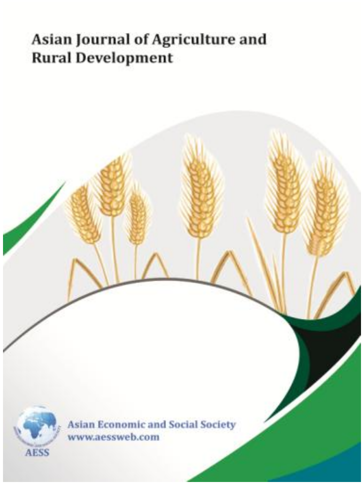
Publisher: Asian Economic and Social Society
ISSN (P): 2304-1455, ISSN (E): 2224-4433
Volume 3 No. 2 February 2013.



The Effective Application Time to Spray *Bacillus Thuringiensis* Subspecies Kurstaki for Managing Bagworm, *Metisa Plana* Walker on Oil Palm

Shahir M. S. (Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Shah Alam, Malaysia)
Mohd Rasdi Z.† (Senior Lecturer, Agriculture Entomologist, Faculty of Plantation & Agrotechnology, Universiti Teknologi MARA Pahang (UiTM), Pahang, Malaysia)
Fauziah I. (Associate Professor/Entomologist; Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Shah Alam, Malaysia)
Md Jamaludin B. (Senior Lecturer; Agribusiness Faculty of Plantation & Agrotechnology, Universiti Teknologi MARA Pahang (UiTM), Pahang, Malaysia)
Fakhrorazi A. (International Business Department, College of Law, Governance, and International Studies (COLGIS), Universiti Utara Malaysia, Malaysia)
Ismail R. (Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Shah Alam, Malaysia)
Mohd Hanysyam M. N. (Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Shah Alam, Malaysia)
Norazliza R. (Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Shah Alam, Malaysia)

Citation: Shahir M. S., Mohd Rasdi Z., Fauziah I., Md Jamaludin B., Fakhrorazi A., Ismail R., Mohd Hanysyam M. N. and Norazliza R. (2013) "The Effective Application Time to Spray *Bacillus Thuringiensis* Subspecies Kurstaki for Managing Bagworm, *Metisa Plana* Walker on Oil Palm", Asian Journal of Agriculture and Rural Development, Vol. 3, No. 2, pp. 72-78.

† Corresponding Author

Asian Journal of Agriculture and Rural Development, 3(2): 72-78

The Effective Application Time to Spray *Bacillus Thuringiensis* Subspecies Kurstaki *f*or Managing Bagworm, *Metisa Plana* Walker on Oil Palm

Author(s)

Shahir M. S.
Universiti Teknologi MARA, Shah Alam, Malaysia
Mohd Rasdi Z.
Universiti Teknologi MARA Pahang (UiTM), Pahang, Malaysia
Fauziah I.
Universiti Teknologi MARA, Shah Alam, Malaysia
Md Jamaludin B.
Universiti Teknologi MARA Pahang (UiTM), Pahang, Malaysia
Fakhrorazi A.
Universiti Utara Malaysia, Malaysia
Ismail R., Mohd Hanysyam M. N., Norazliza R.
Universiti Teknologi MARA, Shah Alam, Malaysia

Abstract

This study was conducted to determine the suitable time to apply the Bacillus thuringiensis sub species kurstaki against the larvae of the Metisa plana oil palm. The aim of this study is to search for the most economical method for using the B. thuringiensis sub species kurstaki. Owing to that matter, the larvae was sprayed at three different times, 0800, 1200, and1600, with 0.68ml of the B. thuringiensis sub species kurstaki product Dipel ES®/200 ml of water, under fully netted, rain sheltered conditions. Results showed the effectiveness of the B. thuringiensis sub species kurstaki in the control of bagworm, with more than 90% larvae mortality in three days after treatment. However there are no significant different in mortality rate and duration killing time for M. plana larvae on these three application times

