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/10.1590/1983-21252024v3712375rc

*Scientific Article*

# Virulence of entomopathogenic fungi in larvae of Lepidoptera: Noctuidae

# Virulência de fungos entomopatogênicos sobre larvas de Lepidoptera: Noctuidae

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**ABSTRACT** - Currently, agricultural cropping systems have adopted integrated pest management (IPM) as a successful model for pest control. The use of entomopathogenic fungi in IPM has increased because of their great potential for reducing arthropod pest populations without causing harm to human health and ecosystems. *Beauveria bassiana* and *Metarhizium anisopliae* stand out among the most used fungi in biological control, with many isolates commercialized worldwide. *Helicoverpa armigera* is an agriculturally important pest in Brazil and worldwide, causing damage to several crops. In this context, the objective of this study was to evaluate the biological control potential of *Beauveria bassiana*, *Metarhizium anisopliae,* and *M. rileyi* on *H. armigera*. The results indicated that the entomopathogenic fungi *B. bassiana* (strain ESALQ PL63), *M. anisopliae* (strain ESALQ E9), and *M. rileyi* (strain UFMS 03), applied using different methods (direct application and dry film) and concentrations ($1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ conidia $mL^{-1}$), resulted in low mortality and no virulence in first-, third-, and fifth-instar larvae of *H. armigera*. The death of all individuals subjected to treatment with *B. bassiana* (strain ESALQ PL63) was confirmed by conidiogenesis. Considering the importance of *H. armigera* as an agricultural pest and the biodiversity of entomopathogenic fungi in Brazil, further investigations on the virulence of fungal strains are necessary to improve the integrated management of lepidopteran pests through microbial control, explore the potential of new strains, and understand the relationships between microorganisms and host defense mechanisms.

**Keywords**: Biological control. *Beauveria. Metarhizium. Helicoverpa.* Pest management.

**RESUMO** - Atualmente, os sistemas de cultivo agrícola adotam o manejo integrado de pragas (MIP) como modelo de sucesso para o controle de pragas. O uso de fungos entomopatogênicos tem aumentado no manejo integrado devido ao seu grande potencial em reduzir as populações de pragas agrícolas sem causar danos à saúde humana e aos ecossistemas. *Beauveria bassiana* e *Metarhizium anisopliae* são as espécies de fungos mais utilizadas no controle biológico, contando com muitos isolados comercializados no mundo. *Helicoverpa armigera* é uma praga de importância agrícola, causadora de prejuízos associados a diversas culturas. Assim, esse estudo teve como objetivo avaliar o potencial virulento de *Beauveria bassiana*, *Metarhizium anisopliae* e *Metarhizium rileyi* no controle biológico dos estágios larvais de *H. armigera*. Os resultados indicaram que em diferentes concentrações ($1 \times 10^7$, $1 \times 10^8$ e $1 \times 10^9$ con.$mL^{-1}$) e métodos de aplicação (DA ou DF) *B. bassiana* (cepa ESALQ PL63), *M. anisopliae* (cepa ESALQ E9) e *M. rileyi* (cepa UFMS 03) causaram baixa mortalidade e não foram virulentos ao primeiro, terceiro e quinto ínstares de *H. armigera*. A mortalidade pelo tratamento com *B. bassiana* (cepa ESALQ PL63) foi confirmada por conidiogênese em todos os cadáveres. Dada a importância de *H. armigera* como praga agrícola e a biodiversidade de fungos entomopatogênicos no Brasil, novas investigações sobre a virulência de cepas fúngicas são necessárias para aprimorar o manejo integrado de pragas através do controle microbiano, o potencial para novas cepas e o entendimento das relações entre microrganismos e os mecanismos de defesa dos hospedeiros.

