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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 in controlling *Oryctes rhinoceros*. This research aims to learn the potency of fungus *M. anisopliae* against *O. rhinoceros* larvae growth and development at the laboratory. The research methods used are T-tests comprising seven treatments and three replications. Fungi were applied in all larvae instar stages starting from the pre-molting of the first larvae instar, post-molting of the second larvae instar, active second larvae instar, pre-molting of the second larvae instar, post-molting of the third larvae instar, active third larvae instar, and pre-pupae stage which were then compared with each instar's own control. The result indicates that fungus *M. anisopliae* is capable of suppressing *O. rhinoceros* growth and development. The fungus induced highest mortality rate of 87% to the third instar larvae and lowest mortality rate of 27% to the pre-molting of the first instar larvae. The fungus also affected the duration of larval stage. At pre-molting of the third larvae instar treated with *M. anisopliae*, the larval duration was 40 days compared to that of control that took 135 days. At the post-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, they were able to last for 15 days. The fungus also affected the success of larva development in becoming pupae in all *O. rhinoceros* larval stage. The lowest success rate was found in the post-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 pre-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 up to 25% damage to mature plant (Fauzana *et al*., 2018). In Indonesia, damages caused by the pest led to an economical loss of 299.3 million USD (Abidin *et al*., 2014).

O. rhinoceros control has been conducted numerous times, such as mechanical control by collecting larva and imago (Pradipta *et al*., 2020), using pheromone trap called ethyl 4-methyloctanoate (Witjaksono *et al*., 2015), and using natural enemies such as insect pathogens. Fungus *Metarhizium anisopliae* is the most commonly used pathogen fungi in controlling pest *O. rhinoceros* (Moslim & Kamarudin, 2014; Bintang *et al*., 2015; Velavan *et al*., 2018).

M. anisopliae with a conidia density of 10^7 conidia/ml induces around 6.6% to 100% *O. rhinoceros* larval mortality (Bintang *et al*., 2015). Besides, treatment with *M. anisopliae* with a dosage of 50 $g.l^{-1}$ (302.4 x 10^6 conidia/ml^{-1}) upon *O. rhinoceros* larva living in oil palm empty bunches in the field caused a mortality of 56% (Fauzana *et al*., 2020).

M. anisopliae infects insect through cuticle. When the fungus penetrates, blastospore will be formed which will then spread inside hemolymph and form secondary hyphae that destroys the tissues inside insect's body. After the insect died, 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 strategy to break the life cycle of *O. rhinoceros* larvae (Paudel *et al*., 2021). Research related to the effect of *M. anisopliae* on the growth and development of *O. rhinoceros* is important for determine the sensitivity of all larval stages, in order to obtain the efficient control of suppressing the development of *O. rhinoceros* larvae. This research aims to learn fungus *M. anisopliae* potency in disturbing *O. rhinoceros* larvae growth and development at the laboratory.

MATERIALS AND METHODS

Oryctes rhinoceros Larva Breeding

O. rhinoceros larvae were collected and bred in a plastic container (30×30×7.5 cm) equipped with window screen contained coconut coir. Larvae were obtained from a pile of rotten oil palm trunk in Triharjo Village, Bantul Regency, Special Region of Yogyakarta. Larvae extracted were the first instar larvae, the second instar larvae, and the third instar larva. Those larvae were put in a 200 g coconut coir as a medium. The medium was replaced once a week along with the distribution of sterile water to maintain its humidity.

Origin of Fungal Isolate

M. anisopliae isolate was obtained from *O. rhinoceros* larvae purified at the laboratory. Isolation and purification of the fungus were conducted using Potato Dextrose Agar (PDA).

On top of that, for the sake of treatment the application of *M. anisopliae* on *O. rhinoceros* larvae, the fungus was propagated onto natural medium such as corn that was sterilized using autoclave at 100–121° C with 15 psi pressure for 30 minutes. The fungus on corn medium will 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 of the fungus on corn medium was calculated before treatment. Based on the calculation, fungus with 10^6 conidia/g spore density was selected. A 100 g coconut coir was put in a tube plastic container (h= 15 cm, d= 15 cm) and treated with fungus *M. anisopliae* with 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 namely, the pre-molting of the first instar larvae (pre L1), the post-molting of the second instar larvae (Post L2), active second instar larvae (L2), pre-molting of the second instar larvae (Pre L2), post-molting of the third instar larvae (Post L3), active third instar larvae (L3), and pre-pupae. As for control, larvae were not treated with fungus *M. anisopliae*. Observation was done within 160–165 days.

Insect mortality test was conducted to learn the mortality rate of *O. rhinoceros* larvae caused by fungus *M. anisopliae*. Insect mortality percentage can be calculated using the following formula Sun and Shepard (1947):

$$M = \frac{\sum n}{\sum N} \times 100\% \qquad (1)$$

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

Oryctes rhinoceros Larva Growth and Development

O. rhinoceros growth and development test was conducted during the process of molting. The observed parameters include duration of larval stage per instar (counted since the pre-molting of the first larvae instar, post-molting of the second larvae instar, active second larvae instar, pre-molting of the second larvae instar, post-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's survivor formation into pupae was calculated using a formula by Mulla and Darwazeh (1975), as follows:

$$P = \frac{p}{N} \times 100\% \qquad (2)$$

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

Statistical Analysis

The data of *O. rhinoceros* larval mortality percentage, the duration of larval life stages, and the percentage of larvae successful pupae formation collected were then analyzed using Paired Simple T-test to compare between that of control and application (Cochran & Cox, 1957).

