BIODIVERSITAS
Volume 24, Number 4, April 2023
Pages: 2384-2392

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240453

Diversity of endophytic fungi of rice plants in Padang City, Indonesia, entomopathogenic to brown planthopper (*Nilaparvata lugens*)

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Manuscript received: 15 March 2023. Revision accepted: 28 April 2023.

Abstract. *Trizelia, Rahma H, Syahrawati M. 2023. Diversity of endophytic fungi of rice plants in Padang City, Indonesia, entomopathogenic to brown planthopper (*Nilaparvata lugens*). Biodiversitas 24: 2384-2392.* Various endophytic fungi can live in plant tissues for all or part of their life cycle without causing damage or disease in their host. Several genera of endophytic fungi have been reported to be entomopathogenic and can be used to control insect pests. This study aimed to determine the diversity of endophytic fungi from rice stems that were entomopathogenic against *Nilaparvata lugens* (Stål, 1854) or Brown Planthopper (BPH). Endophytic fungi were isolated from rice stems of IR42 and Cisokan varieties grown in Padang City, West Sumatra Indonesia. Isolated fungi were first evaluated for their pathogenicity against *Tenebrio molitor* larvae. Subsequently high pathogenicity showed isolates were tested on nymphs and adults of BPH. The results showed that the entomopathogenic fungi isolated from IR 42 and Cisokan were the members of genus *Aspergillus, Trichoderma,* and *Beauveria*. The entomopathogenic fungi from IR 42 were more diverse than Cisokan, and *Beauveria* sp. was the most common isolate and had a better ability to kill the BPH. The highest mortality of BPH was found in PIR 1.3 isolate, but the mortality was still relatively low (53.33% nymphs and 34.99% adults). Further study is needed to increase the effectiveness of these endophytic fungi against BPH directly or indirectly by inducing resistance.

Keywords: *Aspergillus, Beauveria,* insect, mortality, mycosis, pathogenicity, rice, *Trichoderma*

INTRODUCTION

The Brown Planthopper (BPH), *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae), is a primary pest and is recognized as the most destructive insect in rice worldwide. BPH attacks rice plants directly by sucking phloem sap from the leaf sheath and indirectly by dispersing plant viruses (Cheng et al. 2013). Nymphs, brachypteran, and macropteran of BPH suck the sap out, resulting in a color change to yellowish and brownish burned or abnormal growth (Listihani et al. 2022). Furthermore, BPH can also damage rice plants by transmitting Rice Ragged Stunt Virus (RRSV) and Rice Grass Stunt Virus (RGSV). Rice infected with RRSV shows stunted growth and dark-colored leaves, whereas rice infected with RGSV shows pronounced stunted growth, leaves become short, erect, and narrow (Helina et al. 2018) due to nutrient deficiency (Listihani et al. 2022). A BPH attack followed by a virus attack together causes heavy damage and high-yield loss (Helina et al. 2020).

The brown planthopper attack can reduce rice production by about 20-80% and causes an annual economic loss of $300 million in Asia (Min et al. 2014). In Indonesia, *N. lugens* attacks have been reported, and their incidence increases every year (Triwidodo 2020). West Sumatra is one of the provinces in Indonesia also attacked by BPH and has seen an increase in attacks since 2015. The highest number of attacks occurred in 2020, reaching 1,103.56 ha (BPTPH of West Sumatra 2021). Syahrawati et al. (2019) found overlapping generations between nymphs and adults (brachypterous and macropterous, males and females) in rice fields, indicating that BPH has adapted there over a long period of time.

Until now, the farmers in Indonesia still prioritize synthetic insecticides to control the population dan attack level of brown planthopper. Unfortunately, excessive, and continuous use of insecticides leads to environmental imbalance and pollution, reduce biodiversity, and increased the resistance of BPH to various insecticides (Matharu and Tanwar 2020; Lamba and Dono 2021). For this reason, it is necessary to provide more environmentally friendly techniques to reduce pesticides' negative impact. Various techniques have been studied, such as using natural enemies such as predators (Syahrawati et al. 2015; Baehaki 2017; Syahrawati et al. 2021a; Syahrawati et al. 2021b) or using entomopathogens *Metharizium anisopliae* (Metschn.) Sorokīn (Tang et al. 2019) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Suryadi et al. 2018).

