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EFFECT OF LIQUID FORMULATION OF *Metarhizium anisopliae* (COMPOST, METANKOS) ON MORTALITY OF *Oryctes rhinoceros* L. LARVAE ON OIL PALM

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ABSTRACT

Palm oil trees are widely cultivated in Riau Province producting large quantities of oil palm empty fruit bunches (EFB) that can be composted and enriched with the biological control agent *Metarhizium anisopliae* Metsch. to manage the pest *Oryctes rhinoceros* L. This study aims to obtain the best dosage of the liquid formulation of *M. anisopliae* in Metankos on the mortality of *O. rhinoceros* larvae in oil palm plants. This research was conducted experimentally using a complete randomized design (CRD). The data obtained were analyzed statistically using the SAS software, followed by DNMRT test at 5% significance level. The results showed that the best dose of *M. anisopliae* liquid formulation on compost media was 210 g/l per bucket with a total mortality of 90%. A dose of 120 g/L per the bucket also produced significant mortality (72.5%), meeting the criteria of a bioinsecticide for controlling *O. rhinoceros* larvae. These findings indicate that the Metankos liquid formulation has strong potential as an effective biological control agent in oil palm pest management.

Keywords: *Elaeis guineensis* Jacq.; *Metarhizium anisopliae* Metsch.; compost; *Oryctes rhinoceros* L.

ABSTRAK

Tanaman kelapa sawit banyak ditanam di Provinsi Riau dan menghasilkan jumlah tandan kosong kelapa sawit (EFB) yang tinggi, yang boleh dikompos dan diperkaya dengan agen kawalan biologi *Metarhizium anisopliae* Metsch. untuk mengawal perosak *Oryctes rhinoceros* L. Kajian ini bertujuan untuk mendapatkan kadar terbaik formulasi cecair *M. anisopliae* dalam Metankos terhadap kematian larva *O. rhinoceros* pada tanaman kelapa sawit. Penyelidikan ini dijalankan secara eksperimen menggunakan Reka Bentuk Rawak Lengkap (CRD). Data yang diperoleh dianalisis secara statistik menggunakan perisian SAS, diikuti dengan ujian DNMRT pada aras signifikan 5%. Hasil kajian menunjukkan bahawa kadar terbaik formulasi cecair *M. anisopliae* pada media kompos ialah 210 g/L bagi setiap baldi dengan jumlah kematian

sebanyak 90%. Kadar 120 g/L bagi setiap baldi juga menghasilkan kematian yang signifikan (72.5%), memenuhi kriteria bioinsektisid bagi kawalan larva *O. rhinoceros*. Penemuan ini menunjukkan bahawa formulasi cecair Metankos mempunyai potensi yang tinggi sebagai agen kawalan biologi yang berkesan dalam pengurusan perosak kelapa sawit.

Kata kunci: *Elaeis guineensis* Jacq.; *Metarhizium anisopliae* Metsch.; kompos; *Oryctes rhinoceros* L.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an Indonesian commodity plant exported to contribute to the state revenue. Certain parts of the plant, such as the fruit, can be used as a source of vegetable oil. This oil, rich in carotenoids, serves as an important raw material for food processing in West Africa (Wahyuni et al. 2017). The Central Bureau of Statistics (2023) notes that in 2022 the Riau province has the largest area of oil palm plantations in Indonesia, reaching 2.9 million hectares and the production of crude palm oil (CPO) reaching 8,969,588 million tons or around 19.62% of Indonesia's total production. Oil palm plantations in Riau province are followed by high production of oil palm which causes a large amount of oil palm waste to be produced which calls for a proper waste treatment.

Production of empty palm fruit bunches ranges from 22% to 23% of the total weight of fresh fruit bunches processed at the oil palm mill (Lestari et al. 2020). Fertilizer can be obtained from empty fruit bunches (EFB); however, it takes a long time to process. Therefore it would be better if the waste is composted to speed up the availability of nutrients for plants (Umar et al. 2022). EFB increases the organic matter content of the soil, therefore the soil structure improves and the soil's ability to hold water improves as well (Purnamayani 2013). EFB are used as active nests and breeding grounds for horn beetles (*Oryctes rhinoceros* L.) from egg to pupa stages. *Oryctes rhinoceros* attacks caused up to 22 deaths, 76% in Hulu on 2-year-old oil palm plants (Handoko et al. 2017).

This gnawing activity causes damage to young leaves that have not yet bloomed, marked by “V” shaped cuts as the leaves begin to grow. The crown of the leaf becomes irregularly damaged, the leaf midrib often breaks in the middle, the tips of the leaves are damaged, and the base of the midrib can break. The damage slows down the growth of the plant, and if it reaches the growing point, it can cause the death of the plant (Widians & Rizkyani 2020). This incident must be controlled to suppress the population of *O. rhinoceros* L. on palm oil plantations in order to remain below the economic threshold.

The recommended environmentally friendly pest control is integrated pest control, as efforts to control attacks by plant-disturbing organisms with control techniques in one unit to prevent economic losses and create sustainable agriculture. One of the recommended Integrated Pest Management (IPM) elements for controlling pests in oil palm plants is biological agents. Biological control agents in all stages of their development can be used for the purpose of controlling plant pests. The advantages of using biological agents are having a high reproductive capacity, short life cycle, being able to form conidia which are durable in nature even under unfavorable conditions. The use of biological agents can be combined with other IPM components, which are not easily resistant and of course easily decomposed by the environment (Salbiah et al. 2013).

