Metarhizium brunneum PETCH FUNGI IS EFFECTIVE AS A BIOINSECTICIDE AGAINST *Coptotermes curvignathus* HOLMGREN TERMITE PESTS IN THE LABORATORY

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Abstract: Nutmeg is a plantation crop and has high economic value. Currently, the cultivation of nutmeg is experiencing many obstacles. One of them is termite pests. So far, the control is carried out using chemical insecticides and hurts the environment. Therefore it is necessary to control other environmentally friendly ways, and one of them is by using the fungi *M. brunneum* as an ecologically friendly bioinsecticide. The purpose of this study was to analyze the effectiveness of *M. brunneum* as a bioinsecticide against the termite *C. curvignathus* as an essential pest in nutmeg plantations. This research using a completely randomized design (CRD). The results showed that the mean percentage viability of *M. brunneum* conidia at each observation time was classified as useful. The germination of conidia increased to 89.78 % after 72 hours of observation. Conidia density 10^3/ mL distilled water. The highest mortality rate of *C. curvignathus* was found at a density of 10^{10} conidia/ mL of distilled water. The mean time of death for *C. curvignathus* after application of conidia density treatment of 10^{10}/ mL of distilled water was 2.15 days and the death of *C. curvignathus* was 3.35 days using conidia density treatment 10^3/ mL of distilled water. 1 day after application reaches 33.20 % and increases to observation at 5 days after application reaches 100 %. The highest food inhibition occurred in treatment 10^{10}/ mL distilled water (65.81 %) which was significantly different from treatment 10^5/ mL distilled water (43.23 %), and 10^3/ mL distilled water (41.61 %) and control (0.00).

Keywords: Bioinsecticide; termite; pest; *C. Curvignathus*; *M. brunneum*

Abstrak: Pala merupakan tanaman perkebunan dan bernilai ekonomi tinggi. Pada saat ini budidayanya mengalami banyak hambatan. Satu diantaranya adalah serangan hama rayap. Selama ini pengendalian hama rayap dilakukan dengan menggunakan insektisida kimia dan berpengaruh negatif terhadap lingkungan. Oleh karenanya perlu pengendalian cara lain yang ramah lingkungan dan satu diantaranya dengan menggunakan cendawan *M. brunneum* sebagai bioinsektisida yang ramah lingkungan. Tujuan dari penelitian ini adalah menganalisis efektivitas *M. brunneum* sebagai bioinsektisida terhadap hama rayap *C. curvignathus*. Penelitian ini dilaksanakan di Laboratorium Hama Tanaman dan Laboratorium Dasar Proteksi Tanaman Program Studi Proteksi Tanaman Fakultas Pertanian Universitas Syiah Kuala, sejak bulan Februari hingga September 2018, dengan menggunakan Rancangan Acak Lengkap (RAL). Hasil penelitian menunjukkan bahwa persentase viabilitas rata-rata dari konidia *M. brunneum* pada setiap waktu pengamatan tergolong efektif. Perkecambahan konidia semakin meningkat

hingga mencapai 89,78 % setelah diamati pada jam yang ke 72. Rata-rata waktu kematian rayap *C. curvignathus* setelah aplikasi perlakuan kerapatan konidia 10^{10}/ mL akuades adalah 2,15 hari dan terjadi kematian rayap *C. curvignathus* 3.35 hari dengan menggunakan perlakuan kerapatan konidia 10^{3}/ mL akuades. Rata-rata mortalitas tertinggi rayap *C. curvignathus* ditemukan pada perlakuan kerapatan konidia 10^{10}/mL aquades. 1 HSA mencapai 33,20 % dan meningkat hingga pengamatan pada 5 HSA mencapai 100 %. Daya hambat makan tertinggi terjadi pada perlakuan 10^{10}/ mL aquades (65,81 %) yang berbeda nyata dengan perlakuan 10^{5}/mL akuades (43,23 %) dan 10^{3}/ mL akuades (41,61 %) serta kontrol (0,00 %).

