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RHAMNOLIPID BIOSURFACTANT AS A POTENTIAL BIOPESTICIDE TO CONTROL TERMITE, *Coptotermes curvignathus* HOLMGREN (BLATTODEA: RHINOTERMITIDAE)

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ABSTRACT

This study was conducted to observe the efficacy of rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* USM-AR2, towards a termite species, *Coptotermes curvignathus*. *Coptotermes curvignathus* was collected from UPM urban areas and reared in laboratory. Rhamnolipid biosurfactant was produced in *P. aeruginosa* USM-AR2 fermentation using mineral salts medium supplemented with waste cooking oil in a stirred tank bioreactor. Eight different concentrations of the compound were tested against *C. curvignathus*; 5% (T1), 10% (T2), 20% (T3), 30% (T4), 40% (T5), 50% (T6), 75% (T7), 100% (T8) and fipronil as positive control (T9). The wood-soak bioassay was used for all treatments, each with three replicates, and termite mortality was assessed based on immobility. In general, fipronil showed the highest efficacy towards *C. curvignathus* with 92.12% of mortality at DAT1, compared to rhamnolipid biosurfactant with 100% of the compound (T8) only resulted on 16.36% of mortality. However, the mortality rate of the species increased tremendously after treated by rhamnolipid at DAT2 with T8 and T7 recorded 67.27% and 66.06% of mortality rate, respectively. As of DAT 3, T8 has recorded 96% of the mortality, followed by T7 (95.15%), T6 (94.55%) and T5 (93.33%). At DAT4, all treatments except T1 caused on 100% of mortality. The LC_{50} for rhamnolipid biosurfactant was 17.68% at DAT 2, and reduced to 2.30% at DAT3 and 0.29% at DAT4. During the similar periods, the LC_{90} of rhamnolipid biosurfactant ranged between 2122.89% and 18.82% at DAT2 and DAT 4, respectively. In conclusion, the compound at different concentrations were lethal to the termite species and the period taken to control the termite was a good indicator that it had a high potential to be used as termiticide due it delays effect which is important in termite control and management.

Keywords: Rhamnolipid biosurfactant; biotermiticide; termites; *Coptotermes curvignathus*

ABSTRAK

Kajian ini dijalankan untuk memerhati keberkesanan biosurfaktan rhamnolipid yang dihasilkan oleh *Pseudomonas aeruginosa* USM-AR2 terhadap spesis anai-anai, *Coptotermes curvignathus*. *Coptotermes curvignathus* telah dikutip dari kawasan bandar UPM dan dipelihara di dalam makmal. Lapan kepekatan sebatian yang berbeza telah diuji ke atas *C. curvignathus*; 5% (T1), 10% (T2), 20% (T3), 30% (T4), 40% (T5), 50% (T6), 75% (T7), 100% (T8) dan fipronil sebagai kawalan positif (T9). Biosurfaktan rhamnolipid dihasilkan melalui fermentasi *P. aeruginosa* USM-AR2 menggunakan medium garam mineral yang ditambah dengan minyak masak terpakai dalam bioreaktor tangki teraduk. Lapan kepekatan berbeza sebatian ini telah diuji terhadap *C. curvignathus*; 5% (T1), 10% (T2), 20% (T3), 30% (T4), 40% (T5), 50% (T6), 75% (T7), 100% (T8) serta fipronil sebagai kawalan positif (T9). Ujian bio-rendaman kayu digunakan untuk semua rawatan, masing-masing dengan tiga replikat dan kematian anai-anai dinilai berdasarkan dengan tiadanya pergerakan. Secara umum, fipronil menunjukkan keberkesanan tertinggi terhadap *C. curvignathus* dengan 92.12% kematian pada DAT1, berbanding biosurfaktan rhamnolipid dengan 100% sebatian (T8) yang hanya menghasilkan 16.36% kematian. Walaubagaimanapun, kadar kematian spesis tersebut meningkat dengan ketara selepas dirawat dengan rhamnolipid pada DAT2, dengan T8 dan T7 mencatatkan kadar kematian masing-masing 67.27% dan 66.06%. Pada DAT3, T8 telah mencatatkan 96% kadar kematian, diikuti oleh T7 (95.15%), T6 (94.55%) dan T5 (93.33%). Pada DAT4, semua rawatan kecuali T1 telah menyebabkan 100% kematian. LC_{50} bagi rhamnolipid biosurfaktan adalah 17.68% pada DAT2, dan telah berkurang kepada 2.30% pada DAT3 dan 0.29% pada DAT4. Pada tempoh yang sama, LC_{90} bagi rhamnolipid biosurfaktan adalah antara 2122.89% dan 18.82% pada DAT2 dan DAT4. Kesimpulannya, sebatian pada kepekatan yang berbeza adalah berbahaya kepada spesis anai-anai dan tempoh yang diambil untuk menghapuskan anai-anai tersebut adalah petunjuk yang baik bahawa ia berpotensi tinggi untuk digunakan sebagai racun anai-anai disebabkan kesan tunda yang penting dalam kawalan dan pengurusan anai-anai.