RESULTS AND DISCUSSION

Metarhizium anisopliae Influence on *Oryctes rhinoceros* Mortality

The results of the test showed that *M. anisopliae* truly affects some of *O. rhinoceros* instar larvae. The application of *M. anisopliae* with 10^6 spore density can cause higher mortality in some *O. rhinoceros* instar larvae compared to control. In post L2 (before entering the second larvae instar), *M. anisopliae* affected larval mortality. Similar results were found in pre L2 (after molting of the second larvae instar), post 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 have the highest mortality rate with 87% and the lowest mortality percentage was found in post L2 instar with 27%. *M. anisopliae* have proven pathogenic 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 has significant impact towards *O. rhinoceros* L2, L3, pre-pupae and pupae. Conducive environment of the pile of palm fronds was created due to its water content reaching around 80% and 27ºC–29ºC in temperature thus increasing sporulation of the entomopathogenic fungi applied.

According to the research conducted, fungus *M. anisopliae* induced as much as 86.6% *O. rhinoceros* larval mortality through the collaboration of fungus *M. anisopliae* application and coir. This fungus attacked *O. rhinoceros* through cuticle. This entomopathogenic fungus has the ability 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* can infect insect through several steps starting by introducing spores to insect's body, sticking and sprouting fungus spore at 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 around 5–8 pH and between a

temperature 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**

M. anisopliae test results showed the average difference of larval stage duration between control and application. *M. anisopliae* impact on the pre-molting of the second instar larvae applied with *M. anisopliae* is by spending 40 days of larval stage while the control took 135 days. At the post-molting of the third larvae instar, larval stage took 25 days while it took 120 days with control. At the third larvae instar, larval stage took 15 days while it took 110 days with control. At prepupae stage, larvae with application only lasted for 6 days while they lasted for 15 days with control. At other larval stages (Pre L1, Post L2, L2), it did not show that much of difference when they were compared with control (Figure 2).

The success percentage of insect's development is one of the aspects that should be studied aside from its mortality aspect. The results of the test indicated that *M. anisopliae* affects the success rate of larvae turning into pupae in all *O. rhinoceros* larval stage compared to control. The lowest success rate was found at the post-molting of the third larvae instar applied with *M. anisopliae* with 7% compared to that of control with 100% success rate whilst the highest success rate was found at the pre-molting of the first larvae instar with 47% compared to its control with 93% (Figure 3).

Villani *et al*. (1999) stated that Scarabaeidae active larvae move underground so that infection probability is lower whereas larvae will not be able to survive when staying in cocoon and larva's opportunity to succeed in its development into pupae phase will get smaller. 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 experience molting from larvae to larvae, larvae to pupae, and pupae to imago. The molting process from larvae to larvae is influenced by 20-Hydroxyecdysone hormone 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 so that when 20 Hydroxyecdysone reaches commitment peak and low

amount of juvenile is found, 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

Fungus *M. anisopliae* can suppress the growth and development of *O. rhinoceros* in all larval stages. This fungus affects *O. rhinoceros* larval mortality, duration of larval stage, as well as success rate of larvae turning into pupae.

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Oryctes rhinoceros [Pressure of *Metarhizium anisopliae* and Pheromone Trap Application to the Population and Damage Caused by *Oryctes rhinoceros*]. *Jurnal Perlindungan Tanaman Iindonesia*, *19*(2), 73–79. https://doi.org/10.22146/jpti.17260

