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EFFECTIVENESS OF *Beauveria bassiana* AGAINST *Coptotermes curvignathus*

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ABSTRACT

Termites are important pests to human life which can cause severe economic loss in Indonesia. Termites are not only attacking the wood products, but also the living plants with wide host distribution. Implementation of biological agents for termites control is one way to reduce the implementation of synthetic termiticide since it is considered to be more environmentally friendly and doesn't cause resistance to termites. This research aimed to analyze the effectiveness of conidial density levels of *Beauveria bassiana* from various media types against *Coptotermes curvignathus* mortality. The research was designed using factorial completely randomized design by combining media types with conidial density levels. *B. bassiana* suspensions were made from three types of liquid media consist of Potato Dextrose Broth (PDB), Malt Extract Broth (MEB) and Sabouraud Dextrose Broth with Yeast Extract (SDBY). The suspension with density of 108, 106, and 104 conidia ml-1 from PDB, MEB, and SDBY media were applied to *Coptotermes curvignathus*. The results showed that all types of nutrients sources (PDB, MEB and SDBY) did not affect the virulence of *B. bassiana* conidial suspensions against *C. curvignathus* mortality. Nevertheless, *C. curvignathus* mortality were affected by the differences of conidial density levels from *B. bassiana* suspension.

Key words: *Beauveria bassiana*, conidial density, media, mortality

INTRODUCTION

Economic loss caused by termites in Indonesia reached 1.67 trillion rupiahs in 1995 and is estimated to reach 2.8 trillion rupiahs in 2015 (Sudiaman 2015). Termites are not only attacking the wood products, but also the living plants with wide host distribution. There are six species of trees that potentially attacked by termites including Rubber, Pine, Eucalyptus, Acacia, Gmelina and Mahogany (Nair 2001). The genus Coptotermes is the most destructive for the living trees, for instance *Coptotermes curvignathus* which could kill 10 – 50% of field-planted *Acacia mangium* saplings. *Coptotermes sp* potentially attack Jabon stands in forest plantation on peatlands (Pribadi 2010). For that reasons, it is necessary to control termite populations by applying the threat of termite attack not only on buildings but also on a living plants with wide distribution.

Pest control using biological agents such as entomopathogenic fungi is in the increasing trend (Kim *et al.* 2013). *Beauveria bassiana* is one of entomopathogenic fungi that has been observed in termites control (Desyanti 2007). *B. bassiana* requires appropriate nutrients to produce optimum biomass, so that media selection is an important thing to be considered in culturing *B. bassiana*. Several studies have been conducted towards the source of the media for culturing entomopathogenic fungi. To develop biological pesticides in mass production scale, culture medium nutrients that can maximize the amount of mycelia and spores at low cost become an important requirement (Gao and Xingzhong 2010). The highest biomass production was obtained in Sabouraud Dextrose Broth with Yeast Extract (SDBY) media followed by Malt Extract Broth (MEB) and Potato Dextrose Broth (PDB) in *B. bassiana* cultivation. This is allegedly due to the presence of peptone as a source of nitrogen in SDBY nutrient composition (Ramdania 2015). Itis important to know whether the differences of nutrient sources will affect the virulence of *B. bassiana* or not. This research aimed to analyze the effectiveness of conidial density levels of *B. bassiana* from various media types against *C. curvignathus* mortality.

MATERIALS AND METHODS

Fungal Culture

Isolates of *B. bassiana* belongs to the culture collection of Center of Seeds and Protection of Plantation Crops (BBP2TP) Surabaya were used. The isolates were obtained from mummified Lepidiota stigma. Subcultured isolates of *B. bassiana* were maintained on different medias include Potato Dextrose Broth (PDB), Malt Extract Broth (MEB) and Sabouraud Dextrose with Yeast Extract (SDBY). PDB, MEB and SDBY for each 50 ml medium were prepared in 250 ml jar of jam. They were later inoculated aseptically with an 8 mm of B. bassiana isolate and incubated for 4 weeks.

Conidial Suspension

Conidia were harvested from fungal isolate in each culture media after 4 weeks of incubation. Conidia were harvested by scrapping the surface of miselium with rod spreader or spatula then added with 5 ml of sterile

distilled water and filtered into a sterile tube using filter paper. The conidial suspensions were serial diluted to obtain the desired range of density. Density of all suspensions were quantified with a haemocytometer and adjusted to final density of 108, 106, and 104 conidia ml-1 with 0.05% Tween 80 in distilled water.

Bioassays

The termites were obtained from Centre for Research and Development on Forestry Engineering and Forest Products Processing Bogor. Total of 30 workers and 3 warriors caste of *C. curvignathus* were placed into a sterile petri dish with sterile filter paper inside which served as feed. Conidial suspension as much as 0.1 ml were dropped on filter paper inside the petri dish. Three replications were maintained for each conidial density levels from all media types. Control groups were treated with distilled water without fungal conidia with a same method such as conidial suspension application. Termites mortality was observed every 24 hours by recording the dead termites in each unit for 11 days.

Statistical analysis

The research was designed using factorial completely randomized design by combining media types with conidial density levels. Analyses of variance (ANOVA) were performed to evaluate levels of significance of the *B. bassiana* virulence against *C. curvignathus.* SAS software for windows version 9.1 (SAS Institute Inc.) and Microsoft Office Excel 2007 (Microsoft Corp., USA) were used to perform the statistical analysis.

