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Exploring the potential of *Metarhizium* species for the control of *Euschistus heros* (Hemiptera: Pentatomidae)

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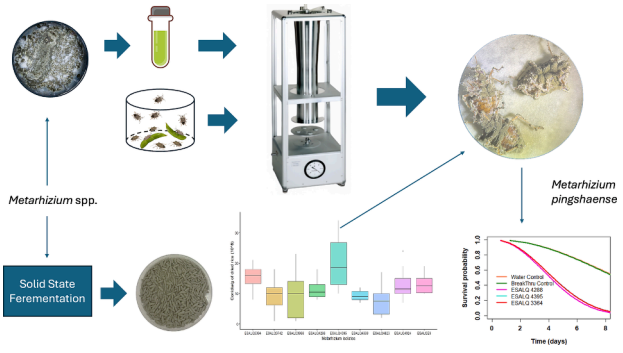
HIGHLIGHTS

- *M. humberi*, *M. pingshaense*, and *M. robertsii* caused high *E. heros* adult mortality;
- *Metarhizium pingshaense* presents high production efficiency by solid fermentation;
- Absence of age-dependent pattern in the mortality of *E. heros* caused by *Metarhizium*;
- High potential of the genus *Metarhizium* for the biological control of pentatomids;
- Less commercially exploited species challenge views of low efficacy for stink bugs.

GRAPHICAL ABSTRACT



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ABSTRACT

Entomopathogenic fungi have been widely used for sustainable agriculture as biological pest control agents. However, commercially available isolates were not efficient in controlling the stink bug *Euschistus heros* (Hemiptera: Pentatomidae), one of the most destructive species in soybean cultivation. There is an increasing demand for exploring new microorganisms that have the potential to control this insect. The present study evaluated the susceptibility of *E. heros* to 15 isolates of different species of the genus *Metarhizium*. A mortality rate of over 95 % was observed in isolates of *M. pingshaense*, *M. humberi,* and *M. robertsii* in 8 days of experiment. The aerial conidia yield of ESALQ 4395 and ESALQ 3364 isolates stood out with a concentration above $1x10^9$ conidia/g of dry rice, which is similar to commercial isolates. The average lethal concentration of these isolates was $5.1x10^5$ conidia/mL for adult insects, with a 95 % confidence interval of $4.7x10^5$ to $5.4x10^5$ conidia/mL for ESALQ 4395 and $4.8x10^5$ to $5.5x10^5$ conidia/mL for ESALQ 3364. However, no age-dependent mortality was observed since mortality among adults was more pronounced than in immature insects. Although low efficiency of entomopathogenic fungi for controlling stink bugs has been associated with the fungistatic and fungicidal action of the volatile compounds present in the alarm pheromone, the data obtained here demonstrated that this insect is

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highly susceptible to *Metarhizium,* especially to species that are not explored commercially in Brazil such as *M. humberi, M. pinghaense* and *M. robertsii*.

1. Introduction

The neotropical brown stink bug, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), is a primary soybean pest in Brazil (Panizzi & Lucini, 2016). The pest appears at the beginning of the crop reproductive phase and can cause damage to various parts of the plant, although the damage is more severe when concentrated in the pods (Panizzi, 2000), which form the preferred attack structure together with seeds.

Since it is a sucking insect, its feeding habit consists of introducing the stylet and injecting saliva into the plant tissues. This saliva carries secretions that facilitate digestion, degrade the tissues, and cause darkening (Tessmer et al., 2022).

The symptoms resulting from *E. heros* feeding on this crop are characterized by deformation, wilting and spots on the grains, which may lose commercial value due to reduced oil content, in addition to delayed maturation and reduced seed vigor (Somavilla et al., 2019; Zerbino & Panizzi, 2019). The perforations left after feeding may also favor infection by pathogenic microorganisms that will be responsible for the development of diseases (Panizzi et al., 2012).

Control measures primarily rely on chemical insecticides (Somavilla et al., 2019). However, resistant populations have been reported in Brazil since the 1990 s (Sosa-Gómez et al., 2001; Sosa-Gómez & Silva, 2010; Sosa-Gómez & Omoto, 2012). In addition, from an environmental perspective, chemical control methods are responsible for harmful effects on non-target organisms. Based on these conditions and considering the growing pressure for residue-free food, there is a need to explore more sustainable control alternatives.

