JPT: Jurnal Proteksi Tanaman (Journal of Plant Protection)
p-ISSN : 2580-0604, e-ISSN: 2621-3141
Accredited: Sinta 3
Publisher: Universitas Andalas, Website: http://jpt.faperta.unand.ac.id/index.php/jpt
Vol. 7 No.2 (2023): 127 – 133

# Virulence of Five Isolates of The Entomopathogenic Fungus, *Metarhizium anisopliae,* Against Brown Planthopper (*Nilaparvata lugens*)

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Received: 11 November 2023 | 1st Revised: 11 December 2023 | Accepted: 14 December 2023 | Published: 15 December 2023

## Abstract

The brown planthopper (*Nilaparvata lugens*) is one of the main pests of rice plants. This pest attack can cause crop failure or puso. Biological control of this pest can be carried out using the entomopathogenic fungus, *Metarhizium anisopliae*. The ability of this fungus to control pests is influenced by the source of the isolate. The purpose of this study was to obtain isolates of *M. anisopliae*, which is virulent to *N. lugens*. This study used an experimental method with a completely randomized design (CRD) in six treatments and five replications. There were five isolates of *M. anisopliae* used and one control. *M. anisopliae* was applied to nymphs and adults of *N. lugens* with a concentration of $10^8$ conidia/mL. The research revealed that all *M. anisopliae* isolates tested were virulent against N. lugens nymphs and adults. The most virulent isolate for the two stages of BPH was Met3B, which was collected from the leek rhizosphere. The isolate caused an adult mortality rate of 52%, nymph mortality of 60% in 10 days after application, and adults formed after application was only 37.33%.

Keywords: Adults, biological control, mortality, nymph, rice

## Introduction

*Nilaparvata lugens* Stal 1854 or brown planthopper (BPH) (Hemiptera: Delphacidae), is Indonesia's primary rice pest (Syahrawati et al., 2019; Iamba and Dono, 2021). This pest damages rice directly by sucking fluid from the stem until the plant dries up and dies. Indirectly, the brown planthopper becomes a vector for spreading grass stunt and grass dwarf diseases caused by viruses. Heavy attacks can cause rice plants to burn and to death (hopperburn) and cause crop failure (Bahagiawat and Rijzaani, 2005; Harini et al., 2013). Yield losses due to attacks of BPH and diseases caused by viruses can reach 70% (Surahmat et al., 2016).

Various efforts have been made to control BPH, such as the use of resistant varieties, but the varieties can only survive for 2–3 seasons. The emergence can break the resistance of rice plants to new biotype BPHs. BPH is a pest with high genetic plasticity and can quickly adapt to existing varieties (Ikeda dan Vanghan, 2004). According to Rugaya

(2013), resistant rice varieties to BPH will be vulnerable if planting is carried out continuously without changing varieties because of a lack of genetic diversity, and his specific abilities were incapable of withstanding attacks from the biotype level known by BPH. Some efforts to control pests using insecticides are also carried out, but they impact environmental pollution and increase the resistance of planthoppers from insecticides.

For this reason, it is necessary to find alternative controls that can reduce the negative impact of insecticides. Integrated Pest Control Technology (IPM) is considered an appropriate and potential technology to BPH, such as exploiting natural enemies as like as predators (Siregar et al., 2023; Utari et al., 2023; Syahrawati et al., 2021a; Syahrawati et al., 2021b; Nasral et al., 2020; Syahrawati et al., 2015), parasitoid (Haryati et al., 2015; Minarni et al., 2018; Abdilah & Susilo, 2020), and entomopathogenic fungus (Trizelia et al., 2023; Hendra et al., 2022a; Hendra et al., 2022b), including *Metarhizium anisopliae*.

*Metarhizium anisopliae* (Ascomycota: Clavicipitaceae) is a facultative fungus, entomopathogenic, soil-inhabiting fungus that has been widely used to suppress a variety of pests in a range of habitats. *M. anisopliae* can infect > 200 species in 17 families of insects and acari worldwide (Zimmermann, 2007). *M. anisopliae* infects susceptible the hosts by penetrating the cuticle directly (Freimoser et al., 2005). The infection process can be divided into: (1) host adhesion and germination, (2) epicuticle degradation, (3) growth as blastospore, (4) killing by various fungal toxins (insecticidal metabolites), (5) immune response as a defense mechanism, and (6) hyphal extrusion and conidiation (Kim et al., 2020).

