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Bacillus thuringiensis*, a green option for controlling oil palm bunch moth *Tirathaba rufivena

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Abstract. A field study to test the efficacy of *Bacillus thuringiensis* subsp. Kurstaki against *Tirathaba rufivena*, the oil palm bunch moth, has been conducted in early mature (5-year-old) oil palm in Kalia Estate, Riau. The experiment was conducted following a randomized complete block design, consisting of five concentration treatments, namely 0, 2, 4, 6, and 8 ml/L of suspension. The *B. thuringiensis* was sprayed directly onto the inflorescences and fruit bunches of selected palm samples every week for a month duration. The result showed that the intensity of *T. rufivena* attack was continuously declined since the first week after *B. thuringiensis* application. The decline of attack intensity is in line with the reduction of the larvae population in the affected fruit bunches. This trial demonstrated that *B. thuringiensis* had high efficacy against the bunch moth with an efficacy value of 74.90 %, 91.18 %, 96.96 %, and 94.77 % achieved at two weeks after application at the concentrations of 2, 4, 6, and 8 ml/L, respectively. This result suggests that *B. thuringiensis* at the concentration of 4-8 ml/L can be used as a sustainable option for controlling *T. rufivena* in oil palm plantations.

Keywords: attack intensity, number of larvae, efficacy value

1. Introduction

Tirathaba rufivena, the oil palm bunch moth, is one of the important pests of oil palm in Indonesia and Malaysia. Larvae of *T. rufivena* feed inside oil palm inflorescences and fruit bunches, causing malformation in fruit bunch development or, in the worst case, early bunch abortion [1, 2, 3]. Infestation of *T. rufivena* mostly happens at the early phase of oil palm maturity, particularly in the first five years of fresh fruit bunches production [2, 4]. Over the last decade, *T. rufivena* has transformed into one of the major pests in oil palm plantations, causing severe damage, especially to those planted in peatland and sandy soil [1, 5, 6]. The population of *T. rufivena* increased mainly due to the abundance of its food sources, such as unharvested ripe bunches and the absence of natural enemies [7]. The outbreak of *T. rufivena* could significantly affect the weight and quality of produced bunches and may lead to 50 % losses in fresh fruit bunch (FFB) production [8].

Synthetic insecticide application, such as cypermethrin and fipronil, has been the standard method to control *T. rufivena* infestation in oil palm plantations [4, 6] since they were capable of rapidly reducing *T. rufivena* population [1, 2]. However, such insecticides also had an adverse effect on other beneficial insects, including predators, parasitoids, and mainly the pollinator insect, *Elaeidobius kamerunicus* [6, 9, 10, 11, 12]. The use of synthetic insecticides also posed a great threat to the environment due to its slow degradation, mainly in peatland [13]. Eco-friendly approaches to control *T. rufivena* using



predators and parasitoids have been conducted with no success [14, 15]. Another biological agent that may benefit oil palm planters in combating *T. rufivena* is *Bacillus thuringiensis*.

Over the past decades, *B. thuringiensis* has been broadly demonstrated to control various oil palm insect pests [16, 17, 18, 19, 20]. Commercial insecticide containing *B. thuringiensis subsp. kurstaki* is available in the market and has been known for its efficacy against various Lepidopteran pests [18, 19]. The bacteria produced Cry protein, namely δ -endotoxin, which had insecticidal characteristics during the sporulation stage [21]. Commercial product of *B. thuringiensis* was mostly available in powder formulation, but recently, a liquid formulation is also available. This research aimed to test the efficacy of the liquid formulation of *B. thuringiensis* against *T. rufivena* infestation in early mature oil palm.

2. Materials and methods

Efficacy of *B. thuringiensis* against *T. rufivena* was conducted in early mature (5-year-old) oil palm in Kalia Estate, Rokan Hilir Regency, Riau. The oil palm planting block used in this experiment was heavily affected by *T. rufivena*, with more than 30 % attack intensity.