One of the biological agents is insect pathogens which have been proven to be quite effective in killing insect pests is *Metarhizium anisopliae* Metsch. The *M. anisopliae* fungus can be mass produced and formulated as a bioinsecticide in solid liquid form. The research results of Fauzana et al. (2023) state that liquid formulation of the *M. anisopliae* fungus was more effective than solid formulations in the process of targeting insect mortality. However, the effectiveness of various *M. anisopliae* preparations, both solid and liquid, will be better if they are adjusted to the method of implementation, which will be more suitable with flush liquid formulations.

Metarhizium anisopliae fungus effectively kills pests in the following order: Orthoptera, Lepidoptera, Homoptera and Coleoptera (Abdullah et al. 2021). Insects can be infected by conidia through the cuticle and form gaps between body segments, by entering the host's body and spreading to the hemocoel (Syazwan et al. 2021). Detecting the initial stage of *M. anisopliae* infection poses a challenge as the infection develops inside the larvae with no noticeable odour changes. However, later stages reveal small dots of white mycelium on the body surface of larvae, which gradually widen and transform into green mycelium over time. *Metarhizium anisopliae* is commonly known as the green muscardine fungus (Rahayuwati & Kusuma 2024). *Metarhizium anisopliae* has larvicidal activity because it produces cyclopeptides, destruxin A, B, C, D, E and desmethyl destruxin B. The effect of destruxin impacts the target cell organelles (mitochondria, endoplasmic reticulum and nuclear membrane), causing cell paralysis and abnormalities in the function of the middle stomach, malpighian tubules, hemocyt and muscle tissue (Archana et al. 2022).

The results of Fauzana and Fadilla (2022) demonstrated that increasing the dose of *M. anisopliae* suspension in compost by sprinkling 75 g/l water, 85 g/l water and 95 g/l water will cause total mortality of *O. rhinoceros* larvae of 72.5±5. 00%, 80. 00±0.00%, and 87.50±9.57%, respectively. A dose of on Metankos media (EFD and sawdust). Building upon these findings, this study aims to determine the optimal dosage of a liquid formulation of *M. anisopliae* in Metankos for the effective control of *O. rhinoceros* larvae in oil palm plants.

MATERIALS AND METHODS

Experimental Design and Treatment Details

The research was conducted at the Laboratory of Plant Pests and Experimental Garden Laboratory, Faculty of Agriculture, University of Riau, Bina Widya Campus Pekanbaru. The study was conducted over five months periods, from June to October 2022. A Complete Randomized Design (CRD) was used, followed by a 5% Duncan's New Multiple Range Test (DNMRT) for mean separation. The experiment consisted of eight treatments with four replications, resulting in a total of 32 experimental units. Each unit contained 10 *O. rhinoceros* larvae and 5 kg of Metankos per bucket.
The treatment can be detailed as follows:

M0: Liquid formulation of *M. anisopliae* dose of 0 g/l per bucket;
M1: Liquid formulation of *M. anisopliae* dose of 30 g/l per bucket;
M2: Liquid formulation of *M. anisopliae* dose of 60 g/l per bucket;
M3: Liquid formulation of *M. anisopliae* dose of 90 g/l per bucket;
M4: Liquid formulation of *M. anisopliae* dose of 120 g/l per bucket;
M5: Liquid formulation of *M. anisopliae* dose of 150 g/l per bucket;
M6: Liquid formulation of *M. anisopliae* dose of 180 g /l per bucket;
M7: Liquid formulation of *M. anisopliae* dose of 210 g/l per bucket.

Compost Preparation and Sterilization

Compost was made from a mixture of chopped EFB organic matter with sawdust and manure on plastic sheeting using a ratio of 3:3:2. To accelerate decomposition, 4 kg of *Trichoderma* sp. culture obtained from the Plant Disease Laboratory was incorporated into the mixture. A 5g of TSP, 5g of urea, 5g of dolomite, 2,5 kg of brown sugar, and water were also added to maintain moisture. The composting process was performed in three layers, in which the mixture was arranged, moistened, and stirred to ensure homogeneous mixing of all ingredients. The compost piles were covered with plastic sheeting and turned weekly to support aeration and decomposition. Compost sterilization was carried out by the tyndallization method or by applying hot steam to the media. Heating the compost was carried out three times using a drum each for 1 hour.

Provision and Preparation of *M. anisopliae*

Re-isolation of M. anisopliae

Re-isolation of *M. anisopliae* isolates on to PDA media was carried out by pouring PDA media in to petri dish that had been sterilized in an autoclave. Each petri dish was filled with 10 ml of PDA media and allowed to solidify first. *M. anisopliae* isolation on PDA media was taken with an ose needle and placed into new PDA media in the LAFC room. The petri dish was tightly covered by plastic wrap and incubated for seven days.

Propagation of M. anisopliae on Cracked Corn Media

Propagation of the *M. anisopliae* fungus on cracked corn media was carried out by washing the broken corn thoroughly and steaming it until it was one third cooked. Cracked corn was put in 0.5 kg size plastic, and sterilized in an autoclave. The *M. anisopliae* isolation growing on PDA media in a petri dish were divided with a sterile knife into nine parts to be inserted into the cracked corn media in the LAFC room, the media was shaken by plastic in order to hence allow the mushrooms to spread evenly. The plastic was glued with a hecter and incubated for seven days.

