Pathogenicity of Entomophatogenic Fungi *Lecanicillium Lecanii* Against Predator Insect *Menochilus Sexmaculatus*

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ABSTRACT

Lecanicillium lecanii is an insect pathogenic fungus that is often used for pest control and has a wide range of hosts. The L. lecanii is capable of infecting several types of host insects including the Order Orthoptera, Hemiptera, Lepidoptera, Thysanoptera and Coleoptera. The extent of this fungus host range was feared to have a negative effect on predator insects Menochilus sexmaculatus. This study aims were to determined the pathogenicity of the fungus L. lecanii against the imago predatory beetle M. sexmaculatus, to know how the predation ability and the number of eggs fecundity of Imago M. sexmaculatus after application L. lecanii. The research was arranged in Randomized Block Design with 4 replications. The conidia density of L. lecanii used were 10^6, 10^7, 10^8, 109 conidia/ml, 1 ml / l of lufenuron insecticide as positive control and sterile distilled water as negative control. The research showed that the mortality percentage of M. sexmaculatus due to L. lecanii application is low and medium. The L. lecanii was not affected for preying ability on imago M. sexmaculatus but gave effect to the number of eggs fecundity of imago M. sexmaculatus.

Keywords: Predator beetle, Biological control, Entomopathogenic Fungus, Lecanicillium

ABSTRAK

Lecanicillium lecanii adalah jamur patogen serangga yang sering digunakan untuk pengendalian hama dan memiliki kisaran inang yang luas. *L. lecanii* dapat menginfeksi beberapa jenis inang dari golongan serangga diantaranya ordo Orthoptera, Hemiptera, Lepidoptera, Thysanoptera, dan Coleoptera. Luasnya kisaran inang jamur ini dikhawatirkan memiliki efek negatif pada serangga predator *Menochilus sexmaculatus*. Penelitian ini bertujuan untuk mengetahui patogenesitas jamur *L. lecanii* terhadap imago kumbang predator *M. sexmaculatus*, mengetahui kemampuan predasi dan jumlah fekunditas telur imago M. sexmaculatus setelah aplikasi *L. lecanii*. Penelitian menggunakan rancangan acak kelompok (RAK) dengan 4 ulangan. Kerapatan kondia *L. lecanii* yang digunakan adalah 10^6, 10^7, 10^8, 10^9 konidia/ml, 1 ml / 1 insektisida lufenuron sebagai kontrol positif, dan air suling steril sebagai kontrol negatif. Hasil menunjukkan bahwa presentase kematian *M. sexmaculatus* akibat aplikasi *L. lecanii* adalah rendah dan sedang. Aplikasi *L. lecanii* tidak mempengaruhi kemampuan *M. sexmaculatus* dalam memangsa, tetapi berpengaruh terhadap jumlah fekunditas telur imago *M. sexmaculatus*.

Kata Kunci: Kumbang predator, Pengendalian hayati, Jamur entomopatogenik, *Lecanicillium*

INTRODUCTION

The use of entomopathogens for insect pest control is now widely introduced to farmers through various programs. It aims to replace synthetic insecticides that are harmful to the environment. One of the most commonly used entomopathogens is *Lecanicillium lecanii* (Zimmerman) which have wide host range (Shinde et al., 2010). *L. lecanii* produces secondary metabolite compounds that are toxic to insects. The secondary metabolite compounds consist of hydrolytic enzymes such as proteases, chitinases and lipases (Hasan, et al., 2013) and toxin compounds such as dipicolinic acid (Claydon and Grove, 1982), vertilecanin-A1, decenedioic acid and 10-hydroxy-8-decenoic acid (Soman et al., 2001). The *L. lecanii* is capable of infecting several insects orders including Orthoptera, Hemiptera, Lepidoptera, Thysanoptera and Coleoptera (Khoiroh, Isnawati, & Faizah, 2014).

The use of *L. lecanii* as a biological control in agroecosystems should be considered to influence beneficial organisms such as predators. The wide range of hosts from this entomopathogen is feared to infect predatory insects. One of the predatory insects potentially infected by *Lecanicillium lecanii*

is *Menochillus sexmaculatus*. M. *sexmaculatus* is one of a kind polyphagous predatory beetle against several insect pests including *Acyrthosiphon pisum* (Harris), *Aphis craccivora* (Koch.), *Aphis fabae* (Theobald), *Aphis gossypii* (Glover), *Aphis ruborum* (Bor), *Myzus persicae* (Sulz), *Rhopalosiphum maidis* (Fitch), *Dialeurodes citri* (Ash), *Diaphorina citri* (Kuw.), and *Tetranychus orientalis* (Mcg) (Irshad, 2001). The wide range of prey of M. *sexmaculatus* makes these predatory beetle found in various agroecosystems of both food crops and horticultural crops (Riyanto et al., 2011).

