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Compatibility of Native Strains of *Beauveria peruviensis* and *Metarhizium* sp. as Strategy for Biological Control of Coffee Berry Borer (*Hypothenemus hampei*, Ferrari)

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Abstract: Coffee is a crop of global importance, and it is especially important in countries such as Peru. However, the presence of the pest *Hypothenemus hampei* represents a significant challenge with a notable economic impact. This study addresses this challenge using entomopathogenic fungi such as *Beauveria peruviensis* and *Metarhizium* sp. The compatibility of three strains of *Beauveria peruviensis* (F5, P19, and P4) and seven strains of *Metarhizium* sp. (MMR-M1, LLM-M2, MHR-M4, PMR-M12, MMR-M15, TOR-M16, and GOR-M18) was evaluated for approximately 2 months. A total of 14 treatments were designed, each consisting of one strain of *B. peruviensis* and one strain of *Metarhizium* sp. The Skott–Knott test ($p \leq 0.05$) revealed that strain LLM-M2 (*Metarhizium* sp. strain) had the highest conidial production (3.75×10^7 conidia/mL). Except for T6 (MMR-M1/F5), which showed a mutual growth type interaction (type A), all other strain combinations showed a type B interaction (mutual inhibition by contact or separation between colony margins (<2 mm)). The combination with the highest germination rate was T10 (MHR-M4/F5) at 89%. In addition, the pathogenicity of the combined strains was evaluated, showing a direct correlation with mortality and mycosis development in the coffee berry borer in treatments T1 (PMR-M12/P19), T10 (MHR-M4/F5), and T11 (MMR-M15/P19), reaching 100% mortality at 72 h with grade 4 mycosis. Regarding mycelial growth, treatments T1 (PMR-M12/P19), T4 (MMR-M1/P19), and T12 (GOR-M18/P19) reached the highest percentages, between 85.8% and 83.10% at 240 h. This study demonstrates the feasibility of using native strains of *B. peruviensis* and *Metarhizium* sp. as a biocontrol strategy against the coffee berry borer in the Amazon department, presenting them as an alternative to traditional chemical methods.

Keywords: compatibility; *Coffea arabica* L.; entomopathogenic fungi; mycosis; Peru

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1. Introduction

Coffee (*Coffea arabica* L.) is a crop of global importance and great economic relevance; in 2021, a world production of 166.2 million 60 kg bags was recorded. Coffee is consumed in various forms and products, the most common being roasted and ground coffee, representing 75% of world consumption, with a high market share in Europe [1]. In Peru, production reached 4200 thousand 60 kg bags, becoming one of the main Arabica coffee-producing countries in 2021, occupying fifth place in world production [2].

Peru stands out for being a coffee-producing rather than consuming country, focusing on the production of coffee beans oriented toward exports and subject to the demands of external markets according to the quality of the product [3]. The economy of farmers

depends on the production and quality of their harvest, which in different stages is affected by the diverse factors that intervene in the development of its cycle [4]. Among these are biotic and abiotic stress, salinity, drought, nutritional deficiencies, and phytosanitary problems; among the latter are the damages caused by pests, and among the most important in terms of economic damage is the coffee berry borer (CBB) because it can spread rapidly, affecting up to 95% of the cultivated areas [5].

The coffee berry borer [*Hypothenemus hampei* (Coleoptera: Curculionidae)] is an important pest worldwide that affects the profitability of the crop; despite being native to Central Africa, it can be distributed in all coffee-producing regions [6]. This pathogen causes the most damage in its adult stage; the females perforate the berry to deposit their eggs in internal galleries, which, in their larval stage, feed on the berry endosperm, spending most of their life cycle inside the berry; consequently, its control is difficult, and the use of chemical control strategies significantly increases production costs [7,8]. The use of agrochemicals limits the ability of farmers to certify their crops as organic, especially in the case of Peruvian specialty coffees. This certification is granted to crops that do not use agrochemicals, allowing them to obtain fairer prices on the world market due to the high demand for these products [9].

