Journal of Physics: Conference Series

Country	United Kingdom - SCIMAGO INSTITUTIONS RANKINGS
Subject Area and Category	Physics and Astronomy Physics and Astronomy (miscellaneous)
Publisher	IOP Publishing Ltd.
Publication type	Conferences and Proceedings
ISSN	17426588, 17426596
Coverage	2005-2020
Scope	The open access Journal of Physics: Conference Series (JPCS) provides a fast, versatile and cost-effective proceedings publication service.

70

H Index

Homepage

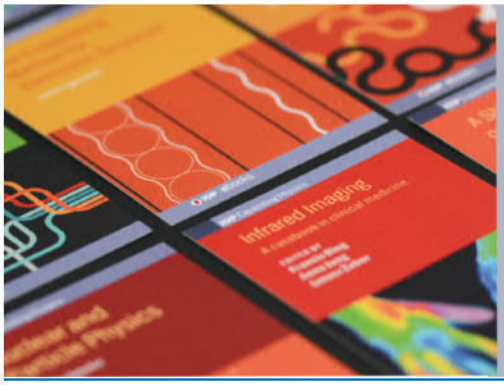
How to publish in this journal

Contact

Join the conversation about this journal

Journal of Physics: Conference Series

Test on Several Concentrations *Metarhizium anisopliae* (Metsch) Sorokin in Palm oil Empty Fruit Bunch Compost (metankos) to Infecting *Oryctes Rhinoceros* Larvae.



Test on Several Concentrations *Metarhizium anisopliae* (Metsch) Sorokin in Palm oil Empty Fruit Bunch Compost (metankos) to Infecting *Oryctes Rhinoceros* Larvae.

Hafiz Fauzana, Febriliani Arda, Nelvia, Rusli Rustam, Fifi puspita

Departement Agrotechnology, Faculty of Agriculture, Riau University, Indonesia

fauzana_hafiz@yahoo.co.id

Abstract. Oil palm is a very important plant because it is one of the world's vegetable oil producing plants. *Oryctes rhinoceros* is one of the important pests that attack oil palm plants. The recommended control of *Oryctes rhinoceros* is used integrate pest control, one of which can use the biological agent of the fungus *Metarhizium anisopliae*. The composition of oil palm empty fruit bunches can be a medium rich in organic matter for the breeding of Fungus *Metarhizium anisopliae*. This research aims to study the best concentration of *Metarhizium anisopliae* in oil palm empty fruit bunch compost (metankos) to infect *Oryctes rhinoceros* larvae. This research was conducted in Laboratory of Plant Pests, Faculty of Agriculture, University of Riau, from April to October 2019. This study is experimental with Complete Randomized Design with 6 treatments and 5 replications. The treatment of *Metarhizium anisopliae* concentration on metankos consisted of : 0 $g.l^{-1}$, 10 $g.l^{-1}$, 20 $g.l^{-1}$, 30 $g.l^{-1}$, 40 $g.l^{-1}$, and 50 $g.l^{-1}$ *Metarhizium anisopliae*. The results show is concentration of *Metarhizium anisopliae* 50 $g.l^{-1}$ water with a total mortality of 56% larvae.

1. Introduction

Palm oil is a very important plantation crop because it is one of the world's vegetable oil producing plants. In Indonesia, oil palm is a plantation commodity that has a high selling value and is the biggest foreign exchange earner for the country compared to other plantation commodities. Riau is a province that has the largest area of oil palm plantations in Indonesia, this is indicated by statistics from the Central Statistics Agency [1] which reported that the area of palm oil plantations in Riau Province in 2017 was 2,260,941 ha, and this area experienced an increase from the previous year, in 2016 the total area of oil palm in Riau Province was 2,012,951 ha or 17.97% of the total area of palm oil plantations in Indonesia. The increase in the area of palm oil plantations is directly proportional to the increase in pest attacks on oil palm. Pests are one of the problems that are often faced in the cultivation of palm oil plants, especially the pests of *Oryctes rhinoceros*.

Oryctes rhinoceros pest is one of the important pests in palm oil plants. *O. rhinoceros* beetle attacks parts of palm oil plants such as palm leaves (young leaves) and growing points as in that palm oil growth is inhibited and in severe attacks palm oil plants will die [2].

The attack of the *O. rhinoceros* beetle in Riau Province cover as much as 12,384 ha. The attack spread in several districts in Riau Province. The heaviest attack was found in Indragiri Hilir Regency with an area of land affected by the *O. rhinoceros* beetle 2,717 ha, Siak 340 ha, Kampar 579 ha, Kuansing 459 ha and the remainder is spread in community palm oil plantations [3].

Oryctes rhinoceros attack can result in a delay in palm oil production up to one year and dead plants can reach 25% in the area of palm oil rejuvenation [4]. *O. rhinoceros* spread almost in all

provinces in Indonesia because of the availability of hosts and piles of organic material in the field as breeding ground and food larvae [5].

Adding organic material to the plantation land is the site for the development of the adult stage *O. rhinoceros* [6]. The organic material of palm oil empty fruit bunches (POEFB) in Riau was deliberately placed to increase nutrient content. According to Prawirosukarto *et al.* [7] the application of palm oil bunches mulch caused problems, namely it is used as a breeding ground for *O. rhinoceros* adult stage.

Palm oil empty fruit bunches (POEFB) are rich source of organic matter, N, P, K and Mg. In addition, they also contain complex organic materials including carbon-rich materials, namely Cellulose 42.7%, Hemicellulose 27.3%, and lignin 17.2%. One of the uses of palm oil empty fruit bunches waste is to make compost [8]. The excess of compost from organic material, the nutrients are in form available for plants [9]. According to Rajan *et al.* [10], organic waste that has been decomposed into compost, cattle manure, and coconut tree trunks that rot is the preferred place for *O. rhinoceros* to lay eggs. According to Yustina *et al.* [11] decomposed waste and oil palm empty fruit bunches were left after harvesting and rotted leaves, this condition is a very suitable place for adult stage development *O. rhinoceros*.

