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Mycoinsecticide capacity of five strains of *Beauveria bassiana* isolated from Misiones (Argentina)

Capacidad micoinsecticida de cinco cepas de *Beauveria bassiana* aisladas de Misiones (Argentina)

Marilyn R. V. Silva[1, 2, *]; Gustavo A. Bich[1, 2]; Marcela A. Sadañoski[1, 2]; María L. Castrillo[1, 2]; Pedro D. Zapata[1, 2]; Laura L. Villalba[1]; María I. Fonseca[1, 2]

1- Laboratorio de Biotecnología Molecular. Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS). Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones. Posadas, Misiones, Argentina.
2- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Buenos Aires, Argentina.
* E-mail: marisr424@gmail.com

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Abstract
This study aimed to isolate and identify entomopathogenic fungal strains of the genus *Beauveria* from agricultural soils and to evaluate their pathogenic potential as mycoinsecticides. Fungal identification was based on a multifaceted approach that included macro- and micromorphological characterization, scanning electron microscopy, and analysis of nuclear ribosomal DNA, ITS, and EF-1α sequences. Pathogenicity tests were conducted against the larval stage of *Tenebrio molitor*. Among all isolates examined, two fungal strains collected from yerba mate plantations in southern Misiones were identified as *Beauveria bassiana* (HEP 32 and HEP MSO2). Larval mortality of *T. molitor* was significantly affected, particularly by the *B. bassiana* HEP MSO2 strain, suggesting its potential as an effective mycoinsecticide for integration into crop pest management strategies.

Keywords: Entomopathogenic fungus, Biocontrol, Pathogenicity.

Resumen
Este estudio tuvo como objetivo aislar e identificar cepas de hongos entomopatógenos del género *Beauveria* a partir de suelos agrícolas y evaluar su capacidad patogénica. La identificación fúngica se basó en un enfoque multifacético que incluyó el reconocimiento macro- y micromorfológico, microscopía electrónica de barrido y análisis del ADN ribosomal nuclear, secuencias ITS y EF-1α. Las pruebas de patogenicidad se realizaron sobre larvas de *Tenebrio molitor.* De todos los aislamientos estudiados, dos cepas fúngicas recolectadas en plantaciones de yerba mate del sur de Misiones fueron identificadas como *Beauveria bassiana* (HEP 32 y HEP MSO2). La mortalidad de las larvas de *T. molitor* se vio significativamente afectada, principalmente por la cepa HEP MSO2, lo que sugiere su potencial como micoinsecticida eficaz para integrar en estrategias de manejo de plagas en cultivos.

Palabras clave: Hongos entomopatógenos, Biocontrol, Patogenicidad.

INTRODUCTION

Insect pest management continues to pose a persistent global challenge (1). Due to their ongoing interactions, fungi and insects have co-evolved over thousands of years. These multifaceted relationships include antagonistic interactions, which render fungi valuable agents for biological control (2). A variety of practices enhance crop management, reinforce soil stability, and support environmentally sound actions, all of which are essential components of Integrated Pest Management (IPM) (3). In this context, entomopathogenic fungi (EPF) are key biological control agents against numerous insect pests, particularly those of significant sanitary relevance when incorporated into integrated management strategies (4).

Unlike other entomopathogens that require ingestion to infect, EPF typically penetrate the insect cuticle actively, irrespective of the insect's feeding behavior (5). The most prominent genera of entomopathogens include *Beauveria*, *Metarhizium*, and *Purpureocillium*, all belonging to the phylum Ascomycota (Order: Hypocreales) (6). These fungi are ubiquitous, saprotrophic filamentous organisms frequently isolated from soil, vegetation, decaying matter, or parasitized arthropods. They exhibit infective and lethal properties against insects across diverse geographic, climatic, and agroecological regions, offering promising prospects for the development of integrated management techniques that provide long-term solutions (7).

Mycobiocontrol, or the use of mycoinsecticides, plays a crucial role in environmental protection and food safety. It represents a valuable tool within IPM by reducing dependence on chemical insecticides. However, the current pathogenic

mechanisms of entomopathogens are relatively slow and require further refinement to enhance their efficacy (8). One strategy to address this limitation involves isolating and selecting fungi from environments that have remained largely undisturbed by chemical agents over extended periods, favoring traits such as rapid growth and heightened virulence.

Biotechnology contributes significantly to understanding the pathogenicity and mechanisms of action of EPF during pest control. Moreover, ecological insights into EPF are essential for designing strategies that enhance their effectiveness in field applications, including determining appropriate bioinsecticide dosages, identifying optimal application sites and timing, and recognizing the onset of insect attacks on crops (9). The vast microbial diversity present in natural ecosystems remains largely untapped and could serve as a valuable reservoir for future EPF candidates (10).

Beauveria bassiana is one of the principal EPF species. It causes white muscardine disease and can infect and parasitize insects until death, making it a highly promising bioinsecticide (11).

The objective of this study was to isolate and identify *Beauveria* strains from agricultural soils in Misiones and to evaluate their pathogenic potential as potent mycoinsecticides.

MATERIALS AND METHODS

Soil sampling

A total of 55 soil samples were collected from eleven Ilex paraguariensis (yerba mate) plantations located in the central and southern regions of Misiones province (Argentina) (Table 1). The area is characterized by a humid subtropical climate, with average annual maximum and minimum temperatures of 21 °C and 10 °C, respectively, rainfall of approximately 79.38 mm, and relative humidity around 77.38%. Soil samples (500 g) were taken at depths of 5–10 cm adjacent to yerba mate plants and 10–15 cm from the surface, using a previously disinfected shovel. Five randomly selected locations were sampled per site. Samples were stored in sterile polyethylene bags and transported to a laboratory in Posadas, Misiones, Argentina, for immediate processing.

Table 1: Sampling sites in yerba mate plantations in Misiones province (Argentina).

