JPPIPA 11(11) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Effect of the Culture Medium of the Pathogenic Entomo Fungus Metarhizium sp on the Growth Rate and Pathogenicity in Larvae of Oryctesrhinoceros in the Laboratory

Sulaiman Ginting[1*], S. Edy Sumantri[1], Dendi Putra Siregar[1]

[1] Department of Agriculture, Agrotechnology Faculty, Universitas Islam Sumatera Utara, Medan, Indonesia

Received: August 29, 2025
Revised: November 18, 2025
Accepted: November 29, 2025
Published: November 30, 2025

Corresponding Author:
Sulaiman Ginting
sulaiman.ginting@fp.uisu.ac.id

DOI: 10.29303/jppipa.v11i11.12695

Abstract: Oryctes rhinoceros is a major pest in oil palm plantations, causing serious yield losses. Biological control using the entomopathogenic fungus Metarhizium sp. is a safer alternative to chemical insecticides. This study aimed to evaluate the effect of different culture media on the growth and pathogenicity of Metarhizium sp. against O. rhinoceros larvae. The experiment used a Completely Randomized Design with seven treatments: sterile aquadest (control), PDA, PDA + O. rhinoceros extract, corn medium, corn medium + O. rhinoceros extract, rice medium, and rice medium + O. rhinoceros extract. Observed parameters were colony diameter, initial time of larval mortality, and larval mortality. The results showed that culture medium significantly affected colony growth and virulence of Metarhizium sp. Corn medium supplemented with O. rhinoceros extract (A4) produced the largest colony diameter (95 mm at 23 days after inoculation) and the fastest onset of mortality (8.33 days). All Metarhizium sp. treatments caused high mortality of O. rhinoceros larvae under laboratory conditions. These findings indicate that nutrient-rich media, particularly corn combined with insect extract, enhance the performance of Metarhizium sp. and support its use as an effective biological control agent against O. rhinoceros.

Keywords: Biocontrol agent; Culture media; Entomopathogenic fungi; Metarhizium sp.; Oryctes rhinoceros

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is one of the most important plantation commodities in Indonesia and plays a vital role as a source of vegetable oil for domestic and global markets. Indonesia is a major producer and exporter of palm oil, and the sustainability and productivity of oil palm plantations are closely linked to effective pest management (Magfira et al., 2022; Maysaroh et al., 2022; Santi et al., 2022; Sormin & Junaedi, 2017). One of the most destructive pests in oil palm plantations is the rhinoceros beetle *Oryctes rhinoceros* L., which has long been recognized as a major constraint to oil palm cultivation (Harahap et al., 2023; Magfira et al., 2022; Manjunatha et al., 2023; Villamizar et al., 2024).

This pest causes both direct and indirect economic losses. Indirect losses arise from damage to fronds and young leaves, which reduces photosynthetic activity and consequently lowers palm oil yields. Reduced photosynthesis also weakens plant vigor, making palms more susceptible to secondary infections and other stress factors (Indriyanti et al., 2018; Indriyanti, Widiyaningrum, et al., 2017; Prastowo et al., 2022). Attacks by *O. rhinoceros* can extend the immature or unproductive period (TBM, *Tanaman Belum Menghasilkan*), and in severe cases palms may never fully recover and produce optimally throughout their life cycle (Magfira et al., 2022; Maysaroh et al., 2022; Santi et al., 2022). Direct losses result from adult beetles boring into the crown region and destroying the apical meristem, often causing the death of young palms

How to Cite:
Ginting, S., Sumantri, S. E., & Siregar, D. P. (2025). Effect of the Culture Medium of the Pathogenic Entomo Fungus Metarhizium sp on the Growth Rate and Pathogenicity in Larvae of Oryctesrhinoceros in the Laboratory. *Jurnal Penelitian Pendidikan IPA*, *11*(11), 1130–1142. https://doi.org/10.29303/jppipa.v11i11.12695

(Indriyanti, Widiyaningrum, et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024). The severity of infestation can be very high, with substantial reductions in yield and stand density reported in various production systems (Gunawan et al., 2024; Harahap et al., 2023; Manjunatha et al., 2023; Maysaroh et al., 2022). These impacts pose a serious threat to both smallholder farmers and large-scale plantation companies, as reduced productivity directly affects national palm oil output and economic competitiveness (Magfira et al., 2022; Santi et al., 2022; Villamizar et al., 2024).

Traditionally, farmers have relied heavily on chemical insecticides to control *O. rhinoceros*, applying them through trunk injection, soil drenching, or spraying at breeding sites. However, over-reliance on chemical insecticides, often at doses exceeding recommended rates and applied continuously over time, has led to environmental contamination and risks to non-target organisms (de Miranda et al., 2024; Hajjar et al., 2023; Nik & Rusae, 2024; Quesada-Moraga et al., 2024). These practices contaminate soil and water, disrupt natural ecosystems, and pose risks to human health through occupational exposure (Astuti et al., 2023; Basri et al., 2023; Mamahit et al., 2024; Pramudya et al., 2021; Supriatno et al., 2023). Indiscriminate insecticide application also disrupts ecological balance by eliminating natural enemies such as predators and parasitoids (Quesada-Moraga et al., 2024; Ramalho et al., 2021). Prolonged use of synthetic insecticides promotes the evolution of resistance in pest populations (Manjunatha et al., 2023; Villamizar et al., 2024; Wang et al., 2019), leading to reduced field effectiveness and frequent control failures (Gunawan et al., 2024; Harahap et al., 2023).

Given these limitations, biological control has gained increasing attention as a safer and more sustainable approach to pest management (de Miranda et al., 2024; Hajjar et al., 2023; Quesada-Moraga et al., 2024). Biological control employs natural enemies such as predators, parasitoids, and pathogens to suppress pest populations to sub-economic levels and is a core principle of Integrated Pest Management (IPM) (Hajjar et al., 2023; Quesada-Moraga et al., 2024). For *O. rhinoceros*, several natural enemies have been identified, including entomopathogenic fungi, bacteria, nematodes, viruses, and predatory insects (Indriyanti et al., 2018; Indriyanti, Putri, et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024).

Among these, entomopathogenic fungi are particularly promising because they can infect and kill insect pests without posing significant risks to humans or beneficial organisms (de Miranda et al., 2024; Li & Xia, 2022; Wang et al., 2019). These fungi infect insects through cuticle penetration or ingestion, proliferating inside the host and ultimately causing death (de Miranda et al., 2024; Li & Xia, 2022; Wang et al., 2019). After the insect dies, fungal mycelia sporulate on the cadaver, spreading infection to other susceptible hosts. The most studied species in pest control include *Beauveria bassiana* and various *Metarhizium* species (de Paula et al., 2021; Gomes et al., 2023; Gotti et al., 2023; Wang et al., 2019; Yousef-Yousef et al., 2022).

Among these species, *Metarhizium* sp. is considered one of the most effective biocontrol agents due to its high virulence, environmental persistence, and compatibility with pest management practices (de Miranda et al., 2024; Li & Xia, 2022; Suryadi et al., 2024). However, its successful application depends largely on efficient and economical mass-production systems. Various media and substrates have been evaluated for large-scale production, including agar-based and grain-based media (Agale et al., 2018; Barra-Bucarei et al., 2016; Rajak et al., 2010; Soriano & Adion, 2023; Sucipto et al., 2025). Agricultural substrates such as rice and corn support high spore yields and good fungal performance (Agale et al., 2018; Barra-Bucarei et al., 2016; Suryadi et al., 2024). The choice of growth medium affects not only fungal growth rate but also conidia viability, pathogenicity, and environmental persistence (Carolino et al., 2021; Ismanto & Sukartana, 2016; Mejía et al., 2024; Yang et al., 2024).

