LABORATORY EFFICACY OF RHAMNOLIPID PRODUCED BY Pseudomonas aeruginosa USM-AR2 AS BIOPESTICIDE AGAINST BAGWORM, Metisa plana WALKER (LEPIDOPTERA: PSYCHIDAE)

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ABSTRACT

The bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) is one of the major pests causes defoliation of oil palm leaves, resulting in severe yield loss of oil palm production. In this study, rhamnolipid, a biosurfactant produced by *Pseudomonas aeruginosa* USM-AR2, was tested for its effectiveness in causing mortality of bagworms. A total of 13 treatments were tested, namely mixtures of different doses of rhamnolipid and neem oil as well as cypermethrin, as chemical insecticides that are commonly used in oil palm plantations, to control bagworms. The results showed that the mixture of 200 ppm rhamnolipid and 30000 ppm neem oil, and a single treatment of 30000 ppm neem oil recorded 83.33% mortality at five days after treatment (DAT). The chemical insecticide treatment by cypermethrin recorded 100% mortality at 2 DAT. The lowest efficacy was observed on the mixture of 100 ppm rhamnolipid and 10000 ppm neem oil, producing 50% at 5 DAT and 73.33% of mortality at the end of the observation. Probit analyses showed that the LT₅₀ score for single treatment of rhamnolipid of 100ppm and 200ppm were 5.22 DAT and 4.61 DAT. Meanwhile, the LT₉₀ scores for 100ppm and 200ppm rhamnolipid were 13.08 DAT and 11.07 DAT respectively.

Keywords: Metisa plana, bagworm, oil palm, rhamnolipid, biopesticides

ABSTRAK

Ulat bungkus, *Metisa plana* Walker (Lepidoptera: Psychidae) merupakan salah satu perosak utama yang menyebabkan defoliasi daun kelapa sawit dan mengakibatkan hasil pengeluaran sawit rendah. Dalam kajian ini, rhamnolipid, racun biologi yang dihasilkan oleh *Pseudomonas aeruginosa* USM-AR2, telah diuji keberkesanannya untuk membunuh ulat bungkus. Sebanyak 13 jenis rawatan telah diuji keberkesanannya seperti campuran rhamnolipid dan minyak neem yang berlainan dos serta cypermethrin, sebagai racun kimia yang biasa digunakan di ladang sawit untuk mengendalikan ulat bungkus. Hasil kajian menunjukkan bahawa campuran 200 ppm rhamnolipid dan 30000 ppm minyak neem dan 30000 ppm minyak neem mencatat 83.33%

kematian ulat bungkus pada lima hari selepas rawatan (DAT). Rawatan racun kimia, cypermethrin mencatatkan kematian 100% pada 2 DAT. Kematian ulat bungkus terendah adalah pada campuran 100 ppm rhamnolipid dan 10000 ppm minyak neem di mana 50% kematian direkodkan pada 5 DAT dan 73.33% kematian diakhir pemerhatian. Analisis probit menunjukkan skor LT50 untuk rawatan 100 ppm dan 200 ppm rhamnolipid adalah 5.22 DAT dan 4.61 DAT. Sementara itu, skor LT90 untuk 100 ppm dan 200 ppm rhamnolipid adalah 13.08 DAT dan 11.07 DAT.

Kata kunci: Metisa plana, ulat bungkus, kelapa sawit, rhamnolipid, biopestisid

INTRODUCTION

Oil palm is one of the most important crops contributing a major economic value in the agriculture sector of Malaysia (Kushairi et al. 2019). Infestations or outbreaks of insect pests are causing huge losses in oil palm production, inevitably affecting the growth of the economy of the country. One such pest is the bagworm. During an outbreak, enormous amounts of photosynthetic leaf areas of oil palm are devoured by these voracious phytophagous pests as the bagworm caterpillars complete their life cycle (Basri & Kevan 1995; Turner & Gillbanks 2003; Priwiratama et al. 2019). Defoliation by bagworms adversely affects oil palm productivity by reducing both number and size of fruit bunches (Basri et al. 1988; Ooi & Kamarudin 2018). Three common species of bagworms that damage oil palm plantations in Malaysia are Metisa plana Walker, Pteroma pendula Joannis and Mahasena corbetti Tam (Chung 1998; Wood & Kamarudin 2019). High prevalence of M. plana was reported in the Peninsular while M. corbetti was more prevalent in Sabah and Sarawak (Basri et al. 1988; Chung 1998). Presently, chemical control is still the principal treatment against bagworm infestations in oil palm plantations. The insecticides commonly used are the phyrethroid insecticides cypermethrin and lambda-cyhalothrin (Chung 1998; Kok et al. 2012; Salim et al. 2015). However, most chemical insecticides are also deleterious to non-target organisms, especially beneficial insects.

