

Pathogenicity of the Entomopathogenic Fungus *Myrothecium roridum* Tode Ex Steudel, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium* sp. from Natural in West Sumatera Indonesia against *Coptotermes gestroi* Wasmann (Blattodea: Rhinotermitidae)

by

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Abstract

Pathogenicity bioassay of the entomopathogenic fungus *Myrothecium roridum* Tode ex Steudel (isolates My-Pd and My-Bk), *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium* sp. originated from West Sumatera Indonesia as bio termiticide to control subterranean termite *Coptotermes gestroi* Wasmann using five level of concentration of each fungus was carried out. The results are *Metarhizium* sp. and *M. roridum* (isolate My-Pd) more pathogenic, the result also showed that there were correlations between conidial amount and termite's mortality of *C. gestroi* 7 days after inoculation. *M. roridum* (isolate My-Pd) and *Metarhizium* sp. had remarkable virulence to *C. gestroi* On 10^6 conidia/ml; *M. roridum* could kill 96.25% with correlation value, $R^2 = 0.39$, $LC_{95} 3.7 \times 10^6$ conidial/ml, $LT_{95} 6.43$ days, and *Metarhizium* sp. could kill 100% with correlation value, $R^2 = 0.78$, $LC_{95} 9.4 \times 10^5$ conidial/ml, $LT_{95} 6.50$ days, whereas *M. roridum* (My-Bk) and *B. bassiana* more lower, *M. roridum* (My-Bk) just could kill 57.50% with correlation value, $R^2 = 0.32$, $LC_{95} 3.9 \times 10^7$ conidial/ml and $LT_{95} 6.88$ days and *B. bassiana* could kill 58,75% with correlation value, $R^2 = 0.36$, $LC_{95} 8,5 \times 10^{11}$ conidial/ml and $LT_{95} 51.72$ days

Key words: Entomopathogenic fungus, pathogenicity, LC, LT and *C. gestroi*

Introduction

Pathogenicity is the qualitative ability of a pathogen to cause disease and it is determined by a variety of factors, including the physiology of the host (e.g. defence mechanisms), the physiology of the fungus (e.g. pathogenicity factors, such as the production of enzymes and toxins) and the end is the environment. Insect death may result from a combination of actions, including depletion of nutrients, physical obstruction or invasion of organs and toxicosis, Such as *B. bassiana* produces a number of toxic compounds including beauvericin, bassianolide and oosporein and destruxins produced by *M. anisopliae* (Butt *et al.* 2001). Toxicity of entomopathogenic fungus beside be influenced by their host condition (physiological and morphological) also by environment factors of natural such as origin of isolate source, and where will they are applied.

In this case, if we need the bio-control agents for control the termite in building, house and another structure in West Sumatera as a province that has been passed by equator, we can collecting of bio-control agents from the same place (in-situ isolates), this is my be more effective than we use the agents from another place. Beside that, the species is also important because they are not always

effective as bio-control for all insect pests, but depend on fungi species and target of insect pests, it is meant that condition mentioned above also need specific fungus for control of termite.

West Sumatera is part of territory of Indonesia, its regency or town each other have been varied weather condition. This territory are harmonizing habitat by some organism of wood degradation such as termite and another insects and pathogen such as fungi. Desyanti *et al.* (2006; 2008; 2009); Zulyusri *at al.* (2010) have collected the entomopathogenic fungus from varied source or host in West Sumatera, the result of screening test for termite control, from 42 isolates (10 species) only 8 species effective as candidate of bio-termiticide, they are *Metarhizium anisopliae* (Metsch) Sorok, *Metarhizium sp.*, *Myrothecium roridum* Tode ex Steudel, *Beauveria bassiana* (Bals.) Vuill, *Aspergillus flavus* Link, *Aspergillus niger* van Tieghem, *Aspergillus sp.*, *Rhizopus sp.*, *Penicillium sp.* and *Acremonium sp.*,

As candidate of bio-termiticide agents, the fungus from the screening test must be going to continue study to know their pathogenicity. In this study based on some case only part of the effective entomopathogenic fungus as candidate of bio-termitisida were continued to evaluation their pathogenicity.

