FORMULATION OF ENTOMOPATHOGEN *Metarhizium anisopliae* var. *anisopliae* BAIT TO CONTROL SUBTERRANEAN TERMITE, *Coptotermes curvignathus* HOLMGREN (BLATTODEA: RHINOTERMITIDAE)

Nyam Vei Ting[1], Joseph Bong Choon Fah[1],
Ong Kian Huat[1] & Patricia King Jie Hung[1,2*]
[1]Faculty of Agricultural Science and Forestry,
Universiti Putra Malaysia Bintulu Sarawak Campus, Malaysia
[2]Institut Ekosains Borneo,
Universiti Putra Malaysia Bintulu Sarawak Campus, Malaysia
*Corresponding author: *patricia@upm.edu.my*

ABSTRACT

Coptotermes curvignathus is well known as the leading pest termite of agricultural and forestry tree crops. Termites are cryptic insects, hence their infestations can go undetected for years and this hinders the planters to carry out effective pest controls timely. This underscores the need for innovative forms of pesticides deployment. The use of bait is becoming a favorable approach that acts as edible 'smart missiles' to knock out the whole population of termites. To execute potent baiting in the field, effective bait matrix that able to preserve the effectiveness of the entomopathogenic agents impregnated and avoid the repellence of the targeted insect is sought-after in designing baits and this study aimed to formulate an entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* based bait. Seven baits were formulated based on the pest feeding preferences and termites' behavior studies. Additional substances were supplemented in the formulas as food sources and attractant to termites. Bait matrix made of dextrin, PVP K90 and skim milk (DPS) was found to be the most palatable to termites than other bait matrix tested. Termite took shorter time to finish the DPS bait with average 11.2 days and consumed significantly greater amount as compared to the other baits. DPS bait impregnated with *M. anisopliae* resulted closed to 100% mortality in 19 days. Based on shelf-life bioassays, all formulated baits were able to maintain consistent termite mortality above 80% for over six months. The laboratory assessment had concluded that the formulated DPS bait was able to retain the effectiveness of the active entomopathogenic ingredient and appear palatable to *C. curvignathus*. Future field work should concentrate on the effectiveness of this bait in deterring pest resurgence.

Key words: Biological control, pest control, subterranean termites

ABSTRAK

Coptotermes curvignathus terkenal sebagai anai-anai perosak utama yang mengancam tanaman pertanian dan perhutanan. Anai-anai merupakan serangga kriptik, oleh yang demikian, serangan anai-anai jarang dapat dikesan pada peringkat awal dan ini menghalang para petani melakukan langkah kawalan perosak yang berkesan dan tepat pada waktunya. Justeru itu, kawalan perosak yang inovatif diperlukan. Penggunaan umpan yang bertindak sebagai 'peluru berpandu pintar' yang berpotensi menghapuskan seluruh koloni anai-anai semakin mendapat perhatian. Untuk menjamin keberkesanan umpan di ladang, matriks umpan perlu dapat mengekalkan keberkesanan agen entomopatogen dan dapat menarik serangga yang disasarkan untuk memakannya. Kajian ini bertujuan untuk menghasilkan umpan berasaskan kulat entomopatogenik *Metarhizium anisopliae* var. *anisopliae* yang berkesan dalam mengawal perosak. Dalam formulasi umpan ini, pemilihan makanan, tingkah laku dan habitat anai-anai dikaji agar formulasi ini lengkap berfungsi sebagai sumber makanan dan mampu menarik anai-anai. Matriks umpan yang dibuat daripada dekstrin, PVP K90 dan susu skim (DPS) didapati paling menarik anai-anai. Anai-anai mengambil masa yang lebih pendek untuk menghabiskan umpan DPS dengan puratanya 11.2 hari, manakala anai-anai juga memakan umpan DPS lebih banyak dari formulasi umpan lain yang diformulasikan mengikut peratusan susu skim, dekstrin, PVP K90 dan kaolin yang berlainan. Umpan DPS yang dicampurkan dengan *M. anisopliae* menghasilkan kematian 100% dalam tempoh 19 hari. Berdasarkan bioasai jangka hayat, semua umpan yang diformulasi dapat mengekalkan kematian anai-anai dengan konsisten dan melebihi 80% selama enam bulan atau lebih. Hasil kajian ini menyimpulkan bahawa umpan DPS lebih menarik untuk dimakan oleh *C. curvignathus* dan dapat mengekalkan keberkesanan bahan entomopatogen aktif. Kajian akan datang seharusnya menumpukan ke atas keberkesanan umpan ini dalam mencegah kemunculan semula perosak.

Kata kunci: Kawalan biologi, kawalan perosak, anai-anai subterranean

INTRODUCTION

Subterranean termites *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae) are one of the key pests in many agricultural landscapes and significantly impacted the positive return of the industry. It causes more than 40 billion USD economic damages annually (Rust & Su 2012; Subekti et al. 2015). Their voracious appetite for crops is highly attributed to their sophisticated digestive system and enzyme repertoire (Hoe et al. 2019; Sia et al. 2020). *Coptotermes curvignathus* leads a cryptic lifestyle that hidden from human detection. This poses great challenges to control them and to effectively deliver sufficient pesticide requires drilling and drenching, or a better alternative is using baits (Chan & Bong 2008; Nur-Atiqah et al. 2017).

Application of bait is presently the best approach to defend against hidden termite infestations (Sajap et al. 2000; Su 1994, 2019). There are several baits that has been developed to control termites (Lenz & Evan 2002; Su 2019; Wan Umar & Abdul Majid 2020). Most of the baits available in the world market has insect growth regulator as active ingredient. However, insect growth regulator is rarely fatal for adult insects. Hence, to effectively control *C. curvignathus* especially the adult worker and reproductive termites, insecticide with other mode of action is needed to be incorporated in the baits.

The past decade has seen an arising interest in entomopathogenic fungus, *Metarhizium anisopliae* var. *anisopliae* to control subterranean termites (Hussain et. al. 2011; Yii et. al. 2016). A useful feature of *M. anisopliae* var. *anisopliae* is that it attacks the insects first by establishing host adhesion, then it germinates and yields epicuticle degradation, and finally kills the insect by various fungal toxins without being less effective either on adult or juvenile. This makes *M. anisopliae* a good candidate to be incorporated in the bait to control termites. Devising a suitable delivery method is essential to ensure the effectiveness of entomopathogenic fungus in controlling termite (Chouvenc et al. 2011). Hence this paper aims to report a formulated bait formulation based on *M. anisopliae* that effectively alluring termites to ingest and carry them back to their colony to reduce termites' infestation.