Kata kunci : Bioinsektisida; rayap; hama; *C. Curvignathus*; *M. brunneum*

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Introduction

Nutmeg is a valuable plantation crop from an economic perspective (Dewi *et al.*, 2018). Nutmeg cultivation is currently experiencing many problems, and one of them is the termite attack. *C. curvignathus* is a termite species that can adapt to unfavourable environments when compared to other termite species **(**Savitri *et al.*, 2016**).** The results of observations from plantations showed that in every 10 individual nutmeg plants it was found that on average 2 individual nutmeg plants experienced physical injuries caused by termite pests or 20 % of the nutmeg plants were attacked by termite pests so that the nutmeg plants experienced injuries at the base of the plant stem (Sayuthi *et al.*, 2016). The optimal environment has a positive effect on the growth and colony development of termite pests (Toni *et al.*, 2015). Termite attacks on nutmeg plants are increasing with the control of a wider range of radius in the environment (Pratiknyo *et al*., 2018). This condition hurts expanding the production of nutmeg to the maximum (Agesta *et al*., 2018).

Therefore, it needs serious attention related to this pest (Sayuthi *et al.*, 2016). Termite attacks on nutmeg need to be controlled immediately so as not to cause physical damage to plants so that they do not hurt crop production (Ngatiman, 2018). The termite colony of *C. curvignathus* has more than 1,000,000 individuals for each colony, with a minimum range of 450 m^2 (Santoso *et al.*, 2015). Damage to the roots can inhibit the translocation of water and nutrients from the soil to all parts of the plant tissue, and cause plants to wither, die until they are not producing. (Hutabarat *et al.*, 2015). The use of synthetic insecticides for termite control can pollute the environment because the residue lasts long and is difficult to wash and hurts humans (Ary *et al.*, 2015).

So far, termite control is carried out using synthetic insecticides and hurts the environment. Therefore, it is necessary to control other methods, and at this

time, there are no effective and environmentally friendly techniques for controlling them. The solution to this problem is through the use of *M. brunneum* as a bioinsecticide as a biological agent against termite pests from the Isoptera order and other pests (Anggriawan et al., 2018). According to Jaber and Enkerli (2016), Metarhizium sp can attach to and penetrate the cuticle of the host to invade internal parts of the host's body to absorb nutrients as a source of energy, so that the host will get sick and die.

These fungi can destroy the host tissue by removing toxins or secondary metabolites to cause abnormal growth of the host. Several types of toxins produced by Metarhizium sp. Such as cytochalasins, destruxin and swainsonine (Aryo et al., 2017). These toxic compounds can cause stomach function disorders and damage the muscle tissue of termites (Irwan, 2016). According to Sayuthi et al. (2018), *M. brunneum* is more effective than *M. anisopliae* and *B. bassiana* as bioinsecticides, and environmental factors largely determine its success. The release of 10 % individual carrier termites infected with *M. brunneum* conidia with a conidia density of 1.21 x 10^6 / mL of water observed at 15 days after the application was able to produce mortality against *C. gestroi* termites up to 90 %.

Based on these considerations, it is necessary to study the pathogenicity of *M. brunneum* as a bioinsecticide against termite pests that damage nutmeg plants. The hope is to contribute information related to alternative technology for termite control that is environmentally friendly. The purpose of this study was to analyze the effectiveness of *M. brunneum* as a bioinsecticide against the termite *C. curvignathus* as an essential pest in nutmeg plantations.

Related With the pathogenicity of *M. brunneum* as a bioinsecticide, it is necessary to study its effectiveness so that that termite pests can be conditioned effectively. Hopefully, it becomes information in the management of environmentally friendly termite pests. The purpose of this study was to analyze the effectiveness of *M. brunneum* as a bioinsecticide against the termite *C. curvignathus* as an essential pest in nutmeg plantations.

