



Pathogenicity of Entomopathogenic Fungi as Bioinsecticides for Controlling Green Leaf Hoppers (*Nephotettix virescens*)

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Abstract : Green leaf hoppers (*Nephotettix virescens*) is the main vector of tungro causing virus. Integrated tungro disease control can involve several components at once including the use of entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana*. The purpose of this study was to determine the potential use of *M. anisopliae* and *B. bassiana* fungi on mortality and time of death of green leaf hoppers as a tungro disease vector. This study used four concentrations of conidia, namely 0^7 (A1), 10^8 (A2), 10^9 (A3) and 10^{10} (A4) for *M. anisopliae* and 10^6 (B1), 10^7 (B2), 10^8 (B3) and 10^9 (B4) for *B. bassiana*. The method of application uses three methods: 1) method of insect spray, 2) method of plant spray and 3) method of insect spray on plants. Parameters observed were mortality and time of death at 12, 24, 48 and 72 hours after application. Differences in mortality of green leafhopper at several conidia concentrations, and without treatment (control) were tested with the chi-square model while LT50 with probit analysis. The results showed that the mortality of green leafhopper with *M. anisopliae* (A4) treatment on insect spray method (76.7%), plant spray (50.0%) and insect spray on plants (86.7%), while with treatment *B. bassiana* (B4), mortality in insect spray method (86.7%), plant spray (53.3%) and insect spray method in plants (93.3%) at 72 hours after application. The LT50 value was found in treatment A4 (24.6 hours) and B4 (18.2 hours).

Keywords : Mortality, *Metarhizium anisopliae*, *Beauveria bassiana*, tungro disease.

I. Introduction

Green leaf hopper (*Nephotettix virescens*) is one of the important pests in rice plants, especially in several countries located in southern and Southeast Asia. Green leaf hopper attack rice plants directly by sucking on plant fluids and indirectly act as transmitter (vector) of tungro virus¹.

There are five types of green leafhoppers that can transmit tungro viruses, namely *N. virescens*, *N. nigropictus*, *N. malayanus*, *N. parvus* and *Recilia dorsalis*². Among species of green leafhoppers there is a difference in the efficiency of transmitting the virus. The efficiency range of virus transmission by the population of *N. virescens* in endemic areas (81%) while in non-endemic areas reached 52%³ compared to *N. nigropictus* whose efficiency range was 0.27%⁴. Other species of green leafhoppers such as *N. malayanus* and

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N. parvus have the ability to transmit viruses 40% and 7% respectively^{5,6}. Thus *N. virescens* is the most important vector because of its highest transmission efficiency^{7,8}, and earlier colonies and faster population development^{9,10}.

High and low transmission of tungro disease is closely related to fluctuations in vector populations¹¹ if the source of the virus is available. Thus understanding of population fluctuations and factors that can reduce the population density of green leafhoppers is very important to complement the understanding of epidemiology in order to form a strategy for tungro disease control in an integrated manner.

Control of plant pests in rice plants must consider the technical, ecological, social and economic aspects so that the desired agricultural products are free from chemical pesticide residues^{12,13}. One of the environmentally friendly alternative controls, namely by utilizing biological agents as bioinsecticides^{14,15}.

Biological control is one of the components of integrated pest control that is safe and environmentally friendly. Biological control by utilizing entomopathogenic fungi needs to be increased to reduce the population density of green leafhoppers as spreaders of tungro disease.

The entomopathogenic fungi of the genus *Metarhizium* and *Beauveria* have been tested for their effectiveness in controlling various types of insect pests¹⁶. *B. bassiana* has the potential to kill pest insects from Order Lepidoptera¹⁷, Coleoptera¹⁸, Hemiptera and Homoptera¹⁹, while fungus *Metarhizium* sp. the potential to control the order of Orthoptera²⁰, Lepidoptera^{21,22}, Homoptera²³, and Coleoptera^{24,25}.

