# PATHOGENITY OF Beauveria bassiana IN COMPOSED MEDIA FOR PALM OIL PEST CONTROL Oryctes rhinoceros L.

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#### ABSTRACT

Oryctes rhinoceros is an important pest of oil palm. Pest problems occur because the provision of Oil Palm Empty Fruit Bunches (OPEFB) compost comes to a breeding site for O. rhinoceros larvae. This study aimed to obtain the best ability of several compost organic materials containing B. bassiana for controlling O. rhinoceros larvae, to obtain the ability of B. bassiana in compost to control various instars of O. rhinoceros larvae, and to obtain the best concentration of Beauveria bassiana in compost in controlling O larvae. rhinoceros. The study was carried out from February-November 2020. Three stages of the research experiment were: 1) testing of several compost organic materials containing B. bassiana at 75 gl<sup>-1</sup> concentration, using a completely randomized design (CRD), Experiment 2 were: test the ability of B. bassiana with 75 gl<sup>-1</sup> concentration of compost at various instars of O. rhinoceros larvae, using a Completely Randomized Design (CRD. Experiment 3 were: concentration test of B. bassiana fungi in compost media against O. rhinoceros larvae, using a Completely Randomized Design (CRD), with 6 treatments 4 replications obtained 24 experimental units, while the treatments were 0 gl<sup>-1</sup>, 15 gl<sup>-1</sup>, 30 gl<sup>-1</sup>, 45 gl<sup>-1</sup>, 60 gl<sup>-1</sup>, and 75 gl<sup>-1</sup>. Compost organic material contains the fungus B. bassiana which has the best ability to control O. rhinoceros, namely OPEFB + sawdust because caused total mortality to reach 90% with conidia number 115.2 x108, initial death 67.2 hours after application, lethal time 50 206, 4 hours after application. B. bassiana fungi in OPEFB + sawdust compost can control O. rhinoceros 3 instar larvae with total mortality of up to 92.5% and control 1, 2, and 3 instars larvae of O. rhinoceros have no effect on the initial time of death and lethal time 50 on each instar. The initial time of death used was 54-75 hours and the lethal time 50 was 198-252 hours. OPEFB compost + sawdust containing the fungus B. bassiana 75 gl-1 had the best ability to control O. rhinoceros larvae with a total larvae mortality of 87% which caused early death 54 hours after application, 50 lethal times of 213 hours, and LC50 of 3.3% or the equivalent of  $33 \text{ gl}^{-1}$ .

Keywords : Oil palm, Oryctes rhinoceros L., Beauveria bassiana

#### INTRODUCTION

Oil palm is an important plantation crop-producing food oil, industrial oil, and biodiesel (Nuryanti.s, 2008). Oil palm plantations in Riau have increased. The area of oil palm plantations in Riau in 2014 reached 2,411,819 ha with a production of 7,761,293 tons while in 2015 the land area increased to 2,424,545 ha with a production of 7,841,947 tons (BPS, 2017) (BPS, 2017)

Palm oil processing produces solid waste in the form of Oil Palm Empty Fruit (TKKS). The Bunches solid waste produced is directly proportional to the number of fresh fruit bunches produced (Rahmadi, et al, 2014) Processing 1 (one) tonne of fresh fruit bunches (FFB) of oil palm will produce

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waste in the form of EFB as much as 23% or 230 kg (Susanto et al., 2017).

Palm oil companies generally process EFB waste into compost. OPEFB compost can add nutrients, increase soil organic matter content so that the soil structure is better and the soil's ability to hold water is better (Veronika et al., 2019). The current problem is that the provision of OPEFB compost becomes a breeding site for O. Rhinoceros larvae. This is reinforced by observations in the field that O. rhinoceros larvae were found in OPEFB compost applied to oil palm plants (Kiki Hidayat, 2019). Pertami, (2016), compost According to media is used as an active nest and also a breeding ground (eggs to pupae) for O. rhinoceros pests.

Oryctes rhinoceros is an important pest on oil palm. O. rhinoceros pest attack in Riau Province covering an area of 12,384.85 ha (Dinas Perkebunan Provinsi Riau. 2014). Candra. et al.,(2019) stated that this pest attack can cause plant death if it attacks oil palm growing points. The population density of O. rhinoceros is influenced by weather factors (temperature, relative humidity, rainfall), biotic factors (natural enemies), and the availability of feed (an organic substance in the soil). Weathering of perfect empty bunches and high micro humidity in empty bunches is a suitable habitat for O. rhinoceros larvae. Larvae are found at empty bunches depth of 10-35 cm (Nuriyanti et al., 2017).

