

Effectiveness *Trichoderma* and *Beauveria bassiana* on Larvae of *Oryctes rhinoceros* On Palm Oil Plant (*Elaeis Guineensis* Jacq.) In Vitro

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Abstract— *O. rhinoceros* horn beetle (Coleoptera: Scarabaeidae) is the main pest attacking oil palm crops in Indonesia, especially in palm oil rejuvenation areas. The study was conducted from April to August 2016 in the laboratory of the Faculty of Agriculture, Al-Azhar University, Medan. The materials used in this research are horn beetle pest larvae (*O. rhinoceros*) originating from PT. Socfin Indonesia, *Trichoderma* sp fungi originating from the Food Crops and Horticultural Fields of Medan and the *B. bassiana* fungi are derived from the Plant Seed Plant Protection Center (PBPTP), aqua pro injection, Tween 80, rice and 96% alcohol. This research uses Completely Randomized Design (RAL) Non Factorial consisting of 13 treatments. The result of mortality of *O. rhinoceros* larvae on 1-14 DAA observation can be seen in appendix 2-43. Based on fingerprint analysis showed that the application of *Trichoderma* sp and *B. basiana* fungi on *O. rhinoceros* larvae had no significant effect on observation of 1 DAA to 9 DAA, but had a very significant effect on observation of 10 DAA to 14 DAA. The results of germination of *Trichoderma* sp and *B. basiana* fungus 4 hours after incubation period can be seen in appendix 61-66. Percentages mortality of the highest larvae of *O. rhinoceros* to *Trichoderma* sp fungus with a dose of 20 gr (96.67%) with the application method spread on the larvae. The highest amount of conidial density is found in *Trichoderma* sp fungi with doses of 60 g x 100-1 ml of aqua pro injection of 7.25×10^6 conidia/ml. Germination level of conidia mushroom highest in *Trichoderma* sp fungi with dose 60 gr x 100-1 ml aqua pro injection that is as much as 91%.

Keywords—Effectiveness, *Trichoderma*, *Bauveria*, *Oryctes*, Oil Palm Oil, Vitro.

I. INTRODUCTION

Palm oil is the most productive plant with the highest production of oil per hectare from other vegetable oil

producers. Indonesia is the second largest palm oil producer in the world after Malaysia. As many as 85% more world market of palm oil is controlled by Indonesia and Malaysia [39]. One of the obstacles in the cultivation of oil palm crops is the pest attack that can cause damage to the plant to result in decreased levels of palm oil production. Pests can attack palm oil from the pre-nursery stage to the producing stage [23].

O. rhinoceros horn beetle (Coleoptera: Scarabaeidae) is the main pest attacking oil palm crops in Indonesia, especially in palm oil rejuvenation areas. These insects bore oil palm shoots which resulted in stunted growth and damage to the point of growing so deadly plants. In palm oil rejuvenation areas, horn beetle attacks can result in delays in palm oil production up to a year and dead plants may reach 25% [23]. Reducing the use of pesticides in agricultural areas requires the availability of other safe and environmentally friendly methods of control, such as by utilizing natural enemies, such as entomopathogenic fungi, predatory insects, and parasitoids [41].

Groups of fungi that infect insects are called entomopathogenic fungi. The famous entomopathogenic mushrooms are *Namuraea rileyi*, *Metarizium anisopeliae* and *B. bassiana*. *B. bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphocreales) is a facultative entomopathogenic fungus with a wide range of hosts, besides this fungus has the potential to control more than 70 insect pests belonging to different orders, especially lepidoptera pests [18].

In addition, the use of antagonistic fungus is also an alternative control option because this method is considered safe for both users, consumers and the environment. An antagonist fungus that has been widely used as a biological controller is *Trichoderma* sp. *Trichoderma* sp mushroom cultures in applicative media such as bran can be given to the planting area and are bio decomposers as well as bio

fungicides. *Trichoderma* sp also has a very effective biocontrol mechanism in suppressing the development of pathogens such as mycoparasitism, antibiosis, and competition [7].

B. bassiana fungi have been tried to control the *O. rhinoceros* pests in oil palm with concentration of 25g ml⁻¹ [32]. While *Trichoderma* sp fungus has been tried to control *P. nicotianae* disease on Deli tobacco plants with concentration of 20g / L. Therefore, it is necessary to do research to find out the concentration of *B. bassiana* and *Trichoderma* sp fungi which is most effective to control horn beetle pest larvae on oil palm crop.

This study aims to determine the effectiveness of *B. bassiana* and *Trichoderma* sp fungi with different doses and methods of application to control horn beetle (*O. rhinoceros*) pest larvae in oil palm crops.

II. METHOD

Place and time of research

This research will be conducted at the Laboratory of the Faculty of Agriculture, Al-Azhar University, Medan. The study was conducted from April to August 2016.

