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MANAGEMENT CONTROL OF THE RED COFFEE BORER, *Zeuzera* spp. (LEPIDOPTERA: COSSIDAE) ON *Eucalyptus pellita* F. MUELL. USING ENTOMOPHATOGENIC FUNGI

Mohamad Suheri[1,4], Ruly Anwar[2*], Noor Farikhah Haneda[3], Jung Yoon Hwa[4] & Shinichi Sukeno[4]

[1]Program Tropical Silviculture
Faculty of Forestry and Environment, IPB University
Jl. Ulin Lingkar Akademik, Dramaga,
Bogor 16680, Indonesia
[2]Department of Plant Protection
Faculty of Agriculture, IPB University
Jl. Meranti, Kampus IPB Dramaga,
Bogor 16680, Indonesia
[3]Department of Silviculture
Faculty of Forestry and Environment, IPB University
Jl. Ulin, Kampus IPB, Dramaga,
Bogor 16680, Indonesia
[4]Research and Development
PT. Korintiga Hutani
Camp Pellita, Nanga Mua,
Kab Kotawaringin Barat 74152, Indonesia
*Corresponding author: *ranwar@apps.ipb.ac.id*

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ABSTRACT

The Red Coffee Borer, *Zeuzera* spp. (Lepidoptera: Cossidae), is one of the major insect pests of *Eucalyptus pellita* plantations. The larvae may cause holes in stems, thereby reducing log productivity and have difficulty to control the larval stage. The use of biological agents has been carried out, however the application of nematode group as agent is still limited. Integrated Pest Management (IPM) strategy, including biological agents by fungus group, may have potential to control *Zeuzera* spp. by providing the optimal results. This study aims to analyze the potential use of entomopathogenic fungi (*Beaveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium majus*) in infecting the larvae of *Zeuzera* spp. in the laboratory and field scale. In the laboratory scale, a non-factorial completely randomized design implemented with 13 treatments and three replications (the total larva of *Zeuzera* was 78 samples). In the field scale, a randomized block design with one factor (species of entomopathogen) and four planting areas. The application of *B. bassiana*, *M. majus*, and *M. anisopliae* with different conidial densities (10^8 conidial ml^{-1}, 10^6 conidial ml^{-1}, 10^4 conidial ml^{-1}, 10^2 conidial ml^{-1}, and control) had a significant effect on the mortality of *Zeuzera* spp. larvae. Entomopathogenic *B bassiana*

at 10^8 ml^{-1} causes the highest infection rate of *Zeuzera* spp. Compare two other fungi. However, there was no significant effect of the application of three species of entomopathogenic fungi on *Zeuzera* spp. Larvae in young *E. pellita* in the field. Results from the analysis concluded that entomophatogens have given positive results and potential to control *Zeuzera* spp. larvae.

Keywords*: Beauveria*, fungus, infection, inoculation, *Metarhizium*

ABSTRAK

Ulat Pengorek Kopi Merah, *Zeuzera* spp. (Lepidoptera: Cossidae) merupakan salah satu serangga perosak utama di ladang *Eucalyptus pellita.* Larva serangga ini membuat lubang pada batang, seterusnya mampu mengurangkan produktiviti kayu balak dan ianya sukar dikawal. Penggunaan agen biologi telah dijalankan tetapi hanya kepada kumpulan nematod dan ianya masih terhad. Strategi Pengurusan Perosak Bersepadu (PBB), termasuk agen biologi daripada kumpulan kulat, berkemungkinan mempunyai potensi untuk mengawal *Zeuzera* spp. dan mampu memberikan hasil yang optimum. Kajian ini bertujuan untuk menganalisis potensi penggunaan kulat entomopatogen (*Beauveria bassiana*, *Metarhizium anisopliae* dan *Metarhizium majus*) dalam menjangkiti larva *Zeuzera* spp. dalam skala makmal dan lapangan. Dalam skala makmal, reka bentuk rawak sepenuhnya bukan faktorial digunapakai. Terdapat 13 rawatan dan tiga ulangan (jumlah larva *Zeuzera* ialah 78 sampel). Dalam skala lapangan, penggunaan reka bentuk blok rawak dengan satu faktor (spesies entomopatogen) dan empat kawasan penanaman. Penggunan *B. bassiana*, *M. majus*, dan *M. anisopliae* dengan kepadatan konidia yang berbeza (10^8 konidia ml^{-1}, 10^6 konidia ml^{-1}, 10^4 konidia ml^{-1}, 10^2 konidia ml^{-1}, dan kawalan) mempunyai kesan signifikan ke atas kematian larva *Zeuzera* spp. entomopatogen *B. bassiana* dengan kepadatan konidia 10^8 ml^{-1} yang menyebabkan tahap jangkitan tertinggi pada larva *Zeuzera* spp. jika dibandingkan dengan dua jenis kulat yang lain. Walau bagaimanapun, tiada kesan signifikan penggunaan tiga spesies kulat entomopatogen pada larva *Zeuzera* spp. dalam *E. pelita* belum matang di ladang. Keputusan daripada analisis menyimpulkan bahawa entomopatogen telah memberikan hasil yang positif dan berpotensi dalam menjangkiti dan mengawal larva *Zeuzera* spp.

