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Characterization, molecular and pathogenicity of *Metarhizium huainamdangense* isolated from Dumoga against the corn leafhopper *Peregrinus maidis*

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Abstract. *Siahaan P, Langanawa M, Mangais RER. 2025. Characterization, molecular and pathogenicity of* Metarhizium huainamdangense *isolated from Dumoga against the corn leafhopper* Peregrinus maidis. *Biodiversitas 26: 6111-6119.* The corn leafhopper (*Peregrinus maidis*) is a major pest of maize, causing significant yield losses and threatening sustainable production. Reliance on chemical pesticides has led to resistance and environmental risks, creating a demand for eco-friendly alternatives. Entomopathogenic fungi, particularly *Metarhizium* spp., have shown strong potential as biological control agents. This study characterized and evaluated the pathogenicity of a local *Metarhizium* isolate from West Dumoga, North Sulawesi, Indonesia. Morphological observations confirmed colony and conidial traits typical of *Metarhizium*. Molecular identification through ITS sequencing placed the isolate within the *Metarhizium huainamdangense* clade with >98% similarity. Conidial density reached 1.39×10^9 conidia/mL with 100% germination after 24 hours, indicating strong physiological fitness. Pathogenicity assays against *P. maidis* demonstrated concentration- and time-dependent mortality, with up to 100% mortality at 10^9 conidia/mL by day 14. However, the high LC_{50} (1×10^9. conidia/mL) and LT_{50} (>8.5 days) values indicate relatively low virulence compared with other *Metarhizium* species. This study confirms the Dumoga local isolate as *Metarhizium huainamdangense* and has potential as a promising biocontrol agent with formulation optimization to improve field effectiveness in integrated pest management.

Keywords: Biocontrol, entomopathogenic fungi, maize pest management, *Metarhizium huainamdangense*, *Peregrinus maidis*

INTRODUCTION

Global agriculture continues to face serious challenges concerning pest control, which is key to sustainability and agricultural productivity (Kalogiannidis et al. 2022). Corn (*Zea mays*), as one of the agricultural commodities, is also not exempt from pest attacks, with one of the major threats to corn plants being the attack by *Peregrinus maidis* (Ashmead, 1890) (Hemiptera: Delphacidae), a type of leafhopper that can cause serious damage to the crop (Hill et al. 2024). In many tropical and subtropical corn-producing regions across the world, *P. maidis*, the corn leafhopper, is one of the most prevalent leafhoppers that attack corn plants (Higashi and Bressan 2013). In Indonesia, *P. maidis* has caused damage to corn plants, resulting in decreased production in several regions such as Lampung Province, West Java, Central Sulawesi, Gorontalo, and North Sulawesi (Hasibuan et al. 2021). Farmers' control of *P. maidis* still predominantly relies on intensive synthetic pesticide use. Negative effects include pest resistance, pesticide residues, and the demise of natural enemies, which might result from the excessive and ongoing usage of synthetic pesticides. Continuous exposure to these chemicals not only leads to the development of resistant pest populations but also causes the accumulation of harmful residues in soil, water, and agricultural products. Furthermore, the indiscriminate application of synthetic pesticides disrupts ecological balance by killing beneficial organisms such as pollinators, predators, and decomposers, ultimately reducing biodiversity and threatening long-term agricultural sustainability (Rahmawasiah et al. 2022).

Sustainable pest control has become a primary focus to preserve environmental integrity and reduce dependence on chemical pesticides (Souto et al. 2021). One of the environmentally friendly pest control agents that has been widely utilized is entomopathogenic fungi. Entomopathogenic fungi are heterotrophic organisms that parasitize insect bodies (Bava et al. 2022). In this endeavor, the entomopathogenic fungus *Metarhizium* shows promise as a biological control agent that is both efficient and eco-friendly (Bamisile et al. 2021). Fungi from the genus *Metarhizium* have proven effective in controlling various leaf-sucking insects, such as the corn leafhopper *P. maidis* (Hasibuan et al. 2021), the species *M. brasiliense,* which infects the corn leafhopper *Dalbulus maidis* (Souza et al. 2021), and the species *M. anisopliae,* which controls the green leafhopper *Nephotettix virescens* (Ibrahim et al. 2021). However, to optimize their use in control strategies, a deep understanding of the biological characteristics, genetic identification, and virulence levels of local *Metarhizium* isolates is required. This understanding is crucial because each *Metarhizium* isolate can exhibit significant differences in morphology, sporulation ability, tolerance to environmental conditions, and infection mechanisms in insect hosts. In addition, genetic analysis plays an important role in ensuring accurate species and strain identification, thereby enabling the selection of isolates with the highest biocontrol potential. Virulence characterization is also necessary to assess the effectiveness of isolates in suppressing target pest populations, while minimizing impacts on non-target organisms and ecosystem balance. Comprehensive information on these biological, molecular, and ecological aspects will form the basis for developing more effective, sustainable, and environmentally friendly *Metarhizium*-based biopesticide formulations.

