The mortality of green planthoppers *Nephotettix virescens* (Homoptera: Cicadellidae) by *Metarhizium anisopliae* Metchn and *Beauveria bassiana* (Bals.) Vuill

T Abdullah¹, S N Aminah¹, Fatahuddin¹ and N W Anisaa²

¹Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia
²Magister Student of Phytopathology, Post Graduate Program, Bogor Agricultural University, Bogor 16680, West Java, Indonesia

E-mail: abdullah_journal84@yahoo.com

**Abstract.** The green planthopper *Nephotettix virescens* Distant (Homoptera: Cicadellidae) is one of the important pests of rice plants in South and Southeast Asia. *N. virescens* caused damages to rice plants by sucking rice fresh fluid and vector virus of tungro disease. The purpose of research is to determine the effectiveness of two types, entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*, in suppressing the population level of *N. virescens* on rice plants. The research activities were held at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar, from September 2018 to June 2019. The research used a Factorial, Completely Randomized Design with eight treatments and five replications. Each treatment used ten individual nymphs of *N. virescens*. The research started from the collection and mass rearing of *N. virescens*, prepared the rice plants, cultured of *M. anisopliae* and *B. bassiana*, application of entomopathogenic fungi *M. anisopliae* and *B. bassiana*. The application of *M. anisopliae* and *B. bassiana* on the green planthoppers nymph was showed the average mortality of *N. virescens* by spraying (89.6%), watering (89.6%), and dipping (89.6%) not significantly. However, the mortality was higher compared to the control (0.50%). Watering treatment used the solution of *B. bassiana* showed very high average mortality compared dipping used *M. anisopliae*. The application of *M. anisopliae* in the spraying and watering treatments showed the average mortality not significantly different compared to the spraying treatment used entomopathogenic *B. bassiana*.

1. Introduction

Rice (*Oryza sativa* L.) is the important staple food for Indonesian people stated in Indonesian Government Law Number 7 the year 1996 about food. Rice is a strategic food crop because food derived from rice as raw material contributes 60% of the total calorie required by the Indonesian population. The need for food from the rice was estimated to increase by around 3% per year, related to population growth. Since 2007, the Indonesian Government has targeted increasing national rice production by around 5% per year through the Increasing National Rice Production Program [1].

The annual obstacle conducting rice farming is the presence of important rice pest green planthopper *Nephotettix virescens* Distant (Homoptera: Cicadellidae) [2]. Green leafhoppers knew as destructive pests of rice plants cultivated in several countries located in southern and southeastern
Asia. Green planthopper attacks rice directly by sucking plant fluids and indirectly acts as a transmitter (vector) of the tungro virus. The interaction between green leafhoppers, tungro virus, and rice plants causing tungro disease, an important disease of rice plants based virus activities [3, 4]. There are five types of green leafhoppers that were transmitting the tungro virus: *Nephotettix virescens*, *N. nigropictus*, *N. malayanus*, *N. parvus*, and *Recilia dorsalis*, respectively [5]. *N. virescens* is the most important vector because it has the highest transmission efficiency [6], forming an earlier colony and faster population development [7]. The efficiency of tungro virus transmission by green leafhoppers in endemic areas reaches 81%, while in non-endemic areas it reaches 52% [8].

According to [9, 10] state that the use of biological control agents to control insect pests is the right choice to suppress the use of chemicals in the agricultural sector. Indonesia is a tropical country with high biodiversity that can be maximally utilized to control Plant Pest Organisms. Few organisms in nature as beneficial organisms playing a role as pathogens, parasitoids, and predators of plant pests. The functional relationship between pests and natural enemies will work well if they met several requirements: 1) natural enemies can find a host or prey; 2) minimum population of natural enemies capable of killing the host or prey; 3) synchronization and phenology between natural enemies and host or prey; and 4) always available feed of biological agents to survive.

*Beauveria bassiana* Vuill is one of the natural enemies microorganism, is an entomopathogenic and potential fungi in Integrated Pest Management techniques. The effectiveness of *B. bassiana* as a biological control for a number of insect pests has been proven through various studies [11, 12, 13, 14]. The use of *B. bassiana* in Indonesia to control green leafhoppers still very limited because farmers rely heavily on chemical pesticides. *B. bassiana* is one of the entomopathogenic fungi that is widely used to control various agricultural crop pests. In America, the entomopathogenic fungi *B. bassiana* was found to infect a variety of immature and adult-stage many insects, including mealybugs, aphids, grasshoppers, termites, Colorado potato beetle, Mexican bean beetle, Japanese beetle, boll weevil, and various other insects from various families [15, 16]. According to [17, 18] reported that *B. bassiana* could infect and cause the death of *Aphis* sp., *Bemisia* sp. as well as various types of insects from the orders Coleoptera, Lepidoptera, and Orthoptera.