N°	Location	Geolocation	Type of management	Date
1	**Garupá**	27°27'10.4"S 55°50'17.0"W	conventional	15/10/2018
2	**Candelaria**	27°28'12.9"S 55°45'56.9"W	organic	2/12/2018
3	**Santo Pipó**	27°09'44.2"S 55°20'12.6"W	organic	7/11/2018
4	**Montecarlo**	26°53'26.0"S 54°76'23.2"W	organic	5/11/2018
5	**Aristóbulo del Valle**	27°02'09.8"S 54°47'26.3"W	conventional	24/7/2018
6	**Colonia Acaraguá**	27°29'24"S 54°50'13"W	organic	12/1/2019
7	**Posadas/El Porvenir I**	27°31'41.8"S 55°58'25.2"W	conventional	2/2/2019
8	**Posadas/El Porvenir II**	27°34'15.5"S 55°50'46.2"W	conventional	2/2/2019
9	**Parada Leis**	27°34'15.5"S 55°50'46.2"W	conventional	15/2/2019
10	**San José I**	27°45'27.9"S 55°46'27.9"W	conventional	15/2/2019
11	**San José** II	27°35'05.2"S 55°50'30.8"W	organic	15/2/2019

Isolation from soil: Soil dilution plating

Fungal isolation was performed using the dilution plate technique. One gram of each soil sample was suspended in 9 mL of distilled water and vortexed vigorously for 5 minutes. Subsequently, 0.1 mL of the suspension was plated at the center of a Petri dish and spread using a sterile glass spreader. Samples were cultured on a semi-selective medium for *Beauveria*, as described by Doberski and Tribe (12), with modifications (13): glucose 40 g/L, peptone 10 g/L, thiabendazole 0.004 g/L, chloramphenicol 0.5 g/L, crystal violet 0.01 g/L, agar 15 g/L, distilled water 1 L, pH 6. The medium was supplemented with 0.2 g/L ampicillin. Plates were incubated at 28 °C for 7–10 days. Colonies with expected morphology were subcultured onto Potato Dextrose Agar (PDA) plates. Axenic strains were identified based

on cultural and morphological characteristics described in the literature and stored on PDA at 4 °C.

Isolation of Beauveria species from insect cadavers

Collected insects were placed in sterile conical flasks and kept at room temperature for transport to the laboratory. Specimens were disinfected with 70% ethanol, and conidia from the surface of infected insects were transferred to the semi-selective medium for *Beauveria* (see section 1.2). Cultures were incubated in 9-cm Petri dishes at 28 ± 2 °C in darkness for 10 days. Pure cultures were then transferred to PDA, maintained at 28 °C, and subcultured every 2–3 weeks. Identification of the isolate obtained from the insect cadaver was performed using microscopy and molecular techniques (PCR and sequencing). Conidial suspensions of this isolate were used in bioassays.

Morphological and microscopic identification

Morphological identification of the isolates was based on macroscopic observation of colony characteristics (color, shape, and size) and microscopic examination of mycelium and reproductive structures. Identification keys by Samson et al. (14), Alves (15), and Humber (16) were used to classify the isolates at the genus level.

Microphotographs of the fungal surface were obtained using Scanning Electron Microscopy (SEM). Mycelium was fixed in a solution of formaldehyde, ethanol, and acetic acid (10:50:5). After critical point drying with CO_2 and gold coating, samples were examined using a JEOL 5800LV scanning electron microscope.

Molecular identification

Genomic DNA was extracted from 7-day-old axenic cultures grown on PDA supplemented with chloramphenicol, following the protocol by Fonseca et al. (17). The ITS and EF-1α regions were amplified via PCR under standard conditions and thermal cycling parameters (Table 2). Amplicons were sequenced bidirectionally by Macrogen (Korea), and consensus sequences were assembled using Chromas and BioEdit Sequence Alignment Editor v7.0.5. Alignments were performed and concatenated using MEGA v7.0, and phylogenetic analysis was conducted using the Neighbor Joining method with the Tamura model and 1000 bootstrap replicates. *Isaria* sp. sequences were used as outgroups. Final sequences were deposited in GenBank, and clades with <50% bootstrap support were collapsed in the consensus tree.

Table 2: Primers used for molecular identification of entomopathogenic fungi

Primer name	Oligonucleotide sequence 5′- 3′	Amplified región	Reference
ITS-1	TCC GTA GGT GAA CCT GCG G	ITS1-5.8 S-ITS2	(18)
ITS-4	TCCTCCGCTTATT GATATGC	ITS1-5.8 S-ITS2	(18)
EF1-7 28F	CATCGAGAAGTT CGAGAAGG	EF1-α	(19)
Tef1-R5	CCTTGGAGATAC CAGC	EF1-α	(19)

Viability tests

Spore viability was assessed prior to each pathogenicity test on *T. molitor* larvae. For each trial, 100 µL of conidial suspension was spread on 60-mm PDA plates using a sterile bent glass rod (Drigalski spatula). Plates were incubated in darkness at 28 °C for 24 hours. Spore viability was evaluated under a compound light microscope (20). One hundred conidia were randomly selected per plate (21) and considered viable if a germ tube at least half the conidial diameter was observed.

