EFFICACY OF BAFOG-1 (S), FORMULATED LOCAL *Bacillus thuringiensis* FOR CONTROLLING BAGWORM, *Pteroma pendula* (Lepidoptera: Psychidae)

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ABSTRACT

A study on the efficacy of Bafog-1 (S), a formulated, local strain of Bacillus thuringiensis *(Bt) applied via fogging was carried out both in the laboratory and the field, against the bagworm species* Pteroma pendula*. Laboratory assay of Bafog-1 (S) using five different treatments showed that the Bafog-1 (S) at the highest dose of 2.36 x 10^{12} cfu ml^{-1} and ratio of Bafog-1 (S):diesel;1:1 (v/v) killed as high as 78.6%* P. pendula *at seven days after treatment (DAT). In field trials, the effectiveness of Bafog-1 (S) in controlling the bagworm was compared with a commercial Bt product. The field trial was conducted in an estate at Hutan Melintang, Perak, covering a total area of 12 ha. Based on a Student's t-test, with one-tailed distribution, the total bagworm populations (larvae + pupae) in Bafog-1 (S) and Dipel ES plots were significantly reduced ($P<0.05$) from 569 bagworms per frond (BPF) and 668 BPF at 0 DAT to 266 BPF and 432 BPF at 14 DAT, respectively. The results indicated that the efficacy of Bafog-1 (S) was comparable to the commercial Bt.*

Keywords: *Bacillus thuringiensis*, Bafog-1 (S), efficacy, fogging.

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INTRODUCTION

In Malaysia, the major insect pests capable for causing outbreaks are bagworms and nettle caterpillars. The economic impact from a moderate bagworm attack of 10%-50% leaf damage may cause 43% yield loss (Wood *et al*., 1972; Basri and Kevan, 1995). The bagworm is a leaf-eating caterpillar concealed within its carrot-shaped bag, which is constructed from bits of material from the plant upon which it feeds (Barlow, 1982). Bagworms are a recurring problem in oil palm plantations, and several factors have been identified as causes for the outbreaks. The continuous use of chemical insecticides to control bagworm outbreaks, the lack of beneficial plants in the plantation to attract natural enemies, and infestation in neighbouring plantations were reported as the contributing factors for bagworm outbreaks (Siti Ramlah *et al*., 2007b). Bagworms outbreaks are also associated with the dry season, and many planters believe that bagworms feed more actively and spread faster in hot and dry weather (Chung and Sim, 1991).

Currently, insecticides or chemical control are being used to control bagworms when the economic

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threshold level is reached. It can provide quick reduction of the pest number. Several types of insecticides have been found to be effective against bagworms in the oil palm plantation when applied by various techniques of spraying such as ground and aerial spraying and fogging (Wood, 1968; Chung, 1988). Recently, several organophosphate, carbamates and synthetic pyrethroids have been recommended for controlling bagworms in the infested area of oil palm plantations (Chung, 1988; 1998). Besides, a stomach poison is also applied via trunk injection and root absorption technique which is more selective, and does not affect non-target insects such as parasitoids, predators and oil palm pollinators.

However, the use of broad-spectrum contact insecticides for bagworm control via ground spraying technique has often disrupted the balance between the insect and its natural enemies such as predators, parasitoids and beneficial insect such as *Elaeidobius kamerunicus*. Furthermore, these chemical insecticides affect the non-target organisms and their residues often persist in the environment (Najib *et al*., 2008). Realising the hazardous effect of chemical insecticides, the *Bacillus thuringiensis* (Bt)-based products have been widely used as a safer alternative method for controlling the pests.

Furthermore, with the depletion of land for cultivation of oil palm in Malaysia, peat land use for oil palm has taken place. Bagworm infestation on oil palm on peat land is common and not easily controlled, such as in Lower Perak (Siti Ramlah *et al*., 2007). In order to overcome this constraint, MPOB has developed a new formulation of Bt product called Bafog-1 (S), suitable to be used via fogging for oil palm on soft peat soil, where the use of a tractor-mounted turbomist blower is not possible. Bafog-1 (S) is a local *B. thuringiensis* isolate, MPOB Bt1, formulated as a solution for controlling bagworms via fogging. The name Bafog-1 (S) is derived from *Bacillus* sp. for fogging in solution form (S). Bafog-1 (S) is produced by vacuum evaporating the fermented liquid culture of MPOB Bt1 at 33^{o}C to produce a concentrated culture, which is further processed by mixing with inert ingredients (Siti Ramlah *et al*., 2009b). The efficacy of Bafog-1 (S) was evaluated in the laboratory and field trial was conducted and reported in this article.

