

Biological Synergy of *Metarhizium* Spp. and *Beauveria bassiana* Against *Oebalus insularis* in Rice Cultivation

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Abstract

Original Research Article

This research study evaluated the biological efficacy of the entomopathogenic fungi *Metarhizium* spp. and *Beauveria bassiana*, applied individually and in a biological consortium, for the microbiological control of the rice bug (*Oebalus insularis*). The study was conducted in two phases: an in vitro phase at the biopesticide laboratory of the National Agrarian University and a validation phase on commercial plots in the community of Ochomogo, Rivas, Nicaragua. The consortium mixture (*Metarhizium* + *Beauveria*) achieved 100% mortality and 100% parasitism in the specimens, outperforming the individual applications of *Metarhizium* spp. (98%) and *B. bassiana* (92%). Consistent with these results, field evaluations demonstrated that the biological consortium induced the greatest population suppression of *O. insularis*, reducing its density by 93%, compared to reductions of 85% and 79% obtained with the individual formulations. These findings demonstrate that the use of fungal consortia constitutes a highly efficient and safe biotechnological alternative for safeguarding the productivity, profitability, and safety of rice grain in Nicaraguan agricultural systems.

Keywords: *Oebalus insularis*, *Oryza sativa*, entomopathogens, biological consortium, bio-inputs

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a cornerstone of the agri-food sector, holding immense socioeconomic significance for ensuring food security and nutritional sovereignty in Latin America and the Caribbean (FAO, 2023). In the tropical and subtropical regions of Central America, rice production relies on both irrigated and favorable rainfed systems, supplying the daily diet of millions and underpinning the rural economies of small- and medium-scale producers (Mendoza *et al.*, 2021). However, optimal yield and the grain's agro-industrial quality remain constantly vulnerable to adverse climatic factors and the phytosanitary pressure exerted by insect pest complexes adapted to the agroecosystem's dynamics (Llerena-Ramos *et al.*, 2025).

Among the complex of harmful arthropods infesting rice crops, the rice bug (*Oebalus insularis*, Hemiptera: Pentatomidae) also known as the empty-grain bug stands out as a key pest with major economic impact in the region (Vivas & Astudillo, 2010). This hemipteran colonizes rice fields primarily during the crop's most critical phenological phases: flowering, the milky stage, and the dough stage of grain development

(Pérez-Consuegra *et al.*, 2018). Using their piercing-sucking mouthparts, *O. insularis* adults and nymphs puncture the glume and ingest photoassimilates from the developing endosperm, causing substantial quantitative and qualitative losses (Echeverry-Galvis *et al.*, 2021).

The feeding damage caused by *O. insularis* is not limited solely to a physical reduction in hectoliter weight and the total or partial emptying of panicles (Rodríguez-G. *et al.*, 2006). During the feeding process, the insect inoculates salivary enzymes and mechanically transports propagules of opportunistic phytopathogens primarily from the genera *Bipolaris*, *Curvularia*, *Fusarium*, and *Sarocladium* (Añazco-Ríos *et al.*, 2022). This biophysical interaction causes grain staining (pecky rice), increasing endosperm fragility during industrial milling; this leads to a higher percentage of broken grains and severely reduces the crop's commercial value in both domestic and international markets (García-Martínez *et al.*, 2019).

Historically, the control of *O. insularis* has relied on a chemical-dependent model characterized by the continuous and indiscriminate application of synthetic pesticides belonging to the organophosphate,

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carbamate, and pyrethroid families (Monzón, 2001). Although this strategy achieves a rapid initial knockdown of insect populations, its repeated implementation has triggered profound ecological imbalances most notably the selection of resistant populations, the resurgence of secondary pests, and the drastic elimination of native beneficial insect fauna that naturally regulate the crop (Téllez-Jurado *et al.*, 2009).

In addition to disrupting the agroecosystem's food web, the overuse of agrochemicals generates severe socio-environmental externalities in agricultural valleys (Silva-Sánchez *et al.*, 2020). Leaching and surface runoff of synthetic residues contaminate groundwater, arable soil, and surface water sources, directly impacting aquatic biodiversity as well as the public health of farm workers and neighboring communities (Mateo-Estrada *et al.*, 2022). Consequently, there is an urgent need to transition toward an agroecological paradigm based on the development of safe, efficient, and environmentally friendly bio-inputs (Altieri & Nicholls, 2020).