The recommended control of *O. rhinoceros* pest is to use integrated pest control (IPM). This biological control technique is one of the environmentally friendly techniques so that the balance of the ecosystem is maintained. Biological agents that have great potential as natural controllers of *O. rhinoceros* are entomopathogenic *Metarhizium anisopliae* and *Beauveria bassiana.* According to Harjaka *et. al* [12] One of the insect pathogens that have been used to control *O. rhinoceros* in most countries in the world is the fungus *M. anisopliae.*

Metarhizium anisopliae has larvicidal activity containing cyclopeptide toxin, destruxin A, B, C, D, E and desmethyldestruxin B. and has been considered as an insecticide for a new generation. The destruxin has effect for the target cells organella (mitochondria, endoplasmic reticulum and nucleus membrane) which causes cells paralysis and abnormalities in the function of the middle stomach, tubules malphigi, hemocytes and muscle tissue [13].

The use of *M. anisopliae* to control *O. rhinoceros* with direct application to the pests has been widely reported, one of which is by Parinduri *et al.* [14] which stated that the concentrate *M. anisopliae* 30 $g.l^{-1}$ water was able to cause mortality *O. rhinoceros* larvae 62,5%. According to sihombing *et al.* [15] which stated that the concentrate 75 $g.l^{-1}$ water was able to cause mortality larvae 89.6%.

According to Wicaksono *et al.* [16] aplication method sprayed of *B. bassiana* on compost was able to cause *B. carambolae* mortality of 25,33%. The use of *M. anisopliae* entomopathogenic fungi given in compost has not been widely reported, POEFB compost applied with suspension of *M. anisopliae* fungus called metankos can be used to control *O. rhinoceros* pests. Compost from POEFB can be medium rich in organic material for the proliferation of *M. anisopliae*. POEPB compost given an inoculums *M. anisopliae* (metankos) which has 2 functions, namely as biopesticide and biological fertilizer.

This research aims to obtain the best concentration of *M. anisopliae* (Metsch) Sorokin in palm oil empty fruit bunch compost (metankos) to infect the *O. rhinoceros* L. larvae.

2. Methodology

This study was conducted at the Laboratory of Plant Pests, Faculty of Agriculture, University of Riau. The research was conducted from April to October 2019. This research was conducted using a Completely Randomized Design (CRD), which consisted of 6 sheets and 5 replications, and there were 30 experimental units. Each treatment unit consists of 10 *O. rhinoceros* larvae. The treatment of *M. anisopliae* concentration on metankos consisted of:

M0 = *M. anisopliae* 0 $g.l^{-1}$ water
M1 = *M. anisopliae* 10 $g.l^{-1}$ water
M2 = *M. anisopliae* 20 $g.l^{-1}$ water

M3 = *M. anisopliae* 30 $g.l^{-1}$ water
M4 = *M. anisopliae* 40 $g.l^{-1}$ water
M5 = *M. anisopliae* 50 $g.l^{-1}$ water

Procedure of the research consisted of: provision of entomopathogenic fungal isolates of *M. anisopliae,* propagation of fungus *M. anisopliae,* preparation of a suspension for treatment, the density calculation conidia, provision POEFB, manufacture metankos, *O. rhinoceros* larvae infestation, suspension applications *M. anisopliae* on Metankos.

Provision of entomopathogenic fungal isolates and propagation of *M. anisopliae.* Entomopathogenic *M. anisopliae* was isolated on PDA media so that the level of pathogenicity remained high. Thus, *M. anisopliae* isolates propagated in the media of broken corn. Corn was washed clean and boiled in saucepan until 1/3 cooked and then cooled. Corn weighed as much as 250 gramsin plastic bag inserted into the size of 500 grams-plastic bag. Inoculum *M. anisopliae* was isolated chopped corn media in the isolation chamber. Starter fungus M. anisopliae were incubated for 7 days so that the fungus would grow and could be applied to metankos.

Preparation of a suspension for treatment. *M. anisopliae* which had been propagated in the chopped corn media weighed as much as 10 g, 20 g, 30 g, 40 g and 50 g using an analytical balance. The fungus *M. anisopliae* were mixed with 1 liter of water in a 1000 ml erlenmeyer, then shaken using a rotary shaker for 24 hours to accelerate cell division. *M. anisopliae* suspension added sugar. Sugar served as a nutrient to the conidia of *M. anisopliae*. Suspension of *M. anisopliae* fungus that had been made according to the treatment was done by conidia density calculation using a haemocytometer.

The density calculation conidia**.** The concentration of *M. anisopliae* suspension treatment dilution was done using serial dilution technique. 1 ml suspension was diluted with 9 ml of water, stirring to obtain 10^{-1}. Then dilution solution back from 10^{-1} by taking 1 ml of the suspension was then put in 9 ml of water, then dilution of 10^{-2} was obtained, until dilution was done for 6 or 10^{-6} times. The taken of a few drops of solution which has been diluted to 10^{-6} conidia were then calculated using a haemocytometer under microscope . if Conidias meet the dilution back to conidia, they were not too tight. Conidia calculations were done using the formula according to Siswanto and Trisawa (17):

$$S = \frac{t \times d}{0{,}25\ x\ n} 10^6$$

Information:
S = Conidia
t = Number of known conidia
n = Number of boxes counted
d = Degree of dilution
0,25 = Correction level

Manufacture Metankos. Media consisting of POEFB was chopped, comparison POEFB and manure that was 3: 1 with the needs POEFB 360 kg, additional 120 kg of manur, and EM4 were active. Activation of EM4 by adding 20 ml of EM4 in 1 liter of solution containing 300 g sugar, solution was then incubated for 24 hours, EM4 which had been active was dissolved in watering pot with 5 liters of water. POEFB was placed on top of the tarp in first layers, as much as 9 kg of manure was then spread evenly, then EM4 was poured slowly on the stack of POEFB and watering EM4 to 3 was repeated until wet evenly, then the same thing was done up to 4 layers of compost, then water was poured on the moist compost. POEFB and compost were mixed evenly and sealed using plastic sheet. The POEFB was inverted every week, and sealed again. Incubation was carried out for 3 months. After POEFB compost matured, compost was sterilized and then put in a plastic bucket in the size of 22 cm high, 28 cm diameter top and 18 cm diameter bottom with a depth of + 20 cm. Suspension *M. anisopliae* then sprayed according to treatment by 300 ml into a bucket evenly obtained from calibration results, and the suspension was stirred evenly. After than the bucket was placed on storage racks and incubated for 7 days.