Management of insect pests in oil palm plantations today presents many challenges. The concept of environmentally friendly management adopted by the oil palm industry in Malaysia has completely changed the approach of any program involving pest control, particularly in oil palm plantations (Ooi & Kamarudin 2018; Wood & Kamarudin 2019). It entails evaluation of the indirect effects in the use of chemical control, especially to non-target organisms and the environment (Chung 2012; Shahir et al. 2013). The increasing awareness of environmentally friendly pest control methods have resulted in increasing occurrence of bioinsecticides used to control insect pests in oil palm plantations (Pierre et al. 2015; Ramlah et al. 2003; Salim & Hamid 2012; Ahmad et al. 2013; Masri & Ariff 2020). In light of this, rhamnolipid has been explored as a potential biopesticide for environmental-friendly agricultural practice. This is especially true as chemical pesticides are mostly derived from petroleum feedstock where toxicity cannot be avoided. Hence, it is not only harmful to the environment, but may also increase the chemical resistance in the target organisms. Rhamnolipid biosurfactants are non-polluting, sustainable, biodegradable and less toxic. They show excellent physicochemical properties, making them attractive for various applications in different industries such as in food processing, as biopesticides in agriculture, in pharmaceutical formulations, oil and gas industry and environmental bioremediation (Chen et al. 2017; Chrzanowski et al. 2012; Mnif & Ghribi 2016; Sachdev & Cameotra 2013).

A study by Kim et al. (2011) highlighted the ability of the rhamnolipid as the main metabolite exhibiting insecticidal against aphids, therefore potentially becoming the pesticide against other insect pests. This was also proven by the study from Silva et al. (2015) on *Aedes aegypti* larvae where at 1000 mg/L, the rhamnolipid was able to eliminate 100% of the mosquito larvae. However, the potential of rhamnolipids as an effective biopesticide against bagworms, *M. plana* has not been explored and become the aims of this study.

MATERIALS AND METHODS

Bagworm Sampling, Rearing and Isolation

The larvae of bagworms were collected from Felda Gunong Besout 3, Sungkai, Perak, Malaysia (3.8418° N, 101.3023° E). The middle fronds of highly infested palms from the sampling site were cut and the live larvae of *M. plana* on the leaves were collected. The collected larvae were transferred into a plastic container (measuring 35cm length x 20cm width x 20cm height) with proper ventilation and fresh oil palm leaflets were placed inside to provide food for the bagworms. The bagworms were then brought back to the Entomology Laboratory in School of Biological Sciences Universiti Sains Malaysia (USM), Penang, Malaysia. In this study, only third instar larvae of bagworms were selected for the efficacy study due to their efficiency in feeding and ease for observation (Ahmad et al. 2013; Basri & Kevan 1995; Masri & Ariff 2020). There were a total of 390 third instar larvae of *M. plana* used in the efficacy test of this study.

Production and Recovery of Rhamnolipid

Rhamnolipid production by *P. aeruginosa* USM-AR2 fermentation was carried out for 5 days in a 3.6 L stirred tank bioreactor (Infors HT Labfors 4) equipped and monitored using IRIS® 6 software. The cultivations were carried out at 28 °C, 400 rpm agitation and aerated at an air flow rate of 0.3 vvm. The minimal salt medium (MSM) used for fermentation contained ingredients as follows per liter: 5.5 g NaNO₃, 0.5 g MgSO₄.7H₂O, 1.0 g KCl, 0.3 g K₂HPO₄ and 1 ml trace elements. At the end of fermentation, the culture broth was centrifuged (Hettich Zentrifugen, Universal-320R) at 8000 \times g for 20 minutes to separate the cell pellet and supernatant containing rhamnolipid. The supernatant was subsequently transferred into a 500 mL shake flask and added with ethyl acetate at 1:1 volume ratio. Then, the supernatant-solvent mixture was shaken at 200 rpm for 1 hour in an orbital shaker (Thermo Scientific, MaxQ-4000). It was subsequently transferred into a separating funnel, where it was left to stand overnight to form separate layers. The upper aqueous layer containing rhamnolipid was carefully transferred into a glass Petri dish and dried in a fume hood chamber overnight. Dried crude rhamnolipid was scraped from the surface of the glass Petri dish and stored at 4 °C until further use. Upon use, rhamnolipid suspension was prepared by dissolving dried rhamnolipid in distilled water. The rhamnolipid concentration was quantified by orcinol assay (Jeong et al. 2004), using rhamnose as a reference.

