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Natural Occurrence of *Metarhizium rileyi* on *Darna diducta* as a Biological Control for Oil Palm Pests in Poso, Central Sulawesi

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ABSTRACT

The nettle caterpillar, *Darna diducta*, is a significant pest in oil palm plantations, causing damage to palm fronds that impacts overall plant health and yield. Traditionally, synthetic insecticides are used to manage this pest; however, such intensive chemical use has led to adverse effects, including pest resistance, resurgence, and environmental degradation. Entomopathogenic fungi like *Metarhizium rileyi* (formerly *Nomuraea*) represent a promising, environmentally friendly alternative for pest control. This study focused on the natural occurrence of *M. rileyi* infections in *D. diducta* larvae within oil palm plantations in East Pamona, Poso Regency, Central Sulawesi, observed in August 2015. Surveys conducted across several villages found a remarkable 100% infection rate of *D. diducta* larvae by *M. rileyi*, with each infected larva exhibiting extensive fungal growth, characterized by white mycelium covering the entire body. The resulting impact on palm frond damage was minimal, with the percentage of plant damage due to *D. diducta* categorized as mild, ranging from only 0.24% to 0.34%. These findings indicate that *M. rileyi* effectively suppresses *D. diducta* populations, reducing pest severity and frond damage. The high infection rate and its impact on pest control underscore *M. rileyi*'s potential as a natural biocontrol agent, offering a sustainable approach to managing *D. diducta* infestations. Thus, integrating *M. rileyi* as a biological control method could significantly benefit oil palm cultivation by minimizing chemical use, promoting ecological balance, and supporting long-term pest management strategies.

Keywords: Biological control, entomopathogen fungi, oil palm, pest

INTRODUCTION

Oil Palm Commodity is a sensibly crucial annual plant because it contains lofty vegetable oil and is one of the world's main oils. Oil palm produces vegetable oil three times higher than coconut oil, seven times higher than rapeseed oil, and almost ten times higher than soybean oil (Nair, 2021). The increasing expansion of oil palm plantations in Indonesia has resulted in extensive monoculture crops. The expansion of monoculture crops is related to the potential for pests to emerge. Oil palm leaf-eating caterpillars are one of the main pests of the Lepidoptera group, such as nettle caterpillars, hairy caterpillars, and bagworms (Prawiratama et al., 2018). The leaf-eating caterpillar population explosion almost always occurs yearly and is generally dominated by nettle caterpillar attacks. Leaf-eating caterpillars of the Lepidoptera family (nettle caterpillars, bagworms, and hairy caterpillars) that attack oil palm trees cause yield losses of up to 50% (Kalidas, 2012).

Integrated Pest Management (IPM) is a strategy to reduce pest populations. Biological control is an effective and environmentally friendly alternative. One of the biological controls is the application method of entomopathogenic fungi (Mantzoukas & Eliopoulos, 2020). Biological control is an alternative to synthetic chemical products, using parasitoid biological agents, predators, and entomopathogenic microorganisms against insect pests (Tiago et al., 2014). Environmentally friendly pest control can help reduce the use of synthetic insecticides, and biological agents in the form of viral microorganisms, bacteria, and fungi can be an excellent alternative control (Samada & Tambunan, 2020). Unlike bacteria and viruses as insect pathogens, entomopathogenic fungi can infect pests by producing enzymes and penetrating the host cuticle. Entomopathogenic fungi are appropriate for biological control (Skinner et al., 2014). According to Lopes et al. (2018), entomopathogenic fungi have high genetic variability, infect their hosts at every stage, work through contact with insect integuments, and have a high propagule dispersal capacity. Entomopathogenic fungi have hyphae to penetrate host organisms (insects) and spores to transmit effectively from one host to the next, and many species produce toxic compounds (mycotoxins). In some insect orders, the nymph and larval stages are more susceptible to fungal infections than the adult stages (Maina et al., 2017).

Entomopathogenic fungi are biological agents that occur naturally in ecosystems, as with the fungus *Metarhizium rileyi*. The fungus *M. rileyi* has a cosmopolitan occurrence that infects insect pests, especially Lepidoptera larvae. *M. rileyi* (Farlow) Samson (Hypocreales: Clavicipitaceae), formerly known as *Nomuraea rileyi* (Kepler et al., 2014), is an entomopathogenic fungus that can effectively control several pests of the order Lepidoptera. In laboratory tests by Stefanelli et al. (2021), *M. rileyi* caused around 60-80% mortality against *Spodoptera litura*. *M. rileyi* has been reported to infect armyworms *Spodoptera frugiperda* that attack corn plantations in Tomohon (Siahaan & Mullo, 2021). Some Lepidoptera pests infected by *M. rileyi* are *Spodoptera litur*a (Liu et al., 2019; Namasivayam & Bharani, 2015), *Spodoptera frugiperda* (Cruz-Avalos et al., 2019; Ramanujam et al., 2020; Montecalvo & Navasero, 2021), *Spodoptera exigua* (Montecalvo et al., 2022a), *Helicoverpa armigera* (da Costa et al., 2015), *Anticarsia gemmatalis* and Chrysodeixis includes (Lopes et al., 2020).