Oryctes rhinoceros larvae Infestation. *O. rhinoceros* larvae used as test insects were the second instar larvae of *O. rhinoceros* taken from a community garden at Jl. Seroja Kulim Village, District Tanayan Raya, Pekanbaru. *O. rhinoceros* larvae used were second instar larvae with a length of 4 cm. The larvae infested were as many as 10 *O. rhinoceros* larvae for each unit of experiments.

Suspension Applications *M. anisopliae* on Metankos. Metankos which had been incubated for 7 days in a bucket, was added by larvae of *O. rhinoceros* as many as 10 animals at the depth of 10 cm metankos. Metankos was then sealed again by as thick as 10 cm, then prepared in accordance with the design used.

Parameter Observation consisted of: Parameters his observations of behavior and morphological changes observed among the larvae, the start time of death, lethal time 50 (LT50), lethal concentrate (LC50; 95), the daily mortality, and total mortality.

Daily mortality data, behavior and morphological changes obtained from the study were analyzed descriptively and displayed in a picture, the data lethal concentrate 50 (LC50) ; 95) were analyzed probit using the program POLO-PC, while the start time of death, lethal time 50 (LT50) and total mortality was analyzed statistically with using analysis of variance. Data analysis results are significant variance followed by a further test Duncan's New Multiple Range Test (DNMRT) at 5% level.

3. Results and Discussion

3.1 The Initial Time of Death (Hour)

The results of variance showed that the treatment of some *M. anisopliae* entomopathogenic fungi concentrations on metankos had a significant influence on the initial time of death of *O. rhinoceros* larvae. The results of the initial time of death of *O. rhinoceros* larvae after a DNMRT test at 5% can be seen in Table 1.

Table 1. Initial Time of Death of *O. rhinoceros* Larvae After Giving Treatment of Several *M. ansiopliae* Concentration on Metankos

Treatment of several *M. ansiopliae* Concentrations on metankos	Initial time of death (hour)
0 $g.l^{-1}$ (without *M. anisopliae*)	504,0 a
10 $g.l^{-1}$ (33,6 x 10^6 conidia.ml^{-1})	153,6 b
20 $g.l^{-1}$ (57,6 x 10^6 conidia.ml^{-1})	148,8 bc
30 $g.l^{-1}$ (62,4 x 10^6 conidia.ml^{-1})	129,6 bc
40 $g.l^{-1}$ (81,6 x 10^6 conidia.ml^{-1})	124,8 bc
50 $g.l^{-1}$ (302,4 x 10^6 conidia.ml^{-1})	40,8 c

The figures on the lane followed by lowercase letters were not significantly different according to the DNMRT test at the 5% level after being transformed into $\sqrt{y}$.

Table 1 shows that some *M. anisopliae* concentrations had a significant effect on the initial time of death of *O. rhinoceros* larvae with a time range of 40.8-504 hours after application. The treatment of *M. anisopliae* concentration in metankos 50 $g.l^{-1}$ water caused the initial time of death of *O. rhinoceros* larvae at 40.8 hours after application and was not significantly different from the treatment of *M. anisopliae* concentration in metankos 40 $g.l^{-1}$ water, 30 $g.l^{-1}$ water, and 20 $g.l^{-1}$ water

amounted to 124.8 hours, 129.6 hours, and 148.8 hours but were significantly different from the treatment of *M. anisopliae* concentration on metankos 10 $g.l^{-1}$ water amounted to 153.6 hours. Treatment of *M. anisopliae* concentration on metankos 0 $g.l^{-1}$ water until the end of observation (504 hours) showed no dead larvae due to the absence of conidia of entomopathogenic fungi in metankos.

The initial time of death of *O. rhinoceros* larvae after the application of entomopathogenic fungus *M. anisopliae* was the tend fastest on the treatment of 50 $g.l^{-1}$ water with the highest amount of conidia density which was 302.4 x 10^6 conidia.ml^{-1} was 40.8 hours. This was due to the large number of conidia in metankos, so the faster the death of *O. rhinoceros* larvae, the higher the concentration, the higher the density of the entomopathogenic fungus conidia and the faster it killed insects. This was in accordance with the opinion of Siswanto and Trisawa [17] which states that the high density of conidia and supported by high germination ability will determine the speed of the fungus *M. anisopliae* killing its host, the high conidia density will result in the insect integument being damaged faster and body fluids more run out quickly so that insects die faster.

The initial time of death of *O. rhinoceros* larvae tended to be the lowest in the treatment of *M. anisopliae* concentration in metankos 10 $g.l^{-1}$ water with a conidia number of 33,6 x 10^6 conidia.ml^{-1} which was 153,6 hours which was also not significantly different from the concentration treatment *M. anisopliae* on metankos 20 $g.l^{-1}$ water, 30 $g.l^{-1}$ water, and 40 $g.l^{-1}$ water. Low concentrations contain a smaller number of conidia, so it took a long time to kill off *O. rhinoceros* larvae, because the conidia were low, the chance of infection was low. This was consistent with the opinion of Yuningsih and Widyaningrum [18] that a high concentration with a higher number of conidia results in more conidia attached to the cuticle of the larvae so that it infects the larvae faster than a low concentration with a smaller number of conidia. In the opinion of Sedighi *et al.* [19] that the *M. anisopliae* fungus takes more than 24 hours to kill test insects.

The difference in the initial time of death of *O. rhinoceros* larvae was caused by differences in the concentration of *M. anisopliae* which were inoculated into metankos, where the higher the concentration given the tendency to more quickly kill off *O. rhinoceros* larvae. According to Susanti [20] that the higher the concentration of infected conidia, the higher the chance of contact between the pathogen and the host. The higher the attack, the faster the process of death of infected insects. In the opinion of Freimoser *et al.* [21] which states that differences in time of death of insects due to the ability of fungal infections are different, both at the time of penetration, growth speed and use of enzymes *M. anisopliae* produces enzymes that play a role in killing insects such as lipase, chitinase, amylase, proteinase, pospatse and esterase, which play a role during penetration or invasion in the insect's body.