Considering this scenario, microbial control of insects, especially using entomopathogenic fungi, has become an excellent tool for sap-sucking insects such as pentatomids, as they have contact action (Lacey & Goettel, 1995). However, commercially available products are almost all based on two strains from the species *Metarhizium anisopliae* (IBCB 425) and *Beauveria bassiana* (IBCB 66), formulated alone or in combination (AGROFIT, 2025). These products are also used to control various insect pests but were not specifically selected for targeting pentatomids. As a result, their performance against pentatomids may vary due to the additional chemical defenses exhibited by these insects. Pentatomids release volatile compounds that function as alarm or defense pheromones (Moraes et al., 2008), and some of them may have fungicidal or fungistatic effects on genera such as *Metarhizium* and *Beauveria* (Borges et al., 1993; Sosa-Gómez et al., 1997; Silva et al., 2015; Lopes et al., 2015).

In addition to the chemical defenses particular to this insect, cuticular components are extremely important for the survival of insects in general. However, just as insects have improved their defenses throughout the evolutionary process, pathogens have been developing structures and mechanisms capable of circumventing this system (Napolitano & Juárez, 1997).

On the other hand, pathogens also need to face other problems, since biotic and abiotic factors are capable of strongly influencing the microorganism's ability to cause higher levels of infection. Environmental conditions such as sunlight incidence, temperature and humidity, soil type, plant nutritional status, agricultural practices and chemical pesticides are some of the factors that are constantly interconnected in the field and can interact in different ways, which makes it difficult to accurately measure how each of them can influence the dynamics of the infectious process of entomopathogenic fungi (Lacey et al., 2015).

Despite the factors that may influence the infection process and the low susceptibility of pentatomids to entomopathogenic fungi, the genus *Metarhizium* presents itself as an interesting microorganism for insect control. Although only one species is being used commercially (AGROFIT, 2025), Brazilian soils have a wide diversity of *Metarhizium* species (Botelho et al., 2019) and the genus presents a great intraspecific variability (Castro et al., 2016; Moreira, 2016; Iwanicki et al., 2019; Botelho, et al., 2019) and high genetic plasticity (Hu et al., 2014). Among the species used in this study, *M. pingshaense* is more predominant in Asia and Australia, *M. anisopliae* in South America and Africa, *M. robertsii* in North America, *M. brunneum* in Europe, however, all were isolated from Brazilian soils and *M. humberi* (the last to be identified among these) is more common in Brazilian soils (Botelho et al., 2019; Luz et al., 2019; Rehner and Kepler, 2017).

Furthermore, the ovicidal effect of *M. anisopliae* and *M. pingshaense* isolates on *E. heros* eggs was proven for the first time (Silva et al., 2025), and other studies highlighted the potential of some fungal species in controlling adult insects (Groth, 2017; Resquín-Romero et al., 2020). These data demonstrate the potential of the genus *Metarhizium* for the control of insect pests (Bilgo et al., 2018; Iwanicki et al., 2020; Marciano et al., 2021) and encourage new studies aimed at advancing the development of bioinsecticides. Despite the mentioned diversity, few species and isolates of this genus are explored as microbial insecticides (Mascarin et al., 2019), since Brazilian products only have isolates of the species *M. anisopliae* as active ingredient. In this regard, this study evaluated virulence of Brazilian *Metarhizium* strains from five species against *E. heros* under laboratory conditions. We assessed the conidia yield and median lethal concentration of *Metarhizium* isolates that caused high mortality as key steps in developing a bioinsecticide for pest management.

2. Material and methods

2.1. Fungal strain and insects

The insects used in the experiments were obtained from the Koppert Biological Systems company (Charqueada – SP). The insects were kept in plastic cages covered with voile, with a natural diet consisting of bean pods and peanuts at 26 °C.

Three isolates from five different species of the genus *Metarhizium* were tested: *M. anisopliae*, *M. brunneum*, *M. humberi*, *M. pingshaense,* and *M. robertsii,* totaling 15 isolates (Table 1). All isolates used belong to the "Prof. Sérgio Batista Alves" Entomopathogenic Microorganisms Collection of the Laboratory of Pathology and Microbial Control of Insects of the Department of Entomology (ESALQ/USP). These isolates were obtained from soil samples from various locations and recovered from the soil using live bait or selective media. After recovery, the species were identified using the 5'-TEF region (GenBank accession numbers – Table 1).

The isolates are preserved in 10 % glycerol and stored in a freezer at −80 °C. Storage was done by cutting out small portions of sporulated culture medium with the species of interest submerged in 1 mL of 10 % glycerol in cryovials. After thawing the cryovials, the isolates were cultivated in PDA (Potato Dextrose and Agar) culture medium and kept at 26 °C with a 12 h photoperiod for approximately 7 days. The sporulated cultures were used to prepare suspensions for the bioassays.

Experimental samples were prepared using 250 mL plastic pots with perforated lids to ensure optimal survival conditions of the insects and fungi. Pieces of green peanuts and peanut kernels were placed at the bottom of each pot to serve as food. A piece of cotton moistened with distilled water was added to increase humidity. The pods were disinfected in 70 % alcohol for 30 s, 0.5 % sodium hypochlorite for 1 min, and washed in distilled water. After assembling the experimental units, 10 adults of *E. heros* were added to it. Solid vaseline was applied to the

edge of the plastic pots to prevent the insects from escaping.

