The effectiveness of the entomopathogenic fungus

*Metarhizium anisopliae* in controlling the green leaf hopper

(*Nephotettix virescens*)

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**Abstract.** Green leafhopper (*Nephotettix virescens*) is one of the major pests on rice plants that can be a vector of tungro disease. Current pest control using insecticides by farmers is very worrying because in general it is not as recommended which can cause disturbances in the balance of the ecosystem, so it is necessary to use more environmentally friendly control alternatives, one of which is the use of entomopathogenic fungi that are targeted and do not cause the death of natural enemies. The potential of entomopathogenic fungi as biological control agents needs to be continuously developed to reduce the use of insecticides, one of which is the fungus *Metarhizium anisopliae* which is a type of entomopathogenic fungus that can kill insect pests. The purpose of this study was to determine the effect of several concentration and application method of *M. anisopliae* on the mortality of the green leafhopper (*Nephotettix virescens*). The study was conducted in the Laboratory and Greenhouse of the Tungro Disease Research Station starting from February - April 2020 using a two-factor factorial design in a completely randomized design (CRD). The first factor was the application method consisting of two treatments, namely the spray method (A1) and dip method (A2) while the second factor was the conidia density of *Metarhizium anisopliae* with 4 levels, namely 0 (C0) as control, conidia density 10⁶ (C1) conidia density 10⁷ (C2) conidia density 10⁸ (C3). Each treatment was repeated three times. The results showed that the concentration of 10⁶ by dipping application showed the fastest average death time of green leafhopper imago, which occurred after 4 days of application, while the fastest average death time of green leafhopper nymph was 3.67 days after application at a concentration of 10⁷ by dipping applications.

1. **Introduction**

Green leafhopper (*Nephotettix virescens*) is one of the major pests on rice plants that acts as the main vector causing tungro disease [17]. Yield losses due to tungro disease very depending on the growth period of the plant when infected, the location and point of infection, the growing season and the type of variety planted [16]. The highest yield loss occurred at the infection stage of 2 - 12 weeks after planting (wap), which ranged from 90 - 20% [15].

The control of green leafhoppers by most farmers generally uses insecticides that are not recommended and even exceed the dose, so that it can disrupt the balance of the ecosystem, ecological pollution and the death of non-target insects which can even interfere with human health and the environment [2]. There is a need to develop alternative strategies such as biological control because of
the negative environmental effects of pesticides [14], one of which is by utilizing the potential of entomopathogenic fungi.

The fungus of *M. anisopliae* is an important entomopathogenic fungus which has been a long-standing model for the study of biological control of pest insects by fungi [20]. *M. anisopliae* is the best characterized entomopathogenic fungi and the most widely used in biological control programs [5], amongst several other species described with the specific objective to suppress pest populations by producing enzymes and toxins[12].

The purpose of this study was to determine the effect of several levels of concentration and application method of *M. anisopliae* on the mortality of green leafhoppers.

2. Methodology
This research was carried out in the laboratory and green house. The tungro disease research workshop took place from 3 to 21 April 2020. The study used the entomopathogenic fungus *Metarhizium anisopliae* UGM isolate. The fungal isolates were grown on PDA media (potato dextrose agar) in a petri dish with a diameter of 9 cm. At the age of 21 days after inoculation (dai), the fungal culture was added with 10 ml of water and then the colonies were scraped with a soft brush to collect the conidia. The conidia suspension was shaken using a vortex for 60 seconds then the conidia density was calculated using a haemocytometer to determine the conidia density $10^6, 10^7, 10^8$ and 0 (control) as treatment. The application methods used two methods, namely the dip method and the spray method. In the immersion method, the tested insects were immersed one by one for 5 seconds in a solution of *M. anisopliae* according to the pesticide density concentration. For dip method use grather cages that have been modified. Then the spray method, the test insects were placed in a test tube and then sprayed using a handsprayer. The dead green leafhoppers were isolated on filter paper/moist tissue for approximately 3 days, after which they were transferred into a petri dish containing PDA media and the colony growth was observed.

This study used a two-factor factorial design in a completely randomized design. The first factor was the application methods consisting of 2 treatments, namely the spray method (A1) and the dip method (A2), while the second factor was the conidia density of *Metarhizium anisopliae* UGM isolates with 4 levels, namely 0 (C0) as control, conidia density $10^6$ (C1) conidia density $10^7$ (C2) conidia density $10^8$ (C3). Each treatment was repeated three times. Each treatment combination was applied to adults and green leafhopper nymphs.

Observation parameters were the time of death of insects observed every day until 7 days after application (dda). According to Karmila (2006) in [19] the time of death of insects is calculated using the formula:

$$W = \frac{\sum (m \times h)}{\sum N}$$

Information:

$W$ = Time of death of insects
$m$ = insects that die on day $i$
$h$ = Day $i$ of dead insects
$N$ = All dead insects

The treatments were significant in further tests using LSD at 5% significance level. The data obtained were analyzed using the SAS (Statistical Analysis System) 9.0 for windows program.
3. Results and Discussion

The entomopathogenic fungus of the genus Metarhizium has been tested to control various types of insect pests. According to [6] fungi from the genus Metarhizium can infect and kill green leafhoppers. The results showed that there was an interaction between the application method and the conidia density of *M. anisopliae* and had a significant effect. The fastest day of death for adult green leafhoppers was the dye method with a conidia density of $10^6$ while in the nymph phase the fastest death day was found in the dyeing method with a density of $10^7$. The results of the research by [17] sprayed green leafhoppers with conidia suspension of *M. anisopliae* ($1.4 \times 10^7$ – $1.4 \times 10^9$ conidia ml$^{-1}$) significantly decreased the mobility of green leafhoppers at 3, 7 and 10 days after application.