Keywords: Metisa plana, Bacillus thuringiensis, Oil palm

Introduction

With the Ninth Malaysian Plan, agriculture has been earmarked as Malaysia's third engine of growth for the economy in the new millennium. Therefore, the oil palm, as the most important and the most produced crop planted in Malaysia, plays a vital role in ensuring that this national aspiration becomes a reality. Taking into account the economic importance of oil palm crops to the country's economy, the productivity of the oil palm must increase, but a key obstacle in this objective is the presence of pests and diseases in the estate. Bagworm (*Metisa plana*) is one of the most serious leaf-eating insect pests of oil palm in Malaysia (Basri and Norman, 2002). The bagworm is a polyphagous insect that feeds on several crops. It belongs to the order *Lepidopera* and is among the family *Psychidae*, which include several species. This insect pest can be recognized by the case or bag that the caterpillar forms, which are made of silk and the plant host's leaves and twigs. The cases are usually hanging onto the oil palm leaves on which it feeds.

The larval stage is the destructive stage of bagworm, because the larvae feed mainly on the palm leaves. High population densities can lead to the complete skeletonization and death of the fronds, which has caused sporadic economic damage and crop losses. The occurrence of these insect pests in the estate leads to the suppressed growth of the oil palms and may eventually cause economic injury. Bacterial pathogens used for insect control are spore forming, rod-shaped bacteria in the genus *Bacillus*. They are commonly found in soil, and most insecticidal strains have been isolated from soil samples (Weinzierl et al., 1997). *B. thuringiensis* is a naturally occurring soil bacterium whose poison causes disease to the insect (PIP, 1994).

The insecticidal properties of *B. thuringiensis* have long been recognized. The Egyptians were aware of the insecticidal properties of what probably was *B. thuringiensis* and used it to control insect pests, while the organism was first isolated about 100 hundred years ago in Japan from silkworm larvae suffering from the disease name "flacherie." The organism was known as *B. thuringiensis* by Berliner in 1991, who isolated it from the diseased flour moth larva (Nester et al., 2002). The crystalline protein inclusion produced by *B. thuringiensis* in the course of sporulation was responsible for the insecticidal action (Nester et al., 2002). Insecticidal products of B.t. were first commercialized in France in the late 1930s. For over 40 years, *B. thuringiensis* has been applied to crops as an insecticidal spray, a mixture of spores and the associated protein crystals.

By 1995, a total of 182 *B. thuringiensis* products were registered by the United States Environmental Protection Agency (EPA), but in 1999, *B. thuringiensis* formulations constituted less than 2% of the total sales of all insecticides. The use of *B. thuringiensis* has increased as insects have become resistant to chemical insecticides. Recently, the use of *B. thuringiensis* in pest control has increased substantially through more widespread use by both conventional and "organic" food producers, as well as in forestry (Nester et al., 2002).

There are several methods to control a bagworm population in a plantation, but mostly all control methods use chemicals, such as trunk injection, knapsack spraying, and mist-blowing. Increasing awareness about the adverse effects of chemical pesticides on human health and the environment put pressure on the plantation sector to replace controlling methods with those that are more environmentally friendly. One of the main replacement methods is using *B. thuringiensis* (B.t.) as a biological insecticide to control bagworm. This study was carried out to determine the effectiveness of *B. thuringiensis* to control a population of bagworm and to identify the most suitable time to apply the *B. thuringiensis*.

This study was carried out by determining and identifying bagworm as either alive or dead after spraying. The samplings and observations were conducted at days 1, 3, 7, 15, and 30 after *B. thuringiensis* application. The data were collected according to the number of bagworms that were dead or alive. The results of the study are important to provide baseline information on the effectiveness of *B. thuringiensis* for controlling the population of bagworm, as well as providing insight into the effective timing to spray *B. thuringiensis* . This is to make sure that a proper control method can be carried out and avoid overuse of *B. thuringiensis,* which can increase cost plantation management. Standard safety measures could be prepared and more extensive studies could be planned for future research following the outcome of this study. The objectives of study are (1) to determine the effectiveness of the *B. thuringiensis* sub species kurstaki for controlling the bagworm population, (2) and to identify the suitable time to spray the *B. thuringiensis* sub species kurstaki.