This study aims to determine the potential use of *M. anisopliae* and *B. bassiana* on mortality and time of death of green leafhoppers as a tungro disease vector.

II. Material and Methods

1. Insect Preparation

This research was carried out in the greenhouse of the Department of Plant Pest and Disease of the Faculty of Agriculture, Sam Ratulangi University, Manado from March to September 2018. Green leaf hoppers which were obtained from the field were bred in greenhouses by collecting them on rice plants, then put in large gauze cages that already contained rice plants. Imagoes were kept until they lay eggs and hatch, and after the nymph stage was performed the application.

2. Preparation of Rice Plants

Rice seeds from Situ Bagendit variety were sown for one week and then the growing rice plants are transferred to buckets of three rice plants each. Rice plants are treated until they are 40 days old to be ready for use in applications

3. Preparation of Conidial Suspension and Calculation of Conidia Concentration

Preparation of conidial suspension of *M. anisopliae* and *B. bassiana* by pouring 20 ml sterile water on several plates, and removing the conidia on the surface of the media by swiping with a flat and thin ose needle, then filtering and centrifuging at 5000 rpm for 10 minutes so that the conidia settles and collects. Calculate the conidia concentration of this suspension with haemocytometer. Calculation of concentration was carried out until the concentration of conidia was 10¹⁰/ml. In this study using isolates from Balai Perlindungan Tanaman Pangan dan Hortikultura, North Sulawesi Provincethat were ready to be use. The treatments that have been carried out are as follows:

- A1 = 10⁷conidia/mLof *M. anisopliae*
- A2 = 10⁸conidia/mLof *M. anisopliae*
- A3 = 10⁹conidia/ml of *M. anisopliae*
- A4 = 10¹⁰conidia/ml of *M. anisopliae*
- B1 = 10⁶conidia/mLof *B. bassiana*
- B2 = 10⁷conidia/mLof *B. bassiana*
- B3 = 10⁸conidia/mLof *B. bassiana*

B4 = 10^9 conidia/mL of *B. bassiana*

Control (without treatment)

4. Application Method

1. Insect spray method
2. Method of plant spray
3. Method of insect spray on plants

5. Testing of Pathogenicity

Thirty nymphs of green leaf hoppers were placed into rice plants for each treatment with three methods used namely insect spray method, plant spray method and insect spray method on plants. The conidia suspension has been sprayed using a hand sprayer.

The percentage of green leafhopper mortality is calculated using the formula referring to²⁶, namely:

$$M = \frac{\sum n}{\sum N} \times 100 \%$$

Where,

M = mortality (%)

n = the number of green leafhoppers that die from fungal infections

N = the number of green leafhoppers tested

Observations were made at 12, 24, 48 and 72 hours after the application. The half-time of death was observed at 10^{10} conidia/ml conidia concentrations for *M. anisopliae* and 10^9 conidia/ml for *B. bassiana*. Both fungi with these concentrations were applied to the nymphs of green leafhoppers which were kept in maintenance cages separately for each conidia concentration. The half-time of death was estimated by probit analysis²⁶.

III. Results and Discussion

1. Insect Spray Method

The results showed that the application of *M. anisopliae* and *B. bassiana* with insect spray methods at various concentrations was significantly different from mortality of green leafhopper nymphs at 12,24,48 and 72 hours compared to controls (Table 1).

Table 1. Mortality of green leaf hopper nymphs using entomopathogenic fungi (*M. anisopliae* and *B. bassiana*) with insect spray method

Treatments	Mortality (%) at hour:			
	12	24	48	72
A1	6,7*	13,3*	23,3*	33,3*
A2	13,3*	20,0*	40,0*	46,7*
A3	13,3*	23,3*	40,0*	60,0*
A4	20,0*	33,3*	56,7*	76,7*
B1	13,3*	20,0*	30,0*	40,0*
B2	20,0*	33,3*	43,3*	56,7*
B3	26,7*	40,0*	53,3*	73,3*
B4	33,3*	53,3*	60,0*	86,7*
Control	0	0	0	0

*) = $p < 0,05$ chi square test was significant with control

Based on the results of Chi-square analysis of mortality of green leafhopper nymphs, *M. anisopliae* and *B. bassiana* fungi were significantly different from control. This information shows that the two fungi with

insect spray methods effectively suppress the green leafhopper nymph population. At the 72nd observation, mortality was higher in B4 (86.7%) than in A4 treatment (76.7%).