Preparation of M. anisopliae Into Liquid Media

Metarhizium anisopliae starter on cracked corn was weighed as much as 0 g, 30 g, 60 g, 90 g, 120 g, 150 g, 180 g, and 210 g using an analytical balance. The *M. anisopliae* fungus was mixed with 1 liter of water in a 1000 ml beaker glass and granulated sugar while stirring. The starter was put in a 1000 ml bottle using a plastic funnel to prevent the corn from getting into the bottle. Then it was shaken using a rotary shaker for 24 hours to accelerate cell division.

Treatment Application

Treatment application was done by watering and mixing methods. Compost weighing 5 kg per bucket was weighed using a scale. The starter was filtered before being put into the fanfare. The liquid formulation was measured as much as 800 ml using a 1000 ml glass beaker according to the flush volume. The liquid formulation was poured into the compost according to the treatment dose and mixed evenly. The compost that had been treated was put in a bucket. The bucket was covered with tile cloth and arranged according to the design used.

Procurement and Infestation of *O. rhinoceros* Larvae

Oryctes rhinoceros instar II was taken from the Sei Kijang oil palm area, Pelalawan Regency, Riau. The larvae were brought to the Plant Pest Laboratory using a bucket containing EFB. Ten larvae were infested in a plastic bucket at a depth of 10 cm and compost was added to a height of 20 cm. The bucket was covered again with a tile cloth. There were a total of 320 *O. rhinoceros* second instar larvae for 32 treatment units used.

Observation Parameters and Data Collection

Changes in behavior and morphology of O. rhinoceros larvae infected by M. anisopliae

Observation of changes in behavior and morphology of *O. rhinoceros* larvae infected by *M. anisopliae* was carried out 12 hours after application. Observations were made by looking at the changes that occurred in the body of *O. rhinoceros* larvae after infection. The changes in characteristics of infected larvae included the larvae turned pale white, the posterior part shrinked, gradually hardened and stiffened, the larval body was filled with powdery white fungal conidia and over time the conidia turned brownish green (Erawati & Wardati 2016) .

Initial time mortality time of O. rhinoceros larvae (hours)

Observations were made by calculating the earliest time needed for the *M. anisopliae* fungus to kill one of the larvae of *O. rhinoceros* in each treatment. Observations were made every 12 hours. *Oryctes rhinoceros* larvae that had been infected by *M. anisopliae* were characterized by a white fungal mycelium that completely covered the larvae and then the larvae turned brownish green in color.

Lethal time 50 (LT_{50}) of O. rhinoceros Larvae (Hours)

Observations were made by calculating the time required for each treatment to kill 50% of the *O. rhinoceros* larvae. Observations were made every 12 hours after treatment to achieve 50% mortality of the larvae for each experimental unit.

Lethal Dose (LD_{50} and LD_{95}) of O. rhinoceros Larvae (%)

Observations were made by calculating the correct dose of *M. anisopliae* in killing 50% and 95% *O. rhinoceros* larvae. Observations were made at the end of the observation.

Daily Mortality of O. rhinoceros Larvae (%)

Observations were made by counting the dead *O. rhinoceros* larvae every day after treatment was given. The percentage of daily mortality can be calculated using the following formula:

$$\mathrm{MH} = \frac{\mathrm{a} - \mathrm{b}}{\mathrm{a}} \mathrm{x}\ 100\%$$

Daily mortality is the number of observed *O. rhinoceros* larvae (a) minus the number of alive *O. rhinoceros* larvae (b) the result showed number of observed *O. rhinoceros* larvae (a) multiplied by 100%.

Total Mortality of O. rhinoceros Larvae (%)

Observations were made by counting the total number *O. rhinoceros* larvae that died after application of *M. anisopliae*. Observations were made every 12 hours starting 2 hours after application. The total mortality can be calculated using the following formula:

$$\mathrm{MT} = \frac{\mathrm{b}}{\mathrm{a} + \mathrm{b}}\ \mathrm{x}\ 100\%$$

Total mortality is the number of dead *O. rhinoceros* larvae (b) divided by sum of total alive *O. rhinoceros* larvae (a) plus the number of dead *O. rhinoceros* larvae (b) multiplied by 100%.

*Conidial Density of M. anisopliae (per g***)**
Calculation of the conidia of the *M. anisopliae* fungus was carried out using the serial dilution method 1 gram of compost media was diluted with 9 ml of distilled water, stirred hence a 10^{-1} dilution was obtained, then dilution was carried out again from a 10^{-1} dilution solution by taking 1 ml of the solution and then it was put into 9 ml of distilled water, then a 10^{-2} dilution was obtained, and continued on until the 10^{-8} dilution was obtained. Conidial density was counted using a haemocytometer under a microscope. Conidial density was calculated using the Nuryanti et al. (2012) formula as follows:

$$J = \frac{t \times d}{0.25 \times n} \times 10^6$$

The density of conidia per ml of solution (J) is the product of the total number of conidia in the sample box observed (t) multiplied by the dilution level (d) divided by product correction actor in small scale sample squares on the haemocytometer (0.25) times the number of square boxes counted (n) times 10^6.

Temperature and Humidity
Temperature and humidity within the compost media were monitored using a thermohygrometer. Measurements were taken daily at three time points: in the morning at 07:00 WIB, at midday at 12:00 WIB, and in the afternoon at 17:00 WIB.

RESULTS AND DISCUSSION

This study was conducted at a compost temperature of 28.65ºC and a humidity level of 83.87%. The results of the chemical analysis of the compost showed a pH 7.35, a moisture content 56%, an organic carbon level of 37.22%. and a total nitrogen content of 0.66%. The macroscopic and microscopic characteristic of the local *M. anisopliae* isolates from Riau are presented in Figure 1.