| Table 1. Time of death of adult and nymph stages in spray and dipping concentrations |
|---------------------------------------------------------------|
| **Treatment** | **Adult** | **Nymph** |
| A1C0 (spray method + M. anisopliae concentration 0) | 2.48 abc | 2.35 b |
| A1C1 (spray method + M. anisopliae concentration $10^6$) | 2.67 ab | 2.55 ab |
| A1C2 (spray method + M. anisopliae concentration $10^7$) | 2.48 abc | 2.35 b |
| A1C3 (spray method + M. anisopliae concentration $10^8$) | 2.79 a | 2.80 a |
| A2C0 (dip method + M. anisopliae concentration 0) | 2.55 abc | 2.33 ab |
| A2C1 (dip method + M. anisopliae concentration $10^6$) | 2.09 c | 2.42 b |
| A2C2 (dip method + M. anisopliae concentration $10^7$) | 2.85 a | 2.00 c |
| A2C3 (dip method + M. anisopliae concentration $10^8$) | 2.22 b | 2.55 ab |
| CV=10.55% | CV=8.05% |

Information:
1. The data has been transformed using $\left(\sqrt{X}+0.5\right)$
2. Numbers followed by letters in the same column mean that they are not significantly different at LSD test level $\alpha=0.05$.

The spray application with a density of $10^8$ gave the longest day of death on the nymph and adult stages of green leafhoppers. This was because the imago and nymphs of the green leafhopper had a very fast movement by flying themselves in case of disturbance. Application by direct spray would disturb the imago or nymph so that it would fly or move to another place that was considered safe.

![Figure 1](image-url)
Figure 2. shows that the dyeing method with a conidia density of $10^6$ caused the death of mature green leafhoppers with an average of 4 days after application. This was because the conidia of *M. anisopliae* at the concentration and method used attach to and enter the insect body tissue. Conidia density and insect mortality have been widely reported. According to [4] to control the nymphal stage of the aphid *Cinara atlantica* (Homoptera: Aphididae), the conidia density of *V. lecanii* used should be $10^8$ /ml. The maximum conidia density of *L. lecanii* to suppressed *R. linearis* egg development was $10^8$ /ml [9]. Differences in sensitivity between insect species to certain bioactive compounds can be caused by differences in the nature of the barrier system for the entry of these compounds into the insect's body and the resistance of the target part or differences in the metabolic ability of insects to decompose and remove toxic substances from their bodies.

Figure 2. Death time of green leafhopper nymphs on various application methods and concentration levels

The dipping application in the nymph phase showed the average mortality on the third day after the application of *Metarhizium* with a concentration of $10^7$ (Figure 2). [1] reported that the density of $10^7$-$10^8$ /ml was able to kill the third instar nymph *Myzus persicae* (Homoptera: Aphididae) up to 100%. [3] controlled of *Aphis gosypii* (Homoptera: Aphididae) at the nymph stage with *V. lecanii* must use conidia density above $10^8$ /ml causing mortality up to 100%. Insect development, age, sex, and size affected the day of targeted insects. According to [10] the difference between immature insects with different ages on bioactive compounds was caused by growth and changes related to the molting process. In the dormant phase (eggs or pupae), anatomic reorganization and changes in metabolism were important factors. Applications of entomopathogenic fungi at the nymph and imago stages need to pay attention to the accuracy of the concentration used because these stages were mobile insects [8].
**Figure 3.** (a) Greenleafhopper imago infected *M. anisopliae* by dipping with concentration of $10^6$ and control; (b) Reisolation of imago greenleafhopper on PDA media and control

Figure 3.a showed that the imago of green leafhoppers which were applied by dipping the entire body surface was dry, rigid, wrinkle and whole body was covered by greenish white mycelium, while the control did no (Figure 3.a.). [13] reported that the insects infected with *M. anisopliae* showed symptoms as follows: insect body was dry and wrinkle, no smell, brittle and outer integument was coated by mycelia having greenish white to dark green. As well as the results of reisolation of imago on PDA media showed that in treatment $10^6$ *M. anisopliae* mycelium grew dark green after incubation of 7 day after inoculation (Figure 3.b.). [11] reported that growth of the fungus *M. anisopliae* was indicated by the presence of white hyphae which are incubated for 5-7 days until dark green spore and conidia form concentric circles around the petri dish.

**Figure 4.** (a) Greenleafhopper nymps infected *M. anisopliae* by dipping with concentration of $10^7$ and control; (b) Reisolation of imago greenleafhopper on PDA media and control

Fig. 4.a. the treatment with a concentration of $10^7$ which applied by spray showed that the nymphs were infected with the fungus and the control treatment did not (Fig.4.a.). [7] reported that spraying application of formulation *M. anisopliae* in 2nd-5th larvae instar of *Spodoptera litura* was high in field application. The results of reisolation of nymphs on PDA media showed that the fungus *M. anisopliae* was overgrown with the treatment at concentration $10^7$ (Fig. 4.b), This is supported by the research of [13] that colony of *M. anisopliae* initially had white color similar to color of *Beauveria bassaiana*, but change into greenish and dark green or dark as fungi become older.

4. **Conclusion**

The fungus of *Metarhizium anisopliae* was a potential biocontrol agent to control green leafhoppers in the adult and nymph stages. Conidia density of $10^6$ with the dye method was the best concentration of
application method in controlling green leafhopper imago, while in nymphs conidia density $10^7$ with the dye method gave the best response on green leafhopper mortality.

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