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Research Article

Effectiveness of *Metarhizium anisopliae* and Entomopathogenic Nematodes to Control *Oryctes rhinoceros* Larvae in the Rainy Season

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Abstract

Background and Objective: *Metarhizium anisopliae* (MET) and entomopathogenic nematodes (EPN) are microorganisms that attack the larvae of *Oryctes rhinoceros*. The effects of MET, EPN and the combination of both on the *O. rhinoceros* larvae were studied during the rainy season in Jepara Indonesia. This study aimed to determine the effectiveness of *Metarhizium anisopliae* and entomopathogenic nematodes to control *Oryctes rhinoceros* larvae in the rainy season. **Materials and Methods:** There were four level doses of MET, four level doses of EPN and four mixture of MET and EPN. The experiment used 72 containers that were placed in the garden with coconut palm shade. Five kilograms of organic soil that was mixed with biological control agents (MET, EPN and MET+EPN) and ten *O. rhinoceros* larvae 3rd instar were put in each other container. The data were analyzed by descriptive analysis. **Results:** Every larvae mortality was observed once a week and observations are for 8 weeks. The result showed that the larval mortality due to MET treatment occurred on 2nd-7th week. Meanwhile, the larval mortality due to EPN treatment took place on 2nd-8th weeks and the larval mortality due to MET+EPN treatment occurred on 1st-5th weeks. **Conclusion:** The combination of MET and EPN was simultaneously effective to control *O. rhinoceros* larvae than separate use of MET or EPN. Result of this study showed that using two agents of biocontrol was more effective, so that it can be beneficial for controlling *O. rhinoceros* larvae in the field.

Key words: Biological control, *Metarhizium anisopliae*, entomopathogenic nematodes, *Oryctes rhinoceros*, coconut (*Cocos nucifera* L.)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is the major producer of coconut (*Cocos nucifera* L.), with an annual production of nearly 15,000 million nuts. In Central Java Indonesia, coconut is one of the valuable commodities with cultivation area up to 288,000 ha and 222,000 t/year production[1]. Many factors hinder the effort of increasing the coconut production. One of the factors is coconut rhinoceros beetle or *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) attack. This pest is considered asone of the most damaging pests for coconut palm in Indonesia. In Jepara Central Java Indonesia itself, the dramatical decrease of coconut palm production in Jepara was also caused by the coconut rhinoceros beetle attack. The rhinoceros beetles are major constraint in the production of coconut in South Pacific[2], in India[3] and in Malaysia[4].

The coconut rhinoceros beetles fly to the central crown of the coconut palm, then bore through the heart of the palm into the unopened fronds, which is unfolding later, revealing tattering and V-shaped cutting of the leaflets. The eggs of rhinoceros beetles are laid, the larvae develop on the tops of dead standing palms, decaying trunks of coconut palm and other woods[2]. Materials like compost, sawdust heaps, rotting logs, decaying vegetable, bridges made of coconut trunk, dead pandanus, old latrines, sugarcane bagasse, rice straws and also humus rich soil also serve as suitable habitats for immature beetles[4].

Oryctes rhinoceros control itself is often neglected due to several reasons: (1) The coconut palm is not a major food crop, therefore, it gets less attention, (2) The coconut palm has a high stem which is consequently difficult to control the rhinoceros beetles, (3) The larvae live in the soil. Hence it is difficult to control and (4) The location of the larvae nest is often found outside the plantation. As a consequence, *O. rhinoceros* attacks which are known after the pest population was high.

Farmers use chemical pesticide to control the *O. rhinoceros*. However, there is considerable interest in reducing pesticide inputs because of the risks they pose to humans and environment as well as the increased resistance in pest populations[5]. Also, the use of synthetic pesticide causes the death of the natural enemies[6]. Therefore, environmentally friendly biological controls such as fungi, nematode, virus and bacteria have paramount importance to overcome those.

Metarhizium anisopliae is the most common entomopathogenic fungi used in microbial control[7]. *M. anisopliae* was applied as a biocontrol agent for *O. rhinoceros* beetles in a program of pest management[8]. In fact, *M. anisopliae* is a soil fungus of most fields which causing the disease in insects[4,9]. The application of entomopathogenic fungi as the biocontrol is proved as the best approach to killing the certain phase of the pest in their life cycles in soil[7,10].