Materials and Methods

Experimental site

The study was conducted in Pusat Perkhidmatan Pertanian Tun Razak (PPPTR). The experimental site is situated at latitude 3º 50'N, longitude 102º 34'E and 76.0 meters above sea level. This study was conducted in the fully netted rain shelter at the Crop Protection Department of PPPTR. The size of the experimental site was 30 meters^2 with a total plant density of 1m x 1m spacing. The age of the oil palms sampled was about 1½ years-old, of the variety Dura x Psifera, or Tenera. The poly bags were arranged using Complete Randomized Design (CRD).

The area inside the rain shelter was cleaned and the grasses were cut and sprayed with herbicide to destroy any potential breeding sites for the pest. The poly bags of oil palm trees at age 1½ years old were arranged according to CRD in the rain shelter, and marked according to the application time of the B.t. and the number of replications [Figures 1 (a)]. The oil palm trees were trimmed to eliminate the small canopy, to avoid the overcrowding the fronds and making the task of counting the bagworms more difficult.

The stock of female bagworm were placed into plastic cages and attached to the oil palm leaves [Figure 1(b)]. Soon after hatching from the eggs, the bagworm larvae were allowed to spread on the oil palm leaves in the host plant [Figure 1(c)]. After the bagworm larvae reached instar two, the larvae were distributed to the experimental unit by cutting the oil palm leaves in the host plant and subsequently attaching the leaves to each experimental unit. When the leaves were dried, the bagworms climbed to the green leaves in the experiment unit in order to access the new leaves for food. In order to get a uniform distribution, the larvae were picked and transferred to other leaves in the experimental unit.

After two days, the pre-census was conducted and the equipment needed were assembled [Figure 1(d)]. The mixture of *B. thuringiensis* kurstaki (Dipel ES) was prepared in the biker [Figure 1(e)]. The 0.68 ml concentration of *B. thuringiensis* was measured using a pipette and poured into the biker. Then, 200 ml of water were added and the mixture was stirred for about 2 minutes to make sure the solution was evenly mixed. Then, the mixture was poured into the hand spray. The *B. thuringiensis* were applied at 0800, 1200, and 1600 [Figure 1(f)]. The mixture needed to be shaken frequently before being sprayed on oil palm trees to prevent the Dipel ES® from accumulating on the bottom of the hand spray. This may affect the concentration of mixture, and also a barrier made of plastic was used to avoid spray moisture travelling to the other experimental unit. One solution was used for only one tree and sprayed uniformly onto the whole tree.

Data collection

The pre- census was made before the plant sprayed by *B. thuringiensis* by counting the number of bagworm larvae in the each plant. The post-census data were collected five times within one month, 6 until July, 5 2009. Data were taken at day 1, 3, 7, 15 and 30 after sprayed with *B. thuringiensis*, then recorded by identified and counted number of dead and alive of bagworm larvae in each plant were calculated. Data on timing sprayed were collected three times a day at 0800, 1200 and 1600. The data were analyzed and the percentage of dead and living bagworm larvae was counted. The study was conducted within the duration of one month. The data were collected five times on day 1, 3, 7, 15 and 30 after treatment. The numbers of dead and alive bagworms were counted, recorded, and analyzed.

Data Analysis

Analyses of variance were performed to show the significant difference in the mean number of bagworms between three applications over time and the rate of mortality achievable.

Results and Discussion

From the ANOVA table, the result shows that there is no significance difference ($p<0.05$) between the time of the applications to the mortality of the bagworm larvae. The interaction between the time of spray and of the sampling date also shows no significance difference ($F=0.78$; $df=8,45$; $p>0.05$). Besides, the percentage of mortality shows the significance difference ($F=922.9$; $df=4,45$; $p<0.001$) between sampling dates.