3.2 Lethal Time 50 (Hour)

Results of variance showed that the treatment of several concentrations of *M. anisopliae* on metankos had no significant effect on the lethal time of 50 *O. rhinoceros* larvae. The lethal time results of 50 *O. rhinoceros* larvae after DNMRT test at 5% level can be seen in Table 2.

Table 2. LT_{50} *O. rhinoceros* Larvae After Giving Treatment of Several *M. anisopliae* Concentrations on Metankos

Treatment of several *M.anisopliae* Concentrations on metankos	*Lethal time* 50 (hour)
0 $g.l^{-1}$ (without *M. anisopliae*)	504,0 a
10 $g.l^{-1}$ (33,6 x 10^6 conidia.ml^{-1})	504,0 a
20 $g.l^{-1}$ (57,6 x 10^6 conidia.ml^{-1})	444,0 a
30 $g.l^{-1}$ (62,4 x 10^6 conidia.ml^{-1})	422,4 a
40 $g.l^{-1}$ (81,6 x 10^6 conidia.ml^{-1})	398,4 a
50 $g.l^{-1}$ (302,4 x 10^6 conidia.ml^{-1})	381,6 a

The figures on the lane followed by lowercase letters were not significantly different according to the DNMRT test at the 5% level after being transformed into $\sqrt{y}$.

Table 2 shows that some treatments of *M. anisopliae* concentration on metankos had no significant effect on the lethal time of 50 *O. rhinoceros* larvae, with a range of 381.6 - 504 hours, these results were not significantly different between fellow treatments. these results were not significantly different between fellow treatments. It was suspected that the body of *O. rhinoceros* larvae was still able to with stand the treatment given so that it did not show significantly different results. In addition, due to the treatment had not experienced the maximum infection process in killing 50% of the *O. rhinoceros* larvae, because the conidia in metankos needed time to stick to the integument and germinate. This was consistent with the opinion of Sihombing *et al.* [15] that although entomopathogenic virulence increased, the resistance of *O. rhinoceros* larvae also increased so that the results would not be obtained significantly different. In the opinion of Nurjayanti [22] that the time of death of insects that are different is not real because the ability of entomopathogenic fungi to cause death to insects is not optimal.

The results of Table 2 also showed that the treatment of 50 $g.l^{-1}$ water (302,4 x 10^6 konidia.ml^{-1}) the fastest tendency in killing 50% of the *O. rhinoceros* larvae was 381.6 hours. This was because high concentrations contain high conidia densities, so the time needed to kill larvae is faster. Opinions of Wicaksono *et al.* [16] that a high conidia density contains more conidia so that penetration, development, and infection by the fungus are faster and lead to death than low concentrations. In the opinion of Gopal et al. [23] that the time needed to kill test insects depends on the conidia concentration given and depends on the weather that supports the activity of the fungus *M. anisopliae*.

The length of time to kill 50% of *O. rhinoceros* larvae with a range of 381.6-504 hours, this is due to several effectiveness factors of the *M. anisopliae* fungus in metankos in killing off *O. rhinoceros* larvae, including pathogenicity and insect resistance to compounds toxin. The low pathogenicity of isolates causes the time to kill 50% of larvae is also low, this can be due to the origin of the isolates, the age of the isolates and the method of application, this causing the time to kill 50% of the *O. rhinoceros* larvae takes a long time. In the opinion of Athifa *et al.* [24] the time taken for entomopathogenic fungi to infect insects is influenced by the isolates used, host type and environmental conditions. Siswanto and Triswa [17] added that the cause of low pathogenicity is due to the application method, the application period is only once and the larvae have skin changes so that the conidia that has been attached to the host's skin come off.

The method of application of conidia suspension on metankos also influences the length of time to kill 50% of *O. rhinoceros* larvae, because conidia *M. anisopliae* was sprayed on compost, indirectly on the larvae cuticle so that *M. anisopliae* fungus took time to reach the larvae cuticle and performed stages of compost to infect the *O. rhinoceros* larvae to kill 50% of the larvae. According to Wicaksono *et. al* [16] the time of death of test insects was faster in the application method of entomopatogen fungus sprayed on test insects than sprayed on compost, and the virulence of entomopathogenic fungi needed time to infect to kill the insect, infection began from attaching conidia, germination and penetration.

3.3 Lethal Concentrate (LC 50; 95) (%)

Based on the results of lethal concentrate (LC) probit analysis using the POLO program, the concentration of *M. anisopliae* showed LC 50 and LC 95, respectively 3.9% and 134.8%. Probit analysis results can be seen in Table 3.

Table 3. *Lethal Concentrate O. rhinoceros* Larvae againts *M. anisopliae.*

Lethal Concentrate	Concentrate (%)	SK Range
LC_{50}	3,9	(2,8 - 9,0)
LC_{95}	134,8	(29,7 - 68489,7)

Table 3 shows that the right concentration to kill 50% of *O. rhinoceros* larvae in metankos was 3.9% or equivalent to 39 $g.l^{-1}$ of *M. anisopliae* water. Meanwhile, the concentration to kill 95% of *O. rhinoceros* larvae in metankos was 134.8% or equivalent to 1348 $g.l^{-1}$ of *M. anisopliae* water.

The result of probit analysis of the exact concentration of *M. anisopliae* to kill 50% of larvae of *O. rhinoceros* was of 3.9% or equivalent to 39 g.l^{-1} of water of *M. anisopliae*, this means it takes 39 g.l^{-1} of water to kill 50% of larvae of *O rhinoceros,* approaching a concentration of 40 g.l^{-1} water (81.6 x 16 conidia.ml^{-1}). This was due to the number of conidia that affects the level of pathogenicity in killing off *O. rhinoceros* larvae. This was by the opinion of Siswanto and Trisawa [17] that a higher concentration of *M. anisopliae* results in the greater number of fungal conidia entering the insect's body compared to the treatment with a smaller convalescence.

