ISSN impresa 0717-3644
ISSN online 0718-221X

Maderas. Ciencia y tecnología 2024 (26): 44, 1-12

DOI: 10.22320/s0718221x/2024.44

TOXICITY AND REPELLENCY OF CHLORPYRIFOS NANOCAPSULES AGAINST SUBTERRANEAN TERMITE *Coptotermes curvignathu*

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ABSTRACT

Chlorpyrifos is widely used in agricultural and building industries to control many insects including termites. However, its low aqueous solubility and tendency to decompose under sunlight results in decrease in its efficiency. In the present study, chlorpyrifos was encapsulated into nano-sized poly (styrene-co-maleic anhydrite) using a mini-emulsion in-situ polymerization method to improve the effective utilization rate. The termiticidal properties of nanocapsules at different concentrations (1 - 25 wt%) were tested against subterranean termite; *Coptotermes curvignathus.* Results of Choice bioassays shows that chlorpyrifos nanocapsules are strongly toxic to *Coptotermes curvignathus*. The filter paper feeding inhibition and repellent bioassay show a significant feeding inhibition (> 60 %) at lowest concentration of chlorpyrifos nanocapsules. The highest mortality rate of termites (90 %) after 24-h exposure was observed in the sample treated with the highest concentration of chlorpyrifos nanocapsules (25 wt%). Results obtained from this study show that chlorpyrifos nanocapsules have a strong termiticidal property against *Coptotermes curvignathus*. This indicates that the nanoencapsulation of biocides chlorpyrifos opens a real potential of new and advanced wood preservation technology.

Keywords: Chlorpyrifos, mortality, nanocapsules, toxicity, repellency, subterranean termite

INTRODUCTION

Termites can cause severe damage to wood and wood-based products causing tremendous economic losses. The silent attack of termites is hard to detect until their sudden appearance of damage exposes building occupants to danger. Termite also attacks standing plant likes palm oil tree, rubber tree and sugarcane (Manager and Singh 2001, Cheng *et al*. 2008). In Malaysia, the subterranean termites, *Coptotermes* spp. (Isoptera: Rhinotermitidae), are the most widespread destructive termites to wood and wood-based products (Kamble and Davis 2005, Abdul Majid and Ahmad 2009). Preservation of wood structure and wood-based products using chemical is an effective technique for preventing termite damage.

Chlorpyrifos is an organophosphate broad-spectrum pesticide introduced in 1965 and used on crops, ani-

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Received: 18.02.2022 Accepted: 08.06.2024

mals, buildings, and other settings to kill many insects (Paul and Lydia 2011, Dick *et al.* 2001) that kills insect pests by disrupting their Central Nervous System (CNS) (Jones and Huang 2003, Srihayu Harsanti *et al.* 2015, Hassan *et al.* 2018, Kurnia *et al.* 2021).

Chlorpyrifos was used as a termiticide in 1980s for termite control including pre- and post-treatment applications by professional applicators (Dyer *et al.* 2001, Anon 2002). However, insecticidal action of this insecticide is relatively short, and it degrades rapidly after application (Budarz *et al.* 2019). High concentration and repeat application of chlorpyrifos are needed to sustain the insecticide function for a long time (Ahmed *et al.* 2017). Moreover, its low aqueous solubility also restricts its application.

Repeated application and high concentration results in increasing residual toxicity and can cause ecological imbalance, a serious threat to human, and the survival of other non-target organisms (Enserink *et al.* 2013). Chlorpyrifos was reported to cause cancer, respiratory problems, abnormal cell growth, asthma and reproductive system injury (Lasagna *et al*. 2020, Sabbouri *et al.* 2020). It is also harmful to the environment (Iram *et al.* 2013, Subekti *et al.* 2019). The direct use of chlorpyrifos contaminates the ground-water thus affects many biological systems (Roy *et al*. 2009, Fan *et al*. 2021).