2.2. Isolate selection by virulence to Euschistus heros

Fungal suspensions were prepared and adjusted to a $5x10^7$ conidia/mL concentration. Distilled water and a 0.05 % Break-thru ® S 255 (Evonik Industries) solution was used as control. Subsequently, 2 mL of each suspension was sprayed into the pot containing the insets and the insect food using a Potter Spray Tower, at a uniform working pressure of 68.94 kPa. After application, the pots were kept at 26 °C with a 12 h photoperiod for 8 days.

The experiment was evaluated daily for 8 days. Dead insects were removed from the pots and superficially disinfected with 70 % alcohol for 30 s, 0.5 % sodium hypochlorite for 1 min, and rinsed in distilled water subsequently. The insect cadavers were placed in humid chambers and kept at 26 °C to confirm mortality due to fungal infection. During the experiment, the green pods and cotton were changed when necessary, and egg masses were removed whenever present. Each treatment consisted of 5 replicates, and the experiment was performed twice at different times.

2.3. Isolate selection by aerial conidial productivity

The isolates that caused the highest *E. heros* mortality were evaluated for the conidia yield in solid-state fermentation. This step used plastic bags with 150 g of parboiled rice hydrated for 50 min. The rice bags were stapled and autoclaved at 120 °C for 20 min. After cooling, 5 mL of the fungal suspension at $5x10^7$ conidia/mL was added to each bag. The rice was kept at 26 °C with a 12 h photoperiod for 10 days. After this period, production was quantified from an aliquot of 10 g of sporulated rice diluted in 90 mL of water with 0.05 % Tween 80. This rice suspension was diluted 3 times following the serial dilution method, and the count was performed in a Neubauer chamber using the third dilution under an optical microscope to obtain the yield of conidial production per gram of rice.

Subsequently, to perform the extraction and estimate the weight of conidia per kilogram of dry rice, the rice was placed in a drying chamber for approximately 48 h, and the water activity was measured (AquaLab). The dry material was sieved for 1 h using a sieve shaker. After this step, the extracted conidia and remaining rice were weighed, and the conidial production per kilogram of dry rice was estimated.

2.4. Dose-response assay for E. heros adults

Bioassays were conducted to determine the median lethal concentration (LC_{50}) of isolates selected according to virulence to *E. heros* and productivity in solid fermentation. These bioassays followed the isolate selection step described above in topic 2.2; however, suspensions were used at different concentrations ($1x10^5$, $1x10^6$, $1x10^7$, and $1x10^8$ conidia/mL).

2.5. Virulence assay with E. heros nymphs

Isolates with greater virulence against adults and greater conidial productivity were selected for the virulence study against nymphs. The insects were separated into 2nd and 3rd instar nymphs and 4th and 5th instar nymphs. The suspension was prepared and applied as described for the adult assay at a $5x10^7$ conidia/mL concentration. Dead nymphs were removed from the jars daily, sterilized in 70 % alcohol for 30 s, 0.5 % sodium hypochlorite for 1 min, and rinsed in distilled water. After sterilization, they were kept in a humid chamber at 26 °C to confirm mortality. Each treatment consisted of 5 replicates, and the experiment was performed twice. Images of the exuviae of treated insects were obtained using Scanning Electron Microscopy (SEM) techniques to verify if the applied conidia remained in the exuvia after the moult. The exuviae were exposed to osmium vapor (OsO_4) for 24 h and then placed in silica for a few hours as part of the method to observe conidial adhesion. After drying, the specimens were fixed to a sample holder (stub) using double-sided carbon tape, metalized with gold in sputtering equipment (Balzers SCD 050), and photographed in a scanning electron microscope (JEOL JSM-IT300 operated at 15.0 kV).

2.6. Statistical analysis

All experiments were performed under laboratory conditions with a completely randomized design. To evaluate the survival of *E. heros* adults and nymphs, a generalized linear model (GLM) with exponential distribution was applied, and the curves were compared with each other based on the Log-Rank test ($P < 0.05$). The LT_{50} of nymphs and adults was also obtained by the generalized linear model (GLM). Regarding conidial productivity, ANOVA was used, and significant differences were evidenced by Tukey multiple comparisons ($P < 0.05$). For the lethal concentration (LC_{50}) data, a generalized linear model (GLM) with a binomial distribution, logit link function, and log-dose transformation were applied. The confidence interval was 95 %. The analyses and graphs were produced by Graph Pad Prism 5.0 software.

