Original Research Paper

Control of Subterranean Termite *Coptotermes Curvignathus* (Isoptera: Rhinotermitidae) by Entomopathogen *Metarhizium Anisopliae* Var. *Anisopliae* Cultured in Liquid State Fermentation

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Abstract: Solid state fermentation has been used for mass production of *Metarhizium anisopliae* many years ago. However, solid state fermentation was time consuming, costly and unable to produce in large amount. We examined the suitability of liquid state fermentation to mass produce M. anisopliae and the effect of culture medium on fungal pathogenicity. Three different liquid medium selected for this study was Jenkins medium, Leland medium and MPOB medium. The fungus was cultured for 5 days to examine the growth rate. Yield of submerged conidia in different medium was assessed on day 3. Fungal virulence was bioassayed on termite *Coptotermes curvignathus*. The results indicated that Jenkins medium yielded significantly lower growth rate and dry weight (1.472 mg mL^{-1}), but the fungus exhibited highest virulence (100% mortality on day 4) among the 3 medium studied. MPOB medium had the highest growth rate and DW yield (1.961 mg mL^{-1}) but achieved 100% mortality only on day 6. The LC$_{50}$ result showed Jenkins medium yielded the most virulent fungus culture compared to Leland medium and MPOB medium.

Keywords: Isoptera, Rhinotermitidae, *Coptotermes Curvignathus*, *Metarhizium Anisopliae*, Liquid State Fermentation, Termite

Introduction

The entomopathogen *Metarhizium anisopliae* has been widely used to control agricultural pests for many years. However, the lack of inexpensive manufacturing technique and the inability to maintain production quality and quantity for a long period have limited the entomopathogen to be used commercially (Jackson *et al.*, 2004; Nelson *et al.*, 1996). Most of the available fungal growth and pathogenicity research were conducted by using fungal surface culture and aerial conidia and very little work was published on using fungal submerged conidia. Robert and Sweeney (1982) reported that fungal submerged culture was less pathogenic compared to aerial conidia. However, Leland *et al.* (2005) and Jenkins and Prior (1993) found that alternation on liquid medium could produce pathogenic submerged conidia. Medium Carbon to Nitrogen ratio, carbohydrate sources, nitrogen sources, yeast extract and other nutrients have been reported to change fungal characteristic and pathogenicity (Mehta *et al.*, 2012; Mustafa and Kaur, 2009; Jackson and Jaronski, 2009; Bharati *et al.*, 2007; Rangel *et al.*, 2006; Shah *et al.*, 2005; Li and Holdom, 1995). The purpose of this study is to identify the suitable medium from three different sources derived from Moslim *et al.* (2009), Leland *et al.* (2005) and Jenkins and Prior (1993) to mass produce pathogenic submerged conidia for control of subterranean termite *Coptotermes curvignathus* and further use to formulate the biopesticide.

Materials and Methods

Fungal Isolate

Local isolate of *M. anisopliae* var. *anisopliae* (TA) originally isolated from peat soil in Bintulu Sarawak was obtained from Entomology Laboratory of Universiti Putra Malaysia Bintulu Sarawak campus (UPMKB). The culture was maintained on Sabouraud Dextrose Yeast Agar (SDYA) added with 0.01% streptomycin and incubated in total dark condition at room temperature (27±1°C).

Preparation of Liquid Medium for Fungal Mass Production

Three different liquid media were derived from Jenkins and Prior (1993) (Jenkins medium); Leland *et al.* (2005) (Leland medium); and Moslim *et al.* (2009) (MPOB medium). Jenkins medium consists of 1% yeast extract and 1% sucrose. Meanwhile, Leland medium consists of 0.4% yeast extract, 0.4% fructose and 0.5% lecithin and MPOB medium consists of 1% yeast extract, 1% dextrose and 1% peptone. The fungal growth rate, the yield of submerged conidia and the effect of medium on the fungal pathogenicity to *C. curvignathus* were determined by using these 3 medium.