APPENDIX

LIST OF FIGURES

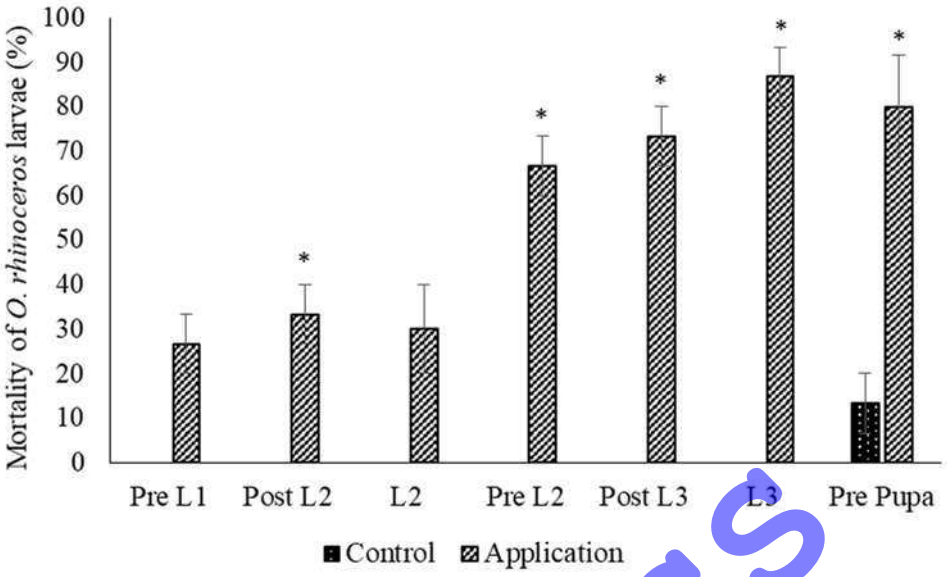

Figure 1. *Metarhizium anisopliae* influence on *Oryctes rhinoceros* larval mortality; asterisk symbol (*) indicates significant difference based on 5% significance level T-test

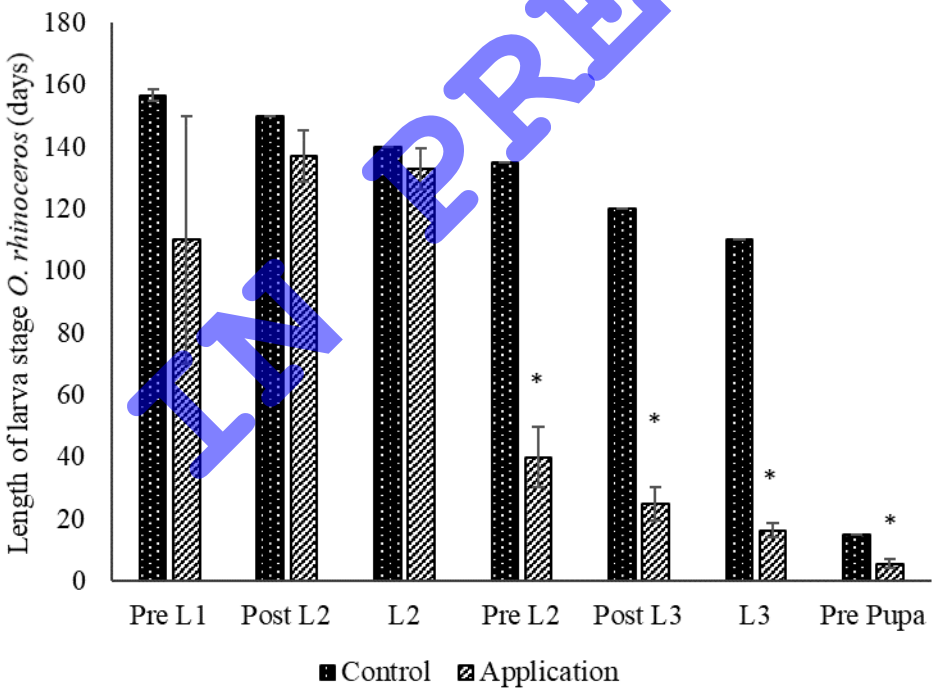

Figure 2. *Metarhizium anisopliae* influence on the duration of *Oryctes rhinoceros* larval stages; asterisk symbol (*) indicates significant difference based on 5% significance level T-test

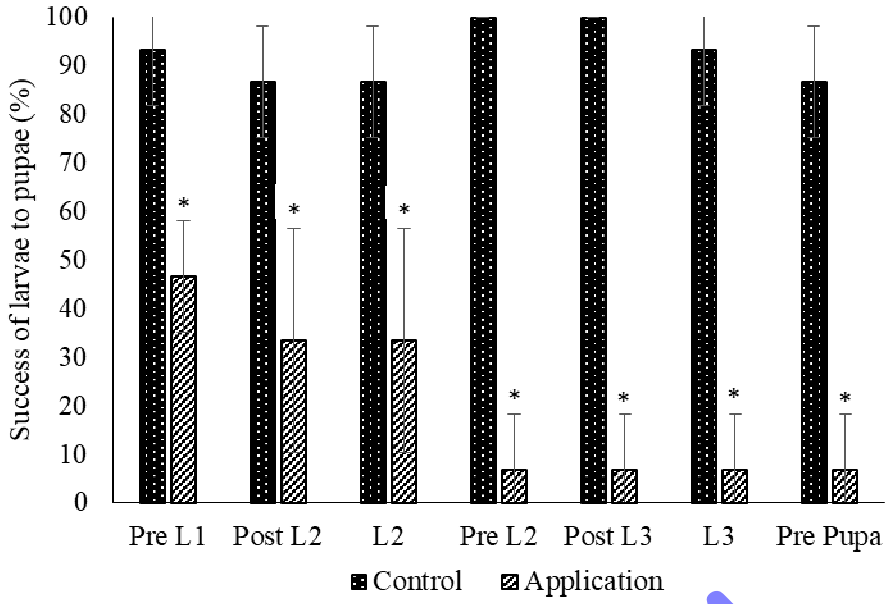

Figure 3. *Metarhizium anisopliae* influence on the success rate of *Oryctes rhinoceros* larva's turning into pupa; asterisk symbol (*) indicates significant difference based on 5% significance level T-test