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The Ability of Compost Materials Containing Entomopathogen *Metarhizium anisopliae* (Metsch.) Sorokin to Suppress *Oryctes rhinoceros* L. Larvae

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ABSTRACT

Oryctes rhinoceros is one of the main pests in oil palm plantations. Biological control using *Metharizium anisopliae* has great potential to control O. rhinoceros larvae which are usually found in organic matter. This study aims to obtain the best organic compost material for the development of *M. anisopliae* as biological control against O. rhinoceros larvae. This research was conducted with a completely randomized design (CRD) consisting of 6 treatments, each treatment was repeated 5 times to obtain 30 experimental units. Each unit contained 10 *O. rhino* larvae. The concentration of *M. anisopliae* was 50 g / L of distilled water. The treatments of organic media consisted of 6 setting media, as follows: Oil Palm Empty Fruit bunch (OPEFB), rice husk, sawdust, ½ part OPEFB + ½ sawdust, ½ part OPEFB + ½ part rice husk, and ½ part rice husk + ½ part sawdust. The observations included the time of initial death, time of death, daily mortality, total mortality, changes in behavior and larvae morphology. Chemical analysis of OPEFB, temperature, and humidity were recorded. The results showed that compost from sawdust media had the tendency as the best media for the development of *Metarizhium anisopliae.* It had ability to infect *O. rhinoceros* larvae which caused larvae infection 72.50%.

INTRODUCTION

Palm oil is a very important plantation crop because it is one of the world's oil-producing plants. Oil palm in Indonesia is one of the plantation commodities which has a high selling value and is the biggest foreign exchange earner for the country compared to other plantation commodities. The palm oil industry contributes to the development of CPO (producing areas *Crude Palm Oil*) (Ministry of Agriculture, 2012). Riau is a province that has the largest area of oil palm plantations in Indonesia. Statistical data from BPS (2018) reports that the area of oil palm plantations in Riau Province in 2017 was 2,260,941 ha, with a total production of 7,722,564 tons, and this area increased, in 2016 the area of oil palm plantations in Riau was 2.01 million hectares. The production of oil palm plantations is 17.97% or about 35.57 million tons of the total area of oil palm

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plantations in Indonesia. The area of oil palm plantations is increasing, directly parallel to the increase in pest attacks on oil palm, especially the Rhinoceros beetle (*O. rhinoceros*).

Oryctes rhinoceros pest is one of the major pests in oil palm plantations. *O. rhinoceros* L. beetle attack in Riau Province amounted to 12,384.85 ha, which spread in several regencies in Riau Province. The heaviest attack was found in Indragiri Hilir Regency, with the area affected by the *O. rhinoceros* L. beetle 2,717 ha, Siak 340 ha, Kampar 579 ha, Kuansing 459 ha and the rest spread in smallholder oil palm plantation (Dinas Perkebunan Riau Province, 2014). *O. rhinoceros* attack in the area of oil palm rejuvenation can result in a delay in the production of oil palm up to one year and the number of dead plants can reach 25% (Winarto, 2005).

The female *O. rhinoceros* beetle lays eggs on organic matter such as trash cans, rotten leaves, manure, coconut trunks, and rotted fruit bunches (Wesi *et al.*, 2014). O. rhinoceros beetles lay eggs on organic matter, one of which is compost (Vander *et al.*, 2002). *O. rhinoceros* pest control in oil palm often encounters problems triggered by the application of oil palm empty bunches (EFB) organic matter to increase soil fertility. OPEFB media of eggs, larvae, and pupae of *O. rhinoceros* for captivity. The solution offered is composting because nutrients are readily available to plants so it is good for soil physical, chemical, and biological properties. The media commonly used for the propagation of *M. anisopliae* are rice husk and sawdust. The organic matter contained in the composted oil palm empty bunches was more than the organic media of *M. anisopliae* fruit and sawdust, the three media were thought to be able to develop *M. anisopliae*. Based on field observations, rhino larvae were found in composted EFB media (Kiki hidayat, FR, Personal Communication). Compost from EFB, rice husks, and sawdust can be a medium that is rich in organic matter. Therefore, the M. anisopliae media was tested on rice husks, sawdust, and OPEFB for the compost medium of M. anisopliae fungi. Compost given inoculum *M. anisopliae* (metankos) has 2 functions, namely as a biopesticide and biological fertilizer.

