Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252025v3812910rc

Scientific Article

Effectiveness of Baits Containing Entomopathogenic Fungi Against *Gryllus assimilis*

Eficácia de iscas contendo fungos entomopatogênicos no controle de *Gryllus assimilis*

Arminda D. Sumbuleiro[1], Maguintontz C. Jean-Baptist[1], Duane B. da Fonseca[2], Edison Zefa[3], Flávio R. M. Garcia[3]*

[1]Graduate Program in Plant Health, Universidade Federal de Pelotas, Pelotas, RS, Brazil. [2]Institute of Biological Sciences, Universidade Federal do Rio Grande, Rio Grande, RS, Brazil. [3]Department of Insect Ecology, Zoology, and Genetics, Universidade Federal de Pelotas, Pelotas, RS, Brazil.

ABSTRACT - The use of entomopathogens, including entomopathogenic fungi, for biological control provides a viable alternative to chemical pesticides for pest management. Entomopathogenic fungi infect and cause epizootics in a wide range of insect orders, including Hemiptera, Lepidoptera, Coleoptera, Diptera, Hymenoptera, and Orthoptera. This study aimed to assess the insecticidal efficacy of the entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea* against *Gryllus assimilis* (Orthoptera: Gryllidae) under laboratory conditions. The experiments were conducted using a completely randomized design with four replications. Two strains of *B. bassiana* (IBCB 66 and PL 63), one strain of *M. anisopliae* (E9), and one strain of *I. fumosorosea* (ESALQ 1296) were evaluated. The highest mortality of *G. assimilis* was observed seven days post-treatment with *M. anisopliae* at 0.15 g, resulting in resulting in over 50% insect mortality.

RESUMO - O controle biológico usando entomopatógenos, incluindo os fungos entomopatogênicos, oferece uma alternativa relativamente adequada para o manejo de pragas. Sabe-se que os fungos entomopatogênicos infetam e causam epizootias em uma ampla gama de ordens de insetos, incluindo Hemiptera, Lepidoptera, Coleoptera, Diptera, Hymenoptera e Orthoptera.. O objetivo do presente estudo foi avaliar a eficácia dos fungos entomopatogênicos *Metarhizium anisopliae, Beauveria bassiana* e *Isaria fumosorosea* sobre a mortalidade de *Gryllus assimilis* (Orthoptera: Gryllidae) em ambiente de laboratório. Os testes foram realizados em delineamento inteiramente casualizado, com 4 repetições. Foram avaliadas duas cepas de *B. bassiana* e uma cepa de *M. anisopliae* e *I. fumosorosea*. 7 dias após o tratamento com *M. anisopliae* 0,15g, foi observada a maior mortalidade de *G. assimilis*, tendo causado mortalidade de mais da metade dos insetos.

Keywords: Biological control. Bioformulations. Insects. Cricket.

Palavras-chave: Biocontrole. Bioformulações. Insetos. Microbiológicos.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: August 27, 2024.
Accepted in: March 31, 2025.

Editor in Chief: Aurélio Paes Barros Júnior
Section Editor: Carlos Henrique Brito

Data Availability: The data that support the findings of this study can be made available, upon reasonable request, from the corresponding author .

***Corresponding author:**
<flavio.garcia@ufpel.edu.br>

INTRODUCTION

Crickets are omnivorous insects that feed on a wide variety of plant and animal material. *Gryllus assimilis* (Orthoptera: Gryllidae), for example, is known to damage eucalyptus plantations, plant nurseries, and seedlings during the first two years after planting, often necessitating replanting.

Pest management in agricultural production areas currently relies primarily on synthetic pesticides, which are widely recognized for controlling agricultural pests and enhancing crop yields (ANANI et al., 2020). However, these pesticides adversely affect soil microbial activity, reducing ecosystem services and the production of plant growth-promoting compounds such as siderophores, nitrogen, and indole-3-acetic acid. Moreover, synthetic pesticides contribute to environmental contamination through various pathways, including volatilization, improper disposal, spray drift, erosion, and leaching. Consequently, non-target plant species may be affected, reducing their photosynthetic capacity and seed production (HASHIMI; HASHIMI; RYAN, 2020). Furthermore, pesticide residues accumulating in water bodies can bioaccumulate in aquatic organisms and then in animals and humans through biomagnification, resulting in severe diseases, including cancer, kidney disease, skin rashes, and diabetes (MANFO et al., 2020). This highlights the importance of developing and implementing alternative pest management strategies that are environmentally friendly, cost-effective, reliable, and sustainable (TUPE et al., 2017).

Entomopathogenic fungi are important biological control agents that have been studied globally for over a century. The earliest experiments were conducted in the nineteenth century by the Russian zoologist Metschnikoff, who investigated the efficacy of *Metarhizium anisopliae* for controlling a beetle species (VEGA et al., 2012).

Biological control using entomopathogenic fungi provides a viable alternative to chemical insecticides for pest management (TUPE et al., 2017).

Entomopathogenic fungi infect and cause epizootics across a diverse range of insect orders, including Hemiptera, Lepidoptera, Coleoptera, Diptera, Hymenoptera, and Orthoptera (SHAH; PELL, 2019; ISLAM, 2021).

Entomopathogenic fungi exhibit parasitic behavior that can be utilized for the biological control of pests and diseases, representing the primary cause of microbial diseases in invertebrates. They comprise a diverse group of nearly 1,000 species, serving as natural mortality factors for insects and arachnids, including ticks and mites (HUMBER, 2008; BOOMSMA et al., 2014; STONE; BIDOCHKA, 2020). Unlike viruses, protozoa, and bacteria, which typically require specific infection pathways such as ingestion, most entomopathogenic fungi infect arthropods by directly penetrating the host's cuticle, primarily functioning as contact pathogens (MASCARIN et al., 2016).

