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Research Article

The Potency of *Metarhizium anisopliae* in Disturbing *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) Growth and Development

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ABSTRACT

Metarhizium anisopliae is one of the most frequently used insect pathogen fungi to control *Oryctes rhinoceros*. This research aimed to learn the effect of *M. anisopliae* on *O. rhinoceros* larvae growth and development at the laboratory. Fungi were applied on all larvae instar stages starting from the post-molting of the first larvae instar, pre-molting of the second larvae instar, active second larvae instar, post-molting of the second larvae instar, pre-molting of the third larvae instar, active third larvae instar, and pre-pupae stage which were then compared with each instar's own control. Results were analyzed using T-tests comprising seven treatments and three replications. Results indicated that *M. anisopliae* was capable of suppressing *O. rhinoceros* growth and development. The fungus induced the highest mortality rate of 87% when applied to the third instar larvae and lowest mortality rate of 27% to the post-molting of the first instar larvae. The fungus also affected the duration of larval stage. Post-molting of the second larvae instar treated with *M. anisopliae* experienced larval duration of 40 days compared to that of control that took 135 days. At the pre-molting of the third larvae instar, the larval duration was 25 days compared to that of control that took 120 days. At the third larvae instar, the larval duration was no more than 15 days compared to that of control that reached 110 days. At pre-pupal stage, the larvae only lasted for 6 days while at control, individuals were able to last for 15 days. The fungus also affected the success rate of larvae development to pupae in all *O. rhinoceros* application stage. The lowest success rate was found in the post-molting of the second larvae and pre-molting of the third larvae instar treated with *M. anisopliae* with 7% compared to its control with 100%. While the highest success rate was found in the post-molting of the first larval instar with 47% compared to its control with 93%.

Keywords: development; growth; *Metarhizium anisopliae*; *Oryctes rhinoceros*; suppress

INTRODUCTION

Coconut palm rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) is considered as a major pest of Palmae, especially, coconut and oil palm tree (Bedford, 2018). *O. rhinoceros* can cause damage up to 25% no mature plant (Fauzana *et al.*, 2018). In Indonesia, damages caused by this pest led to an economical loss of 299.3 million USD (Abidin *et al.*, 2014).

Oryctes rhinoceros control is conducted using numerous techniques, such as mechanical control by collecting larva and imago (Pradipta *et al.*, 2020), using pheromone trap with ethyl 4-methylitaconate (Witjaksono *et al.*, 2015), and applying natural enemies such as insect pathogens. *Metarhizium anisopliae* is the most commonly used fungal pathogen for controlling pest *O. rhinoceros* (Moslim & Kamarudin, 2014; Bintang *et al.*, 2015; Velavan *et al.*, 2018). Previous research showed that *M. anisopliae* with conidia density of 10^7 conidia/ml induced 6.6% to 100% *O. rhinoceros* larval mortality (Bintang *et al.*, 2015). In addition, *M. anisopliae* application at dosages of 50 $g.l^{-1}$ (302.4×10^6 $conidia.ml^{-1}$) on *O. rhinoceros* larva living on empty bunches of oil palm in the field caused mortality of 56% (Fauzana *et al.*, 2020).

Metarhizium anisopliae infect insects through their cuticle. When the fungus penetrates, blastospore will be formed and then spread inside the hemolymph,

form secondary hyphae that later destroys the inner tissues of insect's body. After insects die, the fungus continues its life cycle in saprophytic phase by colonizing the host's body and producing infectious spores (Boucias *et al*., 1988; Ment *et al*., 2010; Aw & Hue, 2017). *M. anisopliae* is considered an efficient control strategy that can be used to disrupt the life cycle of *O. rhinoceros* larvae (Paudel *et al*., 2021). Research on the effect of *M. anisopliae* on the growth and development of *O. rhinoceros* is important to determine the sensitivity of all larval stages, and efficiently control or suppress the development of *O. rhinoceros* larvae. This research aims to determine fungus *M. anisopliae* potency in disturbing *O. rhinoceros* larvae growth and development at the laboratory.