This study aims to obtain the best compost organic material for the development of *M. anisopliae*, which is indicated by its pathogenicity properties against *O. rhinoceros* larvae.

MATERIALS AND METHODS

This research method was carried out with a Complete Randomized Design (CRD) consisting of 6 treatments and each treatment was repeated 5 times to obtain 30 experimental units. Each experimental unit each contained 10 larvae *O. rhinoceros.* The concentration of *M. anisopliae* was 50 g/l aquades. The treatment was the difference in compost organic material media consisting of 6 treatment media as follows; OPEFB media, rice husk, sawdust, ½ OPEFB + ½ sawdust, ½ OPEFB + ½ rice husk, and ½ rice husk + ½ sawdust.

Compost organic materials included OPEFB, sawdust, and rice husk were prepared. Each organic material media was placed on plastic sheeting, chicken manure was added, with a ratio of organic media and manure 3: 1, added by 100 ml EM4, dolomite, stirred, and covered with plastic sheeting. The media was flipped every week. Incubation was performed for 3 months.

Isolation of inoculum was *M. anisopliae* taken from soil media, as a fishing rod, Hongkong caterpillars (*Tenebrio Molitor)* were used. The infected HongKong caterpillars were transferred to the moist chamber, then grown on the PDA media. Fungi were identified macroscopically and microscopically using a light microscope isolates *M. anisopliae* propagated on broken corn media. The corn was washed thoroughly and then boiled in a pot until it was 1/3 cooked, then it was cooled and put into the plastic. Inoculum *M. anisopliae was* inoculated on chopped corn media and incubated for 7 days.

Suspension *M. anisopliae* for application prepared using application concentration was

50 g/l. The *M. anisopliae* suspension that had been set was put into a bottle and then shaken using a rotary shaker for 24 hours to speed up cell division and 25 g of sugar was added. Then the conidia density was calculated using a hemocytometer ready.

Compost of each of the-set organic media was put into a plastic bucket as much as ± 4 kg. The compost was then added with suspension *M. anisopliae* applied to the compost media at every 500 ml organic media treatment and incubated for 1 week. Furthermore, the larvae of *O. rhinoceros* instar II were infested by 10 birds in each treatment at a depth of 10 cm, the top of the plastic bucket was covered with gauze.

Observation parameters included initial death time, lethal time, daily mortality, total mortality, changes in behavior and morphology of larvae, chemical analysis of metankos, temperature, and humidity

RESULTS AND DISCUSSION

Chemical Analysis of Metankos

Compost of several treatment organic materials which became the growth medium of *M. anisopliae* (metankos) was observed in its nutrient content. The results of their chemical analysis can be seen in Table 1.

The results of the chemical analysis of all organic metankostic materials in Table 1 show that the pH of metankos in the range 5.81-7.04 is classified as neutral. Metankos organic matter from EFB has higher pH and nutrient content, namely pH 7.04, N 0.72%, P 0.82 mg / 100g, K 4.32 mg / 100g. The results of the analysis ½ EFB + ½ sawdust obtained metankos which had the second-highest pH and nutrient content with pH 7.00, N 0.73%, P 0.54 mg / 100g, K 4.76 mg / 100g.

The development of entomopathogenic fungi in several types of media is related to nutrient content in the media. According to the report by Herlinda et al., (2008) nutritional media is needed by fungi for vegetative growth and spore formation. The spores formed germinate faster and have high virulence. This causes the killing power of the fungus to be higher.

Early Death Time Oryctes rhinoceros (hour)

The results of variance showed that the treatment of several types of organic material containing entomopathogenic *M. anisopliae* (metankos) had a real effect on the initial time of death of *O. rhinoceros* larvae. The average results of the initial time of death of *O. rhinoceros* larvae after DMRT test at 5% level can be seen in Table 2.

Table 2 can be seen in all treatments showing that the treatment of several metankos organic materials, truly affected the initial time of death of larvae *O. rhinoceros*. Death occurs 36 hours to 84 hours after application. The fastest time for the beginning of death was ½ OPEFB + ½ nrice husk metankos at 36 hours. The use of several metankos organic materials causes different growth of *M. anisopliae*, causing a difference in the initial time of death of *O. rhinoceros* larvae. The highest chemical analysis of metankos was OPEFB and ½ OPEFB + ½ sawdust. Heriyanto and Suharno (2008) stated that the sporulation of *M. anisopliae was* affected by the nutrient content of the growing media used.