Table 1: Two-way ANOVA on Bagworms, *Metisa plana* Mortality after Treated *Bacillus thuringiensis*

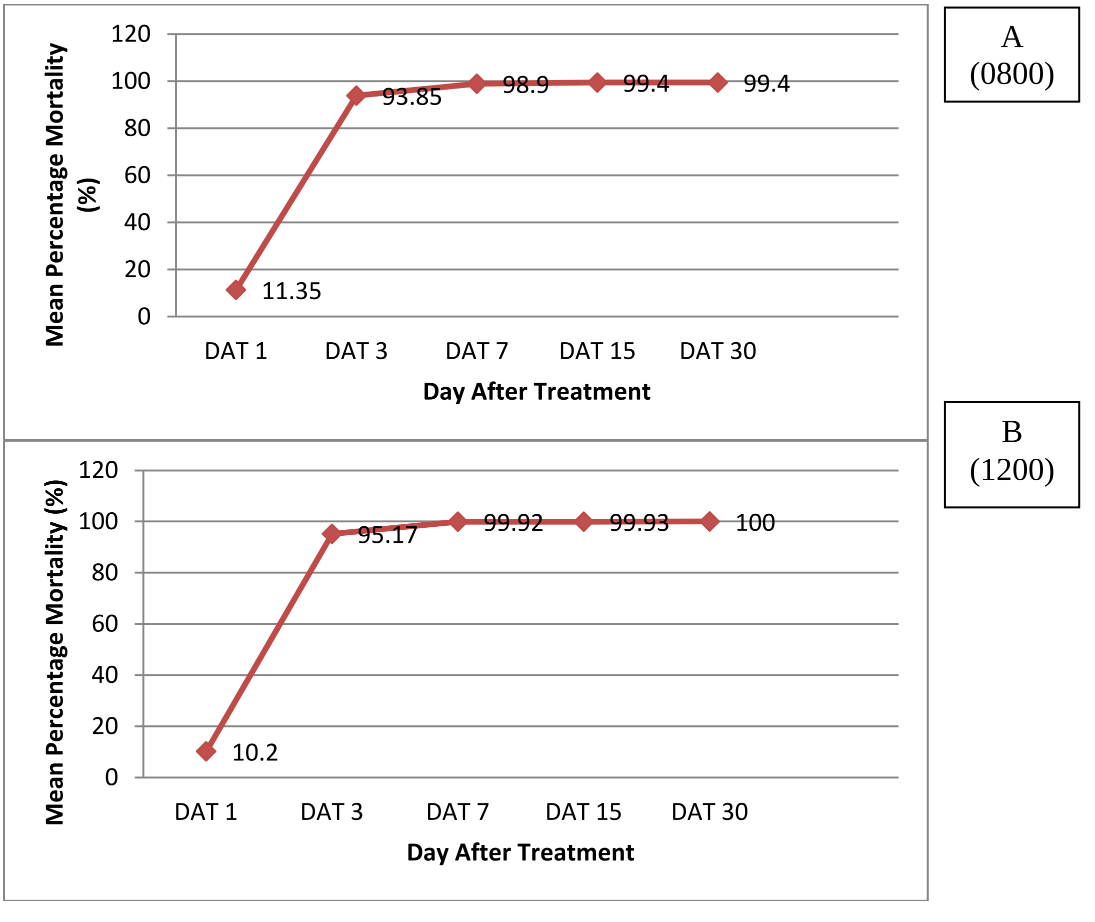
Source	df	SS	MS	F	P
Time (T)	2	20.8	10.4	0.55	0.581
Sampling Date (D)	4	70106.6	17526.6	922.9	0.000
(T) x (D) Interaction	8	118.5	14.8	0.78	0.623
Error	45	854.6	19		
Total	**59**	**71100.5**			