MATERIALS AND METHODS

Oryctes rhinoceros Larva Breeding

Oryctes rhinoceros larvae were collected and reared in a plastic container (30×30×7.5 cm) with a window screen and contained coconut coir inside them. Larvae were obtained from a rotten oil palm trunk in Triharjo Village, Bantul Regency, Special Region of Yogyakarta. Larvae collected were the first, second, and the third instar larvae. Those larvae were placed in a 200 g coconut coir used as a medium. The medium was replaced once a week along with the application of sterile water to maintain humidity.

Origin of Fungal Isolate

Metarhizium anisopliae isolates were obtained from *O. rhinoceros*' larvae and purified at the laboratory. Isolation and purification of the fungus were conducted using Potato Dextrose Agar (PDA). Fungus were propagated on the corn that was sterilized using autoclave at 100–121° C with 15 psi pressure for 30 minutes. The fungus on corn medium was incubated for 10–14 days in the laboratory until the medium was filled with green spores.

Metarhizium anisopliae Mortality Test upon *Oryctes rhinoceros* Larvae

Before conducting mortality test, spore density on corn medium was counted. Based on this, fungus with 10^6 conidia/g spore density was selected. A 100 g coconut coir was placed in a plastic tube (h = 15 cm, d = 15 cm) and treated with *M. anisopliae* at 10^6 conidia/g spore density. Each container was inserted with 5 larvae according to their instar to avoid cannibalism. *M. anisopliae* application was performed to each treatment group, including the post-molting of the first instar larvae (post L1), the pre-molting of the second instar larvae (Pre L2), active second instar larvae (L2), post-molting of the second instar larvae (Pre L2), pre-molting of the third instar larvae (Pre L3), active third instar larvae (L3), and pre-pupae. As for the control, larvae were not treated with fungus *M. anisopliae*. Observation was done within 160–165 days.

Insect mortality was tested to determine mortality rate of *O. rhinoceros* larvae by fungus *M. anisopliae*. Insect mortality percentage was calculated using the following formula from Sun and Shepard (1947):

$$M = \frac{\sum n}{\sum N} \times 100\%$$

M = insect mortality percentage (%), n = number of dead insect (insect), N = number of insect tested (insect).

Oryctes rhinoceros Larva Growth and Development

Oryctes rhinoceros growth and development was tested during the molting. The observed parameters included duration of larval stage per instar (counted since the post-molting of the first larvae instar, pre-molting of the second larvae instar, active second larvae instar, post-molting of the second larvae instar, pre-molting of the third larvae instar, active third larvae instar, to pre-pupae stage) as well as the success rate of larvae turning into pupae. The percentage of larvae to reached pupae was calculated using a formula by Mulla and Darwazeh (1975), as follows:

$$P = \frac{p}{N} \times 100\%$$

P = percentage of pupae formed, p = total number of larvae turning into pupae, N = total initial number of larvae tested.

Statistical Analysis

Oryctes rhinoceros larval mortality percentage, duration of larval life stages, and percentage of larvae that successfully reached pupae formation were then analyzed using Paired Simple T-test to compare differences between that of control and respective treatment (Cochran & Cox, 1957).

RESULTS AND DISCUSSION

Metarhizium anisopliae Influence on *Oryctes rhinoceros* Mortality

The results showed that *M. anisopliae* affected mortality and development of certain instar of *O. rhinoceros* larvae. The application of *M. anisopliae* caused higher mortality of some instar of *O. rhinoceros* of larvae compared to the control. At pre L2 (before entering the second larvae instar), *M. anisopliae* affected larval mortality. Similar results were found in post L2 (after molting of the second larvae instar), pre L3 (before entering the third larvae instar), the third larvae instar and pre-pupae stage. *M. anisopliae* also affected *O. rhinoceros* mortality. From several *O. rhinoceros* instar larvae infected with *M. anisopliae*, the third instar larvae had the highest mortality rate with 87% and the lowest mortality percentage was found in post L1 instar with 27%. In addition, *M. anisopliae* showed pathogenic properties towards nearly all life phases of this insect (Figure 1).

