

## PATHOGENICITY OF TWO SPECIES OF ENTOMOPATHOGENIC AGENTS TO SUBTERRANEAN TERMITE *Coptotermes gestroi* WASMANN (ISOPTERA: RHINOTERMITIDAE) IN LABORATORY

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### RINGKASAN

#### Patogenisitas dua spesies mikrob entomopatogen terhadap rayap tanah *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae)

Patogenisitas dua spesies mikrob entomopatogen *Metarhizium anisopliae* dan *Bacillus thuringiensis* A dan B diuji terhadap rayap tanah *Coptotermes gestroi* Wasmann di laboratorium. Tiga kelompok rayap pekerja masing-masing dipaparkan pada kertas saring yang telah dikontaminasi dengan salah satu mikrob patogen selama satu menit. Dua puluh lima rayap pekerja yang telah terkontaminasi dan tiga perajurit kemudian dimasukkan ke dalam tabung plastik yang telah diisi kertas saring basah. Percobaan disimpan di ruang gelap dan lembab pada suhu kamar selama 14 hari. Tiap perlakuan diulang lima kali. Pengamatan dilakukan tiap hari untuk mencatat dan mengeluarkan rayap yang mati. Hasil penelitian menunjukkan bahwa cendawan *M. anisopliae* lebih bersifat patogen daripada kedua jenis *B. thuringiensis*. Perlakuan dengan *M. anisopliae* menyebabkan mortalitas seluruh rayap dalam waktu 8-10 hari, sedangkan dengan *B. thuringiensis* A dan B tidak menimbulkan kematian yang berarti, bahkan tidak berbeda dengan kontrol, dengan mortalitas kurang dari 3% hingga akhir percobaan.

Kata kunci. Cendawanpatogen, *Metarhizium anisopliae*, *Bacillus thuringiensis*, mortalitas rayap

### ABSTRACT

#### Pathogenicity of two species of entomopathogenic agents to subterranean termite *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae) in laboratory

Pathogenic effects of two species of entomopathogenic agents, *Metarhizium anisopliae* and *Bacillus thuringiensis* A and B, were tested to subterranean termite *Coptotermes gestroi* Wasmann in laboratory. Three groups of the termite workers were each exposed to a treated filter paper with one of the entomopathogenic agents for one minute. Twenty-five termite workers and three soldiers were then transferred to a wetfilter paper disk that had been installed in a plastic vial. The test materials were stored in a dark and humid at room temperature for 14 days. Five replicates were provided. Observations were conducted daily to determine termite mortality and take out dead termites. Results showed that the fungus *M. anisopliae* was more pathogenic than *Bt. A* and *B*. After being exposed to treated paper with the fungus, all test termites died within 8-10 days. Treatments with *B. thuringiensis* A and B did not produce high mortality, and seemed to be not different from the control, only causing less than 3% termite mortality until the end of the tests.

Key words: Entomopathogenic fungi, *Metarhizium anisopliae*, *Bacillus thuringiensis*, termite mortality

### INTRODUCTION

Subterranean termite *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae) could be a species that might be confused with *Coptotermes curvignathus* (Yoshimura *et al.* 1998) as formerly

identified by Tarumingkeng (1971). However, most species of this termite genus are very dangerous, causing damage to wood structure, living trees in the forest, and estate crops. Some living trees, especially pine and agathis, in Bogor Botanical Garden and Experimental Forests in Cikampek and

Janlapa were severely destroyed by this insect (Sukartana 1997). Some wood structures and official documents of the Forest Products Research Center Bogor are also heavily damaged.

Chemical termiticides are commonly used to prevent termite infestation. Saturating the soil around building with the toxic chemicals, which is called soil treatment, is designated to provide poisonous barrier preventing termite invasion from infested areas. Wood preservation, especially in tropical region, is a measure to improve durability of timbers against termite attack. However, because of the awareness to environmental risk, uses of the poisonous chemicals often face with considerable resistance from the public. In addition, since termite colonies can contain millions of individuals (Esenther 1980; Su & Scheffrahn 1988) and live cryptically, it is impossible to treat directly the entire individuals in a colony using conventional termiticides.

Recently, studying on biological insecticides to control termites are becoming of great interest to reduce chemical insecticides uses. Entomopathogenic fungus *Metarhizium anisopliae* is known virulent to Australian termite *Nasutitermes exitiosus* (Hanel and Watson 1983) and Formosan subterranean termite *Coptotermes formosanus* (Jones *et al.* 1996). Infesting termite with pathogenic fungi can be potentially employed to transfer the pathogen to healthy members of a colony (Kramm *et al.* 1981). Some studies have also been carried out on the pathogenicity of *Beauveria bassiana* (Lai *et al.* 1982; Zoberi & Grace 1990), *B. brongniartii* (Yoshimura & Takahashi 1998), *Bacillus thuringiensis* (Smythe & Coppel 1965; Khan *et al.* 1977), and *Conidiobolus coronatus* (Yoshimura *et al.* 1992).

However, it seems that the study on these insect pathogens for termite control has not been developed yet in Indonesia even though there are many destructive termite species to wood structures and other cellulosic materials. In this study we try to evaluate termiticidal effectiveness of two species of entomopathogenic microbes to the subterranean termite *C. gestroi*. In this paper, the pathogenicity of *M. anisopliae* and two strains of *B. thuringiensis* to the termite species will be discussed.