3. Results

3.1. Isolate selection based on virulence to Euschistus heros

All tested isolates caused mortality above 75 % in *E. heros* adults, while isolates *M. anisopliae* (ESALQ 4884 and 4931), *M. humberi* (ESALQ 3742, 3996 and 4823), *M. pingshaense* (ESALQ 3364, 4288 and 4395),

Table 1
Information on the location and collection method of the strains used.

Specie	Origin	Isolation method	Strain	Source Publication	Genbank acession number
M. anisopliae	Boca da Mata (AL)	*Mahanarva posticata*[*]	E9	Commercial isolate	AE15227
	Soil – Native vegetationSinop (MT)	IB – *Galleria mellonella*	4884	Botelho et al., 2019	MH719479
	Soil – Maize cropSinop (MT)	Selective medium	4931		MH719528
M. brunneum	Soil – Sugarcane cropUsina Iracema (SP)	IB – *Galleria mellonella*	5022	Couceiro et al., 2022	MZ611899
	Soil – Sugarcane cropUsina Iracema (SP)	Selective medium	5181	Iwanicki et al., 2019	MH596826
	Soil – Sugarcane cropUsina Iracema (SP)	IB – *Tenebio molitor*	5286		MH596828
M. humberi	Soil – Soybean cropRio Verde (GO)	IB – *Galleria mellonella*	3742	Botelho et al., 2019	MH719676
	Soil – Soybean cropRio Verde (GO)	IB – *Galleria mellonella*	3996		MH719677
	Soil – Soybean cropRio Verde (GO)	Selective medium	4823		MH719709
M. pingshaense	Soil – Banana cropSinop (MT)	IB – *Tenebrio molitor*	3364	Botelho et al., 2019	MH719670
	Soil – Banana cropSinop (MT)	Selective medium	4288		MH719692
	Soil – Maize cropSinop (MT)	IB – *Tenebrio molitor*	4395		MH719703
M. robertsii	Soil – Native vegetationAceguá (RS)	Selective medium	4669	Botelho et al., 2019	MH719541
	Soil – Native vegetationSinop (MT)	Selective medium	4791		MH719544
	Soil – Native vegetationSinop (MT)	IB – *Tenebrio molitor*	4924		MH719522

[*] Isolated from infected insect; IB: insect bait.