Materials and methods

Termites

The termites as object in this research are subterranean termite *C. gestroi* from society housing in Padang West Sumatera Indonesia.

Entomopathogenic fungi

Species of fungus were used are: *Metarhizium sp.* and *Myrothecium roridum* (My-Pd and My-Bk) and *Beauveria bassiana*. These species of fungi were selected from the early research of exploration and screening test. The selected species were store in room temperature before used. More detail those species show in Table 1 and Figure 1.

Table 1. Species of entomopathogenic fungus from nature in West Sumatera Indonesia

Isolates	Isolates Source	Species of fungi	Town origin (year)
1. My-Pd	Sand	<i>Myrothecium roridum</i>	Padang (2006)
2. My-Bk	Soil	<i>Myrothecium roridum</i>	Bukittinggi (2009)
3. Bb-Bk	Soil	<i>Beauveria bassiana</i>	Bukittinggi (2009)
4. Metar	Soil	<i>Metarhizium sp.</i>	Bukittinggi (2009)



B. bassiana

M. roridum

Metarhizium sp.

Figure 1. Colonies of entomopatogenic Fungus (*B. bassiana*, *M. roridum*, *Metarhizium sp.*) from West Sumatera 3 weeks old after culture on Saboraud Dextrose Agar with Yeast Extract (SDAY) medium

Culture procedure

For bio-assay, the Species of fungus were cultured on SDAY medium in Petri dish, and than it was incubation for 3 weeks in room temperature

Preparation of conidia suspension

After the fungus were 3 weeks old, suspensions of fungi were prepared by additional of 2 ml sterilized aquadest contained 0,05% Tween 80. The Petri dish was sought to get the conidia and dilute in the sterilized aquadestilata to get the dilution. The haemocytometer was used to count the total of conidia. Conidia suspension from each species of fungi was prepared with concentration 10^5 , 5.10^5 , 10^6 , 5.10^6 , 10^7 conidia/ml.

Bio-assay

After prepared the suspension mentioned above, and than the 20 individual of termite in colony or unit of treatment infected by suspension of each concentration of conidia of entomopatogenic fungus. The filter paper was placed in a Petri dish (Θ 8 cm) together with total individual in colony (20 workers and 2 soldiers) of termite *C gestroi*. The termite according with the treatments take place in each of colony in laboratory. The Petri dish was placed in a plastic container and keep in dark condition for 7 days. The dead termites were evaluated every day and termite's mortality was calculated. Beside mortality, LC and LT also evaluated.

Lethal Concentration (LC) and Lethal Time (LT)

LC and LT are Concentration and the time were needed by entomopathogenic fungus to kill an amount definite organism in population and is obvious in percentage (%). For known connection regression between the concentration (LC) and applications time (LT) with mortality were used probite analysis (Finney 1971).

Results and discussion

Mortality test

The entomopatogenic fungus *M. roridum*, *B. Bassiana* and *Metarhizium* sp. Originated from west sumatera Indonesia that potential as bio-termiticide were tested their pathogenicity as bio-control of *C. gestroi*.

In this research, *M. roridum* isolate My-Pd and *Metarhizium* sp. after 7 days inoculated can caused mortality *C. gestroi* 96,25% - 100% on treated with 10^6 conidia/ml. those fungus species have higher pathogenicity as bio-control of *C. gestroi*. After use DUNCAN analysis, species of *M. roridum* isolate My-Pd and *Metarhizium* sp not significant difference on those concentrations (Table 1).

Species of *M. roridum* isolate My-Bk also caused mortality of *C. gestroi* high and not significant difference with *M. roridum* isolate My-Pd and *Metarhizium* sp on treatments of concentration 10^7 , 5.10^6 , 5.10^5 and 10^5 conidia/ml, but also not significant difference with another species that caused mortality lower. Species of *B. bassiana* shown it's ability lowest as bio-control of *C. gestroi* on all level of concentration, the highest mortality only 58, 75%. In this study resulted that species of *M. roridum* isolate My-Pd and *Metarhizium* sp. more effective as agents bio-control *C. gestroi*, their pathogenicity highest compared with *M. roridum* isolate My-Bk and *B. bassiana*. (Table 2 and Figure 2).