Methods

Materials

The materials used were the termite species *C. curvignathus*, the fungi *M. brunneum*, potatoes, dextrose, kitchen gelatin, aquadest, and tween 20.

Equipment

The equipment used is the Scanning Microscope Electron (SEM) type jsm 5310 lv japan, optical microscope (Olympus brand, model CX21FS1), haemacytometer (Neubauer Improved brand), test tube, measuring cup, Erlenmeyer, Petri dish, vortex and gas stove.

Research Implementation

This experiment was conducted to collect *C. curvignathus* termite species from Nutmeg Plantation, Meukek District, South Aceh Regency from one colony. Then taken to the laboratory and maintained in Petri dishes measuring 90 mm x15 mm containing cardboard paper as food then wrapped in newspaper and placed in a dark room. The entomopathogenic fungi *M. brunneum* used is a collection of the Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB). The fungi were cultured on PDA (Potato Dextrose Agar) media, with a composition of 200 g of potato, 20 g dextrose, and 20 g of kitchen agar dissolved in 1 litre of water. The fungi culture was stored in an incubator at a temperature of ± 25 ° C.

The conidia density of *M. brunneum* used in the experimental treatment was a fungus that was 21 days old. Conidia that have grown on PDA media are taken with a small, sterile brush. Then the conidia were transferred into a test tube filled with pure water by adding Tween 20 with a concentration of 0.025 mL per 50 mL of pure distilled water. The suspension is shaken for 30 seconds using a vortex. The conidia density of *M. brunneum* was calculated using a haemacytometer (Brand NeubauerImproved). This is a way to produce conidia densities 10^3/ mL, 10^5/ mL 10^7/ mL, 10^9/ mL, 10^{10}/ mL and conidia calculations observed using an optical microscope (Desyanti et al., 2007).

Revirulence of *M. brunneum*

M. brunneum virulence is the restoration of the ability of *M. brunneum* to inflict disease on its host. The M brunneum collection has long been preserved so that its ability to infect its host is thought to be ineffective. Therefore, the *M. brunneum* needs to be re-refracted so that its pathogenicity against the host will be effective again. Virulent isolates generally need a faster time to kill their host, when compared to isolates are not less virulent (Permadi et al., 2018). The virulence process was carried out by collecting 55 individual *C. curvignathus* termites (50 worker castes and 5 soldier castes).

The termites were immersed in a suspension of *M. brunneum* conidia which had been prepared in a petri dish at a density of 10^6 conidia / mL, and placed in another petri dish filled with wet cardboard as food. Then wrapped in paper to protect from light, and incubated at room temperature for three weeks. Termite cadavers that have been infected with the fungi *M. brunneum* are transferred to a sterilized test tube. Purification is done using PDA media. Kondia from *M. brunneum* that has been virulent then reproduced on PDA media. After the conidia grow and germinate, they are tested for termite pests in the laboratory.

Revirulence of *M. brunneum*

Revirulence of *M. brunneum* is an attempt to recover from *M. brunneum* to infect its host. This collection of *M. brunneum* has long been stored in the

laboratory, and the power to infect its host is thought to have decreased. Therefore, M. brunneum needs to be reviulsified again for its pathogenicity to the host to be effective too. Virulent isolates took a shorter time to kill the host, compared to isolates that were low virulent (Permadi et al., 2018). Revirulence was carried out by collecting 55 individual *C. curvignathus* termites (50 worker castes and 5 soldier castes). The termites have immersed in a conidia *M. brunneum* suspension that had been prepared in a petri dish at a density of 10^6 conidia / mL, and placed in a petri dish containing wet filter paper as feed. Then wrapped in newspaper to protect it from light, and incubated at room temperature for three weeks. The termite cadaver which has been infected with the fungi M brunneum is transferred to a sterilized test tube. Purification is done using PDA media. The virulent condition of *M. brunneum* was reproduced on PDA media. Conidia that grow and germinate are then tested on termite pests in the laboratory.