Pathogenicity tests

The *B. bassiana* strains under study were used. *T. molitor* insects (Piantieri Insect Farm, Argentina) were reared weekly in plastic trays containing approximately 200 individuals (eggs, larvae, pupae, and adults). Insects were maintained in a germination chamber at 25 ± 2 °C, 65% humidity, and a 14:10 h light-dark photoperiod. Larvae measuring 18–22 mm were selected under laminar flow conditions and placed in sterile cylindrical plastic containers with four lateral perforations and screw-on lids. Containers were refrigerated at 4 °C for 24 hours to reduce larval metabolism and prevent molting during the bioassay. Forty-eight larvae were selected per treatment, with fifteen treatments performed in triplicate. Larvae were transferred to sterile plastic flasks containing sterile carrot slices and inoculated with *B. bassiana* at concentrations of 1×10^5, 1×10^6, and 1×10^7 conidia/mL using an airbrush connected to an air compressor at a distance of 10 cm. Control groups were inoculated with distilled water. Flasks were kept in a climate chamber at 25 °C, with a 14:10 h photoperiod and 65% humidity, and monitored daily for 14 days. Dead larvae were recorded, and mortality percentages for each isolate were calculated. Deceased larvae were placed on PDA plates supplemented with chloramphenicol (10 mg/L) at 28 °C, and fungal development was monitored daily.

Larvae disinfection protocol

To eliminate external microbiota, *T. molitor* larvae were disinfected following a modified protocol by Cummings et al., involving sequential washes with ethanol, bleach, and sterile water. Disinfection was verified by culturing the final rinse and

performing an imprint on PDA. Larvae were dried on sterile paper and incubated at 28 °C in darkness to confirm mycosis.

Statistical analysis

Data were analyzed using Statgraphics Centurion software (StatPoint, Inc., version 15.2.05). Analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) post-hoc test at $p<0.05$ was used to determine the significance of fungal strain tolerance to the three fungicide concentrations.

The time required to cause 50% and 90% mortality (T_{50} and T_{90}) of insect larvae in laboratory conditions was determined for the most pathogenic entomopathogenic fungus using simple regression analysis in Statgraphics. The equation used was $Y = a + b \cdot X$.

RESULTS

Eleven samples were collected from yerba mate plantations in Misiones province between 2018 and 2019. Approximately 72% of the fungal isolates were identified at the genus level. Of the total fungi recovered, 63.4% were non-entomopathogenic, and only 9% were entomopathogenic (Figure 1).

Among the non-entomopathogenic fungi, the most frequently identified genera were *Aspergillus* (17.5%), *Fusarium* (10.3%), *Mucor* (6.3%), *Penicillium* (19%), *Rhizopus* (3.2%), and *Trichoderma* (7.1%). The entomopathogenic fungi identified included *Clonostachys* (1.6%), *Purpureocillium* (5.6%), and *Beauveria* (1.6%) (Figure 1). The isolates of interest for this study, belonging to the genus *Beauveria* (HEP MSO2 and HEP 32), were collected from yerba mate plantations located in Candelaria and Colonia Acaraguá, in the southwest and southeast regions of Misiones province, Argentina, respectively.

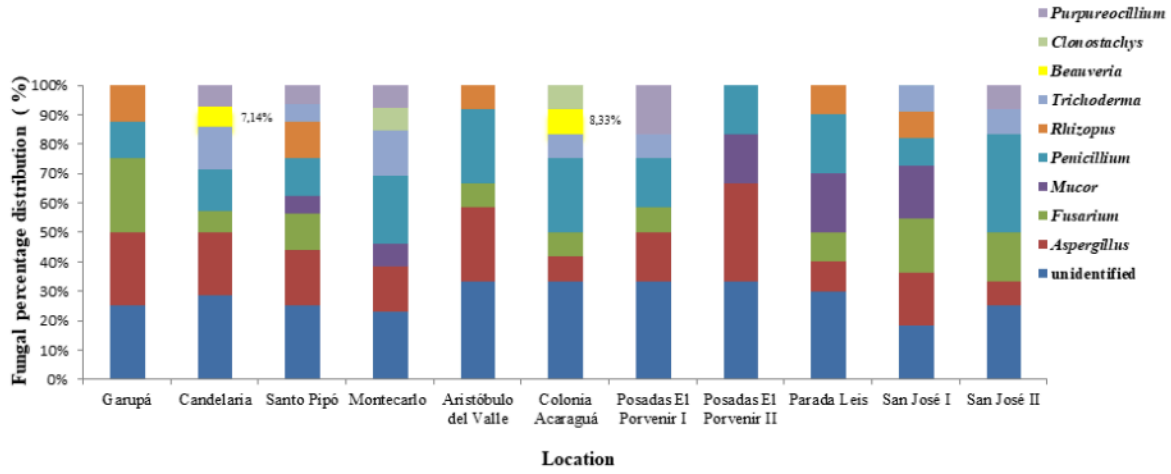

Figure 1. Percentage distribution of the fungal community at the genus level in soil samples and leaf litter from yerba mate plantations located in Misiones province.

Morphological identification of entomopathogenic fungi

Among the samples collected from yerba mate plantation soils, only the HEP MSO2 strain exhibited the typical characteristics of the *Beauveria* genus. HEP MSO2 was isolated from the Colonia Acaraguá plantation, an organic cultivation site (Figure 2a). This isolate displayed white to cream-colored colonies with irregular edges and a powdery texture, which are macroscopic traits characteristic of the *Beauveria* genus (Figure 2b). Microscopic observations revealed reproductive structures and conidia with morphology, size, and coloration consistent with the species *B. bassiana*. The conidiogenous cells measured 5.5 to 8.8 µm (SD 0.5 to 1.1 µm) × 2.0 to 2.7 µm (SD 0.1 to 0.8 µm), featuring a broad base and a tapered apex from which multiple conidia emerged in chains along a zigzag-shaped rachis. The conidia were hyaline and smooth, with a globose to subglobose structure. Their diameters ranged from 1.7 to 2.3 µm (SD 0.5 to 0.6 µm) (Figure 2c). Under scanning electron microscopy, hyphae with diameters of 1.12 to 1.41 µm and spherical conidia measuring 1.32 to 1.71 µm were observed on conidiophores with zigzag phialides (Figure 2d).