Supplementation with specific nutrients or host-derived materials can enhance fungal virulence by improving conidial quality and infection efficiency (Barra-Bucarei et al., 2016; Mejía et al., 2024; Rajak et al., 2010; Sirait et al., 2023; Yang et al., 2024). These findings strengthen the need to develop media formulations that improve field performance while remaining cost-effective.

The novelty of this study lies in evaluating corn- and rice-based media supplemented with *O. rhinoceros* extract to enhance the growth and pathogenicity of *Metarhizium* sp. (Agale et al., 2018; Magfira et al., 2022; Maysaroh et al., 2022; Quiroga-Cubides et al., 2024; Soriano & Adion, 2023). Therefore, this research aims to evaluate the growth performance and pathogenicity of *Metarhizium* sp. cultured on different substrates, including PDA, corn, and rice media, with and without supplementation of *O. rhinoceros* extract, to support the development of sustainable pest management strategies in oil palm plantations.

Method

Data Types and Sources

This research was conducted at the Laboratory of the Center for Seeds and Protection of Plantation Crops (Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan/BBPPTP), Jalan Asrama No. 124, Cinta Damai Village, Medan Helvetia District, Medan,

Indonesia. The study utilized both primary and secondary data. Primary data were obtained directly through laboratory experiments and observations, while secondary data were collected from relevant literature, including journals, books, and official reports related to *Oryctes rhinoceros*, entomopathogenic fungi, and the application of *Metarhizium* sp. (de Miranda et al., 2024; Indriyanti, Widiyaningrum, et al., 2017; Magfira et al., 2022; Prastowo et al., 2022).

Research Method

This study was carried out at the Laboratory of the Center for Seeds and Protection of Plantation Crops (Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan/BBPPTP), Medan, Indonesia. The research was designed to evaluate the effect of different culture media on the growth and pathogenicity of the entomopathogenic fungus Metarhizium sp. against larvae of the rhinoceros beetle, Oryctes rhinoceros. The methodological approach was based on controlled laboratory experiments, which allowed for systematic observation of fungal development and its bio-efficacy on the target pest.

Experimental Design

The experiment employed a Non-Factorial Completely Randomized Design (CRD), which is commonly used in agricultural and biological research due to its simplicity and statistical reliability (Falah et al., 2024; Harahap et al., 2023; Nik et al., 2023; Nik & Rusae, 2024; Supriatno et al., 2023). A total of seven treatments were tested, with each treatment replicated three times to ensure statistical validity and minimize experimental error. The treatments consisted of:

A0 = Sterile aquadest (control)
A1 = Potato Dextrose Agar (PDA) medium
A2 = PDA medium + O. rhinoceros extract
A3 = Corn medium
A4 = Corn medium + O. rhinoceros extract
A5 = Rice medium
A6 = Rice medium + O. rhinoceros extract

The inclusion of different media types, both with and without supplementation of insect extract, was intended to evaluate the influence of nutritional composition on fungal growth and virulence. Each treatment was inoculated with isolates of Metarhizium sp. and incubated under controlled environmental conditions (25 ± 2 °C, ambient laboratory humidity).

Preparation of Culture Media

PDA was prepared following commercial formulations. Corn and rice media were formulated by milling each substrate into flour, mixing with agar and dextrose, and sterilizing at 121 °C for 20 minutes. The suitability of grain-based substrates for *Metarhizium* mass production has been well established (Agale et al., 2018; Barra-Bucarei et al., 2016; Rajak et al., 2010; Soriano & Adion, 2023; Sucipto et al., 2025; Suryadi et al., 2024).

Media supplementation was applied by adding crude extract prepared from homogenized third-instar larvae at a 1:1 ratio with sterile aquadest, then filtered. Host-derived enrichment has been shown to enhance conidial quality and virulence (de Miranda et al., 2024; Li & Xia, 2022; Quesada-Moraga et al., 2024; Wang et al., 2019).

Inoculation of Fungal Isolates

Pure *Metarhizium* sp. isolates were sourced from the BBPPTP culture collection. Plugs (1 cm) from actively growing PDA cultures were aseptically transferred to the test media. Plates were sealed with parafilm and incubated at room temperature. Colony diameter was measured daily across perpendicular axes (Agale et al., 2018; Gomes et al., 2023; Rajak et al., 2010).

Preparation of Insect Test Material

The target pest used in this study was the third-instar larvae of O. rhinoceros. Larvae were collected from decaying oil palm residues and reared in sterilized rotten palm trunks, which served as a natural substrate. Only healthy, active, and uniform-sized larvae were selected to minimize variability. Each larva weighed approximately 10–14 g and measured 7–8 cm in length. The selection of instar III larvae was based on their high feeding activity and susceptibility to fungal infection (Prastowo et al., 2022).

Pathogenicity Test

Pathogenicity tests were conducted using the dipping method. Fungal suspensions were prepared by adding sterile distilled water (15 ml) to the surface of sporulating cultures and gently scraping conidia with a sterile spatula. The resulting suspension was filtered through sterile cheesecloth and adjusted to a concentration of 10^7 conidia/ml using a hemocytometer (Prastowo et al., 2022). Each larva was immersed in the suspension for 20 seconds, air-dried for five minutes, and then transferred to individual rearing containers lined with sterilized palm trunk pieces. The control group (A0) was treated with sterile aquadest only.

Observation and Data Collection

Observations were conducted daily for 21 days. The recorded parameters included colony diameter growth (mm), which was measured at regular intervals from the point of inoculation until the fungal colony reached the edge of the petri dish; the initial time of larval mortality (days), defined as the number of days after treatment until the first larval death was observed in each replicate; and larval mortality percentage (%), calculated as the

cumulative number of dead larvae expressed as a percentage of the total larval population in each treatment. Larvae that died during the experiment were surface-sterilized with 70% ethanol and placed in moist chambers to confirm fungal infection by observing characteristic mycelial growth on cadavers (Li & Xia, 2022).

Data Analysis

Data obtained were analyzed using analysis of variance (ANOVA) following the model of a Completely Randomized Design (CRD) as described Freeman et al. (1985):

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \quad (1)$$

Where:

Y_{ij} = observed value for the i-th treatment and j-th replication
μ = general mean
α_i = treatment effect
β_j = replication effect
ϵ_{ij} = experimental error

When significant differences were detected, the means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level (Harahap et al., 2023).

Result and Discussion

Diameter Growth of Metarhizium sp. Colonies

The results of the variance analysis demonstrated that the type of culture medium exerted a significant influence on the growth diameter of *Metarhizium sp.* colonies during the incubation period. This finding highlights the crucial role of nutrient composition and physical properties of the growth substrate in supporting or constraining fungal development. The average colony diameters measured at daily intervals revealed distinct differences among treatments, which are summarized in Table 1: Average Colony Growth Diameter of *Metarhizium sp.* (mm) at 3–23 Days After Inoculation (DAI).