2. Plant Spray Method

The application of *M. anisopliae* and *B. bassiana* by plant spray method was not significantly different between treatments A1 and B1 with controls at the 12th hour observation while treatments A2, A3, A4, B2, B3, and B4 were significantly different from controls (Table 2).

The results of Chi-square analysis showed that with the plant spray method it turned out that the treatment of the use of *M. anisopliae* and *B. bassiana* fungi was less effective in controlling green leafhopper pests. At the 72nd observation, the mortality was treated in A4 (50.0%) while treatment B4 (53.3%). So with the plant spray method the use of both fungi requires a higher concentration to achieve mortality > 50%.

Table 2. Mortality of green leafhopper nymphs using entomopathogenic fungi (*M. anisopliae* and *B. bassiana*) with plant spray method

Treatments	Mortality (%) at hour:			
	12	24	48	72
A1	6,7*	13,3*	23,3*	33,3*
A2	13,3*	20,0*	40,0*	46,7*
A3	13,3*	23,3*	40,0*	60,0*
A4	20,0*	33,3*	56,7*	76,7*
B1	13,3*	20,0*	30,0*	40,0*
B2	20,0*	33,3*	43,3*	56,7*
B3	26,7*	40,0*	53,3*	73,3*
B4	33,3*	53,3*	60,0*	86,7*
Control	0	0	0	0

*) = $p < 0,05$ chi square test was significant with control

3. Insect Spray Method in Plants

The results of the Chi-square analysis showed that the insect spray method on plants showed significant differences between treatment and control at 12, 24, 48, and 72 observations (Table 3).

Table 3. Mortality of green leafhopper nymphs using entomopathogenic fungi (*M. anisopliae* and *B. bassiana*) with Insect Spray Method in Plants

Treatments	Mortality (%) at hour:			
	12	24	48	72
A1	6,7*	20,0*	33,3*	43,3*
A2	13,3*	26,7*	53,3*	63,3*
A3	20,0*	40,0*	63,3*	70,0*
A4	46,7*	56,7*	70,0*	86,7*
B1	16,7*	23,3*	40,0*	53,3*
B2	23,3*	40,0*	53,3*	76,7*
B3	40,0*	56,7*	76,7*	83,3*
B4	53,3*	63,3*	83,3*	93,3*
Kontrol	0	0	0	0

*) = $p < 0,05$ chi square test was significant with control

Observations show that the use of entomopathogenic fungi (*M. anisopliae* and *B. bassiana*) with insect spray methods in plants is very effective in controlling green leaf hopper. With the treatment of A4 at the 24th hour observation, it was able to turn off 56.7% while treatment B4 at the 12th observation turned off 53.3%.

The method of application of insect spray and insect spray on mortality plants is higher than that of the plant spray method because the conidia fungus will be active when it touches the insect's body directly. Conidia that attaches to the insect's body in a suitable microenvironment will germinate which begins with the formation

of a sprout tube. The excellent microenvironment for conidium germination is at 23-25°C and relative humidity is 92 %. The sprout tube will extend through the insect's skin to haemocoel, and develop into hyphae and then follow the blood flow, hyphae spread to all parts of the insect's body. Hyphae develop to form stalks of conidia (blastospores) which emit toxins which cause death of insect cells. Damage to the structure of cell membranes causes cells to lose a lot of water so that the insects die¹⁰.