The Dumoga region in North Sulawesi, Indonesia, is an important center for rice and corn cultivation. In this area, farmers often face attacks from major pests such as brown planthoppers on rice plants and stem borers (e.g., *Ostrinia furnacalis*) on corn plants. The presence of these two types of pests is not only a serious threat to agricultural yields but also an important ecological indicator. That is because stem borers serve as natural hosts for the entomopathogenic fungus *Metarhizium* spp., which is known as the natural enemy of these insects (Peng et al. 2020). Therefore, the presence of stem borers indirectly indicates the potential for natural populations of *Metarhizium* in the surrounding environment. This condition makes the Dumoga region highly potential for exploration and development of *Metarhizium*-based biological agents. The use of this fungus as a biological control agent is expected to serve as an environmentally friendly alternative in pest management, while also supporting sustainable agricultural systems in the region. This study is important because it provides the first report of *M. huainamdangense* infection in *P. maidis* in Indonesia and aims to characterize, identify, and evaluate the virulence of local isolates to support their potential use as biological control agents.

MATERIALS AND METHODS

Study area

This research was conducted from April to May 2025. The fungus of *Metarhizium* sp. was collected from Wangga Baru Village, West Dumoga, Bolaang Mongondow District, North Sulawesi, Indonesia (Figure 1). Isolation stages, morphology analysis, and genetic analysis were conducted at the Laboratory of Advanced Research in the Department of Biology, Universitas Sam Ratulangi, Indonesia. Pathogenicity tests against *Peregrinus maidis* were conducted at the Biological Agents Laboratory, Kalasey, North Sulawesi, Indonesia.

Procedure

Fungus isolation

Metarhizium spp. fungal isolates were obtained from Wangga Baru Village, West Dumoga, by collecting naturally infected leafhoppers covered with green mycelium. The infected specimens were surface sterilized with 70% ethanol and sterile distilled water, then cultured on Potato Dextrose Agar (PDA) and incubated at 27°C for 7 days (Wang et al. 2020). Pure cultures were produced through repeated subcultures and mass-produced on autoclaved rice grains to produce conidia for formulation. The isolate (code: MHPS) was stored at the Advanced Biology Laboratory, Department of Biology, Universitas Sam Ratulangi, and stored on slanted PDA at 4°C.

