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Research Article

In Vitro Biological Activity of *Beauveria bassiana*, *Beauveria peruviensis*, and *Metarhizium* sp. against *Hypothenemus hampei* (Coleoptera: Curculionidae)

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Coffee (*Coffea arabica*) is the main commodity in Peru and is the economic support for thousands of small farmers. However, coffee production is affected by the coffee berry borer (*Hypothenemus hampei*). Currently, *H. hampei* is the most important pest in whole coffee-growing regions in Peru. This study aimed to evaluate *in vitro* biological activity of *Beauveria bassiana*, *Beauveria peruviensis,* and *Metarhizium* sp. against *Hypothenemus hampei* in two trials at different times. Conidia production, Conidia viability, and pathogenicity against *H. hampei* were evaluated at three concentrations (1×10^5, 1×10^7, and 1×10^9 conidia/mL^{-1}). In addition, lethal times (LT_{50} and LT_{90}) and lethal concentrations (LC_{50} and LC_{90}) were calculated. There were significant differences in conidia production ($P < 0.001$) and conidia viability ($P < 0.041$). The highest conidia production and conidia viability were reached by *B. bassiana* and *B. peruviensis*, respectively. Likewise, there were differences in the pathogenicity of the strains in the two tests carried out (test 1: $P < 0.0009$ and test 2: $P < 0.0001$). The highest mortality occurred in the treatments of *B. bassiana* 1×10^9 conidia/mL^{-1}, *B. bassiana* 1×10^7 conidia/mL^{-1}, and *B. peruviensis* 1×10^9 conidia/mL^{-1}. The treatments with lower LT_{50} and LT_{90} were *B. bassiana* 1×10^9 conidia/mL^{-1} and *B. peruviensis* 1×10^9 conidia/mL^{-1}, and the strains with the lowest LC_{50} and LC_{90} were *B. peruviensis* and *B. bassiana*. The *in vitro* characteristics shown by *B. bassiana* and *B. peruviensis* conditions suggest they should be evaluated in the field to determine the capability of these strains to reduce populations of *H. hampei*.

1. Introduction

Coffee (*Coffea arabica* L.) is the main commodity in Peru, with relevant economic, social, cultural, and ecological importance. Coffee is grown on 432 400 hectares and generates more than two million jobs in its agricultural production chain [1]. This crop is cultivated in 11 states of Peru, while Amazonas state contributes in 12% to national production. According to MINAGRI [1], in 2019, Amazonas had a production of 71,622 TM of coffee.

In Peru, as in many other coffee-producing countries, production is affected by the coffee berry borer (*Hypothenemus hampei*), an insect native to Central Africa [2]. *H. hampei* was first reported in Peru in 1960 Amaral [3] and

is currently the main pest for coffee production in all coffee-growing regions. This insect lives in the internal tissues of the coffee fruit; thus, the damage is caused by larvae and adults due to their feeding and reproduction process in the endosperm, reducing the yield and quality of coffee production [4]. In addition, *H. hampei* has a cryptic life cycle because it spends most of its life inside the endosperm, which hinders the effectiveness of control strategies [5, 6].

Chemical insecticides were used as a measure to control the coffee berry borer (CBB), being endosulfan the most widely used. Other formulations with effectiveness against *H. hampei* are pirimiphos methyl, fenitrothion, chlorpyrifos, and fenthion. However, the irrational use of insecticides may cause resistance and adverse effects on the coffee ecosystem. Therefore, their use is recommended under an integrated pest management scheme [7–9]. Thus, the most recommended control measures are cultural and biological control [6].

Cultural control strategies include the timely harvesting of ripe fruit, harvesting of residual fruits, pruning, elimination of young shoots, selective weed control, and use of silos to dry coffee with heat [9]. For biological control, the conservation of natural enemies of *H. hampei* and the use of exotic natural enemies have been recommended. The parasitoids *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae), *Prorops nasuta* (Hymenoptera: Bethylidae,) and *Phymastichus coffea* (Hymenoptera: Eulophidae) have shown effectiveness in different countries. Similarly, some entomopathogenic fungi have been used efficiently in the control of *H. hampei*, where *Beauveria bassiana* (Bals) Vuill (Hypocreales: Cordycipitaceae) is the most used fungi in the CBB control [10].