MATERIALS AND METHODS

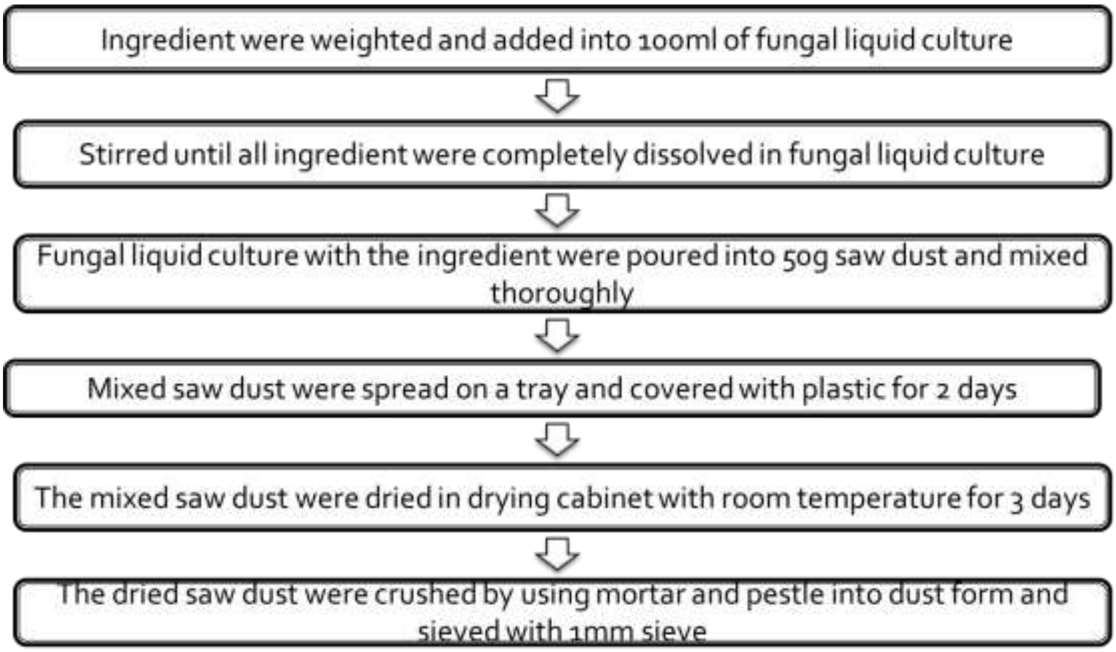
Preparation of *Metarhizium anisopliae* var. *anisopliae* Conidia as Active Ingredient

Metarhizium anisopliae var. *anisopliae* isolate was obtained from the Entomology Laboratory of UPM Bintulu Campus. The Jenkins medium (Jenkins & Prior 1993), contained 1% sucrose and 1% yeast, was chosen as culturing medium for mass production of the fungus. The medium was found to yield higher submerged conidia and more virulent culture as compared to fungus cultured in MPOB medium and Leland medium (Nyam et al. 2015).

A 5 mm diameter mycelia disk of *M. anisopliae* var. *anisopliae* was inoculated on Sabouroud Dextrose Agar with 1% yeast extract (Merck) and 0.01% streptomycin sulphate. The fungal culture was grown at room temperature (27±1°C) for 14 days for optimum sporulation. Conidia were harvested by flooding 0.05% Tween 80 into the plate and scrapping off all the conidia on the plate. The harvested conidia were vortexed for 15 minutes and filtered through 2 layers of cheese cloth. The conidia suspension was diluted to 1×10^6 conidial mL^{-1} via serial dilution. The prepared conidia suspension was inoculated into 100 mL of Jenkins medium for mass production. Inoculated liquid mediums were incubated at room temperature (27±1°C) on 180 round per minute (rpm) rotary shaker for 4 days.

Formulation of Baits

Several ingredients that include dextrin, polyvinyl pyrrolidone (PVP K-90), skim milk and kaolin were used in the bait formulation. A total of eight formulas were developed and coded according to their content: D for bait with dextrin only matrix (acted as control), DK for bait with matrix contained dextrin and kaolin, DP for baits made up of dextrin with PVP K90, DPK for bait matrix contained dextrin, kaolin and PVP K-90 (DPK), DPS for bait with dextrin mixed with PKP K-90 and skim milk matrix, DS for bait with dextrin mixed with skim milk, PS for bait contained PVP K-90 and skim milk; and PK for bait with PVP K-90 mixed with kaolin matrix (Table 1). The baits were prepared based on the procedure shown in the flow chart as follow.



Source: Nyam et al. (2015)

All the ingredients were weighed and added into prepared 100 mL fungal liquid medium (Nyam et al. 2015) and stirred with magnetic stirrer until completely dissolved in fungal liquid cultures. Fungal culture with the ingredients were poured into 50 g of rubber wood sawdust and mixed thoroughly to form a paste. The sawdust paste was spread on a tray and covered with a plastic sheet for two days in an incubator at room temperature (27±1°C) for the fungus to adapt and grow in the bait paste. It was then dried in a drying cabinet at room temperature for three days. The dried bait was crushed by using mortar and pestle into dust form. The dust was sieved with 1 mm sieve and stored in an air-tight plastic container until use.

Table 1. The bait ingredients with sawdust as base

Ingredient	**Code**
10g Dextrin (control)	D
10g Dextrin+10g Kaolin	DK
10g Dextrin+5g PVP K-90	DP
10g Dextrin+5g PVP K-90+10g Kaolin	DPK
10g Dextrin+5g PVP K-90+10g Skim milk	DPS
10g Dextrin+10g Skim milk	DS
5g PVP K-90+10g Kaolin	PK
5g PVP K-90+10g Skim milk	PS

Pathogenicity of Formulated Baits

A day prior to bait application, 20 termites (15 workers and 5 soldiers) were introduced into a 90 mm Petri dish filled with a thin layer of moist sand for conditioning. Bait amounted 0.1 g each were placed in the center of Petri dish and the plates were incubated in dark at room temperature. The bioassay was repeated four times with eight replicates each.

Termite Preference Test

The experiment was conducted by using the multiple choices feeding chamber. The design of experiment was modified from Chan & Bong (2008). A 15 cm Petri dish and nine 9 cm petri dishes were used. Eight straw-size holes were punctured at the perimeter of the 15 cm Petri dish which acted as a harborage for the termite (Figure 1a). The 9 cm petri dish which acted as the feeding chamber (FC) was punctured with a hole at the perimeter and moist sand were filled as base. The feeding chamber were arranged randomly around and connected to the harborage via a bridge (plastic drinking straw) 7 cm long with 0.5 cm diameter. A day before treatment, 50 termites were transferred into the harborage. Eight types of baits (Table 1) were used with 500 mg of each bait weighed into a pre-weighed small plastic bag. Each bait bag was placed in a feeding chamber which was connected to the harborage (Figure 1b) and connected the termites to harborage. Each feeding chamber contained one type of bait. The control treatment (c) was the bait without inoculated with fungus and made from sawdust with dextrin. One week after feeding, the bait bag was removed, wiped clean of sand on the surface and dried in a drying cabinet for one day. The bait bag was then weighed, and total loss of bait was recorded. The test was repeated five times with eight replicates each.

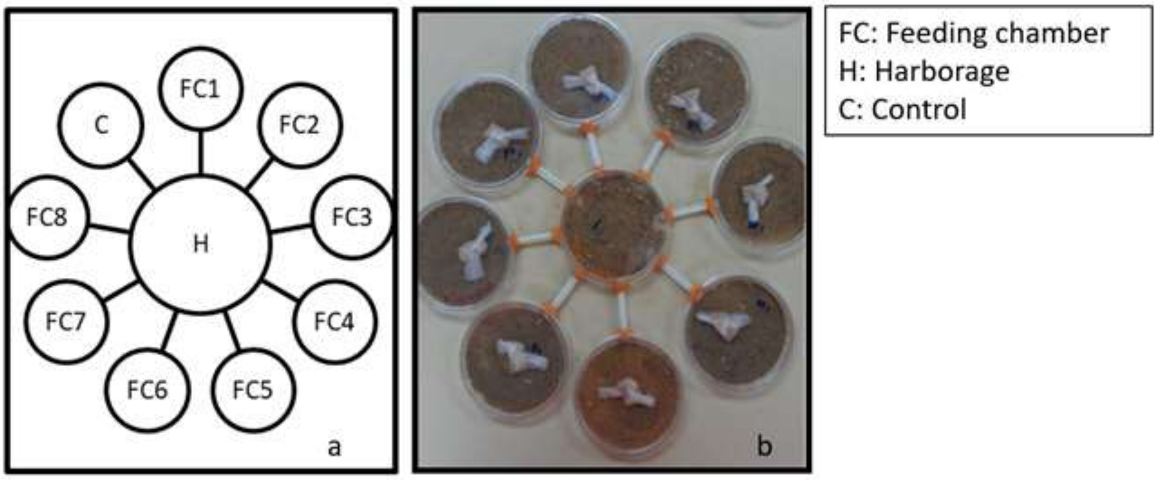


Figure 1. a-Experiment layout for termite preferences test; b-The picture of termite preferences test