Nowadays, *B. bassiana* is not only used as an entomopathogen but also to promote plant growth promoter through its role as endophytic fungi (Mantzoukas et al. 2021; Nchu et al. 2022). Endophytic fungi generally can live in plant tissues, colonize them and infect pests concurrently without damaging that plant. The colonization in plant tissue affects the presence of pests and pathogens (Hilarino et al. 2011; de Andrade et al. 2022). They also can function as soil nutrient distributors, abiotic stress, and drought tolerance enhancers in plants (Bamisile et al. 2018). Using endophytic fungi for pest control is more profitable because of their high reproducibility, specific targets, and short generation time. Endophytic fungi are also classified as saprobic to survive longer without a host (Mantzoukas and Eliopoulos 2020). One endophytic fungus can form tripartite relationships that influence the flow of nutrients between fungi, plants, and pests (Branine et al. 2019). Endophytic fungi were reported as entomopathogens for aphids, thrips, and other cosmopolitan insects, including larvae of lepidopterans (Akutse et al. 2014).

Many researchers have reported the endophytic fungi as pathogenic to insect. Trizelia and Winarto (2016) found three genera of endophytic fungi living in cacao plant tissues that are pathogenic to the insect, such as *Beauveria*, *Aspergillus*, and *Fusarium*. Trizelia et al. (2021) found three endophytic fungi from the genus *Fusarium*, *Aspergillus*, and *Trichoderma* from shallot plants that are pathogenic to insects. Endophytic fungi found in rice tissue are divided into some genera, namely *Fusarium*, *Aspergillus*, *Curvularia*, *Penicillium*, *Gilmaniella*, *Arthrobotrys* (Zakaria et al. 2010), *Metarhizium*, *Trichoderma* (Sopialena et al. 2019), *Beauveria* (Shaalan et al. 2021), and *Nigrospora* (Su-han et al. 2019). According to Lau et al. (2013), some factors that influence the presence of endophytic fungi on plants are the sampling location and variety of rice. There has been no report on the diversity of endophytic fungi in rice plants from Padang City, West Sumatra. This study aimed to know the diversity of endophytic fungi from stems of the rice plant that are pathogenic to insects (entomopathogenic) to control *N. lugens*.

MATERIALS AND METHODS

Endophytic fungi isolation

This research was conducted in of Padang City, West Sumatra, Indonesia. The rice stem samples were taken directly from a farmer's rice field in Pisang Village (0°56'14.23"S; 100°24'16.40"E), Pauh Subdistrict. with an area of about 1 ha, and planted with IR 42 and Cisokan rice varieties, aged 30 days after planting. Five clumps of each variety were selected from the area with best growth as compared to other rice clumps, characterized by higher growth, thicker clumps, and without attacks by pests and diseases. A total of 3 rice stems were chosen from each selected clump, with the criteria of having wider stem and leaf diameters. All rice stems were collected and brought to the Biological Control Laboratory, Faculty of Agriculture, Universitas Andalas. While, brown planthoppers were collected from an IR 42 ricefield in Kuranji Village, Kuranji Subdistrict (0°52’45.0”S, 100°25’25.1”E) and a Cisokan ricefield in Limau Manis Village, Pauh Sub-district (0°55’42.0”S, 100°27’00.0”E) (Figure 1),

All stems were cleaned and then cut into 1 cm size. The stem pieces were sterilized in 70% ethanol for 60 seconds, followed by 3% NaOCl for 60 seconds, and 70% ethanol for 30 seconds. The sterile stem was rinsed four times with sterile distilled water, dried on sterile filter paper, then placed into a petri dish containing Malt Extract Agar (MEA) media, and incubated for one week at room temperature. Fungi obtained from plant tissues were purified on PDA and incubated at room temperature for 15 days.