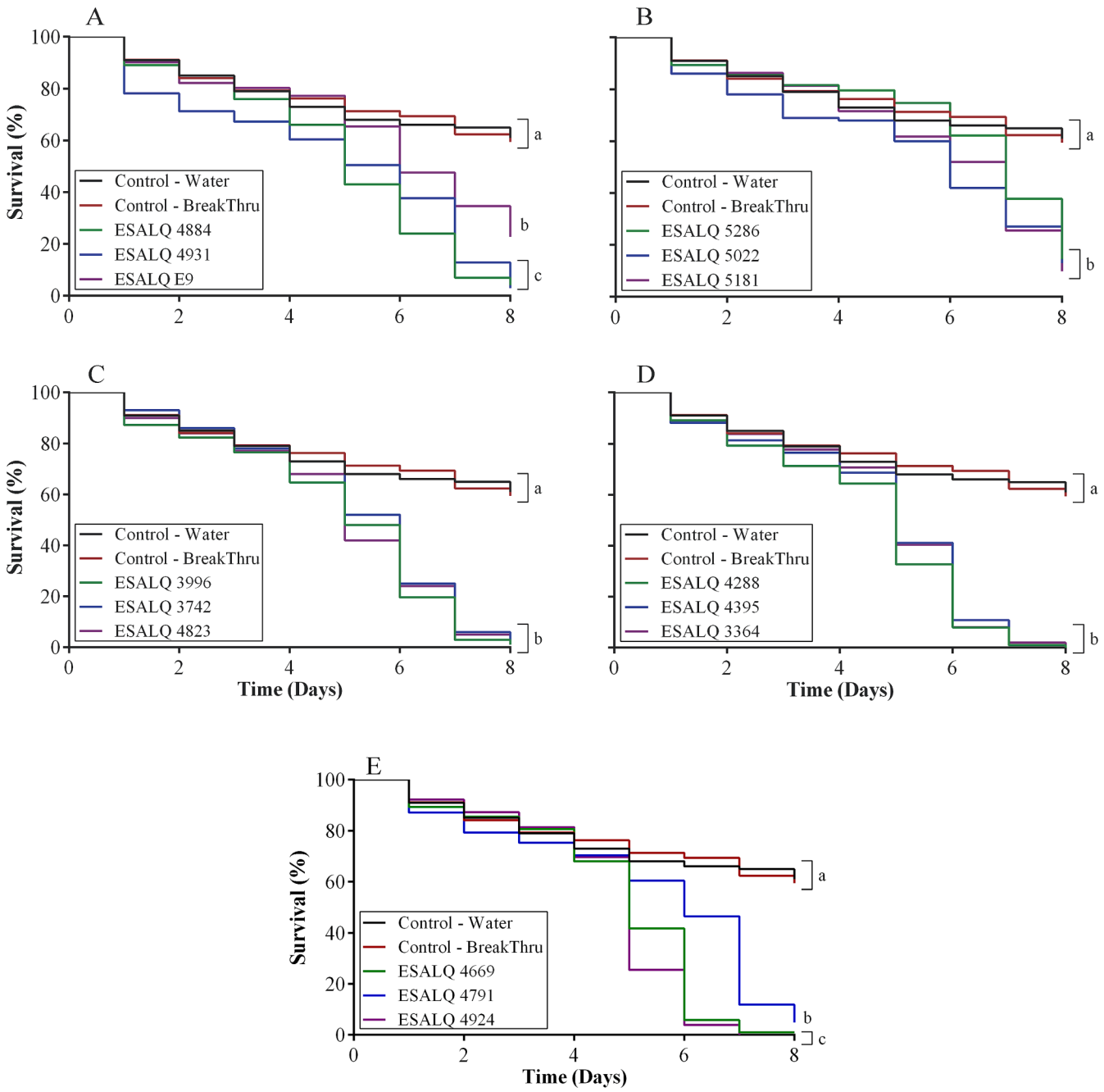

Fig. 1. Survival rate of *Euschistus heros* adults after exposure to 15 *Metarhizium* isolates. A) *M. anisopliae*; B) *M. brunneum*; C) *M. humberi*; D) *M. pingshaense*; E) *M. robertsii*. *Different letters indicate statistical difference ($p < 0.05$).

and *M. robertsii* (ESALQ 4699, 4791 and 4924) standing out due to reaching almost 100 % mortality (Fig. 1). This value was significantly higher than the mortality caused by the commercial reference isolate ESALQ E9 (*M. anisopliae*), which had the worst performance among the isolates (77 %) and the control (water) with 39 % ($p = 0.00015$). In addition to the high mortality caused by the isolates, the percentage of sporulated insects (confirmed mortality) was above 49 %, which indicates that the leading cause of insect death was infection by the microorganisms (Table 2). In addition, the species that presented greater virulence also had a median lethal time (LT_{50}) equal to or less than 6 days (Table 2).

3.2. Isolate selection by aerial conidial productivity

Conidial productivity was assessed using isolates that caused high mortality in adult insects, and isolate ESALQ E9 was used as a commercial reference. This step shows that not all isolates that caused high mortality have desirable characteristics for large-scale production. Some isolates have better physical aspects, such as looser conidia, which allows for better extraction and yield. Therefore, considering both production yield and extraction aspects, two isolates of *M. pingshaense* were selected: ESALQ 4395 ($1.8x10^9$ conidia/g of dry rice) and ESALQ 3364 ($1.5x10^9$ conidia/g of dry rice). Both isolates produced higher concentrations than ESALQ E9 ($1.2x10^9$ conidia/g of dry rice), which is known for commercial production capacity (Fig. 2). For extraction efficiency, the calculation was obtained only for the three isolates mentioned in

Table 2
Percentage of total and corrected mortality of *E. heros* adults and dead insects that presented conidiogenesis (Sporulated cadavers), and median lethal time (LT_{50}) of *Metarhizium* isolates.

Specie	Strain	Total mortality (%)	Sporulated cadavers (%) *	LT_{50}	Lower CI 95 %	Upper CI 95 %
Distilled water		39 ± 0.25	0	NA	NA	NA
Break-thru 0.05 %		41 ± 0.25	0	NA	NA	NA
M. anisopliae	E9	77 ± 0.23	49	8	7	NA
	4884	96 ± 0.20	80	6	5	7
	4931	97 ± 0.25	75	6	6	7
M. brunneum	5022	87 ± 0.25	64	6	5	7
	5181	90 ± 0.22	71	7	6	7
	5286	85 ± 0.23	65	7	6	8
M. humberi	3742	97 ± 0.19	90	5	5	6
	3996	99 ± 0.19	82	5.5	4	6
	4823	97 ± 0.19	96	5	5	6
M. pingshaense	3364	100 ± 0.18	92	6	5	6
	4288	100 ± 0.18	91	5	5	6
	4395	99 ± 0.18	95	6	5	6
M. robertsii	4669	99 ± 0.16	90	5	5	6
	4791	95 ± 0.23	86	6	5	7
	4924	100 ± 0.14	87	5	5	5

* Values relative to the total number of insects.