Research implementation

This experiment was carried out at the Basic Laboratory of Plant Protection, Plant Protection Study Program, Faculty of Agriculture, Syiah Kuala University, from January to October 2018 using a completely randomized design (CRD). This experiment consisted of 5 treatments (10^3 / mL, 10^5 / mL, 10^7 / mL, 10^9 / mL 10^{10}/ mL, and control) and 4 replications. Each treatment consisted of 4 replications. Each repetition of each treatment used 55 *C. curvignathus* individuals (50 worker castes and 5 soldier castes). Termites were inserted into each treatment with *M. brunneum* conidia suspension for 4 seconds, and the control used aquadest (Desyanti et al., 2007).

Each treatment and each replicate was stored at room temperature (25-28 °C) with humidity (70 % - 95 %) in dark conditions. The observed variables are; (1) viability of conidia or germination of conidia, (2) Mortality of termites observed from the day after an application today 5. (3) Time of death was observed with time intervals of 1 day to day 5, (4) Percentage of inhibiting feeding This is done by weighing the cardboard paper and drying it in an oven to prepare it for termite bait. Cardboard paper is weighed up to 1.30 g. Then the cardboard is moistened with distilled water for the test termite bait. After the experiment, the remaining cardboard that is not eaten by the termites was collected and using an oven. The cardboard material was dried. The calculation of the percentage of food retardants is done by weighing the initial weight of the boxes before treatment and the weight of the remaining packages. The value of the initial weight and the final weight of the cardboard boxes is included in the formula to determine the percentage of inhibiting food, namely: (Prijono, 2003).

$$PM = (Bk-Bp) / Bk \times 100\ \% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Percentage of inhibitor feeding (PM), the initial weight of feed (Bk), weight of final feed (Bp).

Data analysis.

The data obtained were analyzed ANOVA completely randomized design using SAS version 6.12

Results and Discussion

Viability of the conidia Metarhizium brunneum.

Mean germination percentage of *M. brunneum* conidia at density 10^7 / mL was observed at 24, 48 and 72 h after inoculation. Conidia density calculations used a haemocytometer and observed using an optical microscope (Table 1).

Table 1. Mean viability of *M. brunneum* at the conidial density of 10^7/ mL observed at 24, 48 and 72 hours after inoculation (JSI).

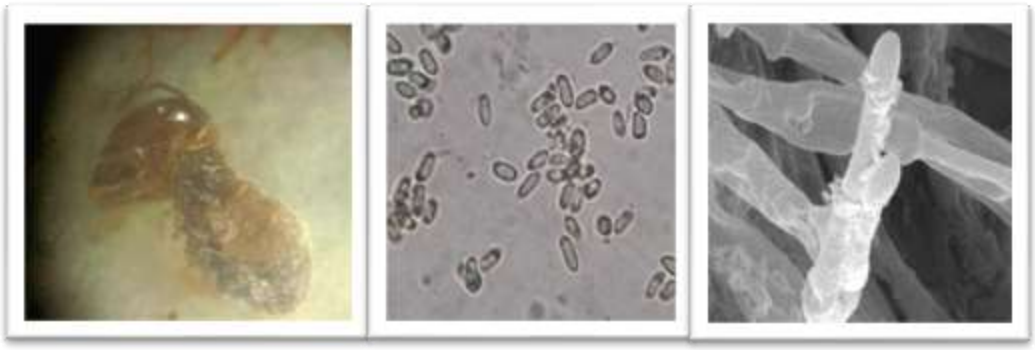
Observation time (hour)	Viability of conidia *M. brunneum* (%)
24	79.31
48	83.69
72	89.78

Table 1. The results showed that the mean germination percentage of *M. brunneum* conidia at 24, 48 and 72 hours after inoculation showed that conidia germination at 24 hours reached 79.31 %. Entering the 48th hour, the germination rate of *M. brunneum* conidia increased to 83.69 %. The germination of conidia increased by 89.78 % or nearly 90 %, which was observed at 72 hours. This indicated that *M. brunneum* conidia germination was effective, and fulfilled the requirements to be used as a biocontrol agent against termite pests.