Kata kunci: Rhamnolipid biosurfaktan; biotermitisid; anai-anai; *Coptotermes curvignathus*

INTRODUCTION

Termites are considered as a highly destructive insect pest, especially the subterranean termites. Some species of subterranean termites are the most damaging termites to wooden structures. Seven genera of subterranean termites such as *Coptotermes*, *Macrotermes*, *Microtermes*, *Globitermes*, *Odontotermes*, *Schedorhinotermes*, and *Microcerotermes* are usually found to inhabit the areas around buildings and structures (Lee et al. 2007). With a vast distribution in Asia, Australia, Africa, and America, they have inflicted a tremendous damage in building, furniture, plant, and agricultural crop. In 2010, subterranean termite destruction accounted for 80% of the $40 billion in global economic losses attributable to termite damage, and nearly $1.6 billion annually in Southeast Asia (Farhan et al. 2021; Rust & Su 2012).

In Malaysia and many countries in the world which are facing the termite infestation problems, *Coptotermes* spp. is highly regarded as one of the most destructing pests (Couvenc et al. 2016), including agricultural sector especially in the main industrial crop like oil palm and rubber (Lim & Silek 2001). As a result, a number of approaches were carried out to control the infestation with chemical termiticide was widely employed by termite control professionals (Kuswanto et al. 2015). However, the majority of chemical pesticides are toxic, which are a threat toward beneficial insects (Kumar 2012) and the residue from the deployment of termiticides will remain in the soil for a longer duration (Sindhu et al. 2011). In addition to being harmful to the environment, it might induce chemical resistance within that particular species (Mnif & Ghribi 2016).

The rising sense of ecologically friendly pest management approaches has led to an increase in the usage of bioinsecticides to control the pests including termites (Chung 2012; Fenibo et al. 2021; Kumar et al. 2021a; Pierre et al. 2015) which consist of bacterial, cyanobacterial, and microalgal agents, and several biopesticides are naturally occurring mixtures of animal, plant, and microorganism-derived substances (Egbuna et al. 2020; Kumar et al. 2021b; Mnif & Ghribi 2016). This is interesting because the biopesticides are made from renewable sources that are environmentally safe and sustainable (Kalpana & Anil 2021). Despite the advantages of biopesticides, their usage has not yet been as prevalent as expected due to several reasons. Kumar et al. (2021a) listed the related problems and issues, including the high cost to produce pesticides because of the expenses associated with evaluating, producing, and obtaining regulatory approval for new biological agents. Biopesticides were also have a short shelf life due to their susceptibility to changes in temperature and humidity and field effectiveness reduction (Quiroz et al. 2019).