Table 1. Average Growth Diameter of Mushroom Colony (mm) 3 – 23 DAI

DAI	A1	A2	A3	A4	A5	A6
3	13.67 c	16.00 b	21.00 b	23.33 a	13.33 c	23.33 a
4	17.33 c	21.00 b	27.00 b	29.33 a	18.33 c	27.33 a
5	21.00 e	26.00 c	32.00 b	34.33 a	23.33 d	32.33 b
6	24.67 e	31.00 c	36.00 b	38.33 a	27.33 d	36.33 b
7	28.00 e	35.50 c	40.17 b	42.33 a	31.33 d	40.33 b
8	32.17 e	38.33 c	44.17 b	46.50 a	35.00 d	45.50 ab
9	37.00 e	41.33 c	48.00 b	50.00 a	38.83 d	49.00 ab
10	41.33 e	44.33 c	51.67 b	54.00 a	42.67 d	53.33 a
11	47.33 d	49.00 c	54.67 b	57.33 a	46.67 d	57.33 a
12	50.67 d	53.67 c	57.50 b	61.50 a	50.67 d	62.00 a
13	53.67 d	57.67 c	60.50 b	65.00 a	53.50 d	65.00 a
14	57.00 d	64.00 c	64.67 b	69.00 a	57.00 d	68.67 a
15	60.33 c	67.33 b	68.33 b	73.33 a	60.67 c	71.67 ab
16	64.50 c	71.00 b	72.17 b	77.00 a	64.67 c	75.17 ab
17	66.83 c	74.67 b	76.00 b	80.33 a	69.00 c	78.33 ab
18	71.50 c	78.00 b	79.00 ab	83.33 a	73.00 c	81.33 ab
19	73.50 c	80.67 b	81.50 ab	86.00 a	74.67 c	83.33 ab
20	75.67 c	82.67 b	84.17 ab	88.67 a	76.67 c	86.00 ab
21	77.17 c	84.50 b	86.67 b	91.00 a	78.83 c	87.67 ab
22	78.67 c	85.83 b	88.33 b	93.67 a	81.33 c	89.00 b
23	79.83 d	87.00 c	88.67 bc	95.00 a	83.00 d	90.67 b

Information: The number followed by different letters in the same observation column differs significantly at the level of 5% based on the DMRT test.

From the data, it is evident that corn medium supplemented with *Oryctes rhinoceros* extract (A4) consistently produced the largest colony diameters across the observation period, reaching 95 mm by 23 DAI. This value was statistically higher than that of the other media, indicating that the synergy between the corn substrate and the insect extract provided an optimal environment for fungal proliferation. Conversely, the smallest growth was observed in PDA medium (A1), with a final diameter of 79.83 mm, which was not significantly different from rice medium (A5) at 83.00 mm. Rice + *O. rhinoceros* extract (A6) produced a diameter of 90.67 mm, comparable to corn medium alone (A3) at 88.67 mm. These results suggest that while rice is rich in carbohydrates, physicochemical changes during media preparation may have reduced nutrient

bioavailability for fungal metabolism (Barra-Bucarei et al., 2016; Agale et al., 2018; Soriano & Adion, 2023; Suryadi et al., 2024; Sucipto et al., 2025).

The superior performance of A4 can be attributed to the balanced nutrient composition and favorable physical structure of corn, which support both vegetative growth and sporulation of *Metarhizium* sp. Grain-based substrates such as corn and rice provide carbohydrates and proteins that act as key carbon and nitrogen sources for fungal development (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018; Soriano & Adion, 2023). Protein serves as an important nitrogen source that supports germination and enhances virulence through increased enzyme and metabolite production (Mejía et al., 2024; Yang et al., 2024; Yousef-Yousef et al., 2022).

Furthermore, the addition of *O. rhinoceros* extract likely enriched the medium with insect-derived compounds such as chitin, lipids, and hemolymph proteins, which are natural substrates encountered by entomopathogenic fungi during infection. Host-derived substrates and specific nutrient profiles have been shown to stimulate the production of hydrolytic enzymes such as chitinases, proteases, and lipases, as well as secondary metabolites essential for host penetration and fungal metabolism (Li & Xia, 2022; de Miranda et al., 2024; Wang et al., 2019; Quesada-Moraga et al., 2024). Thus, A4 provided both macronutrient sufficiency and host-mimicking signals, accelerating fungal colony expansion.

Despite the high carbohydrate content of rice, the growth performance of *Metarhizium* sp. colonies on A5 and A6 was inferior to A4. This paradox can be explained by the preparation process: heating and subsequent cooling of rice-based media can cause starch gelatinization and restructuring, leading to larger particle aggregates and a denser matrix that limit the surface area accessible to fungal hyphae (Barra-Bucarei et al., 2016; Soriano & Adion, 2023; Yousef-Yousef et al., 2022). Reduced accessibility of soluble carbohydrates and altered substrate microstructure have been reported to constrain nutrient uptake and slow colony expansion in entomopathogenic fungi (Rajak et al., 2010; Agale et al., 2018; Mejía et al., 2024).

Regression analysis further confirmed the strong relationship between incubation time and colony growth across treatments. The coefficients of determination (R^2) were high, ranging from 98.32% in rice medium (A5) to 99.17% in corn + *O. rhinoceros* medium (A4). This indicates that time progression accounted for nearly all variability in colony expansion, although the slope of the regression lines varied according to substrate quality. The steepest slope was observed in A4, reflecting rapid growth rates throughout incubation, whereas A5 exhibited the slowest trajectory.

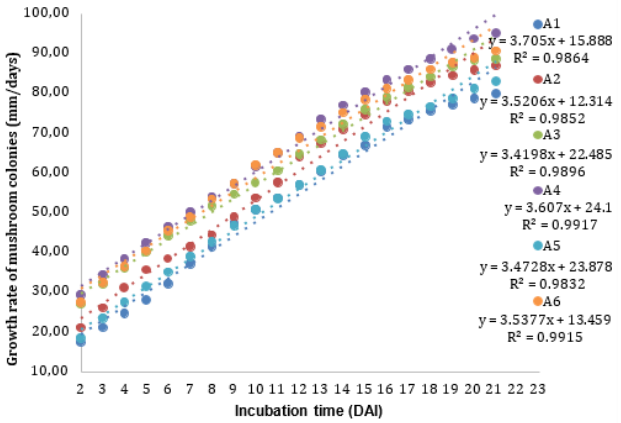

Figure 1. Regression Analysis of Colony Growth Rates of Metarhizium sp. at Different Media Types

Figure 1 illustrates these differences and shows that corn + insect extract provided the most conducive growth environment. Nutrient-rich, well-structured media not only increase final colony size but also accelerate the early exponential growth phase, which is critical for fungal establishment (Yousef-Yousef et al., 2022; Mejía et al., 2024; Soriano & Adion, 2023).

These findings are consistent with earlier studies emphasizing the importance of substrate composition for entomopathogenic fungi. Grain-based and agro-industrial substrates have been reported as particularly favorable for *Metarhizium* mass production due to their balanced nutrient profiles and suitable physical properties, which promote both vegetative and reproductive growth (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018; Soriano & Adion, 2023; Suryadi et al., 2024; Sucipto et al., 2025). The current results corroborate these conclusions, as A4 yielded the most vigorous colony expansion.

By contrast, PDA (potato dextrose agar), although widely used for fungal isolation and maintenance, was less effective as a bulk growth substrate. Agar-based media such as PDA are generally formulated for clear morphological observation rather than maximized biomass production and therefore lack the nutrient density and structural heterogeneity of grain-based substrates (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018). This helps explain the smaller colony sizes recorded in A1 compared with corn- and rice-based media.

Environmental factors such as temperature and humidity can interact with substrate quality to determine fungal performance. However, in this study, incubation conditions were standardized, suggesting that the observed differences are primarily attributable

to media composition and structure rather than external environmental variability (Yousef-Yousef et al., 2022; Quesada-Moraga et al., 2024).

