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Effectiveness Of The Entomopathogenic Fungus *Metarhizium brunneum* Petch As Biotermitisida Against Termite Termites *Coptotermes curvignathus* Holmgren On The Nutmeg Plantation in Aceh Province

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Abstract

Nutmeg is one of flagship commodities in aceh province due to its high economic value. One out of obstacles in nutmeg cultivation is termite attacks that injure the base of nutmeg plant stems. Control of this pest is mainly dependent upon synthetic insecticides. Efforts to control this termite attack are continuously performed such as by studying effects of *M. brunneum* use as environmentally safe biotermiticide. This study aimed to investigate efectivity of *M. brunneum* as biotermiticide on pest termite *C. curvignathus* in nutmeg plantation areas in South Aceh regency by adopting *triple mark recapture technique*. The results showed that 35 colonies of *C. curvignathus* termite were found in 3 different vegatation habitats. Aplication of *M. brunneum* fungi on pest termite *C. curvignathus* by conidia density of 10^9/mL in 250 mL suspension was able to result in mortality rate upto 95.23% or 873,962 *C. curvignathus* individuals were died from initial population size of colony, 917,681 individuals (100%) and 4.77% or 43,719 individuals were still survived.

Keywords: Biotermiticide, entomopathogen, Metarhizium brunneum, C. curvignathus, nutmeg, pest

Background

Nutmeg plant (*Myristica fragrans* Houtt) is one of non-oil and gas export commodities increasing foreign exchange and has high economic value. More than 60% global nutmeg demands are supplied from Indonesia. South Aceh is regency has the second biggest nutmeg production in Indonesia after Moluca Province (Noviar, 2015).

Nutmeg cultivation until now faces many obstacles, one of them is the attack of pest termite that is difficult to detect. Termite infestation is generally noted after a twin tube is formed and attachs to the nutmeg plant stem. Attack of this pest usually injures the stem base and roots of the plant. This condition is assumed to cause reduced absorption of nutrients by roots of the plant from the soil, that inturn result in bad impacts on the ability of the plant to survive from pest and disease attacks (Sayuthi *et al.* 2011).

Infestation of pest termite on a nutmeg plant is predicted to enhance opportunity for other pathogens to enter tissue of the nutmeg plant to cause infection. This condition has resulted in some troubles to nutmeg farmers in the nutmeg plantation areas since the survival of nutmeg plantation of the farmers is directly related to their prosperity (Sayuthi *et al.* 2012).

Control of pest termite so far is done by using synthetic termiticide that might results in pollution on the environment and toxicity to the consumers. Biological agents naturally exist in the nutmeg plantation, especially in Meukek Subdistrict of South Aceh Regency, but do not cause positive impacts on the control of pest termites. Empowerment of these biological agents might require human intervention. Management of species of this pest, however, needs to be studied further. Information about population size of colony is also important to implement effective control technology that can minimize economic loss.

Results of previous study reported that *M. brunneum* has higher pathogenicity and virulence on pest termite *Macrotermes gilvus* in jatropha plantation in Sukabumi, West Java (Sayuthi *et. al.* 2011), and in dan pada penelitian ini di pelajari *mode of action* of *M. brunneum* as biotermiticide on pest species *C. curvignathus*, important pest in the nutmeg plantation in South Aceh Regency of Aceh, was investigated in this study.

Materials and Method

Place and Time

This study was done from January to September 2016 at the Laboratory of Insect Pathology of Department of Plant Protection of IPB, Laboratory of Plant of Plant Pest Study Program of Agricultural Faculty of Syiah Kuala University, and nutmeg plantation areas in Meukek Subdistrict, South Aceh Regency of Aceh have habitat vegetations as the following: 1. Habitat dengan vegetasi beragam (gulma berdaun lebar dan sempit), 2. Habitat dengan vegetasi semak belukar Habitat, 3. dengan vegetasi alang-alang.

Setlement of Observatory Station

Observatory station was assembled by using 3 inch-polivinylchoride (PVC). Wood baits were pine planted in planted in excavated soil with a depth of 15 cm and a diameter of 17 cm. Next, the woods were inserted into each prepared hole in order to separate the soil from the walls of the room formed. Observatory station was then vertically burried in the hole with a depth of 15 cm and 5 cm out of the soil surface. Interval between observation stations varied (5m, 10m, 15m, 20m). Outer part of PVC pipe surface was closed with water strainer fited to the pipe surface. After one month evaluation was done to determine observatory station in the nutmeg plantation attacked by termite pests.

Termite pest *C. curvignathus*

Termite used in this study was *C. curvignathus*, collection of nutmeg plantation of South Aceh Regency of Aceh Province. Identification was done at the Laboratory of Plant Pest of Department of Plant Protection, Faculty of Agiculture, Syiah Kuala University.