Although the primary mode of infection is through the integument, evidence suggests that *B. bassiana* can also infect insects via ingestion, particularly those with chewing mouthparts (FENG; POPRAWSKI; KHACHATOURIANS, 1994). *Metarhizium anisopliae* (Metsch) Sorokin (Ascomycota: Clavicipitaceae) is a naturally occurring entomopathogen, parasitizing insects across diverse habitats, and its use as a biological control agent in pest-infested areas has become widespread in Brazil (ALMEIDA, 2020).

Entomopathogenic fungi are important biological pest control agents capable of colonizing a diverse range of pests and causing epizootic diseases that lead to mortality or disrupt feeding and reproduction in insects and mites (SHARMA; SHARMA; YADAV, 2023; ROHRIG, 2021). These fungi typically infect insects by penetrating their cuticle, regardless of the insect's feeding habits. As the insect cuticle serves as the primary barrier to fungal infection, entomopathogenic fungi produce several extracellular enzymes that degrade proteins, chitin, and lipids, which are the main components of the cuticle (PEDRINI; CRESPO; JUÁREZ, 2007; VEGA et al., 2012).

This study aimed to assess the insecticidal efficacy of the entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea* against *Gryllus assimilis* (Orthoptera: Gryllidae) under laboratory conditions. The efficacy of the tested fungi in causing infections by contact and ingestion was evaluated using different pathogen concentrations and post-treatment times.

MATERIAL AND METHODS

Gryllus assimilis rearing

The *Gryllus assimilis* population, reared at the Federal University of Pelotas (UFPEL), in Pelotas, RS, Brazil, was sourced from the Terrestrial Invertebrate Vivarium, Institute of Biological Sciences, Federal University of Rio Grande (FURG). The *G. assimilis* population was maintained at the Insect Biology Laboratory, Department of Ecology, Zoology and Genetics, Biology Institute (DEZG/IB/UFPEL), following the protocols of Limberger, Nery and Fonseca (2021), under controlled temperature of 25 ± 2 °C, relative humidity of 65 ± 10%, and a 12-hour photoperiod. Crickets were provided ad libitum access to commercial cat feed (Golden, PremieRpet® São Paulo, Brazil) from egg hatching and supplied with water on wet cotton, which was replaced daily.

Bioinsecticide product formulations and treatments

Microbiological insecticides, formulated as emulsifiable concentrates (EC) or wettable powders (WP), were supplied by Koppert do Brasil Holding Ltd., Piracicaba, SP, Brazil. The products included: (i) Boveril Cana®, containing 30 g kg^{-1} of the active ingredient (AI) *Beauveria bassiana* (Bals.) Vuill. strain IBCB 66 (1.5 × 109 CFU g^{-1}); (ii) Boveril® WP, containing 50 g kg^{-1} of the AI *B. bassiana* strain PL63 (1 × 10^8 viable conidia g^{-1}; (iii) Metarril® WP E9, containing 50 g kg^{-1} of the AI *Metarhizium anisopliae* (Metsch.) Sorokin strain E9 (1.39 × 10^8 viable conidia g^{-1}); and (iv) Challenger® EC, containing 85 g L^{-1} of the AI *Isaria fumosorosea* Wize strain ESALQ 1296 (2.5 × 10^9 viable conidia mL^{-1}) (Table 1).

Table 1. Microbiological insecticides used in laboratory bioassays against *Gryllus assimilis*.

Active ingredient	RR	Trade name	Chemical group
Beauveria bassiana strain IBCB 66	1.2 g L^{-1}	Boveril Cana® 30 WP	Microbiological Insecticide
Beauveria bassiana strain PL 63	1.2 g L^{-1}	Boveril® 50 WP	Microbiological Insecticide
Metarhizium anisopliae strain E9	1.2 g L^{-1}	Metarril® 50 WP	Microbiological Insecticide
Isaria fumosorosea strain ESALQ 1296	1.2 mL L^{-1}	Challenger® 85 EC	Microbiological Insecticide

RR = rates recommended by the manufacturer expressed in g or mL of the commercial product per liter of water; IBCB = Brazilian Institute of Biological Control; EC = Emulsifiable Concentrate; WP = Wettable Powder.

Bioassay

Effect of entomopathogenic fungi on mortality of *Gryllus assimilis* adults through ingestion

The experiment was conducted at the Insect Biology Laboratory (DEZG/IB/UFPEL). The four microbiological insecticides (Table 1) were tested at concentrations of 5%, 10%, and 15%, with distilled water serving as a negative control. A completely randomized design with 13 treatments and four replications was used, resulting in 52 experimental units. Each experimental unit consisted of a 700 mL plastic container with a 1 cm diameter hole on the side, covered with a lid. The container held a plastic cup (4 cm height and 4 cm diameter) containing 5 g of commercial feed mixed with the entomopathogenic fungi product. Four unsexed adult *G. assimilis*, aged seven to ten days after preimaginal molting, were placed in each container, totaling 208 individuals. Adults were fasted for 24 hours to encourage rapid feeding.

Solutions were prepared using the microbiological

insecticides at the concentrations tested, with 0.05, 0.10, or 0.15 g (WP), or 0.05, 0.10, or 0.15 mL (EC) diluted in 50 mL of distilled water. The solutions were applied to the feed surface by spraying 2 mL of the solution per 5 g of feed, which was then offered to the insects. Mortality due to fungal infection was assessed daily for eight days, with the dead insects collected and incubated in a biochemical oxygen demand (BOD) chamber at 25 °C, 60% relative humidity, and a 12-hour photoperiod for seven days to confirm mycelium growth.