Moslim *et al.* (2007) reported that *M. anisopliae* applied to a pile of palm fronds caused significant mortality on *O. rhinoceros* L2, L3, pre-pupae and pupae. Conducive environment of palm fronds had water contents reaching 80% and temperatures of 27ºC–29ºC thus increasing sporulation of *M. anisopliae.*

According to the results, *M. anisopliae* caused mortality of 86.6% against *O. rhinoceros* larvae. This fungus attacks *O. rhinoceros* through the cuticle and is able to produce chitinase, protease, lipase, esterase, endoprotease and enzymes which significantly affect its infection to the host insect (Santi *et al.*, 2010; Aw & Hue, 2017). *M. anisopliae* infect insects through several steps starting by introducing spores to insect's body, sticking and sprouting fungus spore in insect's integument through hydrophobic mechanism, forming appressorium at insect's cuticle, forming sprouting tube and piercing through insect's integument. Appressorium grows well at pH of around 5–8 and temperatures of 25–30ºC. Lastly, penetration will form blastospores which will then spread within hemolymph and form a secondary hyphae in order to attack the tissues inside insect's body (Boucias *et al.*, 1988; Altinok *et al.*, 2019; Bava *et al.*, 2022).

Metarhizum anisopliae Influence on *Oryctes rhinoceros* Larva Growth and Development

Results showed difference of larval stage duration between control and ones that received applications. *M. anisopliae* impacted the post-molting of the second instar larvae to last 40 days while the control took 135 days. At the pre-molting of the third larvae instar, larval stage took 25 days while it took 120 days for the control. At the third larvae instar, larval stage took 15 days while it took 110 days for the control. At prepupae stage, larvae treated with *M. anisopliae* only lasted for 6 days while untreated ones lasted for 15 days. At other larval stages (Post L1, Pre L2, L2), it did not difference compared with control (Figure 2).

The success of insect's to reach a certain development stage is another aspect that should be studied

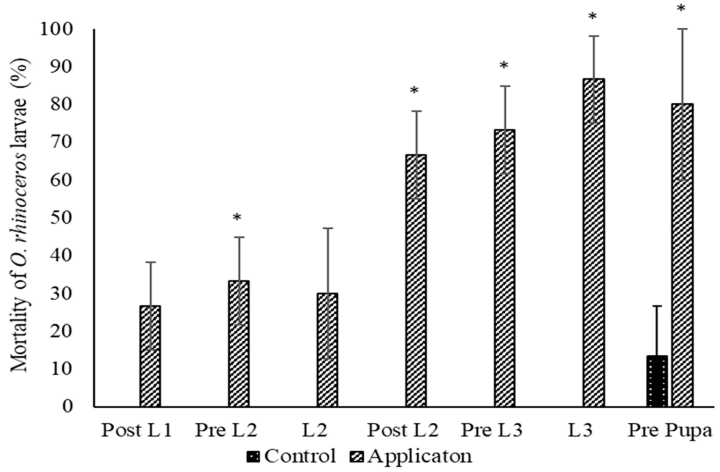

Figure 1. *Metarhizium anisopliae* effects on *Oryctes rhinoceros* larval mortality; asterisk (*) indicates significant difference between the control on treated treatment based on 5% significance level T-test