Generally, control the of O. rhinoceros imago is carried out by farmers using synthetic insecticides, but it has many bad effects on humans and the environment. So it is necessary to strive for environmentally-friendly control using biological agents. The advantages of using biological agents, one of which is entomopathogenic fungi, is that they have a high reproductive capacity, short life cycle, can form spores that are durable even under unfavorable conditions, are relatively safe, selective, relatively easy to produce, and are very unlikely to cause pest resistance.

Beauveria bassiana is a fungus that can be used as a biological control material for many insect pests. In addition, the entomopathogenic fungus B. bassiana has a wide diversity of infections ranging from eggs, larvae, pupae to imago. B. bassiana penetrates the host's body with the help of mechanical pressure and the help of the beauvericin toxin released by the fungus. Insects can become infected with conidia through the cuticle, or through the gaps between their body segments, then germinate bv forming a germination tube so that the fungus can enter the host's body and spread to the haemocoel. Furthermore, the fungus infects the food tract and respiratory system so that the insects die (Pramesti & Toto Himawan, 2014).

The research results of Purnomo et al., (2017) stated that the media of compost, soil, bran, or a mixture thereof can be used as a medium for the growth of the fungus B. Bassiana. Similarly, research results of Hasyim and Azwana cited in al., 2013) showed (Thalib et that administration of 5 ml suspension of B. bassiana against banana borer Cosmopolites sordidus Germar with conidia 3,2x10 ° conidia/ ml provides the highest mortality outcomes in observation (15 HSA) was obtained at the larval stage 2 (100%) and the lowest was at the imago (76.67%). Research stage results of Sihombing et al., (2014) showed that the mortality percentage highest of O. rhinoceros larvae at 18 DAP observations (100%) was found in B. bassiana 75 g/l in *B.* and the lowest (72.93%)bassiana 25 g/l treatment.

The target for *O. rhinoceros* control is the *breeding* site of the pest. The preferred media for O. rhinoceros to lay eggs and where the larvae live are weathered cow dung, weathered sawdust. weathered rice husks. and weathered coconut stalks.

This study aimed to obtain the best ability of some organic compost containing *Beauveria bassiana* in controlling the larvae of *Oryctes rhinoceros* L.

### **RESEARCH METHOD**

The research was carried out from February to November 2020 at the Plant Pest Science Laboratory for pathogenicity analysis and the Experimental Garden Technical Implementation Unit for composting, Faculty of Agriculture, University of Riau.

The research was conducted in the form of an experiment using a Completely Randomized Design (CRD) consisting of 7 treatments with 5 replications, with compost organic material treatment. namely empty oil palm fruit bunches (TKKS), sawdust, rice husks, EFB+ sawdust. EFB+ rice husks. sawdust + rice husk, and rice husk + sawdust + OPEFB. Each treatment was given a of B. bassiana suspension with a concentration of 75 gl<sup>-1</sup>. The parameters observed were the number of conidia, temperature and humidity, early time of death. Lethal Time (LT <sub>50</sub>), the percentage of daily mortality, and the mortality. The percentage of total percentage of daily mortality data were analyzed descriptively and presented in graphical form, while other data were analyzed using SAS version 9.1.3 and later ANOVA followed by Duncan rangetestwithlinearmodelsR Definition A smack L

### **RESULT AND DISCUSSION**

#### Chemical Analysis of Some Compost Organic Materials

Some of the organic compost that the growth medium became for Beauveria bassiana was observed for nutrient content. The its results of chemical analysis in the form of levels of pH, organic C, and N can be seen in Table 4.1. The results of the chemical analysis of all compost organic matter at the pH of the compost range from 5.46 to 6.76. Sawdust + rice husk compost has the highest pH, namely pH 6.76 and the lowest in rice husk compost is pH 5.46. OPEFB compost has the secondhighest pH with pH 6.53. The results of the chemical analysis of sawdust + rice husk + EFB had the third-highest pH with a pH of 6.21. The results of the analysis of sawdust compost have a lower pH and nutrient content with a pH of 6.10. The results of the analysis of OPEFB + rice husk are compost which has a lower pH and nutrient content than sawdust with a pH of 6.05. The results of the analysis of OPEFB + sawdust compost which has a lower pH than the OPEFB + rice husk compost with a pH of 5.84. Rice husk compost has the lowest pH with a pH of 5.46.