Entomopathogen Fungi *M. brunneum*

M. brunneum is an entomopathogen fungi from collection of Laboratory of Insect Pathology of IPB used as biothermicide for managing pest termite at nutmeg plantation of South Aceh Regency.

Prediction of population size of *C. curvignathus colony*

This study adopted *triple mark recapture technique* method (Marini and Ferrari 1998), with the following working steps:

Step I

Baits infected by termite *C. curvignathus* were collected, separated from soils, and subjected for termite number quantification. Quantified termites were feed using tissue paper (Whatman No 1) pre soaked with *neutral red* 0.25% and *nile blue* A 0.05% staining solutions (Sayuthi *et al*. 2012). Tissue papers were given for 3 days until blue and red termites were obtained. The numbers of blue and red termites were quantified before they were returned back to their original observatory stations or termite collections. A week later, all blue and red termites were recollected from observatory stations and quantified.

Step II

Termite collections captured in the step I were restained before released again to their original observatory stations or collections and reevaluated a week later. Time interval between the first and second steps was 10 days.

Step III

In this step all procedures done in the first and second steps were repeated. Population size of termite colonies was predicted using Begon method (Marini dan Ferrari1998):

$N = (\sum M i . n i)/[(\sum m i)+1]$
$SE=N/\{[1/(\sum m i)+1)]+\{(2/((\sum m i)+1)+[(6/(\sum m i)+1)3]\}\}1/2$

Where:
N = population numbers, SE = Standard error,

N = total numbers of termites captured at the collection of i,
m*i*= numbers of marked termites capture at the collection of i,
M= total numbers of marked termites capture at the collection of i

Efectivity of entomopathogen fungi *M. brunnuem* as biotermiticide against *C. curvignathus* termite

Results of study in the laboratory that *M. brunneum* fungi with conidia density of 10^9 conidia/mL (prepared in 250 mL of aquadest) was effective to cause mortality of *C. curvignathus* termite. This density is used as basic reference to be applied in the field by watering observatory station or every colony settled at the habitat in every observatory station. Aplication of *M. brunneum* with this conidia density is carried out once a week for three weeks starting from 17.00 wib until done. Prediction of *M. brunneum* efectivity as biotermiticide against pest termite *C. curvignathus* was done by adopting *triple mark recapture technique* method as the following: (1) capture, (2) mark, and (3) recapturing marked and unmarked termites. Marking, releasing and capturing of *C. curvignathus* were done three times (Begon *et al*. 2005).

$N = (\Sigma\ Mi\ ni) / [(\Sigma\ mi) +1]$
$SE = N\ \{[1/(\Sigma mi + 1)] + [(2/(\Sigma mi + 1)^2]+ [(6/(\Sigma mi +1)^3]\}^{1/2}$

Where:
N = Population size
SE = Deviation standard
n_i = Numbers of termite captured at the i capturing activity
M_i = Total marked termites until the i capturing

Results and Discussion

Estimation of population size of *C. curvignathus* termite pest

1. Habitat with a variety of vegetations (broad and narrow leafy weeds) at nutmeg plantation

Results of observation on habitats had a variety vegetation (Figure 1) showed that total population size of *C. curvignathus* colony at the nutmeg plantation was 489,037 individuals, and from 14 colonies found there was a size difference for every colony population. As shown in Figure 1 there were 80% large colonies and 20% small colonies. Colonies had more than 20,000 individuals were classified large and those have less than 20,000 were clasifed small (Nandika *et al.* 2003).

High percentage of large colonies was assumed related to the number food sources available and the age of *C. curvignathus* termite settled at the location. Other supporting environmental factors were optimum temperature and humidity. These factors altogether facilitate *C. curvignathus* termite to survive in the location. It is predicted that habitat had vegetation variation was optimum for the survival of termites because environmental aspect was very supportive for suvival of termite.

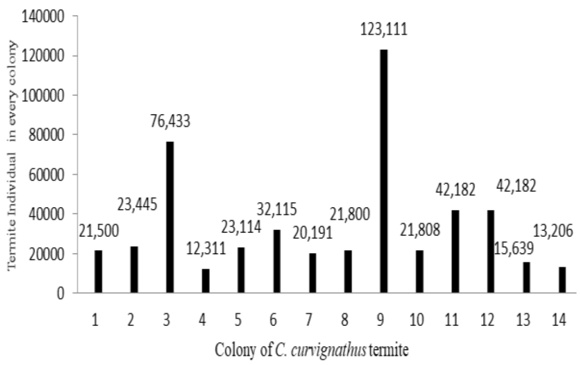

Figure 1. Population size of every *C. curvignathus* colony had a variety vegetation s (broad and narrow leafy weeds) in nutmeg plantations

2. Population size of *C. curvignathus* colony in habitat of shrubs at nutmeg plantation

Figure 2 showed that total population of all colonies presence at habitat with shrubs vegetation were 390,498 individual (100%). There were 7 large colonies (63.64%) and 4 small colonies (36.36%). It was assumed that termites occurred in habitat had shrub vegetations were undergoing growth and development process. These conditions might relate to the availability of supporting factors that have positive impacts on termite population living in the location such as food sources, appropriate humidity and temperature.