Given the growing concern about the environmental impact of traditional pest control methods in coffee crops, a specific strategy has been promoted: biological control of the coffee berry borer (*H. hampei*) with antagonistic fungi [10]. Among the fungal antagonists of *H. hampei* are *Beauveria bassiana* and *Metarhizium* sp., which act through the adhesion of the spore to the host cuticle without being ingested by the host. This occurs in three stages: adhesion of the spore to the surface through the recognition of specific receptors, which are glycoproteins present in the insect; then, union or consolidation occurs at the point of contact between the pregerminated spore and the external layer of the insect. Finally, germination and development begin, culminating in the formation of appressoria to initiate the penetration phase, which causes the host's death [11–13].

From the Amazonas department in Peru, Chuquibala-Checan et al. [14] evaluated the in vitro biological activity of *B. bassiana*, *B. peruviensis*, and *Metarhizium* sp. against *H. hampei.* The results indicated that *B. bassiana* and *B. peruviensis* produced the highest conidia production and viability. Regarding pathogenicity, the highest mortality was reached at concentrations of 1×10^9 with *B. bassiana* and *B. peruviensis* strains, with percentages close to 100%; the lowest mortality was observed for *Metarhizium*.

Based on previous evidence supporting the beneficial effects of these two fungal species [15–17] and their efficiency as biological control agents, this study was conducted to evaluate the compatibility between strains of *B. peruviensis* and *Metarhizium* sp. in the control of coffee berry borer (*H. hampei*) populations at the laboratory level. The objective is to present this research as an effective and economical alternative that can benefit the productive sector, the environment, and health in general.

2. Materials and Methods

2.1. Study Area

The research and sample processing were carried out at the Plant Health Research Laboratory (LABISANV) of the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES) of the National University Toribio Rodriguez de Mendoza of Amazonas, located in Chachapoyas province, Amazonas, Peru, at an altitude of 2335 m a. s. l., with an average rainfall of 2489 mm, a warm temperate climate, and an average annual temperature of 15.2 °C.

2.2. Entomopathogenic Fungal Strains

2.2.1. Selection of *B. peruviensis* Strains

Three strains of *B. peruviensis*, a new species, were selected; these isolates were confirmed by multilocus phylogeny and molecular markers [18] (Table 1).

Table 1. Characteristics of the *B. peruviensis* strains.

N°	Code	District/Area of Collection	% Pathogenicity
1	F5	Omia (La Primavera)	53%
2	P19	Huambo (Dos Cruces)	73%
3	P4	Omia (Líbano)	97%

2.2.2. Selection of Strains of *Metarhizium* sp.

Seven strains of *Metarhizium* sp. were selected according to their percentage of pathogenicity; these strains belong to the mycotheca of entomopathogenic fungi of the Plant Health Research Laboratory (LABISANV) of the National University Toribio Rodriguez de Mendoza of Amazonas (UNTRM) [14] (Table 2).

Table 2. Degree of pathogenicity of the strains of *Metarhizium* sp.

N°	Code	District/Collection Area	% Pathogenicity
1	MMR-M1	Mariscal Benavides (Michina)	100%
2	LLM-M2	Longar (Longar)	70.70%
3	MHR-M4	Huambo (Miraflores)	91.50%
4	PMR-M12	Mariscal Benavides (Pilancon)	83.31%
5	MMR-M15	Milpuc (Milpuc)	96.67%
6	TOR-M16	Omia (Gebil)	100%
7	GOR-M18	Omia (Gebil)	76.67%

Both species were collected from infested berry borers in the coffee agroecosystem of the Rodriguez de Mendoza province of Amazonas, Peru.

2.3. Conidial Concentration

The methodology used was based on the proposal of Gómez et al. [19], which consisted of preparing a stock solution. This was prepared by adding 1 g of rice with conidia of the selected *Metarhizium* sp. and *B. peruviensis* strains to Twin 80 at 0.01%. The mixture was shaken for one minute. Successive dilutions were then made until 10^{-3} was reached. The concentration of conidia was estimated by taking 10 µL of the dilutions, counting them in a Neubauer chamber, and observing them under a Leica® microscope with a 40× objective. The concentration of conidia was calculated according to the formula of Lipa and Slizynzki [20].

$$C = (Cc)(4 \times 10^6)(\frac{Fd}{80}) \quad (1)$$

where

C = number of conidia. mL^{-1}.