Preparation of Conidia for Liquid Medium Mass Production

Conidia of *M. anisopliae* var. *anisopliae* were harvested from 14 days old culture in SDYA by using sterile water containing 0.05% Tween 80. The conidia suspension was further adjusted to 1×10^6 conidia mL^{-1}. Sterile liquid medium at 100 mL per was inoculated with 1 mL conidia suspension and incubated on rotary shaker with 180 rpm at room temperature for 5 days.

Fungal Growth Rate on 3 Different Medium

Fungal growth rate was determined daily until day 5. Growth rate were collected daily by filtering the culture in liquid medium into pre-weighted filter paper (Whatman no. 1) through water filter vacuum pump to discard the liquid medium. The fungal culture on filter paper was dried at 60°C for 1 week. One day prior to weighing, the fungus was transferred into a desiccator. It was weighed using an analytical balance (Satrious) until a constant weight was obtained. Each treatment contained 4 replicates with 4 times repetition.

Submerged Conidia Yield on 3 Different Medium

The inoculated mediums were incubated as before for 3 days. Prior to harvesting, each medium was vortexed for 20 min to separate the submerged conidia from the phialides. The culture medium was then filtered through 2 layers of cheese cloth (pore size ≈ 75 µm) and concentration of submerged conidia in the filtrate was determined using a haemacytometer (Petroff-Hausner). The morphology of submerged conidia was confirmed by using description from Jenkins and Prior (1993) and Leland *et al.* (2005) as references. Each treatment had 4 replicates and repeated 4 times.

Viability Assays

After 24 h incubation, all submerged conidia treatments recorded more than 90% of submerged conidia were viable There was no varied significantly (p<0.05) in the Colony Forming Unit (CFU) for all 3 different submerged treatment.

Virulence of Fungal Mycelia with Submerged Conidia and Submerged Conidia Alone from Different Medium

Fungal submerged conidia and mycelia were prepared from 3 days old liquid culture. Submerged conidia were prepared as before and the filtrate was adjusted to 7 different concentrations. For fungal mycelia with submerged conidia, the culture was pipetted into a 15 mL sterile falcon tube and diluted to 7 different concentrations and the respective dry weights are shown in Table 1.

Bioassay for Virulence

C. curvignathus was collected from an infested oil palm plantation in Bintulu. Termite cultures were maintained on humid black PVC tank with rubber wood as food source. A day before treatment, termites were transferred into a Petri dish which contained a moist filter paper (food sources) for conditioning (80% relative humidity). For the virulence of submerged conidia alone, 1 µL of freshly harvested submerged conidia was topically inoculated on to the termite's thorax and termite mortality was recorded daily until 100% mortality. In the bioassay of mycelia with submerged conidia, 1 µL of culture was topically inoculated on to the termite's thorax and the termite mortality was recorded daily until 100% mortality. Each treatment contained 8 replicates at 10 termites per rep with 2 soldiers and 8 workers per replicate. Bioassays were repeated four times.

Statistical Analysis

All data were analyzed by SAS software version 9.0 and treatment means were separated by Duncan New Multiple Range Test.

Table 1. The dry weight of mycelia with submerged conidia in percentage from different medium

Treatment (Percent fungal culture)	Dry weight (mg)		
	Jenkins medium (J)	MPOB medium (M)	Leland medium (L)
100%	29.44	36.40	39.22
50%	14.72	18.20	19.61
10%	2.94	3.64	3.92
4%	1.18	1.46	1.57
2%	0.59	0.73	0.78
1.3%	0.38	0.47	0.51
1%	0.29	0.36	0.39

*Dry weights were calculated based on growth rate at Day 3 from Fig. 1

Results

Growth Rate

Fungal growth was significantly (p<0.05) affected by the use of different liquid culture medium (Fig. 1). Fungus cultivated in MPOB medium produced the highest growth compared to Leland medium and Jenkins medium. Medium for fungus liquid state fermentation had significant effect on the fungal growth. During day 1 and day 2 fungal cultures in Jenkins medium showed significantly lower dry weight yield compared to Leland medium, but no significant difference was observed when compared to MPOB medium.