The growth of *M. anisopliae* is affected by the environmental factors such as pH, temperature, moisture and nutrition. In fact, Indonesia has dry and rainy seasons. Based on this fact, the control of *O. rhinoceros* larvae using *M. anisopliae* in the dry season is not optimal because of conidia germination failure. On the contrary, the support for the optimum growth of *M. anisopliae* in rainy season is available. Therefore, it is suggested to control the larvae in the rainy season. Regarding this, so far, the application of *M. anisopliae* on the control of *O. rhinoceros* larvae in rainy season has not been investigated in Jepara.

In addition to fungi, there are entomopathogenic nematodes (EPN) from the *Steinernematidae* and *Heterorhabditidae* families which can be applied as biocontrol agents[11]. The EPN are microscopic nematodes, non-segmented worms that exist naturally in most soil that has the symbionts bacteria lives in its body. It acts as the parasite on insect pests that typically have a larval or pupal stage in its life cycle in soil. It is also known to be the parasite the stages of adults, nymphs and larvae on the soil surface. The EPN infects the host body and will enter its prey through body openings. It kills the prey by EPN lethal bacteria injection[12]. This EPN has been successfully used against a wide range of soil-inhabiting pests. EPN is known conclusively to be used to control Order Lepidoptera[13,14] and Coleoptera[15,16] but has not been widely used to *O. rhinoceros* larvae.

According to information from the Estate Crop office in Jepara Regency, *O. rhinoceros* control by *M. anisopliae* has been conducted but not the control with nematode (EPN). Although *M. anisopliae* was already applied, the evaluation has not been done especially during the rainy season. The reasons for choosing the rainy season is because of the high larval population during the season. Therefore, this study was aimed at investigating the effectiveness of three treatments, MET, EPN and a combination of both during the rainy season.

MATERIALS AND METHODS

Study area: The study was conducted at a coconut plantation in Jepara in March-August, 2015, where *O. rhinoceros* attacked more than 75% Coconut palm *O. rhinoceros.* Jepara Regency is located on the Northern coast of Java Island, Indonesia. The region has the tropical climate and two

seasons: Rainy and dry season. The average temperature in Jepara is 23.0°C and its altitude is 700 m. According to the Central Bureau of Statistics Jepara regency, the rainfall rate was 3295 mm/year, average 2,314 mm/year with 131 rainy days/year[17]. Here, the coconut palms grow along the coast of Jepara.

Fungal strain, density and viability conidia *M. anisopliae* (MET): *M. anisopliae* was obtained from Estate Crop Protection Board (ECPB) in Salatiga, Central Java Province, where the ECPB scientist isolated the MET from infected *O. rhinoceros* larvae and it was grown on maize flour media. Kaolin powder was used as the carrier of MET. Both Conidia density and viability of MET were determined in the laboratory. The conidia density was determined by observing the number of conidia using a microscope with the magnification of 400X. At the end of observation, the conidia density is found as much as 5×10^7 conidia mL^{-1}. On the other hand, the viability of conidia was observed by the germination of conidia on the PDA (Potato Dextrose Agar) media and the viability of conidia was 93%.

Entomopathogenic nematodes (EPN): Entomopathogenic nematodes (EPN) were obtained from biopesticides "*Coleonema*," species *Heterorhabditis*. One pouch of *Coleonema* contained 10×10^6 EPN. It was produced by Faculty of Agriculture, University of Jember. Recommended dosage of 14 L water was required to dissolve one pouch of *Coleonema*. This study used 4 doses, namely: N14 = One pouch of *Coleonema* was dissolved in 14 L water, N7 = One pouch of *Coleonema* was dissolved in 7 L water, N3.5 = One pouch of *Coleonema* was dissolved in 3.5 L water and K = No nematode (only water). The application of EPN was conducted in the afternoon (4 PM) to avoid direct exposure to sunlight.

Insects: The third instar *O. rhinoceros* larvae were collected from Jeruk Wangi village, Jepara Regency. The larvae size was 7-10 cm long and 9-11 g/larva. The total numbers larvae were 720, which were divided into three parts; MET (240 larvae), EPN (240 larvae) and a combination of both (240 larvae).