The HEP 32 isolate was obtained from parasitized insects of the genus *Brassolis* (Figure 2e), extracted from the soil surface and leaf litter of an organic yerba mate plantation in Candelaria (Table 1), surrounded by pine trees (*Pinus* spp.). This isolate exhibited macroscopic characteristics similar to those of HEP MSO2 (Figure 2f), although its colonies were slightly more flattened. Under electron microscopy, hyphae with

diameters ranging from 1.25 to 1.55 µm and spherical conidia measuring 1.1 to 1.2 µm were observed on conidiophores with zigzag phialides (Figure 2h). Both isolates produced and exuded structures known as synnemata as they aged.

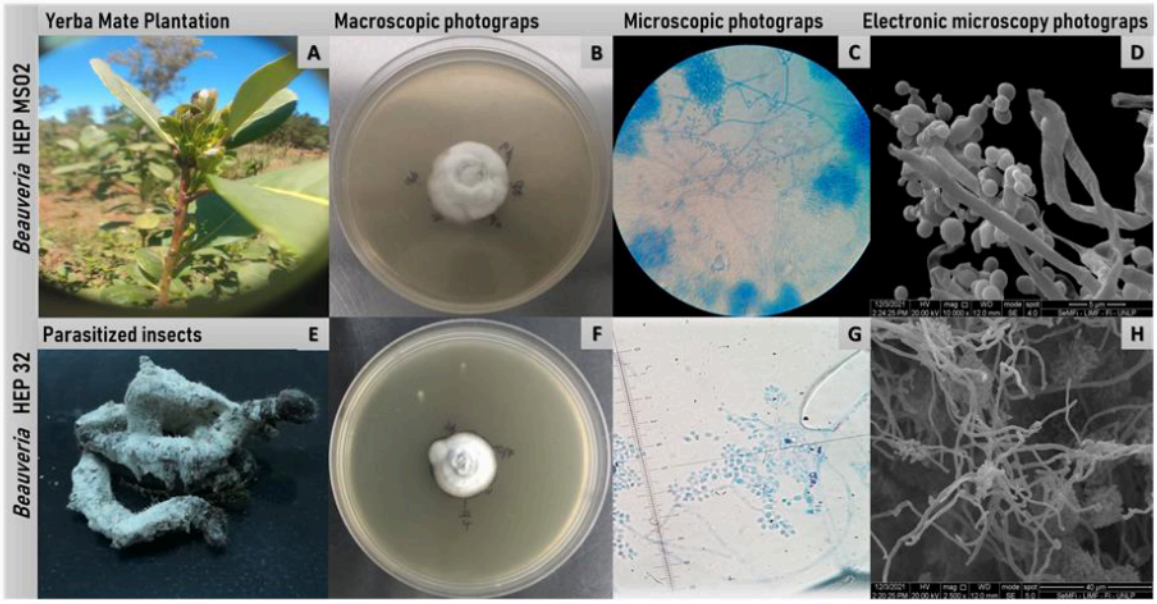

Figure 2. Fungal strains isolated from yerba mate plantation soil and parasitized insects. A) Fungi extracted from soil sample. B and C) Macroscopic and microscopic images of *B. bassiana* strain HEP MSO2. Photographs of colonies grown on PDA medium (Scale bar = 1 cm). Microphotographs stained with lactophenol cotton blue. E) Sample of parasitized insect (Lepidoptera). F and G) Macroscopic and microscopic images of *B. bassiana* strain HEP 32. D and H) Scanning electron microscopy images.

Molecular identification of entomopathogenic fungi

PCR amplification of the ITS and EF1-α regions from the DNA of the isolated entomopathogenic fungi yielded fragments of approximately 555 bp and 986 bp, respectively. Sequence identity analysis of the strains HEP MSO2 and HEP 32, which had been morphologically identified as *B. bassiana*, confirmed their taxonomic classification. The identity indices were 99.65% and 100% for the ITS regions (MK049987.1; MN428795.1), and 99.63% and 100% for the EF1-α regions (MK550625.1; KJ500423.1).

Phylogenetic tree analysis revealed that the fungal strains HEP MSO2 and HEP 32 clustered with other *B. bassiana* strains and their reference sequence (NR_111594), with bootstrap support values exceeding 99% (Figure 3).