Another entomopathogenic fungi, *Metarhizium anisopliae*, has been widely used to control insect pests. The entomopathogenic fungi *M. anisopliae* can isolate from infected soil and insects. *M. anisopliae* is able to survive in soil, especially when its propagules come in contact with the susceptible host. In the soil, *M. anisopliae* roles as a saprophyte [19]. The entomopathogenic fungi *M. anisopliae* is able to penetrate into the host's body in the presence of mechanical pressure and the help of toxins released by the fungi. Insects become infected by fungi spores through their cuticles and the gaps between their body segments. The fungi germinate to form a sprout tube that enters the host's body and spread to the haemocoeel tissue. Furthermore, the fungi infect the food tract and respiratory system, causing insect death. Infective spores of entomopathogenic fungi are immediately formed on the outside of the host's body and are ready to be dispered by wind and water [20]. The purpose of the research is to determine the effectiveness of two types of entomopathogenic fungi *M. anisopliae* and *B. bassiana* in suppressing the population level of *N. virescens* on rice plants.

2. Methodology

2.1. Site of research

The research activities were held at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar, from September 2018 to June 2019.

2.2. The research procedures

The research used a factorial, completely randomized design with a total of eight treatments (four treatments for every entomopathogenic fungi) and five replications. Each treatment used ten individual nymphs of *N. virescens*. The research started from the preparation of rice plants and their growing
media, collection and mass rearing of *N. virescens*, cultured of *M. anisopliae* and *B. bassiana*, application of the entomopathogenic fungi *M. anisopliae* and *B. bassiana*.

The treatments for *M. anisopliae* describe as Control of *M. anisopliae* (only rice plant and nymph of green planthoppers without application of entomopathogenic fungi); Soaking of *M. anisopliae*: the rice seeds soaking used solution 8 g spores of *M. anisopliae* mixed well in the 1,000 ml water. Keep in 24 hours then rice seeds planting in the soil; Spraying of *M. anisopliae*: the spraying in the rice plant used solution 8 g spores of *M. anisopliae* mixed well in the 1,000 ml water. Spraying methods used dosage 10 ml/replication in the plant surfaces; Watering of *M. anisopliae*: the watering method used solution 8 g spores of *M. anisopliae* mixed well in the 1,000 ml water. Watering methods are applied to rice plants about 8 – 11 days after planting. The treatments for another entomopathogenic fungi, *B. bassiana*, used similar methods with *M. anisopliae*.

2.3. Growing media for rice seeds
The growing media used in the research as clean soil free from gravel and stones. They are put in the plastic container as the place for rice seeds development.

2.4. Collection and mass rearing of green planthoppers *N. virescens*
The green planthoppers were held in rice fields used insect nets. The insects’ mass rearing on the cages collected nymphs (about 80 individuals of similar ages). Every two weeks, the damaged rice plant substitute with the new plant. The rice plant (height 10 – 20 cm) is the food source and development place of green planthoppers.

2.5. Preparing the rice plant test
The rice seeds used Ciherang variety. Rice seeds cleaning from the stone and not given fungicide treatment. Rice seeds soaked for 24 hours used clean water and cover with a cloth for 48 hours to support the development of rice sprouts. The rice plants used in the treatment age in 15 days after planting. After 15 days after planting, the rice plants were cleaned from the attached soil, then transfered into a plastic container test. The roots of the rice plant are wrapped used wet cotton.

2.6. Preparing entomopathogenic fungi and their application
The isolates entomopathogenic fungi *M. anisopliae* and *B. bassiana* used in the research as the powder form. *M. anisopliae* used a concentration of $10^7$ cfu/ml, *B. bassiana* used a concentration of $4.5 \times 10^{10}$ cfu/ml. Before application, the entomopathogenic spores weighed used analytical tools with a dosage of 0.8 g/100 ml water.

The application of *M. anisopliae* and *B. bassiana* used eight clear plastic containers (height 24 cm, diameter 6 cm) for all treatments. The side of the bottle is given a hole and covered with gauze to allow air exchange. At the bottom of the bottle, given cotton keeping moisture content. In every treatment, used a total of 10 green leafhoppers nymphs into a clear plastic container with 20 rice plants. The application methods in every treatment: the soaking technique used rice seeds have been soaked by *M. anisopliae* and *B. bassiana* for 24 hours. Spraying treatment used solution of *M. anisopliae* and *B. bassiana* in the sprayer and covered surface of the rice plants. Five days later, the rice plants were sprayed again, then watering treatment soil as growing media, rice plant 15 days after planting watering by the solution of *M. anisopliae* and *B. bassiana*. The control treatment contains rice plants and nymphs of green planthoppers. Every treatment has five replication.

