The effect of *Beauveria bassiana* on the larvae of *Oryctes rhinoceros*

D R Indriyanti*, D Wijayanti, N Setiati

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

*Corresponding author: dyahrini36@gmail.com

**Abstract.** This study aims to analyze the effect of *B. bassiana* on *O. rhinoceros* larvae. The samples used were 30 healthy 3rd instar *O. rhinoceros* larvae weighing 10-16 gr with a body length of 7-10 cm that were obtained from the field. The study consisted of two treatments with 10 replications, namely P1 (2 gr *B. bassiana* kaolin formulation / 200 gr media) and P0 (Control). Observations were made every 2 days until all treated larvae was dead. The results showed that the larvae of *O. rhinoceros* infected with *B. bassiana* in the kaolin formulation died in a stiffness state and were covered with white powdery fungal hyphae on the surface of the larval body. Larvae died from *B. bassiana* on the 8th day after treatment and all larvae died (100%) on the 20th day after treatment. None of the *O. rhinoceros* larvae in the control treatment experienced death until day 20 after treatment.

1. **Introduction**

*Oryctes rhinoceros* is an important pest of coconut palm. Female imago lays its eggs in weathered plants or organic soils. The larvae live below the soil surface until they become pupae. Imago attacks the tip of the stem and damages the young leaves in the form of V-shaped cut. The control of this pest can be achieved by several methods, one of which is with a safe and environmentally friendly method by using biological agent such as entomopathogenic fungi. *Metarhizium anisopliae* is commonly used to control *O. rhinoceros* larvae in recent studies. Other than *M. anisopliae*, *Beauveria bassiana* fungus also has the potential to control pest infestation. Pest control using *B. bassiana* has been done for the other pests such as *Cylas formicarius* in sweet potato [1], *Cosmopolites sordidus* in banana corm [2], *Nezara viridula* in vegetables [3], *Oryctes rhinoceros* larvae in coconut and palm oil [4], *Aphis glycines* in soybean [5], *Symphyllid in pineapple root* [6], *Helopeltis sp. in cacao* [7], and *Spodoptera litura* larvae in tobacco [8]. The objective of this study was to analyze the effect of *B. bassiana* administration on *O. rhinoceros* larvae. Results of this study were expected to provide an alternative method of pest control using entomopathogenic fungus *B. bassiana*.

2. **Methods**

This study was conducted in Biology Laboratory of Universitas Negeri Semarang on March-June 2018. Population in this study was *O. rhinoceros* larvae obtained from coconut palm plantation of Jerukwangi Village, Bangsri Sub-district, Jepara Regency. A total of 30 healthy *O. rhinoceros* third instar larvae
were used as samples. The larvae were 10-16 gr in weight and 7-10 cm in length with creamy white color, tight and soft outer skin, and active movement.

Larvae were maintained on manure. The manure was also as the food source for *O. rhinoceros* larvae. *B. bassiana* in flour-kaolin formulation were obtained from Balai Perlindungan Tanaman Pangan Hortikultura dan Perkebunan (BPTPHP), Salatiga. Larvae were grouped into treatment (P1) and control (P0) group. Larvae in P1 were maintained in 200 gr manure+2 gr flour-kaolin formulation of *B. bassiana* spore. The dose used (2 gr *B. bassiana* formulation) was based on a study [8]. Plastic cups with the diameter of 10 cm and height of 13 cm were used as a container. Holes were made on the lid of the cups for air circulation. Each treatment was replicated 10 times with 1 larva for each replication.

Containers were placed inside the box and covered with newspaper to avoid direct sunlight exposure. Observation was conducted every two days until all of the larvae was dead by putting the larva in a tray and directly observing the symptoms of infection. Data obtained were analysed descriptively. The parameters observed included color changes, body size, larval movement, any symptom of infection, and the death of the larvae. Mortality data is presented in the form of graph. Abiotic data obtained (temperature, humidity, and water content of medium) were used as supplementary materials.

3. Result and Discussion

Infected *O. rhinoceros* larvae by *B. bassiana* were indicated by the color changes and wrinkled body. Observation on the day 4 showed that the color of larval body changed from clear white to dull. On the 6th day, the alive larvae were turning brown with black necrotic spots on their cuticles (Figure 1). This condition is in accordance with a study by Indriyanti [10] that stated that the color change of larval body into black is a sign of melanization, a body defense mechanism in response to pathogen infection. Melanin is formed by phenoloxidase.

The other symptoms of infection on the 4th day was the slow movement, decreased appetite, and the larval body that remained motionless and bent into a C shape. This is also in accordance with a study by Wahyudi [11] that reported that entomopathogenic fungi will release toxin that causes a paralysis in larval body. Paralysis causes a loss of body coordination resulted in irregular and slow movement that will end up with larval mortality. Conclusion

Based on the results of the research and discussion above, it can be concluded that the Covid-19 pandemic has an impact on all aspects of life including education. The policy to learn from home using online learning is not without problems. Because it is carried out due to compelling conditions, it is natural that there are obstacles. Therefore, it is necessary to evaluate and improve so that online learning can run more effectively, not disturbing children's development, especially for elementary school aged children. Likewise, the impact on cognitive, social, emotional and language development of children needs attention. Teachers, parents, and government certainly play a big role in this.