Materials and tools

The materials used in this research are horn beetle pest larvae (*O. rhinoceros*) originating from PT. Socfin Indonesia, *Trichoderma* sp fungi originating from the Food Crops and Horticultural Fields of Medan and the *B. bassiana* fungi are derived from the Plant Seed Plant Protection Center (PBPPTP), aqua pro injection, Tween 80, rice and 96% alcohol.

The tool used in this research is haemocytometer, microscope, glass object, glass cover, hand sprayer, test tube, jar, kassa, erlemeyer, name label, beaker glass, scales, tube, aluminum foil and stationery.

Research methods

This research uses Completely Randomized Design (RAL) Non Factorial consisting of 13 treatments.

Where :

T1 = *Trichoderma* sp dose 20 gr B1 = *B. bassiana* dose 20 gr

T2 = *Trichoderma* sp dose 40 gr B2 = *B. bassiana* dosage 40 gr

T3 = *Trichoderma* sp dose 60 gr B3 = *B. bassiana* dose of 60 gr

1 = Application method is dispersed 2 = Application method is sprayed

(Control) = Provision of aqua pro injection

T11 = *Trichoderma* sp dosage 20 gr pertoples of dispersed application method.

T12 = *Trichoderma* sp dosage 20 gr x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

T21 = *Trichoderma* sp dosage 40 gr pertoples of dispersed application method.

T22 = *Trichoderma* sp dosage 40 gr x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

T31 = *Trichoderma* sp dose 60 gr pertoples of dispersed application method.

T32 = *Trichoderma* sp dose 60 gr x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

B11 = *B. bassiana* with a dose of 20 gr peroples of dispersed application method.

B12 = *B. bassiana* with a dose of 20 gr x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

B21 = *B. bassiana* with a dose of 40 pertoples of dispersed application method.

B22 = *B. bassiana* with a dose of 40 g x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

B31 = *B. bassiana* with a dose of 60 gr peroples of dispersed application method.

B32 = *B. bassiana* with a dose of 60 gr x 100 ml⁻¹ aqua pro injection pertoples application method is sprayed.

Based on the treatment performed, the number of replications can be determined by the following formula (Hanafiah, 2010):

$$t(r-1) \geq 15$$

$$13(r-1) \geq 15$$

$$13r - 13 \geq 15$$

$$13r \geq 15 + 13$$

$$13r \geq 28$$

$$r \geq 28/13$$

$$r \geq 2.15 = 3 \text{ replications [12].}$$

The amount of treatment is 13 treatments, among others:

T11 T12 T21 T22 Controls

T31 T32 B11 B12

B21 B22 B31 B32

But to further strengthen the results of research, then the replication used in the study is as much as 3.

Based on the above description can be arranged the treatment unit as follows:

Treatment amount: 13 treatments

Number of repetition treatment: 3 replications

Number of research units: 39 units

Number of larvae per jar: 10 heads

Number of larvae tested: 390 heads

Distance between jars: 1 cm

Distance between replicates: 15 cm

Number of jars of *Trichoderma* sp: 18 jars

Number of jars of *B. bassiana*: 18 jars

Number of control jars: 3 jars

Total jars total: 39 jars

The mathematical model used in this research is the Non Factorial Completely Randomized Design (Hanafiah, 2010) which is assumed as follows:

$$Y_{ij} = \mu + N_i + \epsilon_{ij}$$

Where:

Y_{ij} : The results of observation of the treatment to the fungus and j-thr

μ : Effect of middle value

N_i : Effect of N treatment on the mushroom stage

ϵ_{ij} : Effect of errors from the treatment of fungi and jth thrust

If the treatment has significant effect, the Duncan test is 5% and 1%.

Implementation of Research

Provision of larvae to be tested

Horned beetle larvae (*O. rhinoceros*) originating from PT. Socfin Indonesia was taken and maintained at Al-Azhar University Laboratory in Medan. The captured larvae are then adjusted in size and size to each other to obtain sufficient quantities to test. The larvae to be tested are instar larvae II.

The supply of entomopathogenic fungi

Trichoderma sp mushroom comes from Balai Proteksi Tanaman Pangan dan Hortikultura (BPTPH) Medan, while the fungus *B. bassiana* is isolated from the pest of *O. rhinoceros* which is derived from Balai Besar Germination Plantation Crop Protection (BBPPTP) Medan. To anticipate the deficiency of the mushrooms that will be used then both mushrooms are propagated in the rice medium each containing 100 grams / pack and incubated at room temperature for 5-7 days. This mushroom removal is done in the incase to avoid contamination. In the event of contamination, re-purification by taking part of the uncontaminated fungus of *B. bassiana* and *Trichoderma* sp. To be grown on rice media until *Trichoderma* sp and *B. bassiana* are completely pure and uncontaminated.

