Toxicity of Bacillus thuringiensis Berl. KJ3P1 and DLM isolates towards pest of oil palm Oryctes rhinoceros [Coleoptera: Scarabaeidae]

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Abstract. Oil palm plants are industrial plants that generate foreign exchange for Indonesia. Some obstacles are leading to production decrease. One of the important pests is *Oryctes rhinoceros* [Coleoptera: Scarabaeidae]. Both larval and imago stadia attack oil-palm in immature plants [IP] and mature plants [MP]. The method of controlling Oryctes often use chemical insecticides, in which continuously applications will cause residues in the environment and mortality of non-target insects. Therefore it is necessary to find an environmentally friendly control method that is easy, inexpensive and safe for the environment. The use of entomopathogenic bacteria *Bacillus thuringiensis* is an alternative. The study was aimed to study the toxicity of two *B. thuringiensis* isolates from the collection of Plant Protection Department, Faculty of Agriculture, Sriwijaya University [KJ3P1 and DLM isolate codes] on mortality of 3rd instar Oryctes larvae. Experiments were conducted at the Entomological Laboratory, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University from March until May 2019. Bioinsecticide-based *B. thuringiensis* was made with 2 types of growth media, namely: 1] cow's bio-urine enriched with 5% molasses, and 2]. Rice washing water enriched with 5% molasses. Treatments were included: 1] DLM5 isolate, 2] KJ3P1 isolate, 3] commercial *B. thuringiensis* and 4]. without treatment [as control]. The test insects used were 25 individuals of 1st instar Oryctes larvae in each treatment. The results showed KJ3PI isolate that propagated in bio-urine media showed the highest production of B. *thuringiensis* [5.69 x 10^6 spores/ml], the highest mortality [76%] and the lowest LT_{50} value [5.28 days] as well. The use of agricultural and livestock waste as a medium to multiply *B. thuringiensis* bacteria may produce high cell and spore production and can be used as bioinsecticides to control *O. rhinoceros*.

1. Introduction

In the cultivation of oil palm plants, there are important pests that attack from the beginning until the mature phase of the plant. The insect pest is the beetle *Oryctes rhinoceros* [Coleoptera: Scarabaeidae]. *O. rhinoceros* is one of the most damaging insects to palms in Asia and the Pacific Islands. They spread almost in all coconut plantations in Indonesia [1]. Adults eat the leaves and burrow into the crown, stunting plant development Typical V-shaped damage to coconut leaves by this beetle [2],[3].

Both larval and imago stadia palm oil attack in immature plants [IP] and mature plants [MP]. As a result, decreased production and severe attacks cause plants to die. This pest attack can take place throughout the year and its population can be influenced by several factors including the breeding grounds of these pests [4].

Several control techniques have been applied to overcome these pest problems in the field. Integrated pest control [IPM] is one of the best alternatives to suppress the pest population [5]. When the pest population has reached the economic threshold, it is permissible to use chemical control to control insect pests. Some negative effects will appear residue in the environment and killing non-target insects. It is necessary to find an environmentally friendly control method that is easy, inexpensive and safe for the environment. The use of entomopathogenic bacteria *Bacillus thuringiensis* is an alternative [6]. The purposes of the research were to investigate 1]. Production of *B. thuringiensis* propagated in Cow's bio-urine enriched with 5% molasses, and Rice washing water enriched with 5% molasses, and 2] Toxicity of *B. thuringiensis* based on bioinsecticide to *Oryctes rhinoceros* larvae.

2. Materials and methods

B. thuringiensis isolates used were isolates with DLM5 and KJ3P1 codes collected from the Entomology Lab of the Plant Protection Department Faculty of Agriculture, Sriwijaya University. Tested insects used were first instar oryctes larvae obtained in the oil palm plantation of the Inderalaya Campus.

The study was designed using a factorial completely randomized design [RALF] with a factor of 1 in the form of *B. thuringiensis* isolates [2 isolates] and a second factor in the form of growth media [2 kinds of media], with 5 times replications.

