A LABORATORY TRIAL ON APPLYING ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* AS A BARRIER FOR SUBTERRANEAN TERMITE *Coptotermes curvignathus*

***(Percobaan Laboratoris mengenai Penggunaan Cendawan Patogen Serangga* Metarhizium anisopliae *sebagai Penyekat Rayap Tanah* Coptotermes curvignathus)**

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ABSTRACT

Control of subterranean termites relies on chemical insecticides that mostly environmentally unacceptable. Biological control, for example using entomopathogenic fungi, is pursued to minimize applying the poisonous chemicals. This experiment was designated to determine effectiveness of 6 strains of entomopathogenous fungus *Metarhizium anisopliae*, obtained from various locations, as a barrier for subterranean termite *C. curvignathus.* Various thickness of the fungi that were cultured in rice media were applied for termite barriers set in glass tubes together with sand substrate and tusam wood blocks *(Pinus merkusii)* of 2 x 1 x 1 cm for bait. 50 termites, containing of 45 workers and 5 soldiers, were introduced into each tube. The tests were incubated for 9 days at room temperature.

Results showed that most termites were able to reach the wood bait, but only termites succeeding in penetrating the fungal barriers of 2 cm thickness or less could feed the bait significantly. Percentage of termite mortality was mostly high at the treatment with fungal barrier of 4 and 5 cm thickness. Fungus strain obtained from Pakem (Yogyakarta) was the most promising, and then consecutively followed by the strains from Jombang (East Java), Gadjah Mada University (GMU) 1, Bogor (West Java), Semarang (Central Java), and Gadjah Mada University (GMU) 2. Barrier thicknesses of 4 and 5 cm generally cused high termite mortality ranging from 80 to 100%.

Key words: Fungal barrier, penetration, termite attack and termite mortality

ABSTRAK

Pengendalian rayap selama ini lebih tergantung pada penggunaan insektisida kimia yang pada umumnya tidak ramah lingkungan. Pengendalian secara biologis, misalnya menggunakan cendawan patogen serangga, sedang dikembangkan untuk mengurangi penggunaan bahan-bahan kimia beracun tersebut. Penelitian ini bertujuan untuk menentukan efektivitas 6 strain cendawan patogen serangga, Metarhizium anisopliae *(Metschnikoff) Sorokin, yang diperoleh dari berbagai lokasi, sebagai penyekat serangan rayap tanah* Coptotermes curvignathus. *Beberapa tingkat ketebalan cendawan yang dibiakkan dalam media beras digunakan sebagai penyekat yang disusun bersama-sama dengan media pasir dan umpan blok kayu tusam* (Pinus merkusii*) dalam tabung reaksi. Rayap tanah sebanyak 50 ekor terdiri dari 45 ekor rayap pekerja dan 5 ekor rayap perajurit dimasukkan ke dalam masing-masing tabung reaksi, dan kemudian percobaan disimpan pada suhu kamar selama 9 hari.*

Hasil percobaan menunjukkan bahwa rayap pada umumnya mampu menembus cendawan penyekat, tetapi hanya rayap yang berhasil menembus penyekat dengan ketebalan 2 cm atau kurang dapat menyerang kayu umpan. Persentase kematian rayap pada umumnya tinggi pada perlakuan dengan ketebalan penyekat 4 dan 5 cm. Strain cendawan yang berasal dari Pakem (Yogyakarta) tampak paling menjanjikan, sementara peringkat di bawahnya secara berurutan adalah dari

Jombang (Jawa Timur), Universitas Gadjah Mada (UGM) 1 (Yogyakarta), Bogor (Jawa Barat), Semarang (Jawa Tengah) dan UGM 2 (Yogyakarta). Ketebalan cendawan penyekat 4 sampai dengan 5 cm pada umumnya dapat menyebabkan kematian rayap yang tinggi, antara 80 sampai dengan 100%.

Kata kunci: Cendawan penyekat, penembusan, serangan dan kematian rayap

I. INTRODUCTION

Termites are social insects found in a wide range of terrestrial environments that are distributed throughout the warmer regions of the world (Becker, 1976). These insects are the most important structural pest and most destructive among other wood destroying insects in Indonesia. *Coptotermes* (Isoptera: Rhinotermitidae) is one of the most economically important pest termite genus that attack wood and wood based materials anywhere in the tropic and subtropics. Serious damage on wood structures and living trees caused by this termite genus is found in Bogor and its around (Sukartana, 2002). *C. curvignathus* Holmgren is one of the most important species in this country.