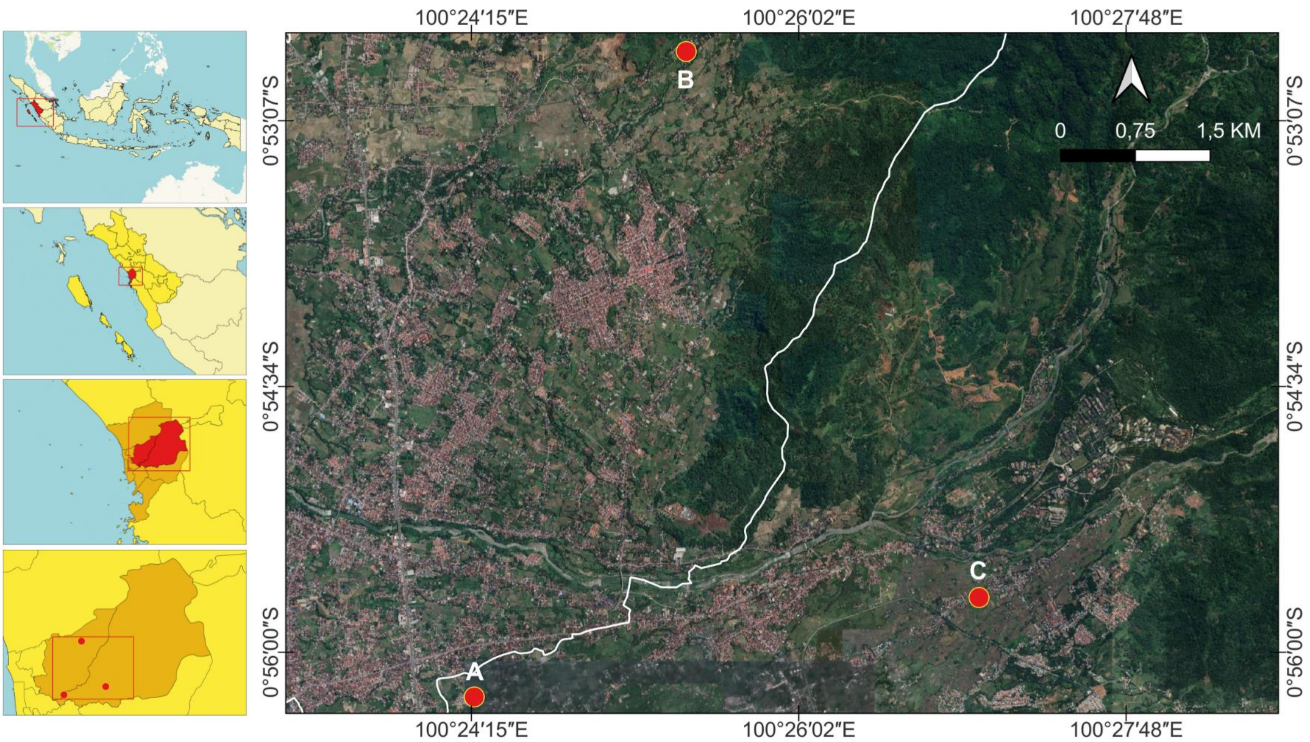

Figure 1. Map of the study site in Padang City, West Sumatra, Indonesia. A. Rice stems sampling location in Pisang Village. B-C. Brown planthoppers collected from Kuranji Village (B) and Limau Manis Village (C).

Pathogenicity test

Pathogenicity test performed on *Tenebrio molitor* Linnaeus, 1758 larvae. Forty *T. molitor* larvae were placed into PDA containing fungal isolate and left to allow contact between conidia and larvae. Besides, for the control treatment, larvae were put into media without endophytic fungi. After 24 hours, all larvae were moved to other petri dishes containing fish pellets. Observations were made on the larval mortality and the number of larvae infected by the fungi one week after application. The dead larvae were put into a petri dish lined with damp tissue and incubated to observe the appearance of fungal conidia on the larval body (mycosis).