Research on the potential of *M. anisopliae* as a biological control agent for insect pests has been widely reported. Paradza et al. (2021) reported that *M. anisopliae* can kill the adults of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Adult mortality ranged from 48.15 to 92.89%, depending on the isolate. *M. anisopliae* isolates: ICIPE 18, ICIPE 62, and ICIPE 69 were the three most virulent isolates that resulted in high adult mortalities (82, 91, 93%), with median lethal times (LT50) (5.20, 5.05, 4.78 days, respectively). Zhao et al. (2023) reported that *M. anisopliae* can kill 2$^{nd}$ instar larvae of *Ectropis oblique* (Lepidoptera: Geometridae) by 83% after 13 days after inoculation.

To increase the success of using *M. anisopliae* as a biological control agent for *N. lugens* in the field, it requires isolates or strains that have high virulence, able to kill insect pests quickly, and can survive in agricultural ecosystems. The research aimed to obtain *M. anisopliae* isolates that were virulent against *N. lugens*.

## Methods

The research was carried out at the Biological Control Laboratory, Faculty of Agriculture, Universitas Andalas during July - August 2022.

### Methods

The experiment was arranged in a completely randomized design (CRD) with six treatments and five replications. The treatment consisted of five isolates of *M. anisopliae* and control. The Conidia concentration of each isolate used was $10^8$ conidia/ml. Each experimental unit consisted of 15 individuals of adult and nymph of *N. lugens*, placed on rice plant seeds.

### Preparing *M. anisopliae*

Five isolates of *M. anisopliae* that used in this study were collected from various plant rhizospheres in West Sumatra, Indonesia, and

grown on sabauraud dextrose agar yeast (SDAY) medium in the Biological Control Laboratory, Faculty of Agriculture, Universitas Andalas (Table 1).

Table 1. Isolates used in this study and the place where they were first collected in

| Code | Rhizosphere | Location |
|---|---|---|
| Met3B | Leek | Padang Luar, Agam |
| C51a | Oil palm | Muaro Kiawai, West Pasaman |
| SRJ | Corn | Balai Malintang, Lima Puluh Kota |
| KRJ | Corn | Kampai, Lima Puluh Kota |
| B22C | Oil palm | Muaro Kiawai, West Pasaman |

**Rearing *N. lugens***

BPH was propagated on IR 42 rice seedlings, soaked in water for 24 hours, then air-dried for 24 hours. The seeds were sown into a plastic jar (top d = 20 cm and bottom d= 17 cm, height 20 cm), and enough water was added into that until the seeds were not submerged. The top of the jar was covered with gauze. Rice seeds, aged seven days after sowing, were ready to be used as host for BPH.

Nymphs and adults of BPH used for treatment came from rice field in the Kuranji, Padang, West Sumatra, Indonesia. The insects are caught using an aspirator. BPH were then bred in plastic jars containing vegetative-stage rice plants as a host and a media to lay eggs. Each two days, the rice plants were replaced with new ones. For the obtained BPH stadia to be uniform, approximately three days after infestation, all adults were removed from plastic jars, while rice seedlings were maintained until the eggs hatched into nymphs and subsequently became adults. The BPH used was adults from the 3$^{rd}$ generation.

**Preparing Conidial Suspension**

Isolates of *M anisopliae* were propagated on SDAY medium in petri dishes at 25$^{o}$C for 21 days. Fungal conidia were harvested by adding 10 ml of sterile distilled water and 0.05% Tween 80 as grading material into the petri dish. Conidia were removed from the medium with a fine brush. The suspension was filtered, and the conidia concentration was calculated using a haemocytometer.

**Virulence Test**

Nymphs and adults of *N. lugens* were inoculated with the conidia of *Metarhizium* spp. The Conidia concentration of each isolate used was $10^8$ conidia/ml. Each experimental unit consisted of 15 individuals of BPH, nymphs and adult, placed on rice plant seeds. Nymphs and adults were then sprayed with a suspension of *M. anisopliae* according to treatment. Application of *M. anisopliae* using a hand sprayer with three sprays per experimental unit. Observations were made on nymph and adult mortality, percentage of adults formed, and symptom of infection.

**Variable Observed**

**Adult and nymph mortality**

Adult and nymph of BPH that died after being applied by *M. anisopliae* in each treatment were counted and observed for 10 days. The mortality was calculated using the following formula:

$$\mathrm{M} = \frac{\mathrm{a}}{\mathrm{A}} \mathrm{x}\ 100 \qquad (1)$$

Notes: M = Mortality (%), a = Number of BPH died, A = Number of all BPH treated

**Adult formed**

This observation was done by counting the number of BPH adults formed from each

treatment. The percentage of adults formed is calculated using the formula:

$$F = \frac{d}{N} x\ 100 \quad (2)$$

Notes: F = Adult formed (%), a = Number of adults formed, A = Number of adults treated

**Symptom of infection**

The data were made by observing all changes in BPH nymphs and adults after applying the *M. anisopliae*, such as shape, and color. The observation was started from the beginning of application until the insect died.