The vigorous growth of *Metarhizium* sp. on A4 medium has broader implications for its pathogenicity. Larger colonies are likely to produce greater numbers of viable conidia, which are critical for successful infection of insect hosts (Magfira et al., 2022; Santi et al., 2022; Maysaroh et al., 2022; Indriyanti et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Gunawan et al., 2024). The high growth rate observed in corn-based media supplemented with insect extract may therefore translate into enhanced field performance when used as a bioinoculant against *O. rhinoceros*. Conversely, the limited growth on PDA and rice media may reduce sporulation potential and compromise biocontrol efficacy.

Moreover, the metabolic activity associated with rapid vegetative growth could support earlier and more intense toxin production. *Metarhizium* sp. produces destruxins and other secondary metabolites that disrupt hemolymph microfilaments, interfere with immune responses, and impair host physiology (de Miranda et al., 2024; Li & Xia, 2022; Wang et al., 2019; Zhang et al., 2024). Enhanced nutrient uptake in A4 may accelerate the biosynthesis of these compounds, further increasing virulence.

Visual examination of colony morphology also supported the quantitative data. Colonies on A4 exhibited dense, evenly distributed mycelial mats with vigorous radial expansion, whereas colonies on A1 and A5 appeared thinner and more irregular. After 23 DAI, colonies in A4 and A6 treatments displayed a characteristic olive-green coloration associated with conidial maturation, suggesting that both vegetative growth and reproductive development were promoted (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018).

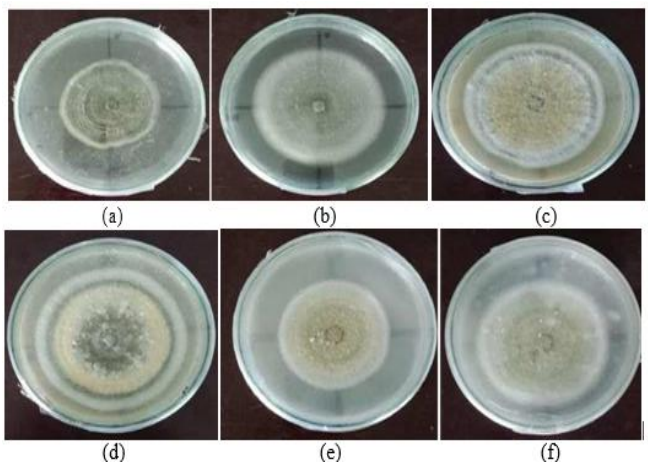

Figure 2. Morphological Appearance of *Metarhizium sp*. Colonies on Six Different Media at 23 DAI

Figure 2 provides photographic documentation of these differences. Such visual contrasts reinforce the notion that substrate quality directly influences growth speed, colony structure, and sporulation potential (Barra-Bucarei et al., 2016; Soriano & Adion, 2023; Sucipto et al., 2025).

The growth curves observed align with the classical microbial growth pattern characterized by lag, exponential, stationary, and decline phases. In nutrient-rich media such as A4 and A6, the lag phase was brief, suggesting that fungi readily adapted to these substrates, while the exponential phase was more pronounced, reflecting sustained colony expansion. Approaching the stationary phase, colony growth in A1 and A5 plateaued earlier, indicating nutrient depletion or physical constraints. By contrast, A4 maintained expansion nearly until the end of the incubation period, consistent with superior nutrient availability and uptake efficiency (Yousef-Yousef et al., 2022; Mejía et al., 2024; Soriano & Adion, 2023).

These dynamics underscore that entomopathogenic fungi require not only macronutrients but also micronutrients, vitamins, and structurally suitable substrates in their culture environment. Substrates that balance these factors accelerate growth and ultimately enhance pathogenic potential (de Miranda et al., 2024; Quesada-Moraga et al., 2024; Wang et al., 2019).

Early Time of Oryctesrhinoceros Larval Death (HSA)

The analysis of variance indicated that the type of culture medium significantly influenced the early time of larval death in *Oryctes rhinoceros*. This finding highlights the profound impact of nutritional and biochemical differences in fungal culture substrates on the pathogenic expression of *Metarhizium sp*.. The results of daily observations, summarized in Table 2, provide clear evidence that the mortality onset varied across treatments, with the corn-based medium supplemented with *O. rhinoceros* extract (A4) producing the earliest lethal effects.

Table 2. Average Early Time of Death of O. rhinoceros Larvae (HSA)

Treatment	Early death (HSA)
A0 = Aquadest steril (control)	0.00
A1 = PDA Media	10.33 a
A2 = PDA Media + O. rhinoceros extract	9.33 ab
A3 = Corn Media	9.00 b
A4 = Corn Media + O. rhinoceros extract	8.33 b
A5 = Rice Media	9.33 ab
A6 = Rice Media + O. rhinoceros extract	9.00 b

Information: The number followed by different letters in the same observation column differs significantly at the level of 5% based on the DMRT test.

The data show that the fastest onset of larval death occurred in A4, with an average of 8.33 HSA. This value was statistically similar to A3 and A6 (9.00 HSA), as well as A5 and A2 (9.33 HSA), but significantly different from PDA medium alone (A1), which delayed mortality until 10.33 HSA. In the control group, no larval mortality occurred throughout the observation period, confirming that death was directly attributable to fungal infection rather than environmental stress.

Beyond quantitative differences, qualitative observations revealed a distinct sequence of infection symptoms. By the fifth day after inoculation, larvae in fungal treatments exhibited reduced mobility, diminished feeding activity, and a tendency to isolate from healthy individuals, behavioral changes characteristic of entomopathogenic fungal infection (Li & Xia, 2022; de Miranda et al., 2024). On the sixth day, external signs of infection became apparent as dark brown patches on the cuticle indicated localized fungal penetration and enzymatic degradation. By the seventh day, larval movement was markedly weakened, and by the eighth day, symptomatic larvae succumbed, with bodies becoming rigid and hardened due to tissue colonization by fungal mycelia (Indriyanti et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023).

The superior performance of A4 in inducing early mortality reflects the synergy between corn as a basal substrate and insect extract as a natural nutrient source. Corn provides carbohydrates and proteins that enhance fungal growth and sporulation, while host-derived components supply additional cues and nutrients relevant to the infection process (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018; Soriano & Adion, 2023; Mejía et al., 2024; Yang et al., 2024). The insect extract contributes biologically relevant compounds such as chitin fragments and hemolymph proteins, which may prime the fungus to produce higher levels of virulence factors, including extracellular enzymes and toxins (Li & Xia, 2022; de Miranda et al., 2024; Wang et al., 2019; Quesada-Moraga et al., 2024).

By contrast, PDA medium (A1), composed mainly of potato-derived carbohydrates with relatively low nutrient density, is less suitable for rapid expression of fungal virulence. Although PDA is widely used for fungal isolation and maintenance, its simplified composition limits biomass and conidial production compared with grain-based substrates (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018). The delayed onset of larval death in A1 therefore reflects slower fungal colonization and toxin production.

Rice-based media (A5 and A6), despite high carbohydrate content, yielded mortality onsets slower than A4. As discussed previously, starch gelatinization and restructuring during preparation can reduce nutrient bioavailability by creating a denser, less accessible substrate matrix (Barra-Bucarei et al., 2016; Soriano & Adion, 2023; Yousef-Yousef et al., 2022). Consequently, fungal growth and conidial virulence were somewhat delayed compared to corn-based substrates.