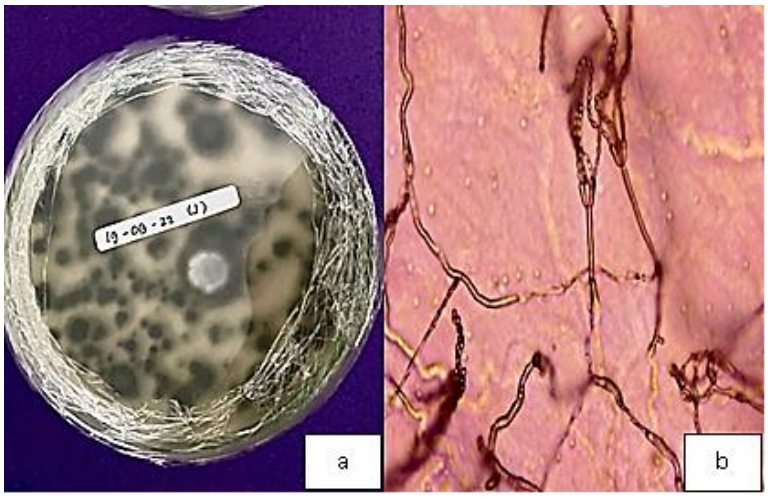

Figure 1. Appearance of *M. anisopliae* isolates local Riau (a) macroscopic. (b) microscopic (400 x magnification)

Changes in Behavior and Morphology Of *O. rhinoceros* Larvae Infected By *M. anisopliae*
Changes in the behavior of *O. rhinoceros* larvae infected by *M. anisopliae* generally occurred on the surface of the Metankos (Figure 2). Litwin et al. (2020) said that infected *O. rhinoceros* larvae resulted in reduced nutrition hence *O. rhinoceros* larvae which was attacked by entomopathogenic fungi would rise to the soil surface. In accordance with Siswanto and Trisawa (2017) infected larvae typically rise to the surface before dying. This behavior is throught to be a defensive mechanism intended to reduce the spread of entomopathogenic fungal infection to other healthy individuals in the population.

After being infected the bodies of the *O. rhinoceros* larvae became paralyzed and eventually died due to the action of *M. anisopliae.* The fungus, produces toxins that disrupt muscle function and impair normal physiological processes. Accroding to Syazwan et al. (2021), insecticides worked by affecting target cell organelles and causing paralysis of muscle tissue, cell paralysis, gastric dysfunction, malpighian tubules, as well as hemocytes.

Figure 2. *Oryctes rhinoceros* larvae climbed to the surface of the compost

Morphological changes in the body of *O. rhinoceros* larvae after the application of *M. anisopliae* fungus were black spots on the cuticle of the larvae after 36 hours of infection (Figure 3a). The larval body turned brown after 48 hours of application (Figure 3b). The body color of *O. rhinoceros* larvae changed to blackish brown after 60 hours of application (Figure 3c). This is in line with Santi et al. (2022) that the characteristic of larvae infected by *M. anisopliae* changes as indicated by the altered color of the cuticle to black-brown which is known as the melanization process. Green conidia will appear on the body of the larva indicating the late stage symptoms of infection with the *M. anisopliae* fungus. The ability of hyphae to appear on the outside of the larva's body depends on the condition of the cuticle on the larvae (Indriyanti et al. 2017a, 2017b).

Based on observations *O. rhinoceros* larvae were found dead without any hyphae appearing on the outside of the body up to the tenth day. This occurred due to the unfavorable conditions for the development of the *M. anisopliae* fungus causing the hyphae of *M. anisopliae* to only exist inside the body of the larvae without penetrating outside the integument. At this stage, the larval tissues appeared severely damaged severely damaged, and the cuticle showed extensive deterioration (Prayogo et al. 2005). According to Indriyanti et al. (2017a, 2017b) the formation of melanin known as melanization is carried out by the enzyme phenol Table 1 shows that the dosage treatment of the liquid formulation of *M. anisopliae* in compost was significantly different from the initial time of the death of *O. rhinoceros* larvae with a range of 57.00-78.00

hours after application. The treatment dose of 0 g/l per bucket (untreated) was significantly different from the doses of 30, 60, 90, 120, 150, 180, and 210 g/l per bucket. This occurred because in the treatment at a dose of 0 g/l per bucket, no larvae died until the end of the observation (240.00 hours) due to the absence of *M. anisopliae* conidia in Metankos. Larvae experience melanization on their lower bodies i.e. chest, stomach, and between bodies. Changes in larval body color indicate decreased movement of the larvae and become lethargic hence kill the larvae.