RESULTS

Conidial suspensions from three different types of liquid media caused differences in the percentage of termites mortality. The highest *C. curvignathus* mortality caused by *B. bassiana* conidial suspension from MEB (44.4%), followed by conidial suspension from PDB (40.4%) and SDBY (21.2%) on the same conidial density level of 108 conidia ml-1 (Table 1).

Table 1 Cumulative mortality of *C. curvignathus* infected by *B. bassiana* from three types of liquid media at various conidial density levels for 11 days

Conidial density levels (conidia ml^{-1})	*C. curvignathus* mortality from each liquid media (%)		
	potato dextrose broth (PDB)	malt extract broth (MEB)	sabouraud dextrose broth with yeast extract (SDBY)
10^8	40.4	44.4	21.2
10^6	29.3	20.2	19.2
10^4	15.2	17.2	17.2
Control	4.0	3.0	5.1

Observation of the treatment showed that termites mortality is characterized by gradually symptoms. The initial symptoms showed that the infected termites weakened, characterized by infected termites were not as agile as healthy termites. The cuticles color of infected termites also changed into darker. Furthermore, some weak and sick termites were eaten by healthy termites. Application of *B. bassiana* suspension at different levels of conidial density resulted in increased mortality of *C. curvignathus* every day. The highest mortality for each type of media was caused by *B. bassiana* suspension with a density of 108 conidia ml-1, followed by the density of 106 and 104 conidia ml-1 (Fig 1). Different types of media did not affect the virulence of *B. bassiana* conidial suspensions against *C. curvignathus* mortality. Nevertheless *C. curvignathus* mortality were affected by the differences of conidial density levels from *B. bassiana* suspension (Table 2).

Table 2. *C. curvignathus* mortality averages due to *B.bassiana* suspension application at various conidial densitylevels

Conidial density (conidia ml^{-1})	Mortality Averages (%)*
10^8	28.94[a]
10^6	19.54[b]
10^4	16.72[b]
Control	4.03[c]

* Values within one column followed by different letters are significantly different at $\alpha = 0.05$ (Duncan's Multiple Range Test)

DISCUSSION

Termites response to *B. bassiana* infection varied. In this study, some cuticles colour of the infected termites were darker than the healthy one. The dark colour on the cuticle showed the penetration site (Boucias and Pendland 1998). One of termites characteristic is cannibalistic, that's why some weak and sick termites were eaten by healthy termites (Nandika *et al.* 2003).

The results show that the virulence of *B. bassiana* conidial suspensions against termites mortality was not affected by the difference of media sources. It means that PDB, MEB, and SDBY media can be used to cultivate *B. bassiana*. Termites mortality were affected by the differences of conidial density levels from *B. bassiana* suspension. The higher the conidial density, the higher the termites mortality. Seemingly, conidia did more contact with the body of termites, which cause a greater chance of conidia to germinate and penetrate the cuticle of termites (Desyanti 2007).

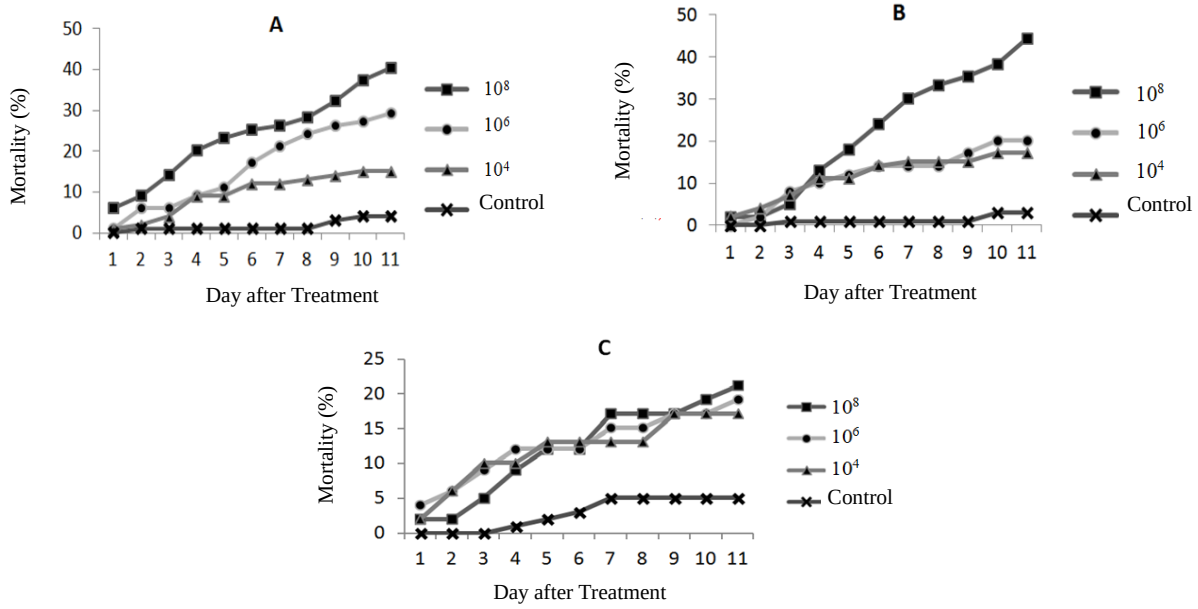

Fig 1 *C. curvignathus* mortality due to application of *B. bassiana* suspension from medium of Potato Dextrose Broth (A), Malt Extract Broth (B) and Sabouraud Dextrose Broth with Yeast Extract (C) at various conidial density levels for 11 days

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