Application of Entomopathogenic Fungi

Application is applied only once in a manner dispersed and sprayed with each type of fungus according to the treatment, on the control only by spraying aqua pro injection alone on instar larvae instar II. Then observed its development.

Observation Parameters

Percentage of Mortality

Observation of larval mortality started one day after application (DAA) weeks 14 days after application. The percentage of larval mortality was calculated by the formula:

$$P = a / b \times 100\%$$

Information:

P = Percentage mortality of *O. rhinoceros* (%)

a = The number of *O. rhinoceros* is dead

b = Total *O. rhinoceros* observed entirely

If on the control found dead larvae then, the percentage of mortality obtained later in the correction using the Abbott's formula is as follows:

$$P = (Po - Pc) / (100 - Pc) \times 100\%$$

Where:

P = Percentage *O. rhinoceros* test that died after correction

Po = Percentage of *O. rhinoceros* test that died at treatment

Pc = Percentage of test dead *O. rhinoceros* in control

Number of Conidia

The number of conidia of entomopathogenic fungi is calculated before application is performed. Conidial density calculation was performed by suspension of conidia from isolation treatment of isolate taken as much as 1 ml. Then the suspension is dropped on haemocytometer. Conidial density was calculated under a 400x magnification binoculars microscope using Gabriel & Riyatno [10] formula as follows:

$$C = t / ((n \times 0.25)) \times 10^6$$

Where:

C: Conidia density per ml of solution

t: Total number of conidia in the sample box observed

n: Number of sample boxes (5 large boxes x 16 small squares)

0.25: Correction factor for use of small-scale sample boxes on haemocytometers

Conidia germination

The conidia granulation from each of the entomopathogenic fungi suspensions used was calculated before application was performed and after 24 hours of inoculation was performed. One drop of suspension of entomopathogenic fungus dripped on the glass object then drops with PDA media and closed with a glass cover, then the amount of

germinated conidia is calculated, so it is not germinated. The calculations are performed on the field of view under a microscope with 400 x magnification, using Gabriel & Riyatno [10] formula as follows:

$$V = g / ((g + u) \times 100\%)$$

Where:

V: Conidia germination (viability)

g: The number of conidia that germinate

u: The number of conidia that does not germinate

III. RESULTS

Percentage of Mortality

The result of mortality of *O. rhinoceros* larvae on 1-14 DAA observation can be seen in appendix 2-43. Based on fingerprint analysis showed that the application of *Trichoderma* sp and *B. bassiana* fungi on *O. rhinoceros* larvae had no significant effect on observation of 1 DAA to 9 DAA, but had a very significant effect on observation of 10 DAA to 14 DAA. For more details can be seen in Tables 1, 2 and 3.

Table.1: Average number of mortality Larva *O. rhinoceros* (%) due to fungus *Trichoderma* sp and *B. bassiana* in the 1-5 Days After Application (DAA)

Treatments	Observation of day to-					Mean
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	
Control	0,00	0,00	0,00	0,00	10,00	2,00
T ₁₁	13,33	23,33	30,00	40,00	40,00	29,33
T ₁₂	10,00	13,33	23,33	30,00	33,33	22,00
T ₂₁	3,33	20,00	23,33	36,67	50,00	26,67
T ₂₂	3,33	6,67	13,33	20,00	23,33	13,33
T ₃₁	6,67	16,67	23,33	26,67	26,67	20,00
T ₃₂	0,00	0,00	0,00	13,33	23,33	7,33
B ₁₁	0,00	6,67	10,00	10,00	20,00	9,33
B ₁₂	16,67	23,33	30,00	30,00	36,67	27,33
B ₂₁	13,33	16,67	23,33	23,33	26,67	20,67
B ₂₂	13,33	13,33	13,33	23,33	26,67	18,00
B ₃₁	0,00	0,00	10,00	13,33	16,67	8,00
B ₃₂	13,33	13,33	20,00	23,33	26,67	19,33
Mean	7,18	11,79	16,92	22,31	27,69	17,18

The average number of mortality larvae of *O. rhinoceros* (%) due to fungus *Trichoderma* sp and *B. bassiana* 6-9 Days After Application (DAA) in Table 2 . While the characteristics of *O. rhinoceros* larvae that died from *Trichoderma* sp and *B. bassiana* fungi can be seen in Figs. 7 and 8.