MATERIALS AND METHODS

Source of Local Bt and Commercial Bt Products

MPOB Bt1 obtained from Malaysian Palm Oil Board (MPOB) culture collection was used for the fermentation process at Microbial Technology and Engineering Center (MICROTEC), MPOB, Bangi, Selangor. The fermented Bt was concentrated using a vacuum evaporator at 33^{o}C and pressure -820 to -850 mbar, then further concentrated by sedimentation technique. Bafog-1 (S) was formulated from the concentrated sediment medium mixed with inert ingredients (Siti Ramlah *et al*., 2009b). For laboratory bioassay and field trial, the commercial Bt product Dipel ES was used side by side with Bafog-1 (S).

Source of Bagworm

The bagworms species *Pteroma pendula* used in this study were collected from the MPOB Research Station, Teluk Intan, Perak. The first larval instars were collected and placed into a cylindrical transparent cage and then brought back to a laboratory for two days rearing. In the laboratory, the active bagworms were temporarily transferred into Petri dishes that were layered with a Whatman filter paper. Each Petri dish was placed with five larvae of bagworm. The larvae were then weighed using an electronic balance to get a consistent weight of the first larval instars of *P. pendula* prior to the treatments. The average weight of individual first larval instars of *P. pendula* used in this study was 0.013 ± 0.010 g (*Figure 1*).

Laboratory Bioassay

Four different treatments were tested against *P. pendula*. Bioassay was conducted in the MPOB laboratory. The treatment design used was completely randomised. A commercial Bt and blank were prepared to compare with each tested product. For each tested product, the experiment was replicated three times. The treatments are as listed in *Table 1*.

The original concentrations of Bafog-1 (S) solution, Bafog-1 (S) powder and commercial Bt used were 2.36 x 10^{12} cfu ml^{-1}, 3.36 x 10^{12} cfu ml^{-1} and

Figure 1. First larval instars of P. pendula *used for laboratory bioassay.*

TABLE 1. PREPARATION OF DIFFERENT TREATMENTS OF *Bacillus thuringiensis* (Bt) PRODUCTS FOR LABORATORY BIOASSAY AGAINST THE BAGWORM, *P. pendula*

Treatments	Compositions	Mixing ratio in 1 litre of diesel
T1	Bafog-1 (S) solution mixed with diesel	At ratio 1:1 (v/v) (50% of a.i.)
T2	Bafog-1 (S) solution mixed with diesel	At ratio 1:3 (v/v) (50% of a.i.)
T3	Bafog-1 (S) powder mixed with diesel	5 g of Bafog-1 (S) well mixed in 1 litre diesel (50% of a.i.)
T4	Commercial Bt, Dipel ES	5 g of commercial Bt mixed well in 1 litre diesel (25% of a.i.)
Control	Untreated (diesel only)	1 litre diesel

1.2 x 10^{12} cfu g^{-1}, respectively. Bafog-1 (S) powder used was 5 g mixed in 1 litre of diesel.

Fogging of Bt products were conducted on a fragment of oil palm leaflet [3 cm (W) x 20 cm (L)]. Prior to treatment, the bottom part of the oil palm leaflet was inserted through a cap of cylindrical plastic vial with a dimension of 4 cm (D) x 8 cm (H), that was half filled with distilled water. The vial with the palm leaflet was then transferred into a 500 ml cylindrical transparent plastic cage. A group of five larvae was then transferred from the Petri dish onto the fragment of palm leaflet to maintain the bagworms after fogging application. The cylindrical cage was then fogged at a flow rate of 20 litres hr^{-1} from the bottom of the cage for about 10 min using a fogging machine model Z-FOG. The treated cages were placed in indoor insectory at temperature between 24°C - 28°C and relative humidity of 50%. Bagworm mortality was recorded at one, three, seven and 13 days after treatment (DAT).