Cc = an average number of conidia counted in the Neubauer chamber.

Fd = dilution factor.

2.4. Strain Compatibility

To test the compatibility between *Metarhizium* sp. and *B. peruviensis*, the methodology described by Elósegui and Elizondo [21] with modifications was used.

The fungal strains were reisolated on potato dextrose agar (PDA) medium for a 5-day incubation period. Then, 4 mm diameter discs were taken from each *Metarhizium* sp. and *B. peruviensis* strain. The locations of the strains were opposite (each at the end of the plate); these were coded and sealed; all these steps were performed in a laminar flow chamber.

Five replicates were performed for each strain combination. Plates were incubated at 26.5 °C for 36 days and measured at two-day intervals [22].

Interaction between Strains

The microorganisms' interaction type was established according to the scale proposed by Magan and Lacey [23] (Table 3). The bioassay lasted approximately two months.

Table 3. The type of interaction to establish the reaction they present.

Interaction Type	Classification Description	Reaction Type
Common growth		A
Mutual inhibition	By contact or spacing between small colony margins (<2 mm).	B
	At a distance (colony spacing > 2 mm).	C
Inhibition of a microorganism	By contact, the inhibitory species continues to grow without changing its growth rate or at a slower rate through the inhibited colony.	D
	At a distance, the inhibitory species continues to grow over the halo produced (resulting in a clear zone), and the inhibited colony may grow at a reduced rate.	E

2.5. Germination Percentage

A dilution of 10^{-2} conidia/mL was selected for the experiment. In a laminar flow chamber, four 10 µL aliquots of each *Metarhizium* sp. and *B. peruviensis* strain were taken, placed together in a Petri plate coated with a thin layer of potato dextrose agar (PDA), and incubated at 27 °C. Each aliquot represented one replicate of each strain. After 14 h of incubation, staining with lactophenol blue was performed to arrest germination and improve contrast for microscopic observation. The PDA medium was removed with a sterile scalpel and placed on a slide for microscopic observation at 60×, after which germinated and non-germinated spores were counted. Germination was considered present when the germ tube exceeded the diameter of the spore. The percentage of germination was calculated using a specific formula [24].

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

where

a = number of germinated conidia.
b = number of germinated conidia.

2.6. Determination of Entomopathogenic Capacity of Native Strains of Metarhizium sp. and B. peruviensis

To determine pathogenicity, 14 treatments were evaluated using *Metarhizium* sp. and *B. peruviensis combinations* after disinfection (Table 4). The insects were immersed for 1 min in Petri plates with 0.5% NaClO [19], and suspensions of 1×10^7 spores/mL per strain were prepared; equal volumes of each suspension were mixed to obtain a total of 20 mL per mixture. The method applied was the immersion method, which consisted of submerging the borers (female and male insects collected from coffee farms in Rodríguez de Mendoza at 1630 m a. s. l. with coordinates 6°25′44″ S and 77°32′16″ W), lodged in a mesh in an aqueous solution with spores of the mentioned strains for one minute.

Next, new petri plates were prepared by placing sterile, pre-moistened filter paper at the base of each plate. Using tweezers, ten inoculated borers and five coffee beans were placed on each plate to avoid cannibalism and starvation among the borers. Each plate was sealed with parafilm and labeled with codes corresponding to the different treatments and replicates, facilitating subsequent counts. These plates were incubated at 27 °C for ten days, after which the filter paper was moistened daily with sterile distilled water, taking care not to saturate the plates. The pathogenicity and virulence of the combined strains of *Metarhizium* sp. and *B. peruviensis* on the coffee berry borer were evaluated for ten days, recording the time of mortality due to pathogenic causes and the fungal cycle, keeping the berry borers alive or dead in the Petri dishes so as not to interrupt the development dynamics of the insect and the fungus [25].

Table 4. *Metarhizium* sp. and *B. peruviensis* strains were evaluated on adults of *Hypothenemus hampei*.

