Effect of Metarhizium anisopliae in Kaolin Formulation and Its Secondary Metabolite on Oryctes rhinoceros Larval Mortality.

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EFFECT OF METARHIZIUM ANISOPLIAE IN KAOLIN FORMULATION AND ITS SECONDARY METABOLITE ON ORYCTES RHINOCEROS LARVAL MORTALITY

DYAH RINI INDRIYANTI, SRI WAHYUNI, PRIYANTINI WIDYANINGRUM, NING SETIATI

Abstract: Pest control of O. rhinoceros can be done using M. anisopliae entomopathogenic fungi. The purpose of this study was to analyze the effectiveness of the M. anisopliae in the kaolin formulation and secondary metabolite solution (MS) of M. anisopliae on the mortality of O. rhinoceros larvae. The population in this study was the O.rhinoceros larvae in from Jerukwangi Village, Bangsri District, Jepara Regency, Indonesia. The samples used were 30 instar 4 of O. rhinoceros larvae with a body weight of 13-16 g. This study used a Completely Randomized Design (CRD), which consisted of three groups with 10 replications. The groups consisted of control group, M group (2 g M. anisopliae in kaolin formulation), and MS group (25 ml of secondary metabolite solution in manure media). Observations were conducted every 2 days for 18 days. The results showed that the O. rhinoceros larvae in M. anisopliae group died in a hardened body state and the body was covered with green fungus hyphae. O. rhinoceros larvae in MS group died in a soft body state and were not overgrown with fungal hyphae on the surface of the larval body. M. anisopliae in kaolin formula kills O. rhinoceros larvae faster that the secondary metabolite solution. Larvae began to die on the 6th day after application and total death (100%) occured on the 18th day after the application. The secondary metabolite solution started to kill O. rhinoceros larvae on the 14th after application (20%) and on 18th day after the application, the death of O. rhinoceros larvae remained 20%.

Keyword: secondary metabolite solution, Metarhizium anisopliae, Oryctes rhinoceros

1. INTRODUCTION

Oryctes rhinoceros is one of the pests that attack the coconut palms. Its attacks are indicated by the V-shaped cuts in the leaves [1]. O. rhinoceros larvae breed in the soil, manure, decayed coconut stems and sawdust close to coconut trees. O. rhinoceros attacks can be controlled using safe and environmentally friendly methods [2]. One of the methods is by using the biological control agent (BCA) such as entomopathogenic fungi, Metarhizium anisopliae [3] [4] stated that M. anisopliae is able to control the insect. About 200 species of soil insect can be controlled by M. anisopliae [5]. M. Anisopliae utilization as pest control agent has been proven to be more environmentally friendly because it does not cause insect resistance as well as the emergence of secondary pests and it also does not affect the user's health [6]. Recently, there is a new way in the application of M. anisopliae by making a solution containing its secondary metabolites. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development and reproduction of organisms, but are formed near the end of the stationary stage of organism growth [7]. Based on the research, it was known that the M. anisopliae secondary metabolite solution can be used to control Anopheles stephensi mosquito larvae, the vector of malaria disease. The secondary metabolite of M. Anisopliae was effective to control all instars of A. Stephensi larvae and 10% mortality of instar 1 larvae was obtained 24 hours after the application [8]. The application of BCA by using the secondary metabolite of M. Anisopliae has never been done in O. rhinoceros larvae. Therefore, this study was conducted to determine the effect of M. anisopliae on O. rhinoceros larvae. The purpose of this study was to analyze the effectivity of M. anisopliae in kaolin formulation and M. anisopliae secondary metabolite on O. rhinoceros larval mortality.