Submerged Conidia Yield

The yield of submerged conidia was significantly affected by different medium. Compared to Leland medium and MPOB medium, Jenkins medium had significantly higher (p<0.0001) total yield of submerged conidia with 2.36×10^9 submerged conidia mL^{-1}, while there was no difference in conidia yield for both MPOB medium (1.87×10^9 submerged conidia mL^{-1}) and Lelaland medium (1.74×10^9 submerged conidia mL^{-1}) (Table 2).

Viability Assays

After 48 h incubation, all submerged conidia treatments recorded more than 90% of submerged conidia were viable There was no varied significantly (p<0.05) in the Colony Forming Unit (CFU) for all 3 different submerged treatment.

Virulence of Fungal Submerged Conidia and Mycelia with Submerged Conidia from Different Medium on C. Curvignathus

In high concentration of submerged conidia (1×10^9 submerged conidia mL^{-1}), there was no 100% mortality over 10 days of assessment (Table 3). Conversely, fungal mycelia with submerged conidia did cause 100% mortality over 10 days of assessment (Table 4). In addition, the 100% of fungal mycelium with submerged conidia (no dilution) from Jenkins medium caused 100% of termite mortality on day 4 post inoculation and 100% mortality on day 7 post inoculation for 50% concentration (1:1 ddH$_2$O dilution of fungal culture with submerged conidia in medium). Probit regression analysis of the mortality data of *C. curvignathus* on day 14 for submerged conidia of *M. anisopliae* var. *anisopliae* on different liquid medium (Table 5), showed that submerged conidia from Jenkins medium had lower LC$_{50}$ compared to Leland medium and MPOB medium. It means that submerged conidia from Jenkins medium were significantly more virulent compared to the other two medium.

Table 2. Effect of liquid medium on submerged conidia yield of *M. anisopliae* var. *anisopliae*

Liquid medium	Submerged conidia concentration (submerged conidia × 10^9 mL^{-1})[1]
Jenkins medium	2.36 ± 0.0006a[2]
MPOB medium	1.87 ± 0.0004b
Leland medium	1.74 ± 0.0002b

[1]Values presented as means ±standard errors

Table 3. Pathogenicity of *M. anisopliae* submerged conidia from different medium on *C. curvignathus*