M. anisopliae conidia will germinate in the

Table 1. Results of chemical analysis of metankos

No	Type of Organic Material Compost	pH (H_2O)	Content Nutrient N (%)	P (mg/100g)	K (mg/100g)
1	OPEFB	7.04	0.72	0.82	4.32
2	Rice husk	5.85	0.72	0.77	1.21
3	Sawdust	5.94	0.55	0.78	1.30
4	½ OPEFB + ½ sawdust	7.00	0.73	0.54	4.76
5	½ OPEFB + ½ rice husk	5.84	0.55	0.74	1.57
6	½ Rice husk + ½ sawdust	5.84	0.85	0.57	1.11

Table 2. Initial time of death of larvae *O. rhinoceros* on several organic materials Metankos

Type of Organic Material Compost	Time of initial death (hours)
OPEFB	48 b
Rice husk	48 b
Sawdust	51 ab
½ OPEFB + ½ sawdust	75 a
½ OPEFB + ½ rice husk	36 b
½ rice husk + ½ sawdust	72 a

Numbers followed by the same lowercase letters are not significantly different according to the DNMRT at 5%

cuticle of the host insect, penetration occurs using the peptidase and chitinase enzymes, then with the help of mechanical pressure the enzyme destroys the cuticle using lysis (Wikardi 2000 *in* Saebani, 2008). Fulfillment of *M. anisopliae's* need for protein from each medium causes the process of enzyme synthesis needed by *M. anisopliae to* run well so that the enzyme performance in larval cuticle penetration is also good and ultimately the time of initial infection is faster.

Lethal time 50 (LT50) _O. rhinoceros_

The results of variance showed that the treatment of several types of organic material containing entomopathogen *M. anisopliae* (metankos) had a real effect on the *lethal time of* 50 *O. rhinoceros larvae*. Average results of *the lethal time* of 50 *O. rhinoceros* larvae after the DNMRT test at a 5% level are shown in Table 3.

Table 3 shows that the organic material of the sawdust metankos tends to kill *O. rhinoceros* larvae more quickly by 50% ie 165 .0 hours and not truly different from rice husk ½ metankos + ½ sawdust 225.0 hours

Table 3. *Lethal time*$_{50}$ of *O. rhinoceros* larvae with the treatment of some organic metankos materials

Type of organic material metankos	*Lethal time* 50 (hours)
OPEFB	243.0 ab
Rice husk	294.5 a
Sawdust	165.0 b
½ OPEFB + ½ sawdust	270.0 a
½ OPEFB + ½ rice husk	273.0 a
½ rice husk + ½ sawdust	225.0 ab

Numbers followed by the same lowercase letters are not significantly different according to the DNMRT at 5%

and OPEFB 243 hours. The fastest tendency to kill 50% *O. rhinoceros larvae* in sawdust metankos organic matter compared to other treatments was influenced by conidia density in each media. Conidia density will affect the ability of fungi to infect test insects. Sawdust media quickly decomposes therefore organic material is quickly available for mushroom development. According to the results of the study of Sudharto *et al.* (1998) higher conidia density will affect the ability of fungi to infect test insect larvae, conidia density is related to the growth of *M. anisopliae* rather than compost organic matter. This will greatly affect the process of infection and the death of larvae. High conidia density will increase the toxin in the form of a cordycepin compound produced to kill larvae (Sinaga, 2010).

The difference in time of insect death caused by the ability of each fungus varies, at the time of penetration (Freimoser *et al.*, 2003), According to Ginting (2008) the time required to infect target pests is affected by the isolates used, host type, and environmental conditions.

Daily mortality of _O. rhinoceros_ (%)

The results of daily observations of *O. rhinoceros larvae* with the treatment of several types of compostable organic material containing entomopathogenic *M. anisopliae* (metankos) showed that the daily mortality of *O. rhinoceros larvae* fluctuated from the first day to the 14th day. Daily fluctuation of *O. rhinoceros* larvae mortality can be seen in Figure 1.