Compost Organia Ingradianta —	Nutrient Level		pН	
Compost Organic Ingredients –	N- Total (%)	C- Organic (%)	H₂O	
TKKS	0.60	27.70	6.53	
Rice Husk	0.51	35.34	5.46	
Sawdust	0.51	40,18	6.10	
OPEFB + sawdust	0.56	30.39	5.84	
TKKS + rice husk	0.53	32.45	6.05	
Sawdust+ Rice Husk	0.55	38.00	6.76	
Sawdust+ Rice Husk+ EFB	0.50	35.15	6.21	

Table 1. Results of Chemical Analysis of Compost Organic Materials

The results of the pH of each compost showed that the pH was classified as neutral. The pH of each compost was optimal for the growth of fungal conidia. The optimum pH for fungal growth is 4-6 (Suprapti & Santoso, 2012).

According to (Sari & Darmadi, 2016) the acidity or pH in the compost pile also affects the activity of microorganisms. A good pH range for composting is around 6.5 - 7.5 (neutral).

The results of the chemical analysis of all compost showed that the N nutrient was in the range of 0.50-0.60%. The results of the chemical analysis showed that OPEFB compost had the highest nutrient content of N 0.60%. EFB+ sawdust compost has the second-highest N with 0.56% N. Sawdust + rice husk compost has the third-highest N element with 0.55% N. OPEFB + rice husk compost has a lower N element than sawdust + rice husk with 0.53% N. Rice husk compost and sawdust compost have the same N element, namely N 0.51% and the lowest N element in sawdust compost + rice husk + EFB N 0.50%. The N content in each compost is not too far in comparison, this shows the maturity of the same compost. (Pratiwi, Sri Hariningsih, 2018) stated that the maturity of the compost also affects the nutrients produced. It is in line with the principle of composting which reduces the value of the C/N ratio of organic matter to the same or close to the soil C/N ratio. The available nutrients determine the survival of the fungus. Menurut (Widarti & Wardhini, 2015) Nutrient N is needed by microorganisms to maintain and form body cells.

The results of chemical analysis showed that sawdust compost had the

highest organic C-nutrient element, which was 40.18% and the lowest was in EFB compost. namely 27.70%. Sawdust + rice husk compost had the secondhighest organic C with 38.00% C. Rice husk compost has the third-highest C with 35.35% C. Sawdust compost + rice husk + EFB has a lower C element than rice husk, namely C 35, 15%. EFB compost + rice husk has a lower C than sawdust + EFB rice husk + with а C of 32.45 %. EFB+ sawdust compost is lower than EFB+ rice husk compost with C 30.29%. OPEFB compost has the lowest C element, namely C 27.70%. Nurul (2015) stated that Gandjar in fungi depend on complex carbohydrates (carbon, hydrogen, and oxygen) as a source of nutrients, carbon sources are needed for energy and structural needs of fungal cells which support the growth of mycelium in fungi.

# The number of conidia *Beauveria* bassiana on compost media

The results showed that the treatment of several organic compost materials produced different amounts of *B. bassiana* conidia in each treatment. The results of the number of conidia can be seen in Table 4.2.

Table 2. The number of Conidia *B. bassiana* in some Compost Organic Materials.

Compost Organic Material Media Media	Number of Conidia (con/ml)	
ТККЅ	96.0 x10 <sup>8</sup>	
Rice Husk	76.8 x10 <sup>8</sup>	
Sawdust	102.4 x10 <sup>8</sup>	
EFB + Husk p adi	70.4 x10 <sup>8</sup>	
EFB+ Sawdust	115.2 x10 <sup>8</sup>	
Sawdust+ Rice Husk	51.2 x10 <sup>8</sup>	
Sawdust+ Rice Husk+ EFB	32.0 x10 <sup>8</sup>	

Table 4.2. showed that the highestnumber of conidia was EFB compost +sawdust at 115.2 x10 ° con/ml and thelowest was sawdust compost + rice husk+EFBat32.0x10 ° con/ml. Compost sawdusthas thesecond-highestnumber of conidia of

102,4x 10 °kon / ml. OPEFB compost had the third-highest number of conidia of 96.0x10 °. Rice husk compost has a lower number of conidia than EFB compost at 76.8x10 °. EFB+ rice husk compost has a lower conide count than rice husk compost by 70.4x10 °. Sawdust + rice husk compost has a lower amount of compost than EFB + rice husk compost by 51.2x10 °.