Submerged conidia mL^{-1}	Daily mortality rate in mean percentage (% mortality)[1]									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
[3]J 1×10^9	42.5±0.29 a[2]	55.0±0.29a	65.0±0.58a	67.5±0.76a	75.0±0.58a	82.5±0.29a	85.0±0.29a	85.0±0.29a	87.5±0.29a	87.5±0.29a
J 1×10^8	32.5±0.76c	42.5±0.29b	45.0±1.00c	47.5±0.29c	50.0±1.00c	55.0±0.58c	57.5±0.29c	60.0±1.53c	62.5 ±0.76c	67.5±0.29c
J 1×10^7	25.0±1.53e	32.5±0.29c	40.0±0.58d	42.5±0.50d	45.0±0.58d	47.5±0.00e	50.0±0.58e	52.5±0.29e	55.0±0.58e	62.5±0.29e
J 1×10^6	22.5±0.76f	27.5±0.50e	32.5±0.29e	37.5±0.76f	40.0±0.58e	42.5±0.76f	45.0±0.58g	45.0±0.58h	47.5±0.29g	50.0±0.58h
J 1×10^5	17.5±0.29g	20.0±0.58h	25.0±0.58h	27.5±0.29i	32.5±0.29g	37.5±0.29g	37.5±0.29i	42.5±0.29i	42.5±0.29i	45.0±0.50j
J 1×10^4	12.5±0.29i	17.5±0.50i	20.0±1.00j	22.5±0.76k	27.5±0.29i	30.0±1.53hi	32.5±0.29j	32.5±0.29l	37.5±0.29j	40.0±1.53l
J 1×10^3	7.5±0.29k	15.0±0.58j	17.5 ±0.87k	20.0±1.00l	22.5±0.29k	25.0±0.50j	27.5±0.76l	32.5±0.29l	32.5±0.58n	35.0±0.58n
L 1×10^9	15.0±0.76h	22.5±0.87g	30.0±1.15f	35.0±0.58g	40.0±0.58e	42.5±0.29f	47.5±0.29f	50.0±0.58f	55.0±0.58e	55.0±0.58g
L 1×10^8	12.5±0.29 i	20.0±1.00h	25.0±0.58h	32.5±0.00h	37.5±0.00f	40.0±1.15f	42.5±0.76h	47.5±0.29g	50.0±0.58f	50.0±0.58h
L 1×10^7	7.5±0.76k	17.5±0.76i	22.5±0.87i	27.5±0.29i	27.5±0.29i	30.0±1.53hi	30.0±1.00k	37.5±0.76k	37.5±0.76j	37.5±0.29m
L 1×10^6	7.5±0.58k	12.5±0.29k	17.5±0.29k	22.5±0.29k	22.5±0.29k	22.5±0.29k	25.0±0.29m	25.0±0.29n	30.0±1.15l	32.5±0.290
L 1×10^5	5.0±0.58l	10.0±0.58l	10.0±1.15m	15.0±1.00n	20.0±1.15l	20.0±1.15l	20.0±1.00n	22.5±0.29o	27.5±0.29m	27.5±0.29p
L 1×10^4	5.0±0.00l	7.5±0.50m	7.5±0.58n	7.5±0.29o	7.5±0.29m	7.5±0.29m	10.0±1.00o	10.0±1.00p	12.5±0.50n	12.5±0.50q
L 1×10^3	5.0±1.00l	5.0±0.58n	7.5±0.76n	7.5±0.00o	7.5±0.00m	7.5±0.00m	7.5±0.00p	10.0±1.00p	10.0±1.00o	12.5±0.76q
M 1×10^9	37.5±0.76b	42.5±0.50b	52.5±0.29b	60.0±1.00b	70.0±1.15b	70.0±1.15b	72.5±0.29b	72.5±0.29b	75.0±0.50b	77.5±0.29b
M 1×10^8	27.5±1.04d	30.0±1.00d	32.5±1.04e	40.0±1.53e	45.0±0.58d	50.0±1.15d	52.5±1.26d	57.5±0.76d	60.0±1.15d	65.0±0.58d
M 1×10^7	22.5±0.29f	25.0±1.15f	27.5±0.50g	32.5±0.29h	37.5±0.29f	40.0±1.00f	45.0±0.58g	47.5±0.29g	50.0±1.53f	57.5±0.50f
M 1×10^6	17.5±0.76g	22.5±0.50g	25.0±0.58h	27.5±0.29i	30.0±0.58h	32.5±0.29h	37.5±0.29i	40.0±1.53j	45.0±0.58h	47.5±0.29i
M 1×10^5	15.0±0.58 h	17.5±0.87i	22.5±0.29i	25.0±0.58j	27.5±0.87i	30.0 ±0.58hi	30.0±0.58k	32.5±0.76l	37.5±0.29j	42.5±0.29k
M 1×10^4	10.0±0.58 j	15.0±0.58j	17.5±0.76k	20.0±1.15l	25.0±0.58j	27.5±0.29i	27.5±0.29l	30.0±1.00m	32.5±0.76k	37.5±0.00m
M 1×10^3	5.0±0.58l	12.5±0.76k	15.0±0.58 l	17.5±0.29m	20.0±1.00l	20.0±1.00l	25.0±1.00m	25.0±0.58n	27.5±0.29m	27.5±0.29p

[1]Means values presented as means ± standard errors
[2]Mean values in column followed by different letters are significantly different using Analysis of Variance (ANOVA) at *P*<0.001 level; (Duncan New Multiple Range Test)
[3]L = Leland medium, J = Jenkins medium, M = MPOB medium, D = Day

Table 4. Pathogenicity of *M. anisopliae* mycelia with submerged conidia from different medium on *C. curvignathus*