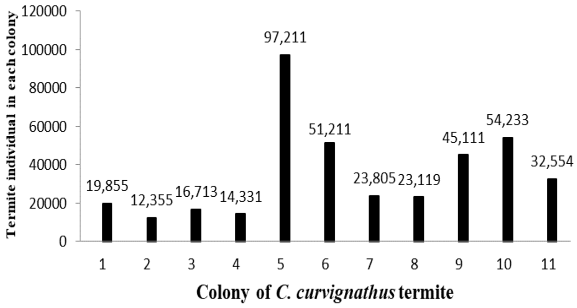

Figure 2. Population sizes of each *C. curvignathus* colony in habitats have shrubs vegetation

This vegetation condition gives opportunity for termite *C. curvignathus* to survive by increasing the size of population under management of queen class termite in order to increase numbers of working class termite to control habitat environment in the location. According to Sayuthi *et. al*. (2012), if environmental condition, especially food sources availability, is effective and optimum, queen class termites manage colony by increasing working class termites to get sufficient foods for the colony. If environmental condition is not conducive for the survival of the colony, queen class termites manage to increase the numbers of soldier class termites to protect the live of colony in normal condition.

3. Population size of *C. curvignathus* colony in habitat of reeds vegetation at nutmeg plantation

Reeds vegetation was dominated by small colony *C. curvignathus* termite (80%) over large colony termite (20%). This situation was predicted related to inopimum environment and insufficient food sources in the habitat. There was a large colony (80,187 individuals) that was predicted outer colony migrate to this location to live. The colony then grew and developed its numbers into a big colony (Figure 3). Control of management activity as done by queen class termites in order to adjust condition of the colony to the new environment, and effort of the queen to strengthen control of the colony by living effectively so that condition of the colony become optimum and sustainable for its survival.

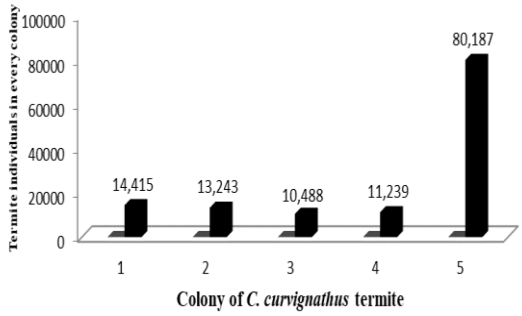

Figure 3. Size population of every *C. curvignathus* colony in reeds vegetation after application of *M. brunneum* as biotermiticide

Reeds vegetation was predicted inoptimum and unefective habitats for the survival of *C. curvignathus* termites. This is related to the observatory results showing that there were only 4 small colonies with total individual termite of 38,146, average number of termite per colony was 9,536.5 individuals. Large colony was only one with population size of 80,187 individuals. This is probably caused by high temperature, low humidity, and limited organic contents in the habitat, all of which bring negative influence on the activity of *C. curvignathus* to survive. According to Nandika *et. al.* (2003), termites prefer low temperature habitats have high organic material contents and humidity. This preference does not mached with the hot, less humid and low nutrient condition of reeds vegetation. Low population size of temite colonies in this vegetation was assumed related to the disinergy of termite habitat with its desired preference, resulting in a low population size of termites species living in the vegetation.

Efectivity of *M. brunneum* as biotermiticide against *C. curvignathus* pest

1. Population size of *C. curvignathus* termite in the different vegetations after application of biotermiticide *M. brunneum.*

Application of entomopathogen fungi *M. brunneun* as biotermiticide with conidia density of 10^9/mL in 250 mL of aquadest in controling *C. curvignathus* termite pest resulted in a reduced population size of colony from initially 489,073 individuals (100%) into 23,083 individuals (4.73 %). The numbers of termite died were 465,990 (95,27%) (Figure 4).

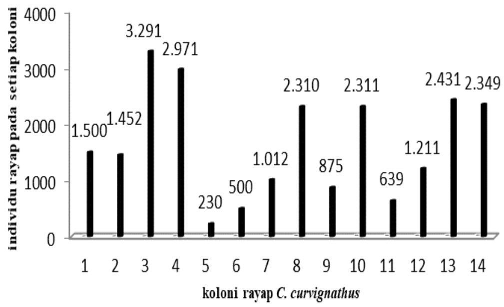

Figure 4. Population sizes of temite colonies in the habitats have different vegetations (boad and narrow leafy weeds) after application of *M. brunneum* biothemicide in the nutmeg plantation.