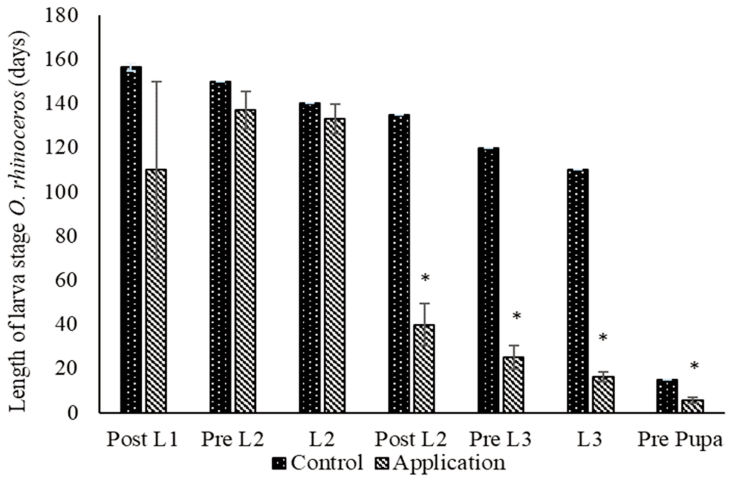

Figure 2. *Metarhizium anisopliae* effects on the duration of *Oryctes rhinoceros* larval stages; asterisk (*) indicates significant difference between the control on treated treatment based on 5% significance level T-test.

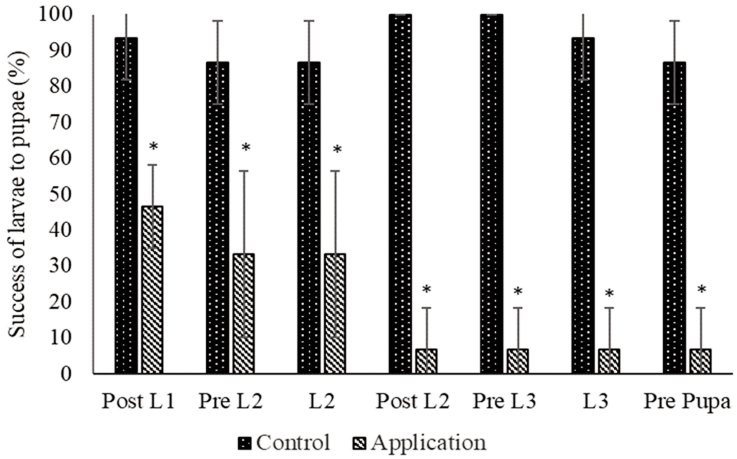

Figure 3. *Metarhizium anisopliae* effect on the success rate of *Oryctes rhinoceros* larva that reach pupa; asterisk (*) indicates significant difference between the control on treated treatment based on 5% significance level T-test

aside from mortality. Results indicated that *M. anisopliae* affected the success rate of larvae turning into pupae of all *O. rhinoceros* larval stage compared to control. The lowest success rate was found at the post-molting of the second larvae and pre-molting of the third larvae instar applied with *M. anisopliae* with 7% compared to the control that reached 100% success rate while the highest success rate was found at the post-molting of the first larvae instar with 47% compared to its control with 93% (Figure 3).

Villani *et al.* (1999) stated that Scarabaeidae larvae move underground causing lower infection probability to be lower and later affecting development. Larvae infected with toxic substance will disturb larval physiology thus resulting in juvenile hormone and ecdysone hormone's roles that affect the molting process. The disturbed molting process will prolong the duration of larval stage and cause larvae's development into pupa to be disturbed as well (Lukman, 2009).

Oryctes rhinoceros larvae experienced molting from larvae to larvae, larvae to pupae, and pupae to imago. The molting process from larvae to larvae is influenced by 20-hydroxyecdysone and juvenile hormone. At the epidermis, 20-hydroxyecdysone hormone and juvenile hormone are found in abundance so that molting from larvae to larvae may occur. The molting process from larvae to pupae is still influenced by 20-hydroxyecdysone

hormone and juvenile hormone. However, the amount of juvenile hormone in this phase is low and when 20-hydroxyecdysone reaches commitment peak, molting from larvae to pupae may occur. On the other hand, molting from pupae to imago is only influenced by 20-hydroxyecdysone since juvenile hormone has been degraded by juvenile hormone esterase (Chapman, 2013). In this research, *M. anisopliae* application brought real impact that caused all larval stages to fail in pertaining to the pupa stage. According to St. Leger (1995), *M. anisopliae* is a fungus that produces cuticle degrading enzyme either in culture or during the process of insect infection.

CONCLUSION

Metarhizium anisopliae could suppress the growth and development of *O. rhinoceros* at all larval stages. This fungus affected *O. rhinoceros* larval mortality, duration of larval stage, as well as success rate of larvae turning into pupae.

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