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Insecticidal activity of supernatant and crude extract of *Bacillus thuringiensis*-based bio-insecticide towards oil palm pests *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. *Oryctes rhinoceros* is an important pest in oil palm plants. Larvae and imago are stadia which attack plants. Control by using synthetic chemical insecticides often create a negative impact on non-target insects and the environment. *B. thuringiensis* can be used as an alternative. The aim of the study was to study the insecticidal activity of *B. thuringiensis* isolates from South Sumatra on the larvae of *Oryctes*. The study was conducted at Entomological Laboratory, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, from March to May 2019. The study was designed by a factorial complete randomized design with two factors in the form of Bt isolates (four isolates) and the presence of spores (crude extract and supernatant) with each repeated 3 times. The treatments were *B. thuringiensis* isolates propagated in Biourin media and applied in the form of crude and supernatant. *B. thuringiensis* propagated in NB media was used as control. Tested insects were 1st instar larvae of *Oryctes*. The results showed application of crude solution of *B. thuringiensis* resulted in higher mortality compared to supernatant treatment. It was assumed in crude solution there was a mixture of spores and proteins compared with the solution of the supernatant which only contains spores. KJ3P1 isolate was able to cause the highest mortality in crude treatment.

1. Introduction

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Oryctes rhinoceros is one of the important pests on oil palm [1] since the prohibition of burning as a method for maintaining estate hygiene in the 1990s [2]. If not controlled, this pest attack will kill young plants or reduce production in old plants. Active stadia are larvae and imago [3]. Control is carried out for larvae of *Oryctes*, starting from mechanically by taking directly from oil palm plantations, using natural enemies by using predators / parasitoids [4,5] and using chemical insecticides. The use of microorganisms as bioinsecticides is getting popular [2] report on the use of viruses, while application of entomopathogenic fungus has also been reported by [6,7]. *Bacillus thuringiensis* as one of the entomopathogenic bacteria, is expected to be a biological control agents for pests *Oryctes*. As a gram-positive bacterium, *B. thuringiensis* produces spores and proteins during sporulation. These spores and proteins are insecticidal acting as stomach poisons. After entering the midgut, the protein is converted by the protease enzyme as a toxin [8]. In suitable growth media such as Nutrient Broth (NB), a certain amount of spore density will be obtained after 72 hours of fermentation. It has been known mode of action of *B. thuringiensis* in killing target insects, which is started from the spores entering the insect



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digestion [9]. Furthermore, the spores enter larval hemocoel, germinate and grow, as well. Increasing number of spores which grow in hemocoel will cause septicemia leading in insect death [10,11]. Being fermented, *B. thuringiensis* spores and protein present in growth media. Both of them play a role in mortality of insects target [12]. The role of spores in killing insects needs to be known specifically those in the supernatant (top layer) by separating from proteins. Therefore a study was conducted to determine the role of spores contained in supernatants in killing oryctes larvae and comparing them with spores that are in crude solution.

2. Materials And Methods

Research has been carried out in Entomological Laboratory of Plant Protection Study Program, Faculty of Agriculture, UNSRI. Research materials were four isolates of *B. thuringiensis* native to South Sumatra (collection of Plant Protection Department) grown in NB media. The experiment was designed in a factorial complete randomized design with two factors in the form of *Bt* isolates (four isolates) and the presence of spores (crude extract and supernatant) with each repeated 3 times.