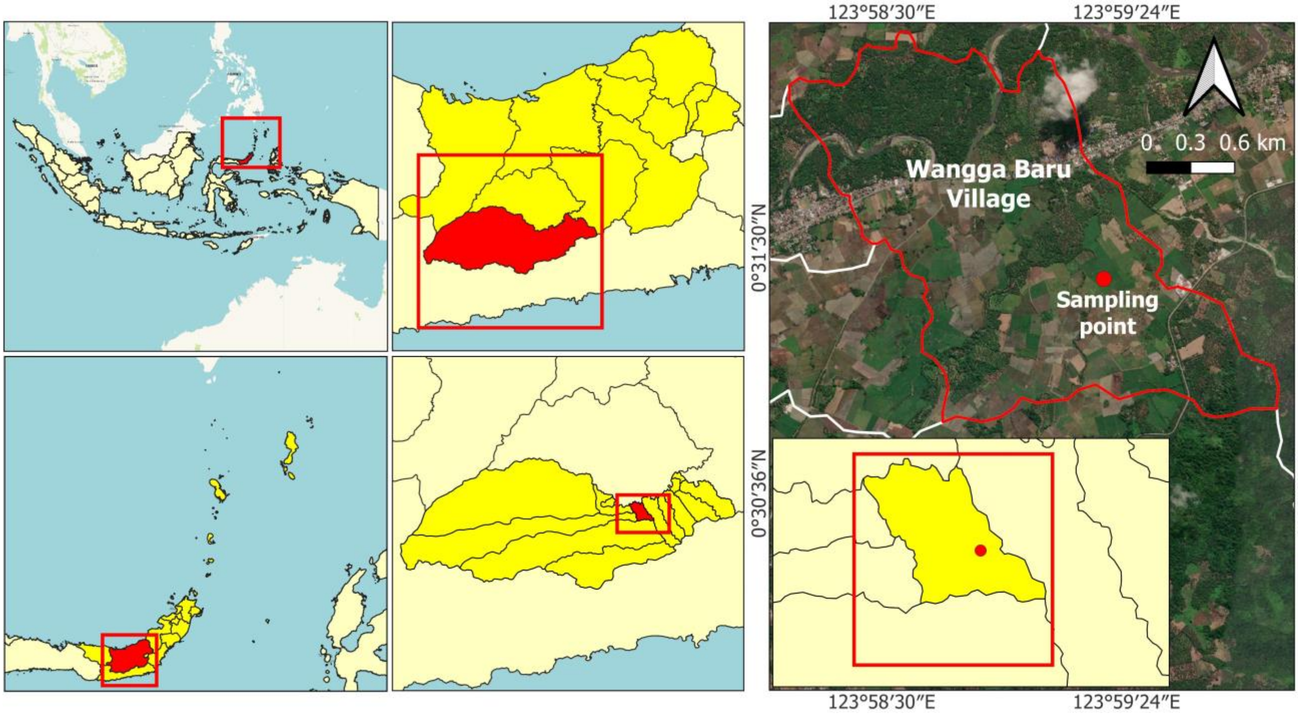

Figure 1. *Metarhizium* sp. fungi exploration location. The red dot marks the sampling location at coordinates 0°31'23.15"N, 123°59'18.99"E

Morphological characterization of fungi

Morphological characterization is based on macroscopic and microscopic characteristics, conidia density, and viability, each performed in triplicate. The morphology of fungi on PDA was observed on day 14, including colony shape, height, texture, and color (top and bottom). Microscopic characteristics, particularly conidia color and shape, were evaluated for preliminary identification according to Mongkolsamrit et al. (2020). Conidia density was determined using a hemocytometer after diluting 100 g of cultivated fungi into 100 mL, while viability was evaluated at 8, 16, and 24 hours of incubation according to Indriyanti et al. (2021).

$$Conidia\ Density = \left(\frac{\bar{X}}{L\ \times t \times d}\right)10^3$$

Where :

$\bar{x}$: Average number of conidia in all five boxes

L : Square area (0.04 mm^3)

t : Depth of calculation (0.1 mm)

d : Dilution factor

10^3 : Calculated suspension volume

$$\text{Conidia viability} = \frac{\text{g}}{\text{g} + \text{u}} \times 100\%$$

Where :

g : Number of germinated conidia

u : Number of ungerminated conidia

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from ~0.5 g of mycelia or conidia using the Plant Genomic DNA Mini Kit (Geneaid, Taiwan) following the manufacturer's instructions. The ITS region was amplified using MyTaq™ HS Red Mix (Bioline, USA) with primers ITS1 (5′-`TCC GTA GGT GAA CCT GCG G`-3′) and ITS4 (5′-`TCC TCC GCT TAT TGA TAT GC`-3′). PCR conditions included an initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 5 min. PCR products were verified by agarose gel electrophoresis and sequenced (First BASE Laboratories, Malaysia) using ITS1 and ITS4 primers to analyze the ITS region comprising ITS1, 5.8S rRNA, and ITS2 (Haque et al. 2020).

Phylogenetic construction

ITS sequences were edited and assembled in MEGA X (Kumar et al. 2018) and compared with GenBank references using the BLASTn algorithm (NCBI, USA) for preliminary identification. Phylogenetic analysis was performed in MEGA X using the Neighbor-Joining method with evolutionary distances computed by the Maximum Composite Likelihood model and clade support assessed with 1,000 bootstrap replicates.

Pathogenicity bioassay against Peregrinus maidis

The pathogenicity of the *M. huainamdangense* isolate (MHPS) against adult *P. maidis* was evaluated using the dipping method. Conidial suspensions (1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/mL) were prepared in sterile distilled water containing 0.05% Tween 80, with the control consisting of Tween 80 solution only. Each treatment had five replicates of 30 insects. Treated insects were transferred to cages with healthy corn plants and maintained under controlled conditions (27±1°C, >80% RH, 12:12 h L:D photoperiod) throughout the observation period to ensure environmental consistency and reliable mortality assessment. Mortality was recorded for 14 days and calculated following Passara et al. (2024):

$$Mortality\ rate = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insects}\ x\ 100\%$$

The Lethal Time (LT_{50}) was calculated based on the accumulated mortality regression value and the time to death of insects using the formula from Fatimah et al. (2020):

$$Y = a + bX$$

Where :

Y : The probit of death

X : The logarithm of dose or time,

a and b : Constants

The lethal concentration (LC_{50}) was calculated based on the regression between the probit mortality value and the logarithm of the treatment concentration using the formula from Saganuwan (2020):

$$P = \alpha + \beta[\log10(\text{Dose})]$$

Where :

P : Probit value of the percentage of deaths

α : The intercept

β : The slope

Dose : The concentration of the test substance.