Entomopathogenic fungi to enter into the group of effective biocontrol agents, the conidia viability must be high so that that termite pest control can be reasonable (Nunilahwati et al., 2012). The entomopathogenic fungi conidia were stated to germinate if the length of the sprouted tube was more than 3µm and the germination was determined mainly by environmental conditions, such as humidity, temperature, light and nutrition (Suprayogi et al., 2015). *M. brunneum* effective in infecting insect pests, the conidia's environment remains humid, and the fungi do not compete with other microorganisms. According to Desyanti et al., (2007), the requirement for an entomopathogenic fungal isolate to be classified as a bioinsecticide, must be rapidly germinated until it reaches more than 80 %.

Conidia *M. brunneum* infection against the termite *C. curvignathus*

Infection of *M. brunneum* conidia that have entered the stage of destruction on the termite integument of *C. curvignathus* (a) and (b) conidia *M. brunneum* which is shaped like a round oval, and (c) germination of conidia (Figure 1).



(a) (b) (c)

Figure 1. Infection of *M. brunneum* conidia against termite *C. curvignathus* on day 5 (five) observed using Olympus Brand optical microscope (cx21fs1) (a), and (b) conidia *M. brunnuem* as oval round (Magnification 400 X), (c) Germination of *M. brunneum* conidia on termite integument was observed on day 3 using SEM.

Conidia of *M. brunneum* after attaching and penetrating the host integument through the natural opening to form a sprout tube (hypha penetrant), then penetrate the hemocele. Mycelia penetrate the host by secreting protease, lipase, esterase and chitinase enzymes. To destroy the host cuticle, the work of secondary metabolite compounds is essential, such as beauvericin, beauverolid, bassianolid, isarolid and oxalic acid. After entering the hemocele, the secondary hyphae of the fungi invade other tissues such as the fatty tissue, nervous system, trachea, and digestive tract (Figure 1a). According to Sukamto and Yuliantoro, (2006) biochemical changes in hemolymph, especially protein content, nutrient deficiency, the presence of toxic compounds in the form of secondary metabolites released by fungi, result in damage to host tissue or cell paralysis until the host dies.

Conidia of *M. brunneum* are infective propagules that infect a host to become sick and die. The death of the host is thought to be due to the activity of chemical compounds or secondary metabolites in the host's body that become toxic, and the host's tissue systems are compromised (Figure 1b). Fungal conidia infect insect pests through several stages, namely conidia inoculating in the insect pest body, then germinating and penetrating until they invade and colonize in homoseol in the host tissue. The time required at each stage varies and is related to the type of fungi, host and environment. The dosage of application and virulence of the isolates much determine the ability of conidia to produce host insect mortality (Effendy et al., 2010). Conidia that have been in the host's body begin to germinate as regeneration in maintaining their life (Ginting, 2008). The germination of the fungal conidia was seen growing on the host integument (1c). The conidia penetrate and form a sprout tube and then penetrate the integument of the host body. Conidia germination can occur by enabling the hyphae for the uptake of nutrients in the hemocele until conidia proliferation until the stage of

destruction. The process of integument penetration by hyphae is a mechanical and chemical process by releasing enzymes such as proteases, lipases, esterases and chitinases and toxins such as beauvericin, beauverolid, bassianolid, isarolid and oxalic acid which help in destroying insect cuticles (Trizelia, 2005).

Mortality of *C. curvignathus* termites

The average mortality of *C. curvignathus* was observed from 1 to 5 Days After Application (DAA) of *M. brunneum* conidia density resulted in the mortality of *C. curvignathus* termites on each observation day of each treatment (Table 2).