Effect of entomopathogenic fungi on mortality of *Gryllus assimilis* nymphs and adults through contact

This experiment was conducted using a completely randomized design with 18 treatments and four replicates, resulting in 72 experimental units. Each experimental unit consisted of a 700 mL plastic container with a perforated lid to ensure ventilation. Four insects were placed in each container, totaling 256 individuals, comprising 128 pre-adult nymphs and 128 adults. The four microbiological insecticides (Table 1) were tested. Solutions were prepared with 20 g and 30 g of each product, using the same method as that described for the previous experiment. The applications consisted of spraying 2 mL of the solution onto the insects in each experimental unit.

Insect mortality due to fungal infection was assessed daily for 6 days, with dead insects incubated as described for the previous experiment. Mortality percentages (M%) were calculated relative to the control, using the formula of Schneider-Orelli (1947: $M\% = 100 \times (Mt - Mc) \div (100 - Mc)$, where *Mt* is the mortality in the insecticide treatment and *Mc* is the mortality in the control treatment.

Data analysis

Mortality data for *G. assimilis*, collected over eight days post-treatment in the first experiment and six days post-treatment in the second experiment, were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using the Bartlett test. When the data did not meet normality criteria, they were transformed using the formula $\sqrt{x} + 0.5$ to satisfy the assumptions of analysis of variance (ANOVA), for each post-treatment evaluation period. Cumulative mortality data were analyzed using ANOVA, with the F-test to evaluate interactions between factors (products and concentrations). The data were then analyzed using polynomial regression in R software (R DEVELOPMENT CORE TEAM, 2023).

RESULTS AND DISCUSSION

Effect of entomopathogenic fungi on mortality of *Gryllus assimilis* adults through ingestion

Mortality rates over time for *G. assimilis* adults, resulting from the application of four entomopathogenic fungal insecticides at different concentrations, generally followed a polynomial distribution. The results indicate that these fungal products are promising biological insecticides against crickets, as incorporation of fungal conidia into feed caused high mortality in *G. assimilis* adults under laboratory conditions.

One day after treatment (DAT), the interaction between factors (product and concentration) was not significant for Boveril Cana® ($F = 1.14$, $Fstat = 1.10$, $p = 0.3034$). Significant interactions were observed for Boveril® ($F = 6.47$, $Fstat = 13.08$, $p = 0.0244$), Metarril® ($F = 8.34$, $Fstat = 4.53$, $p = 0.0127$), and Challenger® ($F = 11.29$, $Fstat = 6.99$, $p = 0.0051$). The highest mortality for *G. assimilis* (18.75%) was observed with Challenger® at a 10% concentration (Figure 1A). These results align with those of Meikle et al. (2005), who evaluated three *B. bassiana* isolates and one *I. fumosorosea* isolate against *Coptotermes formosanus* in Hong Kong, China, under laboratory conditions. In preliminary bioassays, *I. fumosorosea* Pfu02031 was the most effective, achieving 100% mortality of *C. formosanus*, followed by *I. fumosorosea* Pfu02031 combined with a commercial *M. anisopliae* isolate (Bioblast®). Although no significant difference was observed among the fungi tested against *C. formosanus*, when infected and uninfected termites were grouped, *I. fumosorosea* Pfu02031 was significantly more pathogenic, resulting in the lowest mean survival of insects.

At two DAT, the interaction between factors (product and concentration) was not significant for Boveril Cana® ($F = 1.16$, $Fstat = 1.12$, $p = 0.3000$). Significant interactions were observed for Boveril® ($F = 154.84$, $Fstat = 84.39$, $p = 0.0001$), Metarril® ($F = 5.22$, $Fstat = 15.82$, $p = 0.0398$), and Challenger® ($F = 9.28$, $Fstat = 5.04$, $p = 0.0094$). The highest mortality rate for *G. assimilis* (25%) was observed with Metarril® and Challenger® at 5% and 10% concentrations, respectively (Figure 1B). Similar results were reported by Lacey et al. (2011), who evaluated *Metarhizium anisopliae*, *Isaria fumosorosea*, and the insecticide abamectin against the potato psyllid (*Bactericera cockerelli* Sulc) under field conditions, observing reductions in egg production and nymph survival compared to the control, which only mitigated plant damage.

At three DAT, the interaction between factors (product and concentration) was not significant for Boveril Cana® ($F = 3.44$, $Fstat = 2.50$, $p = 0.0861$). Significant interactions were observed for Boveril® ($F = 0.82$, $Fstat = 72.29$, $p = 0.0381$), Metarril® ($F = 9.77$, $Fstat = 18.94$, $p = 0.0080$), and Challenger® ($F = 53.56$, $Fstat = 29.18$, $p = 0.0001$). The highest mortality rate for *G. assimilis* (37.5%) was observed with Boveril® at a 5% concentration. Challenger® at 5% and Metarril® at 15% also caused significant mortality rates of approximately 31.25% (Figure 1C).

At four DAT, significant interactions between factors (product and concentration) were observed for Boveril Cana® ($F = 4.75$, $Fstat = 4.75$, $p = 0.0210$), Metarril® ($F = 20.20$, $Fstat = 16.88$, $p = 0.0012$), and Challenger ($F = 35.56$, $Fstat = 65.29$, $p < 0.0001$). The highest mortality rate for *G. assimilis* (approximately 37.5%) was observed with Boveril Cana® at a 10% concentration. In contrast, no interaction was observed for Boveril® ($F = 0.51$, $Fstat = 44.95$, $p = 0.4876$) (Figure 1D).