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METHODS

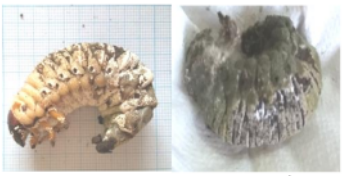
The study was conducted on April to May 2018. The population in this study was O. rhinoceros larvae that live in Jerukwangi Village, Bangsri District, Jepara Regency, Indonesia. The samples used were 30 O. rhinoceros instar 4 larvae weighed 13-16 gr with 7-10 cm of body length. The medium used as a living medium for O. rhinoceros larvae was manure. Abiotic factors that were observed included temperature and humidity of the air. In this study, there were three groups, i.e. control group, M. anisopliae in kaolin formulation (M), and the solution of M. anisopliae secondary metabolite (MS). The containers used for treatment were plastic containers (diameter= 8.4 cm, height= 11 cm). The containers used were equipped with perforated container cover that functions for air exchange. Each treatment was conducted with 10 times repetitions. So that, 10 plastic containers were needed for 10 O. rhinoceros larvae (1 larva/container). In control group, the plastic container was filled with 200 grams of medium that had been mixed with 25 ml of water (the addition of water served to moisturize the medium). Then, 1 larva of O. rhinoceros was put into the container. M. anisopliae in kaolin formulation and secondary metabolite solution (MS) were obtained from Estate Crop Protection Board in Salatiga, Central Java Province, Indonesia. The conidia that were cultured in PDA medium and propagated on cracked corn media had conidia density of 2.84 x 10^8/gr and viability of 94.6%. The dosage used in M group referred to [9], 2 gr /200 gr medium. The plastic containers were filled with 200 grams of medium that had been mixed with 2 grams of M. anisopliae and 25 ml of water. Then, 1 larva of O. rhinoceros was put in each plastic container. The secondary metabolite solution of M. anisopliae was processed from 15 liters of rice washing water, 5 liters of old coconut water, 20 tablespoons of granulated sugar and 1 kg of M. anisopliae fungus cultured on cracked corn medium. The plastic containers were filled with 200 grams of medium that has been mixed with 25 ml of M. anisopliae secondary metabolite solution and 25 ml water. Then, 1 O. rhinoceros larva was placed in each plastic container. O. rhinoceros larvae were first contacted to the secondary metabolite

solution for 5 minutes before being put into a plastic container, so that the destruxin compounds contained in the secondary metabolite solution can absorb more into the body of O. rhinoceros larvae and can work more effectively in killing O. rhinoceros larvae. The 10 plastic containers were placed in a 32 cm x 20 cm x 23 cm container to avoid direct exposure to sunlight. O. rhinoceros larvae were observed every two days for 18 days. Abiotic factors observed included temperature and humidity of the air. Symptoms observed included changes in morphology, movement of larvae, body color, and body texture. Obtained mortality data is presented in the form of larval mortality graph for each treatment. Abiotic factor data was used as supporting data.

RESULTS AND DISCUSSION

O. rhinoceros larva infected by M. Anisopliae

O. rhinoceros larvae infected by the fungus M. anisopliae are characterized by the formation of black spots (necrotic points) at the site of conidial penetration. According to [10], black spots (necrotic spots) formed are the sites of fungal conidia penetration. Necrotic symptoms are formed due to the self-defense process carried out by larvae against fungal infections, which is called the melanization process. Image of O. rhinoceros larvae infected with M. anisopliae is presented in Figure 1.



a b

***Figure 1**. O. rhinoceros larvae infected by M. anisopliae*

White hyphae on the body surface of O. rhinoceros larvae (a) appeared on 10th after application. Green hyphae on the body surface of O. rhinoceros larvae (b) appeared on day 14th after application. Other symptoms are characterized by decreased movement of larvae. According to [11] , it was because the fungus M. anisopliae produced destruxin compounds which could cause depolarization of the muscle membrane of the insect by influencing $Ca2^{+}$ ion transport that resulted in muscle paralysis. Muscle paralysis causes larval movement to decrease, making it difficult for larvae to move and looking for food. The condition of the larvae that becomes weak makes them unable to obtain nutrients, resulting in decreased feeding activity, eventually causing the larvae to die. The body surface of O. rhinoceros larvae that were dead due to infection of M. anisopliae were covered with white fungus hyphae. As time goes by, the hyphae of the fungus turned green and cover the entire surface of the larva's body. The hyphae covering the body of O. rhinoceros larvae began to form colonies and turned green on day 14 after application. It was in accordance with [12] , that on the surface of the body of O. rhinoceros larvae, the white hyphae of M. anisopliae began to grow on the 10th day after the application and on the 13th day after application, the fungus that grows on the surface of the larva's body turn green. O. rhinoceros larvae infected with M. anisopliae died in a hard body condition. Hardening of the dead O. rhinoceros larvae is because all the fluids in the body of O. rhinoceros larvae is used by the fungus M. anisopliae which grew into the cells of the larvae. The hardened dead larval body is consistent with the results of research conducted by [1]. This study showed that the death of O. rhinoceros larva was faster than the appearance of fungal hyphae on the surface of the larval body. Larval death begins on day 6 after application, whereas the hyphae appears on the surface of the larval body on day 10 after the application. This is consistent with the statement that the fungus takes a longer time to bring up the hyphae around the host's body because it has to go through several stages of infection [13]. According to [14], the mechanism of M. anisopliae fungus in infecting a host occurs through four stages. The first stage of inoculation is contact between the fungus propagules and the body of the insect. The second stage is the process of attaching and germination of fungus propagules on the insect integument. The third stage is penetration and invasion through the integument and forming a sprout tube (appresorium). The fourth stage is destruction at the point of penetration and the formation of blastospores which then circulates into the hemolymph and form secondary hyphae to attack other tissues in the insect's body. Fungal hyphae will absorb all the fluids found in the insect's body, thereby causing the insect to die in a dry and hardened state. After the insect dies, the fungus will continue to cycle in the saprophytic phase, which is the formation of fungal colony around the host's body.