Concentration-mortality data were analyzed by probit analysis to estimate LC_{50} and its 95% confidence interval, while LT_{50} values were derived from time-mortality data. Corrected mortality at day 14 was subjected to one-way ANOVA, and means were separated using the LSD test at a 5% significance level. All analyses were performed in SPSS v.22. Dead insects were removed and placed on moist cotton in Petri dishes to confirm fungal infection.

RESULTS AND DISCUSSION

Morphological characteristics

The morphological characteristics of the *Metarhizium* sp. isolate from West Dumoga (MHPS) were determined through macroscopic and microscopic observations. Colony growth is observed with a radial pattern formed through the scratch method (Figure 2.A). The olive-green color dominating the colony surface is produced by active sporulation, while the characteristic pigmentation of *Metarhizium* sp. is also clearly visible on the colony surface. The texture of the colony changes from dense and smooth white in the early phase to granular and slightly rough as conidia form. Intense sporulation is indicated by the accumulation of spore masses on the colony surface. The conidia are observed to have an oval to cylindrical shape, smooth walls, and are arranged singly, which are the characteristic microscopic features of *Metarhizium* sp. The *Metarhizium huainamdangense* (MHPS) isolate exhibits morphological characteristics consistent with the description by Mongkolsamrit et al. (2020). Colonies on PDA after 14 days are pale cream in color with hairy edges, irregular, and a surface that is finely hairy to thickly hairy, turning pale yellow to dark green in the center due to conidia production. Conidia have smooth walls, are dark green in color, cylindrical with rounded ends, and characteristic of the genus *Metarhizium*. These characteristics confirm the morphological identity of the MHPS isolate as *M. huainamdangense*.

The density and viability of conidia greatly influence the virulence of the pathogenic fungus. The calculation result of conidia density four weeks after isolation was 1.39×10^9 conidia/mL, which is not significantly different from the findings of Indriyanti et al. (2017), who found the density of *M. anisopliae* conidia isolated from *Oryctes rhinoceros* to be 1.81×10^8. The obtained results are very good, as they have exceeded the quality standards of *Metarhizium* fungus as a biological control agent.

The conidial germination of the *M. huainamdangense* isolate MHPS was exceptionally high, reaching 100% within 24 hours (Table 1). This result demonstrates that the isolate has robust physiological fitness and potential for effective infection under optimal conditions. Conidial viability is a fundamental parameter in evaluating the pathogenic potential of entomopathogenic fungi, as it directly influences the ability of the fungus to initiate infection in host insects. Successful germination, cuticle penetration, and subsequent colonization of host tissues are all ensured by high viability (Lei et al. 2022).

Molecular analysis

Amplification of the ITS region yielded a single fragment of approximately 585 bp. This result is consistent with the typical amplicon size (500-700 bp) for fungi using ITS1 and ITS4 primers (Siddique et al. 2022). BLASTn analysis revealed that the ITS sequence of isolate MHPS shared 98.31-99.58% identity with reference sequences of *M. huainamdangense* in the GenBank database (Table 3). Phylogenetic analysis was conducted to examine the relationship of the fungus with reference samples obtained from the Gene Bank (Dissanayake et al. 2020). The construction results confirmed that the *Metarhizium* West Dumoga isolate is a species of *M. huainamdangense*. That is indicated by the fact that the isolate is in the same clade as four other isolates of *M. huainamdangense* identified with accession numbers MN781898.1, MN781900.1, MN781899.1, and MN781901.1. The presence of the West Dumoga isolate within the same clade as these isolates indicates that they have a close relationship.

Phylogenetic analysis revealed that the West Dumoga isolate belongs to a monophyletic clade of *M. huainamdangense*, confirming its taxonomic placement with high bootstrap support. Its close clustering with reference strains suggests shared ecological and entomopathogenic traits. These findings provide strong molecular evidence for classifying the West Dumoga isolate as *M. huainamdangense* and highlight the value of phylogenetics in validating species identity.