The early mortality in corn-based treatments is closely linked to the infection mechanism of *Metarhizium* sp., which involves conidial adhesion, germination, penetration, and invasion. Penetration is facilitated by enzymes such as chitinases, lipases, and proteases, while toxins including destruxins disrupt hemolymph and immune functions (Li & Xia, 2022; Wang et al., 2019; de Miranda et al., 2024; Zhang et al., 2024). Nutrient-rich media such as A4 likely accelerate enzyme and toxin synthesis, enabling the fungus to overcome host defenses more rapidly.

Previous studies on entomopathogenic fungi have reported that isolates grown on nutrient-rich media produce more vigorous conidia with faster infection cycles and higher mortality rates in target insects (Magfira et al., 2022; Santi et al., 2022; Maysaroh et al., 2022; Indriyanti et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Gunawan et al., 2024). The behavioral and morphological patterns observed in this study are in line with these findings and with detailed descriptions of infection kinetics in *Metarhizium*–insect systems (Li & Xia, 2022; de Miranda et al., 2024; Wang et al., 2019).

Biologically, earlier larval death implies more effective suppression of pest populations, as larvae are eliminated before causing substantial feeding damage. Practically, culture media that promote early mortality may enhance the efficacy of *Metarhizium* sp. formulations used as bioinsecticides in oil palm plantations. Rapid onset of death can reduce the reproductive potential of *O. rhinoceros* populations, thereby limiting future outbreaks (Magfira et al., 2022; Santi et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Gunawan et al., 2024).

Larval Mortality O. rhinoceros (%)

The variance analysis revealed that culture media of Metarhizium sp. did not significantly affect larval mortality at 8 days after inoculation (HSA), but from 9 to 18 HSA the effect became highly significant. This indicates that the fungus required an initial adaptation phase before expressing pathogenicity, but once established, the growth environment provided by different media strongly shaped the infection outcomes. The average mortality data are summarized in Table 3.

Table 3. Percentage of Larval Mortality of *O. rhinoceros* (%) across Treatments at 8–18 HSA

HSA	A0	A1	A2	A3	A4	A5	A6
8	0.00b	0.00c	3.33c	0.00c	6.67	0.00c	0.00c
9	0.00c	0.00c	6.67bc	10.00ab	20.00a	10.00bc	16.67ab
10	0.00c	10.00bc	23.33ab	26.67ab	33.33a	26.67ab	33.33a
11	0.00c	30.00bc	36.67a	36.67a	40.00a	36.67a	36.67a
12	0.00b	46.67a	50.00a	50.00a	53.33a	50.00a	63.33a
13	0.00b	56.67a	70.00a	66.67a	60.00a	66.67a	70.00a
14	0.00b	63.33a	70.00a	73.33a	80.00a	66.67a	70.00a
15	0.00b	66.67a	70.00a	83.33a	86.67a	70.00a	73.33a
16	0.00b	70.00a	80.00a	83.33a	90.00a	70.00a	80.00a
17	0.00b	70.00a	80.00a	83.33a	96.67a	76.67a	83.33a
18	0.00b	70.00a	80.00a	90.00a	96.67a	76.67a	83.33a

Information: The number followed by different letters in the same observation column differs significantly at the level of 5% based on the DMRT test.

The data indicate that larval mortality first appeared at 8 HSA, with A4 producing the earliest deaths at 6.67%. PDA + insect extract (A2) produced 3.33% mortality at the same time point, while other media and the control showed no mortality. Between 9 and 10 HSA, mortality levels began to diverge sharply from the control, which consistently recorded 0% mortality throughout the observation period.

From 11 HSA onwards, all fungal treatments showed significantly higher mortality than the control, although no statistically significant differences were detected among the media themselves. Descriptively, A4 consistently produced the highest mortality rate, culminating at 96.67% by 18 HSA and approaching 100% in some replicates. Corn medium alone (A3) followed with 90%, rice + insect extract (A6) reached 83.33%, PDA + insect extract (A2) 80%, rice medium (A5) 76.67%, and PDA alone (A1) 70%. These trends are in line with reports that *Metarhizium*-based formulations can cause high mortality in coleopteran pests, including *O. rhinoceros* and other insect pests of oil palm and vegetable crops (de Paula et al., 2021; Falah et al., 2024; Gomes et al., 2023; Gunawan et al., 2024; Indriyanti et al., 2018; Indriyanti, Widiyaningrum, et al., 2017; Lei et al., 2023; Magfira et al., 2022; Manjunatha et al., 2023; Maysaroh et al., 2022; Mejía et al., 2024; Prastowo et al., 2022; Santi et al., 2022; Villamizar et al., 2024).

The mortality curve (Figure 3) visually illustrates the contrast between treatments and the control. Corn-based treatments, particularly A4, exhibited a steep upward trajectory beginning at 8 HSA, surpassing 90% by day 16 and reaching the highest final mortality by day 18. PDA-based treatments increased more gradually, stabilizing between 70–80%, while rice-based media showed intermediate performance. The control line remained flat at 0%, reinforcing the conclusion that larval death resulted from fungal infection.

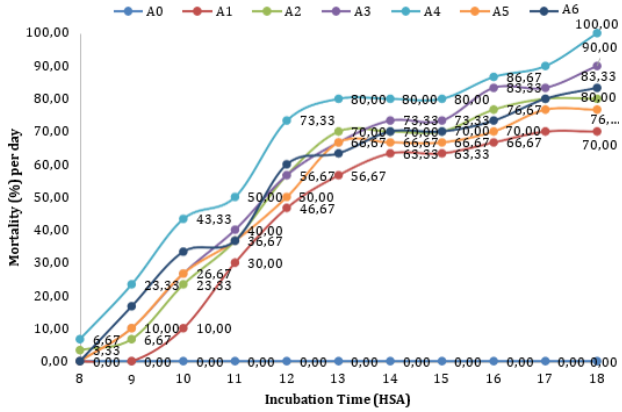

Figure 3. Larval mortality graph of *O. rhinoceros*

The pattern shows a rapid increase in mortality between 9 and 15 HSA, after which mortality plateaued as most larvae succumbed. This exponential increase reflects the typical infection kinetics of entomopathogenic fungi: once conidia germinate and penetrate the cuticle, fungal growth within the hemocoel rapidly overwhelms the host (Li & Xia, 2022; de Miranda et al., 2024; Wang et al., 2019; Quesada-Moraga et al., 2024).

The superiority of A4 can again be attributed to corn's balanced carbohydrate–protein composition and favorable physical characteristics as a solid substrate, which support vigorous fungal growth and sporulation (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018; Soriano & Adion, 2023; Suryadi et al., 2024; Sucipto et al., 2025). Supplementation with *O. rhinoceros* extract may have provided host-specific cues—such as chitin fragments and hemolymph components—that stimulated the production of cuticle-degrading enzymes and virulence factors (Li & Xia, 2022; de Miranda et al., 2024; Wang et al., 2019; Quesada-Moraga et al., 2024).

PDA medium (A1), with its relatively simple and less nutrient-dense formulation, was nutritionally inferior. Although PDA is widely used for fungal

maintenance, it did not foster the highest virulence in *Metarhizium* sp., leading to delayed mortality onset and the lowest final mortality rate. The addition of insect extract in A2 improved mortality outcomes relative to A1, but was still unable to match corn or rice-based media (Rajak et al., 2010; Barra-Bucarei et al., 2016; Agale et al., 2018).

Rice-based treatments (A5 and A6) showed intermediate performance. Despite rice's suitability as a carbon source, heating and gelatinization during medium preparation likely reduced nutrient bioavailability by creating a more compact substrate matrix (Barra-Bucarei et al., 2016; Soriano & Adion, 2023; Yousef-Yousef et al., 2022). As a result, fungal growth and conidial virulence were somewhat delayed compared to corn-based substrates.