2.1. Treatment

The experiment used a Randomized Complete Block Design with five treatments and six replications. The treatment consisted of five concentrations of *B. thuringiensis*, i.e., 0, 2, 4, 6, and 8 ml/L of suspension. The treatment plot consisted of twelve palms, where four palms in the middle were selected as sample palms. Each treatment plot was separated by two rows of oil palm to avoid cross-contamination between treatments. Arrangement of treatment plots was determined based on preliminary observation towards the intensity of pest infestation and population of bunch moth in sample palms.

2.2. Application of *B. thuringiensis*

B. thuringiensis was sprayed directly onto the inflorescences and fruit bunches of selected sample palms until wet. The application was conducted using a mist blower with a spraying volume equal to 400 L/ha. Spraying of *B. thuringiensis* was performed once a week for a month duration.

2.3. Observation

The observation was performed a day before application, 7, 14, 21, and 30 days after the *B. thuringiensis* application. The parameter consisted of attack intensity level and larvae population of the bunch moth *T. rufivena*. The intensity level of *T. rufivena* attack was calculated following the scoring of damage due to *T. rufivena* on every single fruit bunch produced by each sample palm (Table 1). Meanwhile, the number of larvae was counted from the sampled fruit bunch after separating its spikelets (Figure 1).

Table.1. Scoring criteria of *T. rufivena* infestation.

Score	Remarks
0	No damage symptom, 0 %
1	Mild symptom, visible damage or larvae feces < 25 %
2	Moderate symptom, visible damage or larvae feces 25-50 %
3	Severe symptom, visible damage or larvae feces 50-75 %
4	Highly severe symptom, visible damage or larvae feces 75-100 %

The attack intensity of *T. rufivena* was calculated based on the following formula:

$$AI = \frac{\sum(n \times v)}{N \times V}$$

Where: AI = attack intensity of *T. rufivena*, n = number of palm in each damage score, v = damage score, N = total number of observed palms, V = highest damage score used in the scoring criteria (4)



Figure 1. Illustration of a visible symptom of *T. rufivena* attack and larvae counting activity. From left to right: Larvae feces visible on the surface of attacked fruit bunch; separation of fruit spikelet from bunch stalk; observable larvae of *T. rufivena* after spikelet separation.

The resulting data from the control and treatment plot were further used to calculate the efficacy value of *B. thuringiensis* following the Abbott [22] formula:

$$Ev = \frac{(Ca - Ta)}{Ca} \times 100 \%$$

Where: Ev = efficacy value of *B. thuringiensis*, Ca = percentage of mortality or damage on control plot, Ta = percentage of mortality or damage on treatment plot.

All data obtained were analyzed using variance analysis followed by Duncan's Multiple Range Test on a 5 % significance level test to determine the efficacy difference between treatments.

3. Result and discussion

Attack intensity of *T. rufivena* significantly decreased at seven days after application (daa) of *B. thuringiensis* in every tested concentration (Figure 2). Interestingly, all *B. thuringiensis* treatments showed zero infestation of *T. rufivena* at the end of observation (30 daa). In contrast, the attack intensity in the control treatment remained constant, with a slight increased at 14 daa. Despite the decline at 31 daa, most of the inflorescences and fruit bunches were still infested by *T. rufivena*.