Table.2: Average number of mortality Larva *O. rhinoceros* (%) due to fungus *Trichoderma* sp and *B. bassiana* in the 6-9 Days After Application (DAA)

Treatments	Observation day to -				Mean
	6 DAA	7 DAA	8 DAA	9 DAA	
Control	10,00	10,00	20,00	20,00	16,67
T ₁₁	53,33	63,33	76,67	76,67	72,22
T ₁₂	43,33	46,67	50,00	56,67	51,11
T ₂₁	50,00	53,33	60,00	66,67	60,00
T ₂₂	30,00	33,33	36,67	56,67	42,22
T ₃₁	36,67	50,00	50,00	53,33	51,11

Treatments	Observation day to -				Mean
	6 DAA	7 DAAA	8 DAA	9 DAA	
T ₃₂	23,33	26,67	26,67	33,33	28,89
B ₁₁	26,67	36,67	56,67	60,00	51,11
B ₁₂	36,67	46,67	53,33	56,67	52,22
B ₂₁	26,67	33,33	43,33	60,00	45,56
B ₂₂	43,33	50,00	53,33	56,67	53,33
B ₃₁	16,67	33,33	46,67	63,33	47,78
B ₃₂	26,67	33,33	43,33	63,33	46,67
Mean	32,56	39,74	47,44	55,64	47,61

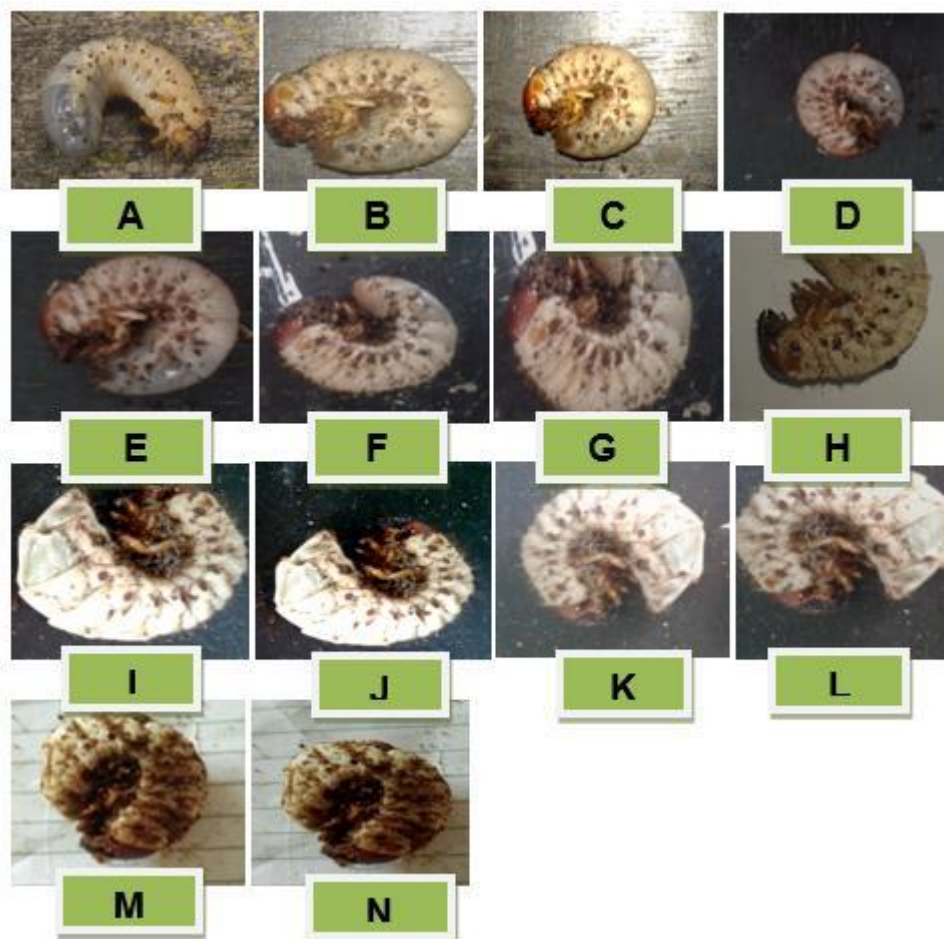


Fig.7: Symptoms of fungal infection of *B. bassiana* on Larvae of *O. rhinocetos*

- A. Larvae attacked by the fungus *B. bassiana* 1 DAA
- B. Larvae attacked by the fungus *B. bassiana* 2 DAA
- C. Larvae attacked by the fungus *B. bassiana* 3 DAA
- D. Larvae attacked by the fungus *B. bassiana* 4 DAA
- E. Larvae attacked by the fungus *B. bassiana* 5 DAA
- F. Larvae attacked by the fungus *B. bassiana* 6 DAA
- G. Larvae attacked by the fungus *B. bassiana* 7 DAA

