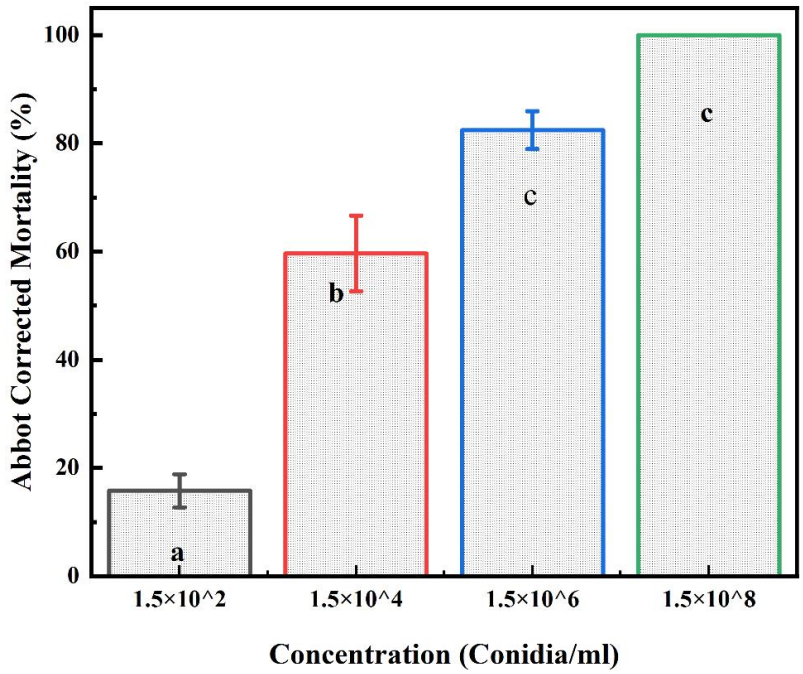

Fig. 2. Tukey's HSD post-hoc comparison of Abbott-corrected mortality (%) of *Blattella germanica* exposed to different concentrations of *Beauveria bassiana* (Mcb_{18}) at day 21. Values represent mean ± SD of three independent replicates. Different lowercase letters (a–c) indicate statistically significant differences among concentrations (α=0.05)

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Discussion

The present study demonstrates the significant pathogenicity of the local *B. bassiana* Rasht strain (Mcb_{18}) against the German cockroach, *B. germanica*, under controlled laboratory conditions. Mortality increased with conidial concentration and exposure time, confirming a clear dose- and time-dependent response. The highest total mortality (100%) was recorded at the highest concentration of *B. bassiana* (1.5×10^8 conidia/ml), while the lowest total mortality (15.79%) was recorded at the lowest concentration (1.5×10^2 conidia/ml). This pattern indicates that this strain exhibits high virulence within an effective concentration range, supporting its potential as an effective biological control agent.

The concentration-mortality relationship was further supported by probit analysis. The results showed that the LC_{50} and LC_{90} values were 4.23×10^3 and 1.59×10^6 conidia/ml, respectively. The relatively low LC_{50} value indicates that the *B. bassiana* Mcb_{18} strain is more virulent compared with several previously reported *B. bassiana* strains. For example, Davari et al. (35) reported LC_{50} values of the *B. bassiana* strains in the range of $3.5–3.6\times10^7$ conidia/ml against the field-collected *B. germanica*. Similarly, Ashbrook et al. (36) achieved 100% mortality in *B. germanica* following contact exposure to *B. bassiana* at concentrations ranging from 8.8×10^5 to 1.3×10^6 conidia/ml. Together, these comparisons support the potential of fungal entomopathogens as effective pest management tools. The lower lethal concentrations identified in the present study suggest that strain-specific genetic characteristics, host susceptibility and experimental factors may account for the differences in reported virulence between studies on fungal pathogens (37).

Based on applied toxicity reference values, LC_{90} is particularly relevant because it estimates the operational dose required for reliable population suppression. The LC_{90} value obtained in this study (1.59×10^6 conidia/ml) is substantially lower than those reported for several other *B. bassiana* strains, indicating that effective control may be achieved without requiring extremely high spore loads. Furthermore, the widespread resistance of cockroach populations to conventional chemical insecticides makes the development of alternative control strategies essential: pathogenic fungi such as *B. bassiana* with their well-established history in pest management (38) represent a promising option. This has motivated continued research into their application for urban cockroach control (39–41). The social behavior of cockroaches also facilitates the transmission of *B. bassiana* within colonies to more widespread and longer-term populations' suppression (36). Additionally, *B. bassiana* shares similar physiological requirements for optimal growth with cockroaches (25–30 °C, high humidity), making it an immediately practical control option in urban environments (42).

The time-mortality analysis revealed an obvious concentration-dependent decline for the LT_{50}, which declined from 19.379 days at 1.5×10^2 conidia/ml to 8.475 days at 1.5×10^8 conidia/ml. This pattern reflects the classical infection dynamics of entomopathogenic fungi, in which higher inoculum densities accelerate spore adhesion, germination, cuticular degradation and hemocoel penetration. Comparable dose-dependent reductions in lethal time have been reported for *Beauveria*, *Metarhizium* and *Isaria* species, where increasing spore loads enhance conidial germination, hyphal proliferation and pathogenic activity, ultimately shortening host survival time (43).