Conidia of *B. bassiana* germinate on the CBB cuticle, grow, and eventually develop appressoria (Figure 1). The pressure exerted by the appressorium and the enzymatic action facilitates the penetration into the cuticle [11]. Fungi mycelia proliferate forming hyphal bodies that invade the procuticle and the epidermal layer (Figure 1). Subsequently, the fungus spreads through the hematocele where it secretes various toxins that facilitate the invasion of the tissues and organs of the host. Physical tissue damage, toxicosis, dehydration of cells, and consumption of nutrients cause the death of the insects. Finally, hyphae emerge on the surface of the insect and initiate the formation of conidia [12].

According to Vega et al. [13], the effectiveness of entomopathogenic fungi in field conditions depends on several factors including adaptations to the environmental conditions of the region of origin, virulence, concentration, and weather conditions, among others. Therefore, one of the first steps in the creation of bioinsecticides is the development of bioassays that allow the selection of strains with the best attributes as biocontrol agents [14]. The effectiveness of entomopathogenic fungi against *H. hampei* should be evaluated under local conditions and native strains [15]. Therefore, this study aims to evaluate the *in vitro* biological activity of the indigenous strains, *B. peruviensis* (P19) and *Metarhizium* sp. (MMR-M1), and the commercial strain, *B. bassiana* (CCB-LE265), on *H. hampei*.

2. Materials and Methods

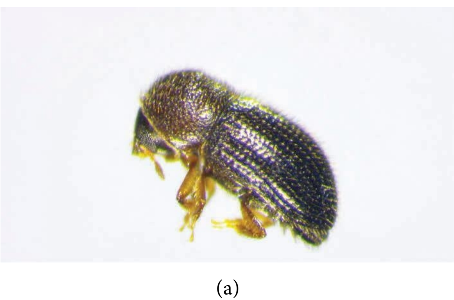
2.1. Microorganisms Acquisition. One strain of each of the three species of entomopathogenic fungi was evaluated: *B. peruviensis* (P19), *B. bassiana* (CCB-LE265), and *Metarhizium* sp. (MMR-M1). These strains are part of the entomopathogenic fungi collection of the Laboratorio de Investigación en Sanidad Vegetal (LABISANV) of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM). *Beauveria peruviensis* is a new species, and the strain was obtained from infected CBB from the coffee agroecosystem of the Rodriguez de Mendoza province, Amazonas state, Peru [16]. *Metarhizium* sp. strain was isolated from soil samples from the coffee agroecosystem in the same region of B. peruviensis, while the B. bassiana strain was acquired from the Servicio Nacional de Sanidad Agraria (SENASA) as a commercial form.

2.2. Physiological Characterization. Two physiological characteristics were assessed in this study, conidia production (CP) and conidia viability (CV).

Conidia production and conidia viability of each species were assessed through the mass production in solid fermentation in rice as a solid substrate. Two hundred grams of pre-cooked rice were placed in plastic bags and sterilized in an autoclave at 121°C for 15 minutes [17]. Five eleven-mm-diameter discs of each entomopathogenic fungi were placed in the sterilized rice bags and disrupted with gentled movements to disseminate the conidia. The bags were incubated at 27°C under dark conditions for 15 days. Five replicates per strain were performed.

After 15 days of incubation, the production of conidia/ mL^{-1} of rice was quantified. To do this, one gram of colonized rice was suspended in 9 ml of Sserile distilled water (SDW) [18, 19]. Then, two serial dilutions were performed. The quantification of conidia was performed in a Neubauer chamber using 0.1 ml from the second serial dilution. The production of conidia per gram of rice was estimated using the formula of [20]. After 15 days of incubation, the production of conidia/mL^{-1} of rice was quantified. The quantification of conidia was performed by serial dilutions up to 10^{-3}. A stock solution was prepared by suspending one gram of colonized rice in 9 ml of sterile distilled water (SDW) [18, 19], plus 1 ml of twin 80 at 0.1%; this solution was homogenized in a vortex agitator (Brand: Velp; Model: MW600) at 3600 rpm for a period of 60 seconds. To obtain the 10^{-1} dilution, 1 ml was extracted from the stock solution and added to a test tube with 8 ml of SDW and 1 ml of twin 80 at 0.1%; again, this dilution was homogenized in a vortex agitator at 3600 rpm for a period of 60 seconds. This procedure was repeated until a 10^{-3} dilution was obtained. Conidia quantification was performed in a Neubauer chamber using 0.1 ml of the second serial dilution. The production of conidia per Gram of rice was estimated by following equation [20]:

$$C = (\mathrm{Cc})\ (4 \times 106) \left(\frac{\mathrm{Fd}}{80}\right), \quad (1)$$


