Density, Viability Conidia And Symptoms of Metarhizium anisopliae infection on Oryctes rhinoceros larvae

by Dyah Rini Indriyanti

Density, Viability Conidia And Symptoms of *Metarhizium anisopliae* infection on *Oryctes rhinoceros* larvae

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Abstract. *M. anisopliae* is parasitic fungus on insect pests; itis used as a biocontrol agent. *M. anisopliae* can be propagated on maize or rice substrate. *M. anisopliae*is currently sold in the form of kaolin powder formulations. Before it is used to check the density, viability and pathogenicityof *M. anisopliae*.However the problem is the kaolin powder very soft, so it difficult to distinguish between kaolin and conidia.This article gives information on how to calculate conidia density, viability and symptoms of *M.anisopliae*infection on *Oryctes rhinoceros* larvae.The study was conducted in the laboratory to determine the density and viability.The pathogenicity testing was done using pots. The Pot is containing soil substrate mixed with *M. Anispoliae* and ten tails *O. Rhinoceros* larvae per pot. The results showed that the density of *M. anisopliae* conidia was 1.81 x 10^8 conidia mL^{-1} and the viability was 94% within 24 hours. The larval mortality began to emerge in the 1st week, and all larvae died at thesixth week. The symptom of *M.anisopliae*infection on *Oryctes rhinoceros* larvae, there was ablack spot on the larval integument. The larvae movements become slow and poor appetite; it will die within 3-7 days. The larvae diehard,and the white hyphae grow on the body surface that turns green.

1. Introduction

Oryctes rhinoceros beetle is one of the major pests attacking coconut crops. The *O. rhinoceros*attack by 2015 in Central Java reaches 10,240.86 Ha [1]. The beetle attacks base of the stem top of the coconut palm which makes the damaged on growing point. The beetle cuts unopened fronds causing the "V" shape cut on open fronds. It is the characteristic of *O. rhinoceros'* attack. The beetles lay eggs on the ground, while the larvae eat the organic matter such as the dirt animal, organic waste, and the trunks of coconuts rotted and the remnants of sugarcane [2].

One of the biological agents to control the larvae of *O.rhinoceros*isis *Metarhiziumanisopliae*,parasitic fungi. *M. anisopliae*can infect many insects. The best host for breeding *M. anisopliae*is the *O.rhinoceros*larvae [3-5].

Laboratory-scale research suggests that *M. anisopliae*is effective in controlling the populations of some insects such as*Spodopteralitura* i.e. [6,7], *Coptotermescurvignathus*[8], *Stibaropusmolginus* [9], *Lepidiota stigma* [10]. The fungi *M. anispoliae* can be grown on corn or rice medium [11]. Currently,*M. anispoliae* sold in the form of kaolin powder formulation. It means that the conidia *M. anispoliae* mixed with kaolin. The density test and viability conidia are needed to find out if the fungus conidia quality is good or not. The problem is kaolin's powder is very soft; it is difficult to distinguish between kaolin's grains and conidia. If the solvent is water, then it is difficult to distinguish. Therefore, this article gives information on how to calculate the conidia density, viability of *M.anispoliae* and *M. anisopliae*infection symptoms on larvae of *O. rhinoceros*.

2. Methods

The density and viability conidia of fungi *M. anisopliae*testingwas done at BalaiProteksiTanaman Perkebunan (BPT-BUN) in Central Java, Salatiga. The infection symptoms of larvae *O.rhinoceros*observed in larvae fed on *M. anisopliae*, the experiment was done at Desa Jeruk Wangi's garden in Bangsri, Jepara, on March – May 2015.

2.1. Preparation of Fungi M. anisopliae

The fungi *M. anisopliae*was obtained from BalaiProteksiTanaman Perkebunan (BPT-BUN) Salatiga with the commercial name ZIUM OR WP. This product was floured, or kaolin media are containing *M. anisopliae*fungiconidia. Treatment doses used were 2 grams. The test of density and viability conidia of *M. anisopliae*fungi were donebefore used and applied to control the larvae of *O. rhinoceros*. There was a simple way to differentiate between conidia and kaolin; the flour was dissolved in a solvent special kaolin called *alkilarilpoliglycolether* (one brand is Agristick).