Field Efficacy Trial

The experiment was carried out in an estate, at Hutan Melintang, Perak with palms aged 18-year-old and 20 m height. The total experimental area was 12 ha. The area was fully treated and equally divided into six sub-blocks, sized 2 ha. Two treatments were tested, which were MPOB Bafog-1 (S) and commercial Bt, Dipel ES (as comparison). Each treatment was replicated thrice in randomised complete block design (RCBD). The field trial started at 7 am in the morning to avoid drift. The duration of the trial per day was 5 hr. Prior to the operation, the fog flow rate was checked and calibrated three times to ensure consistency of the fog flow rate and pattern of the smoke. The observation was done for about 5 min after starting up the machine. During operation, when there was wind from any direction, the operation will be stopped immediately. The wind speed and direction was monitored closely before starting the operation again. The original concentration of MPOB Bafog-1 (S) and Dipel ES was 1.6 x 10^{12} cfu ml^{-1} and 1.2 x 10^{12} cfu ml^{-1}, respectively. The product amount of 3.5 litres was mixed with diesel at 1:1 ratio (v/v) and then poured into the 7-litre tank of a fogging machine (Model FOG-Z). Census to monitor the population of bagworm was conducted at zero, seven, 14 and 30 DAT. The number of larvae that survived per frond was recorded. Ten palms were randomly selected in each sub-block for census. One frond at 45° position will be cut from each palm for census.

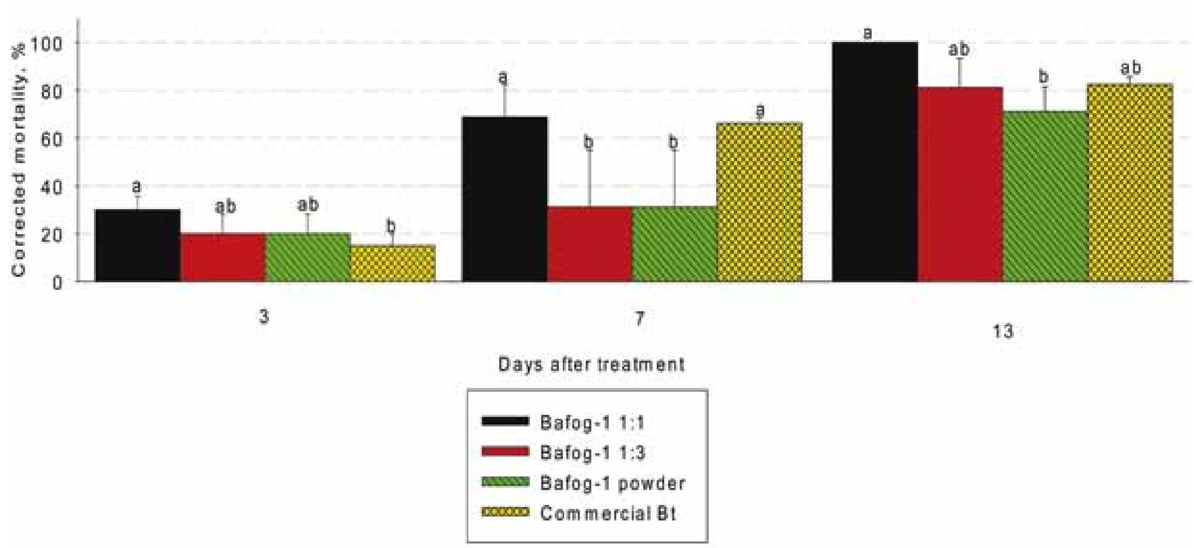
Data Analysis

For laboratory bioassay, data on mortality was corrected for control mortality using the Abbot's Formula, then, analysed using one-way analysis of variance (ANOVA). In this experiment, there was only one factor involved. The interaction being investigated was the difference in response between the treatments (as one factor) that contributed to bagworms mortality. Therefore, the one-way ANOVA analysis was used to analyse data of the laboratory assay. The means were separated using Least Significant Difference (LSD) analysis (SPSS version 11.5). For the field experiment, data on bagworm survival per frond was converted to $\log_{10}$ X using Microsoft Excel version 2007, then, analysed using a Student's t-test, with one-tailed distribution.