(a)

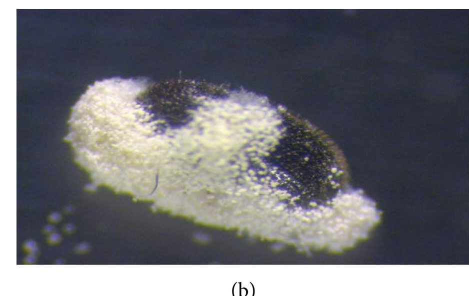

(b)

Figure 1: (a) Adult female of *Hypothenemus hampei.* (b) Adult female of *Hypothenemus hampei* colonized by the fungus *Beauveria bassiana*.

where C represents the concentration (conidia mL^{-1}), Cc represents the average number of conidia counted in the Neubauer chamber, and Fd represents the dilution factor.

To determine the conidia viability from the second dilution, 70 μl aliquots were placed at a concentration of 5×10^6 conidia/mL^{-1} and distributed in 90 mm PDA Petri dishes [21]. Three replicates per strain were performed.

A sterile coverslip was placed on the medium with the inoculated conidia and incubated for 18 h at 27°C. The coverslip was mounted on a slide and stained with lactophenol blue. In each mounting, the reading consisted of 100 conidia [22]. Conidia were considered germinated when the germ tube reached the length of half of the conidium. The following formula was used to determine the germination percentage:

$$\% \text{ germination} = \frac{a}{(a + b)} \text{ x } 100, \quad (2)$$

where a represents the number of germinated conidia and b represents the number of conidia without germination.

2.3. Pathogenic Characterization. A completely randomized assay was set up to measure the pathogenicity. This CRD experiment had 9 treatments as described in Table 1 with three repeats for treatment; a control treatment with SDW was also considered.

Adult female berry borers were used for the study, as they are generally more abundant than the males that never leave the berries [23, 24]; therefore, this pest is considered an obstacle for the development of coffee growing since it can cause great economic losses when it reproduces inside the coffee fruit [25]. Female CBB adults were collected from coffee farms from Mulpic, Rodriguez de Mendoza, Amazonas (6°29′49.25″S and 77°25′59.80″N) at 2537 m.a.s.l. Warm humid weather is typical in this location, with minimum temperatures reaching 12°C and maximum temperatures of 30°C, and an average annual rainfall of 3000 mm [26]. CBB individuals recovered from physiologically mature coffee fruits with visible signs of CBB damage. The damaged coffee fruits were dissected to expose the active CBB adults [27]. These CBB adults were sterilized by immersion in 1% sodium hypochlorite solution for two minutes before the pathogenicity test in order to avoid cross-contamination by other microorganisms that may influence the final result.

The evaluated strains were multiplied by solid fermentation in rice as substrate. The multiplication was performed in the same way as described above. The inoculum was made by conidia suspension in SDW + Tween 80 (0.1% v/v). This suspension was diluted to reach the correct conidia concentration for each treatment, and each strain was evaluated according to the experimental design.

CBB adults were inoculated by immersion in the conidia suspension correspondent for each treatment for 2 min. Inoculated and control insects were placed in Petri dishes, and each experimental unit consisted of ten *H. hampei* adults. Four coffee fruits at harvest maturity were deposited inside the boxes to serve as shelter and food. To maintain humidity inside the box, water was introduced in cotton swabs. Three replicates per treatment were set up, and the boxes with the inoculated insects were incubated at 27°C. Mortality was recorded every 24 hours until at least one treatment reached 100% mortality. Dead insects were placed individually in a humid chamber for sporulation. This experiment was carried out in duplicates on different dates.