Kata kunci: *Beauveria*, fungus, infection, inoculation, *Metarhizium*

INTRODUCTION

The development of plantation forests help satisfy demand for wood resources which cannot be supported by the limited increment of natural forests (Yudistira et al. 2019). Problems arise, one of them by the most destructive insect pests, *Zeuzera* spp. (Tavares et al. 2020). The Red Coffee Borer, *Zeuzera* spp. is a group of insects from the Cossidae family in the larval stage, which becomes stem borers of their host plants including *Eucalyptus pellita* (Suheri et al. 2020). The newly hatched larvae immediately search for young shoots and branches and then begin to enter by boring and damaging from the inside (Monteys 2015). Insect attacks cause host plants to wither and die (Almanoufi et al. 2012). This insect significantly impacted loss and damage to *E. pellita* plantation. However, their position in the stem is very difficult to control (Ibrahim et al. 2019). In this regard, a strategy is needed to manage these insect pests.

Integrated Pes Management (IPM) control is one of the concepts of controlling insect pests by integrating various insect pest control methods, one of which is using biological agents and monitoring activities (Arnaudov et al. 2012). The monitoring output is to find information on trends and pest insect populations for management decision-making (Suheri et al. 2022). Using the entomopathogens fungi are an alternative to control insect of agriculture and urban pests (Nyam et al. 2021). This reason of using this method are sustainable and environmentally friendly and tends to be more specific to target insects. Biological agents not only control insect pests at this time but conidial will survive in locations that support and cause natural epizootic and control pest outbreak conditions (Caron et al. 2023). Although entomopathogens can control insect pests less than chemicals, their effectiveness still has potential and allows long-term use (Sabbahi et al. 2022). However, research on potential entomopathogens against *Zeuzera* spp. larvae is still sparse. This condition is the background for us to conduct this research. This study aimed to analyze the potential use of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Metarhizium majus* in infecting *Zeuzera* spp. larvae. in *E. pellita* plantation forest.

MATERIALS AND METHODS

Time and Place of Research

Research on fungal entomopathogens against *Zeuzera* spp. on *E. pellita* was carried out from May 2022 to November 2022. The research locations were in Block planting 5, Block planting 6, and the Laboratory of Pests and Diseases Perizinan Berusaha Pemanfaatan Hutan (PBPH) PT. Korintiga Hutani, West Kotawaringin, Central Kalimantan, Indonesia (1110 51' 29.859"-20 11'29.814").

Collecting Larva *Zeuzera* spp. and Propagating Entomopathogenic

Zeuzera spp. larvae from various stages were collected from *E. pellita* trees and reared in chopped fresh *E. pellita* stems in plastic containers (25 cm x 30 cm x 8 cm). The fungus *B. bassiana* (East Java), *M. majus* (West Kalimantan), and *M. anisopliae* (West Kalimantan) were grown on potato dextrose agar (PDA) medium until to fill the entire petri dish area (14 - 21 days). Furthermore, three isolates of fungal entomopathogens were given 20 ml of aquadest, and the contacted between *Zeuzera* spp. and entomopathogens suspension lasted 3 minutes. The larvae of *Zeuzera* spp. larvae returned to container by providing fresh chopped *E. pellita* wood. Infected larvae were surface disinfected with 70% alcohol and isolated directly on PDA media as material propagates. Propagated entomopathogenic isolate from *Zeuzera* spp. larvae on PDA medium using sterile ground corn media with 200 grams/pack packaging. Entomopathogenic fungus was cultured on ground corn media, and it can be used for testing at 21 days of age (Figure 1).

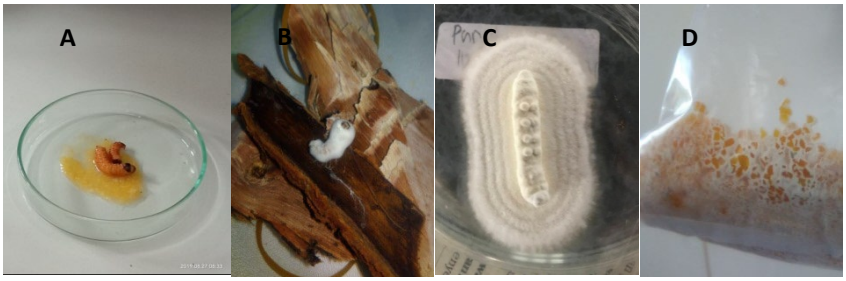

Figure 1 A). Entomophatogenic fungus isolated from infected *Zeuzera* spp., B). *Zeuzera* spp. larvae infected with the entomopathogenic fungus *B. bassiana* with signs that the

whole body is covered with powdery white mycelia. C). Larvae infected with *B. bassiana* were isolated on PDA media to obtain f0. D). Propagation of *B. bassiana* isolates from *Zeuzera* spp. on sterile ground corn media.

Application of Entomopathogenic Fungi with Different Conidial Density

This study was used a completely randomized non-factorial design. There were 13 treatments with 3 replications on *Zeuzera* spp larvae. namely, *B. bassiana* 10^8 conidial ml^{-1}, *B. bassiana* 10^6 conidial ml^{-1}, *B. bassiana* 10^4 conidial ml^{-1}, *B. bassiana* 10^2 conidial ml^{-1}, *M. majus* 10^8 conidial ml^{-1}, *M. majus* 10^6 conidial ml^{-1}, *M. majus* 10^4 conidial ml^{-1}, *M. majus* 10^2 conidial ml^{-1}, *M. anisopliae* 10^8 conidial ml^{-1}, *M. anisopliae* 10^6 conidial ml^{-1}, *M. anisopliae* 10^4 conidial ml^{-1}, *M. anisopliae* 10^2 conidial ml^{-1}, and control. The experimental unit was *Zeuzera* spp. larvae test insect. The total experimental unit of test insects in this experiment was 78 *Zeuzera* spp. larvae. The variables of this experiment were the growth and development of the entomopathogenic fungi on *Zeuzera* spp. larvae, the percentage of mortality, and the symptoms and signs of insects infected with the entomopathogenic fungi (Figure 2 and Table 1).