O. rhinoceros Larvae Conditions Due to the Treatment Using Secondary Metabolite Solution

Dead O. rhinoceros larvae from MS group had the characteristics of soft and brownish colored body. The body surface of the larvae was not covered with M. anisopliae hyphae. In this treatment, 20% larvae were dead, 20% larvae were still alive, while the other 60% developed into pupae. The development of 60% of the larvae into pupae was due to the larvae used in this study were instar 4 larvae, which will soon enter the pupa phase. In addition, 60% of O. rhinoceros larvae developed into pupae because the dose of secondary metabolite solution used in this study is less than optimum. As a result, the antifeedant effect produced by the destruxin compound was low and the larvae still had an appetite. This condition caused the growth and development of larvae to continue into the next phase. Based on research conducted by [15] , the antifeedant effect of test extracts on the larvae of Crocidolomia pavonana increases with increasing concentrations of extracts. The antifeedant effect contributes to the longer time needed for the development of C. pavonana instar II larvae to become the next instar [15]. The nutritional needs that does not meet the need of larvae resulted in the inhibited physiological process. As compensation, the compounds that are supposed to be used for growth and development are allocated to maintain larval survival and the larval development to instar becomes slow. According to [16], inhibition of development in insects can be caused by an inhibition of feeding activity or the attack from toxicity of active compounds. The destruction produced by M. anisopliae has an antifeedant effect, which is the activity of inhibiting the larvae to eat, so that it can accelerate the death of larvae because they are starving [17]. The destruxin produced by the fungus M. anisopliae can cause abnormalities in the function of their stomach. This condition results in the disruption of metabolic processes followed by energy decreases. The drop in energy produced is not proportional to the activity of O.

rhinoceros larvae, so that the larvae will die [18]. The secondary metabolite solution used in this study had no more fungal conidia because the fungal conidia had ruptured due to the shaking process. According to [7], in the process of processing secondary metabolites, the shaking stage is carried out. Shaking was done using an orbital shaker for 7 days at a speed of 120-180 rpm. The shaking process that lasted for 7 days resulted in the conidia of the M. anisopliae fungus to rupture so that the secondary metabolites presented in the conidia of the M. anisopliae were released. That is what causes the O. rhinoceros larvae to die in a soft body condition because the larvae are not overgrown with conidia of the fungus M. anisopliae. Another symptom due to the treatment of a solution of secondary metabolites is a decrease in motion activity. Secondary metabolites in fungi can cause paralysis in the limbs of insects. Paralysis causes loss of coordination of the motion system, so that the movements of the insects are irregular and weak, resulting in total paralysis which results in the death of the insect. Toxins contained in fungi also cause tissue damage in the digestive tract, muscles, nervous system, and respiratory system [19]. According to [12], destruxin can prevent insect immune responses through the mechanism of hemocyte inhibition. Hemocytes are cells that play a role in providing protection against the attack from foreign cells, pathogens and parasites on the body of the insect. The small number of O. rhinoceros larvae that died as a result of treatment with secondary metabolites solution (MS) allegedly because the dose used in this study was less than optimal. The dosage used in this study was 25 ml of secondary metabolite solution/200 gr medium. The larvae used in this study had body weights ranged from 13-16 g and body lengths ranged from 7-10 cm. O. rhinoceros larvae used in the study were old larvae, namely instar 4 larvae that have thick cuticles, so it is possible that a solution of secondary metabolites is not well absorbed in the body of O. rhinoceros larvae. As a result, the antifeedant effect produced by destruxin does not work optimally, so, there were many larvae that did not experience the effects of hunger.