2.4. Data Analysis. Conidia production, conidia viability, and mortality data were evaluated under a completely randomized experimental design. Before analysis, conidia production data were transformed to log (x-1); conidia viability and mortality data were transformed to arcsine of the root square of the proportion. Data from each experiment were subjected to an ANOVA and tested for separation of means by Tukey ($\alpha = 0.05$) using SAS® [28]. Daily mortality data were used to estimate the lethal time 50 (LT_{50}) and lethal time 90 (LT_{90}) for each treatment. Also, with the data on mortality per evaluated dose, the lethal concentration 50 (LC_{50}) and lethal concentration 90 (LC_{90}) of each evaluated strain were estimated. LT_{50}, LT_{90}, LC_{50}, and LC_{90} were calculated by logistic regression using SAS® ($p \leq 0.05$) [28].

3. Results and Discussion

Significant differences ($P < 0.0012$) were found in conidia production in rice among the evaluated strains. The fungus with the highest conidia concentration was *B. bassiana* with 9.23×10^8 conidia/mL^{-1}, with no statistical difference with *B. peruviensis* (Figure 2). The strain with the lowest production was *Metarhizium* sp. with 3.83×10^8 conidia/mL^{-1}.

Table 1: Isolates of entomopathogenic fungi evaluated against *Hypothenemus hampei* and treatments used.

Strains	Strain code	Origin	Treatments (conidia mL^{-1})
Beauveria peruviensis	P19	Perú	*B. peruviensis* 1×10^5 *B. peruviensis* 1×10^7 *B. peruviensis* 1×10^9
B. bassiana	CCB-LE265	Colombia	*B. bassiana* 1×10^5 *B. bassiana* 1×10^7 *B. bassiana* 1×10^9
Metarhizium sp	MMR-M1	Perú	*Metarhizium* sp. 1×10^5 *Metarhizium* sp. 1×10^7 *Metarhizium* sp. 1×10^9

The conidia production obtained by *B. bassiana* and *B. peruviensis* is close to values reported by Torres et al. [29] for native strains of *B. bassiana* in Tabasco, Mexico. However, the conidia production obtained with *Metarhizium* sp. is lower than that reported by Alcantara-Vargas et al. [30], who reported a conidia production of 9×10^8 conidia mL^{-1} of the substrate by *M. anisopliae*. According to Fargues et al. [31] and Alcantara-Vargas et al. [30], conidia production by *Metarhizium* strains can be influenced by the nutrient content of the culture medium and the production method. Currently, no evaluations of conidia production of other strains of *B. peruviensis* have been made. According to Badilla [32], conidia production is a basic aspect to consider when selecting entomopathogenic fungi for commercial scale production.

Likewise, there were significant differences ($P < 0.0418$) in the conidia viability produced on rice. Viability ranged from 88 to 95% at 18 h of incubation. Conidia of the fungus *B. bassiana* presented higher viability with 95.67%, with no significant differences with *B. peruviensis* (92.33%) (Figure 3). The conidia of the fungus *Metarhizium* sp. showed the lowest viability at the measurement time. These results are similar to those reported by García et al. [33], who recorded 95% germination of *B. bassiana* strains at 20 h. To our knowledge, there are no viability evaluations of *B. peruviensis*. The germination found for the fungus *Metarhizium* sp. is similar to the germination range reported by Khashaveh et al. [34], who recorded 89 to 94% germination of conidia of the fungus *M. anisopliae* at 24 hours. According to Fargues et al. [31], germination is a determining factor of virulence that can be affected by the nutritional conditions.

The evaluated strains showed pathogenicity towards *H. hampei* at all assessed doses. One hundred percent of the insects in the control treatment remained alive throughout the evaluation. For each evaluated strain, an increase in mortality with increasing dose was observed in both trials (Figure 4). There were significant differences in mortality between treatments at 96 h after inoculation in the two trials (trial 1: $P < 0.0009$ and trial 2: $P < 0.0001$) (Figure 4). In trial 1, mortality ranged from 60 to 100%, in which the highest mortality occurred in the treatments of *B. bassiana* 1×10^9 conidia/mL^{-1}, *B. bassiana* 1×10^7 conidia/mL^{-1}, and *B. peruviensis* 1×10^9 conidia/mL^{-1}. The treatment with the lowest mortality was *Metarhizium* sp. 1×10^5 conidia/mL^{-1}. In trial 2, mortality ranged from 43.3 to 96.6%. Again, the highest mortality occurred in the treatments *B. bassiana* 1×10^9 conidia/mL^{-1}, *B. bassiana* 1×10^7 conidia/mL^{-1}, and *B. peruviensis* 1×10^9 conidia/mL^{-1}. Also, the treatment with the lowest mortality was *Metarhizium* sp. 1×10^5 conidia/mL^{-1}. In addition, Figure 4 shows that the control shows no percentage of mortality, i.e., all the drills subjected to this treatment survived for the entire duration of the trials.