MA8.2	BB2.2	MM6.3	K.1	MA8.1	MM4.2	MA2.2	MM4.1	MA8.3	MM8.1	MA2.3	MM6.1	MA6.3
BB4.3	BB6.2	BB4.2	MM2.3	MM4.3	MM2.1	MA4.1	BB6.1	BB2.3	BB8.1	K.2	BB6.3	MM2.2
BB8.3	BB8.2	MA6.2	K.3	MA6.1	MM6.2	MM8.3	MA4.2	BB2.1	MA2.1	MA4.3	BB4.1	MM8.2

Figure 2. Chart of experimental design

Table 1. Detailed treatment of *Zeuzera* spp. by entomophatogenic fungus

Code	Treatment
BB8.1	*B. bassiana* 10^8 conidia ml^{-1}
BB8.2	*B. bassiana* 10^8 conidia ml^{-1}
BB8.3	*B. bassiana* 10^8 conidia ml^{-1}
BB6.1	*B. bassiana* 10^6 conidia ml^{-1}
BB6.2	*B. bassiana* 10^6 conidia ml^{-1}
BB6.3	*B. bassiana* 10^6 conidia ml^{-1}
BB4.1	*B. bassiana* 10^4 conidia ml^{-1}
BB4.2	*B. bassiana* 10^4 conidia ml^{-1}
BB4.3	*B. bassiana* 10^4 conidia ml^{-1}
BB2.1	*B. bassiana* 10^2 conidia ml^{-1}
BB2.2	*B. bassiana* 10^2 conidia ml^{-1}
BB2.3	*B. bassiana* 10^2 conidia ml^{-1}
MM8.1	*M. majus* 10^8 conidia ml^{-1}
MM8.2	*M. majus* 10^8 conidia ml^{-1}
MM8.3	*M. majus* 10^8 conidia ml^{-1}
MM6.1	*M. majus* 10^6 conidia ml^{-1}
MM6.2	*M. majus* 10^6 conidia ml^{-1}
MM6.3	*M. majus* 10^6 conidia ml^{-1}
MM4.1	*M. majus* 10^4 conidia ml^{-1}
MM4.2	*M. majus* 10^4 conidia ml^{-1}
MM4.3	*M. majus* 10^4 conidia ml^{-1}
MM2.1	*M. majus* 10^2 conidia ml^{-1}
MM2.2	*M. majus* 10^2 conidia ml^{-1}

MM2.3	*M. majus* 10^2 conidia ml^{-1}
MA8.1	*M. anisopliae* 10^8 conidia ml^{-1}
MA8.2	*M. anisopliae* 10^8 conidia ml^{-1}
MA8.3	*M. anisopliae* 10^8 conidia ml^{-1}
MA6.1	*M. anisopliae* 10^6 conidia ml^{-1}
MA6.2	*M. anisopliae* 10^6 conidia ml^{-1}
MA6.3	*M. anisopliae* 10^6 conidia ml^{-1}
MA4.1	*M. anisopliae* 10^4 conidia ml^{-1}
MA4.2	*M. anisopliae* 10^4 conidia ml^{-1}
MA4.3	*M. anisopliae* 10^4 conidia ml^{-1}
MA2.1	*M. anisopliae* 10^2 conidia ml^{-1}
MA2.2	*M. anisopliae* 10^2 conidia ml^{-1}
MA2.3	*M. anisopliae* 10^2 conidia ml^{-1}
K.1	Control
K.2	Control
K.3	Control

Application of Entomopathogenic Fungi on Young *E. pellita* Trees (4 month) in The Field

The experimental design used was a randomized block design with one factor: species of entomopathogen (*B. bassiana*, *M. anisopliae*, and *M. majus*) with four plot locations (249G, 226E, 233E, and 216F). Spraying application using kap sprayer was carried out on the entire part of *E.pellita* plant at 2 - 3 months. The application time was carried out in the morning with cloudy sunny weather conditions (± 29^0 C and humidity ± 76%). The concentration was four packs of entomopathogenic cultures on sterile milled corn media (200 grams/pack) for one solo cap with 14 liters. Suspension volume sprayed on plants ± 50 ml each tree. The application plot measures 6 rows x 8 plants (48 trees) with an observation plot of 4 rows x 4 plant (16 observation trees) with three replications (total 48 observation plots) (Figure 3). Observational indicators included symptoms on infested plants (swollen stem, broken top-crown) and signs (frass, tunnels, *Zeuzera* spp. larvae), and target fungi infected insects.