$S=4.358$; $R^2=98.8\%$; R^2 adj=98.42

Mortality Rate Patterns of Larvae *Metisa Plana*

Figures 2(A) to 2(C) show the mean percentage mortality of larvae *Metisa plana* derived from three different times of application on five different sampling dates at days 1, 3, 7, 15, and 30 after treatment (DAT). Results showed that *B. thuringiensis* can effectively control the early instar of bagworm larvae, as it gave higher than a 90% kill at 3 DAT. The curves were fitted to show the patterns of the mortality rate of the bagworm larvae at 1 DAT and up to 30 DAT. One day after treatment, the mean percentage mortality was low with only 11.35% of larvae killed at the 0800 application time and 10.2% at 1200. At the 1600 application time, the mean percentage mortality was 17.45%, which was slightly higher compared to the mortality of larvae at 0800 and 1200.

These finding are probably due to the physiological characteristics of bagworm larvae, in which active feeding is in the evening; therefore, greater numbers of bagworm larvae have already consumed *B. thuringiensis*. However, at 3 DAT, the mortality of bagworm larvae increased drastically and more than 90% of the total larvae were killed. The highest mortality was noted at 1200, with the total mortality rate of bagworm larvae at 95.18%, followed by 93.85% at 0800, and 92.59% at 1600 (Figure 2C). At 7 DAT, the mortality of bagworm larvae was continuously increased at a decreasing rate. Only 5.05% of an increase was noted at 0800, with 4.75% at 1200, and 7.4% at 1600. The total mortality of bagworm larvae was 98.9%, 99.2%, and 99.99% at 0800, 1200, and 1600 respectively. At 15 and 30 DAT, no major differences between the mean percentage mortality and three application times were found. At 15 DAT, very small numbers (0.57%) of bagworm larvae were still alive at 0800, and these larvae were still alive until 30 DAT. Perhaps the residual activity of *B. thuringiensis* has been already lost. Some larvae were also observed still alive at 1200 for 15 DAT, but all these bagworm larvae were killed at 30 DAT. At the 1600 application time, 100% mortality was noted at 15 DAT.



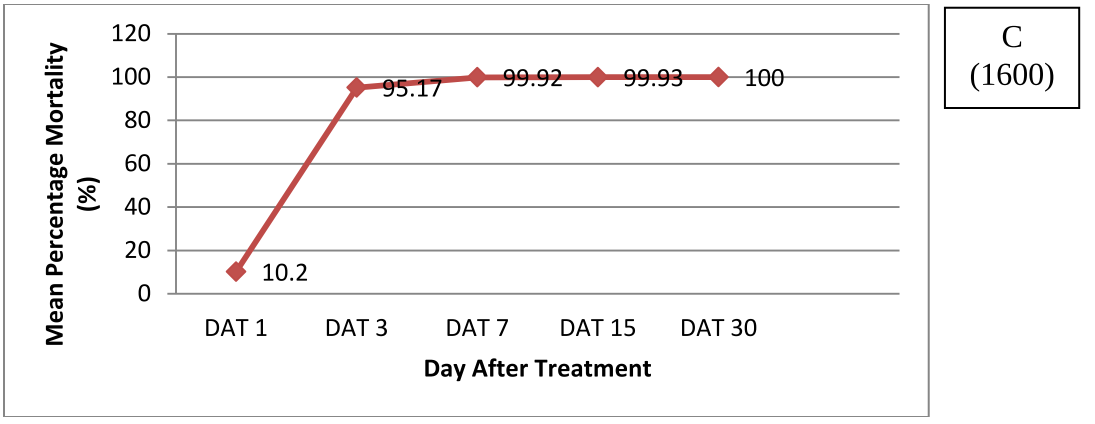


Figure 2: Mean Percentage Mortality of Larvae *Metisa plana* at three different times 0800 (2A), 1200 (2B) and 1600 (2C) of application

Numbers of Larvae Between Sampling Dates

Result shows that, a significance difference between sampling at day 1 after treatment, with day 3, 7, 15 and 30 after treatment. Findings are also noted that no significance difference in mean number of larvae dead between day 3, 7, 15 and 30 after treatment. The results are summarized in Figure 3, where the histograms with same letter indicate no significance different between sampling dates. *Vice-versa*, histogram with different letters shows the significance in mean numbers of bagworm larvae mortalities.