![Figure 1. O. rhinoceros larva infected by entomopathogenic fungi B. bassiana](image.png)
[8], that stated that the white powdery layer on the surface of larval body is the conidia of \( B. bassiana \) growth hyphae. Indriyanti [10] also stated that the stiffness of larval body is because the body fluid of \( O. rhinoceros \) larvae is absorbed by the fungi to live inside the cells of larval body.

\( O. rhinoceros \) larvae in control group did not show any symptoms as shown in treatment group. On the day 1 until day 20 after treatment, all larvae in control group remained alive with normal movement and white body color without any necrotic spots.

Data of \( O. rhinoceros \) larval mortality due to the infection of \( B. bassiana \) is presented in the figure below:

![Figure 2. Percentage of \( O. rhinoceros \) larval mortality due to the infection of \( B. bassiana \) compared to control group](image)

Based on the data in Figure 2, it can be seen that the larvae in control group remained alive until the 20th day after treatment. Meanwhile, \( O. rhinoceros \) larva treated with \( B. bassiana \) in kaolin formulation with the dose of 2 gr (conidial density and viability of 3.06x10^8 conidia/ml and 92.92% respectively) started to die on the 8th day after treatment. The larval mortality continued to increase and reached 100% on the day 20 after treatment.

\( O. rhinoceros \) larvae treated by \( B. bassiana \) need 8 days to die which is considered long time. This condition occurred because \( B. bassiana \) entomopathogenic fungi infect their host through 4 stages. The first stage is inoculation, a contact between \( B. bassiana \) propagule with host’s body. The second stage is the process of attachment and germination of \( B. bassiana \) propagule on insect’s integument. The third stage is penetration and invasion, the fungal penetration to the integument by forming germ tube or appressorium. The fourth stage is the destruction on the penetration spot and the formation of blastopore that will circulate in the hemolymph. Secondary hyphae is then formed to attack the other tissues. Insect infected by \( B. bassiana \) will eventually die and on a suitable condition the host’s dead body will be covered by the spore and hyphae of fungi [12]. The big size of \( O. rhinoceros \) larva (10-16 gr in weight and 7-10 cm in length) also affects the infection process of entomopathogenic fungi \( B. bassiana \) on larval body that needs a long time to completely kill the larva. As a result, larva started to die on the 8th day and completely died on the 20th day after treatment.

\( O. rhinoceros \) larval mortality was also influenced by the abiotic factors such as temperature, humidity, and water content of the medium. These factors affected the activity of \( B. bassiana \) in kaolin formulation. In this study, the temperature used were 29–31.5°C with humidity of 57%-80% and medium water content of about 51%. That the optimum germination, growth, and sporulation of \( B. bassiana \) occur on temperature of 25–30°C and relative humidity of 100%, so that the optimum temperature and humidity is necessary to make the fungi grow optimally. Optimum medium water content needed for entomopathogenic fungal growth for infecting \( O. rhinoceros \) larva is around 40-60% [13]. Result of this study showed that \( B. bassiana \) in kaolin formulation can kill \( O. rhinoceros \) larvae. It can be implied that \( B. bassiana \) in kaolin formulation can be used as biological control agent to control \( O. rhinoceros \) larva.
4. Conclusion

_O. rhinoceros_ larva infected by _B. bassiana_ in kaolin formulation died in body stiffness and was covered by white powdery fungal hyphae. The infected larvae started to die on the 8th day and completely died (100%) on the 20th day after treatment.

References

[1] Artanti D, Trimulyono G and Prayogo Y 2005 *Mengendalikan Telur Hama Penggerek Ubi Jalar (Cylas formicarius)* (Surabaya: Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Negeri Surabaya)

[2] Hasyim A 2006 *Balai Penelitian Tanaman Buah Tropika* **16** 202–210.

[3] Sably H 2012 *J. Floratek*. **7** 13–24.

[4] Sihombing R H, Oemry S and Lubis L 2014 *J. Online Agroekoteknologi* **2** 1300–1309.

[5] Pertiwi S P, Hasibuan R and Wibowo L 2016 *J. Agrotek Tropika* **4** 55–61.

[6] Simarmata D R N, Wibowo L and Affandi 2016 *J. Agrotek Tropika* **4** 62–65.

[7] Indriyanti D R, Faizah S N and Slamet M 2017 *Int. J. Sci. Technol. Res*. **10** 14-17.

[8] Yuningsih and Widyaningrum T 2014 *JUPEMASI-PBIO* **1** 26-32

[9] Indriyanti D R, Mahmuda S and Slamet M 2017 *Int. J. Sci. Technol. Res*. **9** 206-210.

[10] Indriyanti D R, Damayanti I B, Setiati N and Maretta Y A 2018 *J. Eng. Appl. Sci*. **6** 2279-2286.

[11] Wahyudi P 2008 *J Ilmu kefarmasian Indones*. **6** 51-56.

[12] Marheni, Hasanuddin, Pinde and Suziani W 2011 *J. Ilmu Pertanian KULTIVAR* **5** 32-40.

[13] Indriyanti D R, Indah N and Slamet M 2017 *Biosaintifika* **9** 363-369.