Rhamnolipid biosurfactant which is a bacteria-based product has been identified as a conceivable biopesticide for environmentally friendly agricultural practices as its chemical properties resemble petroleum-derived surfactants. Rhamnolipid compound consists of a combination of rhamnose units (a sugar) and β-hydroxy fatty acids (a lipid) that serves as the active ingredient with potential pesticidal properties. It is a natural compound produced by bacteria, which is biodegradable and pose a low environmental risk, offering a significant advantage over synthetic surfactant. Many researchers have proved that rhamnolipids have diverse and unique properties including sequestering, detergency, emulsifying, wetting, vesicle forming, and phase dispersion that could be an alternative to synthetic chemicals used in controlling plant pathogens, hence, they have been recognized as a safe biocontrol agent (Oluwaseun et al. 2017). In addition, rhamnolipid is reported for its high surface activity, biodegradability, low toxicity, stability at extreme conditions, and possessing antimicrobial activities, making them valuable in various industrial applications (Sinumvayo 2015). For example, bacteria such as *Pseudomonas aeruginosa* has produced rhamnolipids with larvicidal ability against *Aedes aegypti* larvae (Silva et al. 2015), *Myzus persicae* (green peach aphid) (Kim et al. 2011) and *Metisa plana* (bagworm) (Muhammad Syafiq et al. 2022). However, up to this point, the rhamnolipid biosurfactant has not been tested against *Coptotermes* sp. Therefore, this study was carried out to determine its efficacy of rhamnolipid biosurfactant as a bio-termiticide to control termite of genus *Coptotermes* sp. in comparison to other chemical commercial termiticides.

MATERIALS AND METHODS

Study Sites

Sampling was conducted in termite infestation areas within an urban landscape. A preliminary survey was carried out to identify the potential *Coptotermes* sp. infestation spot. The *Coptotermes* sp. were subsequently collected from a location within the Universiti Putra Malaysia (UPM) campus in Serdang, Selangor. The sampling site were situated at coordinates of 2°98' N, 101°71' E and 2°99' N, 101°72' E.

Sampling of *Coptotermes curvignathus*

Coptotermes curvignathus was collected from the urban areas in UPM and was microscopically confirmed of the species using identification keys of (Tho 1992). The species was collected using monitoring stations as described by (Abdul Hafiz & Abu Hassan 2009). Cellulose from old paper boxes were used in this study to maximise and accelerate the foraging activities inside the above-ground monitoring stations (Wang & Henderson 2012). The size of the paper box was standardized into 13 cm long x 6 cm width, and was stacked and compacted into a hollow, rectangular disposable plastic container (14.8 cm long x 5.5 cm width x 9.5 cm high). Before the monitoring stations were installed at the infestation spot, the paper boxes were moisturised. The container was installed on the overactive termite's mud tubes on the infested tree's trunk. The monitoring stations were then covered with black plastic to reduce loss of moisture from inside the box and minimise light penetration. The stations were inspected for the presence of termites on a weekly basis by (Abdul Hafiz & Abu Hassan 2009). The infested monitoring stations were transported back to the laboratory and carefully disassembled. The termites were removed from the box and placed in a plastic tray. Using the method from (Tamashiro et al. 1973), the termites were separated from debris by exposing them to a stack of wood block that was soaked with water for 24 hours prior to the collection of the samples. The termites were reared in the laboratory for further investigation against rhamnolipid biosurfactant.

Production and Quantification of Rhamnolipid Biosurfactant

Rhamnolipid biosurfactants produced *Pseudomonas aeruginosa* USM-AR2 (Nur Asshifa et al. 2012) were supplied by Bioprocess Laboratory, School of Biological Sciences, Universiti Sains Malaysia. The fermentation process was carried out using mineral salt medium (MSM) supplemented with waste cooking oil in a 3.6 L stirred tank bioreactor (Infors HT Labfors 4, Switzerland) at 28°C, 400 rpm agitation and aerated at an air flow rate of 0.3 vvm for 5 days (Samsu et al. 2014). At the end of fermentation, the culture broth was centrifuged to separate the cell pellet from the supernatant containing rhamnolipid. The rhamnolipids in the supernatant were extracted using ethyl acetate at a 1:1 volume ratio. The upper aqueous layer containing rhamnolipid was transferred into a glass Petri dish and dried in a fume hood chamber overnight. Dried crude rhamnolipids were scraped from the surface of the glass Petri dish and stored at 4°C until further use (Çakmak et al. 2017).