Treatments	Binding Code	Strain Interaction
T1	PMR-M12/P19	*Metarhizium* sp./*B. peruviensis*.
T2	PMR-M12/P4	*Metarhizium* sp./*B. peruviensis*.
T3	PMR-M12/F5	*Metarhizium* sp./*B. peruviensis*.
T4	MMR-M1/P19	*Metarhizium* sp./*B. peruviensis*.
T5	MMR-M1/P4	*Metarhizium* sp./*B. peruviensis*.
T6	MMR-M1/F5	*Metarhizium* sp./*B. peruviensis*.
T7	LLM-M2/P19	*Metarhizium* sp./*B. peruviensis*.
T8	LLM-M2/F5	*Metarhizium* sp./*B. peruviensis*.
T9	MHR-M4/P4	*Metarhizium* sp./*B. peruviensis*.
T10	MHR-M4/F5	*Metarhizium* sp./*B. peruviensis*.
T11	MMR-M15/P19	*Metarhizium* sp./*B. peruviensis*.
T12	GOR-M18/P19	*Metarhizium* sp./*B. peruviensis*.
T13	GOR-M18/F5	*Metarhizium* sp./*B. peruviensis*.
T14	TOR-M16/P19	*Metarhizium* sp./*B. peruviensis*.

Classification of Insects with Mycosis

The characterization of the different treatments was performed using scales of systemic subcutaneous mycosis according to the degree of damage by intervals (Table 5), defining representative qualities for the adaptability of the strains of *Metarhizium* sp. and *B. peruviensis* [26–29].

Table 5. Parameters for the classification of insects with mycosis.

Characterization	Scale
Evidence from death to mycelium production in the body of the CBB in a percentage between 1 and 25%.	Grade 1
Shows mycelial coverage on the body of the CBB in a percentage between 25 and 50%.	Grade 2
It presents conidiogenesis in the body of the CBB in a percentage between 50 and 75%.	Grade 3
Conidial release in the body of the shoot is at a percentage between 75 and 100%.	Grade 4

2.7. Experimental Design

A completely randomized design (CRD) was used. It was distributed in 14 trials of combined strains of *Metarhizium* sp. and *B. peruviensis*, with three replicates, making each one a total of 42 experimental units (each experimental unit represented a Petri dish of 9 cm in diameter). The population to be studied consisted of a total of Petri dishes. The in vitro effect of combined *Metarhizium* sp. and *B. peruviensis* strains with biocontrol potential on the coffee berry borer (*H. hampei*) was evaluated.

2.8. Data Analysis

The data obtained were first subjected to a Shapiro Wilks normality test and the Levene test to check the homogeneity of variances; data that met these assumptions were analyzed by analysis of variance (ANOVA) and the Skott–Knott multiple comparisons test; data that did not present a normal distribution were transformed with the square root function; data that still did not fit the normal distribution were processed with the non-parametric Kruskall Wallis test. All tests were performed at 5% significance in the InfoStat/Professional version 2018p statistical program.

3. Results

3.1. Concentration of Conidia of Entomopathogenic Fungi

The concentration of conidia is presented in Table 6. The strains that reported the highest concentrations of spores/mL were LLM-M2 (*Metarhizium* sp.) and two strains of *B. peruviensis* (P19 and P4) and were significantly different from the others. On the other hand, the strain with the lowest conidial production was GOR-M18, which is also associated with *Metarhizium* sp. Among the *B. peruviensis* strains, P4 and P19 showed similar results to LLM-M2, with the second and third most abundant conidia.

Table 6. Number of conidia produced by *Metarhizium* sp. and *B. peruviensis* strains assigned to treatment combinations (mean ± standard deviation).