Table 2. Mortality of *C. gestroi* (%) treated by level of concentration of entomopathogenic fungus *M. roridum*, *B. bassiana* and *Metarhizium sp.* originated from West Sumatera

Isolate	Species of Fungi	Concentration of conidia (conidia/ml)				
		10 ⁵	5.10 ⁵	10 ⁶	5.10 ⁶	10 ⁷
My-Pd	<i>M. roridum</i>	20 ef	78,75 abc	96,25 a	95 a	92,50 a
My-Bk	<i>M. roridum</i>	78,75 abc	73,75 abcd	57,50 cd	90 ab	100 a
Bb-Bk	<i>B. Bassiana</i>	23,75 ef	46,25 de	58,75 bcd	56,25 cd	46,25 de
Metar	<i>Metarhizium sp.</i>	67,50 abcd	80 abc	100 a	100 a	100 a

Mean followed by the same letter are not significantly different at 0.05 level of confidence according DNMRT test. (Control was 0%). This treatments Mean with 4 replications

The effective of entomopathogenic fungus as bio-termiticide, besides they were caused by ability of species through invasion and with different toxin compound (depend of species) also caused by level of concentration (conidia/ml) of each fungi species.

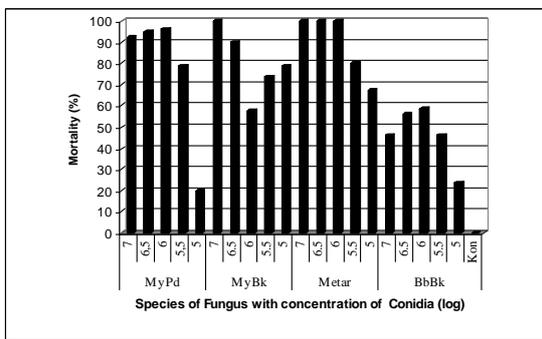


Figure 2. Mortality of *C. gestroi* (%) treated by level of concentration of entomopathogenic fungus *M. roridum* (isolate My-Pd and My-Bk), *Metarhizium sp* (isolate Metar) and *B. Bassiana* (isolate Bb-Bk) from nature in West Sumatera

Mortality of *C. gestroi* was influenced by variation of conidium concentration and species of entomopathogenic fungus, those are showed at differences of Mortality that resulted by each treatment. Each species of entomopathogenic fungi has a level of its pathogenicity, so it needs a concentration level of a conidia for effectiveness as bio-control subterranean termite of *C. gestroi*

Neves and Alves (2004) mentioned that LT of insects are influenced by application concentration or doses and virulence of each isolate. Toxicity level of pathogenic fungi for causing mortality depends on varies factors, such as by host and fungi physiological characteristic and their secondary metabolite such as enzyme and toxin also by influenced of environment (Butt *et al.* 2001). Another item of pathogenisity, species of fungus my be have correlation with their enzyme and mycotoxins produce on period of processing of insects infection such as in period contact with cuticle and in hemoel (Tanada & Kaya 1993).

Level of conidia concentration shown significant different to mortality of *C. gestroi*, generally there are correlation between level of conidia concentration with mortality, the higher concentration of conidia can caused higher mortality of *C. gestroi* (Fig. 3).

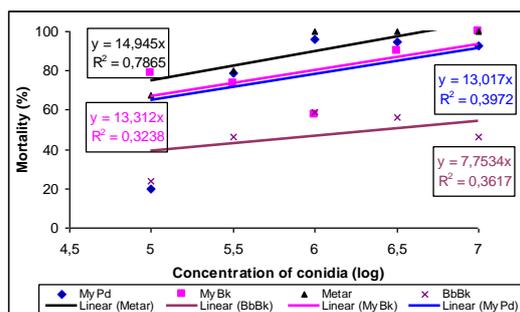


Figure 3. Correlation between Mortality (%) of *C. gestroi* with variation of concentration of conidium's (log) of entomopathogenic fungus *M. roridum* isolate My-Pd and My-Bk, *B. bassiana* isolate Bb-Bk and *Metarhizium sp.* isolate Metar

On Figure 3 also shown that correlation between mortality with concentration of conidia from each species were indicated by R² value, isolate *Metarhizium* its R² value= 0, 78. This positive correlation indicate, the higher concentration of conidium's for each species of fungi in this study caused higher mortality of *C. gestroi*, based R² value, species of *Metarhizium* sp. has correlation more high than another species.