Table 1. Conidial density and germination viability of the *M. huainamdangense* isolate MHPS from West Dumoga

Conidia density 4 weeks after isolation (Conidia/mL)	Viability (%)		
	8 hours	16 hours	24 hours
1.39×10^9	35.33	76.77	100

Table 2. Top BLASTn hits for the ITS sequence of the *Metarhizium* sp. isolate MHPS from GenBank

Scientific name	Max score	Total score	Query cover	E-value	Per. Ident	Acc. Len	Accession
Metarhizium huainamdangense	861	861	100%	0.0	99.58%	607	MN781898.1
Metarhizium huainamdangense	856	856	100%	0.0	99.37%	604	MN781900.1
Metarhizium huainamdangense	856	856	100%	0.0	99.37%	598	MN781899.1
Metarhizium huainamdangense	828	828	100%	0.0	98.31%	599	MN781901.1

Note: Access Date 28 July 2025

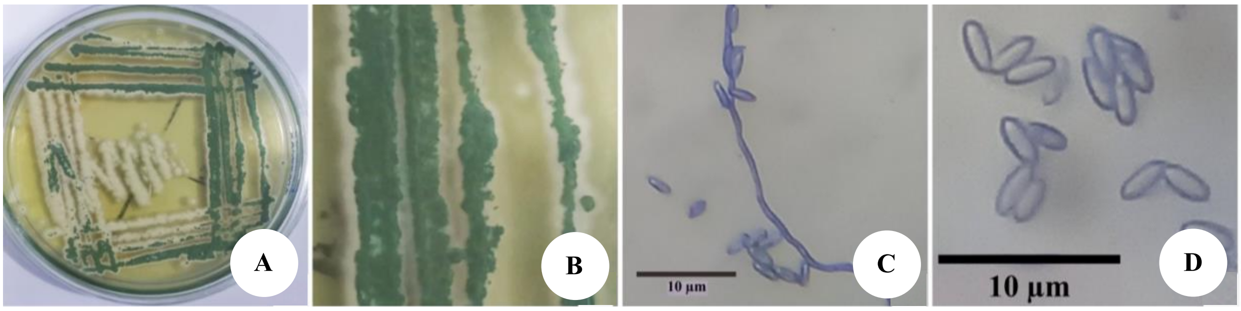

Figure 2. Morphological characteristics of the *M. huainamdangense* isolate MHPS from West Dumoga. A and B. Colony obverse and reverse views on PDA after 14 days, C. Hyphae and conidiophores, D. Conidia (100× magnification)

Calculated lethal time, lethal concentration, and mortality response

The observation results indicate varying percentages of mortality for each treatment. These results are presented in Figure 4, which demonstrates the variation in percentage mortality values for each treatment during the 14-day observation period.

The pathogenicity of *M. huainamdangense* isolate MHPS against *P. maidis* was concentration and time dependent (Figure 4). No mortality was observed in the control treatment throughout the experiment. Mortality was first recorded on day 4 post-application. By day 14, the highest mortality (100%) was achieved at the concentration of 10^9 conidia/mL, while the lowest concentration (10^6 conidia/mL) resulted in 91.11% mortality.

Insect mortality increased with both conidial concentration and exposure time. Mortality reached 91.1% at 10^6 conidia/mL and 100% at 10^9 conidia/mL by day 14, while intermediate concentrations (10^7-10^8) also caused >98% mortality. These results demonstrate the strong potential of *Metarhizium* as a biological control agent against *P. maidis*, with effectiveness enhanced at higher doses and longer exposure periods.

Statistical analysis shows that the treatment influenced the mortality of *P. maidis* ($p<0.05$). The results of the Least Significant Difference (LSD) test showed that concentrations of 10^9, 10^8, and 10^7 were different from 10^6, and all four concentrations were different from the control (Figure 5). The results obtained are consistent with the study conducted by Zheng et al. (2024), who applied *M. anisopliae* at various spore concentrations, resulting in statistically different mortality rates.

The median Lethal Time (LT_{50}) values ranged from 8.5 to 9.2 days across the concentrations tested (Table 3). LT_{50} obtained shows that *M. huainamdangense* is less virulent against *P. maidis*, where *Metarhizium* spp. is said to have high virulence if $LT_{50}<5$ days (Gebremariam et al. 2021). The median lethal concentration (LC_{50}) value was estimated to be 1 × $10^{9.4}$ conidia/mL (Table 4), higher than the concentration treatments applied in this study, indicating that *M. huainamdangense* is less virulent against *P. maidis*. That is in line with the statement by Qasim et al. (2021) that a high LC_{50} value indicates that a high concentration is needed to kill 50% of insects, which indicates low virulence.