Foraging Behaviour and Consumption of Bait by *Coptotermes curvignathus*

The time taken by termite to consume 1 g of the different formulation of baits was assessed. One gram of bait was placed in the centre of the 15 cm Petri dish which was connected to several 9 cm petri dishes by drinking-straw bridges (Figure 2). Fifty termites (40 workers and 10 soldiers) that had been preconditioned for a day before treatment were introduced into a one of the mini-harborage, and there after incubated in the dark. At the end of one week, 50 fresh termites were again introduced into a new mini-harborage. The procedure was repeated weekly until all baits was consumed. The time taken (days) for all bait to be consumed was recorded. Control bait contained sawdust and dextrin.

Besides investigating the time taken for the termite to consume all the baits, this experiment was set up also to study i) whether the termites' feeding was affected by the presence of dead termites (killed by the fungus in the formulated bait), ii) whether there was any bait repellence by the fresh termites, iii) whether there was repeated and continuous consumption of the bait over a period of time, iv) whether there was any burial of the bait, and v) whether there was any entombment of dead termites.

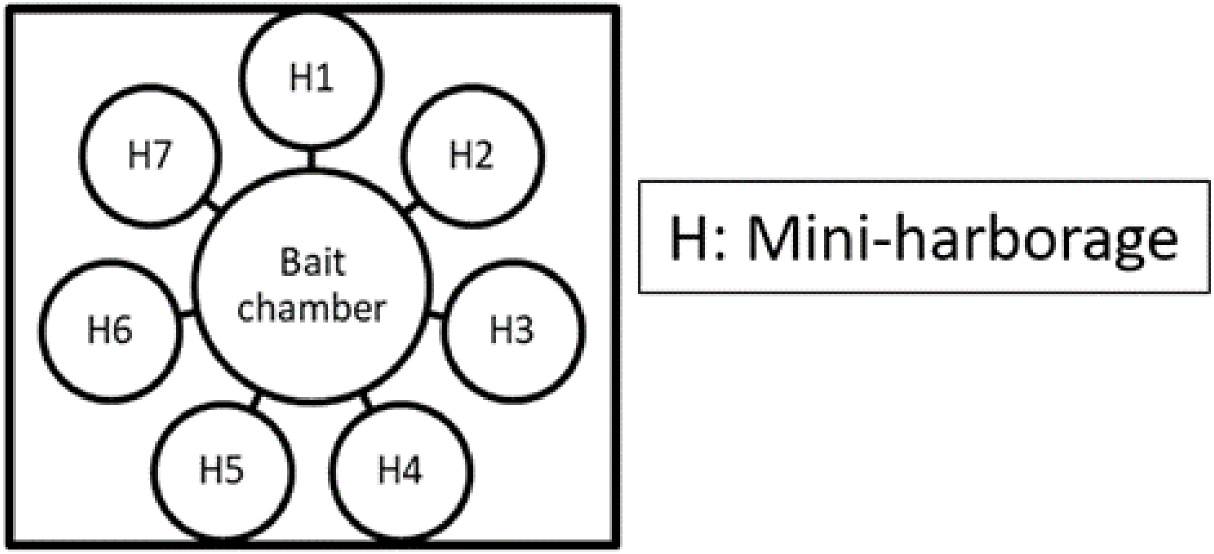


Figure 2. Experiment layout for duration of consumption of formulated baits by *Coptptermes curvignathus*

Evaluation of Bait Shelf Life and Bait Virulence on *Coptotermes curvignathus*
In the virulence test, 1 g of bait was placed in the center of a 9 cm Petri dish followed by 20 termites (15 workers and 5 soldiers) and incubated in the dark at room temperature. Mortality was accessed daily for 20 days for each bait. Cadaver of dead termites were removed and separately incubated for confirmation of death due to *Metarhizium anisopliae* var. *anisopliae*. The experiment was repeated 5 times with 8 replicates per treatment. Sample of different baits were taken every month for 6 months and test for the virulence on *Coptotermes curvignathus*.

Data Analysis
Experiment was conducted using Completely Randomized Design (CRD) and repeated five times with eight replicates each. All data were subjected to Analysis of variance using SAS software, Version 9.2 of the SAS System for Unix. Copyright © 2008 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. Treatment means were partitioned by Duncan New Multiple Range Test (DNMRT) at $P<0.05$.

RESULTS

Bait Pathogenicity
All eight formulated baits were found to effectively kill *C. curvignathus*. Dead termites were covered with *M. anisopliae* var. *anisopliae* mycelia (Figure 3a & 3b) indicated that *M. anisopliae* was the causative agents. Baits DPS (dextrin, PVP K-90 and skim milk) had the highest pathogenicity against *C. curvignathus* among all baits, and exerted 100% mortality on day 19 (Table 2). Baits DS and PS were the second most effective baits that resulted 99.82% and 99.64% mortality respectively, on day 20 after treatment (Table 2).

Figure 3. a-Termites *Coptotermes curvignathus* cadaver covered with *Metarhizium anisopliae* var. *anisopliae* mycelium; b-Cadaver of *Coptotermes curvignathus* covered with proliferating *Metarhizium anisopliae* var. *anisopliae* under 40x magnification