Substrate: Larvae required the substrate to live. The substrate consisted of organic soil, manure and sawdust of coconut trunk (1:1:1). The substrate is not sterilized. Five kilograms media was put into the black container whose diameter was 34 cm and height was 22 cm. The bottom of each container was perforated to discharge water. Ten larvae were put into the container. There were 24 containers for each treatment; 72 containers in total.

***Metarhizium anisopliae* (MET) treatment:** There were 4 doses of MET (abbreviated M) per container. They were: 0 g (Control = K), 1 g (M1), 2 g (M2) and 4 g (M4). Doses 1, 2 and 4 g of MET were used depending on the preliminary test conducted at the laboratory. Each dose of the fungus was mixed with 5 kg media/container. Each unit container was given with ten *O. rhinoceros* larvae. Each treatment of fungus was repeated six times (total 240 larvae). After completion, the surface of the container was perforated then covered by a plastic cover to protect them from wild animals. Containers were put in the shady place of a garden. The observation of mortality of larvae was conducted once a week for 8 weeks. Dead larvae were observed in the laboratory to ensure whether the cause death was from MET or not. Larval mortality was illustrated in the graph and was analyzed descriptively every week.

Entomopathogenic nematodes (EPN) treatment: The EPN dose from recommendation biopesticide was one pouch dissoluble in 14 L water. There were 4 treatment doses of EPN (abbreviated N) namely: 1 pouch of EPN was dissolved in 3.5 L of water (N3.5); 1 pouch of EPN was dissolved in 7 L of water (N7); 1 pouch of EPN was dissolved in 14 L of water (N14) and Control (K) with no EPN, just water. Each dose of nematodes was taken only for 1 L and poured into the container containing 5 kg media. Each container was given with ten *O. rhinoceros* larvae and for each treatment of nematodes was repeated six times for the total of 240 larvae. After completion, the container surface was covered with perforated plastic to protect it from wild animals. The containers were put in a shady place of a garden. The observation of larvae' mortality was conducted once a week in 8 weeks. The dead nematode larvae were observed in the laboratory to ensure the death of nematodes. Larval mortality was illustrated in the graph and was analyzed descriptively every week.

***Metarhizium anisopliae* (MET)+Entomopathogenic nematodes (EPN) treatment:** Both *M. anisopliae* (M) and entomopathogenic nematodes (N) are biological control agents of insect pests. *M. anisopliae* has been used in experiments to control the larvae of *O. rhinoceros* in Jepara, while EPN has never been employed in any experiment before. The MET doses 1, 2 and 4 g/5 kg substrate was used depending on preliminary test results at the laboratory[18].

The process was conducted in the same way as MET and EPN treatment; both were mixed in a container with ten larvae. There were 4 treatment combinations of the dose: K (control), M1N14 (low doses), M2N7 (middle doses) and

M4N3.5 (high doses). Observations of mortality of larvae were conducted once a week for 8 weeks. Dead larvae were observed in the laboratory.

Data analysis: Data larval mortality was illustrated in the graph and was analyzed descriptively every week.

RESULTS AND DISCUSSION

Oryctes rhinoceros has a complete metamorphosis stage, i.e., egg, larvae, pupa and imago as shown in Fig. 1. The larval stage was used in this research.

Effect of *M. anisopliae* (MET) on *O. rhinoceros* mortality: The mortality of *O. rhinoceros* larvae caused by the treatment of MET for 8 weeks is presented in Fig. 2.

The result of the study revealed that there was a correlation between MET doses and mortality of *O. rhinoceros.* Figure 2 shows that there was no larva died during the 1st week. The larvae died in the 2nd week after application as can be seen on the graph of control (K) and treatment M1 and treatment M4. On the control treatment (K), there was 3-20% of the total larvae died (Fig. 2). It indicated that without the MET, there were existing pathogenic organisms in the soil which attacked the larvae. The three treatments (M1, M2 and M4) caused 100% larvae mortality for 7-8 weeks. The larvae mortality percentage of M2 and M4 (Fig. 2) treatments are higher than M1 in the 4th week. According to Erixon *et al.*[19], the higher the concentration of *M. anisopliae* conidia, the more it is attached to the integument larvae. Thus, simplifying the process of infection, causing disturbed metabolism system, so as to accelerate larval mortality.

