

## Development of bio-pesticides from bio-oil of oil palm biomass waste (palm kernel shell) against *Metisa plana* Walker bagworm (Lepidoptera: Psychidae)

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### Abstract

Despite the abundance of palm-based residues generated, the by-products from thermochemical processing such as bio-oil may create value-added products to the palm industry. The palm-based derived bio-oil contains high aromatic compounds, which are active ingredients in the bio-pesticides formulation. Therefore, this study investigated the formulation of the bio-pesticide from this bio-oil and determines their effect on insect-pest in oil palm such as *Metisa plana* walker bagworm. Prior the formulation, preliminary evaluation of the compatibility between bio-oil and surfactants such as Tween 20 and Tween 80 as the bio-pesticides ingredient were evaluated using the ternary phase diagram. The compatibility results showed the best formulation is at 20% of surfactant. Based on these conditions, the experiment was formulated using an active ingredient (AI) called azadirachtin extracted from neem seed. The formulated bio-pesticide was tested for its effectiveness towards the mortality of the bagworm. The results showed that the formulated bio-pesticide was able to repel 50% of the bagworm population, with a lethal concentration (LC50) of 22.1 g/mL showing a good indicator as an effective repellent. Hence, this study provided new knowledge for waste management towards zero waste strategy for a better environment and sustainability.

## 1. Introduction

Various palm biomass such as palm kernel shells (PKS), empty fruit bunches, and mesocarp fibers have been accumulated in numerous palm oil mills in the nation and pose challenges in waste management (Kabir *et al.*, 2018). For this reason, proper utilization of solid biomass waste is necessary for environmental and economic reasons (Mushtaq *et al.*, 2015). Thus, it is vital to explore the gaps in making a more sustainable oil palm industry (Khatun *et al.*, 2017). Therefore, palm biomass's valorization into other useful products such as bio-oil through thermochemical processes such as pyrolysis (Baloch *et al.*, 2018). In general, bio-oil from pyrolysis was used in boilers, diesel engines for power generation or industrial boilers, and kilns to replace fossil fuels (Kim *et al.*, 2010). Recent studies also reported that the conversion of palm kernel shells (PKS) to bio-oil under thermal processing provides a more significant benefit. It minimizes the disposal problems associated with the agricultural wastes (Ogunkanmi *et*

*al.*, 2018). Bio-oil from palm kernel shell is the focus of this study due to the compounds it poses, such as phenol and methyl ester (Chan *et al.*, 2018). These compounds are vital as methyl ester is known to be one of the carriers in the formulation of pesticides (Massaguni *et al.*, 2016). Recently, more researches explored to produce eco-friendly carrier for pesticide formulation rather than relying on petroleum-derived products (Chin *et al.*, 2012). Significantly, pesticide formulations also comprise active ingredients in charge of the pesticidal effect and inert ingredients such as surfactants in order of improving product performance, stability, and usability (Megat Nabil Mohsin *et al.*, 2017). Based on previous studies, botanical insecticide active ingredient, i.e. azadirachtin has the highest insecticidal activity (Zheng *et al.*, 2018).

The major insect pests that cause outbreaks in the oil palm plantation are bagworms (Ahmad *et al.*, 2013). Bagworms such as *Metisa plana* Walker (Lepidoptera: Psychidae) are caterpillars fed on oil palm leaflets

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(Kamarudin et al., 2010). Over time the population of bagworms will increase above its threshold level (Ahmad et al., 2017). Despite extensive efforts, effective management of this pest in the oil palm industry remains unresolved (Halim et al., 2018). Hence, appropriate pest management strategies control bagworms and maintain a healthy oil palm plantation (Kok et al., 2012). This paper highlights the investigation on the formulation of bio-pesticide from bio-oil and its' effect on insect-pest in oil palm such as *Metisa plana* bagworm.

## 2. Materials and methods

The formulation of bio-pesticide consists of three stages, namely compatibility testing on the components (bio-oil and surfactants), analysis of the potential of active ingredient (AI) (azadirachtin), and bio-assay studies of the formulated bio-pesticide on bagworm, *Metisa plana*.