Lethal Concentration (LC) and Lethal Time (LT)

Lethal concentration (LC) is fungus ability to kill an amount definite termite in their population. Lethal time is the time is needed by entomopathogenic fungi to kill the sum of individual of *C. gestroi* in their population.

Table 3. Lethal Concentration (LC) and Lethal Time (LT) of Entomopathogenic Fungus *M. roridum*, *B. bassiana*, and *Metarhizium* sp. as Bio-control of *C. gestroi*

Species	Isolate	LC (conidia/ml)			LT (days)		
		95%	50%	25%	95%	50%	25%
<i>M. roridum</i>	My-Pd	3,7 x 10 ⁶	2,06 x 10 ⁵	6,2 x 10 ⁴	6.43	2.86	2.05
<i>M. roridum</i>	My-Bk	3,9 x 10 ⁷	1,4 x 10 ⁴	5,4 x 10 ²	6.88	1.59	0.87
<i>B. bassiana</i>	Bb-Bk	8,5 x 10 ¹¹	2,5 x 10 ⁶	1,3 x 10 ⁴	51.72	8.69	4.18
<i>Metarhizium</i> sp.	Metar	9,4 x 10 ⁵	5,6 x 10 ⁴	1,7 x 10 ⁴	6.50	2.89	2.07

Lethal Concentration (LC)

LC_{95, 50} and ₂₅ of *M. roridum* (isolate My-Pd and My-Bk), *B. bassiana* and *Metarhizium* sp. (Table 3) in this research is varying and not consistent, but the lower of LC₉₅ was gotten from species of *Metarhizium* sp. (Metar): 9,4 x 10⁵ conidia/ml and than followed by species of *M. roridum* (isolate My-Pd): 3,7 x 10⁶ conidia/ml. Those indicated that *Metarhizium* sp. and *M. roridum* (isolate My-Pd) more high toxic than species of entomopatogen *M. roridum* (isolate My-Bk) and *B. bassiana* to bio-control of *C. gestroi*. Those are have correlation with mortality that resulted by both species of fungus.

Lethal Time (LT)

Letal Time (LT₉₅) entomopathogenic fungus *M. roridum* (isolat My-Pd) is lowest, then followed by *Metarhizium* sp., *M.roridum* (isolat My-Bk) and *B. Bassiana* (Tabel 3). The results are same with mortality, *M. roridum* (isolate My-Pd) and *Metarhizium* sp. also caused mortality the highest. The fungi that have higher level of mortality resulted LT lower or it can caused mortality more quickly.

Conclusions

Pathogenicity test of entomopatogenic fungus *Myrothecium roridum* (isolate My-Pd and My-Bk), *Beauveria bassiana* and *Metarhizium* sp. originated from West Sumatera that potential as bio-termiticide were resulted: *M. roridum* (isolate My-Pd) and *Metarhizium* sp. after 7 days inoculation, caused mortality of *C. gestroi* 96,25% - 100% on treatment of conidia concentration 10⁶ conidia/ml. Species of *B. bassiana* showed its lowest ability to kill *C. gestroi* on the all treatments, it can caused the highest mortality is 58, 75% only. *Metarhizium* sp. Have the best correlation is R² = 0, 78, LC₉₅ = 9, 4 x 10⁵ conidia/ml and LT₉₅ = 2.07 days.

Acknowledgement

We would like to thank for Gaverment of Republic Indonesia, This study was financially supported by Hiba Bersaing DP2M –DIKTI Ministry of National Education Republic of Indonesia period 2009

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