Treatment Concentration (%)	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
[3]J 100%	42.7±0.17a[2]	75.3±0.17a	96.7±0.21a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0 ±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
J 50%	38.7±0.17b	59.3±0.10c	80.0±0.12b	92.0±0.12c	96.0±0.12c	99.3±0.12b	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
J 10%	28.0±0.58e	52.0±0.06e	72.7±0.12e	84.7±0.10d	90.0±0.10d	91.3±0.15e	96.7±0.10c	97.3±0.10d	100.0±0.00a	100.0±0.00a
J 4%	23.3±0.12g	47.3±0.15g	60.7±0.10h	75.3±0.15h	82.7±0.10h	86.7±0.10g	92.0±0.06e	95.3±0.06f	97.3±0.06c	99.3±0.06b
J 2%	24.7±0.12fg	46.7±0.12h	55.3±0.10k	66.0±0.12j	73.3±0.10j	80.7±0.10j	88.7±0.10g	92.0±0.06i	93.3±0.15f	97.3±0.15d
J 1.3%	20.0±0.58h	44.0±0.12i	52.0±0.10m	60.0±0.10m	68.7±0.06l	73.3±0.15m	82.0±0.06i	88.7±0.10k	92.7±0.10g	96.0±0.10f
J 1%	16.0±1.15j	32.0±0.10n	44.0±0.12p	52.0±0.06p	59.3±0.15p	66.0±0.06o	71.3±0.10l	76.7±0.10o	84.0±0.06k	88.0±0.06i
L 100%	32.7±0.15d	60.7±0.17b	73.3±0.06d	82.0±0.10 e	88.7±0.10e	96.0±0.06d	98.7±0.10c	100.0±0.00a	100.0±0.00a	100.0±0.00a
L 50%	23.3±0.10g	43.3±0.12j	56.7±0.10j	75.3±0.12 h	81.3±0.12i	87.3±0.12f	92.0±0.06e	96.7±0.06e	99.3±0.15b	100.0±0.00a
L 10%	23.3±0.12g	44.0±0.10i	53.3±0.06l	61.3±0.15 l	66.7±0.10m	79.3±0.15k	83.3±0.15h	92.7±0.06h	96.0±0.12e	98.7±0.06c
L 4%	21.3±0.47h	39.3±0.15k	52.0±0.06m	56.0±0.06o	64.0±0.06o	70.7±0.15n	76.7±0.15k	90.7±0.15j	93.3±0.10f	96.0±0.06f
L 2%	17.3±0.47ij	36.0±0.06l	46.0±0.12o	51.3±0.10q	56.7±0.10r	65.3±0.10p	69.3±0.10m	78.7±0.15n	85.3±0.12j	87.3±0.12j
L 1.3%	16.0±0.58j	32.0±0.10 n	41.3±0.10q	51.3±0.10s	53.3±0.10s	62.7±0.06r	68.7±0.06n	76.0±0.12p	82.0±0.06l	86.0±0.06k
L 1%	16.0±1.00j	25.3±0.06 p	30.0±0.06s	40.0±0.06s	50.0±0.06t	58.7±0.12s	64.0±0.06o	66.7±0.10r	73.3±0.10n	76.0±0.10m
M 100%	35.3±0.15c	57.3±0.10d	77.3±0.06c	94.0±0.06b	97.3±0.10b	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
M 50%	34.7±0.06c	50.0±0.06f	69.3±0.15f	80.0±0.06f	87.3±0.15f	92.7±0.06d	98.7±0.06b	99.3±0.15b	100.0±0.00a	100.0±0.00a
M 10%	29.3±0.15e	50.0±0.00f	66.7±0.10g	79.3±0.15g	84.0±0.12g	86.0±0.06h	90.7±0.15f	94.7±0.15g	96.7±0.15d	98.7±0.15c
M 4%	25.3±0.10f	46.7±0.10h	58.0±0.06i	73.3±0.10i	81.3±0.06i	85.3±0.06i	90.7±0.06f	92.0±0.10i	93.3±0.12f	96.7±0.06e
M 2%	18.0±1.00i	44.0±0.06i	56.7±0.06j	62.7±0.10k	70.7±0.10k	77.3±0.10l	82.0±0.12i	86.7±0.10l	89.3±0.10h	93.3±0.10g
M 1.3%	16.0±0.58j	35.3±0.10m	47.3±0.10n	57.3±0.10n	66.0±0.06n	70.7±0.12n	78.0±0.10j	84.0±0.10m	86.7±0.12i	88.7±0.12h
M 1%	10.7±0.21k	30.7±0.12o	39.3±0.10r	48.0±0.12r	58.7±0.10q	63.3±0.12q	68.7±0.10n	74.0±0.21q	80.0±0.06m	84.7±0.10l