- H. Larvae attacked by the fungus *B. bassiana* 8 DAA
- I. Larvae attacked by the fungus *B. bassiana* 9 DAA
- A. Larvae attacked by the fungus *B. bassiana* 10 DAA
- K. Larvae attacked by the fungus *B. bassiana* 11 DAA
- L. Larvae attacked by the fungus *B. bassiana* 12 DAA
- M. Larva attacked by the fungus *B. bassiana* 13 DAA
- N. Larva attacked by the fungus *B. bassiana* 13 DAA

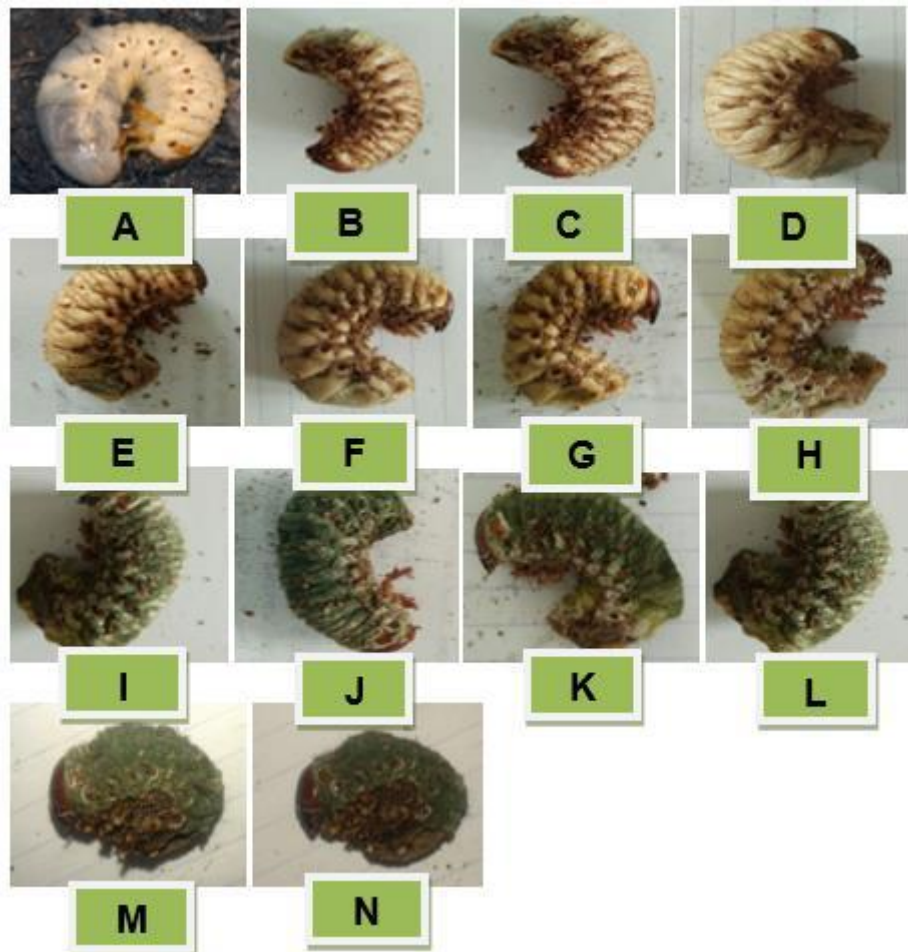


Fig.8: Symptoms of fungal infection of *Trichoderma* sp on Larvae *O. rhinocetos*

- A. Larvae attacked by the fungus *Trichoderma* sp 1 DAA
- B. Larvae attacked by the fungus *Trichoderma* sp 2 DAA
- C. Larvae attacked by the fungus *Trichoderma* sp 3 DAA
- D. Larvae attacked by the fungus *Trichoderma* sp 4 DAA
- E. Larvae attacked by the fungus *Trichoderma* sp 5 DAA
- F. Larvae attacked by the fungus *Trichoderma* sp 6 DAA
- G. Larvae attacked by the fungus *Trichoderma* sp 7 DAA
- H. Larvae attacked by the fungus *Trichoderma* sp 8 DAA
- I. Larvae attacked by the fungus *Trichoderma* sp 9 DAA
- A. Larvae attacked by the fungus *Trichoderma* sp 10 DAA
- K. Larvae attacked by the fungus *Trichoderma* sp 11 DAA

- L. Larvae attacked by the fungus *Trichoderma* sp 12 DAA
- M. Larva is attacked by the fungus *Trichoderma* sp 13 DAA
- N. Larva is attacked by the fungus *Trichoderma* sp 13 DAA

From Table 3 it was found that in 10 DAA of *Trichoderma* sp and *B. bassiana* in T11 treatment was the highest mortality (86.67%) and the lowest was in control (20%). But the different T11 treatment was not significant with the treatments B21, B22, B31, B32, T12, T21, T22, T31, T32, B21, B22 and T11 differed markedly by control.