The mortality caused by *B. bassiana* 1×10^9 and *B. bassiana* 1×10^7 on *H. hampei* agrees with Bastidas et al. [35] who reported 90% mortality of *H. hampei* caused by *B. bassiana* strains at times from 106 to 129 h. Likewise, Torres et al. [29] reported 100% mortality of *H. hampei* by native strains of *B. bassiana* in Mexico. However, these authors reported mortality at 144 h and a concentration of 1×10^7. Similarly, Cárdenas et al. [36] reported mortality of 73 to 100% of *H. hampei* by strains of *B. bassiana* at 192 h of evaluation. *B. bassiana* is considered the main entomopathogenic fungus of *H. hampei* [10], which was corroborated in this study. However, *B. peruviensis* caused mortality on *H. hampei* in a similar degree to the mortality caused by *B. bassiana*. According to Tanada and Kaya [37], virulence is a relevant characteristic in the selection of strains of entomopathogenic fungi for biological control purposes. The pathogenicity shown by *B. bassiana* and *B. peruviensis* against *H. hampei* highlights the importance of identifying new strains of *B. bassiana* and new species with high potential to be used as biocontrol agents of CBB. In this regard, Wraight et al. [38] reported the naturally occurring of *B. bassiana* on *H. hampei* on Hawaii Island.

3.1. Lethal Time 50 and 90 (LT_{50} and LT_{90}). For each evaluated strain, a decrease in LT_{50} and LT_{90} was observed with increasing doses in the two trials conducted (Table 2). In trial one, the treatment that required the shortest time to kill 50% of the *H. hampei* population was *B. bassiana* 1×10^9 conidia/mL^{-1} followed by *Metarhizium* sp. 1×10^9 conidia/mL^{-1} and *B. peruviensis* 1×10^9 conidia/mL^{-1}, with times of 60, 61, and 62 h, respectively (Table 2). The treatment with the highest LT_{50} was *Metarhizium* sp. 1×10^5. On the other hand, the treatments with the lowest TL_{90} were *B. bassiana* 1×10^9 conidia/mL^{-1} followed by *B. peruviensis* 1×10^9 conidia/mL^{-1}. The treatment with the highest LT_{90} was *Metarhizium*

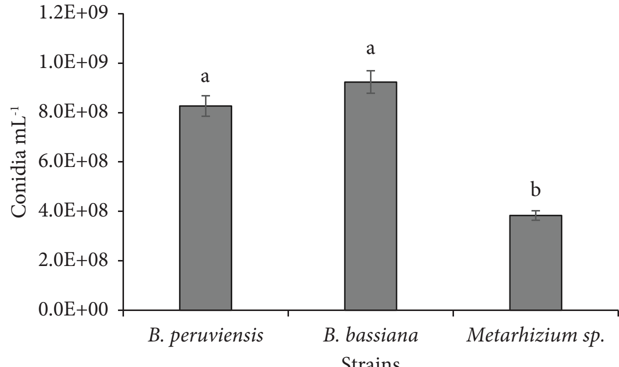


Figure 2: Conidia production in rice (conidia mL^{-1}) of the entomopathogenic fungi *Beauveria peruviensis*, *B. bassiana,* and *Metarhizium* sp. Means with different letters were statistically different (Tukey $P = 0.05$).