Wang et al. (2005) reported that the crude toxins have low toxicity against beetle larva of *Delphastus catalinae* (with LC_{50} values of 1942 (1393–2710) and 2471 (1291–4731) p.p.m., respectively (approximately 10- and 12-fold of field rate of application 200 p.p.m.). The adult beetles had less sensitivity to crude toxins with LC_{50} values of 4260 (3376–5375) and 4426 (1734–11298) p.p.m., respectively (approximately 20- and 22-fold of field rate 200 p.p.m.). The consumption and foraging capacity was significantly impaired especially in the second-instar larval beetles which took longer time (more than twice of the control beetles) to consume whitefly eggs after *D. catalinae* exposure to toxins. The study about the impact of *L. lecanii* application on predatory beetle M. *sexmaculatus* has not been widely reported, so further research is needed on the side effects of *L. lecanii* application on M. *sexmaculatus*. The benefit from this research is the information about the impact of application of *L. lecanii* on mortality of adult M. *sexmaculatus*. The preying ability of M. *sexmaculatus* and fecundity of adult M. *sexmaculatus* after application of *L. lecanii*.

MATERIALS AND METHODS

Places and Time

The research was conducted from February to October 2016 at the Pest Laboratory and Biological Control Laboratory of Plant Pest and Disease Department, Faculty of Agriculture, University of Brawijaya.

Research Preparation

Preparation of research is the collection and propagation of predatory beetles M. *sexmaculatus* taken directly from the field of rice crops, corn, beans, and chili. M. *sexmaculatus* obtained was maintained using a cage and fed *Aphis* sp.

Propagation of *L. lecanii* are done on two mediums, solid medium (PDA) and liquid medium (DPE). Propagation of *L. lecanii* on liquid medium using a shaker orbital with 120 Rpm for 48 hours. Isolate *L. lecanii* obtained from the collection of insect pathogens Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya.

Implementation of Research

The pathogenicity test of *L. lecanii* on M. *sexmaculatus* using Randomized Block Design (RBD) 6 treatment with 4 replications. Each treatment there was 5 adults of M. *sexmaculatus* consisting of 2 males and 3 females. A total of 120 adults M. *sexmaculatus* were sprayed by *L. lecanii* suspension with concentration 10^6, 10^7, 10^8, 10^9 conidia/ml, sterile Aquades (negative control) and IGR pesticide with lufenuron as the active ingredient (positive control).

Variable Observations

The Mortality of adult M. sexmaculatus applied by L. lecanii

The observations were made by counting the number of M. *sexmaculatus* died until 7 days after application of *L. lecanii*.

The preying ability of adult M. sexmaculatus

The preying ability of M. *sexmaculatus* was observed by counting the number of prey (*Aphis* sp)

consumed by M. *sexmaculatus* after application of *L. lecanii*. The amount of *Aphis* sp used as the feed was 20. Observations start 1 day after application and were made daily for 7 days after application. The percentage of imago mortality rate was calculated using the formula:

$$P = \frac{x}{y} \times 100\% \quad (1)$$

P is the percentage of the mortality, x is imago die, y is the total number of imago observed. If in the control of mortality occurs greater than 0% and 20% less than the treatment then mortality corrected by the formula (Abbot, 1987) :

$$P = \frac{p'-c}{100-c} \times 100\% \quad (2)$$

P is the percentage of corrected mortality rate, p 'the observed mortality rate on each treatment, and c is the mortality rate in the control.

Fecundity of adult M. sexmaculatus

Fecundity of M. *sexmaculatus* females was observed in insects that did not die in each treatment, observations were made by mating male and female of adult M. *sexmaculatus*. after the copulation occurs, the number of eggs produced first then counted.

Data Analysis

The observed data were analyzed using F test at 5% level. If the response of the treatment significantly different, then proceed with BNT test at 5% error level. Concentration and time of death of M. sexmaculatus imago were analyzed using probit software analysis (Chi, 1997) to calculate *median lethal concentration* (LC_{50}) and *median lethal time* (LT_{50}).

RESULTS AND DISCUSSION

Patogenicity of *L. lecanii* on adult *M. sexmaculatus* Mortality

The adult of M. *sexmaculatus* infected by *L. lecanii* showed symptoms of reduced activity before death. Symptoms that appear after death are the growth of white mycelium on the body of M. *sexmaculatus* incubated for 2 days after death (Figure 1). According to (Barson, 1976) thats reported *Scolytus scolytus* (Coleoptera: Curculionidae) infected by *L. lecanii* softened shortly before death, and changed color from white to pale or yellow cream very pale. The death larvae are covered in white mycelium.