The process of infection of *M. anisopliae* to *O. rhinoceros* larvae was conducted in the laboratory (Fig. 3) by Rani[20]. In the laboratory (room condition), it showed that the testing of 1 and 2 g MET doses on *O. rhinoceros* caused the larvae to die in 7 days, whereas, the dose of 4 g MET took 5 days for the larvae to die.

The first contacted process of *M. anisopliae* was followed by conidia attachment on the larvae integument. In the beginning, conidia were initially dry and dormant. Then, conidia made contact with the moist soil substrate which caused, Conidia to begin to absorb water and to be actively germinated. *M. anisopliae* conidia required 19 h to grow[1].

Figure 3a shows the early symptoms of *M. anisopliae* infection on larvae integument (brown spots). According to Gabarty *et al.*[9], during fungal infection, the first step before penetration fungi is the host cuticle. Here, there are three successive stages: (1) Adsorption of the fungi propagules (conidia) to the cuticular surface, (2) Adhesion of the interface between propagules and epicuticle and (3) Fungi germination and development at the insect cuticular surface, until appressoria are developed to start the penetration stage.

Figure 3b and c showed the fungal colonies multiplied and the process of penetration and invasion began in the internal organs of insects. According to Milner *et al.*[21], *M. anisopliae* entered into the insects body through the cuticle instead of the food channel. They multiplied themselves through the formation of hyphae in epidermal tissue and other tissues throughout the body until the larvae were filled with mycelia. Tampubolon *et al.*[22] stated that the deep penetration was done chemically with the release of MET fungal toxin causing destruction to an entire organ in *O. rhinoceros* larvae and then the larvae died. The larval body turned green after a week while the fungus had been formed conidia ready to infect another host.

Infected the *M. anisopliae* into the body of *O. rhinoceros* until the larvae died required 7 days in the laboratory[23] or 6.4 days[24]. However, in Fig. 2 the larvae started to die in the 2nd week till the 8 week. The reason for different results

Fig. 1(a-d): Metamorphosis of *O. rhinoceros*, (a) Egg, (b) Larvae, (c) Pupa and (d) Imago
Source: Research documentation

was considerable because of the location took place in the garden of the coconut palm. While the study was conducted, the garden received very high rainfall intensity, especially at night. The heavy rainfall lasted up to 2 weeks every day. The temperature was 23-31°C with (RH) 76-95% of humidity. The high rainfall intensity affected penetration of fungi on larval integument. The growth of the MET conidia is not optimal; the possibility of fungi damaged by stagnant water or conidia does not stick to the skin larvae. It required 2-8 weeks until the larvae died (Fig. 2).

Compare with the same test conducted in another place, the results of this study were different from the same preliminary study using larvae *O. rhinoceros* and MET. As for example, the study conducted in early September, 2014 (transitional drought and rain) in the village Telogoweru, Demak Central Java[25] showed that mortality of larvae at a dose of 1 g MET treatment started in the first week (29%) and in the third week all larvae are all dead infected by the fungus. There

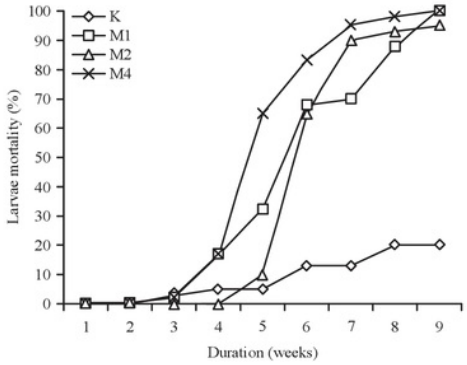

Fig. 2: Percentage of *O. rhinoceros* larval mortality caused by *Metarhizium anisopliae* with the dosage of 0 g (K), 1 g (M1), 2 g (M2) and 4 g (M4). Treatment for eight weeks (average of 6 replicates)

was low-intensity rain at the time of the study in Telogoweru with the temperature of 34.5-39.5°C and humidity level of 7-75%. The differences of these results showed that there were effects of temperature, humidity and precipitation at the rate of *O. rhinoceros* mortality. According to Hussein *et al.*[26], the temperature and RH have a crucial role in the occurrence of fungus infection on insects. The temperature and humidity are the main factors that affect the ability of the fungus to survive, spread, infect and kill the host[27]. Low temperatures, high humidity and low rainfall intensity were an optimal climate for the fungus to grow. Consequently, they could infect larvae quickly, resulted in a lot of larvae dead for one week.