A recent study about biocides encapsulations indicate that this procedure could be appropriate to overcome the drawbacks of chlorpyrifos mentioned above (Huang *et al.* 2018, Bakry *et al*. 2016). The encapsulation of biocides is an innovative strategy to control the release rate, increase service lifetime of biocide and reduce biocides concentration during application (Maia *et al*. 2012, Bilenler *et al*. 2015, Oliveira *et al*. 2018, Ruggiero *et al*. 2019). Encapsulation is the technique that physically entraps the biocides into the protective matrix at the micro- or nano-size range (Karadeniz *et al*. 2018, Ferreira and Nunes 2019). The properties of capsules can be engineered based on their end application. In this way, biocides could be slowly released from the capsules due to pH (Song *et al*. 2019), temperature (Zheng *et al*. 2019), water (Liang *et al.* 2020) or light (Ye *et al.* 2015).

This study aimed to investigate the efficacy of chlorpyrifos nanocapsules to control subterranean termites *Coptotermes curvignathus*. Different bioassays were carried out to assess chlorpyrifos nanocapsules' effectiveness against the *Coptotermes curvignathus*.

MATERIALS AND METHODS

Preparation of chlorpyrifos nanocapsules

We used chlorpyrifos (Certified Reference Material, Supelco), hydroxypropyl cellulose (average Mw ~100000, powder, 20 mesh particle size, 99 % through, Sigma-Aldrich), poly (styrene-co-maleic anhydrite) (average Mv ~65000, Sigma-Aldrich) and distilled water without further purification to prepare chlorpyrifos nanocapsules using miniemulsion in-situ polymerization method. The water phase consisted of hydroxypropyl cellulose (0,06 g) and distilled water (36 ml) was mixed together using a magnetic stirrer. The oil phase was prepared by mixed poly (styrene-co-maleic anhydrite) (0,16 g), chlorpyrifos (0,1 g) and 0,04 g polyvinyl alcohol (PVA). The oil phase was added to the water phase. The mixtures were pre-emulsified mechanically using a high-speed homogenizer (IKA®T25 digital ULTRA-TURRAX®). The miniemulsion was obtained using a sonicator (ultrasonic bath, SB 3200 DTN, 40 KHz, Loyal Key Group Shanghai Branch Co., Ltd). The miniemulsion was continuously stirred with a magnetic stirrer at room temperature for 24 h. The chlorpyrifos nanocapsules stored in a sample bottle before physico-chemical and in-vivo termite bioassays. The encapsulation efficiency of chlorpyrifos nanocapsules (EE %) was calculated using Equation 1 as below:

$$EE(\%) = \frac{W_2}{W_1} \quad (1)$$

Where:

W_1 is the total amount of chlorpyrifos added initially during encapsulation process.

W_2 is the concentration of chlorpyrifos detected in the nanocapsules.

Preparation of different concentrations of chlorpyrifos nanocapsules

The chlorpyrifos nanocapsules obtained in this study were in the form of nanoparticles dispersed in water (Figure 1). The chlorpyrifos nanocapsules is assumed 100 % in purity. Nanocapsules were re-dispersed using water to obtain the concentration of 1 - 25 wt % based on the Equation 2 below,

$$wt(\%) = \frac{w_1}{w_2} \times 100 \quad (2)$$

Where:

w_1: the weight of salute (chlorpyrifos nanocapsules) (g).

w_2: the weight of solvent (water) (g).

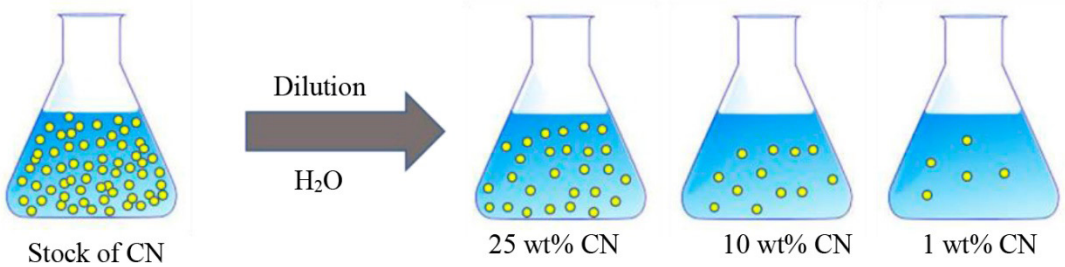

Figure 1: Illustration of nanocapsules stock solution and preparation of different concentration of nanocapsules.