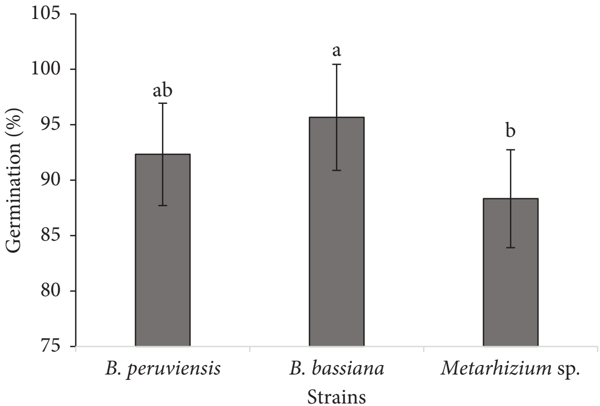


Figure 3: Conidia viability of the entomopathogenic fungi *Beauveria peruviensis*, *B. bassiana,* and *Metarhizium* sp. Means with different letters were statistically different (Tukey $P = 0.05$).

sp. 1×10^5 conidia/mL^{-1}. In trial two, the treatments with the best LT_{50} and LT_{90} were the same as in trial 1. Likewise, the treatment with the lowest LT_{50} and LT_{90} was *Metarhizium* sp. 1×10^5 conidia/mL^{-1} (Table 2). In the two trials, the treatments with the highest consistency in both LT_{50} and LT_{90} were *B. bassiana* 1×10^9 conidia/mL^{-1} and *B. peruviensis* 1×10^9 conidia/mL^{-1}. These results agree with Torres et al. [29] who reported LT_{50} from 71 to 103 h and LT_{90} from 91 to 132 h in *B. bassiana* strains in Mexico, at a dose of 1×10^7 conidia/mL^{-1}. However, in our study, the dose of 1×10^9 conidia/mL^{-1} reduced LT_{50} to values from 60 to 62 h. On the other hand, López-Blanco [39] reported LT_{50} of 57 to 74 h for *B. bassiana* strains on *H. hampei*, when using a dose of 1×10^7 conidia/mL^{-1}. Our results differ from Suárez and Mejía [40] who reported LT_{50} of 168 h for *B. bassiana* against *H. hampei*, using a concentration of 1×10^9 conidia/mL^{-1}. This difference between strains of the same species may be due to the virulence of each strain. In this regard, Cruz et al. [41] reported intraspecific genetic diversity among *B. bassiana* strains, where virulence was associated with the genetic group. On the other hand, Fargues et al. [31] reported the influence of the composition of the culture medium on the lethal time of *M. flavoviride*.

3.2. Lethal Concentration 50 and 90 (LC_{50} and LC_{90}). In trial one, the strains that required the lowest concentration to kill 50% of the *H. hampei* population were *B. peruviensis* and *B. bassiana*, with 6.1×10^2 and 9.6×10^2 conidia/mL^{-1}, respectively (Table 3). *Metarhizium* sp. requires the highest LC_{50} with 1.1×10^4 conidia/mL^{-1}. Similarly, *B. peruviensis* and *B. bassiana* were the strains that required the lowest concentration to kill 90% of the insect population evaluated (Table 3). Also, *Metarhizium* sp. was the strain that required the highest LC_{90}. In trial two, the behavior of the fungal species evaluated was similar to the behavior shown in trial one. However, LC_{50} and LC_{90} were higher (Table 3). Thus, in both trials, the strains with the lowest LC_{50} were *B. peruviensis* and *B. bassiana*, with an average LC_{50} of 1.1×10^3 and 1.3×10^3 conidia/mL^{-1}, respectively. These results differ from De la Rosa et al. [42], who reported LC_{50} of 2.2×10^6 condia/mL^{-1} for *B. bassiana* strains against *H. hampei*. Also, Neves and Hirose [43] reported an LC_{50} of 2.5×10^6 conidia/mL^{-1} for *B. bassiana* strains against *H. hampei*. According to Ferron [44], virulence, measured in terms of lethal concentration, will depend significantly on the strain of fungus used. In accordance with Hayden et al. [45], the variation in virulence of strains is related to