2.1. Insect test preparation

Oryctes rhinoceros larvae were taken from the experimental oil palm state in the Indralaya area of Ogan Ilir Regency, South Sumatera then maintained at the Entomological Laboratory of the Faculty of Agriculture, Sriwijaya University. The 1st instar larvae will be used as tested insects for the application,

2.2. Propagation of B. thuringiensis

Seed culture was prepared by taking an ose needle from petridish, put in 50 ml media Nutrient Broth [NB] placed in a glass bottle. The media was fermented using a shaker for 12 hours at a speed of 200 rpm and room temperature. Ten ml of media was taken and mixed with 50 ml NB, then fermented again using a shaker for 12 hours at a speed of 200 rpm. Seed culture was ready to use.

Medium [bio-urine, molasses, and NB] was sterilized at 121 ° C at 1 atm for 20 minutes in an autoclave. This sterilization process is intended to kill all microorganisms in the media and fermentors with the results that it does not affect the growth process of *B. thuringiensis* during the fermentation process. The treatments were 1] 50 ml bio-urine + 5% molasses, 2] 50 ml rice washing water + 5 ml% molasses and 3] 50 ml NB. Additionally, 5 ml of culture seeds were prepared for each treatment. Seed culture was poured into prepared media. All the glass bottles were installed in the fermenter [200 rpm at room temperature for 72 hours]. Bioinsecticide was ready to use.

2.3. Counting of density spores

Spores density of *B. thuringiensis*-based bioinsecticides was done by making a serial dilution of spores. The results of the last dilution were taken to calculate bacterial spore density in a haemocytometer. Observation of bacterial spores was carried out at 3 times of observation, namely 24 hours, 48 hours, and 72 hours, respectively.

2.4. Bioassay

Thirty g of empty soil bunches were weighed and placed in plastic petridish [20 x 10 x 10 cm]. Fifteen ml of bioinsecticide was sprayed into the soil. Wait until the air dries. After that, 25 larvae of oryctes were inserted. Observation of test insect mortality was carried out for 7 days.

2.5. Calculation of insect mortality

Observation of larval mortality was carried out every day for 7 days. To calculate the percentage of mortality the following formula was used:

$$P = a / b \times 100\%$$

In which:
P = Percentage of larval or imago mortality [%]
a = Larvae or imago that ed infected by *B. thuringiensis*
b = total number of observed larvae and imago

2.6. Data analysis

Spore density and mortality data were analyzed by ANOVA, while LT50 values were analyzed using probit analysis.

3. Results and discussion

3.1. Spores density of Bacillus thuringiensis

Propagation of *B. thuringiensis* in biourine media results in higher spore density compared to rice washing water media. The highest spore density was obtained in KJ3P1 isolate which was 5.69 x 10^6 spores/ml. The complete data is presented in Figure 1.

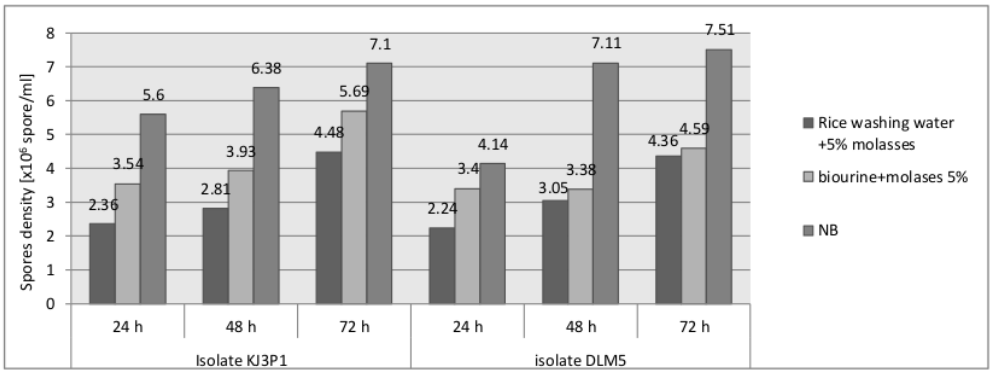

Figure 1. Spores density of *Bacillus thuringiensis* isolates propagated in two media [rice washing water and Biourine enriched with 5 % molasses]

In the calculation of spores after 72 hours of fermentation, the highest spore density was showed in KJ3P1 isolates on biourine media enriched with 5% molasses. Biourine is one of livestock waste that still contains carbon and nitrogen [7]. At the time of *B. thuringiensis* bacterial propagation, C / N component is required. According to Nuraini and Asgianingrum [8], after a 28-day fermentation process, the C / N ratio in biourine reaches 11, while according to Valicente et al [9] the C / N ratio is needed to reach at least 2.5. Therefore the use of bio-urine can meet these needs. In this case, the addition of molasses also further increases the Carbon content. Thus the use of bio-urine and molasses, which are both livestock and agricultural waste, will increase efforts in making bt-based bioinsecticides that are cheap and safe for the environment.