The Effectiveness of M. anisopliae and Its Secondary Metabolite Solution for O. rhinoceros Larval Mortality

Summary of three group of treatments (Control, M, and MS) is presented in Figure 2.

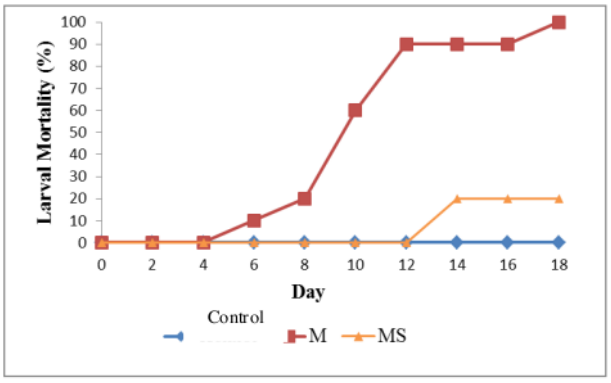


***Figure 2**. The percentage of O. rhinoceros larval mortality in control, M and MS group.*

Based on Figure 2, it can be seen that in the control treatment, all O. rhinoceros larvae were still alive. Larvae treated with M. anisopliae at a dose of 2 gr (conidia density 2.84 x 108 / g and fungal viability of 94.6%) began to die on the 6th day after application. The mortality of O. rhinoceros larvae in the administration of M. anisopliae in this kaolin formula reached 100% mortality on the 18th day after application with a temperature of 29 °C - 31.5 °C and humidity of 57% - 80%.Based on Figure 2, it can also be seen that in the control treatment, larvae are still alive until the 18th day of observation. O. rhinoceros larvae treated with a solution of secondary metabolites at a dose of 25 ml / 200 gr medium began to die on day 14 after application. Mortality of O. rhinoceros larvae treated with a solution of secondary metabolites with a temperature of 29 °C - 31.5 °C and humidity of 57% - 80% is fairly low, because until the last day of observation (day 18) only 20% of the larvae died of a total of 100% larvae. The treatment using M. anisopliae (M) is more effective in killing the O. rhinoceros larvae compared to the treatment using the solution of secondary metabolites (MS). This indicates that hyphae found in M. anisopliae (M) are more effective in infecting larvae, in addition to giving effect to paralysis of the larval muscle, fungal hyphae also absorbs fluid from the larva's body so that the larvae become infected and die faster. Based on Figure 2, it can be seen that the administration of secondary metabolite solution is not effective in killing O. rhinoceros larvae in this study. The dosage used in this treatment was 25 ml of secondary metabolite solution/200 gr medium. The dose is thought to have no effect on the mortality of O. rhinoceros larvae. The mortality of O. rhinoceros larvae is also influenced by abiotic factors such as temperature and humidity. The action of M. anisopliae in kaolin formulation and secondary metabolite solutions depends also on the surrounding environmental conditions. In this study, the temperature of the treatment ranged from 29°C to 31.5°C with humidity ranged from 57% to 80%. Based on [20], generally, the optimum temperature for germination, growth and sporulation of entomopathogenic fungi ranged from 20-30 °C. The optimum humidity for conidia germination of M. anisopliae is 98% and the pathogenicity of M. anisopliae will decrease at 86% humidity [21]. The fungus M. anisopliae has a death point at a hot temperature of 40°C for 15 minutes. At temperatures below 40°C, fungal cells usually survive but cannot develop [22].

CONCLUSION

M. anisopliae in the kaolin formulation is more effective at killing O. rhinoceros larvae compared to the solution of secondary metabolites. M. anisopliae in kaolin formulation can kill O. rhinoceros larvae starting on the 6th day after application and total death (100%) was reached on the 18th day after application, while the secondary metabolite solution kills O. rhinoceros larvae starting at 14th (20%) day and on day 18 after the application, the death of O. rhinoceros larvae remained 20%.

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