4. Half Time Mortality

Based on the results of the chi-square analysis of the green leafhopper nymph with a concentration of 10⁴conidia/mL(A4), mortality in the 12th, 24th, 48th, 72nd hours of observation was significantly higher than the control (Figure 1).The results of the analysis with the chi-square of the green leafhopper nymphs with a concentration of 10⁹conidia/mL(B4) showed that the observations at the 12th, 24th, 48th, 72nd hours were significantly higher than the controls (Figure 2).

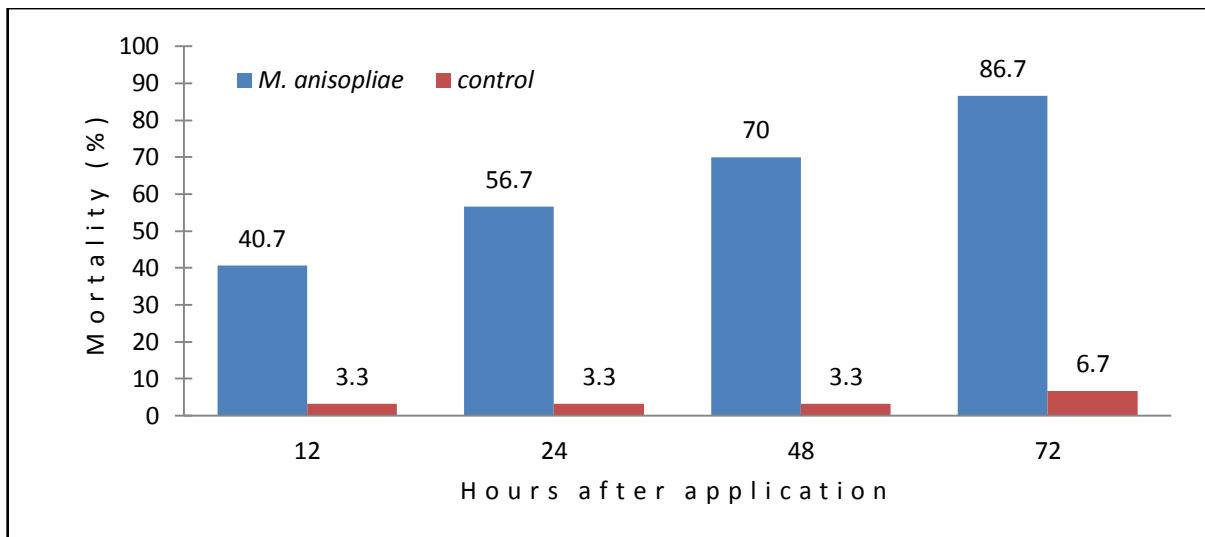


Figure 1. Mortality of green leafhopper in M. anisopliae treatment

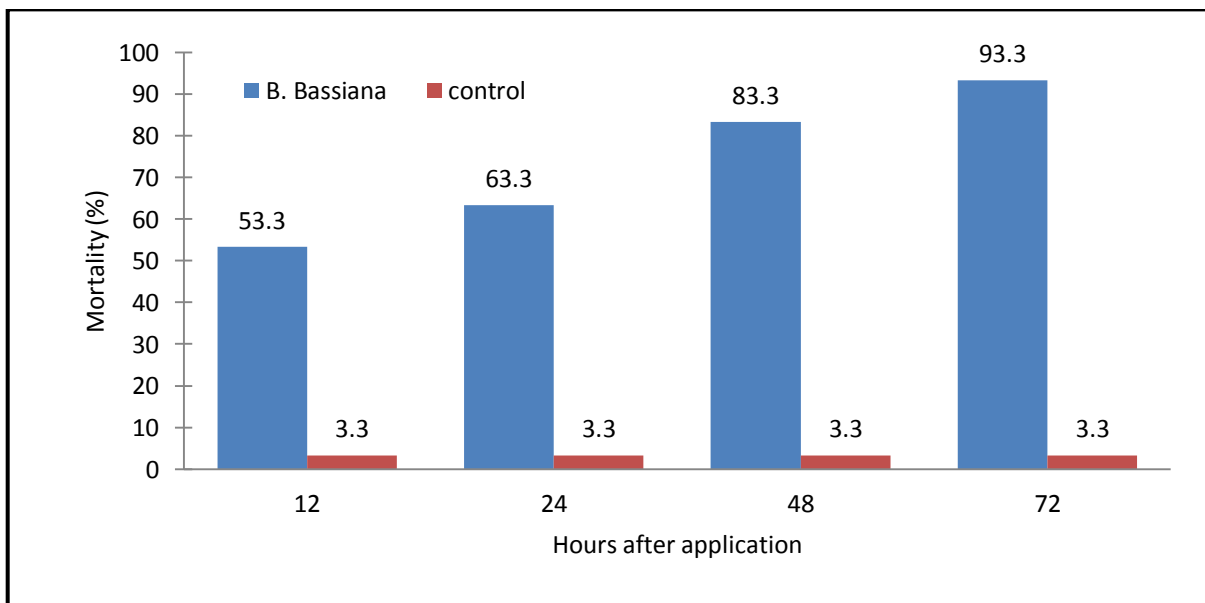


Figure 2. Mortality of green leafhopper in B. bassiana treatment

The results of the analysis show that the higher the concentration of the two entomopathogenic fungi can accelerate the death of the green leafhopper nymph. The results showed that M. anisopliae and B. bassiana were able to cause the death of green leafhopper nymphs starting at the 12th hour after application.

The pathogenicity test of fungus *M. anisopliae* and *B. bassiana* showed that there was an increase in the death rate of green leafhoppers infected with both fungi along with the higher concentration of conidia. The high concentration of conidia applied, increases the chance for conidia to stick, germinate, penetrate and increase the number of hyphae that enter the insect's body, so that more and more blastospores is formed in the insect's body²⁷. Blastospores will spread rapidly throughout the tissues so that it can accelerate the process of tissue damage and increase the point of damage to insect tissue which can cause dead insects^{28,29,30,31}.