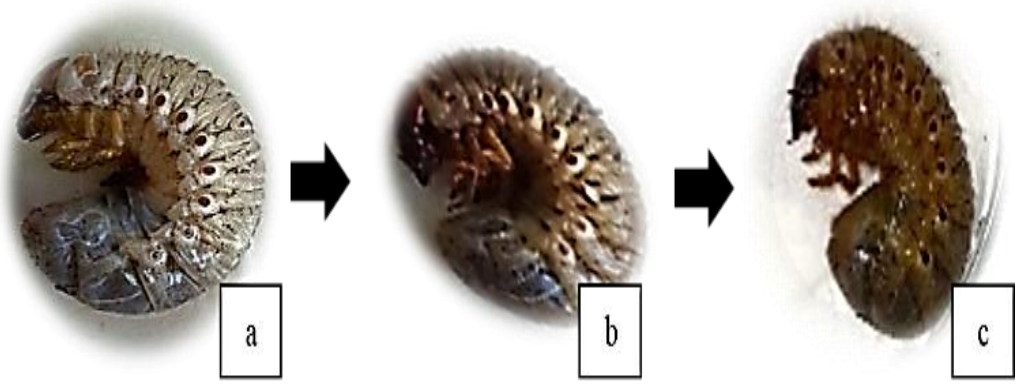

Figure 3. Changes in the morphology of *O. rhinoceros* larvae. (a) There are black stains on the cuticle of the larvae (36 hours of infection) (b) The body of the larvae turns brown (48 hours of infection). (c) The body turns blackish brown (60 hours of infection)

Initial Mortality Time of *Oryctes rhinoceros*

The results of variance showed that the dose treatment of *M. anisopliae* liquid formulation in compost had a significant effect on the initial time of death of *O. rhinoceros* larvae. The results of the initial death of *O. rhinoceros* larvae after the DNMRT test at 5% significance level can be seen in Table 1. Table 1 shows that the dosage treatment of the liquid formulation of *M. anisopliae* in compost was significantly different from the initial time of the death of *O. rhinoceros* larvae with a range of 57.00-78.00 hours after application. The treatment dose of 0 g/l per bucket (untreated) was significantly different from the doses of 30, 60, 90, 120, 150, 180, and 210 g/l per bucket. This occurred because in the treatment at a dose of 0 g/l per bucket, no larvae died throughout the end of the observation (240.00 hours) due to the absence of *M. anisopliae* conidia in Metankos.

Table 1. Average initial time of death of *O. rhinoceros* larvae after several doses of *M. anisopliae* liquid formulation in compost

Treatment Dose of *Metarhizium anisopliae* Formulation Liquid in Compost	**Initial Time of Death (Hours)**
0g/l per bucket	NIL a
30g/l per bucket	78b
60g/l per bucket	72b
90g/l per bucket	69b
120g/l per bucket	66b
150g/l per bucket	63b
180g/l per bucket	60b

210g/l per bucket	57b

Numbers in columns and rows followed by the same lowercase letters are not significantly different according to the DNMRT follow-up test with a 5% transformation level
NIL: No mortality observed for 240 hours

Treatment of the liquid formulation dose of *M. anisopliae* 210 g/l per bucket in compost caused an initial time of death of *O. rhinoceros* larvae subsequent to 57 hours after application and was not significantly different from the dose treatment of liquid formulation *M. anisopliae* in compost of 180, 150, 120, 90, 60, and 30 g/l per bucket for 60, 63, 66, 69, 72, and 78 hours after application. This occurred due to the different resistance of *O. rhinoceros* larvae to *M. anisopliae*. Although the dose of *M. anisopliae* increased, the resistance of *O. rhinoceros* larvae also increased. Thus, there was an insignificant difference in time of death. This is in line with the statement by Islam et al. (2021) that *M. anisopliae* requires time for the target pest to become infected, which is influenced by the isolate used, the host species, and environmental conditions. Fauzana et al. (2020) explained that the time of death of *O. rhinoceros* larvae did not differ significantly when the virulence of *M. anisopliae* increased. This occurred because the resistance of *O. rhinoceros* larvae increases as virulence increases.

Metarhizium anisopliae liquid formulation was dose of 30 g/l per bucket with an initial death time of 78 hours. Evidently a dose of 30 g/l per bucket was able to kill the larvae and was not significantly different from doses of 60, 90, 120, 150, 180 and 210 g/l per bucket with an initial death time of 72, 69, 66, 63, 60, and 57 hours after application.

Lethal Time (LT_{50}) of *Oryctes rhinoceros* Larvae

The analysis of variance showed that treatment with several doses of *M. anisopliae liquid formulation* in compost had a significant effect on the lethal time of 50 of *O. rhinoceros* larvae as can be seen in Table 2.

Table 2. LT_{50} of *O. rhinoceros* larvae after multiple doses of *M. anisopliae* liquid formulation in compost

Treatment Dose of *Metarhizium anisopliae* Formulation Liquid in Compost	**Lethal Time 50% (Hours)**
0 g/l per bucket	NILa
30g/l per bucket	168b
60g/l per bucket	159bc
90g/l per bucket	156bc
120g/l per bucket	147bc
150g/l per bucket	132bc
180g/l per bucket	132bc
210g/l per bucket	120c

The numbers in the columns and rows followed by the same lowercase letter are not significantly different according to the DNMRT follow-up test with a 5% transformation level.
NIL: No mortality observed for 240 hours

Table 2 shows that the dose treatment of *M. anisopliae* liquid formulation in compost had a significant effect on lethal time 50 in killing 50% of *O. rhinoceros* larvae in the range of 120-168 hours after application. Treatment at a dose of 0 g/l per bucket did not result in 50% death until the end of the 240-hour observation due to the absence of *M. anisopliae* conidia in

Metankos. Treatment of liquid formulation doses of *M. anisopliae* 210 g/l per bucket on compost for 120 hours after application showed no significant different effect with doses of 180, 150, 120, 90, and 60 g/l per bucket with 50% mortality for 132, 132, 147, 156, and 159 hours due to infection of the larvae and contact of the fungus and the larvae. According to Jiang et al. (2020) the more contact that occurs between the fungus and the larvae, the more the fungus will stick to the cuticle of the insect hence the pests will die faster. The dose of 210 g/l per bucket was significantly different from the dose of 30 g/l per bucket within 168 hours after application because the dose of 30 g/l per bucket had not been able to infect optimally in killing 50% of *O. rhinoceros* larvae since it takes a while for conidia in the compost to be attached to the insect integument and germinate.