At five DAT, significant interactions between factors (product and concentration) were observed for Boveril Cana® ($F = 9.86$, $Fstat = 6.27$, $p = 0.0078$), Metarril® ($F = 9.75$, $Fstat = 8.57$, $p = 0.0085$), and Challenger® ($F = 51.83$, $Fstat = 28.23$, $p < 0.0001$). In contrast, no significant interaction was observed for Boveril® ($F = 0.56$, $Fstat = 49.13$, $p = 0.4684$). The highest mortality rate for *G. assimilis* (43.75%) was observed with Boveril Cana® at a 10% concentration,

followed by Boveril® at 15%, with 37.5% mortality (Figure 1E). Similar results were reported by Pelizza et al. (2019), who evaluated *B. bassiana* using various baits against the locust *Dichroplus maculipennis* under field conditions, in cages. They observed that bait containing wheat bran and *B. bassiana* conidia caused significant mortality in locusts, and mixtures of Boveril Cana®, Boveril®, Metarril®, and Challenger® with feed acted as toxic baits, leading to death of *G. assimilis* individuals.

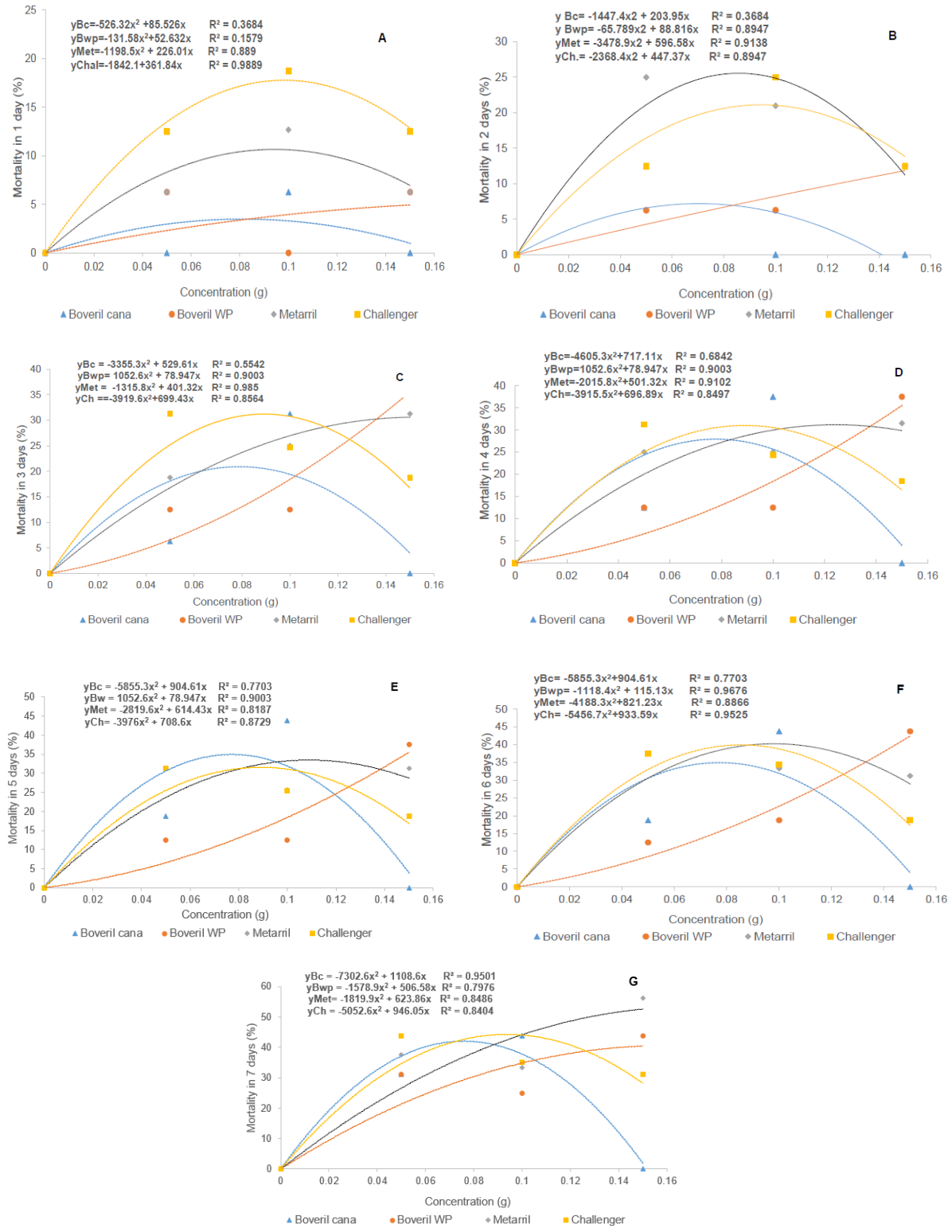

Figure 1. Daily mortality of *Gryllus assimilis* adults subjected to different concentrations (5%, 10%, and 15%) of entomopathogenic fungi-based products (Boveril Cana®, Boveril®, Metarril®, and Challenger®), under laboratory conditions, assessed at 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F), and 7 (G) days after treatment.