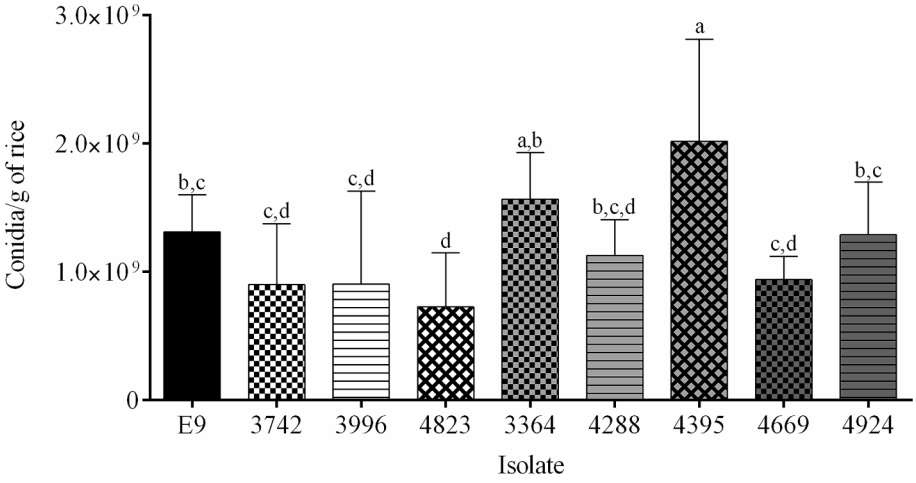

Fig. 2. Conidial production obtained from solid fermentation of 9 different *Metarhizium* isolates. Isolates of *M. anisopliae* (ESALQ E9), *M. humberi* (ESALQ 3742, ESALQ 3996, ESALQ 4823), *M. pingshaense* (ESALQ 3364, ESALQ 4288, ESALQ 4395) and *M. robertsii* (ESALQ 4669, ESALQ 4924). *Different letters indicate statistical difference ($p < 0.05$).

terms of concentration, and the isolates ESALQ 4395, 3364, and E9 produced an average of 45, 42, and 37 g of conidia/kg of dry rice, respectively.

3.3. Dose-response assay for E. heros adults

At this stage, increasing concentration resulted in higher mortality, and the performance of the selected isolates was very similar. The median lethal concentration (LC_{50}) was $5.1x10^5$ conidia/mL for isolates ESALQ 4395 (95 % ci 4.7–$5.4x10^5$ conidia/mL) and ESALQ 3364 (95 % ci 4.8–$5.5x10^5$ conidia/mL) (Table 3).

Table 3
Mortality of *E. heros* adults subjected to different concentrations of *M. pingshaense.*

Treatment	Concentration	Mortality (%)
Distilled water	–	31 ± 0.48
Break-thru solution	0.05 %	35 ± 0.47
ESALQ 3364	$1x10^5$ conidia/mL	45 ± 0.49
	$1x10^6$ conidia/mL	69 ± 0.46
	$1x10^7$ conidia/mL	81 ± 0.39
	$1x10^8$ conidia/mL	87 ± 0.33
ESALQ 4395	$1x10^5$ conidia/mL	48 ± 0.50
	$1x10^6$ conidia/mL	70 ± 0.45
	$1x10^7$ conidia/mL	78 ± 0.41
	$1x10^8$ conidia/mL	93 ± 0.26

3.4. Virulence assay with E. heros nymphs

The virulence of *Metarhizium* isolates for *E. heros* nymphs differed from adults. Contrary to expectations, no age-dependent pattern was observed since the maximum mortality for all isolates in this test did not exceed 67 % ($p = 0.033$) (Fig. 3). This fact may be related to the reduction of the infectious process due to the loss of conidia adhered to the exuvia during molting as observed in Fig. 4.

Furthermore, the conidiogenesis process in nymphs was less than 33 % for all isolates (Table 4). The main difference in virulence was observed for 4th and 5th instar nymphs, where isolate ESALQ 3364 (67 %) was more virulent than isolate ESALQ E9 (59 %) ($p = 0.000023$) (Fig. 3). Finally, the LT_{50} for both 2nd and 3rd instar nymphs and 4th and 5th instar nymphs ranged from 7-8 days for all isolates tested (Table 4).