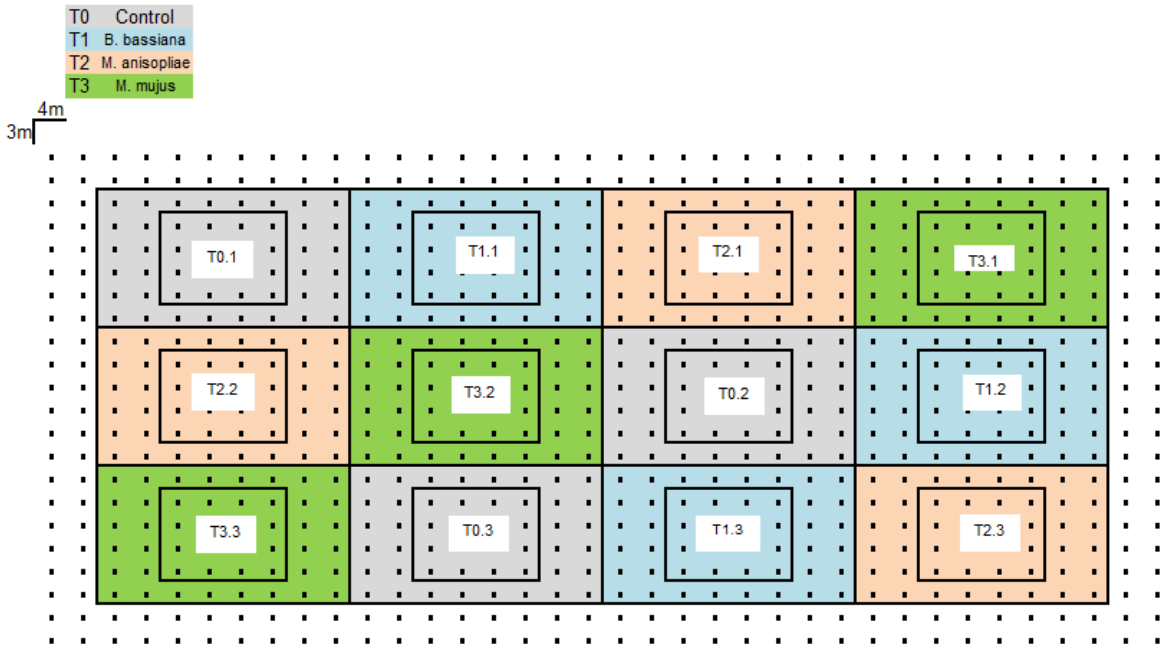

Figure 3. Layout for the application of entomopathogens in the field

Data Analysis
The percentage of mortality of *Zeuzera* spp. after treatment (Balse 1985):

$$\%\ \textbf{mortality} = \frac{\textbf{Number of infected } \boldsymbol{Zeuzera} \textbf{ spp. larvae}}{\textbf{Number of observed } \boldsymbol{Zeuzera} \textbf{ spp. larvae}} \textbf{ x 100 } \%$$

Conidial density/ml (Badan Standardisasi Nasional 2014):

$$S = \frac{x}{L \times t \times d} \times 10^3$$

S = density of conidial (ml^{-1})
x = average number of conidia in boxes a, b, c, d, e;
L = calculating box area 0.04 m^2
t = depth of field calculated 0.1 mm
d = dilution factor
10^3= calculated suspension volume (1 ml = 10^3 $mm^{3)}$

Application data of entomopathogenic fungi with different conidial density and in the field were analyzed using IBM SPSS Statistics 23 to determine significantly treatment affected to response. The Tukey test at α=5% was carried out when the treatment had a significant effect on the treatments to determine the effectiveness of each treatment on the variables in the study.

RESULTS AND DISCUSSION

Application of Entomopathogenic Fungi with Different Conidial Density
The results of the ANOVA test show that the entomopathogenic species and the density of conidial have a significant effect on the mortality of *Zeuzera* spp. larvae (Table 2). Meanwhile, the treatment did not affect to the day of *Zeuzera* spp. infected. The treatments show promising potential of using entomopathogenic fungi to control *Zeuzera* spp. larvae. Based on the results of observations of the mortality of *Zeuzera* spp. larvae by entomopathogens, it is known that the highest mortality occurs on day 5 after inoculation. According to Herlinda et al. (2020), the insect phase is infected by entomopathogens depending on the insect species, but symptoms usually begin on days 3 – 5, such as decreased activeness, reduced appetite, and death.

Table 2. ANOVA results of each treatment on the response to insect tests

Treatments	Respones	
	Mortality of *Zeuzera* spp. larvae	The day start to infection
Entomopathogenic with different conidia density	**	NS

**Significant (P<0.05); NS: Non-significant (P≥0.05)

Tukey test results showed that several entomopathogen treatments: MA 10^8 ml^{-1}, MM 10^8 ml^{-1}, BB 10^6 ml^{-1}, BB 10^8 ml^{-1}, and BB 10^4 ml^{-1} were effective to suppress *Zeuzera* spp.

larvae (Table 3). The BB 10^6 ml^{-1} treatment was good enough for the mortality of *Zeuzera* spp. larvae. (100%) was not much different from the treatment of MA 10^8 ml^{-1}, and MM 10^8 ml^{-1}. In addition, the BB 10^4 ml^{-1} treatment was good enough to cause the mortality of *Zeuzera* spp. (83.33%). The reason is that the species of *B. bassiana* was collected from coffee ground exploration from BBPPTP Mojoagung Surabaya, East Java. One of the key insect pests that attack coffee plant is the coffee borer, *Z. coffeae*. It is suspected that the isolate is quite specific to the *Zeuzera* spp. group. According to Meyling et al. (2009) that *B. bassiana* is a facultative entomopathogen with a wide host range (more than 700 arthropod species), so it allows adaptation and higher infectious power than other fungus.

Tukey test results showed that the conidia density of 10^8 ml^{-1} of three entomopathogenic fungi provide the best results causing 100% mortality of *Zeuzera* spp. The higher the density of conidia makes the higher the chance of infection of the test insects. According to Shapiro-Ilan et al. (2006) that the standard for applying entomopathogens in the field also depends on the species of insect pest. In the case, the standard of conidia density was 10^{13} to 10^{14} conidia/ha for control Hemiptera group. The higher of conidia density affected to the infection rate of the target insect (Table 11).

