Toxicity of *Bacillus thuringiensis*-based Bio-insecticide on *Coptotermes curvinagthus* (Isoptera: Rhinotermidae) in Laboratory

Yulia Pujiastuti
Plant Protection Department, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia
Email: ypujiastuti@unsri.ac.id

Rohwati[1], Suwandi[1], Dwi Probowati[2], Suparman[1], and Arsy[1]
[1]Plant Protection Department, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia
[2]Soil Science Department, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia
Email: rohwati67@gmail.com, suwandi@fp.unsri.ac.id, suparman@unsri.ac.id, dwi_probowati@yahoo.co.id, arsy_unsri@yahoo.com

***Abstract*—Rubber tree termites *Coptotermes curvinagthus* (Isoptera: Rhinotermidae) is an important pest on rubber plantation. Control should be conducted. *Bacillus thuringiensis*-based bio-insecticide was proposed. The treatments were consisted of 4 serial of spore concentrations, i.e. 10^5, 10^6, 10^7 and 10^8 spores/ml, respectively. Chlorphyrifos 0.1% and aquadest were used as control. The objective of the study was to investigate toxicity of *B. thuringiensis* isolates SMR-04 to worker and soldier castes of *C. curvinagthus* in laboratory. The study was designed in Completely Randomized Design with 6 treatments and 5 replications. Results showed highest mortality in worker caste was achieved in the treatment of 10^8 spores/ml (65.77%) and thus in soldier caste was 47.20%. Consumption of feed on workers and soldiers were ranged from 2.2 to 5.2 g and 0.08-0.5 g respectively. Soldier caste cannot afford to eat naturally because the mouth part was reduced, therefore they must be assisted by the worker caste.**

***Index Terms*—termites, *Bacillus thuringiensis*, bio-insecticide, toxicity**

I. Introduction

Rubber tree termite *Coptotermes curvinagthus* (Isoptera: Rhinotermidae) is an important pest on rubber plantation. They live in the forests of Sumatra and Malaysia especially in lowland and regional areas with uneven rainfall. The nest can be found in stalks that have died either below or above the ground and usually make the tunnel 6 mm - 90 mm in length and the depth is 30-60 cm. The termites attack many tree plants such as cultivated teak, rubber, palms, oil palm, coconut, cashew, coffee, chili and sugarcane [1]. In Indonesia, the damage caused by termites could reach to 100 - 300 billion rupiahs every year [2]. Joshi et al. (2005) reported maize losses in India was about 15-25 % and 35.12 USD per year [3]. Moreover, in South Africa, they responsible to 3-100 % of crop losses [4]. Termites are long-lived social insects which made colony both in the soil and on the soil surface. The colonies consist of reproductive forms, sterile workers and soldiers in a colony, usually there is one pair of primary reproductive forms. The sterile castes, workers and soldiers are wingless and lack of eyes [5]. Due to its high population, termites have high feed requirements. Therefore they will be easy to adapt to any environments [6]. Termites often cause damage to rubber plants by grating the stem from the end of the stem to the roots, as a result the eye grafting cannot grow anymore. Termites also eat the roots caused plant growth weakens and eventually dies. The results of land surveys in five districts in South Sumatra stated that most termite attacks were found in less maintenance plants.

The control of termites in rubber plantations is generally conducted conventionally, i.e. by prioritizing insecticides in scheduled applications without monitoring of termite populations. In addition to spending a lot of money, it will also cause adverse effects of environmental pollution. One effort to overcome the problem is to find alternative means of control the pest effectively but environmentally friendly. *Bacillus thuringiensis*-based bio-insecticide is proposed for controlling termite. The advantages of using *B.thuringiensis*-based bio-insecticide are 1) easy to apply, 2) kill target insects, and 3) no residue in environment. Entomopathogenic bacteria *B. thuringiensis* is a bacterium which produce spores and protein crystals during sporulation. The bacteria are known to cause death against insects when spores and / or crystal proteins enter into digestive tract of insects. Bravo *et al.* [7] mentioned that *B. thuringiensis* was able to kill insects because it had endotoxin, the form of protein crystals produced within sporulation. The toxin works as a stomach poison therefore when it is applying, the protein crystals must enter into digestive system. Pujiastuti *et al.* [8] reported protein crystals contained in *B. thuringiensis* subsp *wuhanensis* had high toxicity to armyworm *Spodoptera litura* but were less toxic to the useful insects *Bombyx mori*. In this paper, it is reported a

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study to investigate toxicity of *B. thuringiensis* isolate indigenous South Sumatra for controlling termites *C. curvignathus* in laboratory.