The results of probit analysis to kill 95% of *O. rhinoceros* larvae in metankos the concentration is 134.8% or 1348 g.l^{-1} water of *M. anisopliae*, if it is connected with the treatment, it is very far compared to the highest concentration of 50 g.l^{-1} water treatment. The opinion of Ayu *et al.* [25] the higher the LC value produced, the lower the pathogenicity of entomopathogenic fungi is and vice versa.

3.4 Daily Mortality (%)

The observation of daily mortality of *O. rhinoceros* larvae with the treatment of several concentrations of *M. anisopliae* showed that the daily mortality of *O. rhinoceros* larvae fluctuated from the first day to the twenty-first day. The daily mortality fluctuation of *O. rhinoceros* larvae can be seen in Figure 1.

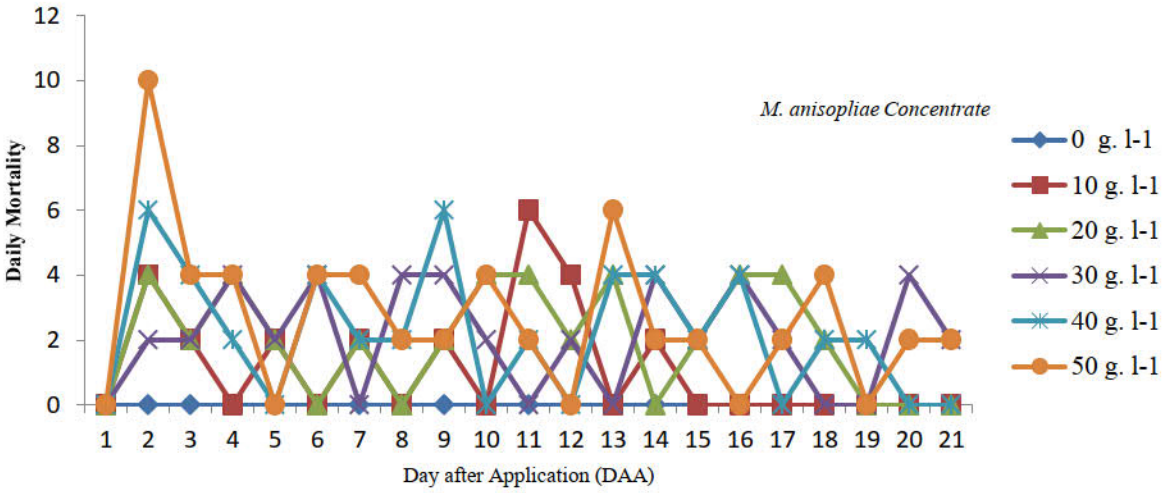


Figure 1. Daily Mortality *O. rhinoceros* after giving treatment of several *M. ansiopliae* concentrations on metankos

Daily mortality of *O. rhinoceros* tends to fluctuate throughout the 21 days of observation. Larvae first infected *O. rhinoceros* on the second day after treatment application *M. anisopliae* concentration on metankos at 10 g.l^{-1} of water, 20 g.l^{-1} of water, 30 g.l^{-1} of water, 40 g.l^{-1} of water, and 50 g.l^{-1} of water, with percentages of 4%, 4%, 2%, 6% and 10%. This was because *M. anisopliae* fungus to kill *O. rhinoceros* larvae took time to infect hosts from entomopathogenic fungi that require several stages of infection, according Yuningsih and Widyaningrum [18] that the fungus *M. anisopliae* in its development requires time stages of infection, mechanism of infection entomopathogen began with the attachment of fungal conidia to the cuticle of the insect, then the conidia germinate and penetrate the insect's body. The next stage, the fungus grew and developed in the blood of insects. The fungus would speed up reproduction by separating the body of the animal to fight the resistance of insects, at the same time, the antibiotic toxin produced by the fungus weakens and kills the insect quickly, then hyphae would grow and fill the entire body of the insect. The fungus began to develop, insects displayed symptoms of pain, such as uncoordinated movements and would eventually cause death.

Treatment of *M. anisopliae* concentration in metankos 50 g.l^{-1} water mortality began to occur and reached the peak of mortality on the second day with a percentage of mortality of 10% and 50 g.l^{-1}

water treatment fluctuated until the end of observation. The peak mortality rate of 50 $g.l^{-1}$ water treatment was the highest percentage compared to other treatments. This is because high concentrations contain a greater number of conidias entering the insect's body, compared to other treatments with lower concentrations and fewer cones. This is in accordance with the opinion of Siswanto and Trisawa [17] that the higher the density of the entomopathogenic fungus conidia, the higher the infection power of the insect. In the opinion of Gabarty *et al.* [26] that when the conidia attach to the cuticle, the insect will germinate and penetrate the insect's skin and then it will produce toxin and damage the insect's immune system.

The treatment of 40 $g.l^{-1}$ water also showed a peak mortality on the second day and tenth day with the same percentage of 6% and fluctuated until the end of the observation. Treatment of 30 $g.l^{-1}$ water reached a peak mortality of 4% and fluctuated until the end of observation. The treatment of 20 $g.l^{-1}$ water reached the peak of mortality with the same percentage as the treatment of 30 $g.l^{-1}$ water which was 4% and fluctuated until the end of observation.

The 10 $g.l^{-1}$ water treatment experienced a peak mortality on the eleventh day with a percentage of 6% and fluctuated until the fourteenth day and decreased on the fifteenth day to the twenty-first day without the death of *O. rhinoceros* larvae. This could be caused by the low factor of conidia that entered the body of the insect, due to the low concentration of metankos given so that it could not match the ability to kill the same as other treatments. This was reinforced by the opinion of Masyitah *et al.* [27] that the number of conidia is also low, and the toxins produced are less resulting in a lower percentage of deaths.

The administration of *M. anisopliae* concentration to metankos gives a lower mortality effect compared to the application method given directly to the pest. This was due to the method of application of spraying the concentration of *M. anisopliae* on compost (metankos) of the fungus *M. anisopliae* requires time to be able to grow and develop on compost before the fungus could contact with larvae and perform several stages of infection. Opinions of Yuningsih and Widyaningrum [18] *M. anisopliae* fungus in their development required time stages of infection, which began with the attachment of conidia to the insect cuticle.