### 2.1 Compatibility of bio-oil and surfactants

The compatibility tests performed to evaluate the suitable composition of bio-oil and surfactant in biopesticide formulation. For this test, the mixture of the theoretical weight of filtered bio-oil and surfactants (a) and deionized water were prepared based on Equation (1) and (2):

$$b = (c / (c + a)) \times 100 \quad (1)$$

$$c = (b a / (100 - b)) \quad (2)$$

Where *a*: weight of oil + weight of surfactant, *b*: percentage of total deionized water (%), *c*: weight of total deionized water content (g) and *d*: weight of deionized water added for each rotation (g).

Later, Equations (3) and (4) were used for each test tube every cycle when deionized water was added in order to calculate the weight of the actual deionized water based on the theoretical weight for each cycle:

$$\text{Theory m (c)} - \text{Theory n (d)} = \text{Theory m (d)} \quad (3)$$

$$\text{Actual m (d)} + \text{Actual n (c)} = \text{Actual m (c)} \quad (4)$$

Where number of cycle,  $n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11$  and  $m = n + 1$ .

Each test tube was then centrifuged and observed for the formation of different phases exist.

The phased presence was evaluated based on the ternary phase diagram (TPD) using Ternary Plot in CHEMIX School software version 7.00 (Arne Standness, Bergen, Norway).

### 2.2 Neem extraction, analysis, and mixture formulations

Neem seeds were washed with running water and let

to be dried at 50°C for 24 hrs. The weight of neem seeds before and after dried was measured. The dried neem seeds were then crushed into 1-2 mm particle size using the conventional blender. Next, 250 g of powdered neem seed was stirred in 1 L of hexane using a magnetic stirrer at 40°C for 2 hrs. After 2 hrs, the residue was filtered, and the extracted oil was kept in a bottle. The defatted neem powder was added into 1 L of methanol and was stirred using a magnetic stirrer at 1500 rpm at 40 °C for 2 hrs. The compositions of AI (azadirachtin) obtained in this extraction process were analysed using UV-Vis spectrophotometer (HACH). Different concentrations of the bio-oil and neem extract were prepared according to the ratio in Table 1.

Table 1. The mixing ratio of bio-oil to neem extract

Samples	Bio-oil: Neem extract
A	10:00
B	8:02
C	5:05
D	2:08
E	0:10

### 2.3 Formulation of bio-pesticide composition

Various combinations of oil (bio-oil and neem extract) and water ratio at constant surfactant percentage (20%) were formulated using TPD as a reference and summarized in Table 2. Each sample was well-mixed using vortex and then centrifuged at 3500 rpm for 30 minutes. Model. The appearance and physical properties of each sample were then analysed and recorded.

Table 2. The composition ratio of bio-pesticide formulation

Formulations	Surfactant	:	Combined oil	:	Water
A <sub>1</sub> ,B <sub>1</sub> ,C <sub>1</sub> ,D <sub>1</sub> ,E <sub>1</sub>	2	:	8	:	0
A <sub>2</sub> ,B <sub>2</sub> ,C <sub>2</sub> ,D <sub>2</sub> ,E <sub>2</sub>	2	:	6	:	2
A <sub>3</sub> ,B <sub>3</sub> ,C <sub>3</sub> ,D <sub>3</sub> ,E <sub>3</sub>	2	:	4	:	4
A <sub>4</sub> ,B <sub>4</sub> ,C <sub>4</sub> ,D <sub>4</sub> ,E <sub>4</sub>	2	:	2	:	6

### 2.4 Bio-assay of *Metisa plana* bagworm

The 3<sup>rd</sup> or 4<sup>th</sup> instar larvae of *Metisa plana* were tested using the formulated bio-pesticides. During the testing, the samples were placed in a plastic cup, fed with some palm oil leaves coated with bio-pesticide cream for 10 s. The leaves kept dried at room temperature and tested on 3 instar larvae for each formulation with deionized water as a control sample. Meanwhile, the instar larvae were maintained under laboratory conditions at 26±2°C. The number of affected bagworms (dead/survive) from the tested formulated bio-pesticides was recorded over 4 days. Larvae that failed to respond to gentle prodding with a fine sable brush were considered as dead. The daily number of bagworms affected, the percentage of mortality and toxicity for

each combination of bio-oil and neem extract were observed and determined.