Particle size distribution and chemical characterization

The particle size distribution and chemicals properties of chlorpyrifos nanocapsules were determined using a dynamic light scattering (DLS) (Malvern Particle Size analyzer) and field emission scanning electron microscope (FESEM). For DLS measurement, the chlorpyrifos nanocapsules were dispersed in distilled water (0,01 w/v%) before analysis. For measurement using FESEM, the dried chlorpyrifos nanocapsules were dispersed on a conductive carbon adhesive tape surface, attached to a FESEM stub, and then goal coated. Gold coating on samples prior to FESEM analysis is necessary to prevent charging of the sample surface and to promote the emission of secondary electrons, which cause the specimen to conduct evenly, and to provides homogenous surface analysis and imaging. Both measurements were carried out at room temperature.

The chemical properties of chlorpyrifos nanocapsules was analyzed using a Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer BX spectrophotometer). The dried chlorpyrifos nanocapsules sample was mixed with KBr and the FTIR spectra were recorded at 280-4000 cm^{-1}. The FTIR analysis was carried out at room temperature.

Termite collection

Coptotermes curvignathus was collected from an active colony that attacked rubberwood (*Hevea brasiliensis* Willd. Muell. Arg) logs placed around the Forest Research Institute Malaysia (FRIM) field 51. The log was cut and taken to the laboratory to separate the termites according to the caste (soldiers and workers). Soldiers and workers were removed from the logs by placing several pieces of filter paper moistened with water and left for a few minutes. Filter paper filled with termites was inserted into the basin to separate soldiers and workers. Only active termites were selected for testing.

Feeding inhibition bioassay

The bioassay method used in the previous study (Roszaini *et al.* 2013) was adopted to evaluate the toxicity of chlorpyrifos nanocapsules against *Coptotermes curvignathus*. Approximately 20 μl of chlorpyrifos nanocapsules solutions (0 wt%; 1 wt%; 3 wt%; 5 wt%; 10 wt%; 15 wt%; 20 wt% and 25 wt%) were pipetted using a micropipette onto filter paper disc weighing approx 30 mg (Advantec, 8 mm diameter and 1,5 mm thickness)

using a micropipette and were dried in a vacuum desiccator for 24 h. All treated and untreated filter papers were oven-dried at 60 °C until a constant weight achieved before and after termite exposure. All weights obtained are recorded. Filter paper treated with 20 μl of copper chrome arsenic (CCA) (3 wt%), chlorpyrifos (3 wt%) and acetone, and untreated filter paper were used as a control. All the filter papers that were tested (either treated or control sample conditioned for seven days). Forty-five active termite workers and 5 soldiers (from active colonies of *Coptotermes curvignathus*) were placed in each Petri dish (90 mm in diameter and 16 mm high) containing 3 g of sterile sand. The dried treated filter paper and untreated filter paper then were placed on the Petri dish. A few drops of water are added periodically to the bottom of each Petri dish. All Petri dishes with lids are placed in an incubator at 22 $\pm$ 2 °C and 65 $\pm$ 5 % RH and number of live termites were counted after every 24 h for 10 days. Each test contained five replicates including the control. At the end of the test, the filter papers were oven dried till constant before weighing. The difference in dry weights before and after the exposure was used to calculate the consumption of the filter papers A dose-mortality line was developed depends on the exposure time(s) and the lethal concentration (LC_{50}) of chlorpyrifos nanonapsules were determined using the Probit method (Finney 1971).

Fumigant activity

The mortality of *Coptotermes curvignathus* workers was tested using the fumigation method following method described by Subekti *et al*. (2019) with following slight modification. Approximately 0,5 ml of each chlorpyrifos nanocapsules concentration (0 wt%; 1.0 wt%; 3 wt%; 5 wt%; 10 wt%; 15 wt%; 20 wt% and 25 wt%), including controls were pipetted on the filter paper which had been glued to the bottom surface of petri dish lid. The control filter paper was treated with 0,5 ml of 3 wt% CCA, 3 wt% chlorpyrifos and only acetone. Then filter papers were left open to evaporate solvent. After that, 20 active workers of *Coptotermes curvignathus* were placed into the petri dish. Then the petri dish was closed and tightly sealed with parafilm. Each test was repeated five times including the control and observation of termite mortality were carried out after 24 h and 72 h of termite release.