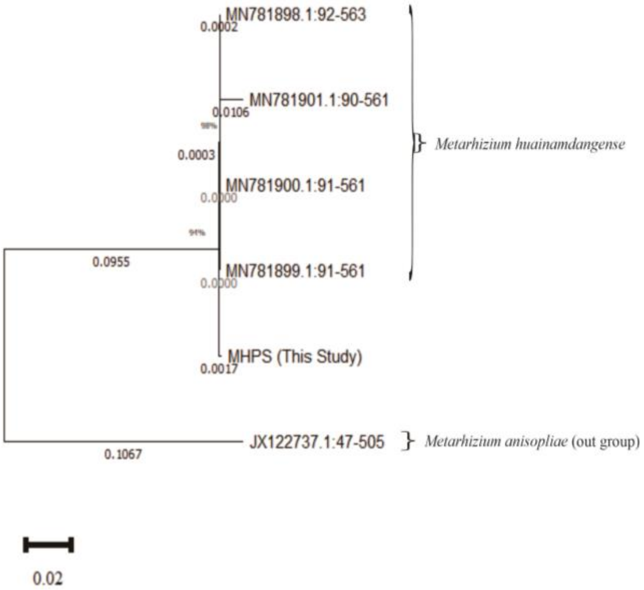

Figure 3. Phylogenetic tree of *Metarhizium* isolates based on ITS sequences constructed using the Neighbor-Joining (NJ) method in MEGA X. The isolate from this study (MHPS) is highlighted in bold. Bootstrap values (1,000 replicates) greater than 50% are shown at the nodes. The scale bar was used to represent the number of nucleotide substitutions per site. *Metarhizium anisopliae* was used as the outgroup

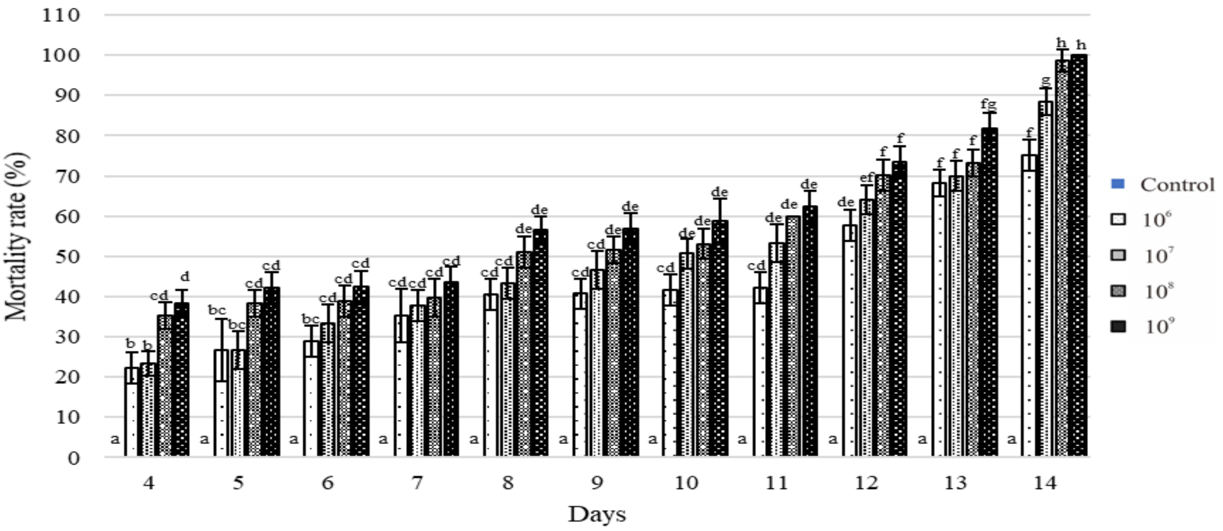

Figure 4. Cumulative mortality (mean±SE) of *Peregrinus maidis* adults treated with different conidial concentrations of *Metarhizium huainamdangense* isolate MHPS over 14 days. No mortality was observed in the first three days or in the control group. From the first to the third day, no insect deaths were found

Insects infected with the entomopathogenic fungus *M. huainamdangense* showed reduced activity (Figure 6.A). Mortality of *P. maidis* insects infected with *M. huainamdangense* began to occur four Days Post-Infection (DPI). At the time of insect mortality, there were no signs of mycelium growth on the bodies of *P. maidis* insects. Fungal mycelium of *M. huainamdangense* first became visible at seven DPI, characterized by white mycelium covering the insect's surface, a hallmark of the vegetative phase of *M. huainamdangense*, and the insect's body hardened like a mummy (Figure 6.B). On day 14, changes were observed on the surface of the insect body where the fungal mycelium had turned green (Figures 6.C and 6.D).