### 3. Results and discussion

#### 3.1 Ternary phase diagram

The ternary phase diagram is a tool used to understand the phase behaviour and stability of bio-oil (Li *et al.*, 2020). The results of these experiments are shown in Figures 1 and 2. From the figures, Tween 20 is more compatible with bio-oil than Tween 80, as more 1 phase (isotropic) appeared when it is mixed with water as well. This is because the solubility of Tween 20 in water is much higher than that of Tween 80 (Podgórska and Agata, 2016). The effect on phase behaviour of aqueous phase, or also known as a mixture of polyoxyethylene sorbitan fatty acid ester (Tween 20 or 80) with oil (such as vegetable oil consisting of soybean oil and rapeseed oil) and water show that only 20% solution was used as the aqueous phase (Prasert and Gohtani, 2016).

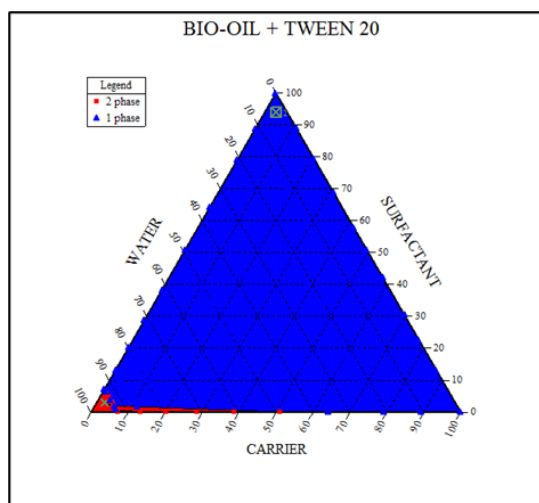


Figure 1. Ternary phase diagram for Tween 20

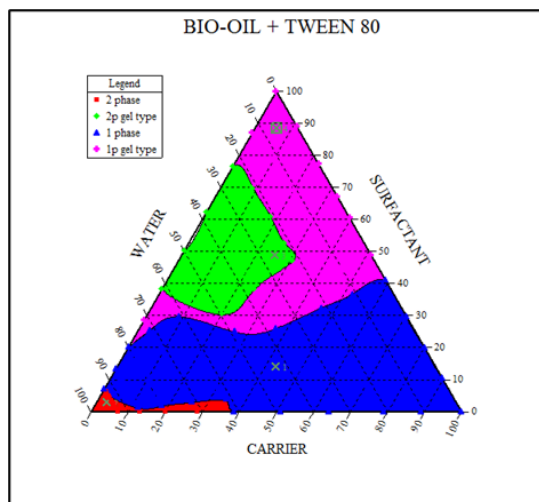


Figure 2. Ternary phase diagram for Tween 80

#### 3.2 Effect of various composition ratio of formulations

Figure 3 shows the pest did not respond quickly to

the bio-pesticide due to the bio-pesticides repellent ability that has been formulated from PKS bio-oil. This is because the bio-oil prior physical form of the liquid is dark brown, free-flowing, and has a strong acrid smell (Abnisa, Arami-Niya, Wan Daud and Sahu, 2013). A strong odour that can be smelled in each formulation shows that bio-oil formulation for A1, A2, A3, and A4 contained some compounds responsible for this odour. The pungent odour release due to the presence of phenol compounds, which can repel the bagworm and cause the bagworm to still alive on day 1. High molecular phenols are the dominant components in bio-oil from the pyrolysis of lignin-rich biomass from PKS (Esohe *et al.*, 2016). Besides, the number of affected bagworms increased on day 2 for all the concentration except for A3. This was because the A3 formulation did not release much odour.

However, one bagworm was dead on the following day, which died because of not consuming. On day 2, the largest number of deaths happened at the highest concentration of bio-oil, which was at A1. All the bagworms were dead on day 4. This shows that the bio-pesticide that has the highest concentration was able to kill most of the bagworm. The production of a large amount of bio-oil showed volatiles' presence during pyrolysis of PKS biomass (Abnisa *et al.*, 2013). Various bio-oil concentrations used in the bio-pesticide formulation for Sample A contain volatile compounds, which help control the bagworm. A high concentration of bio-oil indicates that the volatile compounds were more elevated and can cause a reaction faster. The volatile compound evaporated more quickly and released a strong odour, which could repel the pest away from the leaves. Thus, A1 of bio-pesticide of Sample A has the highest volatile matter cause the two out of three bagworms death quickly on the 2<sup>nd</sup> day compared with other concentrations and all the pest death at the end of the day. The higher concentration of the sample, the larger the number of deaths. Therefore, A1 of bio-oil was the most efficient bio-pesticide when compared with other formulation. The bio-oil that acts as repellent and antifeedant towards the *Metisa plana* larvae ensures the bagworm's fatality is able to achieve.