2.1. Propagation of *B. thuringiensis* on NB media

A loopful of *B. thuringiensis* isolate from NGKG agar was put into 100 ml of Nutrient Broth (NB), was shaken using a shaker for 12 hours at 200 rpm. After that 10 ml is taken to put in 100 ml of NB, then shaken again using a shaker for 12 hours at a speed of 200 rpm. Furthermore, seed culture is ready to be used for making bioinsecticides. As much as 10 ml of seed culture was put into 100 ml of NB media which had previously been added 50 mg CaCl₂, 50 mg MgSO₄, 50 mg K₂HPO₄ and 50 mg KH₂PO₄. The fermentation process is carried out by fermented for 72 hours at a speed of 200 rpm at room temperature. In order to separate supernatant and pellet, centrifugation was carried out at a speed of 3000 rpm for 3 minutes. Spore density was calculated in the supernatant and the initial solution (crude extract). The *B. thuringiensis* solution was ready for bioassay treatment/

2.2. Test insect preparation

Larva *O. rhinoceros* was taken from various oil palm plantations both owned by farmers and companies. It was randomly taken and brought to laboratory to be maintained in boxes with length x width x height: 2 m x 0.5 m x 0.8 m, using oil palm empty fruit bunches as their feed. Spraying was done every day with water so the humidity was available in accordance with the needs of life oryctes larvae. The larvae used were first instar larvae.

2.3. Bioassay and mortality observation

Fifty grams of soil (from oil palm empty fruit bunches) was prepared in a container (d=9 cm). Five ml of supernatant or crude extract of *B. thuringiensis* was sprayed on the surface of soil in each container. A total of 10 first instar larvae were put into the container. Each treatment was repeated 3 times. Observation of larval mortality was carried out during 13 days. Data on larval mortality were analyzed using ANOVA.

3. Results And Discussions

3.1. Spores density of crude extract and supernatant of *Bacillus thuringiensis* isolates

From the propagation of *B. thuringiensis* isolates using NB media and centrifugation at a speed of 3k rpm, a supernatant and pellet were produced. Spores density of supernatant and crude extract were calculated. The results showed density spores in the crude extract was higher than that of the supernatant. The highest density was in KJ3 p1 isolate with a value of 10.64 x 10¹² spore / ml, while the lowest was in SASU isolate which was 4.47 x 10¹² spores / ml. In supernatants, the highest number of spores was KJ3P1 (6.0 x 10¹² spores / ml) while the lowest density was found in MSP isolates of 3.62 x 10¹² spore / ml (Fig. 1).

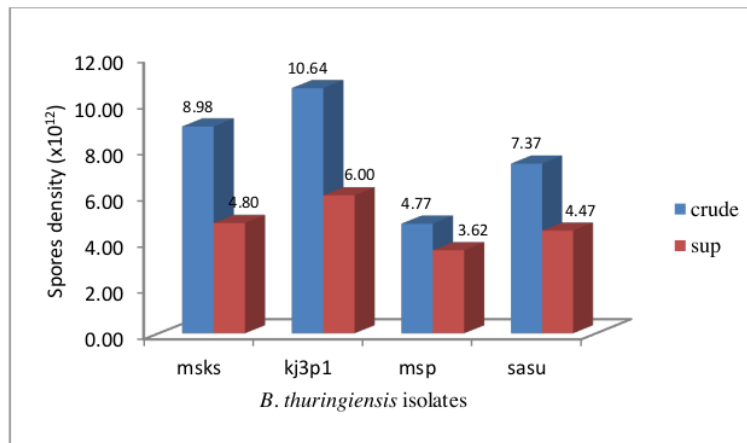


Fig. 1. Spores density of crude extract and supernatant of *Bacillus thuringiensis* isolates

Spore density in both crude extract and supernatant was found highest in KJ3P1 isolates. The lowest spore density in MSP isolates. Density of spores was very dependent on the nature of the isolates and their compatibility with the propagation media. Nutrient Broth was one of the appropriate media for the growth of *B. thuringiensis* bacteria. In condition of limited nutrition, *B. thuringiensis* immediately sporulates and produces spores and proteins [12]. In addition, Ref. [13, 14] stated that the concentration of nutritional components in the growth media of *B. thuringiensis*, such as glucose and mineral salts, could increase the spore production.