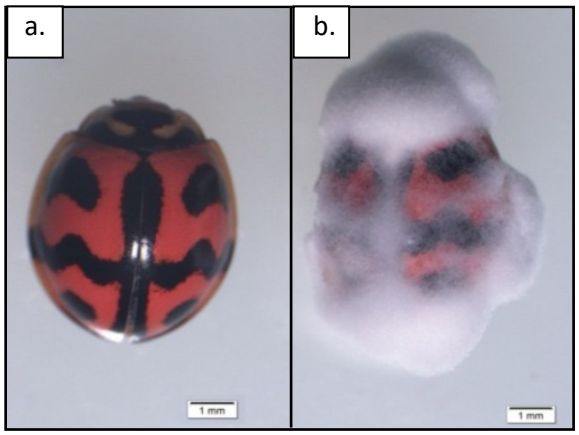

Figure 1. (a) Healthy *M. sexmaculatus* (b) Infected *M. sexmaculatus* by *L. lecanii*

The application of *L. lecanii* with various conidia density did not significantly affect the mortality of M. *sexmaculatus* (Table 1). The *L. lecanii* application was capable of infecting and causing death on adult of M. *sexmaculatus*. However, the mortality of M. *sexmaculatus* based on the classification by (Thungrabeab, Blaeser, & Sengonca, 2006) pathogenicity of entomopathogenic fungi was low and medium, ranging from 15 to 38.75%.

The death of M. *sexmaculatus* suspected due to the influence of secondary metabolite compounds produced by *L. lecanii*. (Claydon and Grove, 1982) and (Soman et al., 2001), states that *L. lecanii* produces secondary metabolite compounds, namely dipicolonic acid, vertilecanin-A1, decenedioic acid and 10-hydroxy-8-decenoic acid which can cause death in some insect pests.

Table 1. Average Mortality of *M. sexmaculatus* 7 days after Application of *L. lecanii*

Treatments	Average mortality of *M. sexmaculatus* (%) ± SE	N
L. lecanii 10^6 conidia/ml	15,00 ± 15,00	20
L. lecanii 10^7 conidia/ml	10,00 ± 05,77	20
L. lecanii 10^8 conidia/ml	22,50 ± 10,31	20
L. lecanii 10^9 conidia/ml	38,75 ± 22,40	20
Positive control (lufenuron 1 ml/l)	25,00 ± 15,00	20

Notes: the data corrected by $P = \frac{p'-c}{100-c} \times 100\%$

the data transformed by $\sqrt{X} + 0.5$
n = total insects observed

Table 2. Median Lethal Time (LT_{50}) *L. lecanii* on Adult *M. sexmaculatus* with Various Density Level of Conidia

Density level (conidia/ml)	Regretion	LT_{50} value (Days after application)
10^6	y = 2,19 + 0,79 x	150,41
10^7	y = -0,67 + 2,08 x	22,32
10^8	y = -1,29 + 2,54 x	12,43
10^9	y = 0,99 + 1,62 x	12,32
Positive control (lufenuron 1 ml/l)	25,00 ± 15,00	20

Notes: Observation conducted until 7 days.

The LC_{50} value of the *L. lecanii* on adult M. *sexmaculatus* mortality was 7.58 x 10^9 conidia/ml. This value indicates that the *L. lecanii* can infect and cause death on adult M. *sexmaculatus* by 50% at the conidia density level of 7.58 x 10^9 conidia/ml. Based on these results, the application of *L. lecanii* does not endanger the population of M. *sexmaculatus*. In general, the density of the *L. lecanii* used by farmers in Indonesia to control insect pests is below 10^8 conidia/ml. However, the application of the *L. lecanii* is advised not to be done too often because of the potential to increase mortality in beneficial insects (Prayogo & Suharsono, 2005).

The higher density of conidia *L. lecanii* applied to adult M. *sexmaculatus* the more rapidly causing the death of adult M. *sexmaculatus*. The density of *L. lecanii* with fastest LT_{50} is at 1 x 10^9 conidia/ml of 12.32 days after application. High conidial concentration affects the speed of penetration on the walls of the insect body to speed up the infection process. (Masyitah et al., 2017) states that insect pathogenic fungi with a greater number of conidia will provide a faster epizootic response to targeted insects.