Table 2. Termite mortality among different formulation of baits

DAT[1]	Bait formulation							
	D	**DP**	**DS**	**DK**	**DPS**	**DPK**	**PS**	**PK**
DAY 1	1.07(±0.01)h[2]	1.16(±0.01)g	3.48(±0.02)a	2.41(±0.02)d	2.50(±0.03)c	2.05(±0.01)e	1.96(±0.01)f	2.68(±0.02)b
DAY 2	2.59(±0.02)h	3.57(±0.01)g	6.52(±0.01)a	6.34(±0.03)b	4.73(±0.01)e	4.38(±0.05)f	5.09(±0.04)d	5.18(±0.03)c
DAY 3	4.11(±0.03)d	8.13(±0.02)c	10.00(±0.66)a	9.38(±0.45)ab	8.93(±0.02)b	9.11(±0.02)b	9.38(±0.48)ab	9.20(±0.22)b
DAY 4	6.61(±0.02)h	12.86(±0.03)f	13.66(±0.01)c	12.68(±0.00)g	14.11(±0.02)a	13.04(±0.02)e	13.21(±0.02)d	13.84(±0.06)b
DAY 5	10.36(±0.05)g	17.95(±0.02)e	19.02(±0.01)a	17.50(±0.07)f	18.48(±0.09)b	18.30(±0.10)c	18.13(±0.09)d	17.50(±0.08)f
DAY 6	12.50(±0.02)h	22.50(±0.01)b	21.96(±0.03)d	20.00(±0.11)g	24.02(±0.03)a	21.79(±0.08)e	22.05(±0.05)c	21.61(±0.08)f
DAY 7	15.36(±0.05)h	28.13(±0.06)b	27.14(±0.07)c	24.02(±0.13)g	29.46(±0.12)a	26.16(±0.03)e	26.61(±0.02)d	25.71(±0.03)f
DAY 8	18.30(±0.03)g	31.61(±0.01)b	31.52(±0.07)c	29.46(±0.05)f	34.02(±0.04)a	30.45(±0.09)e	31.61(±0.11)b	30.80(±0.08)d
DAY 9	22.05(±0.04)d	36.25(±0.60)b	37.32(±0.58)b	31.07(±0.10)c	42.95(±0.08)a	36.16(±0.78)b	38.39(±0.74)b	35.54(±0.95)b
DAY 10	25.54(±0.02)g	42.05(±0.03)c	44.02(±0.07)b	36.34(±0.05)f	50.45(±0.04)a	40.00(±0.12)e	44.02(±0.01)b	41.07(±0.06)d
DAY 11	29.38(±0.02)h	47.50(±0.01)e	52.86(±0.03)b	41.96(±0.02)g	60.80(±0.05)a	45.89(±0.08)f	50.80(±0.06)c	47.68(±0.07)d
DAY 12	32.95(±0.01)h	53.13(±0.05)d	61.61(±0.07)b	47.68(±0.03)g	69.91(±0.09)a	53.04(±0.06)e	58.30(±0.09)c	52.41(±0.05)f
DAY 13	39.11(±0.04)h	58.48(±0.09)f	69.64(±0.04)b	54.29(±0.08)g	78.31(±0.01)a	60.80(±0.09)d	67.86(±0.11)c	58.75(±0.10)e
DAY 14	43.57(±0.07)g	63.39(±0.10)e	77.50(±0.48)b	60.98(±0.08)f	84.46(±0.05)a	69.29(±0.01)c	76.70(±0.62)b	65.80(±0.03)d
DAY 15	49.29(±0.10)h	70.36(±0.13)f	84.02(±0.09)c	67.05(±0.10)g	90.71(±0.02)a	78.39(±0.03)d	84.20(±0.03)b	74.64(±0.05)e
DAY 16	54.46(±0.08)h	75.27(±0.8)f	90.18(±0.05)c	72.77(±0.04)g	94.82(±0.04)a	86.25(±0.07)d	90.36(±0.01)b	82.14(±0.04)e
DAY 17	60.80(±0.11)h	84.20(±0.05)f	93.66(±0.03)c	79.82(±0.06)g	97.95(±0.07)a	92.41(±0.08)d	95.36(±0.05)b	88.93(±0.07)e
DAY 18	68.39(±0.07)h	90.36(±0.08)f	96.52(±0.04)c	86.52(±0.03)g	99.73(±0.10)a	96.43(±0.04)d	97.77(±0.07)b	94.46(±0.10)e
DAY 19	79.38(±0.08)h	94.64(±0.04)f	98.84(±0.04)c	92.05(±0.09)g	100.00(±0.00)a	97.59(±0.01)e	99.02(±0.02)b	97.95(±0.06)d
DAY 20	87.32(±0.06)h	96.25(±0.03)f	99.82(±0.02)b	96.07(±0.02)g	100.00(±0.00)a	98.66(±0.02)e	99.64(±0.05)c	99.38(±0.09)d

[1]DAT = Days after treatment
[2]Means values followed by different letters within row are significantly different at *P<0.05* by DNMRT

Termite Preference Test

In the preference test, bait formulated from DPS had the highest percentage of consumption with 32.0 mg mean weight of bait consumed (Table 3). The second highest preferred baits were PS and DS which each had approximately 23.5 mg and 22.7 mg mean weight of bait consumed respectively. Baits DP and D were the least preferred bait matrix.

Table 3. Quantity of bait consumed by termite in one week

Treatment	Mean weight of bait consumed (mg)	Percent consumption in 7 days (%)
D	9.6(±0.02)e[1]	1.9
DK	13.0(±0.04)d	2.6
DP	10.3(±0.05)e	2.1
DPK	17.0(±0.07)c	3.4
DPS	32.0(±0.03) a	6.4
DS	22.7(±0.08)b	4.5
PK	12.7(±0.02)d	2.5
PS	23. 5(±0.01)b	4.7

[1]Means values followed by different letters within column are significantly different at *$P<0.05$* by DNMRT

Foraging Behaviour and Bait Consumption

The speed of *C. curvignathus* to consume 1 g of bait is shown in Table 4. Termites consumed one gram of DPS bait in an average of 11.2 days, which was the shortest consumption time among all tested baits. D bait had the longest consumption time (25.8 days) hence it is the least preferred bait. The second shortest time was recorded in PS (12.8 days) and DS (14.2 days) baits, followed by DPK (16.2 days), DK (17.6 days), PK (19.6 days) and DP (22 days).

In this study, we observed that the dead termites were initially undisturbed by the other living termites in the harborage. The termites continued foraging towards the bait despite presence of dead termites without any apparent repellence against the bait. The termites' behavior continues to indicate that they did not suspend any potential harm from their food (bait) or the dead body of termites. It was only with the emergence of fungal mycelia (Figure 3b) on the cadaver that alarmed the termites and initiated entombment of the dead termites. However, termites did not bury the baits and continued to consume them.

Bait Shelf Life

The virulence of baits with different storage age ranging from 1 to 6 months were tested against *C. curvignathus* and presented in Figures 4 to 11. There was no significant difference in the virulence trend for each bait over a storage period of 6 months.

Table 4. Mean days taken by Coptotermes curvignathus to consume one gram of formulated bait

Treatment	Mean days
D	25.8(±0.07)f[1]
DK	17.6(±0.02)c
DP	22.0(±0.05)e
DPK	16.2(±0.04)c
DPS	11.2(±0.05)a
DS	14.2(±0.03)b
PK	19.6(±0.02)d
PS	12.8(±0.02)b

[1]Means values followed by different letters within column are significantly different at *P<0.05* by DNMRT

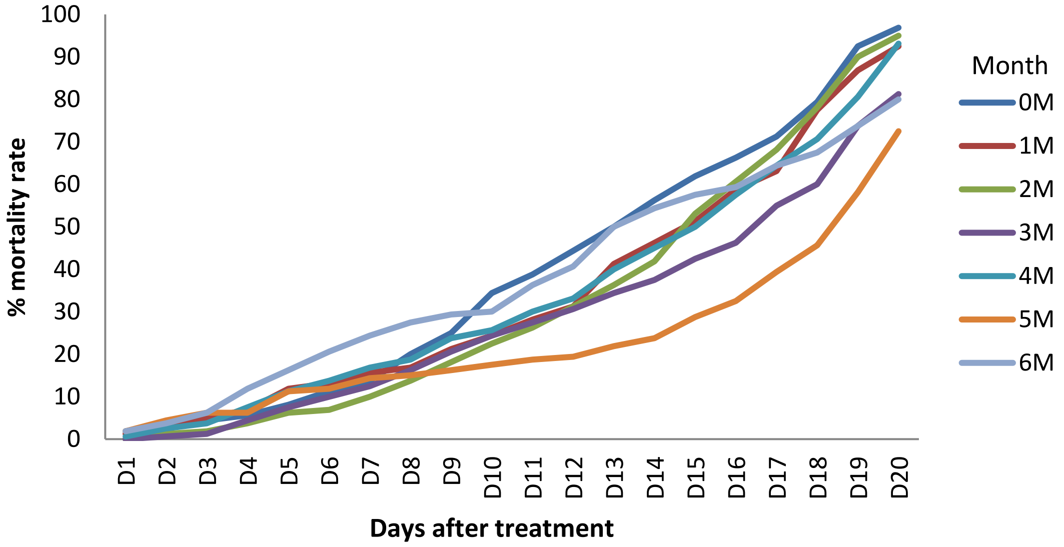


Figure 4. Daily mortality rate for D baits (*Metarhizium anisopliae* var. *anisopliae* baits aggregate with dextrin) with different storage age in month