Finally, *Metarhizium rileyi* is safe for the environment and does not risk non-target insects so that it can be used as an alternative integrated pest control program. This study examines the occurrence of natural control by the fungus *M. rileyi* and plant damage due to nettle caterpillar *Darna diducta* attacks on immature oil palm.

METHODS

Time and Place of The Study

This study was conducted in the periode August 2015 in the oil palm plantation area in East Pamona District, Poso Regency. Entomopathogenic fungi were isolated and identified in the Laboratory of the Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang, East Java.

Sampling and Observation

This study was a survey that determined sample plants by purposive sampling. The survey was conducted randomly and systematically, exploring the oil palm plantation area in each location (block), and then 30 sample plants were determined per location. The age of the oil palm plants as samples was five years. The survey locations were Matialemba, Taripa, Petiro villages, Pamona Timur District, and Poso Regency. We observe living and dead *Darna diducta* larvae due to entomopathogenic fungal infection on each oil palm leaf sheath. Furthermore, isolation and identification of entomopathogenic fungi that infect *D. diducta* larvae were carried out in the Laboratory of the Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang, East Java.

Calculate the percentage of *D. diducta* larvae infected with entomopathogenic fungi at each location to determine the incidence of infection due to entomopathogenic fungi.

Conducting non-absolute (varied) plant damage calculations. Damage to oil palm leaf sheaths due to *D. diducta* larvae bites. Wagiman (2011) and Lahati & Saifudin (2022) stated that the level of damage is divided into five criteria: healthy, mild, moderate, severe, and very severe. The scale value of damage to each oil palm plant is determined in Table 1. Non-absolute damage is damage to parts of the plant, such as leaves, flowers, fruit, twigs, branches, and stems. To calculate non-absolute plant damage, you can use the formula:

$$I = \{(\Sigma n \times v) \div (Z \times N)\} \times 100\%$$

Remarks:

I= Damage Intensity (%)
n= Number of plants or plant parts with the v-scale
v= Plant damage scale value
N= Number of sample plants or plant parts observed
Z= Highest damage scale value.

Entomopathogenic fungi were observed by observing *D. diducta* larvae that died from infection by pathogens on oil palm leaf sheaths. Samples of larvae infected with pathogens were taken to the laboratory to be isolated and identified morphologically using a binocular microscope, based on Dutta et al. (2014).

Table 1. Leaf Damage Intensity Scale

Attack category	Symptomps of leaf sheath damage	Scale
Healthy	There was no damage to leaf shealth	0
Mild	There is damage to the leaf shealth with an intensity of 1 – 25%	1
Moderate	There is damage to the leaf shealth with an intensity of 25 – 50%	2
Severe	There is damage to the leaf shealth with an intensity of 50 – 75%	3
Very severe	There is damage to the leaf shealth with an intensity of 75 – 100%	4

RESULTS AND DISCUSSION

Isolation and Identification of Entomopathogenic Fungi Infecting Darna (Ploneta) diducta

Entomopathogenic fungi were successfully isolated from *D. diducta* larvae. Based on the development of entomopathogenic fungi in potato dextrose agar (PDA) media and morphological characteristics, the fungal pathogen that infects *D. diducta* larvae is *Metarhizium (Nomuraea) rileyi* (Figure 1). Entomopathogenic fungi are insect pathogens that can infect their hosts by attaching to the host's body surface and then penetrating through the cuticle and orally (Mannino et al., 2019). Morphologically, *M. rileyi* is septate, transparent, and has branched hyphae. Branched conidiophores are formed near the septa, with 2-6 phialides. Phialides are short cylindrical, have smooth walls, and are colorless (transparent). Conidia appear in chains, are very oval, have smooth walls, and are transparent. These results are in line with the research of Dutta et al. (2014). *M. rileyi* has macroscopic morphological characteristics with white colony color in the early phase of vegetative growth and is rounded in shape. During the generative phase the colony color changes to dark, the shape of the conidia is oval; the conidiophores are straight and septate, branches grow near the septate, smooth-walled and hyaline ((Siahaan & Mullo, 2021). Furthermore, Montecalvo et al. (2022b) stated that the entomopathogenic fungus *M. rileyi*, which was successfully isolated from *Spodoptera frugiperda* larvae, has morphological characteristics of white to olive green, oval, and transparent conidia.