High-virulence fungi such as *Metarhizium* sp. cause host mortality by penetrating the cuticle with chitinase, protease, and lipase, and by secreting secondary metabolites in the hemolymph, including destruxins and other toxic compounds that disrupt hemolymph microfilaments, interfere with circulation and immune function, and ultimately cause systemic failure (Wang et al., 2019; Li & Xia, 2022; de Miranda et al., 2024; Zhang et al., 2024). The ability to synthesize these compounds is strongly influenced by the nutritional environment; thus, the nutrient-rich A4 medium likely supported faster and more abundant toxin production, resulting in rapid and complete larval mortality.

Larvae exposed to fungal treatments exhibited clear behavioral and morphological symptoms of infection starting at 4–5 HSA. Early behavioral changes included sluggish movement, loss of appetite, and isolation behavior. As infection progressed, larvae became paralyzed and immobile. Morphologically, the first visible signs were darkened patches on the cuticle, followed by yellowing and stiffening of the body. Similar infection patterns and symptom progression have been reported in other studies involving *Metarhizium* spp. and various insect hosts (Indriyanti et al., 2017; Indriyanti et al., 2018; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Lei et al., 2023; Quiroga-Cubides et al., 2024; Falah et al., 2024; de Paula et al., 2021; Gomes et al., 2023).

The infection stages of *O. rhinoceros* larvae (Figure 4), captured through photographic documentation, complement these observations. One day post-mortality, larvae displayed pale-yellowish cuticles; by day two, white mycelial outgrowth was evident across the integument. On day three, mycelial density increased substantially, followed by near-complete coverage and initial green sporulation by day four. By day five, the entire cadaver was enveloped in green conidia, a classical signature of *Metarhizium* sporulation on insect hosts (Indriyanti et al., 2017; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Magfira et al., 2022; Santi et al., 2022; Maysaroh et al., 2022).

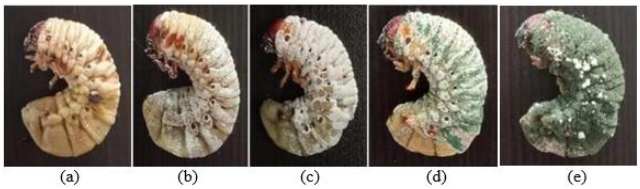

Figure 4 Infection stages of *O. rhinoceros* larvae post-inoculation with *Metarhizium sp*.

The high mortality rates observed in all fungal treatments (70–100%) reinforce the categorization of *Metarhizium* sp. as an effective bioinsecticide. Numerous studies have shown that *Metarhizium*-based formulations can achieve high levels of control against *O. rhinoceros* and other economically important pests in both laboratory and field conditions (Magfira et al., 2022; Santi et al., 2022; Maysaroh et al., 2022; Indriyanti et al., 2017; Indriyanti et al., 2018; Prastowo et al., 2022; Villamizar et al., 2024; Manjunatha et al., 2023; Gunawan et al., 2024; Lei et al., 2023; Quiroga-Cubides et al., 2024; Falah et al., 2024; de Paula et al., 2021; Gomes et al., 2023; Yousef-Yousef et al., 2022; Quesada-Moraga et al., 2024). Although PDA medium (A1) produced slightly lower mortality than the other treatments, most media—especially A4, A3, and A6—achieved high mortality levels, underscoring the fungus's potential for field application against *O. rhinoceros*.

At the same time, research on entomopathogenic microbes such as Metarhizium can serve as an authentic science learning resource to enhance students' literacy on agricultural biotechnology and biological control in formal education settings (Arfan et al., 2025).

Moreover, the fact that mortality was significantly different from the control but not among media indicates that while all tested media can support effective fungal infection, certain substrates (particularly corn + insect extract) accelerate mortality and ensure near-complete suppression. This suggests that substrate optimization can enhance the field efficacy of fungal bioinsecticides, especially under tropical plantation conditions where pest outbreaks are rapid and severe. These results align with broader perspectives on the role of entomopathogenic fungi as key components of integrated pest management in perennial cropping systems (Hajjar et al., 2023; de Miranda et al., 2024; Quesada-Moraga et al., 2024).

Future field programs could be strengthened by quantitative monitoring of M. anisopliae in soil and cocoon samples using specific qPCR primers, allowing more precise tracking of fungal persistence and spread in oil palm ecosystems (Saragih et al., 2025).

Conclusion

The type of culture medium significantly influenced the growth rate and pathogenicity of Metarhizium sp. colonies against Oryctes rhinoceros larvae. Corn medium supplemented with O. rhinoceros extract (A4) produced the fastest and most extensive colony growth (95 mm at 23 days after inoculation), the earliest onset of larval mortality (8.33 days), and the highest final mortality (up to 100% in laboratory conditions). In contrast, PDA and rice media without insect extract supported slower growth and lower overall performance, although all tested media were able to cause high larval mortality. These results indicate that nutrient-rich substrates, particularly corn combined with insect extract, enhance fungal virulence and are more suitable for the mass production of Metarhizium sp. as a biocontrol agent. Practically, the use of such alternative media can support more cost-effective and environmentally friendly management of O. rhinoceros within integrated pest management programs in oil palm plantations.

Acknowledgments
Thank you to the Laboratory of the Center for Seeds and Protection of Plantation Crops (BBPPTP) and the research team who contributed to this study. In addition, thank you to the Head of the Agrotechnology Program and the Dean of the Faculty of Agriculture, University of Islam Sumatera Utara.

Author Contributions
Conceptualization, S.G.; methodology, S.G. and S.E.S.; validation, S.E.S.; formal analysis, S.G. and D.P.S.; investigation, D.P.S. and S.E.S.; resources, S.E.S.; data curation, D.P.S.; writing—original draft preparation, S.G.; writing—review and editing, S.G. and S.E.S.; visualization, D.P.S.; supervision, S.E.S.; project administration, S.G. and S.E.S. All authors have read and agreed to the published version of the manuscript.

Funding
This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest

References

Agale, S. V., Gopalakrishnan, S., Ambhure, K. G., Chandravanshi, H., Gupta, R., & Wani, S. P. (2018). Mass Production of Entomopathogenic Fungi (Metarhizium anisopliae) using Different Grains as a Substrate. *International Journal of Current Microbiology and Applied Sciences*, *7*(1), 2227–2232. https://doi.org/10.20546/ijcmas.2018.701.268

Arfan, A., Ratnawati, R., & Hasmari, H. (2025). Identification and Characterisation of Entomopathogenic Microbes: An Innovative Science Learning Resource to Improve Student Science Literacy on the Topic of Agricultural Biotechnology. *Jurnal Penelitian Pendidikan IPA*, *11*(10 SE-Research Articles), 1132–1138. https://doi.org/10.29303/jppipa.v11i10.13173

Astuti, R. R. U. N. W., Illahi, A. N., Umri, W. N. S., & Falah, A. A. (2023). Potency of Secondary Metabolites from Salacca zalacca, Sonchus arvensis, and Carica papaya against Aedes aegypti L. *Jurnal Penelitian Pendidikan IPA*, *9*(7), 4931–4937. https://doi.org/10.29303/jppipa.v9i7.4129

Barra-Bucarei, L., Vergara, P., & Cortes, A. (2016). Conditions to optimize mass production of Metarhizium anisopliae (metschn.) sorokin 1883 in different substrates. *Chilean Journal of Agricultural Research*, *76*(4), 448–454. https://doi.org/10.4067/S0718-58392016000400008