**Palavras-chave**: Controle biológico. *Beauveria. Metarhizium. Helicoverpa.* Manejo de pragas.

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



**Received for publication in:** December 14, 2023.
**Accepted in:** March 08, 2024.

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## INTRODUCTION

*Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), known as Old World bollworm, cotton bollworm, or corn earworm, was previously considered a quarantine pest in Brazil. It was first reported in Brazilian regions during the 2013/2014 agricultural year, causing yield losses in several economically important crops (CZEPAK et al., 2013). Chemical control has been the main management strategy for *H. armigera* in different cropping systems, although biological control is also a viable alternative for controlling *H. armigera* (FATHIPOUR; SEDERATIAN, 2013). This can be connected to the lower production costs of chemical control, the lack of proper information on integrated pest management (IPM), and potential field applicability (PARRA, 2023).

Microbial control is an effective biological control practice. Entomopathogenic fungi are a diverse group of species that infect and cause diseases in arthropods (LOPES et al., 2023). They are responsible for approximately 80% of the diseases occurring in insects, presenting advantages over other fungi in terms of genetic variability, infection at different host development stages, penetration via integument, and propagules with high

dissemination capacity (FARIA et al., 2022). The genera *Aspergillus, Beauveria, Metarhizium, Cordyceps, Isaria,* and *Pandora* stand out as agents in the microbial control of arthropod pests (GOTTI et al., 2023). Studies with entomopathogenic fungi for pest control under laboratory and field conditions have shown that strains of the species *Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch.) Sorok, and *Metarhizium rileyi* (Farlow) Samson have high virulence in different instars of *H. armigera* larvae (FITE et al., 2019; BORGES et al., 2021; MONTECALVO; NAVASERO, 2021).

The first report of the natural action of entomopathogenic fungus *M. rileyi* on *H. armigera* in Brazil was in cotton crops in 2015 (COSTA et al., 2015). In the state of Mato Grosso do Sul, epizootics and enzootics of *M. rileyi* in *H. armigera* were reported in the 2017 and 2019 soybean crop seasons (DIAS et al., 2019). Although there are reports of the natural action of *M. rileyi* on *H. armigera*, there is no commercial formulation containing this fungus with sufficient studies for production in rice and determination of the hydration level (LOUREIRO et al., 2019). The in vitro mass production, storage, and formulation of entomopathogenic fungi are processes that have restricted increases in their use as biological control agents (DIAS et al., 2020).

Modern agriculture has increasingly incorporated entomopathogenic fungi into integrated pest management due to the great potential of these microorganisms for reducing arthropod pest populations (PARRA, 2023) while causing no harm to human health and ecosystems (VIANNA et al., 2020). Considering the importance of fungal species as biological control agents for agricultural pests, the objective of this study was to evaluate the pathogenicity of *Beauveria bassiana* (strain ESALQ PL63), *Metarhizium anisopliae* (strain ESALQ E9), and *Metarhizium rileyi* (strain UFMS 03) against first-, third-, and fifth-instar larvae of *H. armigera*.

## MATERIAL AND METHODS

### Entomopathogenic fungi

Commercial formulations based on the *Beauveria bassiana* strain ESALQ PL63 (BOVERIL®) and the *Metarhizium anisopliae* strain ESALQ E9 (METARRIL®) were used. BOVERIL® targets mainly *Bemisia tabaci*, *Hypothenemus hampei*, *Gonipterus scutellatus*, and *Tetranychus urticae*, whereas METARRIL® is commonly used against *Mahanarva fimbriolata.*

No commercial formulation containing *Metarhizium rileyi* is available; thus, the *M. rileyi* strain used was UFMS 03, which belongs to the entomopathogenic bank of the Entomology Laboratory at the Federal University of Mato Grosso do Sul (UFMS), in Chapadão do Sul, Mato Grosso do Sul (MS), Brazil. *M. rileyi* was cultured in Sabouraud culture medium, following the methodology of Loureiro et al. (2019).

### Mass rearing of *Helicoverpa armigera*

Adults and larvae of *H. armigera* of different ages were collected from soybean fields in (2022/2023 crop season), free of chemical phytosanitary product spraying, at the UFMS Experimental Area, Chapadão do Sul Campus, MS, Brazil.