The results showed that different compost organic matter affected the number of conidia of the fungus *B. bassiana*, this indicates that the nutrients present in the compost organic matter affected the growth of the fungus *B. bassiana*. The results showed that OPEFB compost, sawdust compost, and OPEFB compost + sawdust had a high number of conidia as well as nutrients N and C in each of these composts, which can be seen in table 4.1.

The growth of *B. bassiana* colonies was influenced by the substrate or media containing nutritional components for the growth of the fungus. Hyphae growth and were influenced by sporulation the presence of nutrients in the medium. Carbon and nitrogen sourced from the components of the medium are the constituent elements of carbohydrates, nucleic acids, proteins, and lipids (Rohman et al, 2017). Results of Wahidah & Saputra, (2015) research showed that on sawdust media the growth of fungal mycelium was faster than rice husk media because of the high content of complex carbohydrates in sawdust planting media which is a source of element C compared to rice husks. The results of the research by Hariadi et al., (2013) showed that rice husks had a C/N ratio of 43.94 and a C/N ratio of 69.33 for sawdust. Table 3. Compost Temperature and Humidity

### Compost Temperature and Humidity

The results showed that the average temperature and humidity of compost and 39.20 %. Table 27.37 °C were 4.3. showed that the average temperature of the compost was 27.37 °C ranging from 27.52 °C. 27.28 to The average temperature of the compost had met the requirements for the growth of the fungus B. bassiana. Soetopo & Indrayani, (2015) stated that the optimum temperature for the development of pathogenicity and survival of fungi is generally between 20-30°C and conidia germination bassiana requires of *B*. humidity of around 50%. The average moisture in some compost organic matter 11.19% ranges from to 70.95%. (Purnama et al., 2003) adding humidity of 58.33% was suitable for growth. The results of the observation that the average humidity of the compost was 39.20%, ranging from 11.19 to 70.95% humidity. High humidity is found in OPEFB + sawdust compost and the smallest moisture is found in rice husk compost. Sawdust compost has the second high humidity of 57.92%. OPEFB compost has the third high humidity of 57.42%. Sawdust compost + rice husk + OPEFB has lower moisture than EFB compost by 49.88%. Sawdust compost + rice husk has lower moisture than sawdust compost + rice husk + EFB by 15.23%. OPEFB + rice husk compost has lower moisture than sawdust + rice husk compost by 11.78%.

Compost Organic Material Media Media	Temperature ( °C)	Humidity (%)
TKKS	27.35	57.42
Rice Husk	27.28	11.19
Sawdust	27.38	57.97
TKKS+ Rice Husk	27.40	11.78
EFB+ Sawdust	27.33	70.95
Sawdust+ Rice Husk	27.52	15.23
Sawdust+ Rice Husk+ EFB	27.33	49.88
Average	27.37	39,20
Observations showed that OPEER	compost + sawdust	had higher moisture

Observations showed that OPEFB compost, sawdust compost, and EFB

compost + sawdust had higher moisture content than rice husk compost or

compost that contained a mixture of rice husks. Rice husk has a low absorption ability so it is difficult to get high humidity. It is supported by Ismayanda & Mulana, (2014) that based on the texture, the ability of rice husks to absorb water is very low, making it difficult for rice straw to retain moisture when compared to sawdust. Widarti et al, (2015) stated that if the environmental conditions of growth are not met (humidity), it will inhibit the growth and spread of fungal mycelium (humidity 40-60% is the optimum range for microbial metabolism. If humidity is below 40%, microbial activity will experience a decrease and will be even lower at 15% humidity.

## Early larval death time (hours)

The results of variance showed that the treatment of several types of organic compost containing *B. bassiana* had a significant effect on the early death time of *O. rhinoceros* larvae (Appendix 1.1). The results of the average early death time of *O. rhinoceros* larvae after the DNMRT test at the 5% level can be seen in Table 4.7.