Microbial Material of *Metarhizium* sp. and *Beauveria peruviensis*	Spore Concentration/mL
F5	$1.48 \times 10^7 \pm 3.31 \times 10^5$ c
GOR-M18	$1.06 \times 10^7 \pm 3.53 \times 10^5$ d
LLM-M2	$3.75 \times 10^7 \pm 3.44 \times 10^5$ a
MHR-M4	$1.75 \times 10^7 \pm 3.05 \times 10^5$ c
MMR-M1	$1.27 \times 10^7 \pm 2.42 \times 10^5$ d
MMR-M15	$1.16 \times 10^7 \pm 3.18 \times 10^5$ d
P19	$3.44 \times 10^7 \pm 3.49 \times 10^5$ a
P4	$3.65 \times 10^7 \pm 2.71 \times 10^5$ a
PMR-M12	$2.53 \times 10^7 \pm 3.45 \times 10^5$ b
TOR-M16	$3.04 \times 10^7 \pm 3.35 \times 10^5$ b

Means with a common letter are not significantly different (Skott–Knott, $p \leq 0.05$).

3.2. Strain Compatibility

Metarhizium sp. and *B. peruviensis* strains were subjected to the combined treatment at different time intervals (Table 7); the first strains to bind were MHR-M4/P4, MHR-M4/F5, LLM-M2/P19 and GOR-M18/F5 at 24 days into the trial, and the last to interact were MMR-M1/P4, MMR-M15/P19 and TOR-M16/P19 at 33 days. Days of coexistence were evaluated after the strains joined until the end of the evaluation at 33 days.

Table 7. Compatibility and interaction of combined treatments of *Metarhizium* sp. and *B. peruviensis*.

Treatments	Bonding Code	Bonding Days	Coexistence Days	Distance (mm)	Interaction Type
T1	PMR-M12/P19	27	9	2.9	B
T2	PMR-M12/P4	27	9	2.6	B
T3	PMR-M12/F5	30	6	2.5	B
T4	MMR-M1/P19	27	9	2.7	B
T5	MMR-M1/P4	33	3	2.3	B
T6	MMR-M1/F5	27	9	1.9	A
T7	LLM-M2/P19	24	11	2.2	B
T8	LLM-M2/F5	30	6	3.1	B
T9	MHR-M4/P4	24	11	2.2	B
T10	MHR-M4/F5	24	11	3	B
T11	MMR-M15/P19	33	3	2.4	B
T12	GOR-M18/P19	27	9	2	B
T13	GOR-M18/F5	24	11	2.2	B
T14	TOR-M16/P19	33	3	2.5	B

After 15 days of evaluation, a decrease in growth was observed, corresponding to the physical proximity of the colony margins of both species. The MMR-M1/F5 treatment showed a common growth-type interaction (A), and the other treatments showed a mutual inhibition-type interaction by contact or separation between colony margins (<2 mm) (B).

3.3. Germination Percentage

Significant differences existed between treatments for this variable, with treatments T1, T3, T6, T9, T10, and T13 achieving the highest germination percentages with percentages between 86 and 89% (Table 8).

Table 8. Germination percentage (mean ± standard deviation) at the treatment level.

Table	Code	Germination (%)
T1	PMR-M12/P19	86 ± 1.4 a
T2	PMR-M12/P4	81 ± 1.4 b
T3	PMR-M12/F5	87 ± 1.4 a
T4	MMR-M1/P19	79 ± 2.8 b
T5	MMR-M1/P4	79 ± 1.4 b
T6	MMR-M1/F5	87 ± 1.4 a
T7	LLM-M2/P19	82 ± 1.4 b
T8	LLM-M2/F5	79 ± 1.4 b
T9	MHR-M4/P4	87 ± 1.4 a
T10	MHR-M4/F5	89 ± 1.4 a
T11	MMR-M15/P19	76 ± 1.4 b
T12	GOR-M18/P19	80 ± 1.4 b
T13	GOR-M18/F5	86 ± 1.4 a
T14	TOR-M16/P19	79 ± 1.4 b

Means with a common letter are not significantly different (Skott–Knott, $p \leq 0.05$).