3.2. Mortality of tested insects

Test insect mortality was known on the third day for isolates DLM5 and KJ3P1. Insects seem to be weak, decreased appetite and eventually found dead. The mortality rate after 7 days of observation is presented in Table 1.

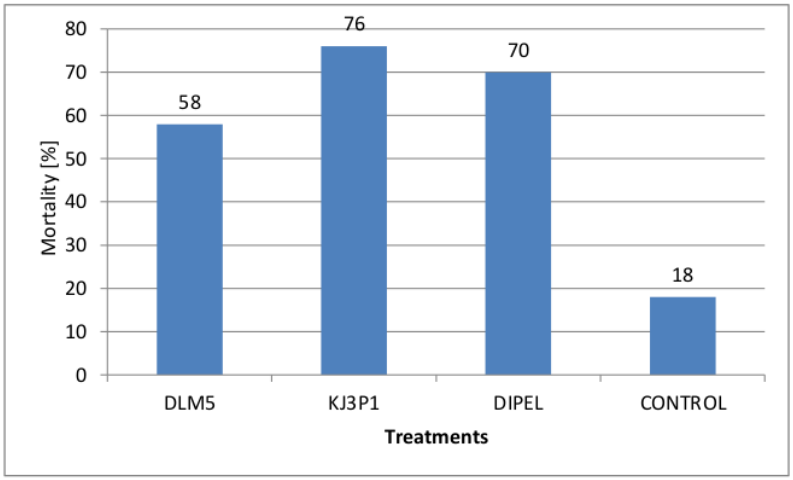

Figure 2. Mortality of 1st instar Oryctes larvae on treatments of *B. thuringiensis*-based

3.3. Bioinsecticide 7 days after application

KJ3P1 isolates that were propagated in bio-urine media enriched with 5% molasses showed a high mortality value of 76%. At the time of bioassay testing, the density of the spores in the bioinsecticide was quite high at 5.69 x 106 spores/ml. This is consistent with the opinion of Pujiastuti et al. [9] who also reported that a high spore density would cause a high mortality rate of test insects as well. Dipel is used as a standard bioinsecticide for *B. thuringiensis* -based bioinsecticide testing because it is known that it contains spores and proteins that can cause death in various insect pests that attack agricultural farming [10].

3.4. LT50 value

To calculate the time needed to kill 50% of test insects, the LT50 value is used. In this experiment, from the calculation results, it is known that the fastest time to kill test insects is 5,280 days. The complete data are presented in Table 1.

Table 1. LT_{50} Value of *Bacillus thuringiensis* isolates towards 1st instar of *Oryctes rhinoceros* larvae

		95% Fiducial Limit		
Treatment	LT_{50} [days]	Lower	Upper	Regression
DLM5	6.976	6.571	7.400	y= 0.793x -3.154
KJ3P1	5.280	5.658	6.514	y= 0.793x -3.233
DIPEL	8.068	8.664	8.491	y= 0.793x -3.227
CONTROL	7.087	6.609	7.619	y= 0.793x -5.622

The time needed from when the *B. thuringiensis* spore is eaten by test insects [oryctes larvae] requires time. It is known that the larvae of oryctes live in soils made from empty bunches and take

their food from the soil as well. If the soil is treated with bioinsecticides containing Bt spores, the spores will enter the digestion and multiply in the midgut. In the process, it takes time to cause the death of test insects. This is supported by research report Pujiastuti [11] who reported that to cause death in the Spodoptera litura test insects, it took more than 3 days.

4. Conclusions

The use of Bt isolates propagated using agricultural and livestock waste can be used to control oryctes larvae on oil palm plants. *B. thuringiensis*-based bioinsecticides are cheap in manufacturing, the medium is easy to obtain and is safe for the environment and non-target insects.

5. Conflicts of interest should be stated clearly.

There is no conflict interest among the authors.

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