Control of termites so far relies on applying chemical insecticides that are used for soil treatment around structures (Su and Scheffrahn, 1990 and Grace *et al.,* 1993). Using insecticides can however not only contaminate our environment but also cause pest resistance so controlling the pest will be more difficult. Alternative methods that are considered environmentally friendlier should be pursued. Biological control as a termite control technology is becoming more desirable. Some entomopathogenous fungi have been studied as bioinsecticides for those purposes (Lai *et al*., 1982; Milner and Staples, 1996, Milner *et al*., 1997; Milner, 2000).

Some preliminary studies on applying bioinsecticides, especially entomopathogenic fungus *Metharizium anisopliae* (Metschnicoff) Sorokin, had been conducted in Forest Products Research and Development Center, Bogor (Sukartana *et al.*, 2000; Sukartana and Rushelia, 2000; Setiawan *et al.*, 2000). These studies should be accelerated to obtain specimen(s) that is(are) cost-effective and environmentally safe alternatives to chemical control for termite control. The objective of this study was to determine effectiveness of various strains of *M. anisopliae* as a barrier for subterranean termite *C. curvignathus* in a laboratory trial.

II. MATERIALS AND METHODS

Six strains of entomopathogenic fungus *M. anisopliae* were used. These fungus strains were obtained from Research Unit for Biotechnology of Crop Plantation, Bogor; Department of Plant Pests and Diseases, Faculty of Agriculture, Gadjah Mada University, Yogyakarta (GMU 1 and 2); Laboratory of Biological Control in Pakem (Yogyakarta), Semarang (Central Java), and Jombang (East Java). The fungus isolates were cultured in PDA media and then transferred into rice media according to the procedures of Jenkins *et al*. (1998).

Tusam *(Pinus merkusii)* wood blocks of 2 x 1 x 1 cm were used as termite bait, and sieved sand using mosquitoes screen wire was used for termite substrate. Each wood block was put in a glass tube of 1.8 cm diameter and 18 cm height. Sand media that had been wetted according to Sornuwat *et al*. (1995) was filled in the tube until 3 cm thickness. Fungus cultured on the rice media was filled with various thickness of 1, 2, 3, 4 and 5 cm above the sand, an on top of the fungus, wetted sand was again filled until 4 cm bellow the lip of the tube (Figure 1). These preparations were kept for three days to make the test arrangement settled.

Termite specimens were collected from municipal area neighbouring of the Centre for Forest Products Research and Development, Bogor. The termites were kept in laboratory for at least two week for acclimatization. Fifty termites containing of 45 workers and 5 soldiers were introduced into each tube. Each tube was loosely caped with aluminum foil to avoid substrate desiccation. The experiments were then incubated at a room temperature for 9 days. Five replicates were prepared.