 Table 4. Early Time of O. rhinoceros Larvae Death in Some Organic Composts

 Containing B. bassiana.

Compost Organic Material Media Media	R Initial time of death (hours)
TKKS	69.6 b
Rice Husk	69.6 b
Sawdust	110.4 ab
EFB + Husk p adi	69.6 b
EFB+ Sawdust	67.2 b
Sawdust+ Rice Husk	141.6 a
Sawdust+ Rice Husk+ EFB	57.6 b

The numbers in the rows followed by lowercase letters that are not the same are significantly different according to the DNMRT test at the 5% level

Table 4. shows the early mortality of O. rhinoceros larvae occurred at 57.6 hours to 141.6 hours after application. The fastest early time of death was sawdust compost + rice husk + EFB at 57.6 hours and not significantly different from EFB compost + sawdust at 67.2 hours, EFB compost + rice husk at 69.6 hours, EFB compost at 69.6 hours, compost rice husk at 69.9 hours, sawdust compost 110.4 hours and significantly different from sawdust + rice husk compost at 141.6 hours. The longest initial time of death was sawdust compost + rice husk at 141.6 hours, not significantly different from sawdust compost at 110.4 hours.

The results showed that different compost organic matter along with the nutrients contained in it caused the growth of *B. bassiana* to be different, so the time needed to infect *O. rhinoceros* larvae was different. Sawdust + rice husk compost has the longest initial time of death, due to the small number of nutrients contained in the compost so that it affects the growth of the fungus B. bassiana accompanied by a lower conidia density than other treatments. Wicaksono & Abadi, (2015) stated that the factors that can affect the effectiveness of the fungus are conidia density, quality of growing media, types of pests controlled, and age of pest stadia. In addition, according to Herlinda & Irsan, (2015), the time required to cause death in test insects depends on the virulence of the pathogen, the nature of host resistance, and microenvironmental conditions in the host's body.

### Lethal time 50 (LT 50) (hours)

The results of the observation of *lethal time* 50 (LT  $_{50}$ ) after analysis of variance showed that the treatment of some organic compost containing *B. bassiana* significantly affected the time required to kill *O. rhinoceros* larvae by 50% (Appendix 1.1). Average yield rata *lethal time* of 50 larvae of *O. rhinoceros* after DNMRT test at 5% level are shown in Table 4. 4.

Table 4.5 shows that time needed to turn off the larvae of *O. rhinoceros* as much as 50% occurred in the 206.4 hours to 463.2 hours after application. A faster time to turn off 50% larvae *O.rhinoceros* on TKKS + sawdust compost is 206.4 hours and significantly different from the composting sawdust + rice husk + TKKS on 381.6 hours, EFB compost at 400.8 hours, rice husk compost at 434.2 hours, EPEFB compost + rice husk at 444 hours, sawdust compost + rice husk at 446.4 hours and sawdust compost at 463.2 hours.

Table 5. Lethal Time 50 Larvae of O. rhinoceros in some Organic Compost Containing B. bassiana.

Compost Organic Material Media Media	<i>Lethal time</i> 50 (LT ₅₀) (hours)
TKKS	400.8 a
Rice Husk	434.2 a
Sawdust	463.2 a
EFB + Husk p adi	444.0 a
EFB+ Sawdust	206.4 b
Sawdust+ Rice Husk	446.4 a
Sawdust+ Rice Husk+ EFB	381.6 a

The numbers in the rows followed by lowercase letters that are not the same are significantly different according to the DNMRT test at the 5% level

The ability of the fastest fungus B. bassiana to shut 50% down of larvae O .rhinoceros contained in the compost TKKS + sawdust compared with other treatments, is in line with the density of conidia compost TKKS + sawdust high of other treatments (Table 4.2). The density of conidia will affect the ability of the fungus to infect the test insects. In addition, the high humidity factor in sawdust + EFB compost, which is 70.95% (Table 4.3) affects the speed of fungal development. This is in line with Mahmud's (1989) statement that the success of pathogenic fungi as pest control is influenced by environmental (temperature, humidity). factors the viabilitv of spores, spore number (germination), and virulent virulence having low infectivity or vice versa. The virulent nature of the fungus is influenced by the production of mycotoxins, in this case, beauvericin, and spore viability.