II. Material and Methods

Research had been carried out in Entomological Laboratory, Department of Plant Pests and Diseases, Plant Protection Study Program, Faculty of Agriculture Sriwijaya University. The study was conducted from September until December 2016

A. Research Methods

The isolates used were *B. thuringiensis* SMR 04 (collection of Entomological Laboratory, Department of Pest and Plant Diseases Faculty of Agriculture Sriwijaya University). It was known to possess high toxicity against *Plutella xylostella* and armyworm *Spodoptera litura* (unpublished data). The experiment was designed in a Completely Randomized Design (CRD). The treatments were consisted of 4 serial of spore concentration, i.e. 10^5, 10^6, 10^7 and 10^8 spores/ml, respectively. 0.1% chlorphyrifos and aquadest were used instead of bio-insecticide, as well. The insects tested were distinguished by the workers' and soldiers' caste.

1) Preparation of seed culture

Seed culture was done by taking isolate *B. thuringensis* SMR-04 as much as 1 ose from slant agar media. Furthermore, it was aseptically transferred into a 250 ml Erlenmeyer containing 50 ml Nutrient Broth (NB) media and sterilized with autoclave at 1 atm, 120˚C for 20 minutes. Erlenmeyer were placed on the shaker and shaken for 12 hours with a speed of 200 rpm. A total of 10 ml of media was retrieved and re-inserted in 250 ml erlenmeyer containing 50 ml of NB. Erlenmeyer were placed on the shaker and reshuffled for 12 hours with a speed of 200 rpm. The result was a seed culture ready to be used for bio-insecticide production.

2) Preparation of B. thuringensis- bio-insecticides

150 ml of NB was added with 50 mg CaCl2, 50 mg MgSO4, 50 mg K2HPO4, and 50 mg KH2PO4 and transferred into a 250 ml erlenmeyer, covered with aluminum foil and sterilized using autoclave for 20 minutes at 120 ℃ at 1 atm pressure. After that, it was added aseptically 5 ml seed culture into erlenmeyer, then closed again. They were be shaken by shaker for 72 - 96 hours with a speed of 200 rpm at room temperature. Spore density calculation was performed before application. Calculation of spores was conducted by taking 1 ml of suspense transferred to haemocytometer and closed by cover glass. Density of spore was calculated under binocular microscope. Suspension of *B. thuringensis* was serially calculated to density levels 10^5, 10^6, 10^7 and 10^8 spores/ml, respectively.

B. Preparation of Test Insects

The test insects used were worker and soldier caste of C. *curvignathus* originated from rubber plantation on Indralaya campus, Sriwijaya University. It was then taken to laboratory and maintained in a maintenance container (d = 15 cm, h = 20 cm). The feed used was rubber wood which started to fragile. Maintenance container is covered with black gauze to keep air circulation for container. Maintenance was performed until termites were ready for examination and be separated between termite workers and termite soldiers. The selected test insects were healthy and the body sizes were similar.

1) Application of B. thuringensis

Suspension of *B. thuringiensis* was prepared with a predetermined density of spores. Application of bio – insecticide was done by spraying suspension on to 50 g feed media (weathered rubber wood). Within 5-10 minutes, *B. thuringiensis* will be absorbed by feed media. Twenty workers termites and soldier caste were separately transferred into container maintenance. Observations were done every 8 hours. Mortality checks were performed every 8 hours after application and observed until the insect tested were died. Termites were declared dead when they were touched with a smooth brush was no response. The percentage of dead termites was calculated using the formula:

$$P = \frac{a}{b} \text{ X } 100\%$$

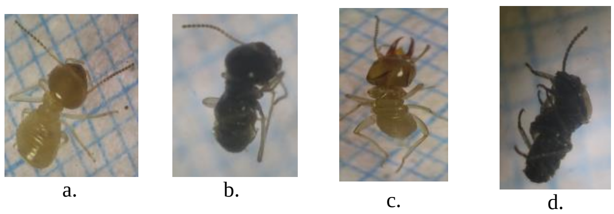
Note:
P: Percentage of termite mortality
a: Number of dead termites infected with *B. thuringiensis*
b: Number of all termites

2) Feeding ability of C. curvignathus

Observation of the feeding ability of *C. curvignathus* was done by weighing the feed at the end of observation. To calculate the weight of feed consumed was used this formula: the initial weight was reduced by the final weight. Termite mortality data and data on feeding ability were analyzed by ANOVA

III. Results and Discussion

Symptoms of termite mortality of workers and termite soldiers due to *B. thuringensis* application were shown in Fig. 1. The body of healthy worker was translucent white and transparent with yellow in head color. Otherwise, dead termites worker were changes in color throughout its body. The abdomen was blackened likewise the thorax and head. In addition, the body texture was softens and decays. The similar thing was happened to soldier termites. In the dead soldier caste, the color of the body was blackened and the head became soft.



a. b. c. d.