Mass breeding was established under laboratory conditions (air temperature of 25±1 °C, relative air humidity of 70±10%, and a 12-hour photoperiod), where larvae were kept in plastic breeding containers with a capacity for 16 individuals (DIAS et al., 2019) and were provided a daily artificial diet of white beans adapted from Greene, Leppla and Dickerson (1976). *H. armigera* pupae were separated by sex. After emergence, the adults were placed in cages, totaling 8 couples per cages.

### Bioassays

The bioassays were conducted under laboratory conditions (air temperature of 25±1 °C, relative air humidity of 70±10%, and a 12-hour photoperiod). Egg masses from the mass breeding were separated for setting up the experiment. After hatching, *H. armigera* larvae sized 1 to 3 mm were individualized in Petri dishes and fed with an artificial diet until the larval stage for the applications. The fungal suspensions were prepared by dilutions (1 g) of each strain in sterile distilled water + 0.01% Tween80® (v $v^{-1}$). Subsequently, the conidia were counted in a Neubauer counting chamber (Boeco®; Lab-Líder, Ribeirão Preto, Brazil) to standardize the concentrations (DIAS et al., 2020).

The treatments (Products) consisted of control with sterile distilled water + Tween80® (P1); and *B. bassiana* strain ESALQ PL63 (P2), *M. anisopliae* strain ESALQ E9 (P3), and *M. rileyi* strain UFMS 03 (P4) at different concentrations: $1 \times 10^7$; $1 \times 10^8$; and $1 \times 10^9$ conidia $mL^{-1}$, adding Tween80® (0.1 mL) to each fungal suspension. The applications were carried out using an adapted Potter tower, with a standard volume of 2 mL per replicate, using two exposure methods: direct application and dry film method to standardize arthropod selectivity tests for chemical substances and or entomopathogens (HASSAN, 1985). The direct application method involved applying the treatments directly to the larvae and Petri dishes (arenas). The dry film method involved applying the treatments only to the arenas; the larvae were placed after excess moisture had dried, following the protocol proposed by the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC), West Palaearctic Regional Section (WPRS).

*H. armigera* larvae at first (L1; > 24 hours after hatching), third (L3), and fifth (L5; > 24 hours after molting to the larval stage) instars were used. Mortality was assessed every 24 hours, with cumulative mortality every 3 days, up to 12 days after application. Dead larvae were placed in a humid chamber to confirm their death and allow conidiogenesis on their bodies (DIAS et al., 2019; BORGES et al., 2021).

### Statistical analysis

A completely randomized experimental design was used, in a 4×3×2 factorial arrangement consisting of 4 treatments (products; P), three concentrations (C), and two application methods (A). Thirty replicates of one *H. armigera* larva per instar were used for each treatment, concentration combinations, and application methods, totaling 600 larvae per instar, including the control treatment. The bioassay was replicated 3 times.

The data were subjected to analysis of variance (ANOVA) and the means were compared by the Tukey's test at a 5% significance level, using the Rbio program.

## RESULTS AND DISCUSSION

### Mortality of first-instar *Helicoverpa armigera* larvae

The products (P), application methods (A), concentrations (C), and interactions between the factors (P×A, P×C, A×C, and P×A×C) had no significant effects on the mortality of first-instar *H. armigera* larvae (L1) at 3, 6, 9, and 12 days after treatment application ($p$>0.05; Table 1). Under the tested conditions, the entomopathogenic fungi *B. bassiana*, *M. anisopliae*, and *M. rileyi* provided low mortality of L1, with no significant differences from the control (Table 2). The death of all larvae infected with *B. bassiana* was confirmed by conidiogenesis.