### Larvae daily mortality (%)

The observation of daily mortality percentage of the larvae of *O. rhinoceros* with several treatments

compost containing *B. bassiana* resulted in mortality of larvae of *O. rhinoceros* different - different. The daily mortality of *O. rhinoceros* larvae can be seen in Figure 1.

Figure 1. shows the mortality of O. rhinoceros in the OPEFB + Sawdust compost treatment which was the highest peak compared to other treatments on the day reaching 14% and then sixth fluctuating the next day. In the treatment of OPEFB compost, the daily mortality of O. rhinoceros larvae reached a peak on the second day reaching 4% as well as rice husk compost reaching a peak on the second day at 4%, OPEFB + rice husk compost reaching a peak on the second day at 6% and sawdust + husk compost rice + EFB by 9%. The treatment of sawdust compost + rice husk mortality of *O. rhinoceros* larvae reached its peak on the tenth day of 8%. OPEFB+ sawdust compost had the highest daily mortality (14%) compared to other composts this was due to the high number of B. bassiaana conidia in OPEFB+ sawdust compost accompanied by fast early mortality of *O. rhinoceros* larvae and 50% larval death time which is fast compared

to other treatments.





Each treatment experienced an increase and decrease in daily mortality of different larvae, the increase and decrease in mortality occurred because the fungus B. bassiana continued to reproduce on the body of O. rhinoceros larvae to obtain nutrients so of O. that the daily mortality rhinoceros larvae could decrease from the previous day and vice versa.

Kurniawan & Panggeso, (2020) stated that an increase in the number of mortality could occur if there was contact between larvae and fungal spores. Upon contact, the spores form a germination tube and secrete enzymes to soften the cuticle of the larvae so that the spores can penetrate the larva's body. The growth of spores in the larval body will disrupt all organ activities and result in the death of the larvae.

## Total larval mortality (%)

Observation of the total mortality of O. *rhinoceros* larvae after analysis of variance showed that the treatment of some organic compost containing *B*. bassiana had a significant effect on the total mortality of O. rhinoceros larvae (Appendix 1.c). The results of the average total mortality of O. rhinoceros larvae after the DNMRT test at a 5% level can be seen in Table 4.5.

 Table 6. Mortality of O. rhinoceros larvae in some organic compost containing B.

 bassiana (%).

Compost Organic Material Media	Total Mortality (%)	
TKKS	44 b	
Rice Husk	42 b	
Sawdust	28 b	
EFB + Rice Husk	26 b	
EFB+ Sawdust	90 a	
Sawdust+ Rice Husk	30 b	
Sawdust+ Rice Husk+ EFB	38 b	

The numbers in the rows followed by lowercase letters that are not the same are significantly different according to the DNMRT test at the 5% level

Table 6 shows that treatment compost TKKS + sawdust containing B. bassiana total mortality of larvae of O. *rhinoceros* best of 90% and was significantly different from other treatments compost. The lowest mortality was in OPEFB + rice husk compost, which was 26% not significantly different from sawdust compost (28%), sawdust compost + rice husk (30%), EFB compost + sawdust + rice husk (38%), rice husk compost (42%) and EFB compost (44%).

OPEFB + sawdust compost containing *B. bassiana* had the best total mortality of *O. rhinoceros* larvae of 90% because OPEFB + sawdust compost had sufficient nutrients for the growth of *B. bassiana* fungus, namely N 0.56%, and C 30.39%. and supported by a high humidity of 70.95% causing a high number of conidia 115.2 x10 ° (Table 2), in addition to a fast initial death time of 67.2 hours and the fastest time required to kill 50% of larvae at 206,4 hours compared to other treatments this causes high total mortality. The higher the number of fungal conidia, the higher the toxin released in the larva's body and the higher the total mortality of the larvae. Hastuti et al., (2017) stated that the higher the conidia density, the higher the contact between the fungus and the test insect, the higher the infection rate that occurs so that the insect death process will be faster.

## CONCLUSIONS

The results of the study concluded that the organic material of OPEFB + sawdust compost containing fungus B. the bassiana had the number of conidia 115.2 x10 <sup>s</sup>had the best ability to control O. rhinoceros larvae which caused early death 67.2 hours after application. *lethal time* 50 206.4 hours after application and the total mortality rate is 90%.

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