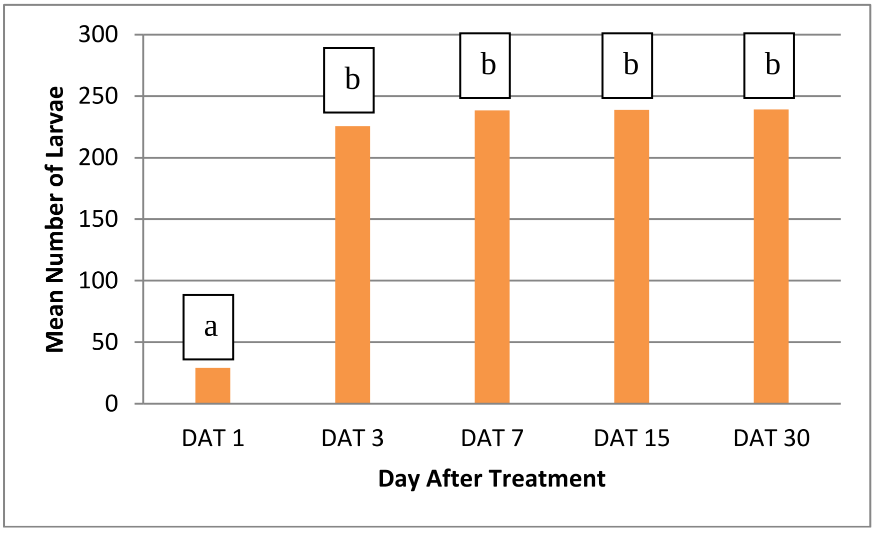


Figure 3: Mean Number of Bagworm larvae, *Metisa plana* at Different Sampling Dates

Discussion

Results from the study show that the *Bacillus thuringiensis* sub species kurstaki (B.t.k) could successfully control more than 90% of *Metisa plana* larvae after 3 days of treatment, and could effectively kill 100% of the larvae after one week. This finding was supported by Tan et al. (2008), which was conducted in a laboratory where the results concerning larvae mortality showed that a pesticide formulation containing *B. thuringiensis* was intrinsically effective on *Metisa plana* larvae and lead to a high larvae mortality rate (70-100%) within seven days after treatment.

One day after treatment, larvae mortality is low (less than 18%). This finding is supported by Rowell (2005), who noted that *B. thuringiensis* does not work like most conventional insecticides in that *B. thuringiensis* has no contact or "knock down" effect; insect pests are not killed instantly after spraying. In order to be effective, *B. thuringiensis* must be eaten by insects in the immature, feeding stage of development referred to as larvae (PIP, 1994). After three days of treatment, larvae mortality rates increased rapidly (>90%). This finding is in accord with what has been reported in pesticide information (2004), which states that the infection causes death within 2 to 3 days. These *B. thuringiensis* proteins paralyze the digestive system, and the infected insects stop feeding within hours. *Bacillus thuringiensis* affected insects generally die from starvation, which can take several days (Cranshaw, 2008). In this study, early instar (instar two and three) were used as the experimental subjects, and the results showed that more than 90% of larvae were killed three days after the treatment was applied. Tan et al. (2008) in their laboratory experiment reported that 3rd instar larvae were more susceptible to *B. thuringiensis* (more than 90% at 3 DAT) than 5th instar, and that the speed of killing was faster for younger larva. Rowell (2005) also suggested that *B. thuringiensis* must be used when insect larvae are small. There are no significant differences in terms of mortality and the speed of killing between treatments at different application times.