Figure 1. Mortality symptom of worker and soldier
a. Healthy worker
b. Dead worker
c. Healthy soldier
d. Dead soldier

Mortality of worker caste ranged from 47.77 to 65.77%. There was no mortality in control. This was meant all mortality may occurred because of *B. thuringiensis* application. At chlorphyrifos insecticide treatment mortality was very high (92.1%) and based on statistical analysis it was significantly different with all treatments. Mortality of termites 100 % was found in the treatment of botanical insecticide *Toona sinensis* stem wood or TSS (MeOH 20%) within 4 days, while another extract (*T. sinensis* stem Bark) or TSSB (20%) was caused 35 % mortality [9]. The usage of chemical in controlling termites was increasing since its ability to kill was very fast and high. Hill [1] reported that nowadays there was widely used of chlorphyrifos because it was very effective in termites control. The highest concentration of *B. thuringiensis* treatment (10^8 spores /ml) resulted in the highest mortality of worker caste (65.77%), significantly different from other treatments. Mortality of soldier caste ranged from 38.33 to 47.20 %. The highest mortality was also found in the concentration of 10^8 spores / ml, however statistical analysis showed in this treatment was not significantly different with the treatments of 10^6 and 10^7 spores / ml. In general mortality of workers was higher than thus in the soldier caste. This was be expected because morphologically, the worker caste had a softer body texture than thus in the soldier caste. In addition, the soldier caste has a hard head and a hardened mandible. This may correspond to their duty as a guardian of the nest [1], [10]. Mortality on soldier caste was also high in control, because soldier caste had no ability to consume rubber wood by themselves. In general, the complete inhabitant consisted of reproductive, worker and soldier castes. Among them, reproductive caste was very small in number, however they were very dominant to control the colony. The queen will chemically understand to produce worker or soldier caste. Number of both castes was different. The worker was much more abundant compare with soldier. Even though their number was little, they had very important tasks to defend their nests from danger. In fact, they must be fed by workers [10]. This showed that soldier caste may not live alone without worker caste. Mortality data was presented in Table I.

TABLE I. MORTALITY LEVEL OF WORKER AND SOLDIERS CASTES OF TERMITE APPLIED BY *BACILLUS THURINGIENSIS*- BASED BIOINSECTICIDE IN LABORATORY WITHIN 96 HOURS

Treatments	Mortality (%)	
	Worker	Soldiers
Control	0.01*a*	35.60*a*
Chlorpyrifos (1ml/l)	92.15*e*	93.13*c*
Bt 10^5 spores/ml	47.77*b*	38.33*a*
Bt 10^6 spores/ml	56.62*c*	44.13*b*
Bt 10^7 spores/ml	59.92*c*	42.40*b*
Bt 10^8 spores/ml	65.77*d*	47.20*b*

Notes: Numbers in the same column followed by different letters (a-e) are significantly different at the level of P < 0.05 according to Tukey's test.

Observation of termite mortality of worker caste was done every 8 hours for 96 hours. In control, there was no mortality. Observation in the treatment of insecticides with active ingredients of chlorphyrifos showed after 8 hours application, the percentage of mortality was 98%. Furthermore, in the second observation (16 hours after application), the percentage of mortality was 100%. Mortality of workers and soldiers were in succession increased with increasing time. Spore and protein crystal *B. thuringiensis* were taken time to kill termites. This was corresponded to a deadly mechanism by *B. thuringiensis* that worked as a stomach poison because *B. thuringiensis* have to enter in the digestion system [7], [11].

Mortality of worker caste was started at the observation of 16 hours after the treatment whereas in soldier caste, mortality was started the observation of 32 hours after the treatment. It was expected in two causes, firstly in worker caste, they consumed directly from the feed that had been exposed by bio-insecticide, whereas in the soldier caste, they could not feed by themselves. This was meant that poison or spore / crystal protein of *B. thuringiensis* in the digestion system of soldier caste was lower than thus in the worker caste. Secondly, it was because the morphological form of worker caste and soldier caste were different. They could be different by hardening of the head and the mandible, as a result this will slower the process of death. Thus far, there was little research revealed the difference in mortality on the workers and soldiers' caste. Most researchers always combine the objects of workers and soldiers because their life in the nest naturally was dependent one caste to other caste [12] Fei and Henderson [13] reported wood consumption of Formosan subterranean termite *Coptotermes formosanus* tended to increase when workers lived with more number of soldier caste. Mortality data of workers and soldiers caste was presented in Fig. 2.