Although faster lethal times (LT_{50} of 2–4 days) have been reported for oral bait formulations of *B. bassiana* (29), this is expected, as ingestion bypasses the cuticular barrier and allows rapid systemic infection via the digestive tract (44). In contrast, the comparatively slower mortality observed in this study reflects its primary infection routes: cuticle-mediated pene-

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tration. This process involves multiple sequential steps in the fungal life cycle, including spore attachment, germination, appressorium formation, enzymatic penetration of the cuticle, and entry into the hemocoele (45). From an applied perspective, identifying a concentration that balances high efficacy with practical feasibility is essential for developing a viable biopesticide formulation. Our data indicate that while the highest concentration tested (1.5×10^8 conidia/ml) achieved complete mortality (100%), its economic viability for large-scale production and field application may be constrained by the costs associated with producing such high spore loads. The LC_{90} value of 1.59×10^6 conidia/ml offers a more pragmatic target, as it theoretically suppresses 90% of the population. Furthermore, the absence of a statistically significant difference in final mortality between 1.5×10^6 and 1.5×10^8 conidia/ml (as revealed by Tukey's HSD test) suggests that concentrations around 1.5×10^6 conidia/ml can achieve mortality levels comparable to a tenfold higher dose. Therefore, we propose that a concentration range of approximately 1×10^6 to 5×10^6 conidia/ml could serve as an optimal starting point for formulating a cost-effective and efficacious product against *B. germanica*. This range balances biological efficacy with economic and practical considerations, though further validation under semi-field and field conditions is warranted to confirm these laboratory findings.

Analysis by one-way ANOVA followed by Tukey's HSD test further clarified the dose–response pattern. While mortality increased with conidial concentration, no significant differences were observed between the two highest concentrations (1.5×10^6 and 1.5×10^8 conidia/ml), indicating a saturation effect. The plateau suggests that beyond a certain point, additional inoculum does not enhance mortality. Practically, this implies that concentrations around 1.5×10^6 conidia/ml could achieve mortality levels comparable to tenfold higher doses, optimizing cost-efficiency and reducing application volume. Similar concentration plateaus have been reported in other cockroach and beetle bioassays with entomopathogenic fungi (46, 47).

Conidiation on cadavers increased with the increasing fungal concentration but remained consistently lower than mortality rates; this indicates that a proportion of infected cockroaches died before external sporulation became visible (48). Such outcomes have been widely reported for *B. bassiana* infections, particularly under conditions of high inoculum pressure or rapid host mortality, where internal colonization and toxin production precede surface conidiation. Although lower levels of visible sporulation may limit secondary distribution of spores in some situations, the presence of conidia on cadavers at higher concentrations is ecologically important for environmental persistence and horizontal transmission within cockroach aggregations (11, 45). Thus, successful sporulation at elevated concentrations remains crucial for ecological sustainability and long-term efficacy of this biological control strategy.

The low mortality in the control group validates the probit model's goodness-of-fit ($\chi^2=2.701$, $p=0.259$) and indicates that the observed effects were primarily attributed to fungal infection, not handling or other stress. This statistical robustness supports the validity of the LC_{50}, LC_{90} and LT_{50} estimates and increases confidence in the reproducibility of the bioassay results. Despite the strong laboratory performance of the *B. bassiana* Rasht strain (Mcb_{18}), several limitations must be considered. First, laboratory-reared cockroach populations are often more susceptible than their wild counterparts. Furthermore, environmental factors such as low humidity, temperature fluctuations and ultraviolet radiation can significantly reduce fungal viability and infection success under field conditions. These limitations, which have been noted in other studies on entomopathogenic fungi for urban pest control (49), underscore the need for semi-field and field-based evaluations. Additionally, for-

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mulation optimization (for example, the use of oil-based carriers or bait systems) is necessary to validate practical efficacy.

This study highlights the *B. bassiana* Rasht strain (Mcb_{18}) as an effective biocontrol candidate for sustainable integrated pest management (IPM) programs. Its application directly to harborages, particularly at lower chemical doses, could improve control efficacy while delaying resistance development and reducing reliance on conventional insecticides.

Conclusion

The indigenous *B. bassiana* (Mcb_{18}) strain demonstrates strong dose- and time-dependent pathogenicity against the German cockroach, *B. germanica*, achieving complete mortality at higher conidial concentrations under laboratory conditions. Its compatibility with cockroach aggregation behavior, cadaver sporulation and shared microclimatic requirements further supports its potential for integrated pest management (IPM). Based on our quantitative analysis, a concentration range of 1×10^6 to 5×10^6 conidia/ml is proposed as an optimal balance between biological efficacy and practical feasibility for formulation development. A strategic combination of *B. bassiana* with low-risk insecticides or targeted environmental management may enhance efficacy and mitigate resistance development. Despite promising laboratory results, semi-field and field validation are essential to confirm performance under variable environmental conditions and to assess economic feasibility for practical applications. Overall, *B. bassiana* (Mcb_{18}) offers a sustainable, environmentally compatible and effective alternative for controlling *B. germanica* populations, contributing to long-term population suppression and healthier indoor environments.

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Ethical considerations

The ethical code IR.GUMS.REC.1394.438 has been registered for this study.

Conflict of interest statement

The authors declare there is no conflict of interest.

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