Basri, M. H., Sulistiyanto, S., & Imaduddin, M. (2023). Implementation of Shallot Pest Trap Model Based on Color Spectrum Using Photovoltaic Energy. *Jurnal Penelitian Pendidikan IPA*, *9*(5), 3833–3838. https://doi.org/10.29303/jppipa.v9i5.3378

Carolino, A. T., Teodoro, T. B. P., Gomes, S. A., Silva, C. P., & Samuels, R. I. (2021). Production of conidia using different culture media modifies the virulence of the entomopathogenic fungus Metarhiziumys against Aedes aegypti larvae. *Journal of Vector Borne Diseases*, *58*(4), 346–351. https://doi.org/10.4103/0972-9062.318315

de Miranda, R. P. R., Soares, T. K. dos A., Castro, D. P., & Genta, F. A. (2024). General aspects, host interaction, and application of Metarhizium sp. in arthropod pest and vector control. In *Frontiers in Fungal Biology* (Vol. 5, p. 1456964). Frontiers Media. https://doi.org/10.3389/ffunb.2024.1456964

de Paula, A. R., Silva, L. E. I., Ribeiro, A., da Silva, G. A., Silva, C. P., Butt, T. M., & Samuels, R. I. (2021). Metarhizium anisopliae blastospores are highly virulent to adult Aedes aegypti, an important arbovirus vector. *Parasites and Vectors*, *14*(1), 555. https://doi.org/10.1186/s13071-021-05055-z

Falah, M. F., Sukorini, H., Septia, E. D., Roeswitawati, D., & Fahmi, I. Z. (2024). Test of Entomopathogenic Fungus Metarhizium rileyi on Mortality of Main Pets of Cabbage (Brassica oleracea var capitata). *Journal of Global Sustainable Agriculture*, 84–92. https://doi.org/10.32502/jgsa.v5i1.303

Freeman, G. H., Gomez, K. A., & Gomez, A. A. (1985). Statistical Procedures for Agricultural Research. In *Biometrics* (2nd ed., Vol. 41, Issue 1). John Wiley & Sons. https://doi.org/10.2307/2530673

Gomes, S. A., Carolino, A. T., Teodoro, T. B. P., Silva, G. A., Bitencourt, R. de O. B., Silva, C. P., Alkhaibari,

A. M., Butt, T. M., & Samuels, R. I. (2023). The Potential of Metarhizium anisopliae Blastospores to Control Aedes aegypti Larvae in the Field. In *Journal of Fungi* (Vol. 9, Issue 7, p. 759). https://doi.org/10.3390/jof9070759

Gotti, I. A., Moreira, C. C., Delalibera, I., & De Fine Licht, H. H. (2023). Blastospores from Metarhizium anisopliae and Metarhizium rileyi Are Not Always as Virulent as Conidia Are towards Spodoptera frugiperda Caterpillars and Use Different Infection Mechanisms. *Microorganisms*, *11*(6), 1594. https://doi.org/10.3390/microorganisms11061594

Gunawan, J. F., Rahayuwati, S., Pratomo, B., & Afrianti, S. (2024). Pengaruh Formulasi Cendawan Entomopatogen Metharizium anisopliae(Metchnikoff) Sorokin dan Beauveria bassiana(Bals.-Criv.) Vuill terhadap Pertumbuhan dan Daya Tahan Hama Penyakit pada Bibit Kelapa Sawit (Pre nursery). *Agroprimatech*, *8*(2), 31–48. https://doi.org/10.34012/agroprimatech.v8i2.5409

Hajjar, M. J., Ahmed, N., Alhudaib, K. A., & Ullah, H. (2023). Integrated Insect Pest Management Techniques for Rice. *Sustainability (Switzerland)*, *15*(5), 4499. https://doi.org/10.3390/su15054499

Harahap, D. E., Wahyuni, S. H., Darwis, M., Mukhlis, & Mahmud, A. (2023). Trap Engineering Against The Effectiveness of Caught Imago Rhinoceros Beetle on Palm Oil Plants. *Jurnal Penelitian Pendidikan IPA*, *9*(3), 1518–1522. https://doi.org/10.29303/jppipa.v9i3.3014

Indriyanti, D. R., Putri, R. I. P., Widiyaningrum, P., & Herlina, L. (2017). Density, Viability Conidia and Symptoms of Metarhizium anisopliae infection on Oryctes rhinoceros larvae. *Journal of Physics: Conference Series*, *824*(1), 12058. https://doi.org/10.1088/1742-6596/824/1/012058

Indriyanti, D. R., Rahmawati, R., Priyono, B., Slamet, M., & Huyop, F. Z. (2018). Ecological studies of oryctes rhinoceros larvae controlled by metarhizium anisopliae and enthomopatogenic nematodes. *Jurnal Pendidikan IPA Indonesia*, *7*(3), 286–292. https://doi.org/10.15294/jpii.v7i3.14239

Indriyanti, D. R., Widiyaningrum, P., Haryuni, Slamet, M., & Maretta, Y. A. (2017). Effectiveness of Metarhizium anisopliae and entomopathogenic nematodes to control oryctes rhinoceros larvae in the rainy season. *Pakistan Journal of Biological Sciences*, *20*(7), 320–327. https://doi.org/10.3923/pjbs.2017.320.327

Ismanto, A., & Sukartana, P. (2016). Uji Efektivitas Isolat Jamur Entomopatogen Metarhizium anisopliae (Metsch.) Sorokin Terhadap Rayap Tanah Pada Pengujian Di Laboratorium Dan Lapangan. *Jurnal Penelitian Hasil Hutan*, *34*(4), 261–268. https://doi.org/10.20886/jphh.2016.34.4.261-268

Lei, C. J., Ahmad, R. H. I. R., Halim, N. A., Asib, N., Zakaria, A., & Azmi, W. A. (2023). Bioefficacy of an Oil-Emulsion Formulation of Entomopathogenic Fungus, Metarhizium anisopliae against Adult Red Palm Weevil, Rhynchophorus ferrugineus. *Insects*, *14*(5), 482. https://doi.org/10.3390/insects14050482

Li, J., & Xia, Y. (2022). Host–Pathogen Interactions between Metarhizium spp. and Locusts. In *Journal of Fungi* (Vol. 8, Issue 6, p. 602). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/jof8060602

Magfira, A. A., Himawan, A., & Tarmadja, S. (2022). Aplikasi Jamur Beauveria Bassiana Dan Metarhizium Anisopliae Untuk Pengendalian Hama Kumbang Tanduk (Oryctes Rhinoceros). *AGROISTA : Jurnal Agroteknologi*, *6*(1), 61–69. https://doi.org/10.55180/agi.v6i1.228

Mamahit, J. M. E., Montong, V. B., & Pakasi, S. E. (2024). Potential of Pangi Leaf Extract For Papaya Mealybug Control (Paracoccus marginatus). *Jurnal Penelitian Pendidikan IPA*, *10*(9), 6690–6694. https://doi.org/10.29303/jppipa.v10i9.9149

Manjunatha, C., Velavan, V., Rangeshwaran, R., Mohan, M., Kandan, A., Sivakumar, G., Shylesha, A. N., Kumar, M. K. P., Pramesh, D., Sujithra, M., Ranganath, H. K., & Sushil, S. N. (2023). Assessment of bio-formulations of indigenous strains of Bacillus thuringiensis, Metarhizium robertsii and Metarhizium majus for management of the rhinoceros beetle, Oryctes rhinoceros L., in field. *Egyptian Journal of Biological Pest Control*, *33*(1). https://doi.org/10.1186/s41938-023-00715-x