Table 2. Observations of the mean mortality of *C. curvignathus* termites from 1 to 5 days after application of the conidia density of *M. brunneum*

Condia Density/mL	Type of Treatment				
	1 DAA	**2 DAA**	**3DAA**	**4DAA**	**5DAA**
10^3	9.67 cd	34.55 c	44.98 c	53.76 bc	92.5 b
10^5	24.52 bc	34.70 c	60.61 cd	67.25 bc	100 a
10^7	28.22 b	37.65 c	62.12 b	68.39 bc	100 a
10^9	27.84 b	44.98 b	72.08 b	72.08 a	100 a
10^{10}	33.20 a	60.09 a	74.11 a	80.75 a	100 a
Control	0.91 d	0.91 d	0.91 d	0.91 c	10 c
LSD 0.05	26.36	12.30	16.22	20.24	20.25

Information: The numbers followed by the same letter in the same column and row are not significantly different at α 0.05 test LSD. Number (0) of the transformation data using Arc sin√x.

Table 2. The average mortality of *C. curvignathus* termites observed from 1 to 5 days after application of the treatment, the density of 1010 *M. brunneum* / mL aquadest has resulted in 33.20 % mortality at 1 DAA. It is increasing to 100 % in observation 5 DAA. Treatment of conidia density 10^3/ mL distilled water at 5 DAA resulted in 92.5 % mortality which was significantly different from other treatments. The level of mortality of *C. curvignathus* termites after application of the *M. brunneum* conidia density treatment was thought to be related to the size of the conidia application density. Conidia *M. brunneum* begins to infect the host by attaching and penetrating. Entering 3 DAA on the host body appears to grow and germinate mycelia from *M. brunneum* conidia. The morphological condition of the host changes to dry and wrinkled and black. Hyphae appear to grow with a whitish colour on the surface of the host integument, and at this stage, the termites have died. To be able to cause an increase in termite mortality is influenced by environmental factors. Conidia density, application frequency and available hosts are essential things that need to be considered in the use of entomopathogenic fungi as biological agents. The application of conidia density as a biological agent has a positive effect on

producing termite mortality (Desyanti et al., 2007). The higher the frequency of application of *M. brunneum* conidia density, the more secondary metabolic compounds are released, such as destruxin cytochalasins, swainsonine. *M. brunneum* attaches and penetrates the host's cuticle until it grows to the internal part of the host's body (hemocoel) to produce nutrients. The infective conidia of *M. brunneum* damages the physiological system of the host by releasing toxins until the host dies.

Mean Time to Death of *C. curvignathus*

Table 3 shows that the meantime of death of *C. curvignathus* after application of the *M. brunneum* conidia density treatment application with several treatments shows that it significantly affects the time of death of *C. curvignathus* termites in each treatment conidia density (10^3/ mL, 10^5/ mL, 10^7/ mL, 10^9/ mL, 10^{10} / mL).

Table 3. Time of death for termites *C. curvignathus* due to the application of *M. brunneum* with multiple conidia density treatments.

Conidia density/ mL	Time of death of *C. curvignathus* termites
	Days After Application (DAA)
K_1 (10^3)	3.35b
K_2 (10^5)	2.98ab
K_3 (10^7)	2.55a
K_4 (10^9)	2.99ab
K_5 (10^{10})	2.15a
LSD 0.05	0.98

Information: The numbers followed by the same letter are not significantly different at α 0.05 test (LSD).

The mean time to death of *C. curvignathus* after application of M. brunneum conidia density with several treatments showed the fastest death rate against *C. curvignathus* occurred at 2.15 days with a conidia density of 10^{10} / mL aquadest. To produce the death of *C. curvignathus* 3.35 days, conidia density 10^3 / mL aquadest was used. The application of *M. brunneum* conidia density to the termite *C. curvignathus* was found to be related to the time required to produce the death of the termite species. The mortality of *C. curvignathus* was related to the conidia density of each treatment. The higher the application of conidia density, the higher the chemical compounds or secondary metabolites produced by *M. brunneum*, such as destruxin cytochalasins, swainsonine and the presence of *chitinase* and *protease* enzymes. These pathogens penetrate directly into the body parts of termites and germinate until they penetrate the host's cuticle to cover the integument of the host's body.