This condition indicated that management of *C. curvignathus* termite pest by using biologic agent *M. brunneum* a biotermiticide was very effective and did not cause negative impacts on the environment. This is inagreement eith the concept of integrated pest control (IPC) that to guarantee the process of agricultural development by promoting environmental sustainability and ensuring human health. The IPC is an agricultural technology that is friendlier to nature, stabilizes the level of production that has been achieved and increases input efficiency. The IPC concept is a system of controlling pests and plant diseases by using an ecological approach, therefore its implication required adequate understanding of the biology and ecology of pests and diseases (Untung 2006).

2. Population size of *C. curvignathus* termite in the shrubs vegetation after application of biotermiticide *M. brunneum* in nutmeg plantation**.**

Observation results showed that colony population size of *C. curvignathus* temite reduced after the application of *M. brunneum* with conidia density of 10^9/mL in 250 mL of aquadest, from initial size of 10,545 individuals (100%) to 9,545 individuals (90.51%) or there was a loss of colony members upto 1,000 individuals (9.49%)

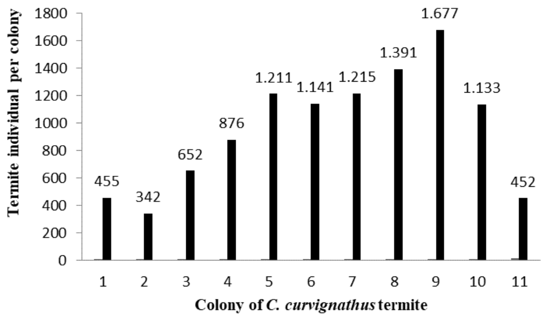

Figure 5. Population size of *C. curvignathus* colony in the habitat with shrubs vegetation after the application *M. brunneum* biothermicide in the nutmeg plantation

This situation was predicted related to effective condition of the habitat with shrubs vegetation for the living of *C. curvignathus* termites due to the availability of food sources required for the optimum growth and development of the termite. It is also assumed that there were a lot of *C. curvignathus* termite colonies in the habitat but they were undetected because they did not visit observatory stations or baits settled during the study. According to Sayuthi *et.al.* (2012), termites stay in certain habitat if environmental condition brings benefits to their live, especially if there is sufficient food souces in the habitat.

3. Population size of *C. curvignathus* colony in the habitat with reeds vegetation after the application *M. brunneum* biothermicide in the nutmeg plantation.

Observation results indicated that after the application of *M. brunneun* with conidia density of 10^9/mL in 250 mL of aquadest by watering on each observatory station settled at habitat had reeds vegetation population size of *C. curvignathus* colony changed from their initial size. This change was predicted related to the mode o action *of M. brunneum* as biotermiticide (Figure 6).
Initial population size of *C. curvignathus* colony was 118,333 individuals in the habitat had reeds vegetation and after

biotermiticide *M. brunneum* application the survival colony population reduced to 11,091 individuals (9.37%). This means that in the colony of *C. curvignathus* termite the population size reduced 107.242 individuals or 90.63% were died. This information showed that *M. brunneum* is an effective biotermiticide against termite pest *C. curvignathus*.

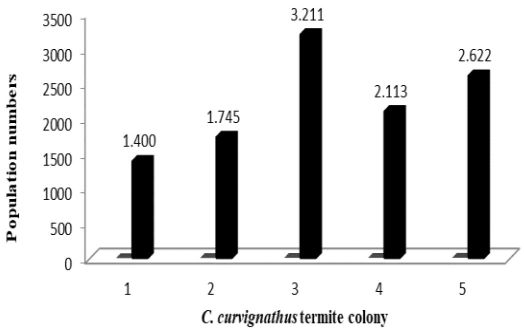

Figure 6. Population size of colony in the habitat with reeds vegetation after application of biotermiticide *M. brunneum* in nutmeg plantation

This is in agreement with results of study by Sayuthi *et. al.* (2011) that *M. brunneum* fungi with conidia density of 1.21×10^6/ mL is able to reduce population size of *M. gilvus* termite colony due to 95.82% of mortality and 4.16 % survival. According to Desyanti (2007), application of *M. brunneum* with conidia density of 1.8×10^5/ mL is able to result in mortality upto 80% on termites compared to other entomophatogen fungi such as *M. anisopliae*, *B. bassiana*, *F. oxysporum* and *A. flavus*.

Conclusion

Tirthy five colonies of *C. curvignathus* termite with initial total population of 917,681 individuals (100%) were found from different habitat vegetations. After the application of *M. brunneum* fungi with conidia density of 10^9/mL the population size of the survival colonies were 43,719 individu (4.77%), and those died were 873,962 individuals (95.23%).

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