3.2. Mortality of *Oryctes* larvae

Mortality of oryctes began on the third day after application. Observation of insect death on the 13th day after application showed the highest mortality was in crude extract of KJ3P1 isolates and the lowest one was in MSP isolate. Likewise with the supernatant, the highest density was at KJ3 P1 and the lowest was at MSP. The complete data was presented in Fig. 2

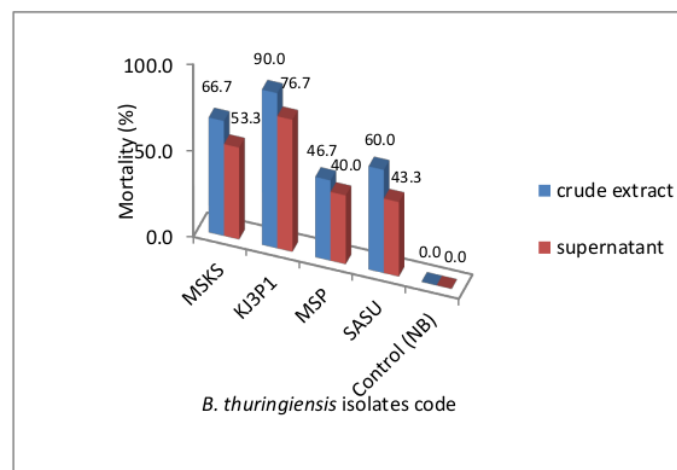


Fig. 2. Mortality of *Oryctes* larvae applied by *B. thuringiensis* propagated in NB media (13 days after application)

Mortality of Oryctes (Coleopteran order) need time compared to Lepidopteran order. It may caused by the hardness of body texture of the oryctes larvae. In order to kill test insects (larvae oryctes), *B. thuringiensis* spores must first be ingested into the midgut. In the midgut protein will be degraded into smaller molecules which are toxic. In crude extracts it still contained spores and proteins so the chance to kill oryctes larvae was bigger [15]. Compared to supernatants, the spore content in crude extract was higher. Therefore it was reasonable to find high mortality in crude extracts.

3.3. Symptom of infected oryctes larvae

At the beginning of application, larvae feed on a mixture of soil a solution of *B. thuringiensis* isolates. Larvae of oryctes infected with *B. thuringiensis* showed symptoms of anxiety, lack of appetite and finally stop eating. Usually it took time between 5-7 days to reach lethal stage. After 13 days of observations, it appeared oryctes larvae turn black in color and soft bodies (Fig. 3).

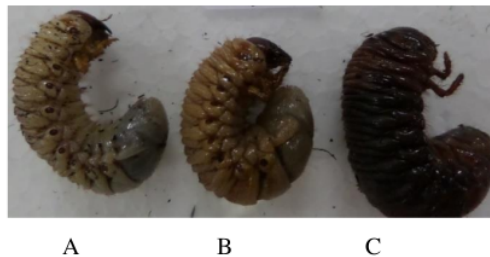


Fig. 3. Mortality symptom of oryctes larvae.

- A. healthy larvae
- B. infected larva 7 days after application
- C. infected larva 13 days after application

Dead oryctes larvae began on the third day, and continue until days of 13. The body tissue of the larvae gradually turn cloudy and eventually becomes black. The body started to change soften, while the larvae lose their appetite and eventually die. Their bodies become soft and eventually die with symptoms of wet rot [16,17]. Consider with the process of killing larvae molecularly, Ref. [8] explained that binding of the toxin to receptor lead to change in the toxin's conformation, allowing toxin insertion into the membrane. The oligomerization of the toxin was followed by forming pore which leading to osmotic cell lysis and larval death.

4. Conclusion

Application of crude solution of *B. thuringiensis* resulting in higher mortality compared to supernatant. *B. thuringiensis* KJ3P1 isolate was an isolate proposed as a candidate for production of bio-insecticides to control oryctes larvae because both solutions (supernatant and crude extract) showed high mortality.

