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DEVELOPMENT OF BIO-PESTICIDES AGAINST *METISA PLANA* WALKER BAGWORM (LEPIDOPTERA: PSYCHIDAE)

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Abstract

The agrochemical business places a high priority on the development of ecologically friendly pesticides for sustainable agriculture. The main hurdles in developing new pesticides are identifying novel active ingredients and optimising the delivery system of a pesticide are the main challenges in developing new pesticides. Currently, it appears that the use of renewable resources, particularly plant oils as pyrolyzed bio-oil has the potential to replace the traditional petroleum-based solvents in liquid pesticide formulations. The palm kernel shell-derived bio-oil contains high concentrations of oil esters compounds (methyl esters) which can effectively encapsulate or bind the pesticide's active ingredients in the material's matrix. These properties drive the selection of this materials to be utilised as carrier (inert ingredients) in the pesticide formulations. This study aims to investigate the viability of using bio-oil to substitute petroleum-based oil as carrier in bio-pesticide formulation against insect-pest such as *Metisa plana* bagworm. Prior to formulation, the ternary phase diagram was used to assess the compatibility between bio-oil and surfactants such as Tween 20 and Tween 80 as bio-pesticide ingredients. According to the compatibility results, the best formulation contains 20% surfactant. Based on these circumstances, the experiment was designed with an active ingredient extracted from neem seeds, azadirachtin. The results showed that the formulated bio-pesticide could repel 50% of the bagworm population, with a lethal concentration (LC50) of 22.1 g/ml. As a result, this study contributes to our knowledge

of waste management and brings us closer to a zero-waste strategy for a cleaner environment.

Keywords

Bio-oil, surfactant, neem extract, azadirachtin, bagworm, bio-pesticides

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1. Introduction

Pesticides are important in agriculture as they increase production and improve pest management¹. Most synthetic pesticides in the market are petroleum-based. Alternative pest management must be implemented to reduce the reliance on petroleum-based oil². Due to limitations of synthetic pesticides, the use of botanical pesticides for crop protection has recently increased. Some commercial botanical pesticides, such as pyrethrum products from *Tanacetum cinerariifolium*, neem products from *Azadirachta indica*, and rotenone from *Derris* and *Lonchocarpus* spp.³, have been traded globally. Pesticide formulations are made up of active ingredients (AI) and inert ingredients. Pesticidal effects are attributed to the former, whereas inert ingredient such as surfactants improve product performance, stability, and usability⁴. Past research⁵ shows that the botanical insecticide AI of Azadirachtin extracted from neem has the most insecticidal activity against insect-pests.

On the other hand, various palm biomass, such as palm kernel shells (PKS), empty fruit bunches, and mesocarp fibers have accumulated in numerous palm oil mills across the country, posing waste management challenges⁶. As a result, it is critical to valorise waste to investigate sustainable options for palm oil processes. This may be done through thermochemical technology such as pyrolysis⁷. According to recent studies, converting PKS to bio-oil via thermal processing provides more significant benefits because it reduces the disposal issues associated with agricultural wastes⁸. Bio-oil from PKS is the focus of this study because of the compounds it contains, such as phenol and methyl ester. These chemicals are important because methyl ester is one of the carriers in pesticide formulation^{9,10}. More research is needed to develop eco-friendly pesticide carriers rather than relying on petroleum-derived products¹¹. This is critical because the carrier is an inert component in pesticide formulation. Surfactants as another inert ingredient are required to dissolve bio-oil in the aqueous phase to replace the petroleum-derived pesticide formulations. Polyoxyethylene sorbitan fatty acid ester, such as Tween 20 or 80, is combined with oil as carrier together in the presence of water for pesticide formulation¹².

Bagworms are the most common insect pests that cause outbreaks in the oil palm plantations¹³. Bagworms caterpillars, such as *Metisa plana* Walker (Lepidoptera: Psychidae), feed on oil palm leaflets¹⁴. The population of bagworms will eventually exceed the threshold level¹⁵. Despite extensive efforts, effective pest management in the oil palm industry remains a challenge¹⁶. As a result, appropriate pest management strategies must be implemented to control bagworms and maintain a healthy oil palm plantation¹⁷. This paper focuses on the development of bio-pesticide derived from bio-oil and its effects on *Metisa plana* bagworm in oil palm (Figure 1).