**Table 1**. ANOVA for mortality of first-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Source of variation | DF | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|---|
| Product (P) | 3 | 0.50 $^{ns}$ | 0.52 $^{ns}$ | 0.62 $^{ns}$ | 0.75 $^{ns}$ |
| Application method (A) | 1 | 0.89 $^{ns}$ | 1.00 $^{ns}$ | 0.88 $^{ns}$ | 0.75 $^{ns}$ |
| Concentration (C) | 3 | 0.95 $^{ns}$ | 0.82 $^{ns}$ | 0.99 $^{ns}$ | 0.98 $^{ns}$ |
| A×P | 3 | 0.88 $^{ns}$ | 0.93 $^{ns}$ | 0.99 $^{ns}$ | 0.94 $^{ns}$ |
| A×C | 3 | 0.99 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 0.99 $^{ns}$ |
| P×C | 9 | 0.95 $^{ns}$ | 0.99 $^{ns}$ | 0.99 $^{ns}$ | 0.98 $^{ns}$ |
| A×P×C | 9 | 1.00 $^{ns}$ | 0.99 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| Residue | 580 | 62.36 | 0.07 | 0.06 | 0.06 |

*Significant at a 5% level by the F test; $^{ns}$ Not significant. DF = degrees of freedom. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

**Table 2**. Mean±standard error for mortality of first-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Product (P) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|
| Control | 0.03± 0.03 a | 0.03±0.03 a | 0.03±0.03 a | 0.03±0.03 a |
| *B. bassiana* | 0.09±0.02 a | 0.09±0.02 a | 0.08±0.02 a | 0.07±0.02 a |
| *M. anisopliae* | 0.07±0.02 a | 0.07±0.01 a | 0.06±0.02 a | 0.06±0.02 a |
| *M. rileyi* | 0.08±0.02 a | 0.08±0.02 a | 0.06±0.02 a | 0.07±0.02 a |
| Application method (A) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| Direct | 0.08±0.01 a | 0.08±0.01 a | 0.06±0.01 a | 0.07±0.01 a |
| Dry Film | 0.08±0.01 a | 0.08±0.01 a | 0.07±0.01 a | 0.06±0.01 a |
| Concentration (C) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| $10^7$ conidia $mL^{-1}$ | 0.09±0.02 a | 0.08±0.02 a | 0.07±0.02 a | 0.06±0.02 a |
| $10^8$ conidia $mL^{-1}$ | 0.09±0.02 a | 0.07±0.02 a | 0.06±0.02 a | 0.06±0.02 a |
| $10^9$ conidia $mL^{-1}$ | 0.08±0.02 a | 0.10±0.02 a | 0.07±0.02 a | 0.07±0.02 a |

Means followed by the same letters in the columns within each factor are not statistically different a 5% level by the Tukey's test. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

### Mortality of third-instar *Helicoverpa armigera* larvae

The products (P), application methods (A), concentrations (C), and interactions (P×A, P×C, A×C, and P×A×C) had no significant effects on the mortality of third-instar *H. armigera* larvae (L3) at 3, 6, 9, and 12 days after application (DAA) ($p$>0.05; Table 3). The mortality of *H. armigera* (L3) found for the effect of the products (P), application methods (A), concentrations (C), and interactions (P×A, P×C, A×C, and P×A×C) at 3, 6, 9, and 12 DAA was similar (Table 4). The death of all larvae infected with *B. bassiana* (strain ESALQ PL63), as well as two larvae infected with *M. rileyi*, was confirmed by conidiogenesis.

**Table 3**. ANOVA for mortality of third-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Source of variation | DF | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|---|
| Product (P) | 3 | 0.43 $^{ns}$ | 0.50 $^{ns}$ | 0.62 $^{ns}$ | 0.70 $^{ns}$ |
| Application method (A) | 1 | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 0.87 $^{ns}$ | 1.00 $^{ns}$ |
| Concentration (C) | 3 | 1.00 $^{ns}$ | 0.97 $^{ns}$ | 0.98 $^{ns}$ | 0.94 $^{ns}$ |
| P×A | 3 | 1.00 $^{ns}$ | 0.98 $^{ns}$ | 1.00 $^{ns}$ | 0.98 $^{ns}$ |
| P×C | 9 | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| A×C | 3 | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| P×A×C | 9 | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| Residue | 580 | 0.08 | 0.07 | 0.06 | 0.06 |

*Significant at a 5% level by the F test; $^{ns}$ Not significant. DF = degrees of freedom. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