3.4. Pathogenicity of the Combination of *Metarhizium* sp. and *B. peruviensis*

Table 9 shows that the mortality percentages at 72 h did not differ significantly among treatments. The percentage of mycelial growth at 240 h determined that treatments T4 (MMR-M1/P19), T1 (PMR-M12/P19), and T12 (GOR-M18/P19) presented percentages close to 100%, which were significantly different from those of the other treatments. The mycosis percentages resulting from these treatments were classified as grade 4 (75–100% conidia on the body of *H. hampei*), and those resulting from the other treatments were classified as grade 3 (50–75% conidia on the body of *H. hampei*).

Table 9. Percentage mortality and mycelial growth (mean ± standard deviation).

Treatments	Combination of *Metarhizium* sp. and *Beauveria peruviensis* Strains	Percentage of Mortality (72 h) [1] (H = 8.37; p = 0.3630)	Percentage of Mycelial Growth (240 h) [2] (F = 5.09; p = 0.0002)
T1	PMR-M12/P19	100 ± 0 a	84.10 ± 4.25 a
T2	PMR-M12/P4	96.6 ± 5.7 a	71.00 ± 5.00 b
T3	PMR-M12/F5	93.3 ± 5.7 a	68.6 ± 2.84 b
T4	MMR-M1/P19	100 ± 0 a	85.80 ± 1.89 a
T5	MMR-M1/P4	100 ± 0 a	76.00 ± 8.18 b
T6	MMR-M1/F5	90 ± 10 a	72.00 ± 1.00 b
T7	LLM-M2/P19	96.6 ± 5.7 a	74.00 ± 3.6 b
T8	LLM-M2/F5	90 ± 10 a	71.10 ± 4.04 b
T9	MHR-M4/P4	96.6 ± 5.7 a	70.10 ± 7.07 b
T10	MHR-M4/F5	100 ± 0 a	76.00 ± 2.00 b
T11	MMR-M15/P19	100 ± 0 a	73.00 ± 4.35 b
T12	GOR-M18/P19	96.6 ± 5.7 a	83.10 ± 3.01 a
T13	GOR-M18/F5	86.6 ± 23 a	73.00 ± 1.00 b
T14	TOR-M16/P19	100 ± 0 a	77.30 ± 3.05 b

[1] Means with a common letter are not significantly different (Kruskall Wallis; $p \leq 0.05$). [2] Means with a common letter are not significantly different (Skott–Knott, $p \leq 0.05$).

4. Discussion

The results of this research underline the efficacy of *Metarhizium* sp. and *B. peruviensis* strains to produce conidia, a key aspect for their practical application in the biological control of coffee berry borer; this ability to produce conidia has been widely documented for *Metarhizium* and *B. bassiana* [30,31]. The strains with the highest concentration of conidia per milliliter were LLM-M2 (*Metarhizium* sp.) with 3.75×10^7 spores/mL and P4 (*B. peruviensis*) with 3.65×10^7 spores/mL. Liu and Bauer [32] obtained lower concentrations when evaluating the susceptibility of *Agrilus planipennis* to *B. bassiana* and *M. anisopliae*, finding that the concentration of conidia between strains varied significantly, ranging between 1.7×10^5 and 1.9×10^7 conidia/mL for *B. bassiana* and between 3.2×10^6 and 1.1×10^7 conidia/mL for *M. anisopliae*. It is important to consider this variable since, according to previous studies, a higher concentration of conidia correlates with greater efficacy in field application, taking into account the multiple factors that limit the efficacy of an entomopathogenic fungus, such as solar radiation and altitudinal floor [33–35].

The most common interaction of the studied treatments of combined strains of *B. peruviensis* and *Metarhizium* sp. was of type B (mutual inhibition by contact or separation of colony margins (<2 mm)). These results differ from those of Elóstegui and Elizondo [21], who found C-type interactions (mutual inhibition at a distance) for interactions between *B. bassiana* and *M. anisopliae*. The understanding of these interactions is important to optimize the formulations of biological control agents [36] since it is important that these two fungi do not repel each other and do not generate allelopathy, taking into account that these two species will coexist in the same solid matrix and when they are applied in the field, both species must be alive and have an effect on the control of the coffee berry borer; If this is not the case, either of the two can dominate in the solid matrix and can reduce the population of the other and even disappear. Under this concept, it would no longer be considered a biological formula.