Rhamnolipid and Neem Oil Preparation

For the toxicity test of rhamnolipid on bagworms, 500 ppm of stock solution of rhamnolipid was prepared for 1L total volume of the solution. From the prepared stock, dilutions were made with distilled water to obtain concentrations of 100 and 200 ppm. The concentration of the treatments were as suggested by Kamal et al. (2012), Kim et al. (2011) and Silva et al. (2015). The treatments used in this study are listed in Table 1. The concentrations (ppm) of neem oil were as recommended by Aliakbarpour et al. (2011) and cypermethrin EC used were based on the recommended dosage for bagworm control in oil palm plantations. Each treatment was diluted with distilled water to obtain the desired concentration needed. Three concentrations of

neem oil (10 000, 20 000 and 30 000 ppm) were added into two concentrations of rhamnolipid (100 and 200 ppm). For Treatment No. 9, 10 and 11 (T9, T10, T11), the surfactant used to mix with neem oil was a commercial petroleum base surfactant. Distilled water served as the control in Treatment No. 13 (T13).

	Table 1. Details of treatments	
Treatment Code	Active Ingredient	Dosage/Application
T 1	Rhamnolipid + Neem oil	100 ppm + 10 000 ppm
Т 2	Rhamnolipid + Neem oil	100 ppm + 20 000 ppm
Т 3	Rhamnolipid + Neem oil	100 ppm + 30 000 ppm
Τ4	Rhamnolipid + Neem oil	200 ppm + 10 000 ppm
Т 5	Rhamnolipid + Neem oil	200 ppm + 20 000 ppm
Τ 6	Rhamnolipid + Neem oil	200 ppm + 30 000 ppm
Т 7	Rhamnolipid	100 ppm
Т 8	Rhamnolipid	200 ppm
Т 9	Neem oil + conventional surfactant	10 000 ppm
Т 10	Neem oil + conventional surfactant	20 000 ppm
T 11	Neem oil + conventional surfactant	30 000 ppm
T 12	Cypermethrin EC	180 ppm
Т 13	Distilled water (Control)	Nil

*EC = Emulsifiable concentrate

Toxicity Test of Rhamnolipid on Bagworms

Leaf-dip bioassays were implemented to evaluate the effectiveness of each treatment in this study. The oil palm leaflets were collected from healthy, insecticide-free oil palm seedlings planted in the Plant House (C14), USM. The middle fronds of the oil palm were chosen and each leaflet was cut into three segments of 2 cm (width) x 10 cm (length). The rectangular pieces of leaflets prepared were individually dipped for 20 seconds into each treatment and air-dried for one hour. After air-drying, the leaflets were placed into individual modified containers with proper ventilation [7 cm height x 10 cm width x 20 cm length].

In the container, the upper side of the leaflets were exposed for larval feeding. After the preparation of the treated leaflets in the containers, 30 viable 3rd instar larvae were carefully released into each of the container using a soft forceps. For each of the treatment, three replicates were set. The leaflets were replaced with fresh pieces every two days for each container. The bagworms were observed, and their mortality were recorded every day until 9 days after treatments (DAT). The larvae were marked as dead if no movement was observed from the larval cases after gentle probing.

Data Analysis

In this experiment, a Probit Analysis was carried out to determine the lethal time for the pesticides to cause the mortality of the bagworms. LT_{50} and LT_{90} refer to the value representing the concentration of the pesticides which were required to kill 50% and 90% of bagworm population. The analyses were computed using the Statistical Package for the Social Sciences (SPSS) version 24.