Discussion

The morphological characteristics of the isolate were consistent with those described for the genus *Metarhizium* (Baró et al. 2022; Prastowo et al. 2022). Based on morphological keys (Mongkolsamrit et al. 2020), the isolate was preliminarily identified as *Metarhizium* sp. Although some colonies exhibited uneven sporulation on PDA (Figure 2.A), the morphological features were still consistent with the genus.

The high conidial density (1.39 × 10^9 conidia/mL) and 100% germination rate within 24 hours (Table 1) indicate the robust physiological quality of the *M. huainamdangense* isolate MHPS under the culture conditions used. This high viability suggests good adaptation to culture conditions and is a promising trait for a biocontrol agent, as it is crucial for successful host infection (Yousef-Yousef et al. 2022). For instance, suboptimal temperatures or prolonged exposure to ultraviolet light can reduce spore viability and infectivity, while adequate moisture and balanced nutrition promote germination and enhance fungal performance. The 100% viability recorded for this isolate suggests that it has adapted well to the culture conditions used and may retain similar robustness under field conditions. This characteristic makes the West Dumoga isolate a strong candidate for development as a biological control agent, particularly in tropical or subtropical environments where temperature and humidity favor entomopathogenic fungal activity.

Molecular identification based on ITS region sequences successfully confirmed the identity of the MHPS isolate as *M. huainamdangense*. BLASTn analysis showed a very high sequence similarity (98.31-99.58%) with the reference strain *M. huainamdangense* in GenBank (Table 3). More significantly in the context of local biodiversity, phylogenetic analysis placed the MHPS isolate in a highly supported clade (high bootstrap value) together with other *M. huainamdangense* isolates, while clearly separating it from geographically and phylogenetically closely related species, such as *M. anisopliae* (Figure 3). This phylogenetic separation indicates an independent evolutionary path and reinforces the status of *M. huainamdangense* as a distinct species.

This finding, confirmed in West Dumoga and supported by previous reports from East Dumoga (Wongkar et al. 2022; Siahaan et al. 2023), collectively establishes the Dumoga region as a natural habitat for *M. huainamdangense*. The presence of this entomopathogenic fungus in Dumoga is closely related to the local microclimate conditions. This region is characterized by a humid tropical climate with stable high humidity, conditions that are ideal for the germination of conidia and infection by *Metarhizium* fungi. Compared to *M. anisopliae* isolates commonly found in regions with drier and more variable climates, the Dumoga isolate of *M. huainamdangense* is thought to have unique physiological adaptations that allow it to thrive in these high humidity conditions, which may be reflected in its virulence against specific hosts in this habitat. In addition to abiotic conditions, the abundance and diversity of the host spectrum in the Dumoga agricultural area is a key supporting factor.

Table 3. Median Lethal Time (LT_{50}) of *M. huainamdangense* isolate MHPS against *Peregrinus maidis* adults at different conidial concentrations

Concentration	LT_{50}±SE	Lower bound	Upper bound	R-squared
10^6	9.2±0.90	8.20	10.84	0.83
10^7	9.1±0.92	8.71	9.75	0.84
10^8	8.6±1.00	8.10	9.29	0.82
10^9	8.5±1.03	6.80	9.25	0.82

Notes: SE: Standard Error

Table 4. Probit analysis of the concentration-mortality response of *Peregrinus maidis* adults to *Metarhizium huainamdangense* isolate MHPS at 14 days post-treatment

Summary of probit analysis	
LC_{50}	1 × $10^{9.4}$
Lower bound	1 × $10^{5.1}$
Upper bound	1 × $10^{16.1}$
Int±SE	3.99±0.27
S±SE	1.03±0.31
ρ-value	<0.05
R-squared	0,85

Note: SE: Standard Error, Int: Intercept, S: Slope

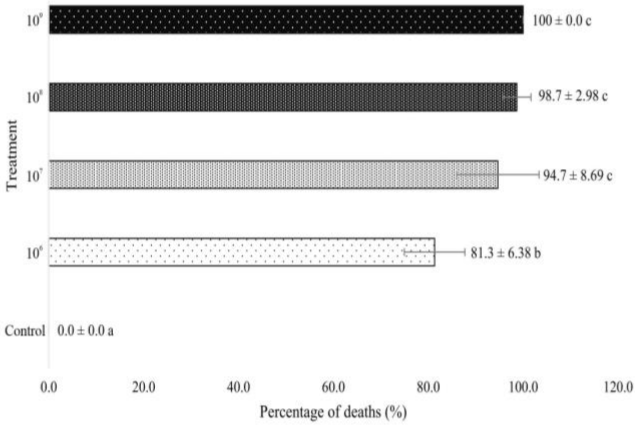

Figure 5. Final mortality (mean±SE) of *Peregrinus maidis* adults on day 14 after treatment with different conidial concentrations of *M. huainamdangense* isolate MHPS. Different lowercase letters above bars indicate significant differences according to the LSD test ($p<0.05$)