Repellent activity

The ability of the chlorpyrifos nanocapsules to repel termites was assessed as described by Roszaini *et al.* (2020). Approximately 1 ml of chlorpyrifos nanocapsules at different concentrations was applied on half of the 9 cm diameter of filter paper. The other half is a control sample. 2 types of control samples were used. One was pipetted with acetone only and the other with distilled water only.

A similar step was used to prepare the control samples. Then, the part of the filter paper treated with chlorpyrifos nanocapsules at different concentrations was taped to one of the control samples. Figure 2 illustrates the treatment carried out on the filter paper for repellence assay. All treatments were replicated five times. The petri dish with 9,1 cm in diameter, was dried in a laminar flow hood for one hour. Then the treated filter paper samples were attached into the petri dish using adhesive tape. Fifty workers of *Coptotermes_curvignathus* were placed in the center of each disc. The number of termites presents in the control and the treated filter paper was assessed after every hour for 4 h.

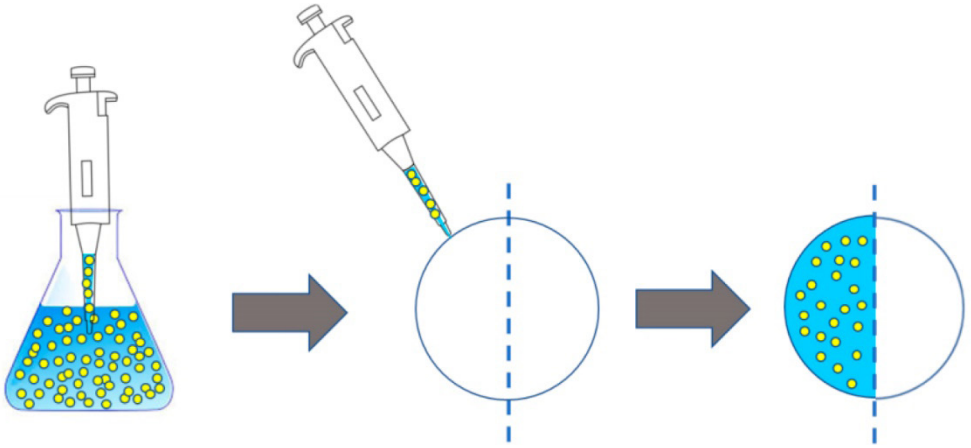

Figure 2: Illustration of treatment carried out on the filter paper for repellence assay.

Statistical analysis

Data obtained was analysed using One Way ANOVA followed Duncan Multiple Range Test. Corrected mortality of termites was calculated using Abbott's formula (Abbott 1925) and median lethal dose was calculated using Probit analysis.

RESULTS AND DISCUSSION

Particle size distribution of chlorpyrifos nanocapsules

The particle size distribution of chlorpyrifos nanocapsules is crucial part for achieving the desired toxicity or repellence properties and essential for its application as a termiticide. Particle size distribution also influences the chlorpyrifos nanocapsules' solubility, stability and reactivity (Liu *et al.* 2002). The smaller the particle size of chlorpyrifos nanocapsules, the higher the surface area (Tontul *et al.* 2017). Table 1 summarizes the particle size of chlorpyrifos nanocapsules determined using DLS and FESEM. The particle size distribution results from FESEM agreed with the results obtained using DLS. The results also indicate that chlorpyrifos nanocapsules are relatively uniform and stable.

Table 1: Particle size distribution of chlorpyrifos nanocapsules determined using DLS and FESEM.

Analysis method	Mean diameter
DLS	210 ± 12
FESEM	219 ± 10

Chemical characterization of chlorpyrifos nanocapsules

The FTIR wavelength absorption give more insight into the structural changes of chlorpyrifos after encapsulation. Table 2 show the FTIR wavelength absorption of chlorpyrifos nanocapsules, capsule without chlorpyrifos and chlorpyrifos. No changes between absorption wavelength of chlorpyrifos nanocapsules, capsule without chlorpyrifos and only chlorpyrifos were observed. The wavelength absorption of chlorpyrifos showed band at 990 cm^{-1} and 750-800 cm^{-1} which are characteristic of the stretching vibration of P-O-P alkyl group and P=S, respectively.