**Table 4**. Mean±standard error for mortality of third-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Product (P) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|
| Control | 0.03±0.00 a | 0.03±0.00 a | 0.03±0.00 a | 0.03±0.00 a |
| *B. bassiana* | 0.10±0.00 a | 0.09±0.00 a | 0.08±0.00 a | 0.07±0.00 a |
| *M. anisopliae* | 0.10±0.00 a | 0.07±0.00 a | 0.06±0.00 a | 0.07±0.00 a |
| *M. rileyi* | 0.10±0. 00 a | 0.08±0.00 a | 0.06±0.00 a | 0.07±0. 00 a |
| Application method (A) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| Direct | 0.09±0.00 a | 0.08±0.00 a | 0.06±0.00 a | 0.07±0.00 a |
| Dry film | 0.09±0. 00 a | 0.08±0.00 a | 0.07±0.00 a | 0.07±0. 00 a |
| Concentration (C) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| $10^7$ conidia mL$^{-1}$ | 0.10±0.00 a | 0.08±0.00 a | 0.03±0.00 a | 0.07±0.00 a |
| $10^8$ conidia mL$^{-1}$ | 0.10±0.00 a | 0.08±0.00 a | 0.07±0.00 a | 0.06±0.00 a |
| $10^9$ conidia mL$^{-1}$ | 0.10±0.00 a | 0.09±0.00 a | 0.06±0.00 a | 0.08±0.00 a |

Means followed by the same letters in the columns within each factor are not significantly different from each other at a 5% level by the Tukey's test. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

### Mortality of fifth-instar *Helicoverpa armigera* larvae

The products (P), application methods (A), concentrations (C), and interactions (P×A, P×C, A×C, and P×A×C) had no significant effects on the mortality of fifth-instar *H. armigera* larvae (L5) at 3, 6, 9, and 12 DAA ($p$>0.05; Table 5). The mortality of L5 were not affected by treatments (P), application methods (A), concentrations (C), and interactions (P×A, P×C, A×C, and P×A×C) at 3, 6, 9, and 12 DAA (Table 6). The death of all larvae infected with *B. bassiana* was confirmed by conidiogenesis.

**Table 5**. ANOVA for mortality of fifth-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Source of variation | DF | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|---|
| Products (P) | 3 | 0.54 $^{ns}$ | 0.23 $^{ns}$ | 0.27 $^{ns}$ | 0.35 $^{ns}$ |
| Application method (A) | 1 | 0.63 $^{ns}$ | 0.76 $^{ns}$ | 0.76 $^{ns}$ | 0.87 $^{ns}$ |
| Concentration (C) | 3 | 0.94 $^{ns}$ | 0.80 $^{ns}$ | 0.89 $^{ns}$ | 0.93 $^{ns}$ |
| P×A | 3 | 0.98 $^{ns}$ | 0.88 $^{ns}$ | 0.94 $^{ns}$ | 0.85 $^{ns}$ |
| P×C | 9 | 0.99 $^{ns}$ | 1.00 $^{ns}$ | 0.99 $^{ns}$ | 0.99 $^{ns}$ |
| A×C | 3 | 0.98 $^{ns}$ | 0.95 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| P×A×C | 9 | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ | 1.00 $^{ns}$ |
| Residue | 580 | 0.06 | 0.07 | 0.07 | 0.06 |

*Significant at a 5% level by the F test; $^{ns}$ Not significant. DF = degrees of freedom. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

**Table 6**. Mean±standard error for mortality of fifth-instar *Helicoverpa armigera* larvae infected with *Beauveria bassiana*, *Metarhizium anisopliae,* and *Metarhizium rileyi,* assessed at 3, 6, 9, and 12 days after application (DAA).