Figure 1: Bagworms sample collection

2. Materials and Methods

The formulation of bio-pesticide consists of experiments of three stages, namely compatibility testing between bio-oil and surfactants, bio-pesticide formulations and bio-assay assessment; the latter involves the formulated bio-assay evaluation of the formulated bio-pesticide on the bagworm, *Metisa plana*.

2.1. Compatibility of bio-oil and surfactants

Compatibility tests were carried out to determine the best combination of bio-oil and surfactant in a bio-pesticide formulation. During the experiment, the percentage of filtered bio-oil and surfactants was calculated for 11 test tubes based on their theoretical weight and denoted as the actual total weight. Following that, deionized water was added to the mixture in accordance with equations (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 (gram)

d : weight of deionized water added for each rotation (gram)

When deionized water was added to each test tube, equations (3) and (4) were used 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 cycles, $n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11$

$$m = n+1$$

Each test tube was then centrifuged, and the formation of different phases was observed. Finally, a ternary phase diagram was created in CHEMIX School software version 7.00 using Ternary Plot (Arne Standness, Bergen, Norway). The same procedures were followed for Tween20 and Tween 80.

2.2. Neem extraction, analysis and mixture formulations

The formulation procedures were as follow:

- 1) Neem seeds were washed under running water and dried at 50 °C for 24 hours.
- 2) The weight of the neem seeds was measured before and after drying.
- 3) Using a conventional blender, the dried neem seeds were then crushed into 1-2 mm particle size.
- 4) Next, 250 g of powdered neem seed was stirred in 1 L of hexane at 40 °C using magnetic stirrer for 2 hours.
- 5) The residues were filtered after 2 hours, and the extracted oil was stored in a bottle.
- 6) The defatted neem powder was added to 1L of methanol and stirred with a magnetic stirrer at 1500 rpm at 40 °C for 2 hours.

The AI (azadirachtin) compositions obtained during this extraction process were analysed using UV-Vis spectrophotometer (HACH) ²¹. Table 1 summarises the different ratios of bio-oil and neem extract combinations that were prepared.

2.3. Formulation of bio-pesticide

The formulation composition was performed using various combinations of oil (bio-oil and neem extract) and water ratios at a constant surfactant percentage (20%), as shown in Table 2. The ternary phase diagram was used to

determine the composition ratio. Each sample had been evenly mixed with vortex being centrifuged at 3500 rpm for 30 minutes. The appearance and physical properties of all samples were evaluated.

Table 1: The mixing ratio of bio-oil to neem extract (combined oil)

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

Table 2: The composition ratio of bio-pesticide formulation

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

2.4. Bio-assay for *Metisa plana* bagworm

Metisa plana larvae in their 3rd or 4th instar were tested with the formulated bio-pesticide. The bagworms were collected from Bukit Rotan Palm Oil Estate. They were placed in plastic cups and fed with palm oil leaves. The latter had a length of 25 cm and had been immersed in the prepared bio-pesticide for 10 seconds with gentle agitation before being dried at room temperature and kept in the plastic cups by replacing the old leaves. For each formulation, the experiments were carried out on three instar larvae. Deionized water was used as the control treatment. The larvae were kept in the laboratory at a temperature of 26±2 °C. For four days, the number of affected bagworms for formulated bio-pesticides was recorded. Larvae that did not respond to gentle prodding with a fine sable brush were deemed dead. The daily number of bagworms that influenced the percentage of mortality and toxicity for each combination of bio-oil and neem extract was observed. The procedures are summarised in Figure 2 and the physical appearance of the formulated samples is depicted in Figure 3.

Figure 2: The bio-assay test set up: (a) dried oil palm leaves immersed with the formulated bio-pesticides, (b) 3rd instar larvae bagworms used for testing and (c) mortality observation set-up

Figure 3: The formulation composition of bio-pesticide based on bio-oil and neem extract

3. Results & Discussions

3.1. Ternary phase diagram

To comprehend the phase behaviour and stability of bio-oil, a ternary phase diagram can be used¹⁸. The compatibility results for the tested surfactants, Tween 20 and Tween 80, are shown in Figure 4. When Tween 20 was

mixed with water, it appeared as a homogeneous phase, indicating that the solubility of the components in the mixture was one phase (isotropic). Figure 4(b), on the other hand, shows that Tween 80 contains more than one phase, indicating that the mixture is heterogeneous¹⁹.