Figure 1 shows that the mortality of *O. rhinoceros* larvae in various metankos organic materials is different. In the sawdust treatment, the mortality of *O. rhinoceros* larvae reached the peak compared to other treatments on the 3rd day, reaching 13% mortality and then went up and down the next day. Each treatment experienced an increase and decrease in daily mortality of different larvae, decreased mortality occurred due to the aging of fungus *M. anisopliae* but it still reproduces itself in the body of *O. rhinoceros* larvae and it gets nutrition, therefore, the daily mortality

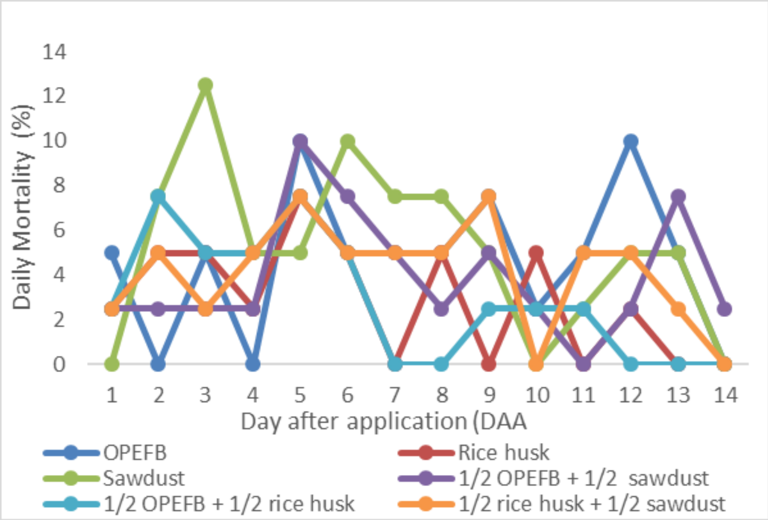

Figure 1. Daily mortality of *O. rhinoceros* in some Metankos organic material

of *O. rhinoceros* larvae can decrease compared to the previous day.

The highest percentage of daily mortality was achieved by sawdust treatment, this shows that the organic material of the sawdust used tends to be the best as the growth medium of *M. anisopliae*. This is presumably because the sawdust media can increase the activity of entomopathogenic *M. anisopliae*. The advantage compared to ½ OPEFB media and ½ rice husk, is that it breaks down quickly therefore nutrients are quickly available for the growth of the *M. anisopliae fungus*. According to Ismail and Waliuddin (1996) that sawdust media contains 53.3% protein and its chemical content consists of 50% cellulose, 25-30% lignin, and 15-20% silica.

Total mortality *Oryctes rhinoceros* (%)

Variance results indicate that the treatment of several types of organic material containing entomopathogen *M. anisopliae* gives a real effect on the mortality of *O. rhinoceros larvae*. The average total mortality of *O. rhinoceros larvae* after DNMRT test at 5% can be seen in Table 4.

Table 4 shows that sawdust treatment causes the total mortality of the *O. rhinoceros* larvae to have the best tendency which is 72.5% and does not differ notably with 65.0% OPEFB, 57.5% ½ rice husk + ½ sawdust and 55.0% ½ OPEFB + ½ sawdust. It is suspected that the nutrient content in each compost media on the growth of fungi used is optimal for entomopathogenic fungi.

Sawdust metankos treatment with 72. *O. rhinoceros larvae mortality* % has been categorized as a bioinsecticide. This is consistent with the opinion of Steinhaus (1963) *in* Hasyim (2007) stating that fungi that can be categorized as bioinsecticides are fungi that have successfully controlled insects with a mortality of 72.5% - 95%.

According to Shegal and Sagar (2006), the nitrogen content in the propagation media will meet the nutritional needs of the fungus. According to Engelkes *et al.* (1997), most entomopathogenic fungi need oxygen, water, carbon sources, organic and inorganic nitrogen as well as several minerals for growth and infectious power (pathogenicity). A good infection power will help the process of penetration of fungi into the body of larvae.

The process of penetration of fungi into the larval body goes through several stages. According to Shegal and Sagar (2006), spores that are on the integument and can hydrolyze the chitin layer of larvae. After infection, hyphae will appear cylindrical in the insect hemocoel, then mycelium will spread until it covers the insect's body.

According to Prayogo (2004) factors that influence the effectiveness of *M. anisopliae* to include the origin of isolates, conidia density, quality of growing media, type of pest controlled, application time, frequency of application, and environmental factors such as temperature, violet ultra-light, rainfall, and humidity.