RESULTS AND DISCUSSION

Laboratory Bioassay

Fogging of Bafog-1 (S) mixed with diesel in the ratio of 1:1 using Bt at the concentration of 2.36 x 10^{12} cfu ml^{-1} caused 79% mortality against the *P. pendula* at seven DAT (*Figure 1*). This mixture was more effective as compared to Bafog-1 (S) mixed with diesel at a ratio of 1:3 and 5 g of Bafog powder mixed with 1 litre of diesel. At seven DAT, fogging of mixture of Bafog-1 (S) and diesel at ratio 1:3 and Bafog powder and diesel did not cause any difference, ($P>0.05$) on mortality of *P. pendula*. Fogging of both mixtures caused only 19.5% mortality on *P. pendula* at seven DAT. At 13 DAT, the mortality of *P. pendula*



Note: Bars in a group with the different letters are significantly different (P<0.05) after LSD test.

Figure 1. Corrected mortality of Pteroma pendula *treated with different rates of Bafog-1 (S) and commercial Bt, Dipel ES in laboratory bioassay.*

in all treatments increased to more than 75%, but no difference (P>0.05) between treatments, except for Bafog-1 (S) powder treatment. The highest mortality of *P. pendula* was recorded in treatment of the Bafog-1 (S):diesel at ratio 1:1 (100% mortality), as compared to Bafog powder mixed with diesel (77% mortality).

The laboratory assay showed that Bafog-1 (S) mixed with diesel in the ratio of 1:1 reached 70% mortality after seven days of the treatment. This result confirmed the finding by Yusdayati (2012) and Chung and Narendran (1996) that Bt gave an effective result after seven days of ground spraying application. Bt gave a satisfactory kill (70%-100%) of the bagworms after one week of application. The Bt on the palm leaflet has to be eaten by the bagworms to activate the Bt spores in the bagworms' stomachs which can cause mortality (Kenny, 1991; Siti Ramlah and Basri, 2002).

Field Efficacy

Application of both Bt products, Bafog-1 (S) and Dipel (ES) by fogging reduced the population of bagworm in the field experimental site. Fogging using Bafog-1 (S) and Dipel (ES) reduced significantly the larval population of *P. pendula* from 451 larvae per frond (LPF) and 585 LPF at 0 DAT to 40 LPF and 37 LPF at 30 DAT, respectively (*Table 2*). At 0 DAT, the pupae population was still high for both plots, Bafog-1 (S) (118 PPF) and Dipel ES (83 PPF) and eggs started to hatch at seven DAT. The effectiveness of fogging using Bafog-1 (S) and Dipel ES significantly reduced the larvae population at 0 DAT from 451 LPF and 585 LPF to 249 LPF and 409 LPF at 14 DAT, respectively. The percentage of larval reduction observed for Bafog-1 (S) and Dipel ES plots at 14 DAT is 45% and 30%, respectively, suggesting that Bafog-1 (S) was more effective (*Table 2*). Such a reduction in larval numbers could be attributed to the conversion of larvae into the pupal stage as evident in *Table 2*. However, based on the total bagworm (larvae + pupae) (*Table 2*), there was a real decline in population (P<0.05). By the 14^{th} day, the decline in numbers was 53% for Bafog-1 (S) and 35% for the commercial Bt, suggesting that Bafog-1 (S) was more effective (*Table 2*). Based on a Student's t-test, with one-tailed distribution, there is an evidence that the mean of total bagworm population between Bafog-1 (S) and Dipel ES treatment at 14 DAT is significantly different as proved by the significant value, P=0.049 (P<0.05). At 30 DAT, Bafog-1 (S) performance was comparable to commercial Bt, at 60% reduction in population, respectively. However, the decline in larval numbers, 60% could be attributed to the conversion of larvae into the pupae stage. The increase in pupal numbers was observed at 30 DAT. Thus, within one cycle, Bafog-1 (S) was able to bring down the bagworm population to less than half the starting population. Ironically, Bt can persist in the oil palm environment for a month, provided of dry season. Furthermore, Bt effect could be clearly seen at seven to 14 DAT, which led to reduction of larvae population as evident in *Table 2*. Yusdayati *et al*. (2012) reported a field trial of *Bacillus thuringiensis* ssp. *kurstaki* in controlling *Metisa plana* which showed effectiveness of Bt at 14 DAT. The mortality caused by Bt is slow and it normally takes two to three days for the larvae to die (Knowles, 1994). This explained the effective mortality of larvae is proven at one to two weeks after treatment.