Tukey test showed that the treatment of MM 10^6 ml^{-1}, MM 10^4 ml^{-1}, MA 10^4 ml^{-1}, MA 10^6 ml^{-1}, MA 10^2 ml^{-1}, MM 10^2 ml^{-1}, BB 10^2 ml^{-1} were not significantly different from the control. The treatment of *B. bassiana*, *M. anisopliae*, and *M. majus* with conidia densities of 10^4 ml^{-1} and 10^2 ml^{-1} were under the standard conidial density procedure for insect testing, according to Badan Standardisasi Nasional (2014). As for the MM 10^6 ml^{-1} and MA 10^6 ml^{-1} treatments, it was found that the origin of the two isolates came from the host insect *Brontispa* spp. (Coleoptera: Chrysomelidae) and *Oryctes* spp. (Coleoptera: Scarabaeidae), so it is suspected to be more pathogenic to their host insects. According to Trizelia et al. (2018), insect mortality by entomopathogens is also influenced by the source of the isolate. The research results of Trizelia et al. (2010) showed that isolates of *Metarhizium* spp. originating from the rhizosphere of several plants, they showed different abilities in infecting *Crocidolomia pavonana* larvae. *Metarhizium* spp. isolates from the rhizosphere of cabbage plants were more virulent against *C. pavonana* than isolates from the rhizosphere of carrot, shallot, and leek plants.

Table 3. Tukey test on the mortality of *Zeuzera* spp. larvae by treatments

Treatments	**Mortality of *Zeuzera* spp. larvae (%)**
MA10^8	100a
MM 10^8	100a
BB 10^6	100a
BB 10^8	100a
BB 10^4	83a
MM 10^6	67ab
MM 10^4	50ab
MA 10^4	33ab
MA 10^6	33ab
BB 10^2	22ab
Kontrol	0b
MA 10^2	0b
MM 10^2	0b

Numbers with the same letter indicate that the treatment is not significantly different at the 5% test level; MM = *M. majus*, MA = *M. anisopliae*, BB = *B. bassiana*, and control.

Application of Entomopathogenic Fungi in The Field

The results of monitoring and analysis show that the specie of entomopathogenic fungi and location had not significantly affected to the incidence of damage from the signs and symptoms of *Zeuzera* spp. and infected target insects on young plants of *E. pellita* (Table 4). It is presumably because the various management methods that have been applied in the field made reducing the population of *Zeuzera* spp. At the moment, the epizootic process in a target insect needs to take a long time. According to Mantzoukas et al. (2022) the application of entomopathogenic fungi that have been applied in the field for many years can indicate a natural epizootic later if the environment supports the growth of entomopathogenic fungi. In addition, direct contact between conidial of entomopathogens and insect pests is the primary trigger condition for infection (Brusselman et al. 2011).

Table 4. Results of ANOVA application of entomopathogenic in the field

Treatments	**Respones**	
	Symptoms	**Signs**
Species of entomophatogens	NS	NS
Locations	NS	NS

NS: Non-significant ($P \geq 0.05$)

Symptoms and Signs of *Zeuzera* spp. Infected by Entomopathogenic Fungi

The results of observations when rearing *Zeuzera* spp. characteristic symptoms and signs were found after the inoculation of entomopathogenic fungi. At the beginning of the inoculation, the larvae were still active in broaching the freshly chopped wood of *E. pellita*. Unless the larvae collected from the field already show signs of illness, they will be different again. Usually, larvae that look unhealthy from the field will be more easily infected with entomopathogenic fungi. *Zeuzera* spp. larvae those starting to be infected with *B. bassiana* will experience loss of appetite, be less active (move less), and be a little restless. The following symptom is that the larvae will be death in a hardened and stiff body conditions. In several cases, dead larvae were found while drilling holes in logs or burrows. Changes in the body skin color of the larvae will turn pale in contrast to healthy larvae, which tend to be bright reddish yellow. According to Sianturi et al. (2014) that the test insects, especially the larvae of the Lepidoptera group, which were infected by the fungus *B. bassiana*, had symptoms such as slow movement, decreased appetite, and eventually died stiff and covered their entire body with *B. bassiana's* typical snow-white mycelia. The dead larvae grow white mycelia from each fall segment until they completely cover it (mummification) (Figure 4).

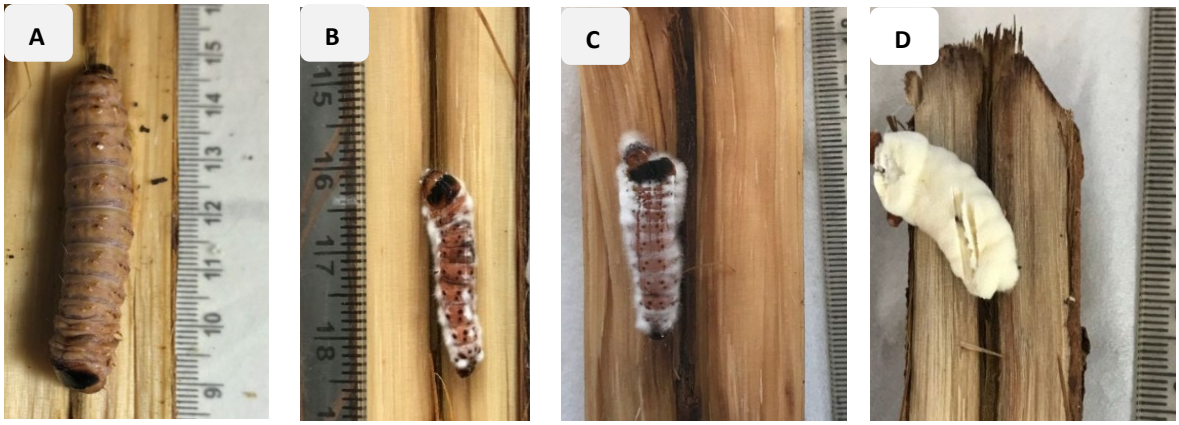

Figure 4. The process of symptoms of Zeuzera spp larvae. infected with *B. bassiana* consisting of; A). Dead larva with stiff and pale (5th day), B). The corner of the larval body begins to grow white mycelia (6th day), C). White mycelia almost wholly covered the larvae's body (7th day), and D). Larvae are covered with typical white mycelia in the form of powder by *B. bassiana* (9th day)