References

- [1] Susanto, A, Sudharto, dan AE Prasetyo. 2011. Informasi Organisme Pengganggu Tanaman Kumbang Tanduk *Oryctes rhinoceros* Linn. Artikel. Pusat Penelitian Kelapa Sawit: Medan
- [2] Ramle, M. , M.B. Wahid, K. Norman, T.R. Glare, T.A. Jackson. 2005. The incidence and use of *Oryctes* virus for control of rhinoceros beetle in oil palm plantations in Malaysia Journal of Invertebrate Pathology 89 (2005) 85–90
- [3] Widyanto, H. Suhendri Saputra, Suryati. 2014. Pengendalian Hama Kumbang Tanduk (*Oryctes rhinoceros* Linn.) Menggunakan Perangkap Feromon Pada Tanaman Kelapa Sawit (*Elaeis guineensis* Jacq.) Di Lahan Gambut Provinsi Riau. Artikel. Balai Pengkajian Teknologi (BPTP) Riau

- [4] El-Shafie, H. A. F, B. M. A. Abdel-Banat and M. R. Al-Hajhoj. 2017. Arthropod pests of date palm and their . CAB Reviews 2017 12, No. 049.
- [5] Priwiratama, H., A.E.Prasetyo & A. Susanto. 2013. Biological Control Of Oil Palm Insect Pests In Indonesia. Indonesian Oil Palm Research Institute (IOPRI). <https://www.researchgate.net/>
- [6] Bedford, G.O. 2014. Advances in the control of rhinoceros beetle, *Oryctes rhinoceros* in oil palm. Journal of oil palm Research. Vol 26 (3): Pp 183-194
- [7] Indriyanti, DR., S.D.Anggraeni and M. Slamet. 2017. Density And Composition Of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) Stadia In Field. Journal of Engineering and Applied Sciences ©2006-2017 Asian Research Publishing Network (ARPN). VOL. 12, NO. 22, www.arpnjournals.com
- [8] Bravo A, Likitvivanavong S, Gill SS. 2011. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochem Mol 41 (2011) 423-431
- [9] Melo, A.L.A., Vanete Thomaz Soccol & Carlos Ricardo Soccol. 2016. *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. Journal Critical Reviews in Biotechnology 36 (2).
- [10] Ramanujam, B., R Rangeshwaran, G Sivakmar, M Mohan And M S Yandigeri. 2014. Management of Insect Pests by Microorganisms. Proc Indian Natn Sci Acad 80 No. 2 Spl. Sec. pp. 455-471
- [11] Mampallil, L.J., MH Faizal and KN Anith. 2017. Bacterial bioagents for insect pest management. Journal of Entomology and Zoology Studies. 5(6): 2237-2244
- [12] Bravo, A., Isabel Gómez, Porta Blanca, Ines García-Gómez, Claudia Rodriguez-Almazan, Liliana Pardo, Mario Soberón. 2013. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microbial technology, 6 (1): 17-26.
- [13] Amin G, Alotaibi S, Narmen YA, & Saleh WD. 2008. Bioinsecticide production by the bacterium *Bacillus thuringiensis*. 1. pattern of cell growth, toxin production and by-product synthesis. Arch. Agron. Soil Sci. 54(4): 387-394.
- [14] Sarrafzadeh MH. 2012. Nutritional Requirements of *Bacillus thuringiensis* during different phases of growth, sporulation and germination evaluated by plackett-burman method. Iran J. Chem. Chem. Eng. 31(4): 131-136.
- [15] Crickmore, N. 2006. Beyond the spore – past and future developments of *Bacillus thuringiensis* as a biopesticide. Journal of applied microbiology. 101 (3): 616-619.
- [16] Johnston, P.R. and N. Crickmore. 2009. Gut Bacteria Are Not Required for the Insecticidal Activity of *Bacillus thuringiensis* toward the Tobacco Hornworm, *Manduca sexta*. Applied And Environmental Microbiology, 75 (15): 5094-5099. DOI: 10.1128/AEM.00966-09
- [17] Broderick, N.A., Kenneth F. Raffa, and Jo Handelsman. 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. PNAS 103 (41) : 15196-15199; <https://doi.org/10.1073/pnas.0604865103>

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