Figure 4: Ternary phase diagram for (a) Tween 20 and (b) Tween 80

(a)

(b)

3.2. Effects of various compositions ratio of bio-pesticides formulations

Since the start of the experiment, the number of affected bagworms, *Metisa plana*, had been collected. Figure 5 depicts the cumulative number of affected bagworms for each formulation. Except for formulas B1, B2, D3, E1 and E4, the formulated bio-pesticides did not indicate significant effect on all formulations on day 1. However, the number gradually increased until Day 4, when all formulations killed 100 percent of the tested bagworms. The odour was caused by a strong acrid which contained phenol compounds. It contributed to the death of the bagworms⁹. The highest death toll occurred on Day 2 at the highest concentration of bio-oil (A1). It demonstrated that a bio-pesticide containing a high concentration of combined oils was capable of killing majority of the bagworms. The high concentration of bio-oil also indicated volatile compound which promoted the evaporation of phenol compounds². As a result, it produced strong odour that could deter pests from the leaves. When compared to other samples, Sample A1 had high death toll. Thus, sample A1 was chosen as the most effective bio-pesticide formulation.

Figure 5 shows that the death toll of the bagworms had been increasing from the start for sample B2. However, it had remained constant for the next two days. It means that the remaining bagworms had not been eating for three days and died on Day 4. Sample B4, with the lowest volatile concentrations, only affected

the bagworms after three days. It indicated a slower reaction towards formulation. This demonstrated that the samples with higher concentrations, such as sample B2 and B1, responded quickly on Day 1. However, sample B1 only affected one bagworm, indicating that the bio-pesticide formulation was inadequate. The lack of water in B1 formulation resulted in poor mixing of oil and surfactant, resulting in a weaker formulation function. However, the water in sample B2 aided the miscible oil and surfactant to function as a good bio-pesticide. The surfactant is used to reduce the tension of the water surface. It aids in the even distribution of A1 in water.

Similarly, various formulations were tested on *Metisa plana*. Among the prepared samples, C2 formulation was the most effective sample, the death toll of bagworms was the highest since Day 2. The bagworms were not repelled by the other formulations. It indicated that the bio-oil and the neem extract compounds were unable to react because their attack time was very short. Despite having the highest concentration, sample C1 formulation did not kill the bagworms for the first two days. This meant that the surfactant with the highest ratio of combined oils (2:8) couldn't make the best combination because using surfactant without water made the bio-pesticide less effective. However, after being starved for two days, at least one bagworm died on Day 3. Sample D2 was the most effective bio-pesticide in the sample D formulation because the death toll of the bagworm had been increasing gradually from

Day 2 and reached the maximum target on Day 4. Unfortunately, sample D2 did not have any effect on the bagworms on Day 1, whereas sample D3 and D4 killed one bagworm. However, the death toll remained constant after Day 1. As stated previously, even the highest concentration of bio-oil could not kill all the bagworms because the bagworms avoid consuming their food.

Finally, the effect on the *Metisa plana* larvae based on different formulations of Sample E in Figure 5 indicated that the E1 formulation was the most effective formulation. Its proclivity to kill all bagworms at the end of Day 2 indicated that E1 had the shortest attacking time compared to other formulations. On Day 1, this formulation was able to kill two bagworms, while E4 formulation killed one and E3 and E2 did not

kill any. Because of the high concentration of AI in the neem extract, E1 had the fastest affecting time. AI is the main component responsible for the antioxidant and toxicity effects of the pests. When being used in the formulation of bio-pesticides vitally, the presence of AI in neem extracts induces hormonal balance in the insects, resulting in failure of moulting or dramatic deformation at immature stages of growth²⁰.