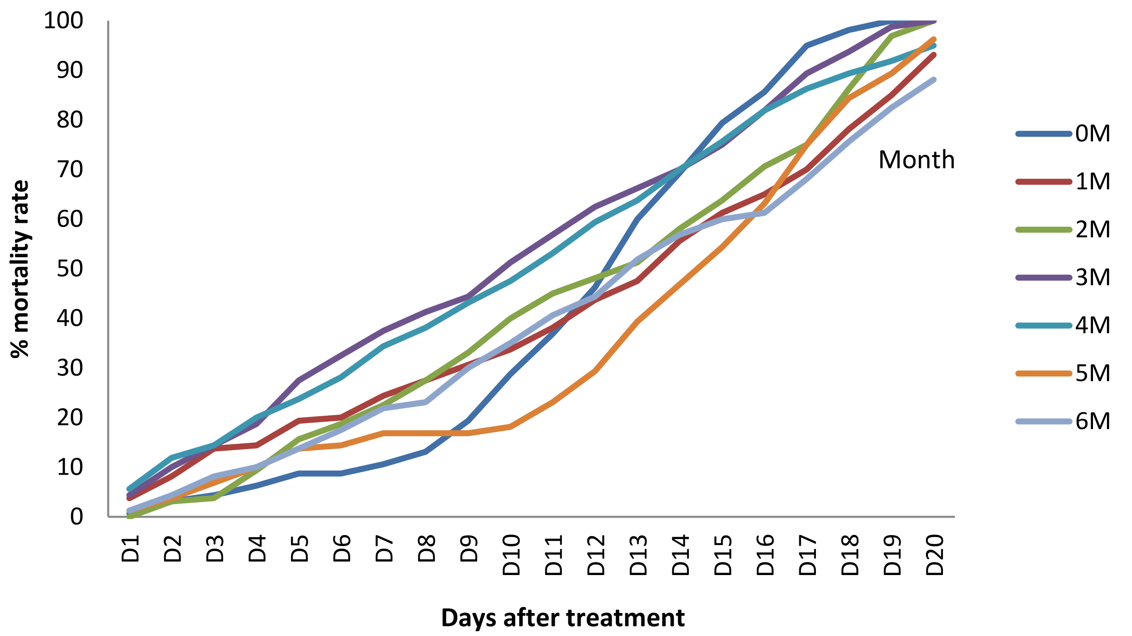


Figure 5. Daily mortality rate for DK baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with dextrin and kaolin) with different storage age in months

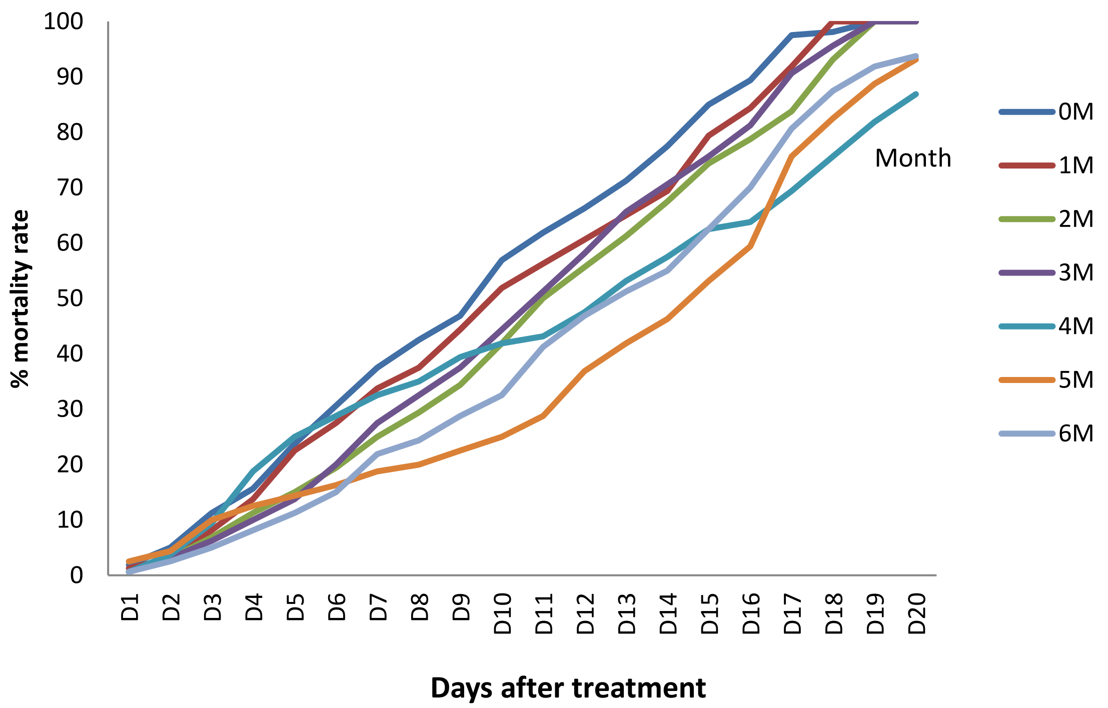


Figure 6. Daily mortality rate for DP baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with dextrin and polyvinyl pyrrolidone K-90) with different storage age in months

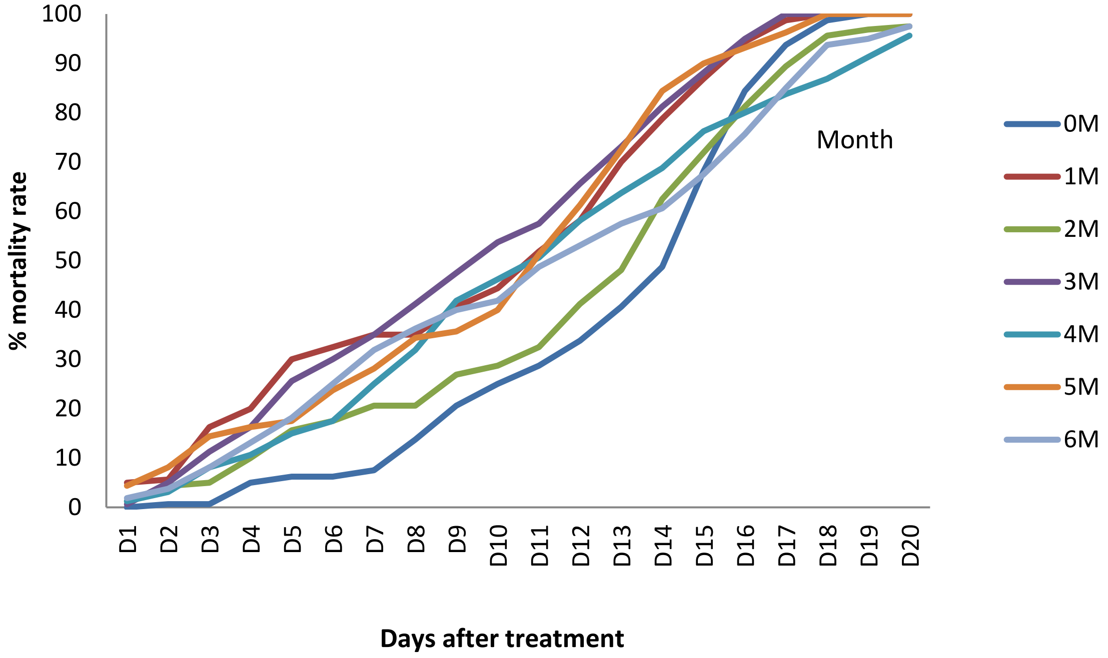


Figure 7. Daily mortality rate for DPK baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with dextrin, polyvinyl pyrrolidone K-90 and kaolin) with different storage age in months