Maysaroh, U., Martono, E., & Harjaka, T. (2022). The Potency of Metarhizium anisopliae in Disturbing Oryctes rhinoceros (Coleoptera: Scarabaeidae) Growth and Development. *Jurnal Perlindungan Tanaman Indonesia*, *26*(1), 51. https://doi.org/10.22146/jpti.71755

Mejía, C., Rocha, J., Sanabria, J., Gómez-Álvarez, M. I., & Quiroga-Cubides, G. (2024). Performance of Metarhizium rileyi Nm017: nutritional supplementation to improve production and quality conidia. *3 Biotech*, *14*(3), 89. https://doi.org/10.1007/s13205-023-03911-6

Nik, N., & Rusae, A. (2024). The Influence of Several Host Types on the Balance of Life Sitophilus Sp. *Jurnal Penelitian Pendidikan IPA*, *10*(4), 1916–1924. https://doi.org/10.29303/jppipa.v10i4.6925

Nik, N., Rusae, A., Tasekab, O., Bano, F., Pais, Y. O., & Tanii, Y. (2023). Identification and Control Model for Pest Organisms in Cabbage Plants. *Jurnal Penelitian Pendidikan IPA*, *9*(SpecialIssue), 1112–1120.

https://doi.org/10.29303/jppipa.v9ispecialissue.7047

Pramudya, M., Hayati, A., Armando, D. S., Wulansari, E., Faridah, N., & Susilo, R. J. K. (2021). Toxicity of copper pollution on sperm quality of Cyprinus carpio. *IOP Conference Series: Earth and Environmental Science*, *718*(1), 12019. https://doi.org/10.1088/1755-1315/718/1/012019

Prastowo, J., Susanto, A., & Hidayat, P. (2022). Patogenisitas Metarhizium anisopliae terhadap larva Oryctes rhinoceros. *Jurnal Perlindungan Tanaman Indonesia*, *26*(1), 14–22.

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. https://doi.org/10.1007/s10340-023-01622-8

Quiroga-Cubides, G., Borrero-Echeverry, F., Jiménez, A. M., Montes-Bazurto, L. G., Pardey, A. B., Gómez, M. I., & Cuartas-Otálora, P. E. (2024). Initial Characterisation Of Metarhizium anisopliae CPMa1502 For The Development Of A Biopesticide Against The Oil Palm Fruit Scraper Demotispa neivai (Coleoptera: Chrysomelidae). *Journal of Oil Palm Research*, *36*(1), 40–50. https://doi.org/10.21894/jopr.2022.0076

Rajak, D. C., Sinha, O. K., & Singh, V. (2010). Effect of Different Media on Growth and Sporulation of Metarhizium anisopliae , a Fungal Bioagent. *Journal of Biological Control*, *24*(1), 80–81.

Ramalho, M. D. O., Kim, Z., Wang, S., & Moreau, C. S. (2021). Wolbachia across Social Insects: Patterns and Implications. *Annals of the Entomological Society of America*, *114*(2), 206–218. https://doi.org/10.1093/aesa/saaa053

Santi, I. S., Ahmad, N. F., Elfatma, O., & Hasanah, N. A. U. (2022). Bio-Use Power of Insecticide Metarizhium anisopliae in Controlling Oryctes rhinoceros in Palm Oil. *Tropical Plantation Journal*, *1*(1), 30–34. https://doi.org/10.56125/tpj.v1i1.5

Saragih, S. A., Takemoto, S., & Kamata, N. (2025). Specific Primer Design for Detection and Quantification of Entomopathogenic Fungi Metarhizium anisopliae using Quantitative PCR (qPCR) in Soil and Cocoon Samples. *Agrivita*, *47*(1), 168–175. https://doi.org/10.17503/agrivita.v47i1.4644

Sirait, D. D. N., Tobing, M. C., & Safni, I. (2023). Genetic diversity of the entomopathogenic Metarhizium anisopliae (Metsch.) from oil palm planting soil based on RAPD markers. *Jurnal Entomologi Indonesia*, *20*(1), 22–39. https://doi.org/10.5994/jei.20.1.22

Soriano, E. A., & Adion, I. M. (2023). Growth response of Metarhizium anisopliae in indigenous media and efficacy to control rice black bug under greenhouse condition. *International Journal of Agricultural Technology*, *19*(2), 721–732. https://doi.org/10.5555/20230213063

Sormin, F., & Junaedi, A. (2017). Manajemen Pengendalian Gulma Kelapa Sawit Berdasarkan Kriteria ISPO dan RSPO di Kebun Rambutan Sumatera Utara. *Buletin Agrohorti*, *5*(1), 137. https://doi.org/10.29244/agrob.5.1.137-145

Sucipto, I., Muhlison, W., & Putri, A. P. (2025). Viability of Entomopathogenic Fungi (Metarhizium anisopliae) in Residual Media Maggots and Pupa Shell Waste. *Jurnal Penelitian Pendidikan IPA*, *11*(1), 978–985. https://doi.org/10.29303/jppipa.v11i1.9603

Supriatno, Jannah, R., Safrida, Hafnati, & Samingan. (2023). Toxicity Test of Shallot Skin Extract (Allium ascalonicum) on Mortality of Leaf Roller Caterpillar (Spoladea recurvalis). *Jurnal Penelitian Pendidikan IPA*, *9*(11), 9474–9480. https://doi.org/10.29303/jppipa.v9i11.4566

Suryadi, B. F., Mustikasari, I., Annisa, Z., Sarkono, & Tresnani, G. (2024). Fishmeal-Based Media Supports Growth and Endospore Production of Locally-Isolated Lysinnibacillus sphaericus and Induces its Toxicity to 3rd Instar Aedes aegypti Larvae in Laboratory Conditions. *Jurnal Penelitian Pendidikan IPA*, *10*(7), 3613–3621. https://doi.org/10.29303/jppipa.v10i7.7361

Villamizar, L. F., Barrera, G. P., Luange, A., Sagata, K., Gende, P., Chris, S., Tsatsia, H., Mudu, F., Weston, M., van Koten, C., Mansfield, S., Jackson, T. A., & Marshall, S. D. G. (2024). Characterization and screening of new Metarhizium isolates to control the coconut rhinoceros beetle in the Pacific islands. *Fungal Biology*, *128*(7), 2127–2138. https://doi.org/10.1016/j.funbio.2024.08.009

Wang, J., Lovett, B., & St. Leger, R. J. (2019). The secretome and chemistry of Metarhizium; a genus of entomopathogenic fungi. *Fungal Ecology*, *38*, 7–11. https://doi.org/10.1016/j.funeco.2018.04.001

Yang, H., Qiu, H., yan Tian, L., Xiao, L.-N., Ling, S., Qin, C., & Xu, J. (2024). The Optimization of Amino Acids in the Culture Medium of Metarhizium Anisopliae Through Liquid Chromatography-Mass Spectrometry (Lc-Ms) and Response Surface Analysis (Rsm). *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.4847535

Yousef-Yousef, M., Romero-Conde, A., Quesada-Moraga, E., & Garrido-Jurado, I. (2022). Production of Microsclerotia by Metarhizium sp., and Factors Affecting Their Survival, Germination, and Conidial Yield. *Journal of Fungi*, *8*(4), 402. https://doi.org/10.3390/jof8040402

Zhang, Q., Wei, X., Fang, W., Huang, X., & Zhang, X. (2024). The secretory protein COA1 enables Metarhizium robertsii to evade insect immune recognition during cuticle penetration. *Communications Biology*, *7*(1), 1220. https://doi.org/10.1038/s42003-024-06827-w