The results of Suryanto research (2020) showed that *O. rhinoceros* larvae infected by the *M. anisopliae* fungus would show spores covering the larvae (appendix 4). *M. anisopliae* spores entered the insect's body through the skin. Spores that had entered the insect's body form hyphae starting from the epidermal tissue causing all insect body tissues to be filled with hyphae. After the host was killed, the hyphae would form primary and secondary spores depending on weather conditions.

Metarhizium anisopliae liquid formulation is a dose of 60 g/l per bucket with a death time of 50% of *O. rhinoceros* larvae for 159 hours or around 6 days. Evidently a dose of 60 g/l per bucket was able to kill *O. rhinoceros* larvae by 50% and was not significantly different from doses of 90, 120, 150, 180 and 210 g/l per bucket with a death time of 50% of *O. rhinoceros* larvae 156, 147, 132, 132, and 120 hours after application. According to Fauzana et al. (2020) the length of time to kill 50% of *O. rhinoceros* larvae ranges from 381.6 to 504 hours at a dose of 0-50 g/l water. This shows that increasing the dose of *M. anisopliae* to 30-210 g/l per bucket can shorten the time to kill 50% of *O. rhinoceros* larvae with a time range of 120-168 hours.

Lethal Dose of 50% and 95% *O. rhinoceros* Larvae

The results of probit lethal dose (LD) analysis using the POLO program. dose of *M. anisopliae* showed LD50 and LD95 which were 6.14% and 26.16% respectively. The results of the probit analysis can be seen in Table 3.

Table-3. Lethal dose of *M. anisopliae* liquid formulation in compost against *O. rhinoceros* larvae

Lethal Dose (LD)	Dose	SK Range (%)
LD_{50}	0.71	0.31-10.36
LD_{95}	12.57	6.72-57.18

SK = Confidence interval.

Table 3 shows that the appropriate dose of *M. anisopliae liquid formulation* to kill 50% of *O. rhinoceros* larvae in compost is 0.71% or equivalent to 71 g/l per bucket. Whereas, the correct dose of *M. anisopliae* liquid formulation to kill 95% of *O. rhinoceros* larvae in compost is 12.57% or equivalent to 1257 g/l per bucket.

Metarhizium anisopliae liquid formulation to kill 50% of *O. rhinoceros* larvae is 0.71% or equivalent to 71 g/l per bucket with a confidence interval of 0.31-10.36, if related to treatment close to a dose of 60 g/l pail. The correct dose of *M. anisopliae* fungus to kill 95% of *O. rhinoceros* larvae is 12.57 or equivalent to 1257 g/l per bucket with a confidence interval of

6.72-57.18, if related to treatment. It is a very far gap from the highest dose of 210 g/l per bucket. This is in accordance with Jiang et al. (2020) that the dose of *M. anisopliae* affects how many conidia can enter the insect's body. The higher the dose, the greater the number of fungal conidia that enter the insect's body hence the higher the lethal dose produced the lower the pathogenicity of the entomopathogenic fungi and vice versa.

A liquid formulation dose of 60 g/l per bucket is recommended because it is close to 71 g/l per bucket which has achieved 50% mortality of *O. rhinoceros* larvae. The results of the study by Simamora et al. (2013) shows that giving the right dose in a minimal amount demonstrates the effectiveness of the dosage of the liquid formulation of *M. anisopliae*. Therefore the low dose of the liquid formulation is accurate enough to be used in killing 50% of the larvae because the dose is more efficient since *M. anisopliae* will continue to grow and develop. In addition it can also survive long-term killing pests.

Daily Mortality of *O. rhinoceros* Larvae

The results of observing the daily mortality of *O. rhinoceros* larvae with a dose of *M. anisopliae* liquid formulation showed that the daily mortality of *O. rhinoceros* larvae increased from the first day to the tenth day. Daily mortality of *O. rhinoceros* larvae can be seen in Figure 4.

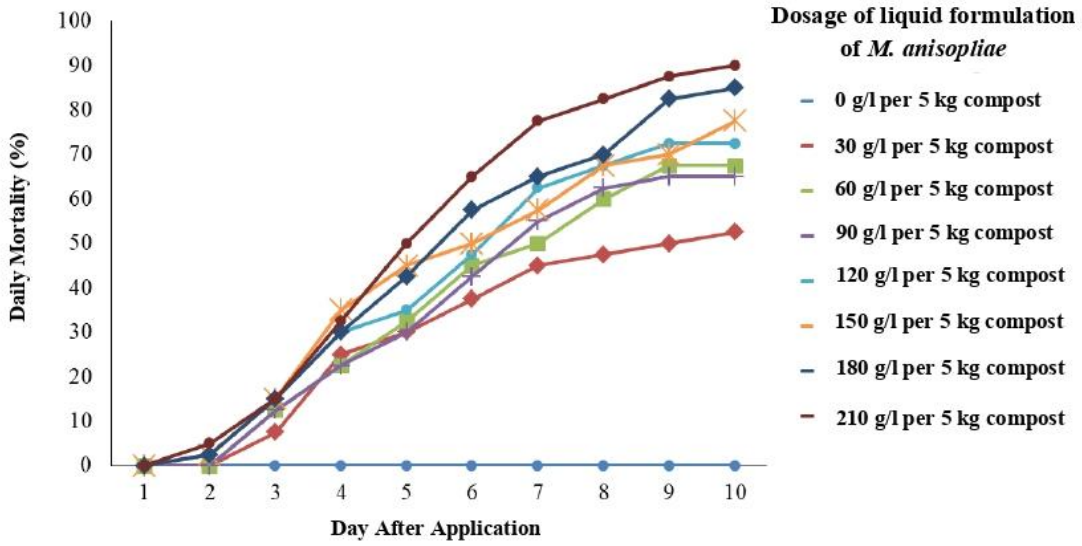

Figure 4. Daily mortality of *O. rhinoceros* larvae after application of liquid formulation of *M. anisopliae* to compost