2.7. Observation and data analysis
Observation of research activities was held by counting the number of dead insects every day for each treatment, started from the first day after application until one of the treatments reached 100% mortality. Green planthopper mortality was calculated used a formula [6]. The mortality data of green leafhoppers were analyzed used analysis of variance and continued with the LSD test at the level $\alpha = 0.05$. 

3
3. Results and discussions

3.1. Mortality N. virescens caused by M. anisopliae

Result application of entomopathogenic fungi M. anisopliae and B. bassiana to N. virescens were observed for five days. Entomopathogenic M. anisopliae and B. bassiana have four different treatments that observed the mortality rate of N. virescens. All treatments showed M. anisopliae and B. bassiana were able to cause higher mortality than control. The observation percentage of N. virescens mortality in rice after application of entomopathogenic fungi M. anisopliae was showed in figure 1.

![Figure 1](image_url)

Figure 1. Entomopathogenic M. anisopliae caused mortality of green planthopper per days after application.

Based on the results of figure 1, M. anisopliae applied in four treatments, the concentration of $10^7$ in the soaking treatment more effective in reducing mortality by more than 85% because of the higher virulence of the entomopathogenic fungi. According to a statement [11] stated that the virulence ability of entomopathogenic fungi is closely related to several types of toxins production. The ability of M. anisopliae to infect and penetrate the insect's body faster due to the presence of toxins. The mechanism to destroy insect tissue started when spores of M. anisopliae germinates in the cuticle of the host when they infect insects. The next step penetrates used peptidase and chitinase compounds. The enzymes very strongly destroy insect skin and other tissues surround it. After the entomopathogenic fungi invade the host insect, its spores rapidly multiply used nutrition of the host, then the spores immediately cover the insect body. The death of the host occurs due to mycelial colonization through the released toxins. According to [19] reported that six enzyme compounds are released by M. anisopliae, including lipase, kithinase, amylase, proteinase, phosphatase, and esterase.

3.2. Mortality of N. virescens by B. bassiana

The research used two types of entomopathogenic fungi that attacked N. virescens. Besides M. anisopliae, another entomopathogenic such as B. bassiana caused mortality of green planthopper per day after the application was showed in figure 2.
Figure 2. Entomopathogenic *B. bassiana* caused mortality of green planthopper per days after application.

Another entomopathogenic fungi, *B. bassiana*, in figure 2, four different treatments with a concentration of $4.5 \times 10^{10}$ for the soaking treatment more effective, increasing the mortality of *N. virescens* more than 80%. The application of *M. anisopliae* and *B. bassiana* on the green planthoppers nymph was showed the average mortality of *N. virescens* by spraying (89.6%), watering (89.6%), and dipping (89.6%) not significantly. However, the mortality was higher compared to the control (0.50%). Watering treatment used the solution of *B. bassiana* showed very high average mortality compared dipping used *M. anisopliae*. The application of *M. anisopliae* in the spraying and watering treatments showed the average mortality not significantly different compared to the spraying treatment used entomopathogenic *B. bassiana*.

*B. bassiana* releases the enzyme or toxin, namely beauvericin, an antibiotic causing disruption of insect hemolymph function, resulting in swelling and hardening that quickly destroys insect body tissue [20]. Eventually, the mycelia will grow to all parts of the insect's body. According to [17] reported that *B. bassiana* as a biological agent is very effective in infecting several types of insect pests, especially from the orders Lepidoptera, Hemiptera, Homoptera, and Coleoptera. *B. bassiana* is a type of bioinsecticide capable of infecting insects by invading the insect host's body through the skin, digestive tract, spiracles, and other natural holes.

4. Conclusions
The conclusion of the research showed the application of *M. anisopliae* and *B. bassiana* on the green planthoppers nymph treatment by spraying (89.6%), watering (89.6%), and dipping (89.6%) not significantly. However, the mortality was higher compared to the control (0.50%). Watering treatment used the solution of *B. bassiana* showed very high average mortality compared dipping used *M. anisopliae*. The application of *M. anisopliae* in the spraying and watering treatments showed the average mortality not significantly different compared to the spraying treatment used entomopathogenic fungi *B. bassiana*.

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