Table.3: Average number of mortality Pests Larvae *O.rhinoceros* (%) due to fungus *Trichoderma* sp and *B. bassiana* 10-14 Days After Application (DAA)

Treatments	Obsert=vation day to-					Mean
	10 DAA	11 DAA	12 HAS	13 DAA	14 DAA	
Control	20,00 bC	20,00 cC	20,00 cC	20,00 bB	20,00 bB	20,00
T ₁₁	86,67 aA	86,67 aAB	90,00 aA	93,33 aA	96,67 aA	91,67
T ₁₂	73,33 abAB	76,67 abAB	83,33 aAB	83,33 aA	96,67 aA	85,00
T ₂₁	80,00 abA	90,00 aAB	90,00 aA	90,00 aA	93,33 aA	90,83
T ₂₂	70,00 abAB	73,33 abAB	76,67 abAB	80,00 aA	90,00 aA	80,00
T ₃₁	60,00 bAB	63,33 bAB	66,67 abB	76,67 aA	83,33 aA	72,50
T ₃₂	60,00 abAB	73,33 abAB	76,67 abAB	80,00 aA	83,33 aA	78,33
B ₁₁	73,33 abAB	80,00 abAB	83,33 aAB	83,33 aA	93,33 aA	85,00
B ₁₂	76,67 abA	80,00 abAB	86,67 aAB	86,67 aA	90,00 aA	85,83
B ₂₁	83,33 aA	86,67 aAB	90,00 aA	90,00 aA	93,33 aA	90,00
B ₂₂	83,33 aA	90,00 aA	90,00 aA	90,00 aA	93,33 aA	90,83
B ₃₁	80,00 abA	86,67 aAB	86,67 aAB	93,33 aA	93,33 aA	90,00
B ₃₂	73,33 abAB	86,67 aAB	90,00 aA	93,33 aA	93,33 aA	90,83
Mean	70,77	76,41	79,23	81,54	86,15	80,83

Description: The numbers followed by the same letter in the same column show different results that are not real based on DMRT at f = 5% and very different 1%.

From observation 11 DAA showed that the highest mortality was in T21 and B22 (90%) treatment and the lowest mortality was in control (20%). This can be seen in Figures 9 and Figures 10 where T21 and B22 treatments differed not significantly on all treatments but differed significantly with controls.

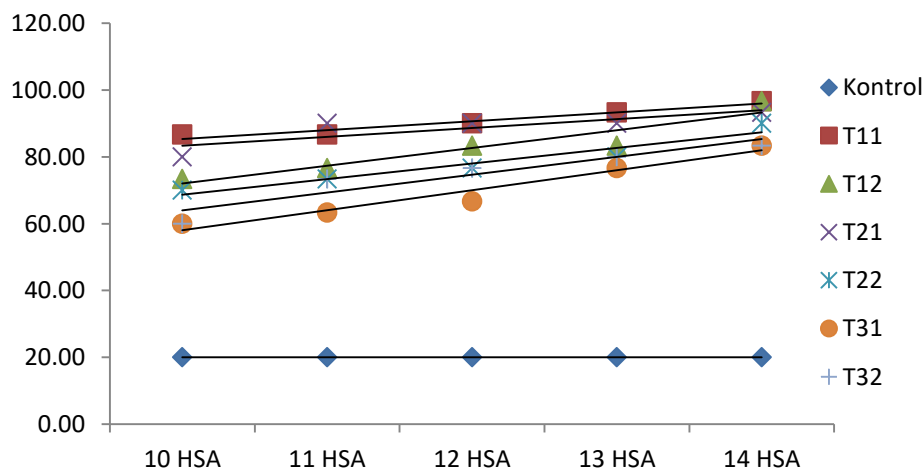


Fig.9: Percentage of Larvae Mortality *O. rhinoceros* After Application of *Trichoderma* sp 10-14 DAA.