Conidia density calculation of *M. anisopliae*was done by 1 gram of *M. anisopliae*thatwas dissolved in 100 ml of aquadest and 1 ml of*alkilarilpoliglycol ether* (Agristick). The addition of *alkilarilpoliglycol ether* (agristick) was used as smoothing agent because of the difficulty to differentiate the kaolin and conidia. The suspension of the fungi *M. anisopliae*stirred with a glass stirrer for 2 minutes. Conidia suspension was taken as much as 1 ml using the eyedropper while stirring. Conidia suspension melted on the haemocytometer and then covered with glass cover and wait up to 1 minute until the location of the conidia stable. Conidia density calculated using a microscope with a magnification of 200 x on the haemocytometer (a + b + c + d + e). The treatment was repeated four times. Density was calculated by using the conidia formula:

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

Description:
S = number of conidia per gram of media culture
t = the number conidia which is calculated on the ' count media " (a, b, c, d, e)
d = degree of dilution
n = the number of small boxes observed (i.e. 5 x 16 = 80 small box)

The calculation conidia viability of *M. anisopliae* calculated with growing 0.05 ml conidia solution on *Potato Dextrose Agar* (PDA) media that has melted on glass objects using the eyedropper tool, wait until solidified (each one drop) and incubation for 8 hours, 12 hours, 24 hours in room temperature. The treatment was repeated three times. Conidia viability were observed and calculated using a microscope at a magnification of 200 x conidia between the life and the dead conidia using the formula:

$$V = \frac{a}{a + b} \times 100\%$$

Description:
V: conidia viability percentage
a : the number of live conidia
b: number of dead conidia

2.2. The Larvae of O. rhinoceros

The *O.rhinoceros*larvae retrieved from the field at DesaJeruk Wangi, Sub-District Bangsri, Jepara. Three instar larvae used as many as 240, long size 7-10 cm long and weighs 9-11 grams per larvae.

2.3. *Media preparation*

The *O.rhinoceros*larvae needed organic soil media for maintenance. The media inserted into the black pot with 34 cm diameter, 22 cm height. Every pot in media content as much as 5 kg of ground mixture, coconut palm' sawdust, and manure fertiliser in the comparison of 1:1: 1.

2.4. Test Execution

Each pot contains 5 kg of mixed media with 2 grams of *M. anisopliae*was giventen *O.rhinoceros*larvae and the treatment was repeated six times. The observation was done every day to see the infection symptoms of *M. anisopliae*on larvae. Data collected: conidia density, conidia viability and infection symptoms of the *O.rhinoceros*larvae due to *M. anisopliae*. Data infection symptoms of *M. anisopliae*on larvae *O.rhinoceros*analysed descriptively.

3. Results and discussion

3.1. Density and Viability of Conidia M. anisopliae

Test results of density conidia *M. anisopliae*on kaolin media will be presented in Table 1.

Table 1.Density conidia *M. anisopliae*in kaolin

Count Field Box Haemocytometer	Time 1	2	3	4
a	10	9	15	8
b	9	2	9	5
c	8	6	8	5
d	11	2	4	7
e	9	10	6	2
Total	47	29	42	27
Density conidia	2.35×10^8	1.45×10^8	2.10×10^8	1.35×10^8
Average	1.81×10^8 conidiamL^{-1}			

Table 1 depicts that density conidia *M. anisopliae* 1.81×10^8mL^{-1}conidia belongs to the good, about the standard quality of the fungi *M. anisopliae*as a biological agency must be $\geq 10^6$conidia/ml [12].Conidia viability of *M. anisopliae*kaolin media for 8 hours, 12 hours, 24 hours of incubation, presented in Table 2.