Besides, Figure 3 also shows that the number of bagworm death has significantly increased from the beginning of the experiment for sample B2 of combined sample B and the result remained constant for the following two days indicated that the remaining bagworm did consume the food for three days and finally died on day 4 onwards. The Sample of B4 with the lower concentration among other samples affected the bagworm after 3 days indicated that the time for the bio-pesticide to attack the bagworm was prolonged. This was

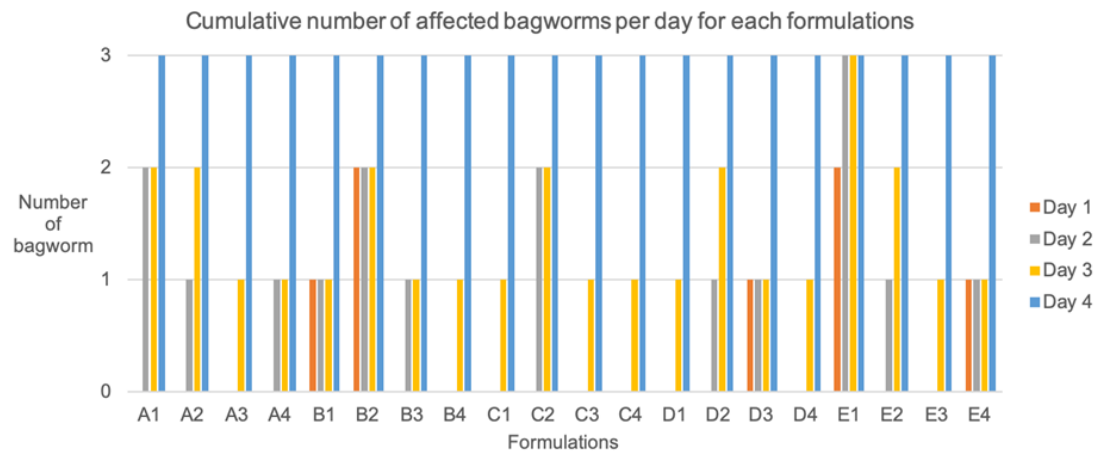


Figure 3. Cumulative number of affected bagworms per day for each formulation

due to the low volatile matters in the B4 that causes the bagworm to respond towards the bio-pesticide slowly. This was proven that higher concentrations such as sample B2 and B1 reacted quickly on the 1<sup>st</sup> day of testing. The higher volatile compounds have a lower attack time for the bagworm. However, Sample B1 only affected one bagworm only when compared with 30 g/mL on the first day. Sample B1 was not an excellent bio-pesticide formulation. The absence of water in the B1 formulation without any water ratio indicates that it was oil-based bio-pesticide as there is no water in the formulation. Since 100% of the sample B1 made up of oil and surfactant, the efficiency was lower than B2. Sample B2 has water, oil, and surfactant, which help the two miscible liquid to mix well. A suitable formulation can be obtained if water, oil as a carrier, and active ingredients with suitable surfactants would produce the best bio-pesticide formulation. Although sample B2 was also known as oil-based bio-pesticide, the efficiency was much higher than sample B1. Therefore, the oil in B1 can be tested straight away without mix it with surfactant. The surfactant is used to reduce the water surface tension by breaking the surface tension of water and helping the active ingredients be evenly dispersed in the water. Sample B2 killed all the bagworms, whereas sample B4 killed two bagworms, and sample B3 killed only one bagworm. Sample B4 killed a larger number of bagworms when compared to the B3 due to the starvation. Therefore, B2 formulation with a ratio of 1:3:1 was the most efficient bio-pesticide formulation for Sample B.