| Product (P) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
|---|---|---|---|---|
| Control | 0.03±0.00 a | 0.03±0.00 a | 0.03±0.00 a | 0.03±0.00 a |
| *B. bassiana* | 0.08±0.00 a | 0.10±0.00 a | 0.10±0.00 a | 0.09±0.00 a |
| *M. anisopliae* | 0.07±0.00 a | 0.08±0.00 a | 0.07±0.00 a | 0.05±0.00 a |
| *M. rileyi* | 0.06±0. 00 a | 0.06±0.00 a | 0.06±0.00 a | 0.07±0.00 a |
| Application method (A) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| Direct | 0.07±0.00 a | 0.08±0.00 a | 0.08±0.00 a | 0.06±0.00 a |
| Dry film | 0.06±0.00 a | 0.07±0.00 a | 0.07±0.00 a | 0.07±0.00 a |
| Concentration (C) | 3 DAA | 6 DAA | 9 DAA | 12 DAA |
| $10^7$ conidia $mL^{-1}$ | 0.08±0.00 a | 0.06±0.00 a | 0.07±0.00 a | 0.07±0.00 a |
| $10^8$ conidia $mL^{-1}$ | 0.08±0.00 a | 0.09±0.00 a | 0.09±0.00 a | 0.08±0.00 a |
| $10^9$ conidia $mL^{-1}$ | 0.07±0.00 a | 0.08±0.00 a | 0.08±0.00 a | 0.06±0.00 a |

Means followed by the same letters in the columns within each factor are not significantly different from each other at a 5% level by the Tukey's test. Bioassay conducted under laboratory conditions: air temperature of 25±1 °C, relative air humidity of 70±10%, and 12-hour photoperiod.

Overall, the strains of entomopathogenic fungi tested at different concentrations and exposure methods showed low virulence across the three larval instars of *H. armigera*. The low mortality found in the present study contradicts results obtained in other experiments. Studies have shown that *B. bassiana and M. anisopliae* have high pathogenicity and virulence against several agricultural pests, including *Plutella xylostella* (AGBOYI et al., 2020; SHEHZAD et al., 2021) *Spodoptera frugiperda* (CRUZ-AVALOS et al., 2019; RAMANUJAM; POORNESHA; SHYLESHA, 2020), and *Helicoverpa armigera* (KALVNADI et al., 2018; FITE et al., 2019; KUMAR; SHARFUDDIN, 2022). However, the efficacy of the fungi varies depending on many factors, such as genetic variability of different strains of entomopathogenic fungi (MONTECALVO; NAVASERO, 2021), characteristics inherent to entomopathogenic fungi on the production of proteases, and cuticle composition and thickness of the pest species (JAFARPOUR et al., 2020).

Variations among strains of entomopathogenic fungi regarding insect pest mortality have been reported in several studies (CRUZ-AVALOS et al., 2019; FITE et al., 2019). Fite et al. (2019), found *H. armigera* mortality ranging from 38% to 91% and from 20% to 73% for different strains of *B. bassiana* and *M. anisopliae*, respectively. Similar results were reported by Cruz-Avalos et al. (2019), who found variations from 28% to 100% in *S. frugiperda* larvae mortality caused by different strains of *B. bassiana*. IDREES et al. (2021) reported that *S. frugiperda* larvae were not susceptible to any of the tested entomopathogenic fungi, which is a similar result to those found in the present study; however, *S. frugiperda* eggs were highly susceptible to the tested fungi.

These differences in pathogenicity and virulence among different isolates of the same species may be connected to the strain genetic variability. Some studies have shown that different strains have different virulence for the same host, and this variability depends on the gene sequence (VIANNA et al., 2020; GASMI et al., 2021; BORGES et al., 2021). According to Gasmi et al. (2021), genetic variability may be an important factor in determining the performance of different strains of *B. bassiana*. In this sense, Vianna et al. (2020) found that the most pathogenic and virulent strains of *B. bassiana* (LPSc1215 and LPSc 1364) against *Helicoverpa gelotopoen* and *Diabrotica speciosa* were grouped in the same cluster, indicating a potential relationship between genetic variability and fungi pathogenicity and virulence.

In addition to the genetic variability of pathogens, the susceptibility of host species may vary. The infection process of entomopathogenic fungi involves adhesion, germination, and penetration of the pathogen through the insect cuticle, overcoming physical and chemical barriers (VIANNA et al., 2020). Chemical barriers are broken down by the production of proteases and chitinases, with a degrading function on the host's cuticle (WANG; FENG, 2014). Thus, the speed at which the fungal reproductive structure penetrates the host's cuticle varies depending on the thickness of the insect's cuticle (KUMAR; SHARFUDDIN, 2022).