Table 2. Conidia viability *M. anisopliae*in the medium of kaolin

Time	Repetition	Total of observed conidia: Not Growing	Grow	Total	Viability (%)	Average (%)
8 hours	1	11	39	50	78	
	2	14	36	50	72	72.67
	3	16	34	50	68	
16 hours	1	7	43	50	86	
	2	8	42	50	84	84.67
	3	8	42	50	84	
24 hours	1	2	48	50	96	
	2	4	46	50	92	94.00
	3	3	47	50	94	

Table 2 shows that the viability of conidia *M. anisopliae*after 24 hours incubation was 94%. The results categorised as a good result, referring to the standard quality of mushrooms *M. anisopliae*as a biological agency (BPTBUN). Conidia viability is affected by temperature, humidity, pH, and the sunshine [13].

3.2. Symptoms of O.rhinoceroslarvae infected with M. anisopliae

The infection mechanism of *M. anisopliae*on*O.rhinoceros*larvae occurred in four stages: 1) the stage of inoculation, 2) snapping and germination, 3) penetration, 4) invasion and destruction at the point of penetration [14]. In inoculation stage, the contact occurred between fungi propagules (conidia) and the

body of the larva. The second stage was the process of snapping and propagule germination of fungus on insects integumentary. The third stage was penetrating and invasion; it penetrated the integumentary and formed a sprouts tube (appressorium). The fourth stage was the destruction at the point of penetration, and the formation of blastospore spread out into hemolymph that formed secondary Hypha to invade other tissues. After insects die, mould will continue to cycle in a phase of continuing depression saprophytic. Mildew will form colonies around the body of the host. After the body of an insect host full of the colony of mould, then conidia will be produced to infect another host [15].

Early symptoms, contact and snapping occurred when fungi *M. anisopliae*mixed in media containing larvae.If humidity and moisture content high on a substrate, then conidia will germinate. Conidia stick to the integumentary germinates,then hypha continued to spread throughout larval' body. Dextrusin toxins released by the fungi *M. anisopliae*will spread throughout the body of the larva. Hypha of *M. anisopliae*fungiutilised the nutrients in the body of the larva [16].

Penetration symptoms and the invasion of *M. anisopliae*on*O.rhinoceros*larvae were indicated by ablackish spot on the abdomen of the larvae. The larvae with blackish spot will be dead within 3-7 days. The blackish spot was the sign that showed larvae had been infected by *M. anisopliae* [11].

Other symptoms of infected larvae could be seen through slow movement of the larvae and appetite reduced. Mould toxic affected on theparalysis of larva's body [17]. The paralysis led larva to lose its motion systems coordination became achaotic movement, gradually weakening the total paralysis occurs later (dead).

The symptoms of infected larvae were characterised with the dead larvae became harder. The surface of the body of the larva grows white hypha become green by the age conidia an increasingly mature. The *O.rhinoceros*larval body became hard, because of the entire system and the larval body fluids have been exhausted utilised by the fungus *M. anisopliae*. As a result, the dead insect's body became hard like Mummy [18].

Temperature Conditions, humidity, light intensity and pH of soil were also greatly affected by the success of the fungi *M. anisopliae*in infecting larvae. The field temperature while the study held was in the range 26 ° C - 28 ° C and 76-87 % humidity. The range of temperature and humidity supported the growth of the fungi *M. anisopliae*, as evidenced by the existence of the mortality of the larvae of *O.rhinoceros*due to the growth of fungi *M. anisopliae*.The optimum temperature for the growth of the fungi *M. anisopliae*occurred at a temperature of 22-27 °C, whereas the formation of sprouts on conidia occurred at humidity 80-92% [13]. It wasby the current condition of research.

4. Conclusion

The level of conidia density of fungi *M. anisopliae*in kaolin media was 1.81 ×**1** ^{8}Conidia/ml with 94% conidia viability in 24 hours. Symptoms of early infection were the blackish spots on the larval integument, larvae dead, and the larvae became hardened and embossed white hypha and gradually turn green.

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