Table 4. Total mortality of *O. rhinoceros* larvae in various types of organic matter (metankos)

Types of organic matter metankos	Total mortality (%)
OPEFB	65.0 b
Rice husk	40.0 a
Sawdust	72.5 b
½ OPEFB + ½ sawdust	55.0 ab
½ OPEFB + ½ rice husk	40.0 a
½ Rice husk + ½ sawdust	57.5 ab

Numbers followed by the same lowercase letters are not significantly different according to the DNMRT at 5%

Changes in behavior and morphology of infected O. rhinoceros larvae

Observation of behavior change in *O. rhinoceros* larvae showed that infected larvae appeared to move slowly and their appetite decreased. Besides that, the larvae also tend to separate themselves from other larvae, then rise to the surface of the compost media. Priyanti (2009) in Suziani (2010) states that the behavioral characteristics that occur (peak disease), namely insects infected with entomopathogenic fungi show behavior in which they will rise to the surface of the plant and place themselves there. This phenomenon is said by some experts to be an attempt to save other healthy populations from entomopathogenic fungal infections.

Morphological changes that occur in larvae of *O. rhinoceros* infected with M. anisopliae are dark or black color changes. This indicates that the fungal propagation has infected the cuticle tissue of the larvae. *O. rhinoceros* larvae infected with the fungus *M. anisopliae* were initially characterized by the appearance of brown spots on the cuticles, a change in the color of the larva's body from pure white to dull brown, then the larvae died with symptoms of hardening of the body. and the surface of the larvae is covered with white hyphae.

The changes seen in the larvae infected with the fungus are overgrown with fungal mycelium, which initially turns white then turns dark. The growth of the mycelium is first seen in the spaces between the larval segments. A few days later the entire surface of the infected larvae will be covered in the mycelium of the original white fungus. After 5 days of larval death, the entire surface of the infected larvae will be covered by fungal mycelium which has turned green. This is in line with the results of research by Prayogo *et al* (2006) which states that larvae are infected by

The fungus *M. anisopliae* will grow green hyphae and the larva's body will harden like a mummy. Kershaw et al (1999) also stated that hyphae generally grow outside the surface of insects through the spiracle, mouth, and intersegmental membrane. The changes in the morphology of *O. rhinoceros* larvae are presented in Figure 2.

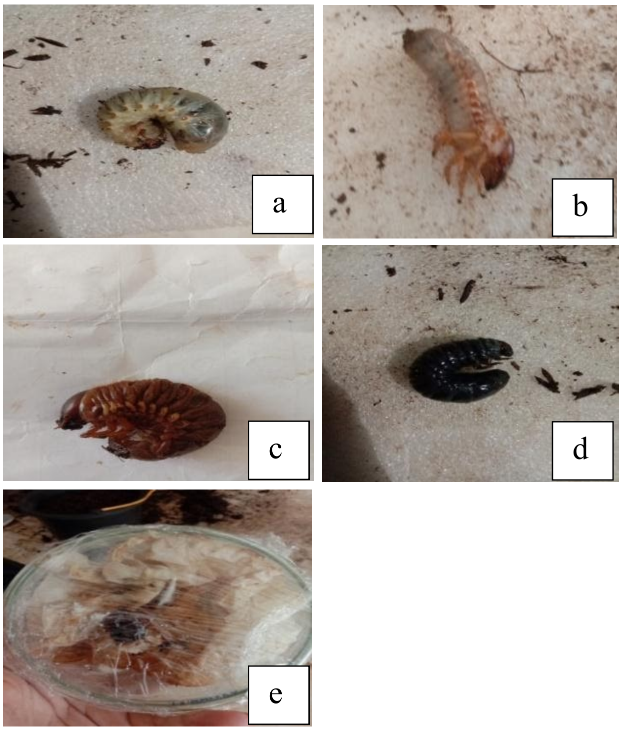

Figure 2. Observations morphological changes of *O. rhinoceros* larvae infected with *M. anisopliae* (a) The *O. rhinoceros larvae which* recently dead, (b) Stiff *O.rhinoceros larvae* (c) *O. rhinoceros* larvae turns into

CONCLUSION

From the research conducted it can be concluded that the best organic compost tendency for the development of *M. anisopliae* in infecting *O. rhinoceros* larvae is sawdust organic matter as a metankos material that can kill *O. rhinoceros* larvae reaching 72.50 %. Pathogenicity of *M. anisopliae* in sawdust metankos against *O. rhinoceros* larvae has been categorized as bioinsecticide.

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