Six treatments achieved germination percentages close to 100%: T10 (89%), T9 (87%), T6 (87%), T3 (87%), T13 (86%) and T1 (86%). In other investigations developed with mixtures of *B. bassiana* and *M. anisopliae* conidia, higher germination percentages were achieved, with means of $98 \pm 0.3\%$, after 20 h of being seeded in 0.1 mL of a conidial suspension that was adjusted to 1×10^6 conidia per milliliter and incubated at 27 °C [21]. The germination rate is an indicator of the viability and pathogenic potential of the conidia [37]. The values close to 100 obtained in this research may have been influenced by temperature since this is a variable that was strictly controlled to be maintained at 25 °C, this being a predominant factor in germination efficiency since there are already many studies that show that at this temperature germination percentages close to 100% can be achieved.

In proportion to mycelial growth, of the 14 treatments evaluated, T4 presented the highest percentage (85.8%) and was classified as mycosis grade 4; this growth is slightly lower than that reported by Cruz et al. [38], who determined the percentage of virulence for a mixture of three strains of *B. bassiana* to be 93%. Despite the differences found in the investigations, a high degree of mycosis of the treatments with combined strains is appreciated, which makes them good green alternatives for the control of *H. hampei*. Factors such as the entomopathogenic capacity of the fungus, dose, environmental conditions, and insect susceptibility are determinants of its infective capacity as a biological control agent [39]. These results are promising for developing integrated management strategies for *H. hampei* incorporating biological control agents as key components.

Under laboratory conditions, the combinations of *Metarhizium* sp. and *B. peruviensis* showed a high percentage of mycosis since the evaluation was carried out in a protected and sterile environment, where conditions are much more favorable than inoculating the fungus in the open field, showing much better results, such as treatment T4 with 85.8%. In this sense, it would be important to consider the application of the strains of treatment 4 and the strains that showed similar results under field conditions in the place of origin of the strains and in the different producing areas of the Amazon department and other departments of Peru, considering the variety of altitudinal levels and climates in which

coffee is grown in the country, since according to some research, the mycosis of combined strains of *B. bassiana* and *M. anisopliae* on the coffee berry borer at different altitudes varies from 14.3% to 40.6% [35].

In the analysis of adult mortality of *H. hampei* at 72 h, the treatments that achieved 100% mortality were T5 (MMR-M1/P4), T4 (MMR-M1/P19), T14 (TOR-M16/P19), T11 (MMR-M15/P19), T10 (MHR-M4/F5) and T1 (PMR-M12/P19). Research such as that of Schapovaloff et al. [40] also showed that the combination of *B. bassiana* and *M. anisopliae* strains is effective in the control of Coleoptera such as *Hedypathes botulinus*; in this particular case, a mortality rate of 81.8% was obtained.

This research provides valuable evidence on the selection and combination of *Metarhizium* sp. and *B. peruviensis* strains for biological control of coffee berry borer. These strains' efficacy, compatibility, and pathogenicity underline their potential as sustainable alternatives to chemical pesticides. However, it is necessary to recognize certain limitations in the methodology since the germination and mortality percentages were not tested individually for each strain studied, which could limit the interpretation of the results of the individual efficacy of each strain. Future research should focus on long-term field trials to validate these findings in real growing conditions and explore methods to optimize the formulation and application of these entomopathogenic strains.

5. Conclusions

The LLM-M2 (*Metarhizium* sp.) and P4 (*B. peruviensis*) strains showed significantly higher conidial production capacity, with 3.75×10^7 conidia/mL and 3.65×10^7 conidia/mL, respectively, highlighting their potential as prime candidates for the development of bioinsecticides against *H. hampei*.

In terms of germination percentage, treatments T10, T9, T6, T3, T13, and T1 achieved the highest germination percentage (100%). The pathogenicity of the coffee berry borer showed a direct proportion with mortality and mycosis in treatments T1, T10, and T11, with 100% mortality at 72 h and grade 4 mycosis, indicating the significant potential for the effective control of *H. hampei* through the application of specific combinations of strains of *Metarhizium* sp. and *B. peruviensis*.

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