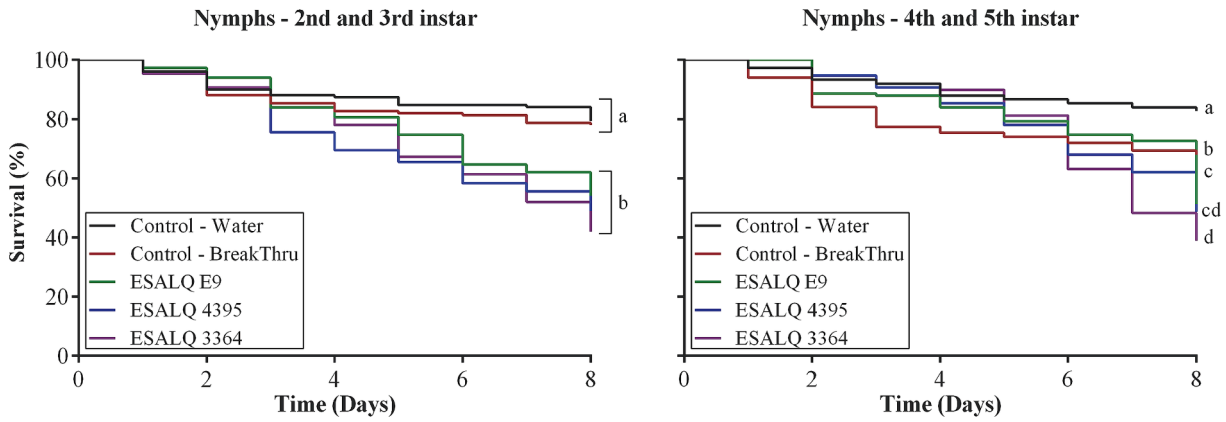

Fig. 3. Survival rate of *Euschistus heros* nymphs after exposure to *Metarhizium* isolates ESALQ E9, ESALQ 4395, and ESALQ 3364. A) 2nd and 3rd instar nymphs B) 4th and 5th instar nymphs. *Different letters indicate statistical difference ($p < 0.05$).

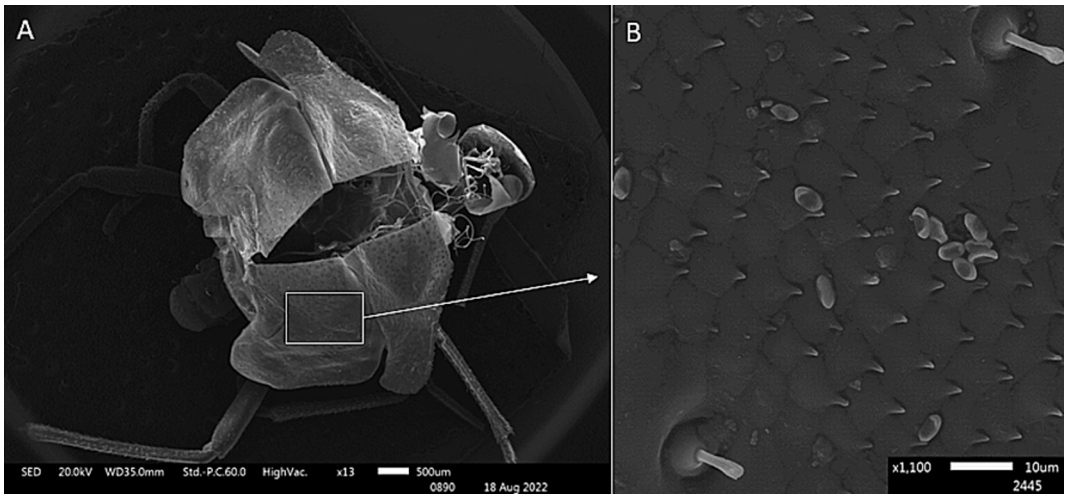

Fig. 4. Scanning Electron Microscopy (SEM) images of exuvia of 3rd instar nymph of *E. heros* after 16 h of application of the ESALQ 4395 isolate of *Metarhizium pingshaense*. A) Exuvia image showing the amplified dorsum area. B) Zoomed-in view of conidia adhering to the exuvia.

Table 4
Percentage of total mortality of *E. heros* nymphs and dead insects that presented conidiogenesis (Sporulated cadavers) and median lethal time (LT_{50}) at different instar nymphs obtained by the experiment to evaluate the virulence of *Metarhizium* isolates.

Instar	Specie	Strain	Total mortality (%)	Sporulated cadavers (%) *	LT_{50}	Lower CI 95 %	Upper CI 95 %
2° e 3°	Distilled water		28 ± 0.16	0	NA	NA	NA
	Break-thru 0,05 %		22 ± 0.17	0	NA	NA	NA
	M. anisopliae	E9	51 ± 0.17	20	8	8	NA
	M. pingshaense	3364	61 ± 0.18	27	7	7	8
	M. pingshaense	4395	55 ± 0.19	33	7	7	NA
4° e 5°	Distilled water		17 ± 0.14	0	NA	NA	NA
	Break-thru 0,05 %		27 ± 0.20	0	NA	NA	NA
	M. anisopliae	E9	59 ± 0.16	27	8	8	NA
	M. pingshaense	3364	67 ± 0.14	28	7	7	8
	M. pingshaense	4395	59 ± 0.15	26	8	8	NA

* Values relative to the total number of insects.