Considering the hypothesis that the fungi *B. bassiana* (strain ESALQ PL63), *M. anisopliae* (strain ESALQ E9), and *M. rileyi* (strain UFMS 03) have low virulence in *H. armigera* due to the cuticle composition of this species, nutrients that improve the its cuticle structure may have provided a protective barrier that prevented the penetration and subsequent germination of the conidia. Melanin stands out among the components that provide structure and strength to the insect's cuticle. It interferes with the penetration of pathogens through physical or mechanical protective barriers and provides the cuticle with important defense mechanisms against pathogens due to chemical properties that increase immunity and antimicrobial activity (KUMAR; SHARFUDDIN, 2022).

Regarding conidiogenesis, mycelial growth was found in 100% of the dead individuals in treatments with BOVERIL® (*B. bassiana* strain ESALQ PL63), 10% in treatments with METARRIL® (*M. anisopliae* strain ESALQ E9), and in two individuals in treatments with *M. rileyi* (strain UFMS 03). The low sporulation found for *Metarhizium* confirm those found in other studies, which showed lower percentages of sporulation for *M. anisopliae* (strain 79) (18.07%) compared to the mycelial growth of *B. bassiana* (strain 124) (67.19%) and *P. fumosoroseus* (strain 14) (84.41%) on *H. armigera*. This may be attributed to the high fungi concentrations, as numerous fungal spores that can

penetrate the host simultaneously may have caused the death of the insect shortly after inoculation due to water loss, before the fungus could effectively grow, thus preventing conidiogenesis in the dead insect (BORGES et al., 2021).

The low susceptibility of *H. armigera* larvae to the tested fungi does not indicate that this species is unharmed by entomopathogenic fungi, as potential side effects may occur in subsequent stages. Studies on the deleterious effects of *M. anisopliae* (strain M14) on *H. armigera* indicate that different sublethal concentrations of the fungus can reduce the growth of the F1 population (TANG; HOU, 2001). Indirect effects on *H. armigera* populations have also been reported for the application of *B. bassiana* (strain DC2), which resulted in adult deformities and infertile eggs (KALVNADI et al., 2018).

The infection mechanism of entomopathogenic fungi is highly specialized and complex, as it explores aspects that are still not fully understood, such as the insect-fungus relationship, the virulence of each strain, and the susceptibility of the host (SEYEDTALEBI et al., 2017). Considering the importance of *H. armigera* as an agricultural pest and the biodiversity of entomopathogenic fungi available in Brazil, further investigations on the virulence of fungal strains are necessary to improve the integrated management of lepidopteran pests through microbial control, explore the potential of new strains, and understand the relationships between microorganisms and host defense mechanisms.

## CONCLUSION

The entomopathogenic fungi *Beauveria bassiana* (strain ESALQ PL63), *Metarhizium anisopliae* (strain ESALQ E9), and *M. rileyi* (strain UFMS 03) evaluated at different concentrations ($1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ conidia $mL^{-1}$) through different application methods (direct application and dry film) were not virulent to first-, third-, or fifth-instar *H. armigera* larvae. The death of all individuals infected with *B. bassiana* was confirmed by conidiogenesis.

## ACKNOWLEDGMENTS

This study was financed in part by the Brazilian Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001, the Women in Science Program 2023 of the Federal University of Mato Grosso do Sul (UFMS); the Foundation for Support of Education, Science, and Technology Development of the State of Mato Grosso do Sul (FUNDECT), PhD scholarship number 59/300.513/2016; and the project funding agency (number 224/2022, 71/027.188/2022 and SIAFEM number 31970).

The authors thank the Graduate Program in Entomology and Biodiversity Conservation (PPGECB-UFGD), Dourados, MS, Brazil; the National Council for Scientific and Technological Development (CNPq); the Federal University of Mato Grosso do Sul (UFMS) in Chapadão do Sul, MS, Brazil; and the members of the LAMIP Research Group.

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