Rhamnolipid suspensions were prepared by dissolving dried rhamnolipid in distilled water. Rhamnolipids were quantified by orcinol assay, using rhamnose as a reference, as rhamnose is a by-product of acid hydrolysis of rhamnolipids (Jeong et al. 2004). In the orcinol assay, 2.7 mL of a 0.19% orcinol solution were added to 0.3 mL of rhamnolipid suspension samples. After heating for 50 min at 70 °C, the mixtures were cooled at room temperature, and the optical density (OD_{421}) was measured using a spectrophotometer (Genesys 20, Model 4001-04, USA). Distilled water was used as a blank, and the samples were subjected to the same procedure as the blank. The rhamnolipid concentrations were calculated from a calibration curve prepared with L-rhamnose and expressed as rhamnolipid equivalents (RE).

Laboratory Investigation of the Efficacy of Rhamnolipid Biosurfactant on *C. curvignathus*

For the toxicity test of rhamnolipid against *C. curvignathus*, stock solution of rhamnolipid was prepared into eight different concentrations. The serial dilutions were made with distilled water to obtain desired concentrations in total of 100ml for each concentration. The concentrations used in this study were listed in Table 1. The dilution for fipronil (Brand Regent, Bayer ®)was prepared according to the recommended rate on the label to control termite infestation. Distilled water was used as the control treatment.

Table 1. Details of treatment

Treatments	Active Ingredient	Concentration (%)
T1	Rhamnolipid	5
T2	Rhamnolipid	10
T3	Rhamnolipid	20
T4	Rhamnolipid	30
T5	Rhamnolipid	40
T6	Rhamnolipid	50
T7	Rhamnolipid	75
T8	Rhamnolipid	100
T9	Fipronil (Positive Control)	0.40
T10	Distilled water (Control)	-

In this study, the wood-soak bioassay method was used, and the reaction of termites towards the treated wood was monitored. Based on the method described by Gautam & Henderson (2011), 20 pieces of woods (*Eucalyptus sp.*) (size of 6.3 cm long x 4 cm width x 0.5 cm height), were dried in an oven at 105 °C for 24 hours and then weighed. Eucalyptus wood was used because the type of wood was one of the most preferable by the termite species (Ameka & Helida 2022). According to Gautam & Henderson (2011), under laboratory conditions, the wood blocks contained 9% to 11% moisture at beginning. After oven-drying, the moisture content of the wood was between 0% and 3%. After that, the woods were soaked in rhamnolipid solution at different concentrations for 24 hours to ensure 100% absorption (Gautam & Henderson 2011). After 24 hours, all the woods were removed from the solutions and air-dried for 30 minutes before being weighted again. These steps were repeated for fipronil and distilled water.

Rectangular plastic containers (size of 14.8 cm long x 5.5 cm width x 9.5 cm height) were used as the treatment container. Eight set of wood blocks which were soaked with rhamnolipid biosurfactant, each consisting of two pieces of wood with toothpick in between the woods (as spacer), were prepared and placed inside eight plastic treatment containers, respectively. Meanwhile, another two set of wood stacks of fipronil and distilled water were placed in two other containers. In each of the treatments, 45 workers (90%) and 5 soldiers (10%) of *C. curvignathus* were released into a plastic container, according to (Gautam & Henderson 2011). In this study, no-choice feeding method was used, and the treatments were repeated in triplicates. Termite mortality was monitored and recorded daily until the sixth day after treatment (DAT). A termite was marked as dead if no movement was observed.