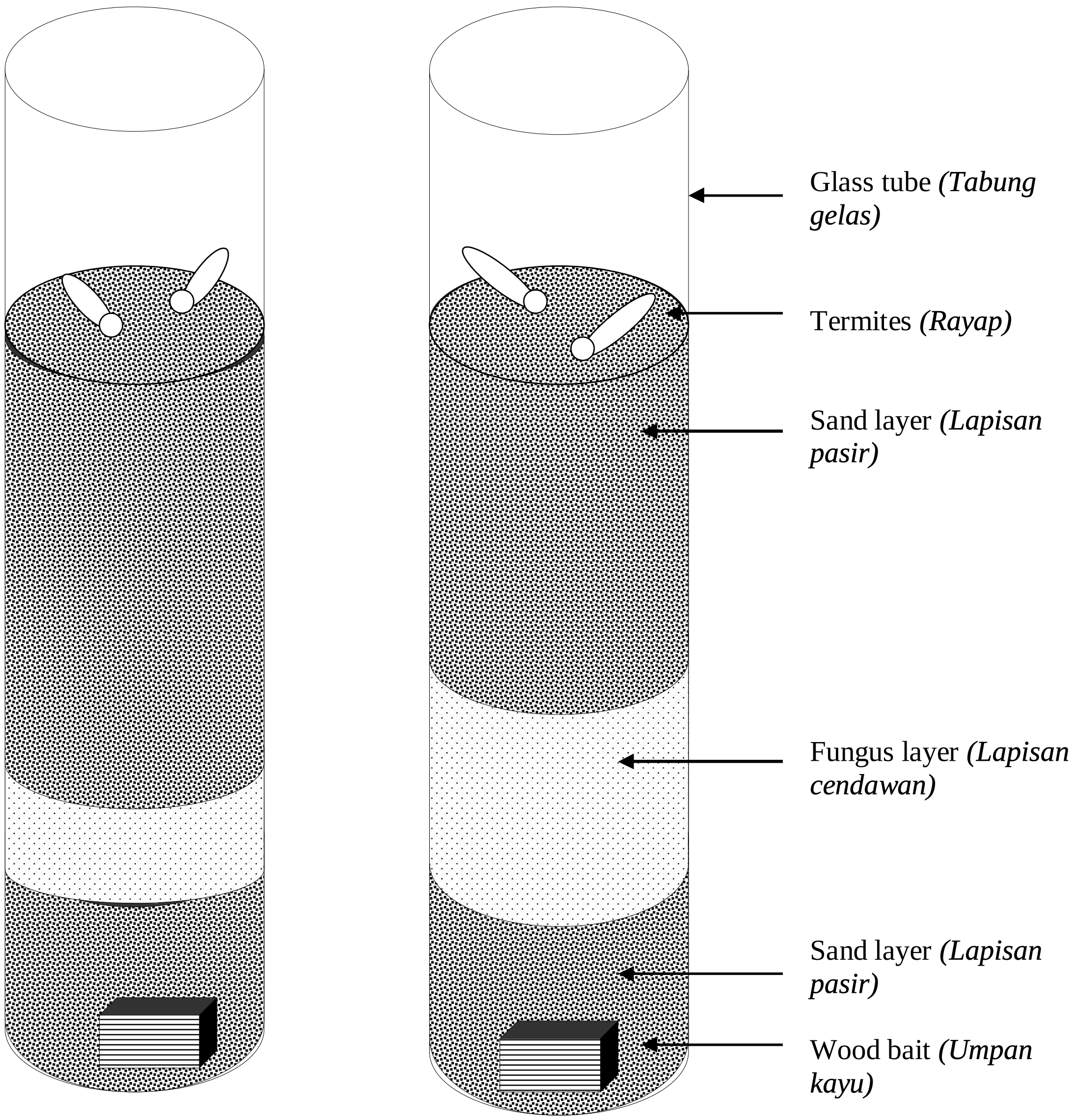

Figure 1. Trial arrangements with two kinds of fungus barrier thickness

Gambar 1. Susunan percobaan dengan dua macam ketebalan cendawan penyekat

Observations were made at interval of 2–3 days to determine the termite activities that were represented by penetration of the termite through the fungal barrier. At the end of experiment, numbers of termite mortality were counted, and rating of termite attack on wood bait was scored according to the standard of ASTM (Anonymous, 1995). After transformed into arcsine of the square root percentage, the mortality data were analyzed using ANOVA and then evaluated their treatment differences according to the procedure of Tukey's *w* Procedure (Steel and Torrie, 1980).

III. RESULTS AND DISCUSSION

Table 1 showed that all termites in each tube had tried to reach the wood bait located in the base of the tube for their food. Several of them failed to reach the bait. To reach the bait, they had to make tunnel through sand covering the wood bait (control), or through sand-fungus-sand barriers (treated). All termites in the control were able to reach the wood bait, and then attacked the bait. Meanwhile, even though the termites succeeded in penetrating the fungal barrier and reached the bait, they did not consume the bait significantly, or even leaved the bait intact particularly on the treatment using fungus barrier thickness of 4 cm or more (Table 2). Therefore, it can be concluded that thickness of the fungal pathogen affected the termite activities.

Table 1. Penetration of termite through *Metarhizium anisopliae* barrier[1]

***Tabel 1. Penembusan rayap melalui penyekat* Metarhizium anisopliae[1]**

Fungus strain *(Strain cendawan)*	Barrier thickness *(Ketebalan sekat)*, cm	Number of substrate/barrier penetrated at day *(Jumlah substrat/penyekat yang ditembus, pada hari ke)*						
		1	2	3	4	7	8	9
Control *(Kontrol*)	0	5	5	5	5	5	5	5
Semarang, Central Java *(Jawa Tengah)*	1	0	2	3	4	5	5	5
	2	0	0	1	3	4	4	5
	3	0	0	1	2	3	3	3
	4	0	0	3	3	5	5	5
	5	0	0	1	4	4	4	4
Pakem, Yogyakarta	1	0	4	4	5	5	5	5
	2	1	2	2	2	2	2	2
	3	0	0	1	1	1	1	1
	4	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
Control *(Kontrol)*	0	5	5	5	5	5	5	5
GMU *(UGM) 2*	1	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5
	4	3	3	5	5	5	5	5
	5	3	4	4	4	4	4	4
GMU *(UGM)* 1	1	2	4	5	5	5	5	5
	2	2	4	4	4	4	4	4
	3	4	4	4	4	4	4	4
	4	3	4	4	4	4	4	4
	5	3	4	4	4	4	4	4
Control *(Kontrol),* 3	0	5	5	5	5	5	5	5
Jombang, East Java *(Jawa Timur)*	1	5	5	5	5	5	5	5
	2	3	5	5	5	5	5	5
	3	3	3	3	4	4	4	4
	4	1	1	1	1	1	1	1
	5	0	0	0	0	0	0	0
Bogor, West Java *(Jawa Barat)*	1	5	5	5	5	5	5	5
	2	5	5	5	5	5	5	5
	3	5	5	5	5	5	5	5
	4	3	4	5	5	5	5	5
	5	0	2	4	4	4	4	4