The wavelength absorption of capsule without chlorpyrifos at 2800-3200 cm^{-1} are due to the presence of O-H stretching, while absorption band at 1600 - 1900 cm^{-1} indicate the presence of C=O vibration. As shown in Table 2, it is evident that FTIR peaks of chlorpyrifos nanocapsules overlap with those peaks of capsules without chlorpyrifos, indicating that chlorpyrifos was successfully encapsulated into the nano-sized poly styrene-co-maleic anhydrite) shell without any chemical interaction between chlorpyrifos and polymer shell.

Table 2: Chemical characteristics of chlorpyrifos nanocapsules, chlorpyrifos, and capsule without chlorpyrifos.

Sample	Band (cm^{-1})	Functional group
Chlorpyrifos nanocapsules	2800 - 3200	Stretching vibration of O-H in polymer capsules (capsule without chlorpyrifos)
	1600 - 1900	C=O vibration in polymer capsules
	995	Stretching vibration of P-O-P alkyl group of chlorpyrifos
	752 - 809	Stretching vibration of P=S of chlorpyrifos
Chlorpyrifos	990	Stretching vibration of P-O-P alkyl group
	750 - 800	Stretching vibration of P=S
Capsule without chlorpyrifos	3042	O-H stretching vibration
	1600 -1900	C=O vibration in poly (styrene-co-maleic) anhydrite

Toxicity and feeding inhibition

Fumigant activity of chlorpyrifos nanocapsules against termites has been extensively studied (Asogwa *et al.* 2009). Table 3 shows the mortality rate of *Coptotermes curvignathus* after it was exposed to filter paper treated by different concentrations of chlorpyrifos nanocapsules. Results indicate that the chlorpyrifos nanocapsules were highly toxic to *Coptotermes curvignathus.* The average consumption of filter paper was significantly lower than in corresponding controls (untreated = 5,87 %, 3 wt% chlorpyrifos = 4,66 %, acetone = 3,42 % of weight loss). This effect was more pronounced for filter papers treated with 25 wt% of chlorpyrifos nanocapsules. There have no significant different on toxicity property of chlorpyrifos nanocapsules against termite at 15, 20 and 25 wt% concentration.

Current studies revealed that chlorpyrifos nanocapsules in a concentration of 15 wt% were found to be sufficient to kill *Coptotermes curvignathus*. The performance of 15 wt% chlorpyrifos nanocapsules is comparable with commercial wood preservatives, CCA at a concentration of 3 %. Results indicated that the toxicity of chlorpyrifos nanocapsules against *Coptotermes curvignathus* was significantly higher than capsule without chlorpyrifos, 3 wt% chlorpyrifos and acetone ($F = 16,526$, $p= 0,001$), ($F = 8,567$, $p= 0,001$) and ($F = 7,125$, $p= 0,001$, respectively).

Table 3: Effect of different concentrations of chlorpyrifos nanocapsules on feeding and mortality of *Coptotermes curvignathus.*

Treatment	C (wt%)	Paper consumption (%)	Feeding-Inhibition (%)	Termite mortality (%)	LC_{50} (wt%)
Control		5,87 (0,44)a		15,65 (1,11)h	
3 % CCA		1,35 (0,27)e		94,53 (2,42)a	
3 % chlorpyrifos		4,66 (0,41)b		40,10 (0,98)f	
Acetone		3,42 (0,33)c		24,72 (0,33)g	
Nanoencapsulated chlorpyrifos	0	3,26 (1,22)c	46,32 (3,13)d	13,45 (2,01)h	21,75
	1	1,76 (0,11)d	66 (1,56)c	18,71 (1,55)gh	
	3	1,37 (0,43)e	71,14 (5,92)bc	43,20 (0,80)f	
	5	1,11 (0,21)f	78,57 (2,56)b	58,44 (0,66)d	
	10	1,17 (0,48)f	76,29 (5,50)b	52,47 (1)e	
	15	0,92 (0,38)g	82,86 (7,28)a	70,69 (0,84)c	
	20	0,89 (0,50)g	83,43 (9,35)a	68,80 (1,22)c	
	25	0,83 (0,38)g	84,80 (6,89)a	88,43 (0,98)b	

Mean (± SD) of 5 replicates for each species. C = Concentration.

Values followed by the same letter are not significantly different in the same group (vertical) at the 0,05 level of probability.

LC_{50} = Lethal Concentration which causes a 50 % reduction in feeding as compared to the non-treated control. FL = Fudicial Limit.