RESULTS

Table 2 shows the efficacy of all the treatments based on the mortality percentages of *M. plana* during the days after treatment (DAT). The highest efficacy was recorded by cypermethrin (T12) where 83.33% of the bagworms died at 1 DAT, and the mortality was 100% at 2 DAT. The second highest efficacy was recorded by T6 (200 ppm rhamnolipid + 30000 ppm neem oil) and T11 (30000 ppm neem oil), which recorded mortalities of 50% at 2 DAT and continuously increased to 83.33% each at 5 DAT. However, at 6 DAT, the mortality of bagworms in T6 was 100% but only 93.33% mortality of bagworms was recorded from T11. At the end of the trial (9 DAT), both treatments showed 100% mortality of bagworms.

	nine da	ys of exp	osure						
Treatmonte	Percentage of <i>M. plana</i> mortality (%)								
Treatments	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6	DAT 7	DAT 8	DAT 9
T1	16.67	16.67	26.67	36.67	50.00	60.00	70.00	70.00	73.33
T2	20.00	30.00	36.67	50.00	56.67	70.00	73.33	80.00	80.00
Т3	30.00	43.33	63.33	66.67	80.00	83.33	90.00	93.33	100.00
T4	30.00	43.33	63.33	73.33	80.00	90.00	93.33	96.67	100.00
Т5	26.67	40.00	53.33	60.00	73.33	80.00	80.00	86.67	96.67
T6	33.33	50.00	66.67	80.00	83.33	100.00	100.00	100.00	100.00
T7	16.67	26.67	33.33	43.33	56.67	66.67	73.33	76.67	80.00
T8	20.00	30.00	40.00	53.33	63.33	73.33	76.67	80.00	90.00
Т9	20.00	33.33	46.67	56.67	66.67	73.33	76.67	83.33	96.67
T10	26.67	43.33	53.33	66.67	76.67	80.00	86.67	90.00	93.33
T11	30.00	50.00	63.33	76.67	83.33	93.33	96.67	100.00	100.00
T12	83.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
T13	3.33	3.33	6.67	6.67	6.67	6.67	6.67	13.33	13.33

 Table 2.
 Efficacy of thirteen different treatments towards the larvae of *Metisa plana* after nine days of exposure

*DAT = Day after treatment*EC = Emulsifiable concentrates

Meanwhile, T3 (100 ppm rhamnolipid + 30000 ppm neem oil), T4 (200 ppm rhamnolipid + 10000 ppm neem oil) and T10 (20000 ppm neem oil) showed similar percentage of bagworms mortality at 2 DAT (43.33%) and increased throughout the observation. Except for T10, T3 and T4 recorded 100% mortality at 9 DAT. During the observation, rhamnolipids (T7, 100 ppm and T8, 200 ppm) required a longer exposure time before the biopesticides were able to achieve more than 80% of bagworms mortality, i.e., T7 at 9 DAT and T8 at 8 DAT, respectively. The lowest efficacy was recorded from T1 (100 ppm rhamnolipid + 10000 ppm neem oil), with mortality at 1 DAT and 2 DAT only being 16.67%, and the mortality increasing to 50% at 5 DAT. At the end of the observation (9 DAT), the mortality of the bagworms exposed to T1 treatment was 73.33%.

Lethal time of all treatments based on LT_{50} and LT_{90} were computed based on the efficacy of every treatment (Table 3). From the analysis, it was clear that the most effective treatment was T12 (cypermethrin), which had the lowest values of LT_{50} and LT_{90} (0.78 DAT and 1.08 DAT). In general, the mixture of 200 ppm rhamnolipid with all doses of neem oil was more effective and faster to result in bagworm deaths in comparison to the mixture of 100 ppm rhamnolipid and all doses of neem oil. The LT_{50} and LT_{90} values were also higher compared

to all single treatments of rhamnolipid and neem oil. In this study, T6 (200 ppm rhamnolipid + 30000 ppm neem oil) and T11 (30000 ppm neem oil) scored LT_{50} of 3.26 DAT and LT_{90} of 5.28 DAT, and LT_{50} of 3.21 DAT and LT_{90} of 5.89 DAT, respectively. Meanwhile, the LT_{50} and LT_{90} values of T4 (200 ppm rhamnolipid + 10000 ppm neem oil) were 3.35 DAT and 6.63 DAT.