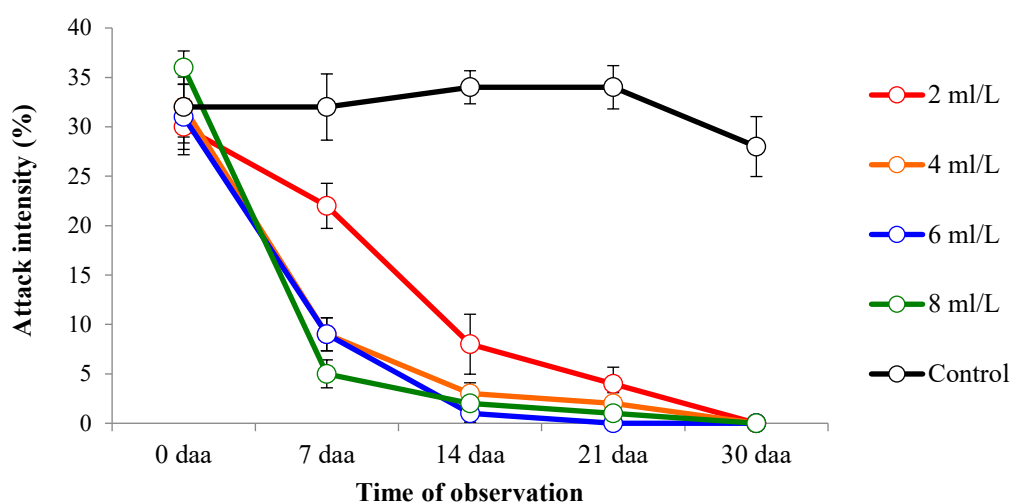


Figure 2. The intensity of *T. rufivena* attack within *B. thuringiensis* treatments.

The decline of *T. rufivena* infestation was indicated by the disappearance of new larvae feces on the oil palm bunch. This is in line with the population measure of *T. rufivena* larvae on bunch sample, where the number of larvae was decreased progressively from 7 to 30 daa on all *B. thuringiensis* treatments (Figure 3). At 30 daa, three concentrations of *B. thuringiensis* (4, 6, 8 ml/L) were capable of eliminating *T. rufivena* infestation on affected palms. On the other hand, *T. rufivena* larvae were actively feeding on untreated bunches, thus becoming an interminable infestation.

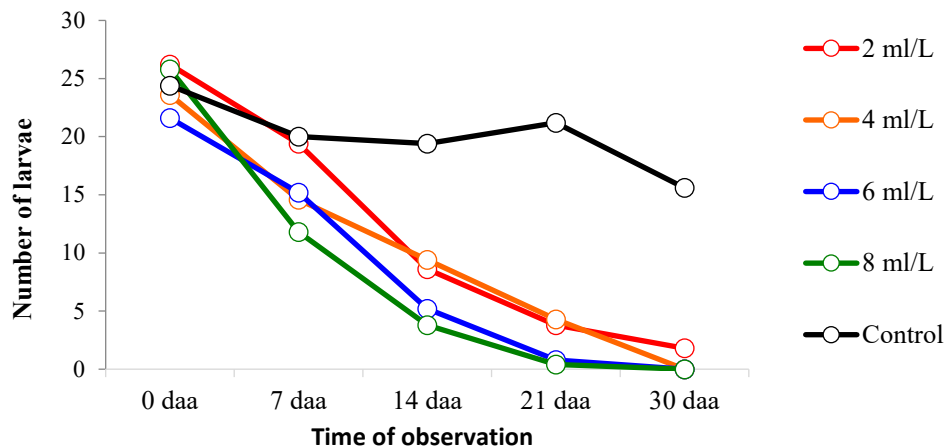


Figure 3. The population of *T. rufivena* larvae in sample bunch within all *B. thuringiensis* treatments.

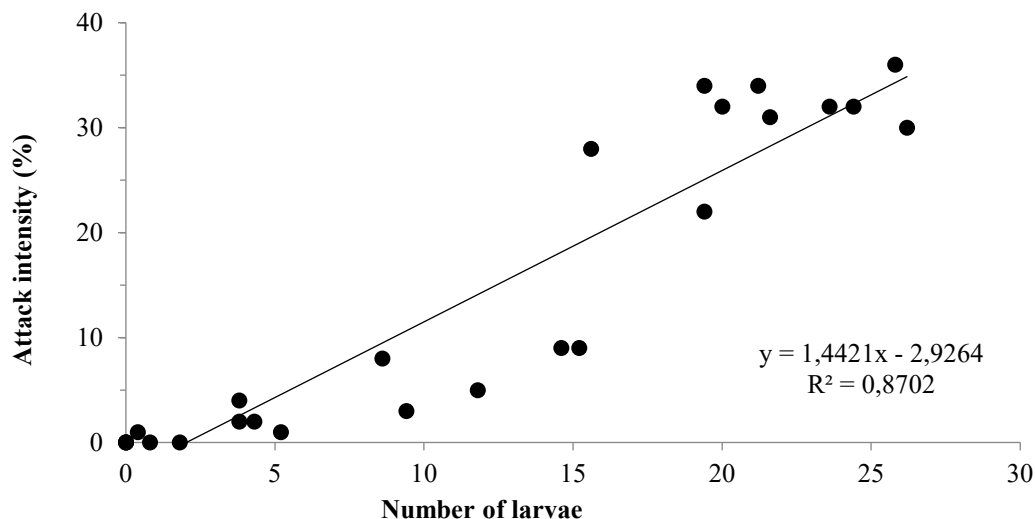


Figure 4. Correlation between the number of larvae and attack intensity level of *T. rufivena*.