At six DAT, significant interactions between factors (product and concentration) were observed for Boveril Cana® (F = 9.74, $Fstat$ = 6.20, p = 0.0081), Metarril® (F = 24.55, $Fstat$ = 16.73, p = 0.0003), and Challenger® (F = 24.27, $Fstat$ = 47.28, p < 0.0001). In contrast, no significant interaction was observed for Boveril® (F = 0.49, $Fstat$ = 58.59, p = 0.4962). The highest mortality rate for *G. assimilis* (43.75%) was observed with Boveril® at a 15% concentration, followed by Metarril® and Challenger® at 5%, each with 37.5% mortality (Figure 1F). These results align with those reported by Konar and Santanu Paul (2005), who evaluated the efficacy of granular insecticides and biopesticides against the mole cricket *Gryllotalpa* spp. (Orthoptera: Gryllotalpidae) in potato cultivars under field conditions. They reported the highest control efficacy for the biopesticides *M. anisopliae* at 1.8×10^9 spores mL^{-1} at 50 g ha^{-1} and *B. bassiana* at 10^8 spores applied at 1,000 g ha^{-1}.

At seven DAT, significant interactions between factors (product and concentration) were observed for Boveril Cana® (F = 26.52, $Fstat$ = 15.68, p = 0.0002), Boveril® (F = 6.67, $Fstat$ = 31.10, p = 0.0227), Metarril® (F = 10.55, $Fstat$ = 24.43, p = 0.0058), and Challenger® (F = 46.42, $Fstat$ = 27.35, p < 0.0001). The highest mortality rate for *G. assimilis* (56.25%) was observed with Metarril® at a 15% concentration (Figure 1G). These results align with those reported by Hanel (1982), who evaluated the insecticidal efficacy of *M. anisopliae* against the termite *Nasutitermes exitiosus* (Hill), using a concentration of 1.29×10^7 conidia mL^{-1} and observed over 95% mortality after 11 days.

At eight DAT, significant interactions between factors (product and concentration) were observed for Boveril Cana® (F = 23.85, $Fstat$ = 12.83, p = 0.0003), Boveril® (F = 11.95, $Fstat$ = 16.50, p = 0.0042), Metarril® (F = 10.85, $Fstat$ = 24.43, p = 0.0048), and Challenger®. The highest mortality rate for *G. assimilis* (37.33%) was observed with Metarril® at a 10% concentration (Figure 2).

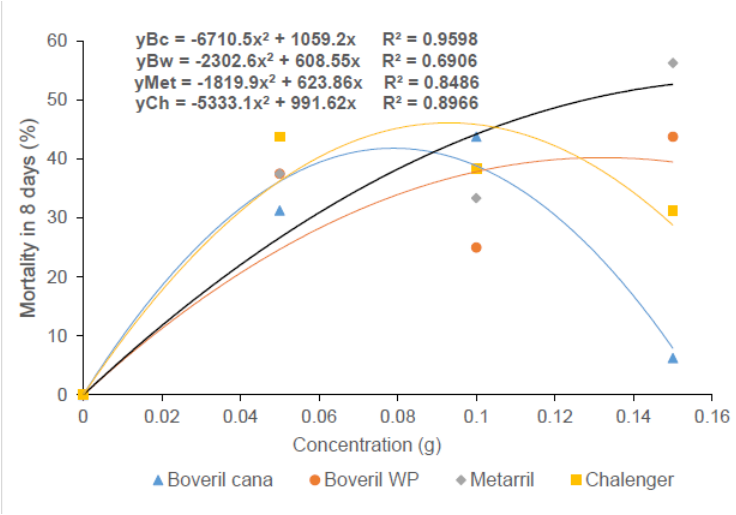

Figure 2. Cumulative mortality of *Gryllus assimilis* adults subjected to different concentrations (5%, 10%, and 15%) of entomopathogenic fungi-based products (Boveril Cana®, Boveril®, Metarril®, and Challenger®), under laboratory conditions, assessed up to eight days after treatment.

Treatments with Metarril® and Challenger® resulted in a gradual reduction in the number of live *G. assimilis* insects. Treatments with Metarril® and Boveril® achieved the highest mortality rates, exceeding 50%, by the end of the experiment. Additionally, the treatment with Challenger® resulted in the third highest mortality rate (43.75%). Reductions in the number of live insects were approximately 43.75% for Metarril® and 68.75% for Boveril Cana®, each applied at 15% concentration (Figure 2).

Dead insects that were collected and incubated in a BOD chamber under controlled conditions exhibited no mycelial growth, regardless of the fungal treatment applied. This may be attributed to mechanisms involved in adhesion, which are not yet fully elucidated. Certain enzymes may be associated with the aggressiveness of specific fungi, and insect mortality results from a complex series of events, making it challenging to correlate fungal treatment with mortality (ALVES, 1986).

Effect of entomopathogenic fungi on mortality of *Gryllus assimilis* nymphs and adults through contact

Nymphs

At one day after treatment (DAT), nymph mortality differed significantly among treatments (F = 15.10, p = 0.0001). The highest mortality rate for *G. assimilis* nymphs (88%) was observed with Boveril® at 30 g, followed by Boveril Cana® at 30 g (57%) and Challenger® at 20 g (51%). The lowest mortality rates were observed with Boveril® at 20 g (39%) and Challenger® at 30 g (48%). Boveril Cana® at 20 g and Metarril® at 20 g and 30 g resulted in no mortality (Table 2).

At two DAT, nymph mortality differed significantly among treatments (F = 4.51, p = 0.0010). However, only Boveril® at 20 g resulted in a significant mortality rate (13%) compared to the control, while the other treatments resulted in

no mortality (Table 2). At three DAT, nymph mortality did not differ significantly among treatments (F = 3.38, p = 0.2993) (Table 2).