The feeding inhibition of chlorpyrifos nanocapsules at different concentrations is summarized in Table 3. Chlorpyrifos nanocapsules at all concentrations exhibited >60 % of the feeding inhibition. The LC50 for individuals value shows that chlorpyrifos nanocapsules require a minimum concentration of 21,50 % wt% to reduce 50 % feeding inhibition compared to untreated samples.

The effectiveness of chlorpyrifos as termiticides against many termite species have been reported in several previous studies (Venkateswara Rao *et al.* 2005, UNEP 2008, Ahmed *et al.* 2015, Chen *et al.* 2015, Salem *et al.* 2020). Chlorpyrifos kills insects by interfering with acetylcholinesterase in the nervous system. This interference causes an increase in levels of the nerve transmitter chemical acetylcholine, leading to over-stimulation of the nervous system and rapid twitching and paralysis of muscles (Subekti *et al.* 2019, Hillock and Bolin 2012).

Mortality

Table 3 also shows that termite mortality is highest on the filter paper treated with 3 % CCA (94,53 %). The performance of this 3 % CCA is better than the other control filter paper (control, 3 % chlorpyrifos and acetone). The percentage of termites killed increases with increasing concentration of the nanocapsulated chlorpyrifos used. The mortality rate was 18,71 % when the filter paper was treated with 1 %, while it further increased to 88,43 % when the concentration of nano-encapsulated chlorpyrifos was increased to 25 %.

This trend is in agreement with previous studies conducted on different termite species tested. Dursban (chlorpyrifos 48 %) caused 80 % mortality of *Microcerotermes eugnathus* at 960 mg/L compared to only 48 % at 120 mg/L after 7 days (Salem *et al*. 2020). The highest concentration (2000 ppm) of chlorpyrifos-ethyl killed 69,33 % of *Ancistrotermes cavithorax* workers in 4 hours of exposure (Akpesse *et al*. 2014). Higher concentration of chlorpyrifos 80 % showed higher mortality (69,61 %) of *Coptotermes heimii* workers than lower concentration (5 % with only 34,97 % of termite mortality) in 3 hours' exposure (Qasim *et al.* 2022).

Fumigant activity

The mortality of *Coptotermes curvignathus* fumigated with different concentrations of chlorpyrifos nanocapsules after 24 and 72 h are presented in Figure 3. In the 24 h exposure, the highest mortality (>30 %) was found in the 3 wt% CCA, 15 wt% and 25 wt% of chlorpyrifos nanocapsules followed by 20 wt%, and 10 wt% (34%), 5 wt% (32%) and 3 wt% (30 %). Mean while, there was an increase in mortality when the fumigation period was increased to 72 h.

Emamjomeh *et al.* (2022) reported that the most practical approach to improve the durability and insecticidal activity of any active ingredient can be achieved by using nanocapsulation methods. Hillock and Bolin (2012) further explained that the high purity and uniformity, small particle size, and good chemical homogeneity result in disrupting the pest's nervous system and subsequently paralyzing brain cells. This causes the pest to stop feeding and die. Apart from this, Mattos *et al.* (2019) reported that nanoparticles release biocides slowly. This is important because termites eat the particles and transfer them to other termites, eventually leading to colony destruction.

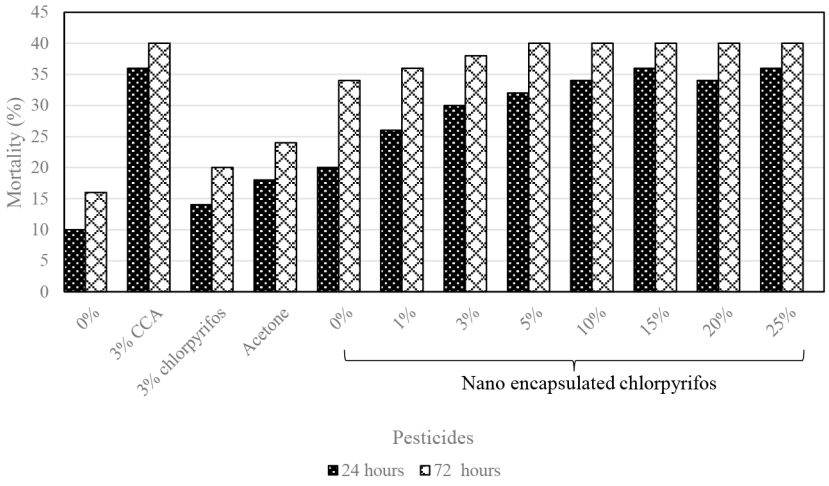

Figure 3: Mortality of *Coptotermes curvignathus* with chlorpyrifos nano capsules treatment for 24 h and 72 h of exposure.