[1]Means values presented as means ± standard errors
[2]Mean values in column followed by different letters are significantly different using Analysis of Variance (ANOVA) at *p*<0.001 level; (Duncan New Multiple Range Test)
[3]L = Leland medium, J = Jenkins medium, M = MPOB medium, D = Day

Table 5. Probit regression analysis of the mortality data of *C. curvignathus* on day 14 for submerged conidia of *M. anisopliae* var. *anisopliae* on different liquid medium

Liquid medium	Regression equation	Chi-square heterogeneity (calculated)	LC_{50}[1]	95% Confidence limits of LC_{50}[1]	
				Lower	Upper
Jenkins medium	Y=0.297X+3.936	3.505	$3.807×10^3$	$3.360×10^2$	$1.786×10^4$
MPOB medium	Y=0.270X+3.846	1.672	$1.904×10^4$	$2.227×10^3$	$8.227×10^4$
Leland medium	Y=0.283X+3.009	1.932	$1.099×10^7$	$3.018×10^6$	$5.681×10^7$

[1]All values of LC_{50} and 95% confidence limits are in submerged conidia/mL

Table 6. Probit regression analysis of the mortality data of *C. curvignathus* on day 2 for mycelia with submerged conidia of *M. anisopliae* var. *anisopliae* on different liquid medium

Liquid medium	Regression equation	Chi-square heterogeneity (calculated)	LC_{50}[1]	95% Confidence limits of LC_{50}[1]	
				Lower	Upper
Jenkins medium	Y=0.419X+6.166	9.199	2.00	1.00	3.00
MPOB medium	Y=0.266X+5.567	6.461	7.00	4.00	22.00
Leland medium	Y=0.338X+5.618	8.a088	15.00	8.00	40.00

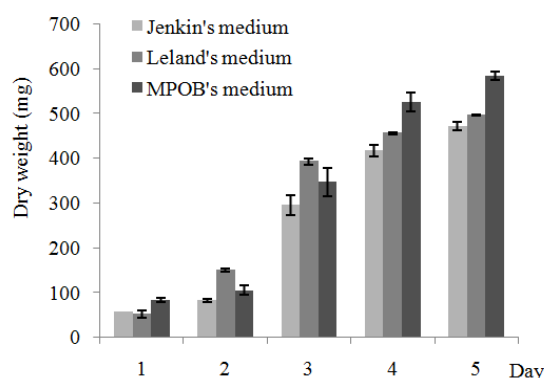[1]All values of LC_{50} and 95% confidence limits are in mg/mL

Fig. 1. Mean dry weight (±SE) of M. anisopliae var. ani-sopliae on different medium over 5 days (p<0.05)

Similarly, the probit regression analysis of the mortality data of *C. curvignathus* on day 2 for mycelia with submerged conidia of M. *anisopliae* var. *anisopliae* on different liquid medium (Table 6), showed fungal culture from Jenkins medium required lower quantity of mycelia with submerged conidia to cause mortality of termites.

Discussion

On day 1 and day 2, fungal spores were adapting in the new environment and thus the fungal growth rate was lower. However, on day 3 the germinated fungal spores started utilizing the nutrient in the liquid medium and growth rate increased rapidly. During the growth phase, the fungus utilized the nutrient in the medium and caused