The number of larvae was highly correlated with the attack intensity level of *T. rufivena* (Figure 4). The higher the number of *T. rufivena* larvae inside a fruit bunch, the higher the damage on the fruit bunch. In *B. thuringiensis*-treated fruit bunches, *T. rufivena* larvae were mainly inactive, probably due to the Cry toxin effect. Thus, no new feces appear on the fruit bunch surface. The effectivity rate of *B. thuringiensis* was affected by the feeding behavior of the targeted pest. To be able to cause mortality, the Cry toxins should be consumed by targeted insects. Once ingested, the Cry toxins travelled into the midgut, where they were activated by the digestive protease, and thus slowly poisoning the insect [21,

23]. Infected larvae had a pale appearance and became inactive [24]. Furthermore, the insect body will be turned brown, blackish, red, or yellow when it got rotten.

Observation at the beginning of the test showed that *T. rufivena* was still in the larvae stage but in overlapped instar conditions with various sizes. Larvae of *T. rufivena* could undergo five instars in 2-3 weeks [5]. In an overlapped condition, a critical point to control the pest was the rate of insecticide application, which was performed once a week in this study. Repeated application of *B. thuringiensis* increases the possibility of *T. rufivena* larvae ingesting the Cry toxins, particularly the bigger larvae that can bore onto the kernel of the fruit.

This study demonstrated that repeated *B. thuringiensis* application had high efficacy against *T. rufivena*. The efficacy level of *B. thuringiensis* was more than 70 % at seven daa and increased to more than 90 % at 14 daa in the concentration of 4 – 8 ml/L (Table 2). On the other hand, *B. thuringiensis* application at 2 ml/L had a slower effect of reducing the attack intensity level despite the efficacy level similar to other concentrations by the end of observation. This suggests that *B. thuringiensis* at the concentration of 4-8 ml/L has better efficacy than the concentration of 2 ml/L.

Table 2. Efficacy level of *B. thuringiensis* against *T. rufivena*..

Concentration (ml/L)	Insecticide efficacy (%)			
	7 daa	14 daa	21 daa	30 daa
8	86.11	94.77	97.39	100.00
6	70.97	96.96	100.00	100.00
4	71.88	91.18	94.12	100.00
2	26.67	74.90	87.45	100.00

Continuous application of *B. thuringiensis* in a short interval, even for a longer time, has no side effect on other beneficial insects living on oil palm inflorescences, such as the pollinator weevil, *Elaeidobius kamerunicus*. The previous study demonstrated that frequent application of *B. thuringiensis*, every two weeks for nine months, was capable of reducing *T. rufivena* infestation and maintaining *E. kamerunicus* population [16]. The study also emphasized that *B. thuringiensis* prevent an adverse effect on insect diversity in oil palm plantations. Earlier studies in Malaysia, performed in a mineral and in a peat soil, also showed that repeated application of *B. thuringiensis* was essential to reduce *T. rufivena* infestation [2, 6]. Meanwhile, repeated application of synthetic insecticide to control *T. rufivena* population was also reported [9, 25, 26, 27]. However, chemical insecticide is not recommended since it had negative impact on the activity and population of *E. kamerunicus* [10, 11, 12, 28, 29].

4. Conclusion

Repeated application of *B. thuringiensis* at the concentration of 4, 6, and 8 ml/L significantly reduced *T. rufivena* infestation on oil palm bunches starting from 7 daa. The efficacy value of these concentrations reached up to 91.18 %, 96.96 %, and 94.77 %, respectively, two weeks after treatment. This experiment suggests that *B. thuringiensis* is one of the sustainable options for controlling *T. rufivena* in oil palm plantations.

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