This finding was in contradiction to Weinzierl et al. (1997), in which they suggested that the application of *B. thuringiensis* in the late afternoon or evening can be helpful because the insecticide remains effective on foliage overnight before being inactivated by exposure to direct sunlight the following day. Treating the oil palms on cloudy (but not rainy) days provides a similar result. In this study, the results showed that in a fully netted rain shelter, the different application time has no effect on the residual insecticidal activity of *B. thuringiensis*. Behle et al. (1997) stated that comparing results for plots covered with clear plastic and those with no covers shows that the clear plastic tent had little effect on the loss of insecticidal activity. Therefore, using clear plastic as a shelter from natural rain with little to no effect relative to light degradation should be possible (Behle et al., 1997).

In this study, the existing fully netted rain shelter was preventing *B. thuringiensis* from being exposed to the variability of weather conditions that may inactivate residual *B. thuringiensis* . During this study, the process of identifying which larvae were alive or dead was facing some difficulties. Tan et al. (2008) stated that the larvae were considered dead when they did not respond to being prodded. In this study, however, the larvae were occasionally inactive during the morning, due to wet conditions caused by early morning dew, and as such the larvae did not respond to prodding. Alternatively, if the eating marks were observed, the eating marks that were still green indicated that a larva was still alive. If the eating marks were dark, it indicates that the larvae have stopped feeding. Then the teasing method was applied – dead larvae produce brownish-black liquid, similar in color to the *B. thuringiensis* product used (Dipel ES®), when they are squeezed. In some cases, the bags of larvae were empty but still attached on the leaves. This decision was made based on report by Rowell (2005), which described that some larvae killed by *B. thuringiensis* may turn blackish in colour. Besides that, dead larvae often shrivel and fall from the plant, where they are not easily observed by farmers.

Conclusions

Bacillus thuringiensis sub species *kurstaki* (Dipel ES®) was effective in controlling early instar of *Metisa plana* under the conditions of a fully netted rain shelter. The speed of killing grew faster (>90% killed) at three days after treatment, and 100% were killed after seven days of treatment. In the fully netted rain shelter setting, the different application time showed no significant difference between the mortality rate and the speed of killing to the larvae. Further study is recommended in a field condition, with UV light intensity taken into consideration, using the equipment such as the luxmeter to determine the effect of UV light on the effectiveness of the residual activity of *B. thuringiensis* .

References

Basri, M. W. and Norman, K. (2002). *Cassia cobanensis* as A Beneficial Plant for Sustenance

Parasitoid in Bagworm Control, *MPOB information series*, 2pp.

Behle, R. W., McGuire, M. R. and Shasha, B. S. (1997). Effects of Sunlight and Simulated Rain on Residual Activity of *Bacillus thuringiensis* Formulations, *J. Econ. Entomol.,* 90(6), 1560-1566.

Cranshaw, W. S. (2008). *Bacillus thuringiensis*, *Colorado State University Extension*, 5, 556. 4pp.

Nester, E. W., Thomashow, L. S., Metz, M. and Gordom, M. (2002). 100 Years of *Bacillus thuringiensis*: A critical scientific assessment, American Academy of Microbiology, 22pp.

Pesticide Information Profile, PIP (1994). *Bacillus thuringiensis*, Extension Toxicology Network, Cornell University, Ithaca, NY.

Rowell, B. (2005). Bt basic for Vegetables Integrated Pest Management. IIPM Newsletter #11. Dept. of Horticulture, University of Kentucky, 8pp.

Tan, S. Y., Yusof, I. and Dzolkhifli, O. (2008). Efficacy of *Bacillus thuringiensis* Berliner Subspecies Kurstaki and Aizawai Against the Bagworm, *Metisa plana* Walker on Oil Palm*. Journal of Bioscience*, 19(1), 103-114.

Weinzierl, R., Henn, T., Koehler, P. and Tucker, C. L. (1997). Microbial Insecticide. *University of Florida publication*, ENY- 275, 173-190.