Figure 1. Active isolate of *Metarhizium rileyi* isolated from *Darna diducta* larvae: (a) Various growths of *M. rileyi* on Potato Dextrose Agar (PDA) media and (b) *M. rileyi* fungus at 400x magnification.

Natural Occurrence of Entomopathogenic Fungal Infection in Darna (Ploneta) diducta Larvae

Symptoms of initial attack by *D. diducta* when the larvae are still young are indicated by the erosion of the epidermis layer from the underside of the leaf; then, further attacks will cause holes and disappear, and finally, only the leaf veins remain. The increase in the population of *D. diducta* caterpillars can cause defoliation and yield losses. The natural occurrence of entomopathogenic fungi infecting *D. diducta* larvae that have been identified as *Metarhizium (Nomuraea) rileyi* reached 100% in Taripa, Petiro, and Matialemba villages (Table 2). From the survey results and observations, no healthy *D. diducta* larvae were found on oil palm leaf sheaths. This natural infection was also reported to occur in *Spodoptera frugiperda* larvae, invasive corn plant pests. Ginting et al. (2020) found a natural occurrence of *M. rileyi* infecting *Spodoptera frugiperda* larvae in corn plantations in Bengkulu.

Table 2. Percentage of Natural Infection Incidents of *Metarhizium rileyi* on *Darna diducta* Larvae and Plant Damage in East Pamona

Location (Village)	Elevation (m)	Coordinate	Average Damage		Infection Incident *M. rileyi* (%)
			%	Category	
Taripa	418	1°55'45"S 120°49'37"E	0.26	Mild	100
Taripa	420	1°56'13"S 120°49'35"E	0.25	Mild	100
Taripa	422	1°56'43"S 120°49'33"E	0.24	Mild	100
Taripa	417	1°57'13"S 120°50'05"E	0.34	Mild	100
Petiro	419	1°56'35"S 120°50'06"E	0.28	Mild	100
Petiro	417	1°56'56"S 120°50'40"E	0.34	Mild	100
Matialemba	421	1°56'18"S 120°51'06"E	0.32	Mild	100
Matialemba	420	1°56'47"S 120°51'14"E	0.30	Mild	100
Matialemba	417	1°57'06"S 120°51'40"E	0.27	Mild	100

Up to this time, the highest natural infection of D. ductus larvae by M. rileyi fungus was 79.0% in Bukit Barisan Village, then in Pulo Geto Baru Village at 26% and Taba Mulan Village, Merigi District, Kapahiang Regency at 5.3%. The lowest natural infection incidence was 1% in Sidomulyo Village, Seluma District, Seluma Regency. The potential of M. rileyi on Lepidoptera was also reported by Mallapur et al. (2018) on Spodoptera frugiperda in corn plantations, with a natural infection incidence reaching 11.87-18.30%. The M. rileyi fungus infects 38% of several instars of S. frugiperda larvae in corn plantations (Visalakshi et al., 2020).

The average damage to oil palm plants due to *D. diducta* ranged from 0.24 - 0.34%, still in the mild category (Table 2). The natural occurrence of *M. rileyi* infection can prevent an early increase in the *D. diducta* population. *D. diducta* that died from natural *M. rileyi* infection were instar 3 - 5 larvae. Larvae infected with entomopathogenic fungi were found in 1 - 3 larvae/oil palm leaf sheath. Montecalvo and Navasero (2020) stated that local isolates of *M. rileyi* originating from *Spodoptera exigua* larvae in corn plantations in the Philippines caused significant mortality in instar three larvae. The time required to kill the host can be associated with increased cuticular defense, which may impact conidiophore attachment, germination, and penetration. The ability of the cuticular defense increases with increasing larval instar (Liu et al., 2019). The cuticle is the main barrier in the infection process of entomopathogenic fungi in host insects (Keyhani, 2018).