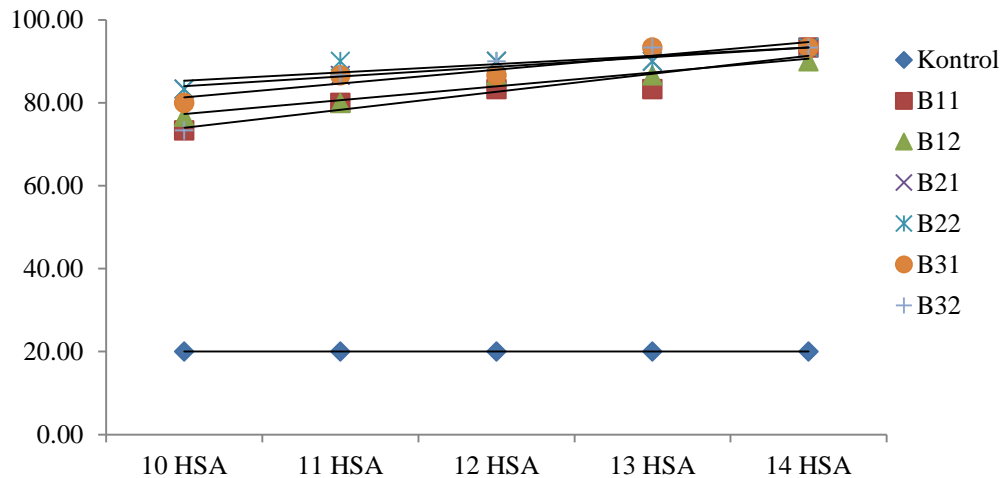


Fig.10: Percentage of Larvae Mortality *O. rhinoceros* after application of *B. bassiana* Fungus 10-14 DAA.

Growth of Conidia

Data from conidial density of entomopathogenic fungi *Trichoderma* sp and *B. bassiana* can be seen in appendix 43-60. After the data were analyzed the density was known that the density of conidia from both entomopathogenic fungi showed a difference of Table 4.

Table.4: Conidial density of *Trichoderma* sp and *B. bassiana* with various doses per ml of aqua pro injection

Types of fungus	Doses	Spore density		
		10 ⁸	10 ⁷	10 ⁶
<i>Trichoderma</i> sp	20 gr x 100 ⁻¹ ml	4,75 x 10 ⁶	5,32 x 10 ⁶	5,85 x 10 ⁶
	40 gr x 100 ⁻¹ ml	5,60 x 10 ⁶	6,22 x 10 ⁶	6,45 x 10 ⁶
	60 gr x 100 ⁻¹ ml	7,25 x 10 ⁶	6,89 x 10 ⁶	5,25 x 10 ⁶
<i>B. bassiana</i>	20 gr x 100 ⁻¹ ml	5,52 x 10 ⁶	4,38 x 10 ⁶	4,22 x 10 ⁶
	40 gr x 100 ⁻¹ ml	5,95 x 10 ⁶	5,12 x 10 ⁶	4,68 x 10 ⁶
	60 gr x 100 ⁻¹ ml	6,33 x 10 ⁶	5,20 x 10 ⁶	4,85 x 10 ⁶

The results of germination of *Trichoderma* sp and *B. bassiana* fungus 4 hours after incubation period can be seen in appendix 61-66. After the data were analyzed, it was found that germination of conidia from both entomopathogenic fungi showed differences in Table 5.

Table.5: Conidia germination of *Trichoderma* sp and *B. bassiana* with various doses per ml of aqua pro injection

Types of fungus	Total spore		
	20 gr x 100 ⁻¹ ml	40 gr x 100 ⁻¹ ml	60 gr x 100 ⁻¹ ml
<i>Trichoderma</i> sp	76%	83%	91%
<i>B. bassiana</i>	77%	80%	85%

IV. DISCUSSIONS

Percentage of Mortality

From Table 1 it can be seen that in observation 1 DAA has seen mortality in *O. rhinoceros* larvae, but the mortality is still relatively low. This low mortality due to fungal conidia that enter and infect is still not maximal, because the fungi entering entomopathogen to infect the insects have

a time span, otherwise the fungus of entomopathogen infects and deadly *O. rhinoceros* larvae is highly dependent on the amount of environmental conidia and nutrients that needed. As stated by [25,29] the ability of entomopathogenic fungi to kill larvae is highly dependent on environmental conditions, nutritional fit, pH, from where the fungus grows and develops.

From Table 2 on observations 6 DAA to 9 DAA mortality of *O. rhinoceros* larvae increased even though the different treatments were not real. But the mortality of larvae is still low, both due to the fungus *Trichoderma* sp and *B. bassiana* slow development of the fungus is due to the nutrients that the body is less available. This is in accordance with the stated by [15] states that the development of entomopathogenic fungi influenced the nutritional content of growing media used.

The Table 3 description of the occurrence of differences in mortality rates against *O. rhinoceros* larvae is caused by the number of conidia or concentration of conidia germination as well as different application methods. In this case the more the number of conidia that enter the body of the larvae and the germination of the high, then the death of the larvae will be faster. In addition, the number of conidia that enter the body of the larvae is also influenced by the method of application. Application with the spread method, then conidia that can enter into the body of the larvae will be more and more. Because the conidia that the application does not exist or very few are not on target. Whereas when the application with spray method the possibility of drift is large enough so that the conidia that concerns the body of the larvae is less. This is consistent with that stated by [32,34] also states that, sooner or later mortality in pests depends on the amount of dose used.