4. Discussion

This study highlighted isolates of *M. anisopliae, M. pingshaense*, *M. humberi* and *M. robertsii*, causing mortality of over 95 % in adult insects in 8 days of experiment, with an average lethal concentration of only 5.1 ± x 10^5 conidia/mL for two strains of *M. pingshaense*. This data contrasts with the literature indicating low susceptibility of pentatomids to entomopathogenic fungi. Some studies support the hypothesis that entomopathogenic fungi have low efficiency in controlling pentatomids due to the adverse effect of alarm pheromones released by these species. Sosa-Gómez & Moscardi (1998) stated that among the bugs that attack soybeans, *E. heros* is one of the most resistant to infections caused by entomopathogenic fungi. Lopes et al. (2015) reinforced this report when they observed that *Diceraeus melacanthus* (Dallas, 1851) was more susceptible than *E. heros* when exposed to the fungus *Beauveria bassiana*. Adult mortality of *Tibraca limbativentris* (Stal, 1860), another

pentatomid, did not reach 40 % using a strain of *M. anisopliae* (Quintela et al., 2013), with a concentration identical to that used in the present study. Nymph mortality was similar to that observed here (approximately 60 %) (Silva et al., 2015). A similar result was observed by Quintela et al. (2013), with reported mortality of less than 50 % in adults of this same species from exposure to isolates of *M. anisopliae*. Raafat et al. (2015) reported LC_{50} concentrations of $3.2x10^8$ and $2.8x10^{10}$ conidia/mL, respectively, for isolates of *B. bassiana* and *Paecilomyces* spp. under the pentatomid *Nezara viridula* (L., 1758).

None of the aforementioned studies have specifically examined the *Metarhizium* species, which is identified here as the most promising. This highlights the need to explore different strains for insect control, especially considering the genetic variability and species richness of the *Metarhizium* genus in Brazilian soils.

The results reported by Resquín-Romero et al. (2020), for example, which uses the immersion exposure method, known for maximum contamination and result in high mortality rates, corroborates this study by using a species that had not been used in commercial products (*M. brunneum*) and demonstrating high mortality rates of *E. heros* (100 %). In contrast, the Brazilian isolates of *M. brunneum* tested here did not perform as well as the other species. The different application methods must also be considered, as they may influence mortality, such as dorsal inoculation (Quintela et al., 2013; Lopes et al., 2015; Silva et al., 2015).

Regarding the mortality of insects at different stages of development, the first instars are typically more susceptible to infection by microorganisms, due to thinner body walls in their initial stages (Kirubakaran et al., 2018). Thus, an age-dependent mortality relationship was expected, but this pattern was not observed in this study. This may occur because individuals in the first nymphal stages can circumvent the infection process through the combination of three factors: smaller body area that results in a smaller number of adhered conidia; time required for conidial germination; and rapid ecdysis, which eliminates the conidia before they can access the host's hemolymph (Kim & Roberts, 2012). This was observed in the present study in the exuvia of some nymphs (Fig. 4).

The confirmation of mortality by conidiogenesis on dead insects followed the same trend observed for mortality among *E. heros* adults and nymphs, being less pronounced among the nymphs. In the external manifestation of the infection, fungal growth began in intersegmental regions, followed by intense conidiation that dominated the entire cadaver (Lopes et al., 2015). Sporulation in cadavers is a fundamental characteristic for determining the success of a biocontrol agent because this allows the dissemination of the pathogen under field conditions, helping it to remain and potentially cause epizootics (Shan & Feng, 2010).

Large-scale production must be profitable for biopesticides to be explored commercially. The conidia production on rice fermentation of selected isolates is high enough to make its industrial use viable. The *M. pingshaense* isolate (ESALQ 4395), presenting the best performance among the isolates evaluated, produces $1.8x10^9$ conidia/g of rice. This yield is close to the best value obtained by other studies. Neves (1998) obtained $1.54x10^9$ conidia/g of rice for isolate of *M. anisopliae* (ESALQ E9). Similarly, Freitas et al. (2014) and Santos (2015) reached concentrations of $1.75x10^9$ to $2x10^9$ conidia/g of rice, respectively.

The high virulence of *M. pingshaense* reported on adults of *E. heros*, combined with its conidia production, demonstrates the potential for its use in biological control to combat one of the main pests of soybeans. From the perspective of the commercial application of entomopathogenic fungi, developing formulations is the next step to ensuring highly efficient products. Efforts must continue in order to bring entomopathogenic fungi to a leading role in contributing to a more sustainable agriculture.

CRediT authorship contribution statement

Aline Nunes-Silva: Writing – original draft, Visualization, Investigation, Conceptualization. **Camila Costa Moreira:** Writing – review & editing, Methodology, Conceptualization. **Janaína Brandão Seibert:** Writing – review & editing, Formal analysis. **Jonathan Rodríguez:** Writing – review & editing. **Italo Delalibera-Júnior:** Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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