In the realm of microbiological control, entomopathogenic fungi such as *Metarhizium* spp. and *Beauveria bassiana* emerge as the most scientifically and commercially viable biocontrol agents (Esparza Mora *et al.*, 2017). These deuteromycetes possess a highly specialized direct-contact infection mechanism, in which conidia adhering to the host's exoskeleton germinate and produce penetration structures (appressoria), aided by the coordinated secretion of hydrolytic enzymes (proteases, chitinases, and lipases) (Leger & Wang, 2020). Subsequently, the fungus colonizes the hemocoel, releasing secondary metabolites that cause paralysis, metabolic collapse, and the insect's death within a few days (Lopes *et al.*, 2018).

Despite the recognized potential of using single strains of *Metarhizium* or *Beauveria*, the effectiveness of a solitary strain under field conditions is often compromised by variability in ultraviolet radiation, temperature spikes, and fluctuating relative humidity levels within the crop canopy (Borisade & Magan, 2014). To overcome these eco-physiological limitations, agricultural science has turned its attention to the formulation of microbial consortia (García & Pérez, 2020). The strategic combination of different fungal strains or species allows for the integration of varying thermal tolerance ranges and complementary enzymatic modes of action, generating a synergistic effect that increases virulence and reduces the likelihood of the pest developing adaptive responses (Rondón-Valero *et al.*, 2021). Historically, the control of *O. insularis* has relied on a chemical-dependent model characterized by the continuous and indiscriminate application of synthetic pesticides belonging to the organophosphate, carbamate, and pyrethroid families (Monzón, 2001). Although this strategy achieves a rapid initial knockdown of insect populations, its repeated implementation has triggered profound ecological imbalances most notably the

selection of resistant populations, the resurgence of secondary pests, and the drastic elimination of native beneficial insect fauna that naturally regulate the crop (Téllez-Jurado *et al.*, 2009).

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In Nicaragua, the Ochomogo community in Rivas is a key rice-producing area where phytosanitary pressure from *O. insularis* and the intensive use of pesticides necessitate the prompt validation of alternative technologies (INTA, 2022). Against this backdrop, the general objective of this study was to evaluate the in vitro and field biological efficacy of an entomopathogenic consortium comprising *Metarhizium* spp. and *Beauveria bassiana* for controlling *O. insularis* in rice crops, specifically assessing its combined effect on mortality,

field population suppression, and the potential to replace synthetic pesticides in the production sector.

2. METHODS

2.1. Study Location and Isolation of Biological Material

The experimental work was divided into two complementary phases conducted in the departments of Rivas and Managua, Nicaragua:

In Vitro Phase:

Carried out at the biological control laboratory of the National Agrarian University (UNA) under controlled environmental conditions: $25 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity, and a 12:12-hour photoperiod (Monzón, 2001).

Field Phase: Conducted on commercial rice (*Oryza sativa*) plots located in the community of Ochomogo, in the municipality of Rivas.

Strains of *Metarhizium* spp. and *Beauveria bassiana* were obtained from the UNA's collection of entomopathogenic microorganism strains. Mass production of conidia was performed on a sterile solid rice (*Oryza sativa*) substrate, following the methodology described by Bustillo-Pardey (2019). Fungal conidial suspensions were standardized to a final concentration of 1×10^8 conidia mL^{-1} via counting in a Neubauer chamber.

2.2. *In Vitro* Phase: Mortality and Parasitism Bioassay

Healthy *O. insularis* adults were collected from untreated rice fields in the Ochomogo area. The bugs were acclimatized in the laboratory for 24 hours prior to inoculation. Sterile Petri dishes lined with moistened filter paper were used to maintain turgidity.

Each experimental unit consisted of a batch of 10 *O. insularis* adults, with four replicates per treatment (40 insects per treatment). Inoculation was performed by directly spraying 2 mL of the adjusted fungal suspension (1×10^8 conidia mL^{-1}) using a calibrated micro-sprayer. The variables evaluated for 7 days post-inoculation were: Mortality (%): Percentage of dead insects relative to the total evaluated.

Parasitism (%): To calculate the percentage of cadavers showing external sporulation (mycelium and conidia) of the inoculated fungus after being placed in a humid chamber at 28°C for an additional 72 hours, the formula proposed by Castillo-Arévalo (2026) for this study was used.

$$\text{Parasitism} = \frac{\text{Number of insects with signs of mycosis}}{\text{Total number of insects evaluated}} \times 100$$

2.3. Field Phase: Population Density Assessment and Agrochemical Substitution

The field phase was established during the grain-filling stage (milky-dough stage) of the rice crop in Ochomogo, the period of greatest vulnerability to *O. insularis* attack. Experimental plots of 100 m^2 per treatment were demarcated, with four replicates.