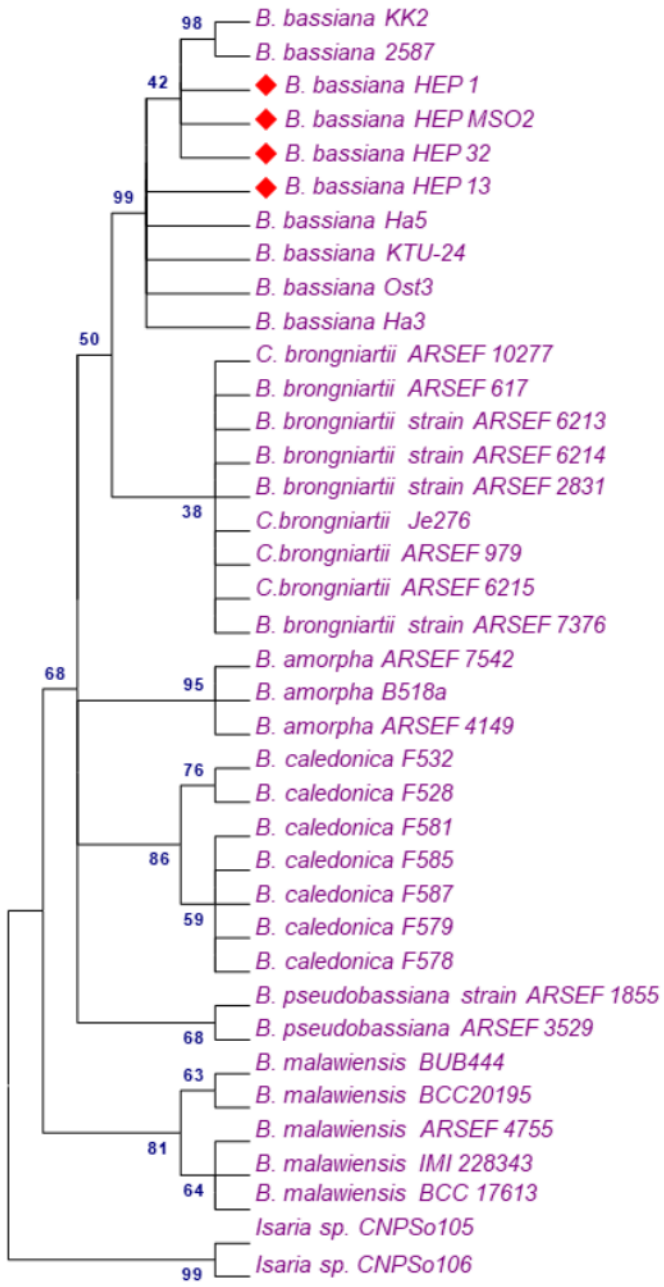

Figure 3. Concatenated phylogenetic tree constructed using the Neighbor Joining method, showing the relationship between the entomopathogenic fungal strains obtained in Misiones province and related species deposited in the GenBank–NCBI database. The sequences used correspond to the ITS and EF1α regions. The tree was built using MEGA 7.0 software. Bootstrap values from 1,000 replicates are indicated next to the branches. *Isaria* sp. sequences were designated as the outgroup to root the tree.

Pathogenicity tests

Spore viability was assessed prior to conducting pathogenicity tests on *T. molitor* larvae, with germination rates exceeding 90% in all trials.

Laboratory pathogenicity assays demonstrated that all five *B. bassiana* strains isolated in Misiones exhibited pathogenic activity, causing mortality in *T. molitor* larvae. Disease symptoms in the larval population varied depending on the fungal strain and conidial concentration. Initial symptoms appeared 48 hours after inoculation, with a marked reduction in insect activity leading to complete rigidity. In some cases, fungal structures emerged from the insect bodies 96 hours post-mortem, a characteristic confirming mortality due to fungal infection (Figure 4).

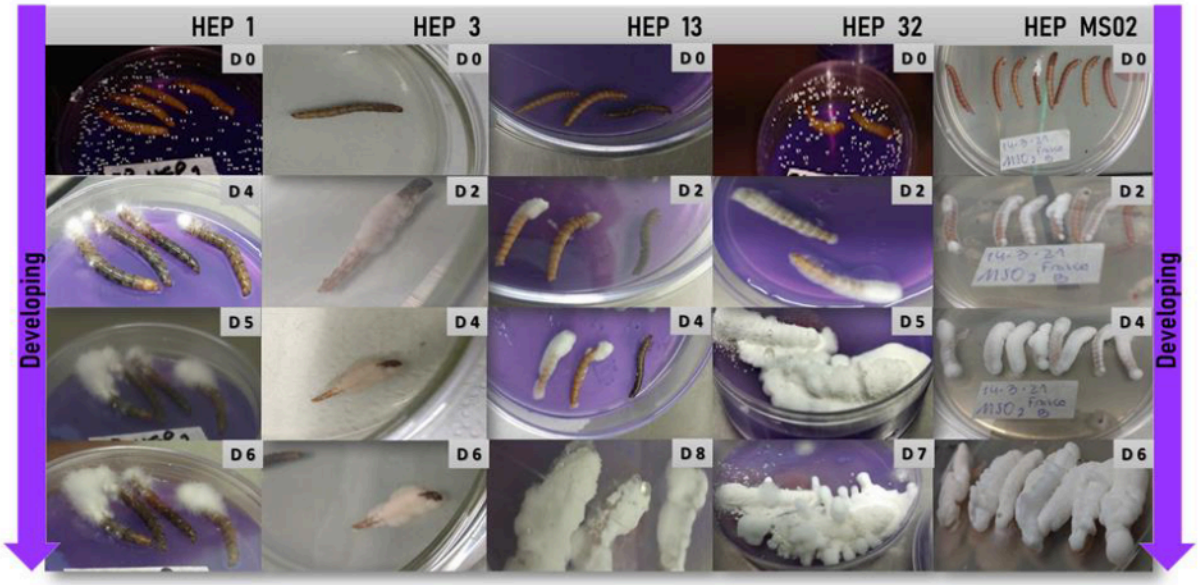

Figure 4. Images of deceased insects and fungal development from different *B. bassiana* strains at a concentration of 10^7 conidia/mL. Day 0 corresponds to the day of insect death. For HEP 1, death occurred on day 3 of the trial; for HEP 3, on day 4; for HEP 13, on day 4; for HEP 32, on day 4; and for HEP MSO2, on day 3.

Larvae disinfection protocol

The disinfection protocol applied at the conclusion of the pathogenicity tests with the five HEP strains successfully eliminated surface microbiota from the *T. molitor* larvae and confirmed that death was caused by pre-mortem mycosis. This procedure validated both the effective disinfection of the larvae and their mortality due to fungal infection. The fungal morphology observed in the insect cadavers was consistent with the colonial growth patterns on PDA medium (Figure 2e, f). Notably, no mycelial growth was detected on the plates used for the insect imprint tests.

Statistical analysis

The mortality rate was dependent on the concentration of *B. bassiana* (Table 3, Figure 5). At a concentration of 10^5 conidia/mL, the strains HEP 3, HEP 13, and HEP MSO2 induced the highest mortality rates in *T. molitor* larvae from the seventh day onward ($p < 0.00$) (Figure 5a). At a concentration of 10^6 conidia/mL, the isolates *B. bassiana* HEP 32 and HEP MSO2 exhibited the greatest larval mortality by the end of the trial ($p < 0.00$) (Figure 5b).