At four DAT, nymph mortality differed significantly among treatments (F = 5.66, p = 0.0002). The highest mortality rate for nymphs (42%) was observed with Metarril® at 20 g (Table 2). At five DAT, nymph mortality did not differ significantly among treatments (F = 0.75, p = 0.6479) (Table 2).

At six DAT, nymph mortality differed significantly among treatments (F = 3.54, p = 0.0060). The highest mortality rate for nymphs (22%) was observed with Challenger® at 20 g, followed by Metarril® at 20 g and Challenger® at 30 g, each with 13% (Table 2).

Table 2. Mean mortality and percentage mortality (M%) of *Gryllus assimilis* nymphs treated with entomopathogenic fungi-based products [Boveril Cana® (BC), Boveril® (BO), Metarril® (ME), and Challenger® (CH)] in contact bioassays using 20 g (20) and 30 g (30) of each product, under laboratory conditions, assessed up to six days after treatment (DAT).

T	C	1 DAT	M%	2 DAT	M%	3 DAT	M%	4 DAT	M%	5 DAT	M%	6 DAT	M%
BC	20	0.71±0.00 c	0	0.71±0.00b	0	0.71±0.00a	0	1.34±0.17a	0	0.84±0.06a	13	0.71±0.00b	0
	30	1.27±0.08ab	57	0.71±0.00b	0	0.84±0.12a	13	0.81±0.06b	13	0.71±0.00a	0	0.71±0.00b	0
BO	20	1.09±0.07bc	39	0.84±0.06a	13	0.84±0.09a	13	0.97±0.06ab	26	0.71±0.06a	0	0.71±0.00b	0
	30	1.58±0.19a	88	0.71±0.00b	0	0.71±0.00a	0	0.71±0.00b	0	0.71±0.00a	0	0.71±0.00b	0
ME	20	0.71±0.00 c	0	0.71±0.00b	0	0.71±0.00a	0	1.12±0.16ab	42	0.84±0.06a	13	0.84±0.06ab	13
	30	0.71±0.00 c	0	0.71±0.00b	0	0.84±0.06a	13	0.84±0.06b	13	0.71±0.00a	0	0.71±0.00b	0
CH	20	1.21±0.10ab	51	0.71±0.06b	0	0.71±0.00a	0	0.71±0.00b	0	0.84±0.06a	13	0.93±0.10a	22
	30	1.18±0.03b	48	0.71±0.00b	0	0.71±0.00a	0	0.97±0.06ab	0	0.71±0.00a	0	0.84±0.06ab	13
CT	30	0.71±0.00 c	0	0.71±0.00b	0	0.71±0.00a	0	0.71±0.00b	0	0.71±0.00a	0	0.71±0.00b	0
DF		8.27		8.27		8.27		8.27		8.27		8.27	
F		15.10		4.51		3.38		5.66		0.75		3.54	
p		< 0.0001		0.0010		0.2993		0.0002		0.6480		0.0060	

M% = percentage mortality calculated relative to the control, using the Schneider-Orelli (1947) formula: $M\% = 100 \times (Mt - Mc) \div (100 - Mc)$, where Mt is the mortality in the insecticide treatment and Mc is the mortality in the control treatment. DF = degrees of freedom. Means followed by the same letter in the columns are not statistically different by Tukey's test at a 5% significance level.

Adults

At one day after treatment (DAT), adult mortality differed significantly among treatments (F = 4.89, p = 0.0008). The highest mortality rate for *G. assimilis* adults (57%) was observed with Boveril® at 30 g, followed by Boveril® at 20 g (26%) and Boveril Cana® at 30 g (22%). A mortality rate of 13% was observed with Challenger® at 20 g and 30 g. Boveril Cana® at 20 g and Metarril® resulted in no mortality (Table 3).

At two DAT, adult mortality differed significantly among treatments (F = 3.54, p = 0.0060). The highest mortality rate for adults (22%) was observed with Metarril® at 20 g, whereas a mortality rate of 13% was observed with Boveril® at 20 g and Challenger® at 20 g. At three DAT, adult mortality differed significantly among treatments (F = 4.51, p = 0.001. However, only Boveril® at 20 g resulted in a significantly different mortality rate (13%), whereas the other treatments resulted in no mortality (Table 3).

At four DAT, adult mortality differed significantly among treatments (F = 4.39, p = 0.0010). The highest mortality rate for adults (51%) was observed with Boveril Cana® at 30 g, followed by Boveril Cana® at 20 g and Metarril® 20 g, each with 26%. Boveril® at 20 g and 30 g, as well as Challenger® at 20 g, resulted in 13% mortality (Table 3). At five DAT, adult mortality differed significantly among treatments (F = 7.05, p = 0.0010). The highest mortality rate for *G. assimilis* adults (26%) was observed with Challenger® at 20 g, whereas Metarril® at 20 g resulted in 22% mortality (Table 3).

At six DAT, adult mortality differed significantly among treatments (F = 3.10, p = 0.013. The highest mortality rate for adults (35%) was observed with Challenger® at 30 g, followed by Metarril® at 30 g and Boveril® at 20 g, each with 26% mortality. Challenger® at 20 g, Metarril® at 20 g, and Boveril Cana® at 30 g resulted in 13% mortality (Table 3).

Dead insects that were collected and incubated in a BOD chamber for seven days exhibited reduced mycelial growth, confirming mortality caused by fungal infection. Microbiological insecticides based on the fungi *M. anisopliae* and *B. bassiana* are effective in controlling insect pests. These fungi are among the most promising microbial control agents, particularly for soil-dwelling insects. According to Feng Poprawski and Khachatourians. (1994), *B. bassiana* is a natural soil-dwelling microbiological agent pathogenic to over 200 insect species. The efficacy of these entomopathogenic fungi depends on their persistence in the pest's microenvironment and the selection of specific strains for suitable hosts (EL-GARHY, 2011).