Metarhizium anisopliae and *Metarhizium majus* have similar symptoms to *Zeuzera* spp. larvae. One of them, they have same green conidia character. *Zeuzera* spp. larvae those infected with *M. majus* and *M. anisopliae* looked restless, seemed to slow down, and black spots like burns were found on the bodies of the larvae (especially those infected by *M. majus*). The next day, the larvae will die in a stiff and pale body. Furthermore, the larvae are covered with thin white mycelia (the sticking and sticking to the larva's body is different from the mycelia belonging to *B. bassiana*, which has a type powder). The thin white mycelia will turn green all over the larva's body. The observation results showed that the *Zeuzera* spp. infected with *M. majus* will have a darker green color compared to *M. anisopliae*, which tends to be young green. According to Tanada and Kaya (1993), larvae infected with *M. anisopliae* show restlessness, lack of active movement, decreased feeding activity, and loss of ability to coordinate with the environment (Figure 5).

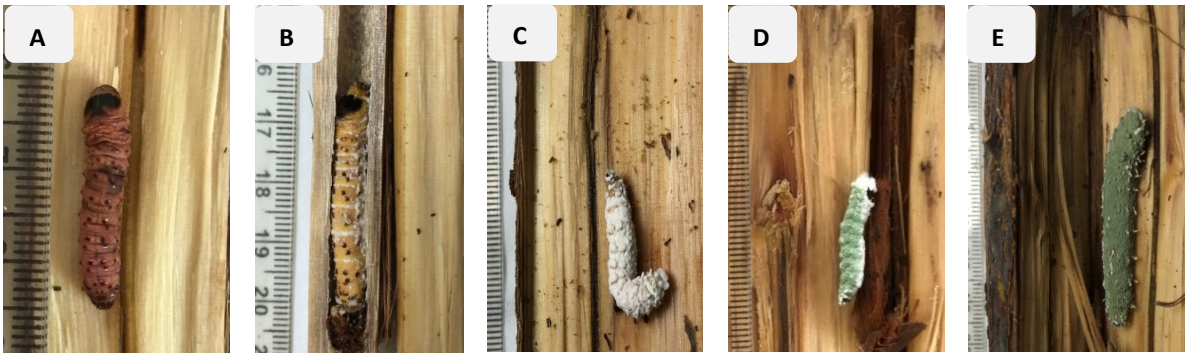

Figure 5. The process of symptoms of *Zeuzera* spp larvae. infected with *M. majus* and *M. anisopliae* consisting of; A). *Zeuzera* spp. larvae dead stiff with black wounds (9th day), B). Larvae body covered with white mycelia attached (10th day), C). White mycelia covered the entire body of the larvae (12th day). D). White mycelia slowly turn green (14th day)

Mortality Rate of *Zeuzera* spp. Larvae According to Species of Entomopathogens and Conidial Density

The results of observations on the mortality rate of entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, and *M. majus*) with conidial density (10^8 ml^{-1}, 10^6 ml^{-1}, 10^4 ml^{-1}, 10^2 ml^{-1}, and control) found that suitable entomopathogenic species of *B. bassiana*, *M. anisopliae*, and *M. majus* with a conidial density of 10^8 ml^{-1} had a high mortality rate killing all tested insects in each treatment (100%) (Figure 6). According to Nelly et al. (2023), using the correct dose and species of entomopathogen can cause high mortality in insects, one of which is high conidial density. *Zeuzera* spp. larvae mortality rate based on the species of entomopathogen, it is known that *B. bassiana* is the best based on the highest accumulation of mortality than *M. majus* and *M. anisopliae* (Figure 7). Lepidoptera group insects are more infected by *B. bassiana* than *M. anisopliae*, a biological agent for insect pests in the soil (Sari et al. 2023). The mortality rate based on conidial density is known that conidial density of 10^8 ml^{-1} is the best because it can cause 100% mortality compared to other conidial densities (Figure 8). Conidial density of entomopathogens influences the mortality of insect pests. (Sopialena et al. 2022). In addition, direct contact is the main trigger requirement for infection (Zhang et al. 2023).

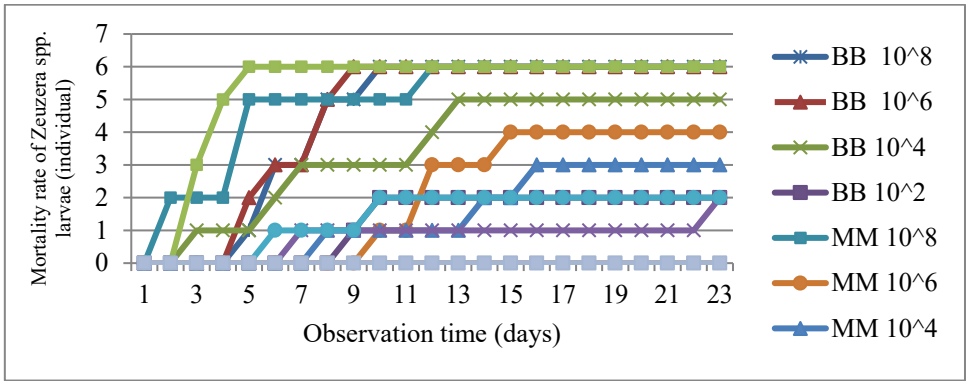

Figure 6. Mortality rate of *Zeuzera* spp. larvae with species of entomopathogens and conidial densities