At the highest concentration tested (10^7 conidia/mL), HEP MSO2 demonstrated the most pronounced pathogenic effect, with mortality observed from day 6 onward ($p < 0.00$) (Figure 5c). All *B. bassiana* isolates at this concentration caused larval mortality exceeding 65% after eight days of treatment. Notably, HEP MSO2 achieved 100% mortality, distinguishing itself as the most virulent strain among those evaluated.

A simple regression analysis was employed to determine the time required to reach 50% and 90% mortality (T_{50} and T_{90}) in larvae exposed to the HEP MSO2 strain. The calculated values were 6.73 days for T_{50} and 14 days for T_{90}, respectively.

Table 3. Fisher's LSD Method, 95.0% Confidence Level

Treatment	Average LS	Sigma LS	Homogeneous Groups			
Control	0	1.12933	X			
HEP 1	29.8245	1.45796		X		
HEP 3	33.3961	1.45796		XX		
HEP 32	35.5135	1.45796			XX	
HEP 13	38.8192	1.45796			X	
HEP MSO2	56.4121	1.45796				X

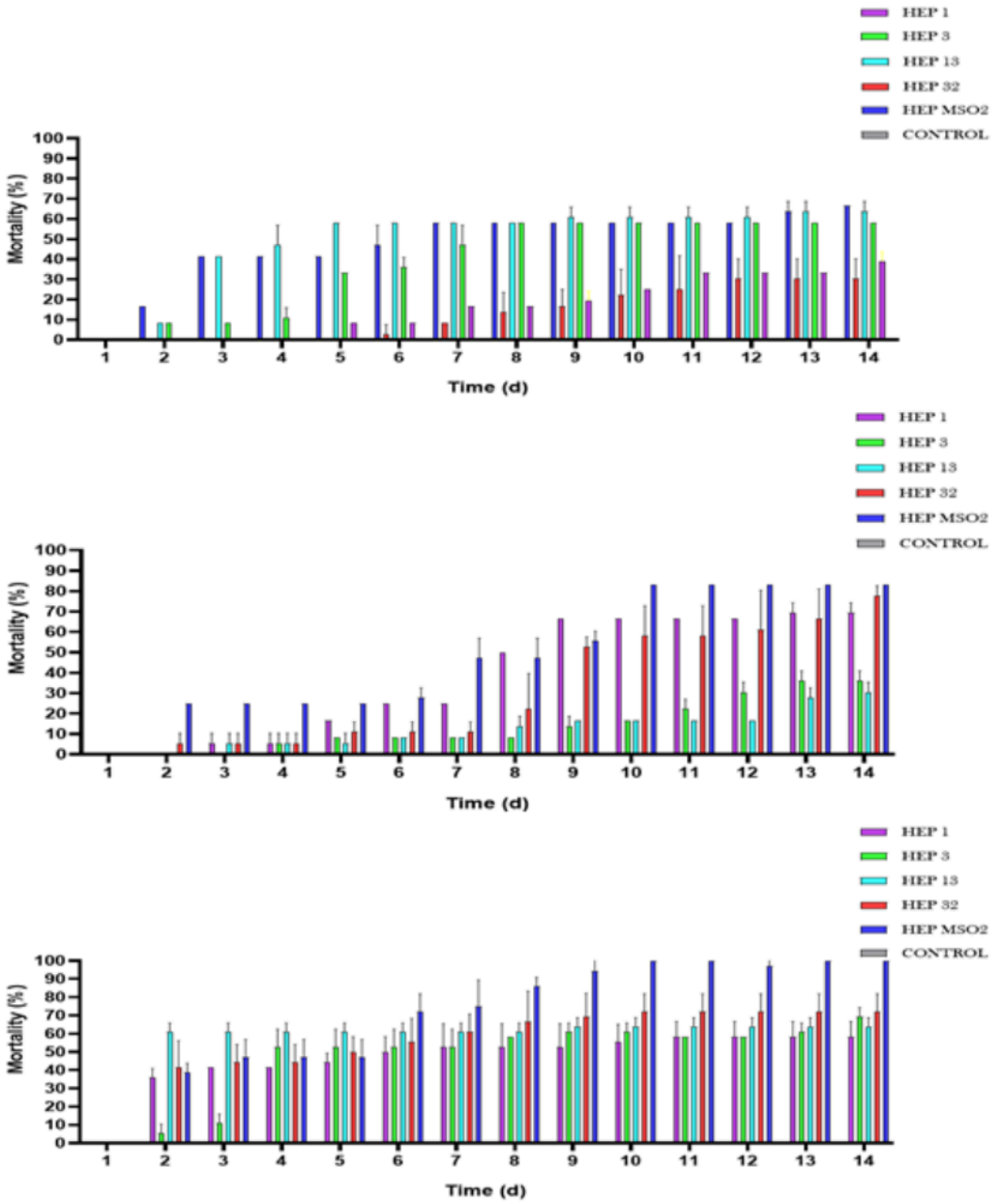

Figure 5. Mortality versus Lethal Time of *B. bassiana* Strains on *T. molitor* Larvae at Different Concentrations. A) 10^5 conidia/mL concentration B) 10^6 conidia/mL concentration C) 10^7 conidia/mL concentration

DISCUSSION

The growing global demand for food has intensified crop production (Lecuona & Posadas, 2019), positioning Argentina among the leading agricultural producers, with Misiones as the foremost global producer of Ilex paraguariensis (22). However, conventional pest control methods in yerba mate plantations, which rely heavily on agrochemicals, have resulted in detrimental effects such as soil degradation and increased pest susceptibility (23), thereby prompting the pursuit of sustainable alternatives.