On the third day to the twenty-first day at the treatment concentration of 50 $g.l^{-1}$ water, 40 $g.l^{-1}$ water, 30 $g.l^{-1}$ water, 20 $g.l^{-1}$ water, and 10 $g.l^{-1}$ water in metankos, the percentage of death of larvae of *O. rhinoceros* continue to experience fluctuations in the range of 2-6%, this could be caused by endurance factors *O. rhinoceros* larvae. In accordance with the opinion of Sihombing *et al.* [15] which stated that although entomopathogenic virulence increases, the resistance of *O. rhinoceros* larvae also increased so that there would not be a large infection difference every day.

3.5 Mortality Total

The analysis showed that *M. anisopliae* in metankos had several significant effects on the total mortality of *O. rhinoceros* larvae. Results for the average of death of *O. rhinoceros* larvae after the DNMRT test at the 5% level can be seen in Table 4.

Table 4. Total Mortality of *O. rhinoceros* Larvae After Giving Several *M. anisopliae* Concentrations on Metankos

Treatments of several *M. anisopliae* Concentration in metankos	Total Mortality (%)
0 $g.l^{-1}$ (without *M. anisopliae*)	0,0 c
10 $g.l^{-1}$ (33,6 x 10^6 conidia.ml^{-1})	24,0 b
20 $g.l^{-1}$ (57,6 x 10^6 conidia.ml^{-1})	42,0 ab
30 $g.l^{-1}$ (62,4 x 10^6 conidia.ml^{-1})	44,0 ab
40 $g.l^{-1}$ (81,6 x 10^6 conidia.ml^{-1})	46,0 ab

50 g.l^{-1} (302,4 x 10^{6} conidia.ml^{-1})	56,0 a

The figures on the lane followed by lowercase letters were not significantly different according to the DNMRT test at the 5% level after being transformed with formula $\arcsin^{-1}\sqrt{y}$.

Table 4 shows that several *M. anisopliae* concentrations on metankos have significant different effect on the amount of total mortality *O. rhinoceros* larvae with 24% -56% range.Treatment concentrations of *M. anisopliae* 0 g.l^{-1} water until the end of the observation (504 hours) showed that no *O. rhinoceros* larvae die and this showed significant difference from other treatments. Treatment concentration of *M. anisopliae* on metankos of 10 g.l^{-1} water total mortality by 24% and no significant with treatment *M. anisopliae* concentration on metankos 20 g.l^{-1} water, 30 g.l^{-1} water, and 40 g.l^{-1} water with a total mortality of 42%, 44% and 46%, but a significant difference was shown by treatment of *M. anisopliae* concentration on metankos 50 g.l^{-1} water with total mortality 56%.

The highest tendency concentration of *M. anisopliae* in metankos was 50 g.l^{-1} water causing a total mortality of 56%. This was due to the higher concentration of entomopathogenic fungi, the more fungal conidia, so that the mortality of *O. rhinoceros* larvae was also higher. This was consistent with the opinion of Susanti [20] which stated that the relationship between the conidia concentration and test insect mortality, the higher the concentration, the higher the test insect mortality. Aw and Hue [28] state that high concentrations that contain large amounts of conidia can cause significant larval mortality.

Giving some concentration of *M. anisopliae* in metankos, concentration of 50 g.l^{-1} water was the best tendency among treatments, which resulted in mortality of 56% with a moderate level of virulence. According to Castrillo *et al.* [29] the level of entomopathogenic fungal pathogenicity is determined by the potential of the host insect and the environment around it, high virulence with a percentage of mortality more than 64.49%, moderate virulence with a mortality percentage of 30.99-64.49% and low virulence with a percentage of deaths less than 30.99%.

The administration of *M. anisopliae* concentration to metankos had not been categorized as a bioinsecticide in killing off *O. rhinoceros* pests, although it could be categorized as moderate virulence level, because the mortality rate is still low at 56%. This was in accordance with the opinion of Steinhaus (1963) in Hasyim [30] fungi that can be categorized as bioinsecticides are fungi that have successfully controlled insects with a mortality of 72%-95%.

The administration of *M. anisopliae* concentration to the mortality metankos was still low compared to the application method given directly to the pest. This was due to the application method of spraying the concentration of *M. anisopliae* on compost (metankos), conidia of the fungus *M. anisopliae* requires time to reach the cuticle of the larvae and carry out stages of infection to infect larvae and kill larvae, so that the resulting mortality percentage would be lower compared to mortality with application method of spraying directly into the pest.

Factors causing low larval mortality are thought to be caused by the application method. Conidia suspension application was given indirectly on the cuticle of the larvae but conidia of *M. anisopliae* was sprayed on compost first, so that the fungus *M. anisopliae* needed time to reach the larvae cuticle and perform steps to infect the *O. rhinoceros* larvae. This was in accordance with the opinion according to Wicaksono *et al.* [16] that insect mortality by the application method sprayed on compost is lower than the application method sprayed on insects.

The factors causing the low mortality of *O. rhinoceros* larvae are also thought to be due to the origin of the isolate and the age of the *M. anisopliae* isolate, the isolates used were not tested for pathogenicity first. Low pathogenicity of isolates causes low mortality. This was in accordance with the opinion of Athifa *et al.* [24] isolate source factors influence the ability of fungi to produce conidia and control target pests.

Various factors affect the effectiveness of fungi in causing the death of target insects, including conidia density, quality of mushroom growing media, type of pest controlled, age of pest status, time of application, frequency of application and environment. The effectiveness of entomopathogenic

fungi was also influenced by the method of application and volume of application that could have a significant effect on the mortality of the pupae *Bactrocera carambolae* [16].