Table 3. Mean mortality and percentage mortality (M%) of *Gryllus assimilis* adults treated with entomopathogenic fungi-based products [Boveril Cana® (BC), Boveril® (BO), Metarril® (ME), and Challenger® (CH)] in contact bioassays using 20 g (20) and 30 g (30) of each product, under laboratory conditions, assessed up to six days after treatment (DAT).

T	C	1 DAT	M%	2 DAT	M%	3 DAT	M%	4 DAT	M%	5 DAT	M%	6 DAT	M%
BC	20	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.93±0.01ab	26	0.71±0.00b	0	0.71±0.00b	0
	30	0.93±0.10ab	22	0.71±0.00b	0	0.71±0.00b	0	1.21±0.10a	51	0.71±0.00b	0	0.84±0.06b	13
BO	20	0.97±0.06ab	26	0.84±0.06ab	13	0.84±0.06a	13	0.84±0.06b	13	0.71±0.06b	0	0.97±0.06ab	26
	30	1.27±0.20a	57	0.71±0.00b	0	0.71±0.00b	0	0.84±0.06b	13	0.71±0.00b	0	0.84±0.06b	13
ME	20	0.71±0.00b	0	0.93±0.10a	22	0.71±0.00b	0	0.97±0.14ab	26	0.93±0.21a	22	0.84±0.06b	13
	30	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.97±0.06ab	26
CH	20	0.84±0.06b	13	0.84±0.06ab	13	0.71±0.00b	0	0.84±0.06b	13	0.97±0.06a	26	0.84±0.06b	13
	30	0.84±0.06b	13	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	1.05±0.13a	35
CT	30	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0	0.71±0.00b	0
DF		8. 27		8. 27		8. 27		8. 27		8. 27		8. 27	
F		4.89		3.54		4. 51		4.39		7.05		3.10	F
p		0.0008		0.0060		0.0010		0.0010		0.0010		0.0129	

M% = percentage mortality calculated relative to the control, using the Schneider-Orelli (1947) formula: $M\% = 100 \times (Mt - Mc) \div (100 - Mc)$, where *Mt* is the mortality in the insecticide treatment and *Mc* is the mortality in the control treatment. DF = degrees of freedom. Means followed by the same letter in the columns are not statistically different by Tukey's test at a 5% significance level.

The strains of entomopathogenic fungi evaluated (*B. bassiana* IBCB 66, *B. bassiana* PL 63, *M. anisopliae* E9, and *Isaria fumosorosea* ESALQ 1296) infect insects across nearly all orders, particularly Hemiptera, Coleoptera, Lepidoptera, Orthoptera, and Hymenoptera (RAMANUJAM et al., 2024; MAINA et al., 2018). They have demonstrated entomopathogenic activity against various arthropods, including *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae), *Dalbulus maidis* (Hemiptera: Cicadellidae), *Gonipterus scutellatus* (Coleoptera: Curculionidae), *Mahanarva fimbriolata* (Hemiptera: Cercopidae), *Diatraea saccharalis* (Lepidoptera: Pyralidae), *Amblyomma cajennense* (Acari: Ixodidae), and *Helicoverpa armigera* (Lepidoptera: Noctuidae) (LOPES et al., 2007).

Overall, mortality rates increased with higher concentrations of the applied products, but average feed consumption varied significantly among treatments and concentrations. Conidia of *M. anisopliae* E9 (Metarril®) were not repellent to *G. assimilis*, and reductions in the number of insects were associated with bait feeding and product concentrations, causing over 50% mortality of *G. assimilis*. Thus, products based on *B. bassiana* and *M. anisopliae* conidia were not repellent and can be incorporated into baits for controlling *G. assimilis*.

CONCLUSION

The results demonstrate that the entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea* cause mortality in *Gryllus assimilis*. The pathogenicity of these fungi, applied at different concentrations, varied over time after application. In contact assays, Boveril® and Challenger® caused the highest mortality rates in *G. assimilis* (88% and 51%, respectively) one day after application. In ingestion assays, Metarril® at 15% resulted in a mortality rate of 56% in insects. Among the evaluated entomopathogenic fungal insecticides, Boveril®, Challenger®, and Metarril® were the most effective. Notably, Metarril® achieved the highest cumulative mortality when applied through ingestion, making it suitable for incorporation into toxic baits for controlling *G assimilis*. Therefore, the use of these entomopathogenic fungi offers an effective and environmentally friendly alternative for controlling *G assimilis*, particularly given the nocturnal habit of crickets, remaining hidden during the day under trunks, tree bark, and soil mulch.

ACKNOWLEDGEMENTS

The authors thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), for granting a master's scholarship to ADS; the Brazilian National Council for Scientific and Technological Development (CNPq) for providing a productivity scholarship to FRMG; the Koppert company and the Federal University of Rio Grande (FURG) for supplying experiment materials; and Daniel Soares Duarte and Lúcia Maciel for their translation assistance.

REFERENCES

ALMEIDA, J. E. M. Biofactories for the production of mycopesticides in Brazil: business opportunities and innovations. **Brazilian Journal of Animal and Environmental Research**, 3: 2444-2557, 2020.