Repellence assay

Chlorpyrifos nanocapsules showed increased repellent activity with increasing concentration, but some treatments were not significantly different from each other (Table 4). Although nanocapsules without chlorpyrifos (0 %) had a strong repellent activity of 22,4 % compared to 3 wt% chlorpyrifos (-17,6 %) and acetone (9,6 %), it still could not overcome the repellent activity equivalent to that shown by the 3 % weight of CCA (35,5 %).

However, the performance of this repellent activity can surpass the performance of 3 % weight CCA after the concentration of encapsulated chlorpyrifor is increased to 10 % weight with a repellent activity of 36,8 %. In general, the increase in repellent activity increases with increasing the concentration of encapsulated chlorpyrifor. Over all, all results suggest that chlorpyrifos nanocapsules are highly toxic against termites in a concentration-dependent manner.

Table 4: Mean percentage of repellent activity for *Coptotermes curvignathus* in a choice test.

Treatments	Concentrations (wt %)/repellent activity (%)							
	Control	1	3	5	10	15	20	25
3 % CCA	35,5a	-	-	-	-	-	-	-
3 % chlorpyrifos	-17,6d	-	-	-	-	-	-	-
Acetone	9,6c	-	-	-	-	-	-	-
Encapsulated chlorpyrifos	22,4db	33,1c	32,8c	33,6c	36,8b	37,4b	48,0a	47,2a

Mean of 5 replicates for each species. Percentage values followed by the same letter in the same colour are not significantly different in the same group at the 0,05 level of probability.

Some studies conducted to control subterranean termites showed promising results, which are also consistent with our present study. Hassan *et al.* (2018) evaluated the persistence of chlorpyrifos (40 EC) in soil against subterranean termites. They found that chlorpyrifos showed a significant increase in mortality of subterranean termite at week 24 (compared to the other two time intervals of 8 and 16 weeks) and suggested that 1-2 liters of chlorpyrifos per 140 ft2 is recommended by pest controllers in Pakistan. They also showed that chlorpyrifos (40 EC) can be used as a termiticide against all types of termites around buildings and also in agriculture. Meanwhile, Kard and Mauldin (1990) reported that a 1% solution of chlorpyrifos can be used effectively against termites.

CONCLUSIONS

By using in-situ polymerization in miniemulsion, chlorpyrifos was effectively encapsulated in nano-sized poly(styrene-co-maleic anhydride), and its structure was verified by FTIR, DLS, and FESEM.

The synthesized chlorpyrifos nanocapsules, with an average diameters of 210 nm ± 12 nm and 219 nm ± 10 nm, respectively, showed consistent and stable structure. At various doses, chlopyrifos nanocapsules also showed good termiticidal activity against *Coptotermes curvignathus*, with an LC_{50} of 14,34 wt%. But when the concentration increases above 15 wt%, the efficacy of the chlorpyrifos nanocapsules becomes insignificant.

To fully understand the function of chlorpyrifos nanocapsules in wood, further research is needed.

Author contributions

R. K.: carried out the experiment, literature search, development of the concept and write-up of the manuscript. T. K.: carried out the experiment, literature search, development of the concept and write-up of the manuscript. M. N. M. A.: carried out the literature search and assisted the development of the concept. S. L.: carried out the literature search and assisted the development of the concept.

Acknowledgements

The authors are grateful to express their gratitude to the staff in Wood Entomology Laboratory, FRIM (Mr. Mohd Saidin Kamaluddin and Mrs. Norziah Ishak) for helped in collecting termites and bioassay test. The authors also would like to thank the Wood Preservation Analytical Laboratory, FRIM (Sabariah Sani, Saharudin Musa and Reduan Zammir Gul) for preparation and physico-chemical characterization of the chlorpyrifos nano capsule. This research was supported by the Ministry of Science, Technology and Innovation (MOSTI) under Science-Fund Project.

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