[1]Remarks *(Keterangan)*: Numbers on each barrier thickness showed number of barrier penetrated by the termites *(Angka-angka pada masing-*

masing ketebalan penyekat menunjukkan jumlah penyekat yang ditembus rayap)

Termite mortality was summarized in Table 3. Analysis of variance of the data indicated that there were significant difference among treatments, but not for the treatment using fungus barrier obtained from GMU 2. Further analysis using Tukey's *w* Procedure ($p < 0.5$) showed that percentage of termite mortalities generally corresponded with penetration of the termite through the fungal barrier (Table 1) and infestation rate of the wood blocks (Table 2). Thus, most percent mortality of the termites was significantly affected by thickness of the fungal barrier, except fungal barrier that was developed from GMU 2 isolate which was least effective.

Figure 2 summarizes the entire resultas. Based on height of the columns, it was shown that fungus strain from Pakem (Yogyakarta) was the most promising and was then, in descendent order, followed by strains originally from Jombang (East Java), GMU 1, Bogor, Semarang and GMU 2. This figure again confirmed with those statistical analysis concerning the less effectiveness of the fungus strain from GMU 2.

Milner (2000) stated that fungus strains affected to tunneling ability and mortality of subterranean termite *C. lacteus.* In his studies, most fungi strains obtained from termites caused higher termite mortality than that from other insects. After 14-18 days experimentation, most termites introduced to that fungi strains had died. Long time culturing of the fungi in artificial media might reduce the fungi virulence. However, re-isolate the fungus through its host or related insect could increase the fungus virulence.

Table 2. Means of infestation rate of termite attack on wood block[1]

Tabel 2. Rata-rata tingkat serangan rayap pada blok kayu[1]

Thickness of fungus barrier *(Ketebalan lapisan cendawan penyekat)*, cm	Infestation rate of wood blocks in connection with fungus origin *(Tingkat serangan pada blok kayu dan hubungannya dengan asal cendawan)*					
	Bogor, West Java *(Jawa Barat)*	Jombang, East Java *(Jawa Timur)*	Semarang, Central Java *(Jawa Tengah)*	Pakem, Yogyakarta	GMU *(UGM)* 2	GMU *(UGM) 1*
Control *(Kontrol)*	6.8	6.8	4.6	4.6	7.8	7.8
1	8.6	8.6	8.6	9	9	9
2	9.2	9.2	9.25	10	9.4	9.8
3	9.8	9.4	10	10	9	9.8
4	10	10	10	10	10	10
5	10	10	10	10	10	10

[1]Remarks *(Keterangan):* Infestation rate was scored according to ASTM Standard (ASTM, 1995*) (Tingkat serangan dinilai menurut Standard ASTM, 1995)*

Termite mortality is caused by penetrating of the fungus pathogen into the body of the termites (Yendol and Paschke, 1965). It seemed, when the termites tunneled the fungus barrier for searching food in the tube base, the fungus infected the termites and consequently caused termites death. A laboratory bioassay showed that fungi *M. anisopliae* and *Verticilium indicum* might cause high mortality to dry-wood termite *Cryptotermes brevis* (Nasr and Moein, 1997). *M. anisopliae* produces spores which kill termites by penetrating the body surface and multiplying throughout the termite's blood and body cavities.

Table 3. Average percent mortality of the termites introduced to 6 strains of fungus barrier

Tabel 3. Rata-rata persentase kematian rayap yang dicoba terhadap 6 strain cendawan

Thickness of fungus barrier *(Ketebalan sekat cendawan)*, cm	Average percent mortality of termites *(Persentase kematian rayap)*[1]					
	Fungal origin *(Asal cendawan)*					
	Bogor, West Java *(Jawa Barat)*	Jombang, East Java *(Jawa Timur)*	Semarang, Central Java *(Jawa Tengah)*	Pakem, Yogyakarta	GMU *(UGM)* 2	GMU *(UGM)* 1
Control *(Kontrol)*	25.2a	25.2a	6.4a	6.4	38.4a	38.4a
1 cm	30.4a	62.8ab	42ab	97.6	37.6a	46.8a
2 cm	79.6a	86.8bc	40abc	100	66.9a	51.2a
3 cm	87.6b	75.2bc	67.5bc	99.6	42.4a	86.8b
4 cm	83.2b	100c	83.2c	100	72.8a	96.4b
5 cm	96.4b	100c	77.2c	100	75.2a	100b