Entomopathogenic fungi (EPF), particularly those isolated from native environments, offer promising potential as biological control agents due to their ability to infect insects via cuticular penetration (24; 25). In the present study, two *B. bassiana* strains (HEP MS02 and HEP 32) were isolated from organic yerba mate plantations in Misiones. Their origin in organically managed systems aligns with previous findings that report greater EPF diversity in soils devoid of chemical inputs (26; 27).

Although *Metarhizium* spp. are generally more frequently recovered from soil (28), *Beauveria* was more prevalent in the samples analyzed. Its presence was restricted to organic soils, corroborating studies that associate fungal

diversity with practices such as cover cropping and minimal soil disturbance (29; 30). Routine cleaning in yerba mate plantations has also been identified as a factor contributing to the loss of soil biodiversity (31). Environmental conditions further influence EPF survival. *B. bassiana* thrives optimally between 23–26 °C and can tolerate temperatures up to 45 °C (32), although conidial persistence is affected by temperature, humidity, and soil composition (33). Its survival is enhanced in temperate regions with continuous host availability (34).

The identification of both strains was supported by morphological traits consistent with previous descriptions (14; 27; 35), including synnemata formation, and was confirmed through ITS and EF1-α sequencing. Phylogenetic analyses placed HEP MS02 and HEP 32 within well-supported clades of *B. bassiana* (35; 36).

Pathogenicity assays conducted on *T. molitor* larvae demonstrated that all strains induced mortality, with HEP MS02 exhibiting the highest virulence (T_{50} = 6.7 days; T_{90} = 14 days). Conidial viability across all strains exceeded 90%, a critical parameter for field efficacy (37). Furthermore, the disinfection protocol (38; 39) ensured that observed mortality resulted from pre-mortem mycosis, thereby validating fungal pathogenicity rather than saprophytic colonization. This step is essential for confirming the onset of infection, as EPF must penetrate the insect cuticle to initiate disease (40).

This study underscores the importance of isolating native EPF strains adapted to local environmental conditions. In particular, *B. bassiana* HEP MS02 emerges as a promising candidate for incorporation into integrated pest management (IPM) strategies in yerba mate cultivation, offering a sustainable alternative to chemical pesticides and contributing to agroecological resilience.

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REFERENCES

1. Quandahor, P., L. Kim, M. Kim, K. Lee, F. Kusi, and I. H. Jeong. 2024. Effects of Agricultural Pesticides on Decline in Insect Species and Individual Numbers. Environments 11(8): 182.

2. Joop, G. and A. Vilcinskas. 2016. Coevolution of parasitic fungi and insect hosts. Zoology 119(4): 350–358.

3. Ohashi, D. V., O. De Coll, M. Mayol, N. Munaretto, R. Escalada, H. Fontana et al. 2018. Propuesta de Manejo Integrado de Plagas para el cultivo de yerba mate. Manejo integrado del cultivo. E.E.A Cerro Azul — INTA. Miscelánea N° 75/2018.

4. Gebremariama, A., Y. Chekol, and A. Fassil. 2021. Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, *Beauveria bassiana* (Balsam) Vuillemin, and *Metarhizium anisopliae* (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide. Heliyon 7. e070912.

5. Sanjuan, T., J. Tabima, S. Restrepo, T. Laessoe, J. W. Spatafora, and A. E. Franco-Molano. 2014. Entomopathogens of Amazonian stick insects and locusts are members of the *Beauveria* species complex (*Cordyceps* sensu stricto). Mycologia 106(2): 260–275.

6. Swathy, K., M. K. Parmar, and P. Vivekanandhan. 2024. Biocontrol efficacy of entomopathogenic fungi *Beauveria bassiana* conidia against agricultural insect pests. Environmental Quality Management 34(1), e22174.

7. Karabörklü, S. 2022. Biocontrol potential of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from Turkey against *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) larvae under laboratory and field conditions. Bioscience Journal 38, e38015.

8. Samal, I., T. K. Bhoi, V. Vyas, P. K. Majhi, D. K. Mahanta, J. Komal et al. 2024. Resistance to fungicides in entomopathogenic fungi: Underlying mechanisms, consequences, and opportunities for progress. Tropical Plant Pathology 49(1), 5-17.

9. Muneli, I. F. 2023. Utilización del hongo entomopatógeno *Beauveria bassiana* en tratamientos de semillas de maíz. Tesis Ingeniería Agronómica. Escuela de Ciencias Agrarias, Naturales y Ambientales. Universidad Nacional del noroeste de la Provincia de Buenos Aires, Pergamino. Pp 37.

10. González-Herrera, and M. Montero-Astúaac. 2021. Identification and phylogenetic analysis of a collection of *Beauveria* spp. Isolates from Central America and Puerto Rico. Journal of Invertebrate Pathology 184: 107642.

11. Chaudhary, R., A. Nawaz, Z. Khattak, M. A. Butt, M. Fouillaud, L. Dufossé, L. et al. 2024. Microbial bio-control agents: a comprehensive analysis on sustainable pest management in agriculture. Journal of agriculture and food research, 101421.

12. Doberski, J.W., and H. T. Tribe. 1980. Isolation of entomogenous fungi elm bark and soil with reference to the ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. Transactions of the British Mycological Society 74: 95-100.