Probit analysis showed that LT50 *M. anisopliae* (A4) was achieved at 24.6 hours after application, and *B. bassiana* was achieved at 18.2 hours after application (Table 4). This information has shown that 50% of the death of green leafhoppers by *B. bassiana* was faster than *M. anisopliae*. Both of these fungi may require different times to breed in the body and kill insects. Many factors cause differences in the speed of deadly insects by entomopathogens including temperature, humidity, the amount of conidia (including viability and virulence) that is sprayed, so the possibility of conidia reaching the target is quite a lot^{32,33}.

Table 4. Part Time (LT50) green leafhopper nymphs with *M. anisopliae* and *B. bassiana* treatments

Fungi	Value of T50 (hour)	Upper Limit (hour)	Lower Limit (hour)
<i>M. anisopliae</i> (A4)	24,6	28,8	18,6
<i>B. bassiana</i> (B4)	18,2	22,7	16,6

The pathogenicity of entomopathogenic fungi is also determined by the host stage when the fungus is applied. Insect status is one of the important factors that influence mortality and death time of insects because each stage has a different skin change pattern. Substitution of insect skin influences the effectiveness of entomopathogenic fungi used³⁴. According to³⁵ that young or nymph insects have less active movements than imago so that the chance for animals to stick to the integument is more and the integument layer is thin and soft makes it easier for the fungus to enter the host's body.

IV. Conclusions

1. *M. anisopliae* and *B. bassiana* were effective as insecticides in controlling green leafhopper.
2. The most effective conidia concentration was 10^{10} with a mortality value of 86.7% for *M. anisopliae*, and 10^9 with a mortality value of 93.3% for *B. bassiana* at 72 hours after application
3. The most effective method of application was the method of insect spray on plants
4. LT50 for *M. anisopliae* was 24.6 hours while *B. bassiana* was 18.2 hours.

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