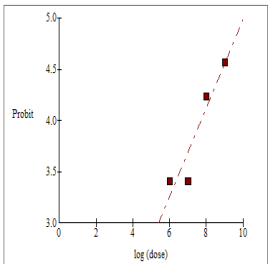

Figure 2. LC_{50} graphic *L. lecanii* on *M. sexmaculatus* Beetle

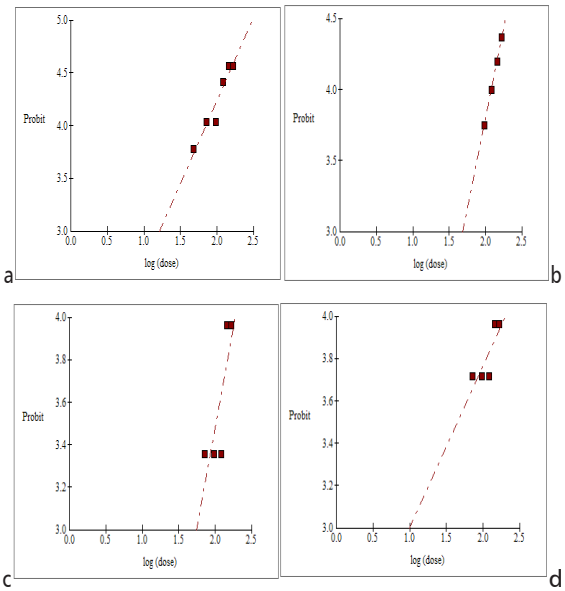

Figure 3. Lethal time graphic of *L. lecanii* with differents concentration on *M. sexmaculatus* Beetle (a) LT_{50} graphic at 10^9 conidia/ml, (b) LT_{50} graphic at 10^8 conidia/ml, (c) LT_{50} graphic at 10^7 conidia/ml, (d) LT_{50} graphic at 10^6 conidia/ml

Table 3. Averages Number of *A. gossypii* that were Preyed by Adult *M. sexmaculatus* until 7 Days After Application

Treatments	Means *Aphids sp* per day ± SE	N
L. lecanii 10^6 conidia/ml	11,67 ± 1,04	17
L. lecanii 10^7 conidia/ml	11,58 ± 0,75	17
L. lecanii 10^8 conidia/ml	12,13 ± 0,89	14
L. lecanii 10^9 conidia/ml	10,49 ± 0,53	12
Positive control (lufenuron 1 ml/l)	11,26 ± 0,75	15
Negative control (Sterill Destilated Water)	11,20 ± 0,97	18

Notes: n = total insects observed.

Table 4. Averages Number of *A. gossypii* that were Preyed by Adult *M. sexmaculatus* until 7 Days After Application

Treatments	Average Number of Eggs + SE	N
L. lecanii 10^6 conidia/ml	4,25 ± 1,44 ab	10
L. lecanii 10^7 conidia/ml	2,00 ± 1,15 a	11
L. lecanii 10^8 conidia/ml	2,00 ± 1,15 a	10
L. lecanii 10^9 conidia/ml	1,00 ± 0,58 a	7
Positive control (lufenuron 1 ml/l)	1,00 ± 0,58 a	9
Negative control (Sterill Destilated Water)	5,25 ± 1,89 b	9

Notes: The number followed by the same letter is not significantly different at the 5% level of the LSD test.

Preying Ability of *M. sexmaculatus*

The application of *L. lecanii* did not have a significant effect on the prey ability of M. *sexmaculatus*. The ability to prey on M. *sexmaculatus* remains high despite the application of the *L. lecanii*, this can be seen from the number of *Aphis* sp. which were preyed on by adult M. *sexmaculatus* showed no difference when compared to controls (Table 3).

Fecundity of adult *M. sexmaculatus*

The application of *L. lecanii* affect on fecundity of adult M. *sexmaculatus*. The higher density level of the *L. lecanii* applied, the number of eggs placed by the female M. *sexmaculatus* decreases. The decrease in the number of eggs placed by M. *sexmaculatus* females reached 80.96% compared with controls. Thats indicates that the application of fungus *L. lecanii* affects the fecundity of M. *sexmaculatus*. (Wang, Huang, You, Guan, & Liu, 2005) also reported that the application of the *L. lecanii* was able to decrease fecundity of the predatory beetle *D. catalinae* (Coleoptera: Coccinellidae).

The decrease number of eggs produced by M. *sexmaculatus* due to the application of *L. lecanii* allegedly because M. *sexmaculatus* lost nutrients in the body that support the process of egg formation. According to (Tanada & Kaya, 1993), after the fungus of the entomopathogen successfully penetrated the cuticle, the hyphae in the insect body will develop and multiply by absorbing the nutrients present in the insect's body. Besides, each insect has a different strategy in dealing with nutritional deficiencies. Especially for the Coccinellidae family the efforts undertaken in the face of nutritional deficiency is to reduce the amount of egg production (Hodek, van Emden, & Honek, 1996).

CONCLUSION

The application of *L. lecanii* at density level 10^6, 10^7, 10^8, 10^9 conidia/ml capable to infecting and causing death on adult of M. *sexmaculatus* with mortality value 15,00, 10,00, 22,50, and 38,75% respectively.The application of *L. lecanii* did not have effect on the prey ability of M. *sexmaculatus*. The application of *L. lecanii* is capable to decrease fecundity of adult M. *sexmaculatus*.

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