Metarhizium anisopliae attacks its host by absorbing fluid from its host body. The fungus grew out of its host body and produced conidia so that the host's body mummified. This was thought to be a result of the start of the operation of the toxin produced by the fungus. The toxin damaged the tissues and absorbs the body's fluid from the larva's body, causing the larvae to dry up and die [27]. The fungus conidia of *M. anisopliae* had the activity of killing larvae because it produced cyclopeptide toxin, destruxin A, B, C, D, E and desmethyl destruxin [13]. According to Aw and Hue [28] on the fungus *M. anisopliae* toxin destruxin, specifically destruxin A and E are more insecticides which are synthesized to suppress the body's immune response of test insects.

3.6 The Changing of Larva Behavior and Morphology

Changes in behavior of *O. rhinoceros* larvae infected with the fungus *M. anisopliae* are infected larvae whose movements become slow and their appetite decreases. This was consistent with the opinion of Sihombing *et al*. [15] that the behavior of the death of larvae infected by entompatogen mushrooms will slow down and their appetite decreases, eventually they become silent and die. According to Utari *et al.* [31] that decreased larval movement due to hyphae emit dextrucine toxins and toxins that damage the cell membrane structure so that cell dehydration will occur, dextrucine effect causes cell paralysis, abnormalities in middle gastric function, malphigi tubules, hemocyt and muscle tissue.

Infected larvae and dead larvae were generally located on the surface of metankos. In accordance with the opinion of Siswanto and Trisawa [17] that dead larvae show symptoms rising to the surface. This is a characteristic of larvae infected by pathogenic fungi known as summit disease. This phenomenon was allegedly an attempt to save other healthy populations from entomopathogenic fungal infections.

Morphological changes in the body of *O. rhinoceros* larvae after application of the fungus *M. anisopliae* i.e. there was a brown spot on the cuticle of the larvae occurred on the second day after application (Figure 2a), then a change in body color of *O. rhinoceros* larvae from yellowish white to blackish brown on the day third (Figure 2b). This was in accordance with the opinion of Siswanto and Trisawa [17] that the change in larval body colour to blackish brown is suspected to be *O. rhinoceros* larvae undergoing the process of melanisation which is a form of insect body defence against pathogens, in contrast to larval bodies without *M. anisopliae* which remained yellowish white. In the opinion of Indriyanti *et al.* [32], the formation of melanin was known as melanisation which was carried out by the enzyme phenol oxidase. Larvae undergo melanisation in the lower body, chest, abdomen, and inter-body segments.

The larvae became stiff and began to mummify on the fourth day (Figure 2c), on the eighth day white hyphae appeared (Figure 2d) and on the eleventh day the fungus would sporulate green (Figure 2e). This wa consistent with the opinion of Indriyanti *et al.* [32] that the appearance of white hyphae outside the larval body indicates that the nutrients contained in the larval body have been depleted. The ability of hyphae to appear on the outside of the larva's body depends on the condition of the cuticles of the larvae, if conditions are dry and moist the hyphae will be able to penetrate the cuticle and cover the body of the insect with hyphae. Some larvae were found dead without hyphae appearing on the outside of their bodies until the 15th day. According to Prayogo *et al.* [33] hyphae do not always grow out of the integument, if the conditions are less favorable, the development of fungal saprophytes will only take place in the larval body without penetrating out the integument and the fungus will form a special structure to survive, namely the arthospora. The cuticle and epidermis appear damaged, the mycelium and conidia were multiplying, and the conidia was ready to infect a new host.

Figure 2. The changing morphology *O. rhinoceros* larvae (a) brown spots on the cuticle of the larvae on the second day (b) larvae runs into melanisation on the day third (c) larvae began to mumification on the fourth day (d) white hyphae starts to appear on the eighth day (e) white hyphae changes to be green on the eleventh day

4. Conclusion

The results of the research is conclusion that concentration of *M. anisopliae* in metankos 50 $g.l^{-1}$ water infected *O. rhinoceros* larvae with a total mortality of 56% but the level of virulence was categorized as medium, because it caused a mortality percentage between 30.99-64.49%. The right concentration to kill 50% of *O. rhinoceros* larvae is 3.9% or equivalent to 39 $g.l^{-1}$ of *M. anisopliae* water, meanwhile, the concentration to kill 95% of *O. rhinoceros* larvae is 134.8% or equivalent to 1348 $g.l^{-1}$ water of *M. anisopliae.*

Acknowledgment

Thank you to the University of Riau DIPA Fund for funding this research.

References

[1] Central Bureau of Statistics 2018 Indonesian Palm Oil Statistics 2017 https: //www.bps.go.id. Accessed January 16, 2019

[2] Palm Oil Research Center 2008 Pest and Disease Control Technology in Palm Oil: Ready for Use and Environmentally Friendly http://pustaka.setjen.pertanian.go.id/publikasi/wr271058.pdf. Accessed October 14, 2018.

[3] Riau Province Plantation Service 2014 Data on Palm Oil Damage in Riau. Oil Palm Plantation Service Pekanbaru

[4] Winarto L 2005 Integrated Palm Beetle Pest Control. http://www.agroindonesia.com. Accessed October 14, 2018

[5] Santi I S and Sumaryo B 2008 Effect of pheromone trap color on the catch of imago *Oryctes rhinoceros* in oil palm plantations. *Indonesian Plant Protection Journal* **14(2)** 76-79

[6] Rahmiaty D P, Wardati and Fauzana H 2018 Provision of hornbill (*Oryctes rhinoceros*) larvae to increase the growth of palm oil seeds (*Elaeis guineensis* Jacq.) In the main nursery *Photon Journal* **8(2)** 45-51

[7] Prawirosukarto S 2003 Pest Control and Coconut Oil Crop Disease. Research Report (Unpublished) Palm Oil Research Center Field

[8] Darnoko D and Sembiring T 2005 Synergy between oil palm plantations and food crop agriculture through the application of OPEFB compost for rice plants. Proc. of the Palm Oil Technical Meeting: Increasing Palm Oil Productivity Through

[9] Hartatik W, Husnain and Widowati L R 2015 The Role of Organic Fertilizers in Increasing Productivity of Soils and Plants. Research Report (Unpublished) Soil Research Institute Bogor

[10] Rajan P, Mohan C and Josephrajkumar A 2010 Integrated pest management in coconut. Proc. of International Conference on Coconut Biodiversity for Prosperity Kerala