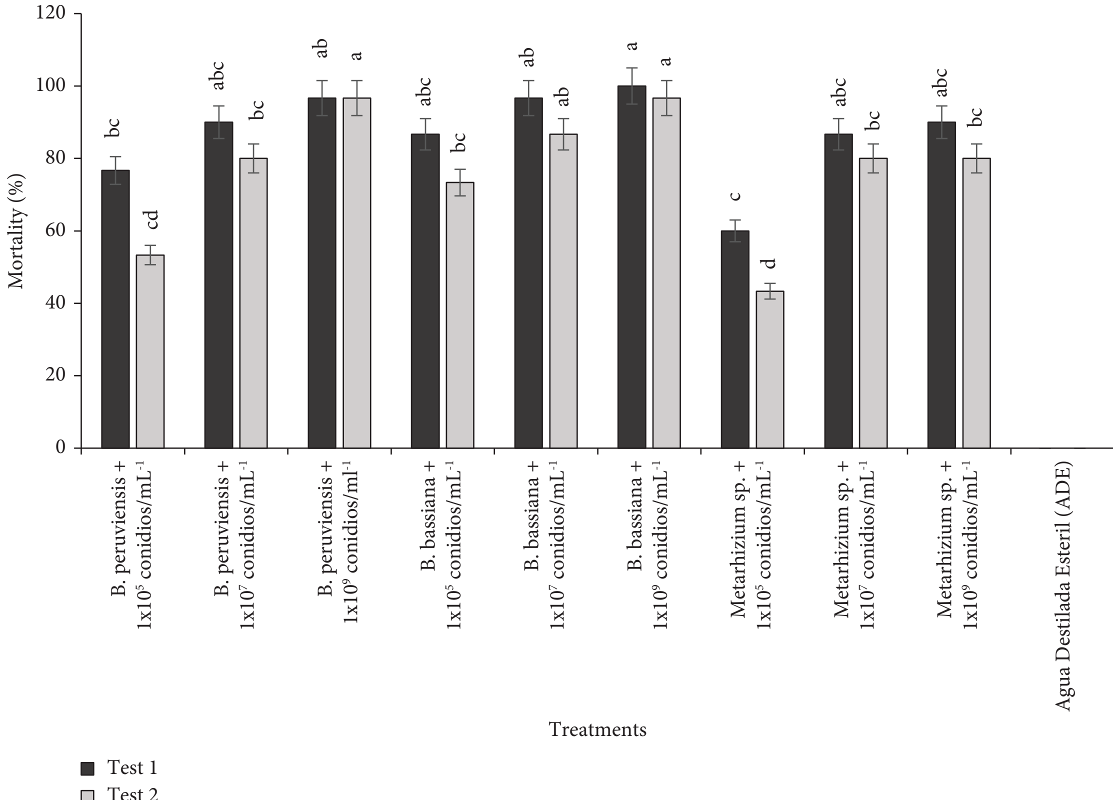


Figure 4: Mortality caused by *Beauveria bassiana*, *B. peruviensis,* and *Metarhizium* sp. against adults of *Hypothenemus hampei in vivo* at doses of 1×10^5, 1×10^7, and 1×10^9 conidia/mL^{-1}. Means with different letters were statistically different (Tukey $P=0.05$).

Table 2: Lethal time 50 and 90 (LT_{50} and LT_{90}) of *Beauveria bassiana*, *B. peruviensis*, and *Metarhizium* sp. on adults of *Hypothenemus hampei* at doses of 1×10^5, 1×10^7, and 1×10^9 conidia/mL^{-1}.