Data Analyses

Probit analysis was performed according to (Muhammad Syafiq et al. 2022) to identify the lethal concentration (LC) of rhamnolipid which caused the mortality of the termites. Meanwhile, lethal time, LT_{50} and LT_{90} values refer to the concentrations of pesticides required to kill 50% and 90% of *C. curvignathus* of post-treatments, respectively. The analyses were computed using the Statistical Package for the Social Sciences (SPSS) version 27.

RESULTS

Figure 1 and Table 2 show the efficacy of all treatments based on the percentage of cumulative mortality of *C. curvignathus*. In general, only fipronil showed a significant difference (*P<0.05*) in mortality rate at DAT 1 until DAT 4.

Table 2. Mean percentage of mortality of *C. curvignathus* for each treatment. All treatments were significantly different at F= 10.81, *P=0.0017*

Treatment	Percentage of *C. curvignathus* mortality (%)					
	DAT 1	DAT 2	DAT 3	DAT 4	DAT 5	DAT 6
T1 - 5% RL	11.52	39.39	79.39	87.27	100.00	100.00
T2 - 10% RL	11.52	43.64	80.00	100.00	100.00	100.00
T3 - 20% RL	11.52	49.70	83.64	100.00	100.00	100.00
T4 - 30% RL	12.73	55.15	86.06	100.00	100.00	100.00
T5 - 40% RL	12.73	57.58	93.33	100.00	100.00	100.00
T6 - 50% RL	13.33	63.03	94.55	100.00	100.00	100.00
T7 - 75% RL	13.94	66.06	95.15	100.00	100.00	100.00
T8 - 100% RL	16.36	67.27	96.36	100.00	100.00	100.00
T9 - 0.4% Fipronil	92.12	100.00	100.00	100.00	100.00	100.00
T10 - Control	0.00	0.00	0.00	0.00	0.00	0.00