**Data Analysis**

The experimental data were processed using analysis of variance at a significance level of 5% and continued with the Least Significant Difference test (LSD). Data were analyzed using the Statistic 8 program.

## Result

**Adults Mortality (%)**

This study showed that all *M. anisopliae* isolates tested were virulent against BPH adults. On the 7 days after application, all *M. anisopliae* isolates caused an increase in BPH adult mortality (18.67 – 37.33%), the same trend was also found 10 days after inoculation (25.33 – 52.00%). The Met3B isolate was able to cause the highest mortality that significantly different with other isolates (Table 1).

Table 3. Adult mortality of BPH after application of *M. anisopliae* with a concentration of $10^8$ conidia/ml

| *M. anisopliae* isolates | Mortality (%) ± SE | |
|---|---|---|
| | 7 days after application | 10 days after application |
| Met3B | 37.33 ± 4.00 a | 52.00 ± 2.49a |
| SRJ | 22.67 ± 2.49 b | 33.33 ± 2.98 b |
| C51a | 18.67 ± 1.33 b | 30.67 ± 2.67 bc |
| KRJ | 21.33 ± 2.49 b | 26.67 ± 2.11 bc |
| B22C | 21.33 ± 2.49 b | 25.33 ± 2.49 c |
| Control | 9.33 ± 1.63 c | 12.00 ± 2.49 d |

**Nymph Mortality (%)**

All *M. anisopliae* isolates were virulent against nymphs. On the 7 days after application, *M. anisopliae* isolates caused an increase in nymph mortality in the range of 32.00 – 40.00%, but the virulence between *M. anisopliae* isolates was not significantly different yet. The effect only became apparent 10 days after inoculation, when there was an increase in mortality. The Met3B and KRJ isolates can cause the highest mortality, reaching 60.00 and 66.67% (Table 2).

Table 2. Mortality of BPH nymphs after *M. anisopliae* application with a concentration of $10^8$ conidia/ml

| *M. anisopliae* isolates | Mortality (%) ± SE | |
|---|---|---|
| | 7 days after inoculation | 10 days after inoculation |
| Met3B | 40.00 ± 3.65 a | 60.00 ± 3.65 a |
| KRJ | 38.67 ± 4.42 a | 66.67 ± 2.98 a |
| SRJ | 34.67 ± 2.49 a | 46.67 ± 3.65 b |
| B22C | 32.00 ± 3.89 a | 44.00 ± 2.67 b |
| C51a | 32.00 ± 2.49 a | 41.33 ± 1.33 b |
| Control | 17.33 ± 1.63 b | 20.00 ± 2.11 c |

**Adult Formed (%)**

Based on the research, the direct application of *M. anisopliae* from various isolate sources to the nymph of BPH has reduced the number of adults formed (32.00 – 77.33%). From the five isolates tested, Met3B and KRJ isolates caused the significantly lowest percentage of adults formed (37.33 and 32.00%) (Table 3).

Table 3. Adult of BPH formed 11 days after inoculation by *M. anisopliae*

| Isolates | Adults formed (%) ± SE |
|---|---|
| Control | 77.33 ± 3.39 a |
| B22C | 56.00 ± 2.67 b |
| C51a | 56.00 ± 1.63 b |
| SRJ | 53.33 ± 3.65 b |
| Met3B | 37.33 ± 4.52 c |
| KRJ | 32.00 ± 2.49 c |

**Symptom of infection**

The *M. anisopliae* isolates caused damage not only at the nymph stage but also at the adult. Some adults of BPH that appeared after the nymphs were treated with *M. anisopliae* were abnormal. An abnormal adult was indicated by less-than-perfect wing formation as if the wings were still wrapped in a membrane that could not be stretched. An abnormal adult grown by *Metarhizium* spp that was occurred after being incubated in a humid room (Figure 1).

a b

Figure 1. a. The healthy of brown planthopper (BPH), b. (A) BPH infected by *M. anisopliae*

**Discussion**

The entomopathogenic fungus, *M. anisopliae*, is considered a potential alternative for controlling *N. lugens* in rice fields. The most important thing during the isolation and screening of entomopathogenic fungi is the selection of virulent isolates. The best isolates often come from target epizootic pests in their natural habitat. In this study, all *M. anisopliae* isolates tested were virulent against nymphs and adults of BPH. *M. anisopliae* causes increased mortality of BPH, adult (Table 1) and nymph (Table 3). It was characterized by the presence of mycelium growing on the nymph's body, initially white and then turns dark green (Figure 1). According to Tanada and Kaya (1993), entomopathogenic fungi can kill insects by releasing toxins, damaging tissue, and causing nutritional deficiencies