3.3. Mortality percentage of *Metisa plana*

The mortality percentage of the bagworms was determined by testing the bagworms with the formulation in Table 1 for each different mixing ratio sample of bio-oil and AI. Sample A was pure bio-oil, Sample E was pure neem extract containing AI, and the other samples were the mixture of the two. The pests were exposed to

Figure 5: Cumulative number of affected bagworms per day for each formulation

Figure 6: Mortality percentage of bagworm

various mixing ratios, sample C and sample D resulting in 42 % mortality, and sample E resulting in 58 % mortality (see Figure 6). Sample E had the highest bagworm fatality rate due to its high concentration of strong AI, making it the most effective bio-pesticides. However, sample E contained higher lethality percentage compared to the other samples. This was also supported by a previous study that AI caused larval and pupal mortality in a Lepidopteran insect²¹. Furthermore, a previous study claimed that AI killed the 3rd instar larvae within 1–4 days, with peak host mortality occurring 2 days after treatment²². The combination of bio-oil and AI, on the other hand, was not the best formulation because the fatality rate was lower than samples A and E. The study showed that the lowest mortality rate was 42%. It demonstrated that the mortality value for samples B, C and D remained constant. This could be due to the fact that all samples had the same concentration. Although the ratio of bio-oil to neem extract had various combinations, the total concentration remained constant at 40 g/ml. The AI of other chemical groups could slow down the development of bio-pesticide resistance. Lowering the mixing or combination of both AI, on the other hand, may contribute to a reduction in formulation efficiency.

3.4. Values of LC50 for *Metisa plana*

The 50 % lethal concentration (LC50) values were determined using Probit Analysis Method, and the results are shown in Figure7. The toxicity after 72 hours was the highest in Sample E, which had the lowest lethal concentration (LC50) of 16.9 g/ml. Because their LC50 values were similar, samples B, C and D were all assigned to the same toxicity group. Observation on the bagworms revealed

that Sample E had the highest toxicity, with the lowest lethal concentration (LC50) of 16.9 g/ml, capable of repelling 50 % of the population. The previous research indicated that including AI in the diet (leaves) can significantly reduce the growth of one of the Lepidopteran insects²³. This demonstrated that the AI in the neem extract would be the most effective repellent for *Metisa plana*. As a result, the toxicity for the bagworm to react to the AI compound was still high at lower LC50; it indicated that a lower amount of AI could kill the bagworm. However, the AI derived from the neem extract is not harmful to both animals and humans. As a result, even in large quantities, its use was not a major issue. According to this study, 22.1 g/ml of LC50 was able to kill 50 % of the bagworm. It indicated that the required concentration of bio-oil (10:0) was much higher than Sample E (0:10). The phenolic compound found in the bio-oil (Sample A) had a distinct odour that was acrid smoke in nature and acted more as a repellent. It also had a bitter, pungent and acrid flavour (1). The odour was due to the present of low molecular weight aldehydes and acids.

The phenolic compound found in bio-oil was the primary reason for Sample A which acted as a repellent to *Metisa plana*. At the end of the experiment, the bagworm died from starvation. The toxicity of the combined bio-oil and AI from neem extract for samples B, C, and D, on the other hand, was the lowest due to their highest LC50 values, 54 g/ml. It showed that massive amount of concentration of combined bio-pesticide was required to affect the *Metisa plana*. As a result, the same total concentration of combined oil with different ratios of bio-oil to neem extract could not produce different LC50 results as samples B, C, and D.

Figure 7: Lethal concentration (LC50) for each sample composition

4. Conclusion

The formulated bio-pesticide developed in this study successfully repelled and killed 50% of the bagworm population while having an acceptable LC₅₀ level of 22.1 g/ml and a 50% mortality rate. The efficient dispersion and delivery of active ingredients (a.i.) during application of liquid pesticide formulations was discovered to be a vital approach for effective biopesticides formulation. The use of pyrolyzed palm kernel shell (PKS)-based oil as carrier has great compatibility with other additives in the formulations (active ingredients and surfactants), is easy to emulsify, and promotes the pesticide stability and thus the insecticidal effects. Thus, this organic-based solvents appear to have a capacity advantage over currently used petroleum-based solvents, which have various drawbacks such as low flash points, high volatility, flammability, phytotoxicity, and inhalation problems to users.

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