[1]Remarks *(Keterangan)*: Numbers in each column followed by same letter not significantly different, Tukey test, P < 0.05 *(Angka-angka pada masing-masing kolom yang diikuti dengan huruf yang sama tidak berbeda nyata menurut uji Tukey, P < 0,05)*

Entomopathogenic fungi produce chitinolytic, lipolytic and proteolytic enzymes that are able to biochemically degrade an insect body (Gabriel, 1968; Leger *et al.,* 1998). Because of their abilities, some insect pathogens are potentially useful for termite control (Grace and Zoberi, 1992, Nasr and Noein, 1997). These laboratory trials also confirmed the similar phenomena about possibility of developing the entomopathogenic fungus, *M. anisopliae,* for termite control in Indonesia.

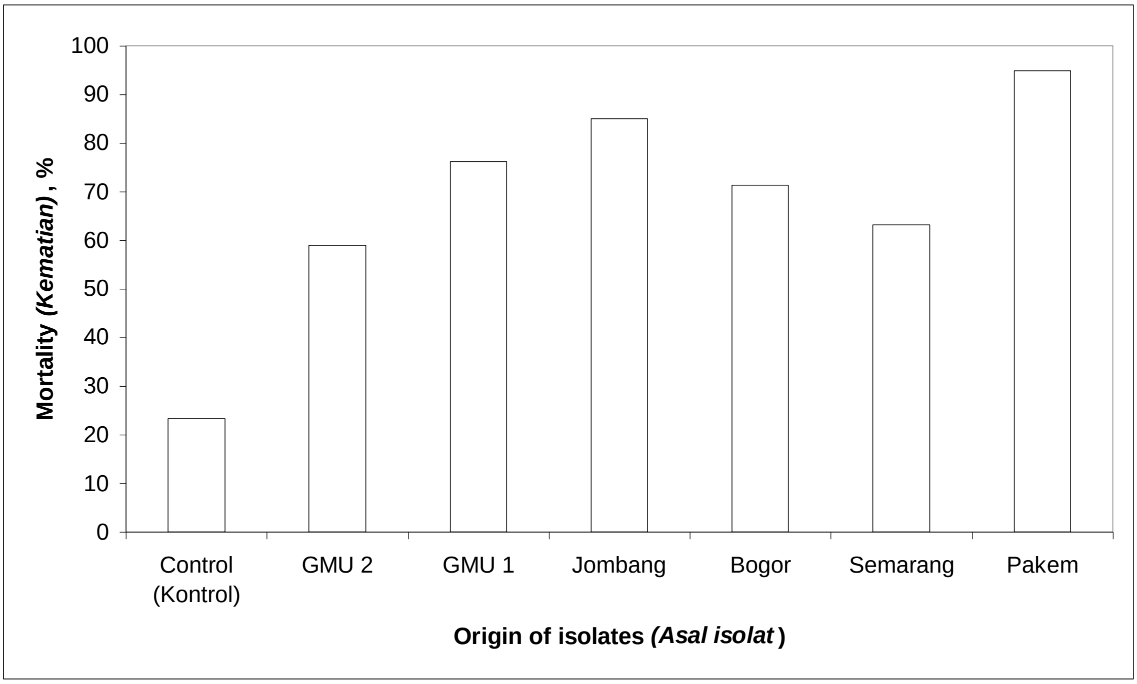

Figure 2. Summarizing of average termite mortality percentage of termite mortality caused by the barriers of the 6 strains of entomopathogenic fungus

Gambar 2. Ringkasan persentase kematian rayap karena perlakuan penyekat 6 strain cendawan patogen serangga

IV. CONCLUSION

It can be concluded that a strain of entomopathogenic fungus, *M. anisopliae,* from Pakem (Yogyakarta) was the most promising for termite barrier than those from others. Fungus barrier that was developed from Pakem isolate of 2 cm thickness or more could cause 100 % termite mortality after 9 days experimentation. The fungus strain from Jombang was less effective. Barrier effectiveness from this fungus can be achieved at the fungus barrier thickness of 4 cm or more. The remains were respectively strains from Gadjah Mada University (GMU) 1, Bogor (West Java), Semarang (Central Java) and GMU 2. Improving fungus effectiveness will be carried out, particularly for the promising fungus strains.

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