Treatments	LT ₅₀		LT ₅₀ ial Limit	– LT ₉₀	LT ₉₀ Fiducial Limit		
		Lower 95% CL	Higher 95% CL		Lower 95% CL	Higher 95% CL	
T1	5.97	4.55	7.16	14.04	10.71	27.48	
T2	5.06	2.79	6.41	13.20	9.98	29.96	
Т3	3.54	1.85	4.60	7.59	6.20	10.34	
T4	3.35	1.99	4.24	6.63	5.51	8.48	
T5	4.02	1.91	5.27	9.58	7.66	15.44	
T6	3.26	1.10	4.15	5.28	4.16	7.16	
Τ7	5.22	3.26	6.46	13.08	10.02	27.27	
Т8	4.61	2.77	5.76	11.07	8.81	18.90	
Т9	4.26	2.35	5.42	10.15	8.18	16.38	
T10	2.95	0.07	5.30	10.95	7.13	21.96	
T11	3.21	1.80	4.06	5.89	4.89	7.35	
T12	0.78	0.00	0.00	1.08	0.00	0.00	
T13	44.86	0.00	0.00	211.40	0.00	0.00	

Table 3.	Lethal time (LT ₅₀ and LT ₉₀) of the treatments towards the <i>M. plana</i> larvae
	during the pesticides exposure periods

Note: CL = Confidence limit; EC = Emulsifiable concentrates

The mixture of 100 ppm of rhamnolipid and 30000 ppm of neem oil (T3) also recorded low LT_{50} and LT_{90} values of 3.54 DAT and 7.59 DAT, followed by T5 (200 ppm rhamnolipid + 20000 ppm neem oil) with LT_{50} and LT_{90} values of 4.02 DAT and 9.58 DAT. For the two single treatments of rhamnolipid (T7 and T8), the values of LT_{50} and LT_{90} varied inversely to the concentration. For T7, the LT_{50} and LT_{90} values were 5.22 DAT and 13.08 DAT. Meanwhile for T8, the LT_{50} and LT_{90} values were 4.61 DAT and 11.07 DAT, respectively. However, all single treatments of 10000 ppm neem oil and 20000 ppm of neem oil showed lower values of LT_{50} and LT_{90} compared to the single treatments of rhamnolipid. Nevertheless, the highest LT_{50} and LT_{90} values were recorded by T1 (100 ppm rhamnolipid + 10 000 ppm neem oil), with LT_{50} of 5.97 DAT and LT_{90} of 14.04, respectively.

DISCUSSION

This study evaluates the potential of rhamnolipid, a glycolipid-type biosurfactant produced by *P. aeruginosa* USM-AR2 (Md. Noh et al. 2014) as a biopesticide toward bagworm, *M. plana* Walker. The experiments described in this study highlight several possible uses of rhamnolipids in pest control by testing its effectiveness as a biopesticide. According to Aliakbarpour et al. (2011), Gahukar (2000) and Shannag et al. (2015), neem oil is practically proven for their insecticidal properties against several insect pests, such as some from the lepidopteran family, while it was practically non-toxic to birds, mammals, bees and plants. Neem oil, being hydrophobic, does not readily mix in water. Meanwhile, rhamnolipid has been reported to enhance pesticide and agrochemical solubility, which suggests its use as adjuvant

in pesticide formulation. The use of the mixture of rhamnolipid and neem oil exemplifies the biosurfactant ability of rhamnolipid to solubilise hydrophobic compounds in an aqueous solution, facilitating dispersion, delivery and wettability. They act as emulsifying, dispersing, spreading and wetting agent that can enhance the efficiency of pesticides (Sachdev & Cameotra 2013). Thus, the use of rhamnolipid offers a green alternative to chemicals in pest management for crop protection (Crouzet et al. 2020).

The assessment for bagworm susceptibility towards the treatments in this experiment were generally estimated from test data using the log-probit model, which is the percentage of mortality expressed as a lognormal function of exposure concentration. Using the log-probit model and the reported or extrapolated 24-hour LT_{50} value and confidence limits or concentration-response slope, the LT_{50} and LT_{90} values were estimated for bagworm exposed to each of the treatments (Paustenbach 2009). The results of this study shows that rhamnolipid has promising insecticidal properties. Based on the LT_{50} and LT_{90} values, the relative susceptibility of the bagworm, *M. plana* to the rhamnolipid was inverse to the concentration of the treatments.

Following the results of previous findings on rhamnolipid as a potential biopesticide, this study was carried to evaluate the efficacy of rhamnolipid, with neem oil as a comparison. In this study, we evaluated the LT_{50} and LT_{90} of the pesticides and the result showed that the values of LT_{50} and LT_{90} varied inversely to the rhamnolipid concentration, i.e., the higher the rhamnolipid concentration, the lower the LT_{50} and LT_{90} values. From the observation, the efficacy of both biopesticides was overwhelmed with the efficacy of commercial chemical pesticides (cypermethrin), which recorded the lowest LT_{50} and LT_{90} scores, resulting from the fastest response on the bagworms. Kim et al. (2011) highlighted the ability of rhamnolipid as a main metabolite, exhibiting insecticidal properties by dehydrating the cuticle membrane on the aphids, therefore potentially becoming a pesticide against other insect pests. This was also proven by the study from Silva et al. (2015) on *Aedes aegypti* larvae; where at 1000mg/L, rhamnolipid was able to eliminate 100% of the mosquito larvae. The rhamnolipids caused the mosquito cuticle to rupture and eventually die. On another study conducted by Kamal et al. (2012), a single 20ppm rhamnolipid could kill 52% of adult *Rhyzopertha dominica* after 72 hours being exposed to the treatment.