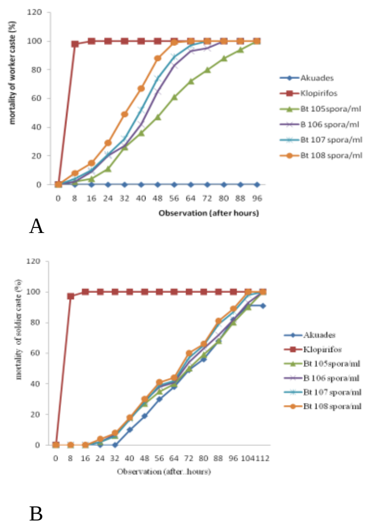


Figure 2. Mortality of *Coptotermes curvinagthus* (Isoptera: Rhinotermidae) applied by *Bacillus thuringiensis*-based bioinsecticide in laboratory. A.worker caste; B.soldier caste.

Feed consumption exposed by *B. thuringiensis* in worker caste was different for all treatments except for treatment of 10^6 and 10^7 spores/ml. Compared to the control and treatment of chlorpyrifos insecticides, the results showed significantly different. Consumption at control was 5.62 g while in synthetic insecticide was 0.88

g. This showed the use of chemicals was very effective because they consumed less feed but resulted in high mortality 92.13% (Table I). The highest consumption level at treatment was 10^5 spores / ml (5.52 g). Statistically analysis showed significantly different consumption level with chemical insecticide but not significantly different with control. *B. thuringiensis* treatment in soldier caste showed the weight of feed was very small and this was not significantly different between the treatment and the control. Yeoh and Lee [14] reported about ability of *C. curvignathus* soldier caste consumed on timber of 0.1787 g per 10 individues. In this paper was reported the use of weathered rubber wood as feed, while Yeoh and Lee [14] was using healthy wood. Due to different feed types, therefore the feed weight consumed by termites was higher in decayed rubber wood compared to healthy wood. Data of feed consumption was presented in Table II.

TABLE II. FOOD CONSUMPTION LEVEL OF WORKER AND SOLDIERS CASTES OF *CONOPTERMES CURVIGNATHUS* TERMITE APPLIED BY *BACILLUS THURINGIENSIS* - BASED IN LABORATORY WITHIN 96 HOURS (N=20)

Treatments	Consumption (g)	
	Worker	Soldiers
Control	5.62*c*	0.58b
Chlorpirifos (1ml/l)	0.88a	0.08a
Bt 10^5 spores/ml	5.52c	0.50b
Bt 10^6 spores/ml	3.14b	0.16a
Bt 10^7 spores/ml	3.40b	0.14a
Bt 10^8 spores/ml	2.24a	0.08a

Caste of soldiers had large heads with highly modified powerful jaws so enlarged they cannot feed themselves. They were fed by workers instead [6]. The statement supported the feed consumption data as shown in Table II, the weight of feed consumed by soldiers was lower than the consumption of the worker caste. Fei and Henderson [12] reported wood consumption of Formosan subterranean termite *Coptotermes formosanus* tended to increase when workers lived with more number of soldier caste.

IV. CONCLUSION

B. thuringiensis application resulted in higher mortality of worker caste (65.77%) than the mortality of soldier caste (47.20%). Feed consumption in workers was ranged from 2.24 to 5.52 g while thus in the soldiers were ranged from 0.08 to 0.5g. The soldier caste could not feed itself because there was modification of the head and mandible, therefore soldier could eat when assisted by the worker caste.

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Yulia Pujiastuti was born in Yogyakarta Indonesia, 18 May 1962. She got her Ph.D degree from Applied Bio-Science Department, Graduate School of Agriculture Hokkaido University, Sapporo Japan in the year 2000.

At presents, she is Associate Professor in Department Plant Protection, Facuty of Agriculture, Sriwijaya University.

Yulia Pujiastuti has interest in Entomology especially in Integrated Pest Management and Biological Control. She is the member of SAFE network and Indonesian Entomological Society.

Rohwati was born in Palembang. She graduated from Dept. of Plant Protection Faculty of Agriculture Sriwijaya University, 2017.

Suwandi was born in Bangka. He is senior lecturer in Dept. of Plant Protection Faculty of Agriculture Sriwijaya University. His major in Plant Pathology.

Dwi Probowati was born in Yogyakarta. She is senior Lecturer in Soil Sciences Department, Faculty of Agriculture Sriwijaya University.

Suparman was born in Banyumas Middle Java. He is senior Lecturer in Dept. of Plant Protection Faculty of Agriculture Sriwijaya University. His major in Plant Pathology.

Arsy was born in Jungkal, South Sumatera, 17 Oktober 1985. He is junior lecturer in Dept. of Plant Protection Faculty of Agriculture Sriwijaya University. His major in Plant Pathology.