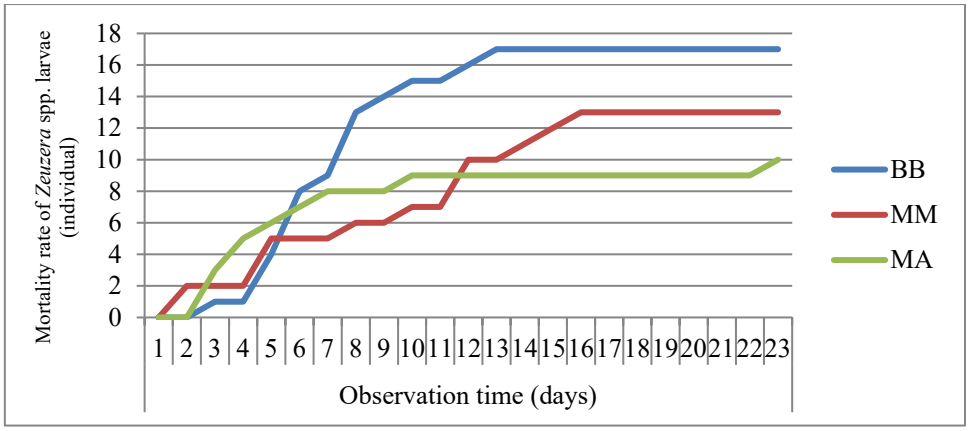

Figure 7. Mortality rate of *Zeuzera* spp. larvae with species of entomopathogens

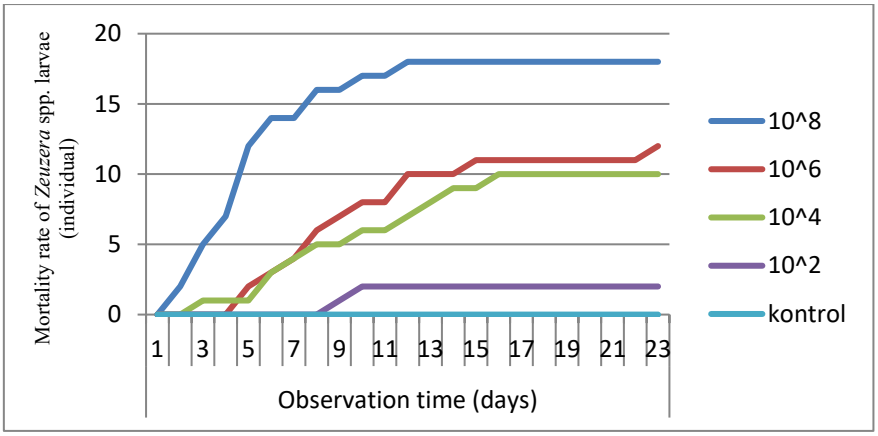

Figure 8. Mortality rate of *Zeuzera* spp. larvae with conidial densities

CONCLUSION

Initiation of entomopathogenic fungi inoculation on *Zeuzera* spp. larvae shows 100% mortality. Application of *M. anisopliae* at 10^8 ml^{-1}, *M. majus* at 10^8 ml^{-1}, *B. bassiana* at 10^8 ml^{-1} , 10^6 ml^{-1}, 10^4 ml^{-1} effective against *Zeuzera* spp. There is no significantly affected of application of three species of entomopathogenic fungi against the early signs and symptoms of *Zeuzera* spp. infected by entomopathogens on young plants of *E. pellita*. Results from the analysis concluded that entomopathogens have given positive results and potential to control *Zeuzera* spp. larvae.

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REFERENCES

Almanoufi, A., Chanan, K., Jama,l M., de Lillo, E., Tarasco, E. & D'Onghia, A.M. 2012. Preliminary experiences in pheromone trap monitoring of *Zeuzera pyrina* (L.) in Syrian Apple Orchard. *Journal of Agricultural Science and Technology* 2: 610-618.

Arnaudov, V., Raykov, V., Davidova, R., Hristov, H., Vasilev, V. & Petkov, P. 2012. Monitoring of pest populations - an important element of Integrated Pest Management of field crops. *Agricultural Science and Technology* 4 (1): 77-80.

Badan Standardisasi Nasional. 2014. *Agens Pengendali Hayati (APH)- Bagian 1: Beauveria bassiana*. Jakarta: BSN.

Balse. 1985. *Field Trial Manual*. Switzerland: Ciba Geigy.

Brusselman, E., Beck, B., Pollet, S., Temmerman, F., Spanoghe, P., Moens, M. & Nuyttens, D. 2011. Effect of the spray application technique on the deposition of entomopathogenic nematodes in vegetables. *Pest Management Science* 68(3): 444-453.

Caron, V., Vila, SP., Gueidan., C. 2023. *Beauveria australis* finds a new host in French dung beetles introduced to Australia. *Journal of Invertebrate Pathology* 197: 107889.

Herlinda, S., Efendi, R.A., Suharjo, R., Hasbi, Setiawan, A., Elfita & Verawaty, M. 2020. New emerging entomopathogenic fungi isolated from soil in south Sumatra (Indonesia) and their filtrate and conidial insecticidal activity against *Spodoptera litura*. *Biodiversitas* 21(11): 5102–5113.