Figure 11 shows that the daily mortality of *O. rhinoceros* larvae after application of the entomopathogen *M. anisopliae* tends to increase every day until the tenth day. A higher increase occurred at a concentration of 210 g/l per bucket. Entomopathogenic infection of *M. anisopliae to O. rhinoceros* larvae for the first time on the second day after application of *M. anisopliae* liquid formulation dosage on compost 120, 150, 180, 210 g/l per bucket with daily mortality of 2.5%, 2.5%, 2.5%, and 5%. While the doses of the liquid formulation were 30, 60 and 90 g/l per bucket, new deaths occurred on the third day with a daily mortality rate of 7.5%, 12.5%, and 12.5%. This is due to the killing of the *O. rhinoceros* larvae by *M. anisopliae*. Infecting the host in several stages is time consuming. This is in accordance with Suprayogi et al. (2015) that the *M. anisopliae* fungus needs a large amount of time to penetrate the

integument of the insect to cause infection and death of the insect. Fungal conidia attached to the cuticle must germinate to form hyphae first so they can penetrate the cuticle (Wahyudi 2002). The length of time needed for isolation of entomopathogenic fungi from fungal infection to the death of larvae ranges from 2-10 days (Herlinda et al. 2005) .

Sihombing et al. (2014) reported that *O. rhinoceros* were first infected by *M. anisopliae* 75g/l treatment, 2 days after application. This indicates that in order to infect the host, entomopathogenic fungi require several stages of infection. Herlinda et al. (2008) added that the time required to cause the death of test insects varies depending on the virulence of the pathogen, host resistance properties, and environmental conditions around the host body.

Figure 4 shows the addition of daily mortality for each treatment from the first day to the tenth day indicating that the lowest increase in mortality occurred at a dose of *M. anisopliae* liquid formulation of 30 g/l per bucket of compost with a final mortality of 52.5%. The highest addition of mortality from the first day to the fourteenth day occurred in the treatment of *M. anisopliae* liquid formulation dose of 21 g/l per bucket in compost with a final mortality of 90%. This occurs because high doses contain a greater number of conidia that enter the body of *O. rhinoceros* larvae compared to low concentrations of *M. anisopliae* entomopatogen which has high spore density that will be able to kill *O. rhinoceros* larvae with high mortality as well. This is in accordance with Hasnah & Susana (2012) that the effectiveness of insect pathogenic fungi to control target pests is highly dependent on the spore density applied.

Total Mortality of *O. rhinoceros* Larvae

The results of variance showed that treatment with several doses of the liquid formulation of *M. anisopliae* had a significant effect on the total mortality of *O. rhinoceros* larvae. The results of the average total mortality 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 multiple doses of *M. anisopliae* liquid formulation in compost

Treatment Dose of *Metarhizium anisopliae* Formulation Liquid in Compost	**Total Mortality (%)**
0g/l per bucket	0.00f
30g/l per bucket	52.50e
60g/l per bucket	57.50de
90g/l per bucket	65.00cde
120g/l per bucket	72.50bcd
150g/l per bucket	77.50bc
180g/l per bucket	85.00ab
210g/l per bucket	90.00a

The numbers in the columns and rows followed by the same lowercase letters were not significantly different according to the DNMRT advanced test with a 5% level of Arc sin transformation.

Table 4 shows that several doses of the liquid formulation of *M. anisopliae* in compost had a significantly different effect on the total mortality of *O. rhinoceros* larvae with a range of 52.5-90%. The treatment dose of *M. anisopliae* 210 g/l per bucket with a total mortality of 90% was not significantly different from the dose of 180 g/l per bucket with a total mortality of 85%, but significantly different from the doses of 150, 120, 90, 60, and 30 g/l per bucket with a total

mortality of 77.5%, 72.5%, 65%, 57.5%, and 52.5%. The difference in the percentage of total mortality of *O. rhinoceros* larvae was caused by the difference in the dosage of the liquid formulation of *M. anisopliae* mixed with compost, which mean the higher the dose of the liquid formulation the faster it takes to kill the *O. rhinoceros* larvae. According to Lin et al. (2017) the higher the dose of conidia given, the higher the probability of contact between the pathogen and the host is. The higher the attack the faster the process of death of infected insects becomes. Temperature and humidity affect the mortality of *O. rhinoceros* larvae (Eilenberg et al. 2019). Optimum temperature and humidity that support the growth and development of the fungus will cause the fungus to infect the larvae faster thus the larvae dies faster. Based on research results, the average compost temperature is 28.65ºC and humidity is 83.87%. The temperature and humidity are optimal for mushroom growth. According to Sun et al. (2022) the optimum temperature for mushroom growth ranges from 22ºC-27ºC and at 80-90% or >90% humidity. Therefore the temperature and humidity in the field have supported the mortality of *O. rhinoceros* larvae to be higher.