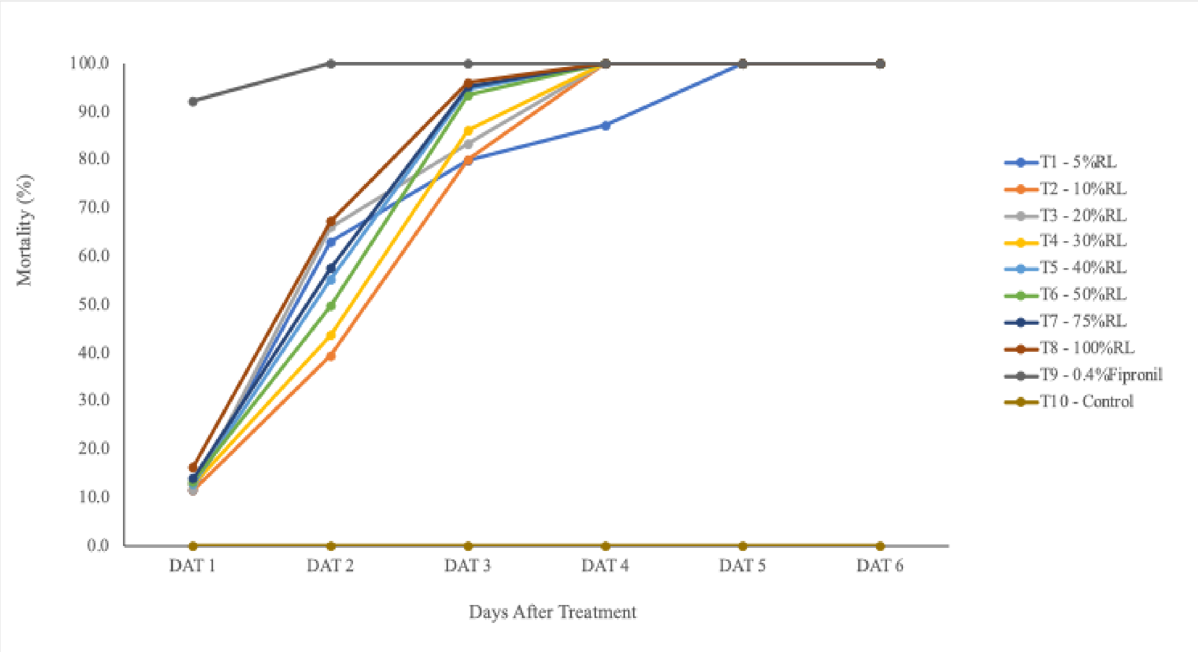

Figure 1. Mean percentage of mortality of *C. curvignathus* for 1,2,3,4,5 and 6 DAT for each treatment

At DAT 1, the mean mortality rate was the highest at T9 (fipronil) with recorded mortality of 92.12%, followed by T8 at 16.36%. The lowest was recorded by RLs (T1) 5%, (T2) 20% and (T3) 30% at 11.52%. Similar pattern was observed in DAT 2, with mortality rate of *C. curvignathus* treated by fipronil was 100%, followed by T8 and T7 in which the mortality rate was 67.27% and 66.06%, respectively. However, at this point, the mortality rate of the termites treated by rhamnolipids, including the fipronil, was significantly different.

On DAT 3, beside fipronil which achieved 100% of mortality, T8 has recorded 96 % of the mortality and followed by T7 (95.15%), T6 (94.55%) and T5 (93.33%). As the previous DAT, T1 scored the lowest mortality rate (79.83%). On DAT 4, all rhamnolipid biosurfactant treatments except T1 eradicated all the termites in each treatment container in which the mortality was 100% at this point. At DAT 3 and DAT 4, the mortality rate of *C. curvignathus* among all treatments was significantly different. T1 achieved 100% mortality rate of *C. curvignathus* at DAT 5, respectively.

LC_{50} and LC_{90} of Rhamnolipid Biosurfactant

Probit analysis was carried out to determine the lethal concentration (LC) of rhamnolipid biosurfactant against *C. curvignathus*, as showed as LC_{50} and LC_{90} in Table 3. However, the LC_{50} and LC_{90} was estimated from DAT2 since the total mortality of *C. curvignathus* in DAT1 was very small compared to the total sample, which lead to inaccurate predictions or unreliable confidence intervals. In this study, LC_{50} and LC_{90} for rhamnolipids biosurfactant treatment was obtained between DAT 2 and DAT4, respectively.

Table 3. Lethal concentration (LC_{50}) of the treatment towards C. *curvignathus* termites during the pesticide's exposure periods

Treatment	Time Exposed (Day)	LC_{50} (%)	LC_{90} (%)	Chi Square
Rhamnolipids biosurfactant	2	17.68	2122.89	0.000
	3	2.30	59.70	0.779
	4	0.29	18.83	0.252

According to the table, the LC_{50} for rhamnolipid biosurfactant was 17.68% at DAT 2, and reduce to 2.30% at DAT3 and 0.29% at DAT4. Meanwhile, during the similar periods, the LC_{90} of rhamnolipid biosurfactant ranged between 2122.89% and 18.82% at DAT2 and DAT 4. According to the result, rhamnolipids have a significant toxicity profile against the *C. curvignathus* population and the toxicity of rhamnolipids was high in accordance with the exposure time. The longer of the exposure with increase the potency effect of the biosurfactant.

DISCUSSION

This study evaluates the efficacy of rhamnolipid, a glycolipid-type biosurfactant produced by *P. aeruginosa* USM-AR2 as a potential biopesticide towards termite, *Coptotermes curvignathus*. Rhamnolipid, a glycolipid biosurfactant of bacterial origin, has gained attention due to its pesticidal activity against a wide range of pests. It is well known for its strong emulsification ability, high surface activity, biodegradability, low toxicity, stability at extreme conditions, and antimicrobial activities, making them valuable in various industrial applications (Sinumvayo 2015). Crouzet et al. (2020) reported that rhamnolipids derived from *Pseudomonas* sp. possess insecticidal properties and may serve as promising agents for the management of other pest species.