decrease of the nutrient. On day 4 there was a gradual decrease in growth as a result of the lower nutrient availability. Fungal growth in Jenkins and Leland medium entered into stationary phase in day 4 while that of MPOB medium which had higher nutrient contents continued on the growth phase till day 5 after which the fungus entered stationary phase (Fig. 1). *Metarhizium anisopliae* growth was greatly dependent on the sources and amount of carbon and nitrogen (Mustafa and Kaur, 2009; Shah *et al.*, 2005). In Jenkins medium, sucrose was a carbon source and yeast extract was a nitrogen source. In Leland medium carbon source was derived from fructose and nitrogen from yeast extract and lecithin. MPOB medium which has longer growth phase in the fermentation process consists of high carbon sources with 2% of dextrose and high nitrogen sources with 1% of yeast extract and 1% of peptone. Although fungal culture in Jenkins medium had significantly lower growth rate compared to Leland medium and MPOB medium on day 3, it had higher submerged conidia yield. In favourable condition with lots of nutrient, the fungus grew vegetatively until most of the nutrients were depleted. Then, in unfavourable condition with high competition for nutrients, the fungus induced spores for reproduction. In Jenkins medium which has lower nutrients, the fungus achieved maximum growth at day 3 producing corresponding highest weight yield of submerged conidia, after which the lag phase set in. For Leland medium and MPOB medium which had higher nutrient contents than Jenkins medium, the fungus continued its growth phase after day 3 with mycelia yield dominating the growth medium. The adaptation and characteristic of fungus culture from different medium had impact on the fungus pathogenicity and nutrition had effect on fungal conidia production and conidia quality (Shah *et al.*, 2005). Hence, liquid fermentation with Jenkins medium can produce *M. anisopliae* var. *anisopliae* cultures pathogenic to termite *C. curvignathus*. Fungal cultures from the liquid state fermentation have previously been reported to have lower pathogenicity compared to aerial conidia produced from solid state fermentation (Roberts and Sweeney, 1982). However, mass production of fungal cultures from liquid fermentation was much easier compared to solid state or two stage fermentation. Furthermore, the former was cheaper and the resources to mass produce were more readily available compared to solid state fermentation. In addition some research showed that the alteration of medium can enhance the pathogenicity of submerged form of fungal culture (Leland *et al.*, 2005).

From the result, submerged conidia alone were less pathogenic to termite. However, the submerged conidia when applied en masse with liquid medium were more virulent to the termite. This may be due to the isolation process that detached the submerged conidia from the fungal phialides which may affect viability of the submerged conidia. This explains why the submerged conidia without removed from the fungal submerged culture were more virulent to termite. Hoe *et al.* (2009) reported that *M. anisopliae* var. *anisopliae* at a high concentration of 1×108 conidia mL^{-1} (aerial conidia) caused 100% mortality in day 3; while in the present study it took 4 days to achieve similar mortality result. However, the time period required to produce that quantity of aerial conidia were much longer compared to liquid state fermentation. In recent years, entomopathogen such as *M. anisopliae* has been reported as not effective or not practical to use for bioncontrol due to the defense mechanisms in allogrooming fungal spores by termites nest mate and cellular encapsulation by termite's own immune system (Chouvenc *et al.*, 2009; Yanagawa *et al.*, 2008). In most or all research on fungus as a biocontrol agent of termites, fungal aerial spores produced in agar plate or from solid state fermentation was the sole infective unit to apply to termite. However, the aerial spores were usually harvested from surface culture with use of surfactant such as Tween 20, Tween 80, Trixton-X or SDS and there was a report that surfactant will reduce spore viability and germination (Mishra *et al.*, 2013). The pathogenicity of aerial spores depended on the active spores; however, topical application using a specific spore concentration did not indicate that all the spores used were viable. Spore reproduction from both sexual and asexual cycle in fungi can either be capable to germinate immediately or stay in dormancy until a favorable condition for germination (Garraway and Evans, 1984). Thus, the inactive, unviable or dormant spores may lead to the termite itself or the nest mates to remove the spores from the body. Consequently, further research is required to evaluate the application and practical use of entomopathogen *M. anisopliae* to control termite. The present study which compares the effectiveness of fungal submerged conidia and submerged culture with both submerged conidia and mycelia may help enhance the effectiveness of the entomopathogenic fungus, although the mechanism of infection in this case remain unknown.

Conclusion

Mass production of fungus on liquid state fermentation was much easier and faster compared to solid state fermentation. From the result has showed even though the fungus cultured in Jenkins medium does not has higher growth rate but it has the higher submerged conidia yield and higher pathogenicity compared to fungus cultured in MPOB medium and Leland medium. Thus, Jenkins medium can produce much batter fungus culture to apply as entomopathogenic fungus.

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Author's Contributions

All authors equally contributed in this work**.**

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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