The doses of *M. anisopliae* liquid formulation on compost of 120, 150, 180, and 210 g/l per bucket with a total mortality of 72.5%, 77.5%, 85% and 90% can be categorized as a bioinsecticide because they have achieved a mortality of 72.5%. This is in accordance with Hasyim (2013) that mushrooms that can be categorized as bioinsecticides successfully control insects with a mortality of 72.5% - 95%.

The dosage of the liquid formulation of *M. anisopliae* tended to be the best among the treatments, which was 210 g/l per bucket which caused a total mortality of 90% and was not significantly different from the dose of the liquid formulation of *M. anisopliae* of 180 g/l per bucket which caused a total mortality of 85%. High doses have a greater number of conidia which can be seen in table 5 hence causing more mortality of *O. rhinoceros* larvae. According to Nasution et al. (2018) the greater the number of conidia is, the more *O. rhinoceros* larvae are infected hence more *O. rhinoceros* larvae deaths occur.

Conidial Density of *M. anisopliae* (per g)

The results of observations of the conidia density of the dose of *M. anisopliae* liquid formulation are presented in Table 5.

Table 5. Density of conidia of *M. anisopliae* in liquid and Metankos formulations

Treatment Dose of *Metarhizium anisopliae* Formulation Liquid in Compost	**Density Conidia**	
	Formulation Liquid	**Metankos**
30g/l per bucket	$1.2x10^8$ conidia	$0.15x10^8$ conidia
60g/l per bucket	$1.6x10^8$ conidia	$0.45x\ 0^8$ conidia
90g/l per bucket	$2.4x10^8$ conidia	$0.75x10^8$ conidia
120g/l per bucket	$2.8x10^8$ conidia	$1.05x10^8$ conidia
150g/l per bucket	$4.4x10^8$ conidia	$1.20x10^8$ conidia
180g/l per bucket	$5.2x10^8$ conidia	$1.50x10^8$ conidia
210g/l per bucket	$5.6x10^8$ conidia	$1.65x10^8$ conidia

Table 5 shows that the highest conidia density was at a dose of 210 g/l per bucket in both the liquid formulation of $5.6x10^8$ conidia and the Metankos of $1.65x10^8$ conidia. The high dose of the given liquid formulation of *M. anisopliae* affected the level of pathogenicity of the test insect larvae (Baleba et al. 2021). The higher the density of conidia, the higher the level of pathogenicity of the test insect larvae becomes. The higher the density of entomopathogenic fungal conidia, the higher the infectivity of insects becomes (Sterkel et al. 2021) .

The high density of conidia causes the ability of the *M. anisopliae* fungus *to* infect *O. rhinoceros* larvae to be higher therefore it can infect *O. rhinoceros* larvae more quickly. This corresponds to the total mortality at the dose of liquid formulation of 210 g/l per bucket with a percentage of 90% which was not significantly different from the dose of liquid formulation of 180 g/l per bucket with a percentage of 85%. The higher the dose of the liquid formulation given, the greater the number of conidia becomes therefore the mortality of *O. rhinoceros* larvae increases.

The dose of *M. anisopliae* in the given compost to the liquid and Metankos formulations had different conidia density. The density of conidia at the dosage of the liquid formulation of *M. anisopliae* was 30 g/l per bucket when the liquid formulation was $1.2x10^8$ conidia while at Metankos it was $0.15x10^8$ conidia. This indicated that the density of conidia when the liquid formulation was higher than when it was at Metankos because the conidia had been mixed with the compost hence after observing the number of conidia, the density of conidia becomes lower. The growth density of the fungus is supported by the nutrients present in the organic media, including carbon and nitrogen, which promote hyphal growth, while proteins enhance spore germination (Triasih et al. 2019).

CONCLUSION

The study demonstrates that the liquid formulation of *M. anisopliae* applied to compost effectively controls *O. rhinoceros* larvae. The highest mortality (90%) was achieved at a dose of 210 g/l per bucket. It can be concluded that the dosage of the liquid formulation of the *M. anisopliae* fungus on compost media in controlling *O. rhinoceros* larvae, is as follow: the dose of *M. anisopliae* liquid formulation on compost media tends to be the best, namely 210 g/l per bucket with a total mortality of 90%. The appropriate dose of liquid formulation to kill 50% of *O. rhinoceros* larvae is 0.71% or the equivalent of 71 g/l per bucket which, if related to treatment, is close to 60 g/l per bucket of *M. anisopliae* with a total mortality of 57%. The dose of liquid formulation of *M. anisopliae* in compost is 120 g/l per bucket because it has caused the death of *O. rhinoceros* larvae by 72.5%. This occurs because biological agents develop in the bodies of pests therefore efficient dosing shows the effectiveness of compost at dosages of liquid formulations.

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AUTHORS DECLARATION

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data availability Statement
This is a Final Year Project (FYP) and the data are currently in the FYP skripsi of Juliana Azzahra (2023).

Author contributions
Hafiz Fauzana (HF) conceptualized this research, designed experiments and wrote the paper participated in the revisions of it ; Nelvia (N), Rusli Rustam (RR), Fifi Puspita (FP) participated in the interpretation of the data; Juliana Azzahra (JA) research and wrote the paper participated in the revisions of it; Mukhlis Ibrahim (MI) Assist of publication. All authors read and approved the manuscrip.

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