[11] Yustina, Fauziah Y and Sofia R 2012 Structure of horn beetle population (*Oryctes rhinoceros*) in the area of oil palm plantations in the Kenantan Village community of Kampar Riau Regency.*J. of Biogenesis* **8(2)** 54-63

[12] Harjaka T, Arif W, Wagiman F X and Muhammad 2011 Pathogenicity of *Metarhizium anisopliae* against *Lepidiota stigma* uret larvae. Nasional Seminar Proc. Vegetable Pesticides IV. Yogyakarta
[13] Widiyanti N L P M and Muyadihardja S 2004 *Metarhizium anisopliae* fungal toxicity test against *Aedes aegypty* mosquitoes. *J. of Health Research and Development Media* **14(3)** 24-30
[14] Parinduri S, Saleh A and Suhanda A 2017 Test the effectiveness of prevention of hornbill larvae (*Oryctes rhinoceros*) using a chemical made from sipermetrin and the fungus *Metarhizium anisopliae. Agro Estate Journal* **1(2)** 128-134
[15] Sihombing R H, Oemry S and Lubis L 2014 Test the effectiveness of several entomopathogens in larvae of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) in the laboratory *Online Journal of Agrotechnology* **2(4)** 1300-1309
[16] Wicaksono A P, Abadi A L and Afandhi A 2015 Test the effectiveness of the entomopathogenic fungus *Beauveriabassiana* (Bals.) Viullemin against the pupa of *Bactroceracarambolae* Drew & Hancock (Diptera: Tephritidae) *Journal of HPT* **3(2)** 39-49
[17] Siswanto and Trisawa I M 2017 Quality Test and Effectiveness of *Metarhizium anisopliae* Isolate Central Kalimantan Against *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). Research Report (Unpublished). Plantation Research and Development Center. Bogor
[18] Yuningsih and Widyaningrum T 2014 Test pathogenicity of *Metarhizium anisopliae* fungal spores to the mortality of *Oryctes rhinoceros* larvae as biology class X high school biology teaching material *JUPEMASI-PBIO* **1(1)** 53-59
[19] Sedighi N, Abbasipour H, Askary H, Gorjan A S and Karimi J 2013 Patogenicity of the enotomopathogenic fungus *Metarhizium anisopliae* var. *Major* on different stages of the sunn pest *Eurygaster integriceps Journal Online*.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4015400/
[20] Susanti U 2013 Tests of Several Concentrations of *Metarhizium anisopliae* (Metsch) Sorokin for Controlling Green Ladybug (*NezaraViridula* L.)Pests in Long Beans (*Vignasinensis* L.). Thesis (Not Published) Riau University Pekanbaru
[21] Freimoser F M, Screen S, Bagga S, Hu G and St. Leger R J 2003 Expressed Sequence Tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals a plethora secreted preotein with potensial activity in insect hosts *Journal of Microbiology* **149** 239-247
[22] Nurjayanti 2017 Tests of Local *Beauveria Bassiana* Vuill Entomopathogenic Fungi Concentration and Local *Cordyseps Militaris* (L: Fr) Against *Sethothosea Asigna* Van Eecke Caterpillars on Palm Oil Plants. Thesis (Not published) Riau University. Pekanbaru
[23] Gopal M A, Gupta and Thomas G V 2005 Prospects of using *Metarhizium anisopliae* to check the breeding of insect pest *Oryctes rhinoceros* L. in coconut leaf vermicomposting sites *J. Biosource Technology* **97** 1801-1806
[24] Athifa S, Anwar S and Kristanto B A 2018 Effect of variant of *Metarhizium anisopliae* fungus on mortality of pest larvae of *Oryctes rhinoceros* and *Lepidiota stigma. J. Agro Complex* **2(2)** 12-127
[25] Ayu A, Salbiah D and Sutikno A 2018 Test several local entomopathogenic concentrations of *Cordyceps militaris* L. Fries fungus to control the caterpillar larvae of *Chrysodeixis chalcites* Esp. in soybean plants *(Glycine max* L. Merrill). Online *J. of Faculty of Agriculture Students* **5(1)** 1-12
[26] Gabarty A, Salem H M, Fouda M A, Abas A A and Ibrahim A A 2013 Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotisipsilon* (Hufn.). *J. of Radiation Research and Appl. Scienc* **7** 95-100
[27] Masyitah I, Sitepu S F and Safni I 2017 Potential of entomopathogenic fungi to control armyworm *Spodopteralitura* F. in tobacco plants in vivo. *J. of USU's FP Agrotechnology* **3 (5)** 484-493
[28] Aw K S M and Hue S M 2017 Mode of infection of *Metarhizium* spp. fungus and their potential as biological control agents. *Journal* Online *of Fungi* **3(2)** 30

[29] Castrillo L A, Roberts D W and Vandenberg J D 2005 The fungal past, present, and future: germination, ramification and reproduction *J. of Invertebrate Pathology* **89** 46-56
[30] Hasyim A 2007 Enhancing infectious capacity of entomopathogen Fungi, *Beauveria bassiana* (Balsamo) Vuill. using various carrier materials in controlling banana corm borer *Cosmopolites sordidus* Germar in the field *Jurnal Horti* **17(4)** 335-342
[31] Utari N M W, Sudiarta I P and Bagus I G N 2015 Effect of media and age of *Metarhizium anisopliae* M. culture on the mortality rate of *Oryctes rhinoceros* L. larvae in the Electronic *J. of Tropical Agricult* **4(2)** 160-169
[32] Indriyanti D R, Damayanti I B, Setiati N and Priyono B 2017 Mortality and tissue damage in every symptom of *Oryctes rhinoceros* L. larvae infection due to the treatment of the fungus *Metarhizium anisopliae J. of Unnes Life Science* **6(1)** 9-17
[33] Prayogo Y, Tengkano W and Marwoto 2005 Prospom of entomopathogenic fungus *Metarhizium anisopliae* to control *Spodoptera litura* gray worm on soybean *Agricult. Research and Development J* **24 (1)** 19-26