Sample C of various formulation were also been tested on the *Metisa plana*. The result obtained in Figure 3 shows that the C2 formulation was the best and most efficient among all those four formulations. The highest death of bagworms on 2<sup>nd</sup> day was obtained from the C2 formulation indicates that this was most effective. Furthermore, the number of deaths of other formulation were unable to achieve at least two bagworm death, denoting that samples C1, C3, and C4 were the weakest

formulation and not recommended. Thus, the lower concentration should not be used to formulate bio-pesticide for sample C combination (1:1). Also, C3 formulation and C4 formulation, which made up from the ratio of 1:2:2 and 1:3:1 respectively, shows that the combination of the compound in bio-oil and the active ingredient that found in the neem extract were unable to produce the best result toward the bagworm as their time to attack the bagworm was very slow. The death of bagworm might occur due to the repellent and antifeedant activity of combined bio-oil to neem extract. In spite of this, the highest concentration of C1 bio-pesticide also unable to kill the bagworm for the following two days implied that the surfactant with 100% combined oils could not make the best combination as the usage of surfactant without water in formulation make the bio-pesticide less competent. However, at least one bagworm is dead on day 3 after been starved for two days. Hence, C2 bio-pesticide was the most effective formulation that could kill the bagworms. However, D2 formulation was the greater effective bio-pesticide as the number of death increased gradually from day 2 and achieved maximum target end of Day 4. Even though sample D2 cannot attack the bagworm on Day 1, sample D3 and D4 cause one bagworm's death. The D3 formulation was the same as a result obtained for D4 formulation of bio-pesticide. However, the number of death remain constant throughout the experiment after day 1. As discussed earlier, the highest concentration could not kill all the bagworms and the reaction was prolonged. In conclusion, D2 formulation at a ratio of 1:3:1 was the most effective bio-pesticide for sample D.

The active ingredient's effect towards the different formulation of Sample E in Figure 3 implies that E1 formulation was the most effective formulation due to its tendency to kill all the bagworms at the end of day 2 indicates that the attacking time for E1 was the fastest when compared with other formulations. This formulation was able to kill two bagworms on the 1<sup>st</sup> day

while E4 formulation killed one bagworm, whereby E3 and E2 did not kill any. The fastest attacking time of the E1 due to its high concentration of azadirachtin in neem extract adept at killing large amounts of bagworms. The azadirachtin is the main component responsible for both antifeedant and toxic effects in pests. Most importantly, this is because the presence of azadirachtin in insects induces hormonal balances, resulting in the failure of moulting or dramatic deformation at immature stages of growth when used in the formulation of pesticides or bio-pesticide vitally (Asaduzzaman *et al.*, 2016). This study proved that the higher the azadirachtin content in the bio-pesticide formulation, the larger the affected bagworm would be. E1 formulation for sample E was the most effective formulation that could be used to kill most of the bagworm in a shorter period of time.

### 3.2 Analysis of azadirachtin from neem extract

The concentration of azadirachtin for each sample has been calculated and summarised in Table 1. Table 1 shows that sample E has the highest concentration of azadirachtin in the mixture since the ratio for sample E was (0:1). Thus, it has been proven that the azadirachtin compound was very high in sample E, whereby the mixture prepared with neem extract only. In conclusion, if the ratio of neem extract was increased from sample A to sample E, the concentration of azadirachtin also increased as in Table 1.

### 3.3 Mortality percentage of *Metisa plana*

Table 1 shows the mortality percentage of the bagworms for each mixing ratio sample of bio-oil and neem extract during testing using the bagworms. Each prepared sample was tested on 12 bagworms. Sample A was pure bio-oil, while Sample E was pure neem extract, which contains azadirachtin, while other samples were the combination of the mixtures. The pest was exposed to different mixing ratios, showing 50% mortality for sample A, 42% mortality for Sample B, Sample C, and Sample D and 58% mortality for Sample E (Figure 3). The highest fatality of bagworms was observed for Sample E due to its strong, active ingredient content, which makes it the most efficient bio-pesticide. Since Sample E was made up of neem extract that has been extracted from neem seeds using alcohol, azadirachtin is the leading cause to produce higher lethality percentage when compared with other samples. This is also supported by a past study that shown that azadirachtin caused both larval and pupal mortality toward a Lepidopteran insect (Rharrabe *et al.*, 2008).

Thus, Sample E was the most effective active ingredient that can be used to formulate a highly efficient bio-pesticide due to its ability to reach a higher lethality

percentage. Sample A containing bio-oil was the second-largest mortality of 50% after sample E. Sample A's fatality was 8% lower than that of Sample E, implying that the active ingredient found in the bio-oil is slightly less vibrant than the azadirachtin compound in neem extract. Moreover, the past study claimed that azadirachtin also killed the fourth instar larvae within 1–4 days, with peak host mortality occurring 2 days after treatment (Nathan and Kalaivani, 2006).