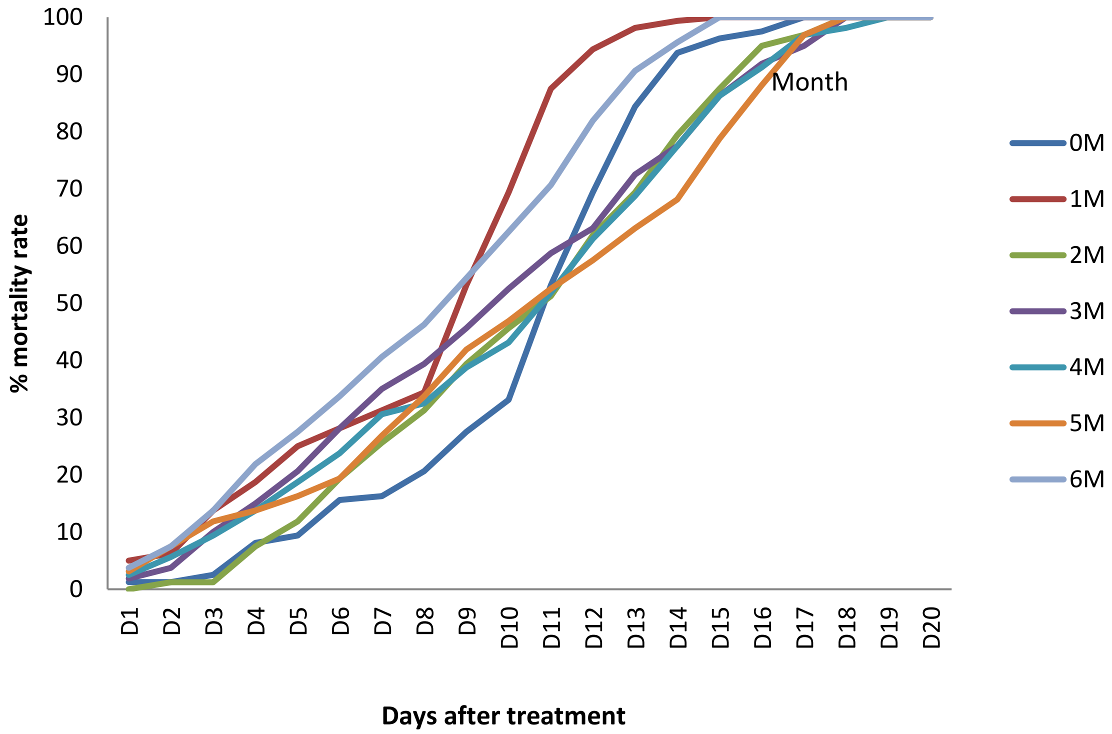


Figure 8. Daily mortality rate for DPS baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with dextrin, polyvinyl pyrrolidone K-90 and skim milk) with different storage age in months

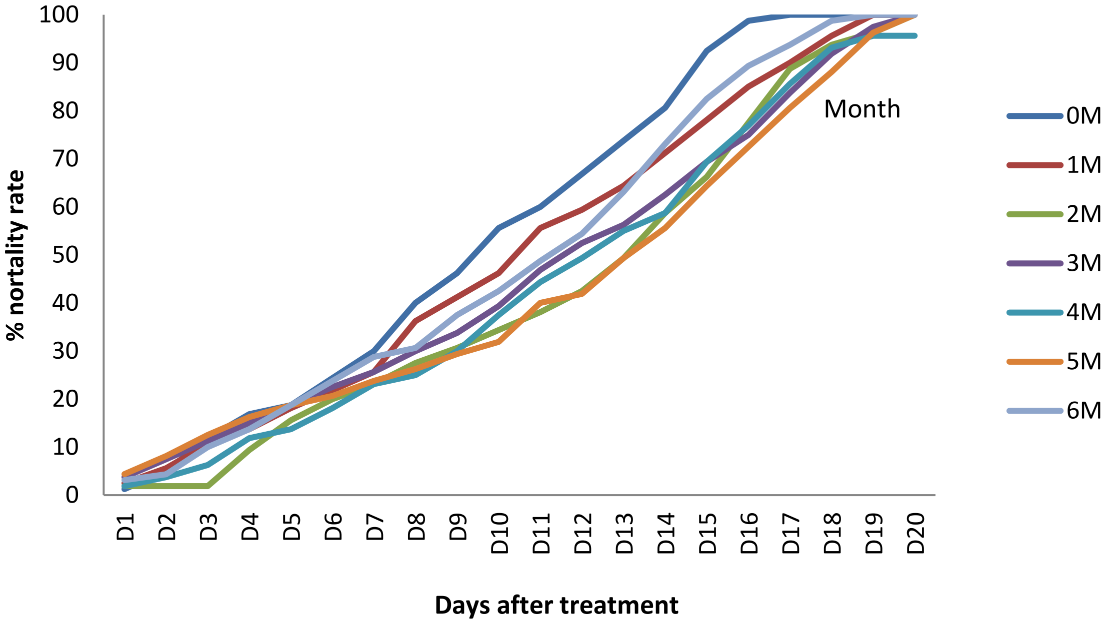


Figure 9. Daily mortality rate for PK baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with polyvinyl pyrrolidone K-90 and kaolin) with different storage age in months

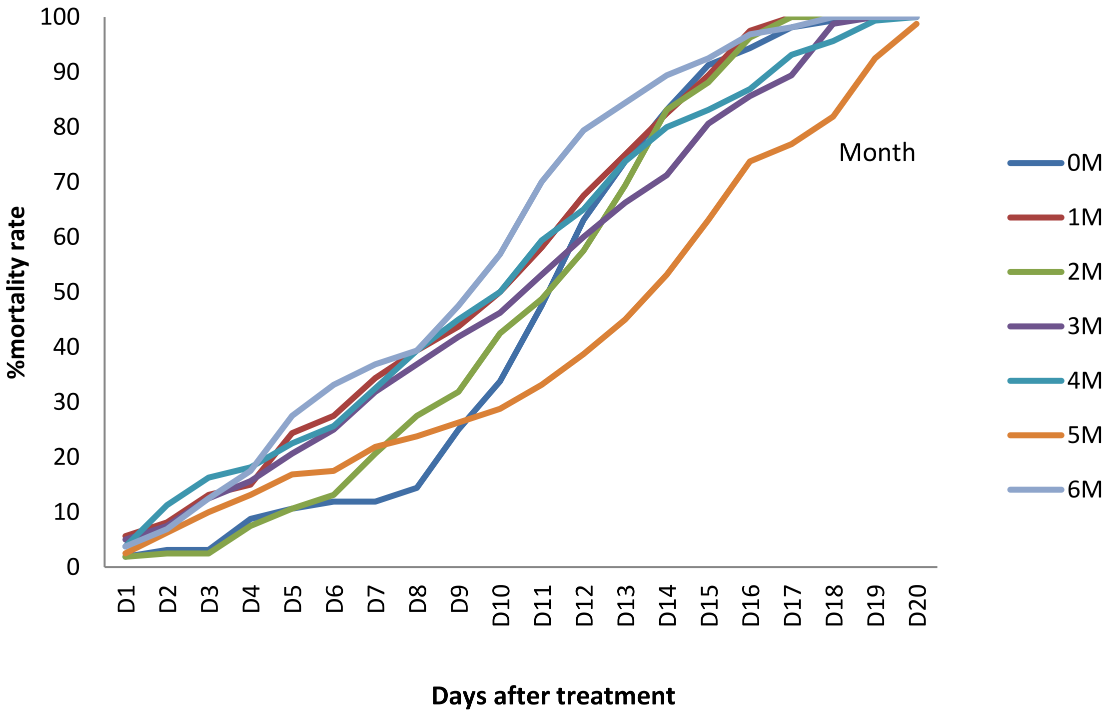


Figure 10. Daily mortality rate for DS baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with dextrin and skim milk) with different storage age in months