Figure 6. Symptoms of *Metarhizium huainamdangense* infection in *Peregrinus maidis*. A. Infected insect showing reduced activity (4 Days Post-Infection (DPI)), B. Mycosis initiation with white mycelium covering the insect's body (7 DPI), C and D. Sporulation stage with green conidia covering the cadaver (14 DPI)

The agricultural environment, which provides a variety of insect pests, creates a diverse host landscape that allows *M. huainamdangense* to survive, reproduce, and proliferate naturally. This host diversity has the potential to drive selection for fungal strains with higher virulence or a broader host range compared to some other isolates that may be limited to specific hosts. Therefore, the confirmation of *M. huainamdangense* in the Dumoga region does not merely add to the geographical distribution list of a species. More than that, this finding positions Dumoga as a highly potential location for the exploration of local biological resources in the development of biological agents. Local isolates such as MHPS, which have adapted to the local microclimate and host spectrum, are promising candidates for development into effective and sustainable bio-insecticides.

The pathogenicity tests demonstrated that *M. huainamdangense* isolate MHPS was pathogenic to *P. maidis*, causing up to 100% mortality at the highest concentration (10^9 conidia/mL) after 14 days (Figure 4). However, the LC_{50} value ($1 \times 10^{9.4}$ conidia/mL) and LT_{50} values (>8.5 days across all concentrations) (Tables 4 and 5) indicate a relatively low virulence compared to other *Metarhizium* species against related pests (Hasibuan et al. 2021; Ibrahim et al. 2021; Hu et al. 2022; Bunsak et al. 2023). This lower virulence could be attributed to several factors. The immune response of *P. maidis* may be more effective against *M. huainamdangense*, or the isolate may lack specific virulence factors (e.g., certain enzymes or genes) required for efficient infection of this host (Umaru and Simarani 2022; Giammarino et al. 2024). Furthermore, although conidial viability was high, other factors such as conidial vigor (germination speed and penetration efficiency), host cuticle composition, and test environmental conditions can significantly influence virulence (Faria et al. 2015; Peng et al. 2022). Despite the relatively low virulence observed, the high sporulation capacity and viability of isolate MHPS suggest its potential for development as a biocontrol agent. Further formulation efforts, such as the use of adjuvants or oil-based carriers to protect conidia and enhance attachment to the insect cuticle, coupled with field evaluation under different environmental conditions, are necessary to fully assess its efficacy against *P. maidis* in integrated pest management programs.

As with the infection mechanism of all *Metarhizium* genera, *M. huainamdangense* primarily infects insect hosts through direct penetration of the cuticle. Infection begins when conidia attach to the insect surface through hydrophobic interactions and adhesive proteins, such as hydrophobin. After germination, the conidia develop appressoria that exert mechanical pressure and secrete a series of cuticle-degrading enzymes, including subtilisin-like proteases (Pr1, Pr2), chitinase, lipase, and esterase, which collectively degrade the structural components of the insect cuticle. After penetration, fungal hyphae invade the hemocoel and differentiate into blastospores, which multiply rapidly and utilize host nutrients, causing host death. The fungus then penetrates the cadaver cuticle and sporulates externally, releasing new conidia that continue the infection cycle. This multifactorial infection strategy highlights the ecological efficiency of *Metarhizium* spp. and supports its potential use as a sustainable biological control agent in integrated pest management systems (Aw et al. 2017; Li and Xia 2022)

In conclusion, the entomopathogenic fungal isolated from West Dumoga is *M. huainamdangense*, as evidenced by morphological, molecular, and phylogenetic analyses. The isolate shows high sporulation capacity and good conidia viability, indicating good physiological quality. Pathogenicity tests showed concentration and time dependent mortality in *P. maidis*, with complete mortality observed at the highest conidia dose. Although *M. huainamdangense* exhibits lower virulence compared to other *Metarhizium* species, its high conidia viability and adaptability indicate strong potential for optimization as a local biocontrol agent, with formulation development and field evaluation to improve its effectiveness and stability under field conditions. These findings provide a basis for developing the use of native entomopathogenic fungi in integrated pest management of *P. maidis* in tropical agroecosystems.

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