It was suggested that the application of MET in the field required consideration for the right season. It sugested not applied MET during the dry season, due to the dry soil which causes the conidia of MET to find difficult to germinate. The best time to control larvae is in the early rainy season, due to the moist soil for the conidia to germinate. As a result, it is easy to infect the larvae. The analysis of conidia *M. anisopliae* in laboratory shows that the number of MET conidia was 5×10^{7} g mL^{-1}. According to Hosang *et al.*[28], conidia at 5×10^{5} conidia mL^{-1} was able to infect the larvae of *B. longissima*. Hashim and Ibrahim[29] stated that EC_{50} for *M. anisopliae* var majus (Metsch.) Sorokin was at 2.0×10^{4} conidia mL^{-1}.

Effect of entomopathogenic nematodes (EPN) on *O. rhinoceros* mortality: *O. rhinoceros* larval mortality due to nematodes for 8 weeks is presented in Fig. 4.

Figure 4 shows that *O. rhinoceros* larvae began to die start in the second week (control and treatment). There were 5% of larvae died in the control group. This result indicated that there were already living microbes which attack larvae before being given with EPN. During the 3rd week, larval mortality was increased in number rather than those in control

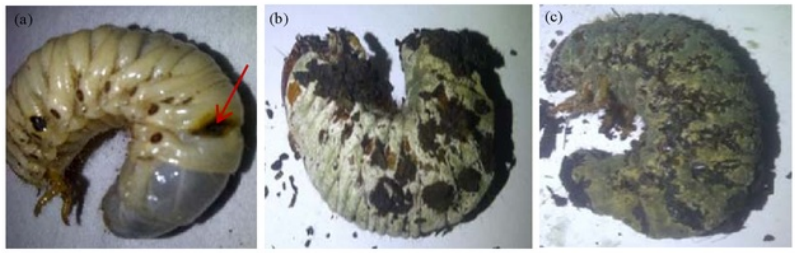

Fig. 3(a-c): Infection process of *M. anisopliae* on *O. rhinoceros* larva by a laboratory experiment, (a) First appearance of infection symptom on *O. rhinoceros* larva after 3 days, (b) Death of *O. rhinoceros* larva after 7 days and (c) Appearance of *M. anisopliae* conidia after 14 days invasion[20]

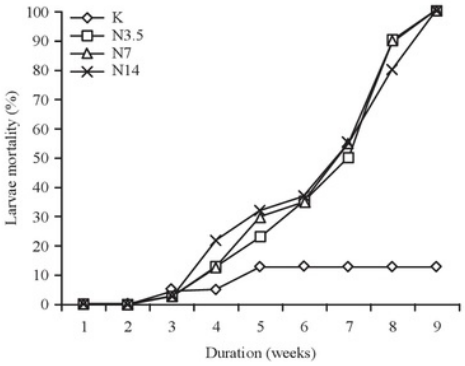

Fig. 4: Percentage of *O. rhinoceros* larval mortality due to entomopathogenic nematodes (EPN)
K: Control, N3.5: One pouch of EPN was dissolved in 3.5 L of water, N7: 7 L of water and N14: 14 L of water. The observation for 8 weeks (average of six replicates)

Fig. 5: The dead larvae *O. rhinoceros* caused by EPN

group. It means controlling larvae with EPN increased larvae mortality (Fig. 4). Thus, EPN is natural enemies (as parasitic) for insect larvae which live in soil but the population is not considerably high. Figure 5 is the example of larvae attacked by EPN.

Comparative studies indicate that EPN can kill the *Cylas formicarius* (Coleoptera: Curculionidae) in 42 h[15], *Crocidolomia binotalis* in 50.7 h and *Spodoptera litura* in 51.6 h[14]. The EPN which infects insect from Lepidoptera died within 24-72 h of infection. However, the larvae of *O. rhinoceros* required the longer time to die (2 weeks). It occurred because, first the larvae *O. rhinoceros* had tough skin and large larval size. This factor contributed to EPN's difficulty in penetrating the larvae skin. Second, the breeding. EPN required relatively longer time compared to the MET. The EPN required 14 days from egg to adult[30], while the MET required 19 h to reproduce. Third, environmental factors such as high rainfall intensity influenced the penetration ability of the EPN on larvae, whereas, rainwater carried the nematodes. The weather condition during treatment was raining with high intensity every day with the temperature of 23-31°C and humidity (RH) of 76-95%.