The mortality of BPH nymphs and adults after 7 days of application by *M. anisopliae* ranged from 32.00–40.00% and 21.33–37.33%, respectively (Table 1, Table 2). The data indicated that the fungal isolates of *M. anisopliae* used showed low virulence. Perwira et al. (2016) showed that the mortality of *Leptocorisa oratorius* adults after *M. anisopliae* application was also low, ranged from 12.33 - 44.67%. Prayogo and Tengkano (2004) reported that *Spodoptera litura* larvae were exposed to conidia *M. anisopliae* at a concentration of $10^7$ conidia/ml causing mortality of up to 83.33% in 12 days after application. Trizelia et al. (2018) reported that applying *M. anisopliae* Met3B to *Nezara viridula* nymphs could cause up to 100% nymph mortality.

Based on nymph and adult mortality data, the nymph stages of N. lugens are more susceptible to fungal infections than the adult. This is because the nymph integument is softer and thin compared to adults, so *M. anisopliae* infects more easily. Lopes and Alves (2011) stated that adults of *Blattella germanica* (L.) (Blattodea: Blattellidae) were more susceptible to M. anisopliae infection

than nymphs. Molting has been shown to represent a significant component of arthropod resistance to fungal infection, particularly in arthropods with short ecdysis intervals (Ekesi and Maniania, 2000).

The nymph and adult mortalities after application with *M. anisopliae* varied among isolates. There were differences in the mortality of nymphs and adults after applying different fungal isolates, thought to be caused by various virulence factors for each fungus, such as toxin production, enzymes, and conidia germination. This is stated by Tanada and Kaya (1993) (7), which states that there are differences in virulence between fungal isolates due to differences in the ability to produce enzymes and toxins during the infection process in insects. The ability of M. anisopliae to kill insects is strongly influenced by physiological and genetic characteristics (Tiago et al., 2014; Schrank and Vainstein, 2010). Schrank and Vainstein (2010) stated that the virulence factors of the entomopathogenic fungus M. anisopliae are the types of enzymes and toxins produced by this fungus. Differences in the mortality of test insects between isolates of M. anisopliae have also been reported by Lubeck et al. (2008). In bioassays, strains E6, CARO14, CG47, and CG97 were highly lethal to *Rhipicephalus (Boophilus) microplus* ticks and caused 90-100% mortality within four days of treatment; strains Nordeste, CARO11, CARO12, CG27, CG30, CG33, CG87, CG125, CG320 and CG374 were not lethal against *R. microplus*. The source of the isolate influenced the death of *S. litura* larvae. Daoust and Roberts (1982) reported no correlation between the host's origin and the isolates' geography and the virulence of *M. anisopliae* isolates against *Culex pipiens* larvae.

The Met3B and KRJ isolates can cause the highest mortality in BPH nymph, reaching 60.00 and 66.67% 10 days after application (Table 2), and cause the lowest adult formation (Table 3). The source of the isolate influence insect mortality. *M. anisopliae* infects susceptible hosts by penetrating the cuticle directly (Freimoser et al., 2005). The mechanism of infection of the entomopathogenic fungus, *M. anisopliae*, in insects can be divided into: (1) attachment of conidia to the host cuticle through hydrophobic interactions and a thin, slimy substance; (2) germination and conidia development; (3) differentiation of germ tubes into appressoria; (4) cuticle penetration; (5) differentiation of hyphae into blastospores/ hyphal bodies in the hemolymph; (6) host colonization; (7) extrusion onto the surface of the host corpse and (8) formation of conidiophores and production of conidia (Schrank and Vainstein, 2010).

### Conclusion

The research revealed that all *M. anisopliae* isolates tested were virulent against *N. lugens* nymphs and adults. The most virulent isolate for the two stages of BPH was Met3B, which was collected from the leek rhizosphere. The isolate caused an adult mortality rate of 52%, nymph mortality of 60% in 10 days after application, and adults formed after application was only 37.33%.

## Declaration

### Author contribution

Trizelia is the main contributor and corresponding author of this paper. Haliatur Rahma dan My Syahrawati are co-authors. All authors read and approved the final paper.

### Funding statement

This research was financially supported by the research grant (T/8/UN16.19/PT.01.03/ PDKN Pangan/2023) of Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology, Indonesia on behalf of Trizelia.

**Competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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How to cite: Trizelia, H Rahma, and M Syahrawati. 2023. Virulence of five isolates of the entomopathogenic fungus, *Metarhizium anisopliae,* against brown planthopper (*Nilaparvata lugens*). JPT: Jurnal Proteksi Tanaman (Journal of Plant Protection) 7(2): 127 – 135.