It was observed that the combination of bio-oil with neem extract was not the best formulation because the fatality percentage was lower than Sample A and Sample E. The lowest value was 42% obtained from the combination of bio-oil and neem extract. The value remains constant for three Sample B, Sample C, and Sample D. This can be explained by the fact that the active ingredient from different chemical groups can slow down the development of bio-pesticide resistance. However, the reduction of its efficiency of the formulation due to the lower mixing or combination of both active ingredients which contribute to reducing the efficiency of the formulation.

### 3.4 Values of LC50 for *Metisa plana*

The data were analyzed accordingly using Probit Analysis Method and the LC50 values were determined. Based on LC50 values, the toxicity of the formulations for 72 hrs after treatment in increasing order were sample E > Sample A > Sample B = Sample C = Sample D. All the Sample B, Sample C, and Sample D were categorized under the same level toxicity group as their LC50 values were the same. The observation of nano-emulsion formulation tested on bagworm shows that Sample E was found to be most toxic with the lowest lethal concentration (LC50) of 16.9 g/mL, is able to repel 50% of the population. This is because a past study highlighted incorporating azadirachtin in the diet (leaves) significantly reduced the growth of one of the Lepidopteran insects (Nathan and Kalaivani, 2005).

This proved that azadirachtin, which contains in the neem extract, can act as the best repellent for *Metisa plana*. Thus, it is noticed that the lower amount could kill the bagworm as the toxicity level for the bagworm to react to the azadirachtin compound was very high at the lower LC50. This phenomenon agrees with other researchers (list of references) where higher active ingredients were used in this study by assuming that the more massive amount of active ingredient in bio-pesticide formulation could kill the bagworm. However, the less purity of the azadirachtin in neem extract resulted in lower impact during the mortality test. Thus, to extract the highly pure Aza-A, a series of steps should be done and therefore prolong the synthesis time to



produce 99% of pure Aza-A. As the produced azadirachtin compounds not a pure Aza A, a large amount was used in the formulation of bio-pesticide in this study. The neem extract is not harmful to the animals and human beings, so the usage of large amounts was not a serious problem since the raw material was neem seed and is not toxic to the user or environment. In this study, the bagworm that has been fed on Sample A (10:0) bio-pesticide formulation shows LC50 of 22.1 g/mL. Sample A could kill 50% of the bagworm at the concentration of 22.1 g/mL indicated that the concentration required was much higher than Sample E (0:10). The phenolic compound found in the bio-oil (Sample A) has a distinctive odour, which an acrid smoky smell. It's a bitter smell or taste that is unpleasant synonym pungent, acrid smoke. The cause of this smell is due to the low molecular weight of aldehydes and acids.

These phenolic compounds found in the bio-oil were the main reason for Sample A to act as a repellent towards *Metisa plana*. This repellent causes the bagworm to be starved and died at the end of this experiment. However, the combined bio-oil and neem extract's toxicity was much lower than bio-oil pesticide and neem extract pesticide because the LC50 was the highest, which was 54 g/mL. This indicated that this massive amount of concentration of combined bio-pesticide required to attack the *Metisa plana*. Therefore, the same total concentration of combined oil with various bio-oil ratio to neem extract could not give a different result of LC50. In consideration of the same LC50, the toxicity level towards the *Metisa plana* was equivalent. Therefore, neem extract bio-pesticide without any combination is highly efficient. This was because of its ability to achieved high mortality of bagworms at lower lethal concentration, LC50.

#### 4. Conclusion

The bio-oil-based pesticide was analyzed in this study as it can act as a repellent towards bagworm due to the LC50 of 22.1 g/mL, which can repel 50% of the population. This indicated that the lower concentration of bio-oil was only needed to kill bagworm with high mortality of 50%. Furthermore, other experiments, such as bio-assay of bagworm, should also be conducted with the use of insect growth regulators (IGRs) such as diflubenzuron (DFB) and teflubenzuron (TFB). This is due to diflubenzuron has been reported to affect bagworm in previous research. Thus, this is vital to observe the mortality of bagworm before bio-pesticide with the active ingredient from azadirachtin, surfactants, and bio-oil as a carrier is formulated to produce a more effective bio-pesticide.

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