13. González, C. E., M. L. A. Encina, G. A. Bich, and M. L. Castrillo. 2021. Aislamiento, identificación y conservación de cepas entomopatogénicas del género *Beauveria* de yerbales orgánicos del Paraguay. Revista

Impacto, 1(1), 1-11.
14. Samson, R.A., H. C. Evans, and J. P. Latgé. 1988. Atlas of entomopathogenic fungi. Springer Verlag Berlin: Heidelberg.
15. Alves, S. B. 1998. Fungos entomopatogênicos. Controle microbiano de insetos. Biblioteca de Ciências Agrárias Luiz de Queiroz 4(11): 289-370.
16. Humber, R. 2012. Identification of entomopathogenic fungi. In: Lacey L, Editor. Manual of Techniques in Invertebrate Pathology. 2nd Edition. Academic Press.
17. Fonseca, M. I., Zapata, P. D., Villalba, L. L., & Castrillo, M. L. (2011). Genetic diversity of Trichoderma isolates from Misiones, Argentina. Revista Argentina de Microbiología, 43(3), 176–185.
18. White TJ, Bruns T, LeeS, Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky .IJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. P 315-322.
19. Samuels, G.J. and A. Ismaiel. 2011. Hypocrea peltata: a mycological Dr Jekyll and Mr Hyde. Mycologia, 103 (2011), pp. 616-630.
20. Ezzati-Tabrizi, R., R. Talaei-Hassanloui, and H. R. Pourian. 2009. Effect of formulating of *Beauveria bassiana* conidia on their viability and pathogenicity to the onion thrips, Thrips tabaci Lind. (Thysanoptera: Thripidae). Journal of Plant Protection Research 49: 93-103.
21. Sun, J., J. R. Fuxa and G. Henderson. 2003. Effects of virulence, sporulation, and temperature on *Metarhizium anisopliae* and *Beauveria bassiana* laboratory transmission in Coptotermes formosanus. Journal of Invertebrate Pathology 84: 38–46.
22. Dohrenwend, A. S. 2019. Socio-Environmental Impacts of Argentine Yerba Mate Cultivation: “El Problema es el Precio Bajo”. URI http://hdl.handle.net/1808/3008423 Barcenilla
23. Bich, G.A., M. L. Castrillo, F. L. Kramer, L. L. Villalba, and P. D. Zapata. 2021. Morphological and Molecular Identification of Entomopathogenic Fungi from Agricultural and Forestry Crops. Floresta Ambiente 28(2).
24. Feng, M., T. J. Poprawski, and G. G. Khachatourians. 1994. Production, formulation, and application of the entomopathogenic fungus. *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology 4, 3–34.
25. de Faria, M. R. and S. P. Wraight. 2007. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43, 237–256.
26. Klingen, I., & S. Haukeland. 2006. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. In An ecological and societal approach to biological control (pp. 145-211). Dordrecht: Springer Netherlands.
27. Schapovaloff, M. E., L. F. Alves, U. M. Angeli and C. C. López-Lastra. 2015. Ocurrencia natural de hongos entomopatógenos en suelos cultivados con yerba mate *(Ilex paraguariensis* St. Hil.) en Misiones, Argentina. Revista Argentina de Microbiología 47(2): 138-142.
28. Bidochka, M. J., J. E. Kasperski, & G. A. Wild. 1998. Occurrence of the entomopathogenic fungi Metarhizium anisopliae and Beauveria bassiana in soils from temperate and near-northern habitats. Canadian Journal of Botany, 76(7), 1198-1204.
29. Gliessman, S. R., V. E. Méndez and V. M. Izzo. 2022. Agroecology: Leading the Transformation to a Just and Sustainable Food System (4th Ed.). New York, USA: CRC Press, Taylor & Francis.
30. Oliveira, I., J. A. Pereira, E. Quesada-Moraga, T. Lino-Neto, A. Bento & P. Baptista. 2013. Effect of soil tillage on natural occurrence of fungal entomopathogens associated to Prays oleae Bern. Scientia horticulturae, 159, 190-196.
31. Wagner, K. A., V. Pauletti, L. B. Gheno & K. M. Cavalieri-Polizeli. 2025. Growth of Yerba Mate Plants (Ilex paraguariensis A. St.-Hil.) Under Soil Compaction. Journal of Soil Science and Plant Nutrition, 1-17.
32. Quesada-Moraga, E., González-Mas, N., Yousef-Yousef, M., Garrido-Jurado, I., & Fernández-Bravo, M. (2024). Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. Journal of Pest Science, 97(1), 1-15.
33. Lingg, A. J., & Donaldson, M. D. (1981). Biotic and abiotic factors affecting stability of Beauveria bassiana conidia in soil. Journal of Invertebrate Pathology, 38(2), 191-200.34. Vänninen
34. Ghorui, M., Chowdhury, S., Burla, S. (2024). The science behind entomopathogenic fungi: Mechanisms and applications. In Entomopathogenic Fungi: Prospects and Challenges Singapore: Springer Nature Singapore. P 3-35.
35. Barbercheck, M. E. (1992). Effect of soil physical factors on biological control agents of soil insect pests. Florida Entomologist, 539-548.
36. Solano-González, S., Castro-Vásquez, R., & Molina-Bravo, R. (2023). Genomic characterization and functional description of *Beauveria bassiana* isolates from Latin America. Journal of Fungi, 9(7), 711.
37. Liu, H., M. Skinner, M. Brownbridge and B. L. Parker. 2003. Characterization of *Beauveria bassiana* and *Metarhizium anisopliae* isolates for management of tarnished plant bug, Lygus lineolaris (Hemiptera: Miridae). Journal of Invertebrate Pathology 82(3), 139-147.

38. Porras, E. M. 2015. Evaluación de la actividad entomopatógena de diversos aislamientos de hongos y cepas de *Bacillus thuringiensis* para el potencial desarrollo de un bioformulado contra las hormigas cortadoras de hojas de la especie *Atta cephalotes*. Tesis de Ingeniería en Biotecnología. Universidad de Costa Rica. Pp 168.
39. Motholo, L. F. (2019). Endophytic establishment of Beauveria bassiana in wheat (Triticum aestivum) and its impact on Diuraphis noxia (Doctoral dissertation, North-West University (South Africa). Potchefstroom Campus).
40. Pereira, A. S. 2014. Fungos associados e ação do imunossupressor ciclosporina A sobre formigas cortadeiras. Maestría en Producción Vegetal. Universidade Federal do Tocantins, Tocantins, Brasil. Pp 81.