In a laboratory bioassay conducted by Yusdayati et al. (2012), the effectiveness of *Bt* towards *M. plana* showed competitive mortality responses with biosurfactants with 200ppm rhamnolipid (T8) used in this study. From the experiment, the bagworm mortality on the first day of *Bt* application was 16.65% compared to the single treatment of 200ppm rhamnolipid (T8) of this study which resulted in 20.00% bagworm mortality. After seven days of treatment application, the bagworm mortality for *Bt* increases to 98.10% and 200ppm rhamnolipid was 76.67%. Another approach to control bagworms by using an entomopathogenic fungi, *Metarhizium anisopliae* was evaluated by Loong et al. (2013) where at $2x10^9$ conidia mL⁻¹, *M. anisopliae* recorded LT₅₀ value of 5.72 days, while in this study, the 200ppm of rhamnolipid recorded lower LT₅₀ value of 4.61 days.

However, the slow response of the biopesticides, especially rhamnolipid, was expected as it coincides with the biopesticide characteristic of slow effectivity (Ahmad et al. 2013; Masri & Ariff 2020). According to Talha et al. (2019), this slow effectiveness response of biopesticides is due to its ability to only suppress the pest population rather than eliminate it within a narrow target range. This characteristic is important for a biopesticide, as it can be used for specific insects so that other beneficial insects will be unaffected. Furthermore, Hogan et al. (2019) reported that biopesticides, such as rhamnolipid, can serve as a green material which is more biodegradable and less toxic than commercial chemical pesticides.

The current practises used to eliminate bagworms in oil palm plantations using commercial pesticides are ineffective due to the development of the pest resistance towards the pesticides (Kok et al. 2012). As a result, a sustainable way to manage pest population in oil palm plantations has to be introduced, and rhamnolipid as a biopesticide together with commercial biopesticides such as neem oil, can reduce the application of chemical pesticides throughout time. Not only will it minimize the insect pest resistance towards pesticides, the application of biopesticides is also harmless to the beneficial insects such as pollinators (Yusdayati & Hamid 2015). The findings of the current study have proved the high potential of rhamnolipid as a biopesticide and further research needs to be carried out in order to be able to fully utilize this bioproduct as an alternative to chemical pesticide.

CONCLUSION

The results of this study provide a promising result on the effectiveness of rhamnolipid as a biopesticide to control bagworms in oil palm plantations. The efficacy test showed that despite of the slow action based on LT_{50} values for a single treatment of 100 ppm and 200 ppm rhamnolipid which is 5.22 and 4.61 respectively compared to cypermethrin with a lower LT_{50} values of 0.78. Rhamnolipid was effective to control the bagworms during the observation period when a single treatment of 100 ppm and 200 ppm rhamolipid recorded 80% and 90% of bagworm mortality at 9 DAT. This alternative biopesticide with favourable toxicological and environmental profile will be an additional tool for oil palm planters to properly manage and control the infestation of bagworms. However, further investigation is needed to provide conclusive evidence of the effectiveness of rhamnolipid as a biopesticide in field settings.