Treatment	Test 1					Test 2				
	LT_{50} (h)		LT_{90} (h)		P	LT_{50} (h)		LT_{90} (h)		P
	Mean	VR	Mean	VR		Mean	VR	Mean	VR	
B. peruviensis 1×10^5	71.3	(61.9–80.2)	114.7	(99.4–146.9)	<0.0001	81.0	(69.1–92.7)	141.9	(118.6–201.2)	<0.0001
B. peruviensis 1×10^7	68.2	(62.4–73.7)	98.4	(89.6–113.0)	<0.0001	69.6	(61.6–77.2)	112.8	(99.3–138.4)	<0.0001
B. peruviensis 1×10^9	62.8	(52.0–72.5)	90.2	(77.4–124.2)	<0.0001	62.8	(52.0–72.5)	90.2	(77.4–124.2)	<0.0001
B. bassiana 1×10^5	69.8	(58.4–79.9)	1007	(86.8–137.3)	<0.0001	76.1	(66.8–84.9)	115.3	(101.1–145.5)	<0.0001
B. bassiana 1×10^7	64.6	(53.5–74.4)	91.3	(78.5–126.0)	<0.0001	69.1	(58.2–78.9)	104.3	(89.7–140.0)	<0.0001
B. bassiana 1×10^9	60.4	(53.5–66.9)	81.9	(73.1–99.8)	<0.0001	56.1	(47.5–63.9)	83.9	(72.6–109.4)	<0.0001
Metarhizium sp. 1×10^5	84.7	(73.6–95.9)	143.2	(121.3–196.4)	<0.0001	86.9	(68.7–106.5)	157.4	(123.1–310.7)	<0.0001
Metarhizium sp. 1×10^7	71.8	(58.5–83.6)	105.7	(89.7–54.0)	<0.0001	65.1	(54.2–75.0)	110.0	(93.1–149.8)	<0.0001
Metarhizium sp. 1×10^9	61.3	(49.3–72.0)	97.7	(81.7–140.2)	<0.0001	59.5	(46.7–70.8)	101.9	(83.7–151.6)	<0.0001

VR = variation range.

Table 3: Lethal concentrations 50 and 90 (LC_{50} and LC_{90}) of *Beauveria bassiana*, *B. peruviensis,* and *Metarhizium* sp. on adults of *Hypothenemus hampei.*

Fungis	Test	LC_{50} (conidia/mL^{-1})		LC_{90} (conidia/mL^{-1})		
		Mean	VR	Mean	VR	P
B. peruviensis	1	6.1×10^2	(2.7×10^1–3.7×10^3)	8.2×10^6	($3.1\times10^6-2.9\times10^7$)	<0.0001
B. bassiana	1	9.6×10^2	(4.6×10^1-4.6×10^3)	2.4×10^5	(1.1×10^5–5.4×10^5)	<0.0001
Metarhizium sp	1	1.1×10^4	(1.7×10^3–4.1×10^4)	7.8×10^8	(2.1×10^8–5.8×10^9)	<0.0001
B. peruviensis	2	1.6×10^3	(1.3×10^2–7.5×10^3)	1.9×10^7	(7.5×10^6-7.6×10^6)	<0.0001
B. bassiana	2	1.6×10^3	(1.3×10^2–7.5×10^3)	1.9×10^7	(7.5×10^6-7.6×10^7)	<0.0001
Metarhizium sp	2	4.6×10^4	($1.3\times10^4-1.1\times10^5$)	5.7×10^8	(1.8×10^8–2.8×10^9)	<0.0001

VR = variation range.

production and enzyme activity during cuticle penetration. On the other hand, the composition of the culture medium where propagules are produced can also influence lethal time [31].

4. Conclusion

The fungi *B. bassiana*, *B. peruviensis,* and *Metarhizium* sp. showed variability in conidial production, conidia viability, pathogenicity, timing, and lethal concentrations against *H. hampei*. The fungi with the highest conidial production and conidia viability were *B. bassiana* and *B. peruviensis*. The three evaluated species showed pathogenicity towards *H. hampei*, and mortality increased with increasing dose. The highest mortality occurred in the treatments *B. bassiana* 1×10^9 conidia/mL^{-1}, *B. bassiana* 1×10^7 conidia/mL^{-1}, and *B. peruviensis* 1×10^9 conidia/mL^{-1}. The treatments with the best LT_{50} and LT_{90} were *B. bassiana* 1×10^9 conidia/mL^{-1} and *B. peruviensis* 1×10^9 conidia/mL^{-1} (Table 2), and the strains with the lowest LC_{50} and LC_{90} were *B. peruviensis* and *B. bassiana* (Table 3). The present study shows that *B. bassiana* and the native strain of *B. peruviensis* have important attributes as control agents of *H. hampei*. It also demonstrates the importance of physiological and pathogenic characterization in the selection of entomopathogenic fungi. The characteristics shown by *B. bassiana* and *B. peruviensis* under laboratory conditions suggest that they have a lot of potentials to mitigate *H. hampei* populations *in situ*; this serves as a precedent for the development of new research that will help to confirm the results obtained in the present investigation.

Data Availability

The data used to support the conclusions of this study are included in this manuscript and can also be requested from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest with respect to the publication of this article.

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