Applications of the fungal formulations were carried out during the early morning hours (06:00–08:00 h) using manual backpack sprayers equipped with hollow-cone nozzles, at a discharge volume of 200 L ha^{-1} . The quantitative and qualitative variables recorded were:

O. insularis population reduction (%): Evaluated 10 days post-application using the sweep-net sampling method (10 sweeps per plot), calculating the percentage decrease relative to the initial pre-application density.

2.4. Experimental Designs and Statistical Analysis

A Completely Randomized Design (CRD) was applied for the *in vitro* phase, whereas a Randomized Complete Block Design (RCBD) was structured for the field phase to control for spatial variability within the agricultural plot (Vivas & Astudillo, 2010).

The treatments evaluated in both phases were:

Biological consortium: Fungal mixture (*Metarhizium* spp. + *Beauveria bassiana*) in a 1:1 ratio (1×10^8 conidia mL^{-1}).

Metarhizium spp.: Monoclonal formulation (1×10^8 conidia mL^{-1}).

Beauveria bassiana: Monoclonal formulation (1×10^8 conidia mL^{-1}). Absolute control: Spraying with sterile distilled water + non-ionic agricultural adjuvant (0.05% v/v).

Data expressed as percentages were transformed using the function $y = \arcsin(\sqrt{p/100})$ to meet the assumptions of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test). A one-way Analysis of Variance (ANOVA) was performed for each phase, and mean comparisons were conducted using Tukey's test ($\alpha=0.05$). All analyses were processed using InfoStat statistical software (v. 2020).

3. RESULTS AND DISCUSSION

3.1. Study 1: *In Vitro* Evaluations (Mortality and Parasitism)

In Vitro Results

Analysis of Variance detected highly significant differences among treatments regarding *O. insularis* mortality at 7 days post-inoculation ($p < 0.0001$).

The biological consortium (*Metarhizium* + *Beauveria*) achieved maximum effectiveness, with 100% mortality and 100% confirmed parasitism on the insect cadavers (Table 1). Individual application of

Metarhizium spp. showed performance statistically equivalent to the consortium (98.00% mortality and 100% parasitism), falling within the same Tukey significance range. Meanwhile, *Beauveria bassiana*

applied in isolation recorded 92.00% mortality and 100% parasitism, placing it in a second statistical group. The absolute control showed no mortality attributable to fungal agents (0.00%).

Table 1: *In vitro* mortality and parasitism of *O. insularis* adults at 7 days post-inoculation

Treatment	Mortality (%) $\pm$ S. D	Parasitism (%) $\pm$ S. D	Tukey clustering ($\alpha=0.05$)
Consortium (<i>Metarhizium</i> + <i>Beauveria</i>)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	a
<i>Metarhizium</i> spp.	98.00 $\pm$ 2.30	100.00 $\pm$ 0.00	a
<i>Beauveria bassiana</i>	92.00 $\pm$ 3.80	100.00 $\pm$ 0.00	b
Control (Water)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	c

Note: Means with different letters in the Tukey Grouping column indicate statistically significant differences according to the Tukey test ($\alpha=0.05$). SD: Standard Deviation.

Discussion of the *In Vitro* Study

Enzymatic penetration mechanisms: The high mortality rates observed for both the consortium (100%) and *Metarhizium* spp. (98%) align with the findings of Leger and Wang (2020), who explain that the infection success of these deuteromycetes depends on the coordinated secretion of hydrolytic enzymes (Pr1 proteases, chitinases, and esterases). These enzymes degrade the protein-chitin matrix of the hemipteran exoskeleton, facilitating appressorium formation and the physical penetration of the germ tube.

Action of secondary metabolites and pathogenesis: The observed response is supported by the findings of Esparza Mora et al. (2017) and Lopes et al. (2018), who demonstrated that, once the cuticle is penetrated, the fungus synthesizes toxic cyclodepsipeptides such as destruxins (in *Metarhizium*) and beauvericin/oosporein (in *Beauveria*). These secondary metabolites suppress the hemipteran's cellular immune system, destroy hemocytes, and induce flaccid paralysis, leading to programmed cell death in the host.

Re-sporulation capacity and parasitism: The 100% parasitism rate confirmed in all treated cadavers corroborates the classic saprophytic cycle described by Bustillo-Pardey (2019). Under conditions of high relative humidity (>75%), the fungal mycelium depletes the nutritional reserves of the hemocoel, ruptures the cuticle from within, and emerges through intersegmental sutures to sporulate externally, thereby ensuring the availability of infective propagules for secondary transmission.