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REFERENCES

- Ahmad M.N., Siti Ramlah A.A., Masri M.M.M. & Wahid M.B. 2013. Efficacy of BaFOG-1 (s), formulated local *Bacillus thuringiensis* for controlling bagwom, *Pteroma pendula* (Lepidoptera: Psychidae). *Journal of Oil Palm Research* 25(2): 228-234.
- Aliakbarpour, H., Che Salmah, M.R. & Dzolkhifli, O. 2011. Efficacy of neem oil against thrips (Thysanoptera) on mango panicles and its compatibility with mango pollinators. *Journal of Pest Science* 84: 503-512.
- Basri, M.W., Abdul Halim, H. & Masijan, Z. 1988. Bagworms (Lepidoptera: Psychidae) of oil palms in Malaysia. Palm Oil Research Institute of Malaysia (PORIM) Occasionnal Paper 23: 27-38.
- Basri, M.W. & Kevan, P.G. 1995. Life history and feeding behaviour of the oil palm bagworm, *Metisa plana* Walker (Lepidoptera : Psychidae). *Elaeis* 7(1): 18-34.
- Chen, J., Wu, Q., Hua, Y., Chen, J., Zhang, H. & Wang, H. 2017. Potential applications of biosurfactant rhamnolipids in agriculture and biomedicine. *Applied Microbiology Biotechnology* 101(23-24): 8309–8319.
- Chrzanowski, L., Lawniczak, L. & Czaczyk, K. 2012. Why do microorganisms produce rhamnolipids? *World Journal of Microbiology and Biotechnology* 28: 401-419.
- Chung, G.F. 1998. Strategies and methods for the management of leaf-eating caterpillars of oil palm. *The Planter* 74(871): 531-558.
- Chung G.F. 2012. Effect of pests and diseases on oil palm yield. In Lai O.M., Tan, C.P. & Akoh, C.C. (eds.). *Palm Oil (Production, Processing, Characterization, and Uses)*, pp. 163-210. USA: AOCS Press
- Crouzet, J., Arias, A.A., Cordelier, S.D., Cordelier, S., Prsic, J., Hoff, G., Gourbeyre, F.M., Baillieul, F., Clement, C., Ongena, M. & Dorey, S. 2020. Biosurfactants in plant protection against diseases: Rhamnolipids and lipopeptides case study. *Frontiers in Bioengineering and Biotechnology* 8(1014): 1-11.
- Gahukar, R.T. 2000. Use of neem products or pesticides in cotton pest management. *International Journal of Pest Management* 46(2): 149-160.
- Hogan, D.E., Tian, F., Malm, S.W., Olivares, C., Pacheco, R.P., Simonich, M.T., Hunjan, A.S., Tanguay, R.L., Klimecki, W.T., Polt, R., Pemberton, J.E., Curry, J.E. & Maiera, R.M. 2019. Biodegradability and Toxicity of monorhamnolipid biosurfactant diastereomers. *Journal of Hazardous Materials* 364: 600-607.
- Jeong, H.S., Dong-Jung, L., Sun-Hee, H., Soon-Duck, H. & Jai-Yul, K. 2004. Rhamnolipid Production by pseudomonas aeruginosa immobilized in polyvinyl alcohol beads. *Biotechnology Letters* 26(1): 35–39.

- Kamal, A., Shaik, A.B., Kumar, C.G., Mongolla, P., Rani, P.U., Krishna, K.V.S.R., Mamidyala, S.K. & Joseph, J. 2012. Metabolic Profiling and biological activities of bioactive compunds produced by *Pseudomonas* sp. Strain ICTB-745 isolated from Ladakh, India. *Journal of Microbiology and Biotechnology* 22(1): 69-79.
- Kim, S.K., Kim, Y.C., Lee, S., Kim, J.C., Yun, M.Y. & Kim, I.S. 2011. Insecticidal activity of rhamnolipid isolated from *Pseudomonas* sp. EP-3 against green peach aphid (Myzus persicae). *Journal of Agricultural and Food Chemistry* 59(3): 934-938.
- Kok, C.C., Eng, O.K., Razak, A.R., Arshad, A.M. & Marcon, P.G. 2012. Susceptibility of bagworm *Metisa plana* (Lepidoptera: Psychidae) to Chlorantraniliprole. *Pertanika Journal of Tropical Agricultural Science* 35(1): 149-163.
- Kushairi, A., Ong-Abdullah, M., Nambiappan, B., Hishamuddin, E., Bidin, M.N.I.Z., Ghazali, R., Subramaniam, V., Sundram, S. & Parveez, G.K.A. 2019. Oil palm economic performance in Malaysia and R&D Progress in 2018. *Journal of Oil Palm Research* 31(2): 165-194.
- Loong, C.Y., Sajap, A.S., Noor, H.M., Omar, D. & Abood, F. 2013. Effects of UV-B and Solar radiation on the efficacy of *Isaria fumosorosea* and *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) for controlling bagworm, *Pteroma pendula* (Lepidoptera: Psychidae). *Journal of Entomology* 10(2): 53-65.
- Masri, M.M. & Ariff, A. 2020. Effect of different fermentation strategies on *Bacillus thuringiensis* cultivation and its toxicity towards the bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae). *Egyptian Journal of Biological Pest Control* 30: 1-10.
- Md. Noh, N.A., Mohd Salleh, S. & Yahya, A.R.M. 2014. Enhanced rhamnolipid production by Pseudomonas aeruginosa USM-AR2 via fed-batch cultivation based on maximum substrate uptake rate. Letters in Applied Microbiology 58: 617-623.
- Mnif, I., & Ghribi, D. 2016. Glycolipid biosurfactants: Main Properties and potential applications in agriculture and food industry. *Journal of the Science of Food and Agriculture* 96(13): 4310 4320.
- Ooi, P., & Kamarudin, N. (2018). The Importance of Sustainable Management for Common Oil Palm Insect Pests. *Oil Palm Bulletin* 77: 14-18.
- Paustenbach, D.J. 2009. *Human and Ecological Risk Assessment: Theory and Practice*. United States of America (USA): Wiley Classics Library.
- Pierre, E.M., Azarae, I., Tsuji, K., Yamane, S. & Norman, K. 2015. Sustainable management of oil palm pests & disease in malaysia (SMOPPD). Technical report LRGS Education Ministry scheme 2015, Universiti Malaya.
- Priwiratama H., Rozziansha T.A., Susanto A., & Prasetyo A.E. (2019). Effect of Bagworm Pteroma pendula Joannis Attack on the Decrease in Oil Palm Productivity. *Jurnal Hama Dan Penyakit Hutan Tropika* 19(2): 101-108.