ALVES, S. B. **Técnicas de laboratório: Controle Microbiano de Insetos**. São Paulo, SP: Manole. 1986. 407 p

ANANI, O. A. et al. Effects of pesticide toxins on food safety: current developments In: MISHRA, P.; MISHRA, R.R.; ADETUNJI, C.O (Eds.). **Innovations in Food Technology**. New York, NY: Springer, 2020. v. 1., cap. 24, p. 313-321.

BOOMSMA, J. J. et al. Evolutionary interaction networks of insect pathogenic fungi. **Annual Review of Entomology**, 59: 467-485, 2014.

EL-GARHY, S. M. M. Impact of certain baits for controlling *Anacardium egyptium* (L.), under laboratory and field conditions, **Fayoum Journal of Agricultural Research & Development**, 25: 238-249, 2011

FENG, M. G.; POPRAWSKI, T. J.; KHACHATOURIANS, G. G. Production, rmulation, and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. **Biocontrol Science and technology**, 4: 3-34, 1994.

HASHIMI, M. H.; HASHIMI, R.; RYAN, Q. Toxic Effects of Pesticides on Humans, Plants, Animals, Pollinators and Beneficial Organisms **Asian Plant Research Journal**, 5: 37-47, 2020.

LIMBERGER, G. M.; NERY, L. E. M.;FONSECA, D. B. Reproductive status modulates mortality rate, lifespan and egg production, but do not physiological aging in the field cricket *Gryllus assimilis* (Fabricius, 1775) (Orthoptera: Grylidae), **Neotropical Entomology**, 50: 237-246, 2021.

HANEL, H. A. Selection of a fungus species, suitable for the biological of the termite *Nasutitermes exitiosus* (Hill) (Isoptera: Termitidae). **Zeitschrift Angewandte Entomologie**, 94: 237-245, 1982.

HUMBER, R. A. Evolution of entomopathogenicity in fungi. **Journal of Invertebrate Pathology**, 98: 262-266, 2008.

ISLAM, W. et al. Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. **Microbial Pathogenesis**, 159: 105122, 2021.

KONAR, A.; SANTANU PAUL, S. P. Efficacy of some granular insecticides and biobiopesticides against mole cricket on potato. **Journal of Applied Zoological Researchers**, 16: 59-60, 2005.

LACEY, L. A. et al. Fungos entomopatogénicos (Hipocreales) para controle do psilídio da batata, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) em em uma área endémica para doença zebra chip da batata, **Controle Biológico**, 56: 271-278, 2011.

LOPES, R. B. et al. Efficiency of formulations of *Beauveria bassiana* and *Metarhizium anisopliae* for the control of nymphs of *Amblyomma cajennense* (Fabricius, 1787). **Revista Brasileira Parasitologia Vetetrinaria**, 16: 27-31, 2007.

MAINA, U. M. et al. A review on the use of entomopathogenic fungi in the management of insect pests of field crops. **Journal of Entomology and Zoology Studies**, 6: 27-32, 2018.

MANFO, F. P. T. et al. Evaluation of the Effects of Agro Pesticides Use on Liver and Kidney Function in Farmers from Buea, Cameroon. **Journal of Toxicology**, 29: 2305764, 2020.

MASCARIN, G. M.; JARONSKI, S. T. The production and uses of *Beauveria bassiana* as a microbial insecticide. **World Journal of Microbiology and Biotechnology**, 32: 1-26, 2016.

MEIKLE, W. G. et al. Evaluation of an entomopathogenic fungus, Paecilomyces fumosoroseus (Wize) Brown & Smith (Deuteromycota: Hyphomycetes) obtained from formosan subterranean termites (Isoptera: Rhinotermitidae**). Journal of Applied Entomology**, 126: 315-322, 2005.

PEDRINI, N.; CRESPO, R.; JUÁREZ, M. P. Biochemistry of insect epicuticle degradation by entomopathogenic fungi. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, 146: 124-137, 2007.

PELIZZA, S. et al. Application of *Beauveria bassiana* using different baits for the control of grasshopper pest *Dichroplus maculipennis* under field cage conditions. **Journal of King Saud University – Science**, 31: 1511-155, 2019.

RAMANUJAM, B. et al. Management of Insect Pests by Microorganisms. **Proceedings of Indian National Science Academy**, 80: 455-471, 2024.

ROHRIG, B. Entomopathogenic fungi in pest control: what they are and how to use them in the crop. **Blog Aegro**, 2021. Available at: https://blog.aegro.com.br/fungos-entomopatogenicos-no-controle-de-pragas/ Access on: Dec. 20, 2022.

R DEVELOPMENT CORE TEAM. **Fundação R para Computação Estatística**, Viena, 2023.

SCHNEIDER-ORELLI, **Entomologisches Praktikum**. 2. ed. Suíça: HR Sauerlander, 1947. 237 p.

SHAH, P. A; PELL, J. K. Entomopathogenic fungi as biological control agents. **Applied Microbiology and Biotechnology**, 61: 413-423, 2019.

SHARMA, A.; SHARMA, S.; YADAV, P.K. Entomopathogenic fungi and their relevance in sustainable agriculture: A review, **Cogent Food & Agriculture**, 9: 2180857, 2023.

STONE, L. B. L.; BIDOCHKA, M. J. The multifunctional lifestyles of Metarhizium: Evolution and applications. **Applied Microbiology and Biotechnology**, 104: 9935-9945, 2020.

TUPE, S. G. et al. Development of *Metarhizium anisopliae* as a mycoinsecticide: from isolation to field performance. **Journal of Visualized Experiments**, 125: 55272, 2017.

VEGA, F. E. et al. ***Fungal Entomopathogens***. Insect Pathology, 2: 171-220, 2012.