According to Nugrohorini[31], the mechanism of EPN pathogenicity occurred through symbiosis with pathogenic bacteria. After reaching insects hemocoel, EPN released bacterial symbionts into the hemolymph, brought to proliferate and produce toxins that kill larvae. Those are the two factors that cause EPN to have the power to kill larvae very quickly. The data in Fig. 4 shows that the percentage of larval mortality in the treatment (N3.5, N7 and N14) were not significantly different. Thus, the recommendation is that N14 can be used to control *O. rhinoceros* larvae.

Effect of *Metarhizium anisopliae* (MET)+Entomopathogenic nematodes (EPN) on *O. rhinoceros* mortality: *O. rhinoceros* larval mortality caused by MET+EPN and nematodes for 8 weeks is presented in Fig. 6. From three treatments of doses (M1N14, M2N7 and M4N3.5); the dose of M4N3.5 was the most lethal one causing larvae to die quicker. The average mortality was not significantly different on the M1N14 and M2N7 doses. All larvae died in the 5th and 6th week.

Figure 6 shows that *O. rhinoceros* larvae began to die in the first week both for treatment and control groups. In the fifth week, the mortality of larvae reached up to 92-100%, while in the control group, 87% of the larvae were still alive.

It indicated that the treatment of mixture between MET and EPN together accelerated larval mortality, compared to MET or EPN separately. Mortality of *O. rhinoceros* larvae that was controlled by MET required 2-7 weeks for all the larvae to die (Fig. 2), 2-8 weeks for EPN (Fig. 4) and 1-5 weeks for the mixture of MET and EPN (Fig. 6). According to Ansari *et al.*[5], there is growing evidence that pest control can be significantly improved by using combinations of biocontrol agents, for example, combining EPN with the fungus *M. anisopliae*. Figure 7 is the example of larvae attacked by MET and EPN.

The synergy of two biocontrol agents (MET and EPN) was more effective to kill the larvae. This finding was confirmed by Ansari *et al.*[5], who stating that the combined application of EPN and *M. anisopliae* synergistically resulted in increasing mortality of larvae. Therefore, it is recommended to use some of the biological control agents to increase larval mortality.

The results showed that the application of the biological control agents during the rainy season required a relatively longer time to kill larvae. It occurred because the rain

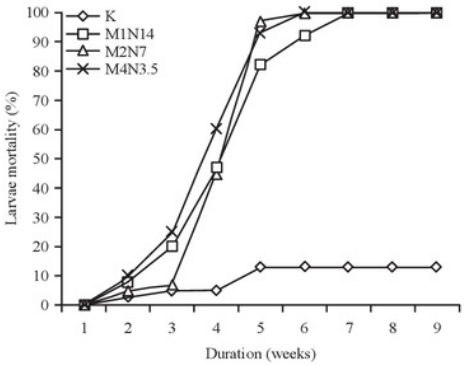

Fig. 6: Percentage of *O. rhinoceros* larval mortality due to MET and EPN

K: Control, M1N14: 1 g of MET and EPN dissolved in 14 L of water, M2N7: 2 g of METand EPN dissolved in 7 L of water, M4N3.5: 4 g of METand EPN dissolved in 3.5 L of water. Treatment for eight weeks (average of 6 replicates)

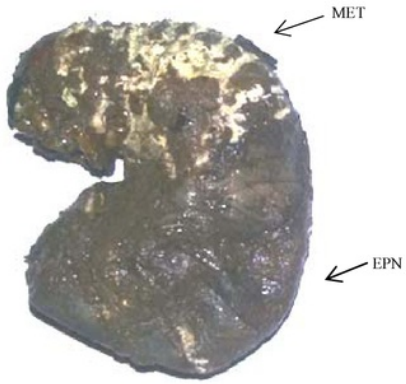

Fig. 7: Dead larvae *O. rhinoceros* due to MET (above) and EPN (bellow)

hampered the dissemination of fungi. The similar phenomenon happened on the EPN. It was soluble by rain resulting in the MET and EPN failed to penetrate the larvae.

Biological control agents are microorganisms which require growth and proliferation. They need the nutrients and environmental factors to allow their growth and development. The growth and development require a moist soil condition and the appropriate time. Therefore, the application of biological control agents requires time and environmental factors to support the growth of these microorganisms.