Investigation of the biosurfactant efficacy as a biopesticide against the termite species is demonstrating another potential application of the compound which could provide a sustainable alternative to chemical pesticides for protecting crops against the termite species (Steinbauer & Peveling 2011). In light to this, the use of waste cooking oil as a low-cost substrate provides a cost-saving strategy for rhamnolipid production by *P. aeruginosa* USM-AR2. This translates to production cost range of RM12 – RM15 per litre at lab scale. Commercial scale production cost is certainly lower due to economy of scale. Rhamnolipid use in agriculture will mitigate environmental contamination by chemical pesticides used against plant pathogens. Its wide agricultural applications include serving as a biocontrol agent, enhancing soil quality, boosting plant immunity, improving plant growth against pathogens, and improving wettability. It is also commonly used for soil remediation to boost soil fertility and further investigated as a component of biopesticides (Largo & Parman 2012).

In this study, *Coptotermes curvignathus* exhibited the highest mortality rate when treated with fipronil at recommended rate, as fast as at DAT 1. This is expected since fipronil as a broad-spectrum insecticide was widely used to control termite infestation (Chen et al. 2015). According to Yii et a. (2015), fipronil was efficiently transmitted across termite workers and caused substantial mortality throughout the recipient individuals, indicating that it has a high toxicity profile. Elango et al. (2012) highlighted that sublethal fipronil controlled *C. curvignathus* up to 99%. Results obtained showed that fipronil achieved 100% mortality at DAT 2.

At day 2 after the treatment, the efficacy of the rhamnolipid showed tremendous increment with T8 and T7 cause mortality to 67.27% and 66.06% of the termites, respectively. Based on the previous study, the biopesticide insecticidal properties started to show decent effect on mortality of test subjects within 1 to 2 days after treatments. In the study, leaf hexane extract of *A. bracteolata*, ethyl acetate extract of *A. paniculata*, *D. metel*, *E. prostrata*, and methanol extract of *A. lineata* and *D. metel* showed considerable termicidal action after 24 and 48 hours of exposure. Similar findings were reported by Seo et al. (2014) in which their essential oils from four plants took two days to show toxicity against the Japanese termite *Reticulitermes speratus*. Interestingly, by day 4 after the treatments, most of the treatments recorded 100% of termite mortality. According to Silva et al. (2019), after 4 days of treatment with 0.075% and 0.1% rhamnolipids, mortality of *Bemisia tabaci* nymphs were also 100%. However, Kim et al. (2011) reported that rhamnolipid took five days to cause the 80% mortality of green peach aphid (*Myzus persicae*). Nevertheless, Muhammad Syafiq et al. (2022) reported that 100ppm and 200ppm of rhamnolipid biosurfactants were not able to achieve 100% mortality of bagworm (*Metisa plana*) even after one week of observation. These findings indicated that the compound was more potent to *C. curvignathus* in comparison to other pests. This study revealed that the LC_{50} and LC_{90} values for rhamnolipid biosurfactant treatments was acquired up to second day of exposure due to 100% of mortality of *C. curvignathus*. Observations indicated that rhamnolipids have a remarkable toxicity profile against the *C. curvignathus* population. In general, the initially high value of LC_{90} suggested that higher concentrations of rhamnolipid were required to overcome variability in the species susceptibility and the relatively slow action of the biosurfactant in causing termite mortality. Factors such as wood absorption and feeding activity may also have reduced uniform exposure. Nevertheless, the decline in LC_{90} over time indicates that rhamnolipid toxicity is time-dependent and becomes more effective with prolonged exposure (Siqueira et al. 2021). The range for LC_{50} in this study, which was between 17.68% on DAT2 and 0.29% on DAT4, was comparable to the range the range of LC_{50} by Mohd Shukri et al. (2011) in their study on larval feeding of diamondback moth (DBM). The LC_{50} of the present study was lower compared to *Azadiractha* biopesticide which was tested on tiger shrimp post-larvae, *Penaeus monodon* with LC_{50} of 54,719.57 ppm (Septiningsih & Permatasari 2021). Meanwhile, Mostakim et al. (2012)