TABLE 2. MEAN POPULATION DENSITY OF *P. pendula* TREATED WITH BAFOG-1 (S) AND COMMERCIAL *Bacillus thuringiensis* (Bt)

Day after treatment	Population density of bagworm (No./frond)							
	Bafog-1 (S)				Commercial Bt			
	Larvae	Pupae	Total bagworm	% Reduction	Larvae	Pupae	Total bagworm	% Reduction
0	451.3±9.7a	118.4±6.8a	569.3±9.9a	-	585.1±9.9a	83.3±8.5a	668.4±10.1a	-
7	365.5±8.5a	0.9±1.3b	367.2±8.5b	35.5	352.4±7.6b	5.0±2.2b	357.2±7.8b	46.6
14	249.2±6.3b	17.2±4.5b	265.7±6.1c	53.3	408.6±8.2b	23.2±3.7b	431.8±8.3b	35.3
30	40.4±2.8c	178.8±7.1c	219.2±5.9c	61.5	37.3±2.7c	230.4±9.8c	267.3±9.7c	60.0

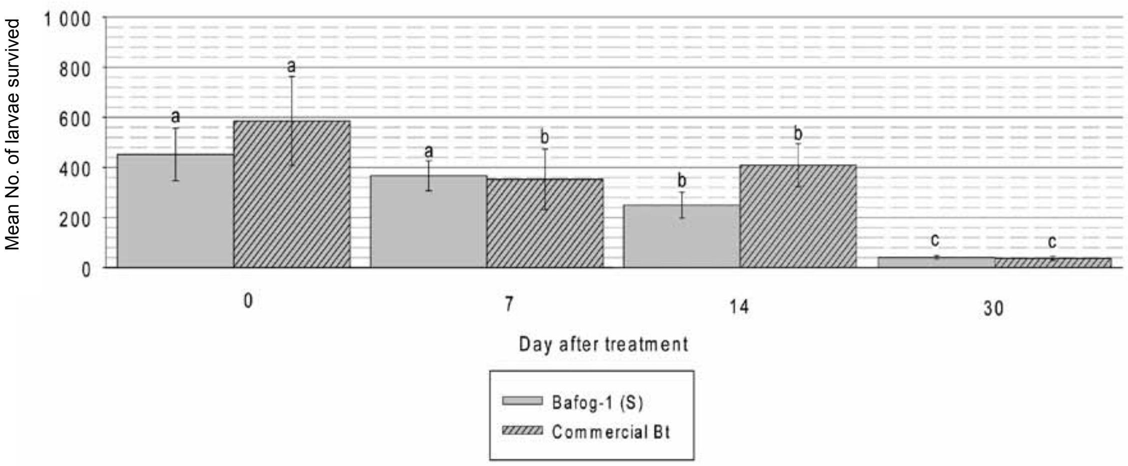
Note: N = 120 and commercial Bt = Dipel (ES) as comparison. Different letters within the same treatment indicate significant difference at P<0.05.

Bafog-1 (S) has proven to be equally effective as commercial Bt, Dipel to control *P. pendula* in oil palm plantations. The application of Bt fogging will be an alternative for ground spraying technique for controlling bagworm outbreaks on oil palm on peat soil. The application of Bt fogging was significantly harmless with no adverse effect on the beneficial insects, especially parasitoids as compared to cypermethrin (Najib *et al.*, 2012). Generally, the application of Bt by using the ground spraying technique was as good as the chemical application (Yusdayati *et al.*, 2012). The use of Bt in IPM programme is able to control bagworm population to below economic threshold level (Siti Ramlah *et al.*, 2012) when it is incorporated with the planting of beneficial plants such as *Cassia cobanensis* and *Turnera* sp. and pheromone trapping by using the receptive female bagworms. Being environmental-friendly, Bt is an ideal alternative for chemical such as monocrotophos that has been banned in some countries of the world (Siti Ramlah *et al.*, 2012).