Based on three treatments of doses (M1N14, M2N7 and M4N3.5), the dose of M4N3.5 was the most lethal one which affected the larvae dying quickly. -All larvae died in the fifth and sixth week.

CONCLUSION

It was concluded that the application of the biological control agents during the rainy season required a relatively longer time to kill *O. rhinoceros* larvae. Controlling the population of *O. rhinoceros* using the mixture of MET and EPN is more efficient than using MET and EPN separately.

SIGNIFICANCE STATEMENT

This study discovers the effectiveness of *Metarhizium anisopliae* (MET) and entomopathogenic nematodes (EPN) mixture to control *Oryctes rhinoceros* larvae in the rainy season. It can be beneficial as a reference in producing the biopesticide that prevent the plants from *Oryctes rhinoceros'*. This study will help the researchers to uncover the critical areas of biopesticide that many researchers were not able to explore.

REFERENCES

1. BPTBUN., 2011. Natural breeding of *Metarhizium anisopliae* mushroom for controlling beetles (*Oryctes rhinoceros*) pest. Leaflet Published by Estate Crop Protection Board in Salatiga, Central Java Province, Indonesia. http:// bptbunsalatiga.com.
2. Bedford, G.O., 1986. Biological control of the rhinoceros beetle (*Oryctes rhinoceros*) in the Southh Paciic by baculovirus. Agric. Ecosyst. Environ., 15: 141-147.
3. Prasad, G.S., V. Jayakumar, H.R. Ranganath and V.R. Bhagwat, 2008. Bio-suppression of coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) by O ryctes baculovirus (Kerala isolate) in South Andaman, India. Crop Prot., 27: 959-964.
4. Manjeri, G., R. Muhamad and S.G. Tan, 2014. *Oryctes rhinoceros* beetles, an oil palm pest in Malaysia. Annu. Res. Rev. Biol., 4: 3429-3439.
5. Ansari, M.A., F.A. Shah and T.M. Butt, 2008. Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus*, control. Entomol. Exp. Applic., 129: 340-347.
6. Sucipto, 2009. Madura's local isolate of entomopathogen nematoda as a environmentally friendly controlling agent of pest in holticulture plants. Agrovigor, 2: 47-53.
7. Sevim, A., I. Demir, M. Hofte, R.A. Humber and Z. Demirbag, 2010. Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. Biocontrol, 55: 279-297.