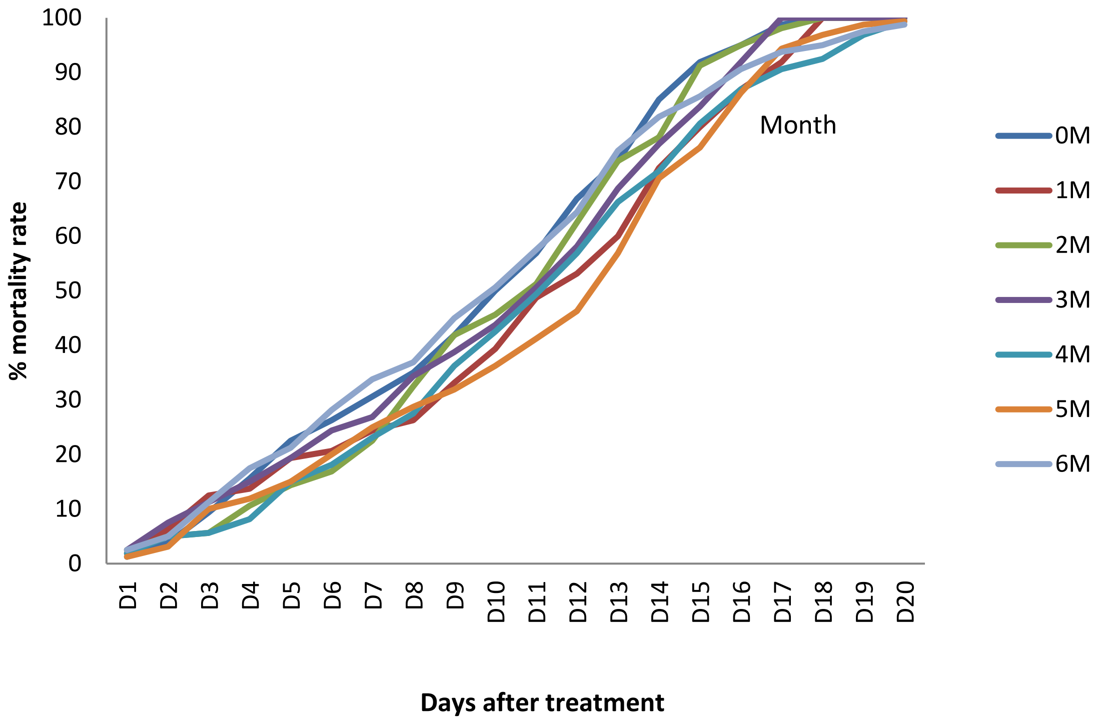


Figure 11. Daily mortality rate for PS baits (*Metarhizium anisopliae* var. *anisopliae* bait aggregate with Polyvinyl pyrrolidone K-90 and skim milk) with different storage age in months

DISCUSSION

The formulated bait was found to kill more than 87% of tested termite (Table 2) in 20 days. During the initial monitoring period, termites were found actively transporting the baits back to their colony. It shows the formulated bait displayed no acute poison effect to the termite. This is important for a bait to function well as this would allow foraging termites to carry more baits back to their colony and spread the poison throughout the colony.

In less than 26 days, termites would consume at least one gram of all formulated baits. This indicated all the bait matrix ingredients that include dextrin, PVP K-90, skim milk, and kaolin had no apparent repellence to termites. Dextrin often use as binding agent (Carvalho et al. 2007) and it was incorporated in the formulated baits as glue to bind all ingredients. Polyvinyl pyrrolidone (PVP K-90) is a stabilizer and gelling agent (Bühler 1998) that was supplemented in this bait formula to stabilize the bait matrix. Skim milk is a common coating agent in food product encapsulation and microbiological coating (Lucas-Clements1981). Kaoline is a diluent clay which is common in manufacturing industry such as paper grading and ceramics (Murray 1961), and serves as insecticide binder, and coating agent. In this study, rubber wood sawdust was used as cellulose source for the baits. This is based on the findings in Chan & Bong (2008) that reported *C. curvignathus* prefers rubber wood than other wood species.

The bait matrix that contained skim milk: DPS, DS and PS (Table 2) were found to exert faster pathogenic effect and took shorter time to reach 100% mortality than baits without skim milk. Skim milk contains high sugar lactose and Pearce (1987) suggested that sugar and amino acids such as proline and lysines in the skim milk were effective feeding stimulants which could tempt the termites to continuously eat the food. Yii et al. (2016) also in agreement to this. The tested termites consumed more of the bait with skim milk, especially DPS bait as can be observed in the termite preferences test (Table 3). Thus, skim milk can be concluded to be an effective attractant in bait formula based on the preference test.

In addition to skim milk, dextrin and polyvinylpyrrolidone (PVP) were found to enhance the palatability of termite bait, which can be seen in bait DPS performance in feeding preference (Table 3). DPS had the highest mean weight of bait consumed. PVP is a hydrophilic polymer widely used as a carrier in the medicine and nutraceutical product (Martins et al. 2013; Rasekh et al. 2014). It has excellent solubilizing and binding properties; on top of that it is known to have stabilizing effect for suspensions and emulsions (Folttmann & Quadir 2008); hence effectively conceal the pathogenic agents in the DPS baits. Dextrin may contribute to the fiber texture of the DPS bait and increase the palatability. Combining all of these qualities, DPS had the highest palatability and lowest termites' repellency among all baits.

In the preference and behavior tests, we observed that the soldier termites scavenged around the harborage (H) then explored the feeding chamber (FC) (Figure 1a and 1b). Once the soldier termite allocates the food (baits), they would return to the harborage (H) and conveyed message to their fellow worker termites. The soldiers' behaviour may suggest that they are responsible to detect any harmful elements. Worker termites foraged for food that were predetermined as safe, either by worker termites themselves or soldier termites and brought back the baits to feed the whole colony. The result of our stimulation study (Table 4) has shown that our formulated baits, especially the DPS bait had excellent attractant then continuously allured the termites to return and feed on it. Continuous consumption of bait was observed throughout the entire monitoring period. The termites were not alarmed by the presence of dead termites until the appearance of mycelia on the cadaver. Termites then entombed the dead termites.

Interestingly, it was observed that the termites did not bury or entomb the bait. It is well established that termite would bury any material that may pose harm to themselves or their colony (Chouvenc et al. 2013, Lenz & Evan 2002). Hence, our observations suggested that the termites did not suspect or recognize the presence of the fungus (submerged conidia and mycelia) in the bait, thereby repeated consumption of the bait was possible. Our formulas were able to deceive the termite hence no sign of repellence.

Other than non-repellence, the success of a bait is highly depended on the survival and subsequent virulence and effectiveness of the active ingredient (*M. anisopliae* var. *anisopliae* in this case). The formulated *M. anisopliae* var. *anisopliae* in this study could kill 90% of termite colony in 17 days and be stored for at least 6 months without compromising on its virulence on termite (Figures 4 - 11). Hence, our bait formulation was a success and has high potential to be used in termite management.