## MATERIALS AND METHODS

*M. anisopliae* was obtained from a laboratory culture on corn media, while the two strains of *B.*

*thuringiensis* were respectively bought from agricultural stores in Bogor (*B. thuringiensis* A) and Bandung (*B. thuringiensis* B). The termite specimen was collected using the method described by La Fage *et al.* (1983) from an infested wood stump.

Three pieces of filter paper disks, about 5 cm in diameter, were prepared. One was contaminated with the fungus by inserting it in culture of *M. anisopliae* and the others were immersed in 10% solution of *B. thuringiensis* A or B for one minute. Prior to insertion in the *M. anisopliae* culture, the paper was wetted with water to make the paper more sticky to the fungus spores.

The papers were taken out and then each put in a petridish. Three groups of termite workers were each exposed on a treated filter paper with one of the entomopathogenic microbes for one minute. Twenty-five termite workers and three soldiers were then transferred to a damp filter paper disk that had been previously installed in a plastic vial. The vials were capped and stored in a covered plastic bowl and then kept in a dark and humid at room temperature for 14 days. Some layers of wetted tissue paper were put in the bowl prior to the vial storage to saturate air moisture in the bowl chamber using the method of Sukartana (1998). Five replicates were provided.

The test was observed daily to determine and take out dead termites. The data of termite mortality, after conversion into percentage, were presented in a graph to figure out the effectiveness of treatments with the pathogenic microbes on the termite.

## RESULTS AND DISCUSSION

The fungus *M. anisopliae* was more pathogenic to control the termite than *B. thuringiensis* A and B (Figure 1). Treatment with *M. anisopliae* produced 100% termite mortality within 8-10 days. Meanwhile, treatment with both strains of *B. thuringiensis* did not cause significant termite mortality, or even similar to the control performance.

Previous experiment showed that treatment with *M. anisopliae* resulted in 100% death of dry wood termite *Cryptotermes cynocephalus* and *Coptotermes gestroi* within less than 3 and about 8-10 days after treatment respectively, but it was less effective to subterranean termite *Schedorhinotermes javanicus* (Sukartana *et al.* 2000). Similar performance is obtained from study on Formosan sub-

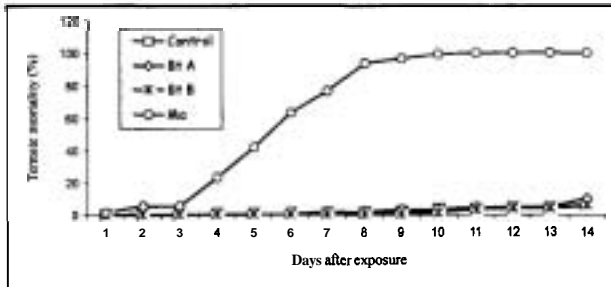


Figure 1. Cumulative termite mortality caused by treatment with an entomopathogenic fungus *M. anisopliae* (Ma) and *B. thuringiensis* A and B (BtA and BtB)

terrestrial termite *C. formosanus*. Termite feeding on agar culture of *M. anisopliae* died within 6 days (Ko *et al.* 1982) and that exposed to the fungus cultures in rolled paper baits died by the fourth day (Delate *et al.* 1995).

This fungus is transmittable from treated termites to the healthy ones. Introduction of termites treated with the fungus sharply increased the level of mortality in previously unexposed population of *Reticulitermes* sp. (Kramm *et al.* 1982) and that of *C. gestroi* (Sukartana *et al.* 2000). Transfer of fungal pathogen *Beauveria bassiana* was also demonstrated in a group containing infected and uninfected subterranean termite *Reticulitermes flavipes* (Grace & Zoberi 1992). Laboratory studies have shown that treatment of only five to 10% can result in 100% mortality (Rath 1995). Termites are social insects. Through their grooming behavior, transfer of fungal spores may occur from infected termites to the healthy ones within a colony. Release recapture technique, a method used for estimating colony size introduced by Esenther (1980), probably can be developed for termite control using insect pathogen.

Meanwhile, pathogenicity of *B. thuringiensis* was very low. Both strains of *B. thuringiensis* showed similar performance and there was no difference from the control. Khan *et al.* (1977) showed that this bacteria caused 100% mortality of termite *Heterotermes indicola* and *Microtermes championi* in a period of more than 20 days. A soluble toxin derived from this bacteria combined with its spore produces about 90% mortality on some termite species after 9 days (Smythe & Coppel 1965). Study on this bacteria for termite control is still rare. There was also no *Bacillus*

species mentioned in a comprehensive review of biological control of termites in Australia (Milner & Staples 1996).

## CONCLUSION AND SUGGESTIONS

Entomopathogenic fungus *M. anisopliae* is obviously more toxic to subterranean termite *C. gestroi* than *B. thuringiensis*. Treatment with *M. anisopliae* resulted in 100% termite mortality within about 8-10 days whereas that with *B. thuringiensis* only caused less than 3%, a number that was closed to the control performance.

The insect pathogen *M. anisopliae* is seemingly potential to be developed as biological insecticide for termite control in Indonesia. Through grooming activities, a treated termite is expected to transfer the disease to other individuals in a colony. Hence, comprehensive studies, for examples; virulence, effectiveness, culturing, formulating, packaging and method of application, should be conducted to provide strong scientific bases prior to using the pathogen for termite control.

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