8. Gopal, M., A. Gupta and G.V. Thomas, 2006. Prospects of using *Metarhizium anisopliae* to check the breeding of insect pest, *Oryctes rhinoceros* L. in coconut leaf vermicomposting sites. Bioresour. Technol., 97: 1801-1806.
9. Gabarty, A., H.M. Salem, M.A. Fouda, A.A. Abas and A.A. Ibrahim, 2014. Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn.). J. Radiat. Res. Applied Sci., 7: 95-100.
10. Soelistijono, R., 2015. Assessment of the effectiveness of lowland and mediumland *Rhizoctonia* sp microryza on severity of Orchid *Phalaenopsis amabilis's* DSI disease against *Fusarium* sp. Biosaintifika: J. Biol. Biol. Educ., 7: 112-119.
11. Indriyanti, D.R. and N.L. Muharromah, 2016. Mass cultivation of entomopathogenic nematode in artificial media. Biosaintifika, 8: 111-118.
12. Acosta, E.W., 2011. Lawn and garden pest control beneficial nematodes. http://www.biconet.com/biocontrol/nemas.html
13. Chaerani and B. Nurbaeti, 2007. Effectiveness test of entomopatogen nematodes (Rhabditida: Steinernema and Heterorhabditis) as natural enemies of non-endemic yellow rice stalk borer (*Scirpophaga incertulas*). J. HPT Tropika, 7: 71-79.
14. Subagiya, 2005. Biological control with local strain entomogenous nematodes *Steinernema carpocapsae* against *Crocidolomia binotalis* pest in Tawangmangu. Agrosain, 7: 34-39.
15. Bunga, J.A., 2004. Exploring entomopathogenic nematodes from Kupang NTT and testing their effectiveness against *Cylas formicarius* (Coleoptera: Curculionidae). Thesis Report. Post Graduate of Institut Pertanian Bogor, Indonesia.
16. Nderitu, J., M. Sila, G. Nyamasyo and M. Kasina, 2009. Effectiveness of entomophatogenic nematodes against sweet containerato *Cylas puncticollis* (Coleoptera: Apionidae) under semi-field condition in Kenya. J. Entomol., 6: 145-154.
17. Climate-Data.ORG, 2015. Jepara's climate. Climate-Data. ORG, https://id.climate-data.org/region/1212/
18. Indriyanti, D.R., B.K. Rani and P. Widiyaningrum, 2015. Pathogenicity of *Metarhizium anisopliae* M. fungus derived from infected larvae to *Oryctes rhinoceros* arvae mortality. Jurusan Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Negeri Semarang.
19. Erixon, M.H., S. Oemry and F. Zahara, 2015. The effectivity of suspension *Baculovirus oryctes* and *Metarhizium anisopliae* (Metch.) Sorokin to control *Brontispa longissima* Gestro. J. Online Agroekoteknol., 3: 124-128.
20. Rani, B.K., 2015. The pathogenicity of *Metarhizium anisopliae* to the of *Oryctes rhinoceros* larvae mortality. Skripsi. Jurusan Biologi, FMIPA Universitas Negeri Semarang.
21. Milner, R.J., P. Samson and R. Morton, 2003. Persistence of 1conidia of *Metarhizium anisopliae* in sugarcane fields: Effect of isolate and formulation on persistence over 3.5 years. Biocontrol Sci. Technol., 13: 507-516.
22. Tampubolon, D.Y., Y. Pangestiningsih, F. Zahra and F. Manik, 2013. Pathogenicity test of *Bacillus thuringiensis* and *Metarhizium anisopliae* against *Spodoptera litura* Fabr (Lepidoptera: Noctuidae) mortality in laboratoratory. J. Online Agroekoteknol., 1: 784-791.
23. Mulyono, 2007. Study of pathogenicity of *Metarhizium anisopliae* fungi against *Oryctes rhinoceros* L. as a pests of coconut plants at various applications time. Thesis Report. Surakarta: Post Graduate of Universitas Sebelas Maret.
24. Manurung, E.M., M.C. Tobing, L. Lubis and H. Priwiratama, 2012. Efficacy of *Metarhizium anisopliae* formulations against *Oryctes rhinoceros* L. larvae (Coleoptera: Scarabaeidae) di Insektarium. J. Online Agroekoteknol., 1: 47-63.
25. Indriyanti, D.R., 2014. Efficacy test of APH Metarhizium anisopliae in Telogoweru Demak. Jurusan Biologi, FMIPA Universitas Negeri Semarang.
26. Hussein, K.A., M.A.A. Abdel-Rahman, A.Y. Abdel-Mallek, S.S. El-Maraghy and J.H. Joo, 2010. Climatic factors interference with the occurrence of *Beauveria bassiana* and *Metarhizium anisopliae* in cultivated soil. Afr. J. Biotechnol., 9: 7674-7682.
27. Xiao, X., X. Wang, X. Zhang, E. Chen and J. Li, 2015. Effect of the quality property of table grapes in cold chain logistics-integrated WSN and AOW. Applied Sci., 5: 747-760.
28. Hosang, M.L., A. Jelfina, C. Alouw and H. Novarianto, 2004. Biological control of *Brontispa longissima* (Gestro) in Indonesia. RAP Publication 2004/29, Report of the Expert Consultation on Coconut Beetle Outbreak in APPPC Member Countries 26-27 October 2004, Bangkok, Thailand.
29. Hashim, N. and Y.B. Ibrahim, 2003. Efficacy of entomopathogenic fungi, *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Metarhizium anisopliae* var. majus against *Crocidolomia binotalis* (Lepidoptera; Pyralidae). Pertanika J. Trop. Agric. Sci., 26: 103-108.
30. Imanadi, L., 2012. Study of pest control with entomopatogen nematodes (*Steinernema* spp and *Heterorhabditis* spp). Balai Karantina Pertanian Surabaya, Surabaya.
31. Nugrohorini, 2007. Toxicity test of *Steinernema* sp. nematoda (Tulungagung isolate) on plant sugar hama (*Brassica juncea*) at the laboratory. J. Pertanian Mapeta, 10: 1-6.

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