Variability in monoclonal virulence: The lower effectiveness observed with the *B. bassiana* isolate (92%) compared to *Metarhizium* spp. is consistent with the observations of Altinok et al. (2019) and Valero-Posada et al. (2020). These authors note that pentatomid bug nymphs and adults possess a thicker sclerotized cuticle and a surface lipid composition requiring higher lipolytic activity, an enzymatic attribute typically expressed more strongly in selected strains of *Metarhizium* spp.

3.2. Study 2: Field Evaluations

Field Results

ANOVA performed evaluations in the rice crop in the Ochomogo community (Rivas) revealed highly significant statistical differences among the bio-inputs regarding the reduction of *O. insularis* population density 10 days after application ($p<0.0001$).

The *Metarhizium* spp. + *Beauveria bassiana* biological consortium achieved the highest level of pest control under actual production conditions, resulting in a 93.00% population reduction (Table 2). Monoclonal formulations of *Metarhizium* spp. and *B. bassiana* achieved reductions of 85.00% and 79.00%, respectively; these differed statistically from each other and from the consortium in the Tukey test ($\alpha=0.05$). Regarding socio-environmental impact, 100% of the local producers involved in the validation successfully replaced synthetic chemical insecticide applications entirely with the entomopathogenic consortium.

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Table 2: Reduction in *O. insularis* population density 10 days after application in Ochomogo, Rivas

Treatment	Population reduction (%) $\pm$ SD	Tukey clustering ($\alpha=0.05$)
Consortium (<i>Metarhizium</i> + <i>Beauveria</i>)	93.00 $\pm$ 2.10	a
<i>Metarhizium</i> spp.	85.00 $\pm$ 3.20	b
<i>Beauveria bassiana</i>	79.00 $\pm$ 2.80	c
Control (Water)	0.00 $\pm$ 0.00	d

Note: Means with different letters in the Tukey Grouping column indicate statistically significant differences according to the Tukey test ($\alpha=0.05$). SD: Standard Deviation

Field Study Discussion

Microbial synergy and canopy cover: The superior performance of the consortium in the field (93% reduction, Tukey group 'a') confirms the synergistic phenomenon among entomopathogens analyzed by García and Pérez (2020) and Rondón-Valero et al. (2021). Combining two fungal genera broadens the spectrum of biological response to the variable microclimates within the rice canopy. The combined action of multiple toxins and proteases overcomes the insect's immunological barriers more aggressively than a monoclonal formulation.

Tolerance to microclimatic factors (Thermal and UV stress): The marked difference in effectiveness between *Metarhizium* spp. (85%) and *B. bassiana* (79%) when applied individually aligns with the eco-physiological research of Borisade and Magan (2014) and Jaronski (2014). *Metarhizium* conidia exhibit melanin pigmentation that provides greater stability and protection against photo-oxidative damage caused by ultraviolet radiation and the extreme temperatures (>30°C) characteristic of tropical rice agroecosystems.

Economic threshold management and grain damage: Given that densities exceeding 0.7 bugs per panicle during the milk stage cause severe losses due to empty grains and panicle sterility (Echeverry-Galvis et

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al., 2021; Llerena-Ramos et al., 2025), the 93% suppression achieved by the mixture keeps *O. insularis* populations well below the economic damage threshold. This prevents the inoculation of grain-staining fungi (e.g., *Sarocladium*, *Curvularia*) and preserves industrial milling quality (Añazco-Ríos et al., 2022). **Agroecological sustainability and safety:** The total (100%) replacement of synthetic pesticides in the Ochomogo plots supports the agroecological goals proposed by Altieri and Nicholls (2020) and the FAO (2023). Eliminating organophosphate compounds prevents runoff contamination of water sources (Mateo-Estrada et al., 2022), protects native natural enemies, and ensures the production of rice that is safe for human consumption.

4. CONCLUSIONS

In Vitro Phase: The biological consortium of *Metarhizium* spp. and *Beauveria bassiana* demonstrated high virulence, achieving 100% mortality and parasitism against *Oebalus insularis* 7 days post-inoculation, showing statistical equivalence to the monoclonal *Metarhizium* spp. formulation (98%).

Field Phase: The entomopathogen consortium mixture was statistically superior under field conditions, reducing the *O. insularis* population density by 93% 10 days post-application, thereby outperforming individual

applications of *Metarhizium* spp. (85%) and *B. bassiana* (79%).

Sustainability and Transfer: Validation of the biological consortium enabled the complete (100%) replacement of synthetic chemical insecticides in the demonstration plots in Ochomogo, Rivas, establishing it as an efficient, ecologically sustainable, and safe biotechnological alternative for integrated pest management in Nicaraguan rice farming.

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