According to Tantawizal and Prayogo, (2013) entomopathogenic fungal conidia infect the host through several stages, namely conidia attaching and penetrating the host's body, then germinating to invade and destroy by colonizing to cover the host's body. According to Sutopo and Indriyani, (2007), high humidity has a significant effect and is an effective condition for fungal conidia to infect termite pests. The behaviour of termite pests or social interactions between individuals in a colony, such as trophallaxis, cannibalism and grooming, is an opportunity for the biological agent *M. brunneum* to transmit its conidia quickly and precisely to other healthy termites until it becomes infected and dies in the colony (Dwiastuti et al ., 2007).

Percentage of Inhibition eat *C. curvignathus* termites

The results of observations after applying several treatments of *M. brunneum* conidia density showed an effect on the feeding resistance of the termite *C. curvignathus* (Table 4).

Table 4. The mean percentage of *C. curvignathus* termite feeding inhibition observed after the application of several treatments of *M. brunneum* conidia density.

The density of Conidia/ mL Aquadest	Inhibition eat *C. curvignathus* (%)
10^3	41.65a
10^5	43.23a
10^7	48.85b
10^9	50.75b
10^{10}	65.81ab
Control	0.00
LSD. 0.05	3.88

Information: The numbers followed by the same letter are not significantly different at α 0.05 test (LSD).

The results of observing the mean percentage of *C. curvignathus* termite feeding inhibitor in each treatment, *M. brunneum* conidia density showed a significant effect on each treatment and control (Table 4). The highest food inhibition was produced by treatment of 10^{10}/ mL of distilled water which was not significantly different from the treatment of 10^9/ mL of distilled water and 10^7/ mL of distilled water, but substantially additional from 10^5/ mL of distilled water, 10^3/ mL of distilled water and control.

Application of the conidia density of *M. brunneum* affects the activity of the termite *C. curvignathus* in consuming food, and it is suspected that the infection caused by *M. brunneum* is in the host's body parts so that the body's tissue systems are disrupted and sick. This condition can negatively affect the

appetite of C. curvignathus, which is getting lower until it experiences death. The size of the consumption power of *C. curvignathus* in each conidia density treatment is thought to be related to the role of the activity of secondary metabolic compounds produced by conidia from *M. brunneum* that have been contaminated to become infected with *C. curvignathus* termites.

The higher the secondary metabolite content produced by M. brunnuem, the more effective its function as a bioinsecticide. According to Desyanti et al. (2007), the effectiveness of *M. brunneum* as a bioinsecticide is related to the secondary metabolic compounds produced. The more secondary metabolite production it produces, the more effective it will function as a bioinsecticide, this is because of the density and quality of conidia as a determining factor for its effectiveness as a bioinsecticide.

Conclusion

The percentage of average treatment viability of the conidia density of *M. brunneum* at each observation time increased to 89.78 % at 72 hours. 2.15 days of distilled water and 10^3 conidia density treatment/ mL of distilled water took 3.35 days to kill *C. curvignathus* termites. The highest mortality rate for *C. curvignathus* was in the treatment of 10^{10} conidia/ mL of distilled water, reaching 33.20 %, which was observed 1 day after application. The mortality rate increased until it reached 100 % of the 5 DAA observations. The highest food inhibition occurred in treatment 10^{10}/ mL distilled water, namely 65.81 % and significantly different from treatment 10^5/ mL distilled water, namely 43.23 % and 10^3/ mL distilled water (41.61 %) and control (0.00).

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