The overall time course reduction in the population was partly attributed to the synergistic effect caused by natural enemies that had been established with the presence of beneficial plants in the experimental sites (Siti Ramlah *et al.*, 2007). It was observed that the estate has planted several species of beneficial plants such as *Antigonon leptopus*, *Turnera ulmifolia* and *Cassia cobanensis* along the roadside. It is well-known that beneficial plants can produce nectar to prolong life-span and sustains the population of natural enemies in oil palm plantations (Basri *et al.*, 1999).

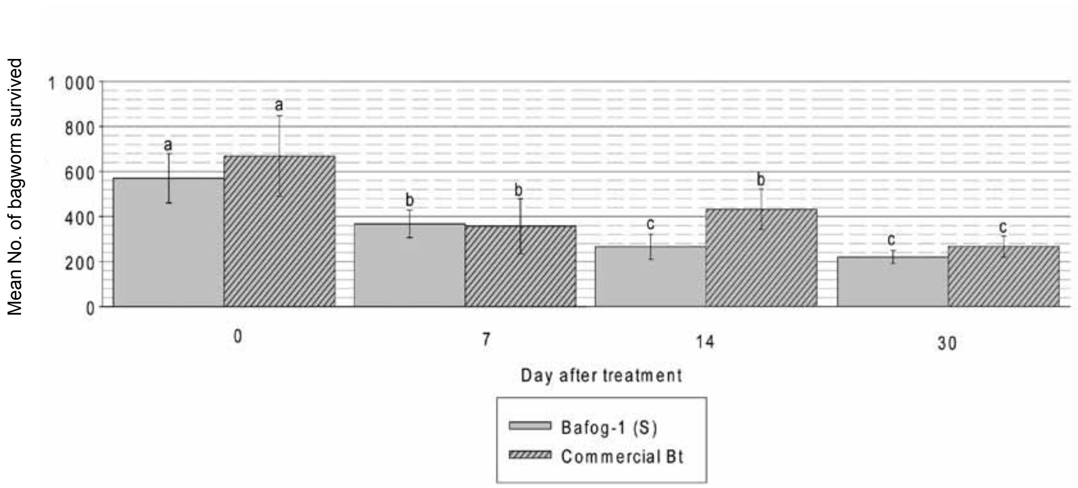
Figure 2 clearly shows that larval population in Bafog-1 (S) plots decreased significantly (P<0.05) from 451 LPF at 0 DAT to 249 LPF (45%) at 14 DAT and 40 LPF (91%) up to 30 DAT. Whereas, in Dipel ES plot, the larval population only decreased from 585 LPF at 0 DAT to 409 LPF (30%) at 14 DAT.

From another point of view, based on total bagworm population (larvae + pupae) in *Figure 3*, the total bagworm populations in Bafog-1 (S) and Dipel ES plots were significantly reduced (P<0.05) from 569 bagworm per frond (BPF) and 668 BPF at 0 DAT to 266 BPF and 432 BPF at 14 DAT, respectively. The census at 30 DAT indicated that the *P. pendula* was



Note: Bars with different letters within the same treatment indicate significant difference at P<0.05 after Student's t-test.

Figure 2. Larvae survival after first fogging using Bafog-1 (S) and Dipel ES at Hutan Melintang.



Note: Bars with different letters within the same treatment indicate significant difference at P<0.05 after Student's t-test.

Figure 3. Total bagworm (larvae + pupae) survival after first round of fogging using Bafog-1 (S) and Dipel ES at Hutan Melintang.

at the pupal stage and was slightly above threshold level of 10 LPF. This suggested that another round of fogging was required for the subsequent generation of *P. pendula*.

CONCLUSION

This study showed that Bafog-1 (S) can be used by the fogging method to control bagworm, *P. pendula* when the population is very high. Laboratory bioassay and field trial results showed that fogging of Bafog-1 (S) reduced the bagworm population. It is proven by Student's t-test that the efficacy of Bafog-1 (S) in field application is comparable to commercial Bt, Dipel ES. This technique of application is simple, convenient and can reach palm canopies for effective control of bagworms for old palms on soft peat soil, where the use of tractor is not possible.

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