This indicates that the application of *Trichoderma* sp and *B. bassiana* fungi with the same dosage applied sprinkled differently is not real by spraying either at the beginning of the observation or until the end of the observation. This is due to conidia applied from both types of fungi the germination and the power of the infection are both high. So the mortality of larvae is also high, as stated by [3, 36, 36] that the higher dose conidia used in the treatment, the larval mortality is also high.

From observation 12 DAA showed that the highest mortality was in T11, T21, B21, B22 and B32 treatment (90%) and the lowest mortality was in control (20%). The treatments T11, T21, B21, B22 and B32 were not significantly different for all treatments but differed significantly with the controls. The high mortality of larvae other than influenced by the amount of conidia is also influenced by temperature/humidity. This is in accordance with the results of [32] study that the optimal temperature for *Trichoderma* sp growth ranges from 22°C-27°C. Meanwhile, according to [1] that germination, growth and optimal sporulation of *B. bassiana* fungi occur at temperatures 25-30°C.

From observation 13 DAA showed that the highest mortality was in T11, B31 and B32 treatment (93.33%) and the lowest mortality was in control (20%). Treatments of T11, B31 and B32 were not significantly different for all treatments but differed significantly with controls. This suggests that the density of conidia effectively controls the mortality of the *O. rhinoceros* larvae. At a higher level of conidia density, more and more mycelium and conidia grow. This is in accordance with [37] assertion that it increases the occurrence of conidial contact with the body of the larvae, thereby providing a better chance for conidia to stick, germinate and penetrate into the body of the larvae. From the 14 DAA observations it was found that T11 and T12 (96.67%) and B11, B21, B22, B31 and B32 (93.33%) were the highest mortality and the lowest was in control (20%). However, the treatments of T11 and B11 differed significantly from the treatment of T12, T22, T22, T31, T32, B12, B21, B22, B31, B31, T11 and B11. This is because the longer the entomopathogenic fungi are in the body of the larvae, the amount of conidia increases. Damage to body tissue larvae due to fungus attacks will be more severe, so that the mortality rate of larvae will be higher. This is in line with the statement [19,38] the longer the contact time of entomopathogenic fungi and host, the more likely the entomopathogenic fungus to infect the host, resulting in an increase in larval mortality.

From the observation in Fig. 9-10 it is also found that infected entomopathogenic larvae indicate a change in which the larval body becomes hard, mummification and the appearance of fungal conidia around the conidia color tube will appear in accordance with the color of the infecting conidial fungus. This is in line with [37] statement that infected insects of entomopathogenic fungi undergo mummification and after a few days will grow a colony of white-colored fungus around the body. At the beginning of death the larvae have not shown any change. While on the third day the larvae have begun to experience a change in color, where conidia mushrooms have begun to grow on the side of the larvae. On the fifth day until the larval larvae infected the body the larvae are fully satisfied by the entomopathogenic fungal conidia.

Growth of Conidia

Conidia density

Table 4 shows that the highest conidial fungus, *Trichoderma* sp with dose of 60 gr x 100-1 ml of aqua pro injection, is 7.25 x 10⁶ conidia / ml and the lowest conidial density is found in fungus *B. basiaana* at a dose of 20 gr x 100-1 ml aqua pro injection as much as 4.22 10⁶ conidia/

ml. If seen from the number of doses can be concluded that, the higher the dose the number of conidia will also be higher.

Conidia germination

Table 5 shows that the number of conidia of *Trichoderma* sp fungi is higher than that of *B. bassiana*. The conidia growth difference of each of these fungi may be caused by unequal nutrients. Each genus or fungal species requires nutrients, pH, water content in medium, optimal temperature, light, aeration, and different incubation periods for growth and development of conidia [24,25,42]. The percentage of conidial germination is also influenced by the amount of conidia and conidial germination. The higher the amount of conidia and higher germination, the faster the mortality of the larvae. As stated by [43] that the higher dose conidia used in the treatment, the more rapid the death.

V. CONCLUSIONS

From result of research can be concluded that:

1. Percentages mortality of the highest larvae of *O. rhinoceros* to *Trichoderma* sp fungus with a dose of 20 gr (96.67%) with the application method spread on the larvae.
2. The highest amount of conidial density is found in *Trichoderma* sp fungi with doses of 60 g x 100-1 ml of aqua pro injection of 7.25×10^6 conidia/ml.
3. Germination level of conidia mushroom highest in *Trichoderma* sp fungi with dose 60 gr x 100-1 ml aqua pro injection that is as much as 91%.

VI. SUGGESTIONS

To know the effectiveness of the fungus *Trichoderma* sp and *B. bassiana* need dilakuakn further research on application fungus of *Trichoderma* sp and *B. bassiana* directly to the field.

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