Therefore, rapid penetration in penetrating the cuticle can affect the speed of time in killing host insects directly from the cuticle so that the time required to kill host insects is shorter; this is very important and related to the pathogenicity of the entomopathogenic fungi themselves (Dar et al., 2017). Areas suitable for fungal development will cause natural and epizootic infections. This is also likely due to environmental conditions supporting entomopathogenic fungi development. Geographical location, climate, habitat, altitude, and soil pH or organic matter affect the presence of entomopathogenic fungal species. Choudhary et al. (2012) reported that temperature, humidity, and rainfall are essential in entomopathogenic fungi's occurrence, distribution, prevalence, and effectiveness. Unlike synthetic pesticide applications, entomopathogenic fungi can survive in nature, thus reducing control applications, saving time, costs, and energy, and maintaining a sustainable environment. The highest incidence of *S. frugiperda* larvae infected with *M. rileyi* is influenced by high rainfall and humidity, which is suitable for the growth and development of the fungus. This causes the highest incidence of *S. frugiperda* larvae infection by *M. rileyi.* In addition, raindrops help conidia reach the leaf surface where insects forage, resulting in a primary infection (Ginting et al., 2022).

Entomopathogenic fungi are living organisms. Therefore, they are susceptible to a series of factors that can affect their effectiveness, such as temperature, humidity, sunlight radiation, and the application of synthetic chemical pesticides. In line with the opinion of Barra-Bucarei et al. (2019), who stated that most entomopathogenic fungi can germinate and develop adequately between 22 and 28 °C, while humidity is the most essential thing for conidia germination, most entomopathogenic fungal species are hydrophilic. Solar radiation has wavelengths, such as UVA and UVB, affecting entomopathogenic fungi's effectiveness. Short wavelengths can delay or suppress conidial germination. Using synthetic chemical pesticides can affect spore production and the performance of entomopathogenic fungi as bioclogi control.

Description of Mycosis in Darna (Ploneta) diducta

Infection of the fungus *Metarhizium (Nomuraea) rileyi* was observed in several *D. diducta* larvae in early August 2015; the fungus *M. rileyi* infected *D. diducta* larvae reaching 100% in instar larvae 3-5. Microscopically, the entire body surface of *D. diducta* larvae is colonized by white *M. rileyi* mycelium. (Figure 2). In line with the results of the study by Visalakshi et al. (2020), stereomicroscopic observations of mycosis have shown complete colonization of the prothorax, mesothorax, metathorax, and all abdominal segments filled with white mycelial growth from the fungus, there were only differences in the head capsule, chest shield, setae, and crotchet of *S. frugiperda* no mycelium and conidia of *M. rileyi* were found. In this study, fungal mycelium and conidia were found to appear on the body surface of *D. diducta* larvae. This revealed that in the early stages, *D. diducta* larvae were colonized by the mycelial growth of the fungus and then invaded insect tissue and produced many conidia in humid conditions. Infected larvae eventually died and often did not reach adulthood. The mycelium had penetrated further into the insect cuticle and other holes and invaded all host tissues.

Most *M. rileyi* isolates are dimorphic hyphomycetes that cause epizootic mortality in various Lepidoptera insect pest species (Sinha et al., 2016). This entomopathogenic fungus infects its host by attaching to the cuticular surface, tissue invasion, enzymatic activity, and toxicosis (Fronza et al., 2017).

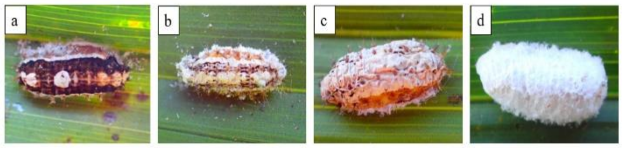

Figure 2. Various Forms of Entomopathogenic Fungal Mycosis *Metarhizium (Nomuraea) rileyi* Naturally in *Darna (Ploneta) diducta* Larvae

CONCLUSIONS

This study demonstrates the potential of *Metarhizium rileyi* as an effective biological control agent against *Darna (Ploneta) diducta*, a leaf-eating caterpillar, in oil palm plantations. The interaction study revealed that natural infection rates of *D. diducta* larvae by *M. rileyi* reached 100% in Pamona Timor, Poso, indicating the fungus's capacity to significantly reduce pest populations without relying on chemical insecticides. This reduction in pest-induced plant damage to a mild category level (0.24% to 0.34%) further underscores *M. rileyi*'s effectiveness as a sustainable alternative within integrated pest management (IPM) strategies for oil palm cultivation.

By minimizing the use of chemical insecticides, *M. rileyi* not only supports environmentally friendly pest control but also reduces potential risks to human health and non-target organisms. Future research should focus on refining the application methods for *M. rileyi*, exploring its efficacy across varying environmental conditions, and determining optimal fungal concentrations and formulations for field application. Additionally, investigating the incorporation of *M. rileyi* into specific IPM practices, such as targeted applications during peak pest infestations, could enhance its impact and longevity as a pest control measure. With further development, *M. rileyi* could become a cornerstone in sustainable pest management for oil palm plantations, contributing to the long-term health of both the ecosystem and agricultural productivity

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