reported that *P. aeruginosa* (IL5) showed LC_{50} and LC_{90} values of 24.0784 µL/mL and 47.3440 µL/mLagainst olive fruit fly (*Bactrocera oleae*) after 72 hours of exposure. Kakka et al. (2021) in their observation of the neem extract against water flea (*Daphnia magna*) reported that the LC_{50} and LC_{90} of the neem extract was 522.86 ppm and 1840.48 ppm (Kakka et al. 2021). The present study also indicated that LC_{50} and LC_{90} values of rhamnolipid biosurfactant decreased over time. According to Connell et al. (2016), based on Habers Rule, the lethal concentration was invertedly correlated with time of exposure and this is very important particularly in relation to health risk assessment and guidelines for exposure to toxicants.

Although the mode of killing of rhamnolipid biosurfactant against *C. curvignathus* was not observed, the property of rhamnolipid might have disturbed the cuticle part of the insect pest. Based on the biology of lower termites such as *C. curvignathus,* gut bacteria such as *Bacillus cereus* is required to digest cellulose (Ramin et al. 2009). According to observation by Kannan et al. (2022), rhamnolipid causes the breakdown of beneficial interaction between the gut and microbes including *B. cereus* in *Bombyx mori* larvae. Therefore, it is safe to speculate that rhamnolipid biosurfactant contributed in reducing the population of gut microbes of *C. curvignathus*, thus leading to the mortality of the species after feeding on the wood treated by the compound. Besides, the compound also showed slow action efficiency compared to fipronil, which was very important in termite control and management. According to Sapkota et al. (2020), delayed mortality of termite after exposure to termiticide is important to provide some time for horizontal transfer of the compound among the members of the colony including the termite queen. This mode of action will perish the whole colony when the compound is able to reach and kill the queen. Therefore, rhamnolipid biosurfactant has potential application against the termite as it acts suitably like the established termiticide. Although this study has documented the preliminary findings of rhamnolipid potential as biopesticide, further investigation is required especially in the field application before it can be widely used to control *C. curvignathus* infestation.

CONCLUSION

This study confirmed the potential of rhamnolipid biosurfactant as a pesticide against *C. curvignathus*. Efficacy was observed at after two days of application, with complete termite elimination by day four. The decreased values of LC_{50} and LC_{90} over the same period, demonstrating the increasing toxicity of the compound. This study showed a promising finding to further investigate this compound to be used as biotermiticide. This compound with favourable toxicological and environmental profile (biodegradable) will be an alternative to control and manage the termite infestation especially the Subterranean termite, *Coptotermes curvignathus* which causes a lot of damage and loss to human. However, further investigation needs to be carried out to provide conclusive evidence on its effectiveness and suitability to be used in the field.

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AUTHORS DECLARATION

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
This is a Final Year Project (FYP) and the data are currently in FYP thesis of Muhammad Adib Shaharuddin (2022).

Authors' Contributions
Muhammad Adib Shaharuddin (MAS) and Wan Mohd Hafezul Wan Abdul Ghani (WMHWAG) conceptualized this research and designed experiments; Nur Asshifa Md Noh (NAMN) and Ahmad Ramli Mohd Yahya (ARMY) prepared and supplied the rhamnolipid biosurfactant for the experiment; MAS, Anis Syahirah Mokhtar (ASM) and WMHWAG participated in interpretation of the data; MAS wrote the paper and WMHWAG, NAMN, ARMY and ASM participated in the revisions of it. All authors read and approved the manuscript.

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