- Ramlah, A.S., Basri, M.W. & Mahadi, N.M. 2003. IPM of bagworms and nettle caterpillars using *Bacillus thuringiensis*: Towards increasing efficacy. *Proceedings of the PIPOC* 2003, pp. 449-474.
- Sachdev, D.P. & Cameotra, S.S. 2013. Biosurfactants in Agriculture. *Applied Microbiology and Biotechnology* 97(3): 1005–1016.
- Salim, H. & Hamid, N.H. 2012. Evaluation of Several chemical control approaches against bagworm, *Metisa plana* Walker (Lepidoptera: Psychidae) in FELDA Oil Palm Plantations. *The Planter* 88(1040): 785-799.
- Salim, H., Che Salmah, M.R., Abu, H.A., & Salman, A.A. 2015. Efficacy of insecticide and bioinsecticide ground sprays to control *Metisa plana* Walker (Lepidoptera: Psychidae) in Oil Palm Plantations, Malaysia. *Tropical Life Science Research* 26(2): 73-83.
- Shahir, M.S., Mohd Rasdi, Z., Fauziah, I., Fakhrorazi, A., Ismail, R. & Mohd Hanysyam, M. 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* 3(2): 72-78.
- Shannag, H.K., Capinera, J.L. & Freihat, N.M. 2015. Effects of neem-based insecticides on consumption and utilization of food in larvae of *Spodoptera eridania* (Lepidoptera: Noctuidae). *Journal of Insect Science* 15(1): 152.
- Silva, V.L., Roberta, B.L., Claudio, J.V.Z. & Jonas, C. 2015. Rhamnolipids : Solution against *Aedes aegypti. Frontiers In Microbiology* 6(88): 1-5.
- Talha, N., Sehroon, K. & Dewen, Q. 2019. *Biological Control of Insect Pest*. Beijing: Pests Insects, Management, Control.
- Turner, P.D. & Gillbanks, R.A. 2003. *Oil Palm Cultivation and Management*. Second Edition. Kuala Lumpur: The Incorporated of Planters.
- Wood, B.J. & Kamarudin, N. 2019. Bagworm (Lepidoptera: Psychidae) infestation in the centennial of the Malaysian oil palm industry – A review of causes and control. *Journal* of Oil Palm Research 31: 364-380.
- Yusdayati, R. & Hamid, N.H. 2015. Effect of Several insecticide against oil palm pollinator's weevil, *Elaeidobius kamerunicus* (Coleoptera: Curculionidae). *Serangga* 20(2): 27-35.
- Yusdayati, R., Hamid, N.H., Hamzah, S. & Mustapa, M.R. 2012. In vivo and in vitro study of Bacillus thuringiensis ssp. kurstaki in controlling Metisa plana (Lepidoptera: Psychidae) in oil palm plantation by using air blast spraying technique. Forth Iopri-Mpob International Seminar 2012- Existing and Emerging Pests and Diseases of Oil Palm Advances in Research and Management, pp. 86-97.