To date, very few *M. anisopliae* based bait have been reported to control termites, even fewer tested against *C. curvignathus*. Wang & Powell (2004) had formulated a cellulose powder *M. anisopliae* bait and tested against *R. flavipes* and *C. formosanus*. They tested effectiveness of various concentrations of *M. anisopliae* but they did not conduct any study on shelf life. However, they have found the cellulose bait had no obvious repellent to the targeted termites. Our previous study (Yii et al. 2016) suggested that *M. anisopliae* bait mixed with chemical pesticide such as fipronil at sublethal concentration may enhance the biopesticide potent. The baits formulated in this study solely depends on the entomopathogenic properties of *Metarihizium anisopliae* without the synergistic effect from the chemical pesticide. However, it did not adversely affect its pathogenicity against termites. Different than Wang & Powell (2004) and Yii et al. (2016) baits, the baits in this study incorporated several additive substances to promote stability and palatability of the baits; thus are more mature formulated bait.

CONCLUSIONS

The baits formulated in this study were based on the knowledge of the pest feeding preferences, and social behavior. From the laboratory trial, the formulated bait was found effective in attracting the consumption of bait, spreading the bait to other nestmates and escaped the grooming behavior of termites that aims to prevent hazardous substance from entering the nest. All formulated baits showed ability to control termite and had consistently causing mortality on termite over 6-month period. There was no repellence of termite towards the treated bait. Termite found favored the bait formulated with dextrin, PVP K90 and skim milk, and this bait also shown capability to result quicker mortality as compared with other bait matrix. The baits formulated in this study is the first study exploring *C. curvignathus* preference on different matrix materials and examining the entomopathogenic effect of *Metarhizium anisopliae* as well as the bait's shelf life. Further field research focuses on comparing chemical bait and *M. anisopliae* bait effectiveness against *C. curvignathus* and pest resurgence is highly recommended.

REFERENCES

Bühler, V. 1998. *Kolidon: Polyvinylpyrrolidone for the Pharmaceutical Industry*. 4th Edition. Germany: BASF. Ludwigshafen.

Carvalho, J., Gonçalves, C., Gil, A.M. & Gama, F.M. 2007. Production and characterization of a new dextrin based hydrogel. *European Polymer Journal* 43: 3050-3059.

Chan, S.P. & Bong, C.F.J. 2008. Susceptibility of planted forest species to *Coptotermes curvignathus* Holmgren. Proceedings of 2nd USM Penang International Postgraduate Convention 2008 and 4th Life Science Postgraduate Conference USM 2008.

Chouvenc, T., Su, N.Y. & Kenneth Grace, J. 2011. Fifty years of attempted biological control of termites-Analysis of a failure. *Biological Control 59*: 69–82.

Chouvenc, T., Efstathion, C.A., Elliott, M.L. & Su, N.Y. 2013. Extended disease resistance emerging from the faecal nest of a subterranean termite. *Proceedings of the Royal Society of London. Series B, Biological* 280: 20131885.

Folttmann, H. & Quadir, A. 2008. Polyvinylpyrrolidone (PVP)-One of the most widely used excipients in pharmaceuticals: An overview. *Journal of Drug Delivery Science and Technology* 8: 22–27.

Hoe, P.K., King, J.H.P., Ong, K.H., Bong, C.F.J. & Mahadi, N.M. 2019. Laccases repertoire of a subterranean termite *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae). *Serangga* 24 (2): 169-197.

Hussain, A., Ahmed, S. & Shahid, M. 2011. Laboratory and field evaluation of *Metarhizium anisopliae* var. anisopliae for controlling subterranean termites. *Neotropical Entomol*ogy 41(2): 244-250.

Jenkins, N.E. & Prior, C. 1993. Growth and Formation of True Conidia by *Metarhizium flavoviridae* in a simple liquid medium. *Mycological Research*. 97(12): 1489-1494.

Lenz, M. & Evans, T.A. 2002. Termite bait technology: Perspectives from Australia. Proceeding of the 5th International Conferences on Urban Pests. Charleston, South Carolina, US, 7-10 July 2002.

Lucas-Clements, R. H. 1981. Use of skimmed milk in human foods in developing countries. *Journal of the Society of Diary Technology* 34: 67-70.

Martins, R.M., Pereira, S.V., Siqueira, S., Salomão, W.F. & Freitas, L.A.P. 2013. Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract. *Food Research International* 50: 657–663.

Murray, H. H. 1961. Industrial applications of Kaolin. *Proceedings of the Tenth National Conference on Clay and Clay Minerals*, pp. 291-298.

Nur-Atiqah, J., Saputra, A., Esa, M.F.M., Shafuraa, O., Billy, A.N.A., Yaziz, N.A.A.M. & Faszly, R. 2017. *Coptotermes* sp. (Rhinotermitidae: Coptotermitinae) infestation pattern shifts through time in oil palm agroecosystem. *Serangga* 22(2):15-31.

Nyam, V.T., Bong, C.F.J. & King, J.H.P. 2015. Control of subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) by entomopathogen *Metarhizium anisopliae* var. anisopliae cultured in liquid state fermentation. *American Journal of Agricultural and Biological Sciences* 1(10): 35-40.

Pearce, M.J. 1987. Seals, tombs, mummies and tunneling in the drywood termite *Cryptotermes* (Isoptera: Kalotermitidae). *Sociobiology* 13:217-226.

Rasekh, M., Karavasili, C., Soong, Y.L., Bouropoulos, N., Morris, M., Armitage, D., Li, X., Fatouros, D.G. & Ahmad, Z. 2014. Electrospun PVP–indomethacin constituents for transdermal dressings and drug delivery devices. *International Journal of Pharmaceutics* 473: 95–104.

Rust, M.K. & Su, N.Y. 2012. Managing social insects of urban importance. *Annual Review of Entomology* 57: 355–375.

Sajap, A.S., Amit, S. & Welker, J. 2000. Evaluation of hexaflumuron for controlling the subterrranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) in Malaysia. *Journal of Economic Entomology* 93: 429-433.

Sia, S.L., King, J.H.P., Ong, K.H., Samsi, I.H. & Sarbini, S.R. 2020. Digestive system of worker termite *Coptotermes curvignathus* Holmgren and its chemical and cellulolytic microbial properties. *Serangga* 25 (3): 45-64.

Su, N.Y. 1994. Field evaluation of hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitdae). *Journal of Economic Entomology*. 87(2): 389-397.

Su, N.Y. 2019. Development of baits for population management of subterranean termites. *Annual Review of Entomology* 64: 115-130.

Subekti, N., Yoshimura, T., Rokhman, F. & Mastur, Z. 2015. Potential for subterranean termite attack against five bamboo species in correlation with chemical components. *Procedia Environmental Science* 28:783–788.

Wan Umar W.A.S & Ab Majid A.H. 2020. Efficacy of Minimum Application of Chlorfluazuron Baiting to Control Urban Subterranean Termite Populations of *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae). *Insects* 11(9):569.

Wang, C. & Powell, J. 2004. Cellulose bait improves the effectiveness of *Metarhizium anisopliae* as a microbial control of termites (Isoptera: Rhinotermitidae). *Biological Control* 30 (2): 523-529.

Yii, J.E., Bong., CF. J. King, J.H.P. & Kadir. J. 2016. Synergism of entomopathogenic fungus, *Metarhizium anisopliae* incorporated with fipronil against oil palm pest subterranean termite, *Coptotermes curvignathus*. *Plant Protection* 53(1): 35-44.