Ibrahim, R., Alahmadi, S., Binnaser, Y.S. & Shawer, D. 2019. Seasonal prevalence and histopathology of *Beauveria bassiana* infecting larvae of the leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae). *Egyptian Journal of Biological Pest Control* 29(1): 1-7.

Mantzoukas, S., Kitsiou, F., Natsiopoulos, D. & Eliopoulos, P.A. 2022. Entomopathogenic fungi: Interactions and applications. *Encyclopedia* 2: 646-656.

Meyling, N.V., Lubeck, M., Buckley, E.P., Eilenberg, J. & Rehner, S.A. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. *Molecular Ecology* 18: 1282e1293.

Monteys, V.S.I. 2015. The leopard moth *Zeuzera pyrina* in Catalonia (Spain) (Lepidoptera Cossidae). Proceeding of the IPM International Fruit Congress 'Smart Fruit', 11 – 12 March 2015, Barcelona.

Nelly, N., Syahrawati, M., Hamid, H., Habazar, T. & Gusnia, D.N. 2019. Diversity and characterization of entomopathogenic fungi from rhizosphere of maize plants as potential biological control agents. *Biodiversitas* 20(5): 1435-1441.

Nyam. V.T., Joseph, B.C.F., Ong, K.H. & Patricia, K.J.H. 2021. Formulation of entomopathogen *Metarhizium anisopliae* var. *anisopliae* bait to control subterranean termite, *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae). *Serangga*

26(4): 42-59.

Sabbahi, R., Hock, V., Azzaoui, K., Saoiabi, S. & Hammouti, B. 2022. A global perspective of entomopathogens as microbial biocontrol agents of insect pests. *Journal of Agriculture and Food Research* 10: 100376.

Sari, J.M.P., Herlinda, S., Suwandi & S., Elfita. 2023. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the growth of *Spodoptera frugiperda* by seed inoculation. *Biodiversitas* 24(4): 2350-2357.

Shapiro-Ilan, D.I., Gouge, G.H., Piggott, S.J. & Patterson, F.J. 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control* 38: 124e133.

Sianturi, B.N., Pangestiningsih, Y. & Lubis, L. 2014. Uji efektifitas jamur entomopatogen *Beauveria bassiana* (Bals.) dan *Metarrhizium anisopliae* (Metch) terhadap *Chilo sacchariphagus* Boj. (Lepidoptera: Pyralidae) di laboratorium. *Jurnal Online Agroekoteknologi* 2(4): 1607 – 1613.

Sopialena, Sahid, A. & Hutajulu, J. 2022. Efektivitas jamur *Metarhizium anisoplae* dan *Beauveria bassiana* Bals lokal dan komersial terhadap hama kutu daun (*Aphis craccivora* C.L. Koch) pada tanaman kacang panjang (*Vigna sinensis* L.). *Jurnal Agrifor* 21(1): 147-160.

Suheri, M., Haneda, N.F., Anwar, R., Jung, Y., Sukeno, S. & Park, J. 2022. Population dynamics of *Zeuzera* spp. (Lepidoptera: Cossidae) on *Eucalyptus pellita* plantation in Central Kalimantan, Indonesia. *Biodiversitas* 23(11): 5782-5789.

Suheri, M., Haneda, N.F., Jung, Y.H., Sukeno, S. & Moon, H.K. 2020. Effectiveness of pheromone traps for monitoring *Zeuzera* sp. (Lepidoptera: Cossidae) population on *Eucalyptus pellita* plantation. *IOP Conference Series: Earth and Environmental Science* 468: 012016.

Tanada, Y. & Kaya, H.K. 1993. *Insect Pathology*. California (US): Academic Pr. Inc.

Tavares, W.de.S., Kkadan, S.K., Hendrik, A.M., Tarigan, M., Asfa, R., Yakovlev, R.V., Tachi, T., Duran, A, Wong, C.Y. & Sharma, M. 2020. Notes on the biology and natural enemies of *Polyphagozerra coffeae* (Nietner, 1861) infesting *Eucalyptus pellita* F. Muell. (Myrtaceae) trees in Riau, Indonesia (Lepidoptera: Cossidae, Zeuzerinae). *SHILAP Revista de Lepidopterología* 48(190): 333-349.

Trizelia, Sulyanti, E. & Suspalana, P. 2018. Virulensi beberapa isolat cendawan entomopatogen *Metarhizium* spp. terhadap kepik hijau (*Nezara viridula*) (Hemiptera: Pentatomidae). *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia* 4(2): 266-269.

Trizelia, Syam, U. & Herawaty, Y. 2010. Virulensi isolat *Metarhizium* sp. yang berasal dari beberapa rizosfer tanaman terhadap *Crocidolomia pavonana* Fabricus (Lepidoptera: Pyralidae). *Journal of Plant Pest and Diseases Management Manggaro* 11 (2): 51-56.

Yudistira, P., Karuniasa, M., Mahardhito, Y. & Wardhana, A. 2019. Model pengelolaan *Eucalyptus pellita* pada hutan industri berkelanjutan. *Jurnal Selulosa* 9(1): 33-38.

Zhang, Z., Guo, W., Lu, Y., Kang, Q., Sui, L., Liu, H., Zao, Y., Zou, X. & Li, Q. 2023. Hypovirulence-associated mycovirus epidemics cause pathogenicity degeneration of *Beauveria bassiana* in the field. *Virology Journal* 20: 255.