Identification of endophytic fungi

Endophytic fungal isolates pathogenic to insects were identified at the genus level on the basis of macroscopic and microscopic structures (Barnett and Hunter 1972; Watanabe 2002). The macroscopic structure was identified by color and shape of conidiophores, and conidia, while macroscopic characteristics were identified by color and shape.

Rearing of *Nilaparvata lugens*

Nilaparvata lugens were reared on the IR42 rice variety. The initial population of brown planthopper (nymphs and adults) was obtained from rice cultivation in the Kuranji and Pauh Subdistricts, Padang City, West Sumatra, Indonesia. The BPH was collected from the rice field using an aspirator, then infested to rice plants aged 1 to 1.5 months at laboratory. BPH rearing was aimed at maintaining the availability of BPH during the study.

Virulence test

Endophytic fungi obtained from the highest mortality of *T. molitor* larvae were tested for virulence against brown planthopper nymphs and adults. The fungus was propagated on SDAY medium in petri dishes at 25°C for 21 days. Fungal conidia were collected by adding 10 mL of sterile distilled water and 0.05% Tween 80 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 hemocytometer.

Nymphs or adults of *N. lugens* were inoculated with a conidial suspension of endophytic fungi. The experiment was arranged in a Completely Randomized Design (CRD) with seven treatments and five replications. The treatment consisted of seven isolates of endophytic fungi and one control. The conidial concentration of each isolate used was 10^8 conidia/mL. Each experimental unit consisted of 15 individuals of BPH. The BPH were sprayed with a conidial suspension of endophytic fungi. Observations on the mortality were carried out every day until seven days after application. Symptoms of fungal infection were also observed in this experiment. The mortality was calculated based on the number of *N. lugens* that died seven days after treatment. Calculations were carried out every 24-hour interval using the following formula Mulyani et al. (2022):

$$M = n/N \times 100\%$$

Where:
M : nymph/adult mortality
n : several dead nymphs or adults
N : a total number of nymphs or adults observed

Statistical analysis

The data (mortality of *T. molitor*, nymph, and adult of *N. lugens*) were analyzed through Analysis of Variance (ANOVA) and continued with the Tukey test (HSD) at a significance level of 5%.

RESULTS AND DISCUSSION

Mortality of *Tenebrio molitor* (one week after application)

Twenty-six isolates of endophytic fungi from rice stems were obtained from both varieties of rice, 20 isolates from IR 42, and 6 isolates from Cisokan. The results showed that not all isolates were pathogenic to larvae of *T. molitor*. Out of 26 isolates, only 14 isolates (53.85%) were entomopathogenic to *T. molitor*, of which 12 isolates were obtained from IR 42. The appearance of symptoms of mycosis on the body of larvae was the presence of infection by the pathogen. The IR 42 variety had a greater diversity of endophytic fungi than the Cisokan. This could possibly be due to the influence of differences in the nutrition given. The IR42 was applied with 0.5 tons/ha of basic compost during tillage process, but the Cisokan was applied with synthetic fertilizers. Various factors can influence the presence of endophytic fungi, including variations in plant varieties, sampling locations, rainfall, and cultivation method (David et al. 2016). The cultivation method is one factor that affects the presence of endophytic fungi (Sopialena et al. 2018). Synthetic fertilizers and pesticides contain ingredients that can affect plant metabolism and the number or variety of endophytic fungi. Reducing the application of synthetic fertilizers and pesticides positively impacts the presence and number of endophytic fungi (Compants et al. 2005).

The mortality of *T. molitor* larvae varied among isolates (Table 1). The analysis of variance showed that endophytic fungal isolate had a significant effect on the mortality of *T. molitor* larvae ($F = 108$; df = 14.59, $P < 0.000$). Endophytic fungi isolated from rice stems can kill *T. molitor* larvae. Larval mortality after the application of endophytic fungi was influenced by body damage due to fungal invasion and the enzymatic activity and toxins produced by these fungi. According to Fingu-Mabola et al. (2021), the mortality of insects due to the application of endophytic fungi is caused by the presence of toxins and secondary metabolites produced by the fungus, which causes the insects to stop consuming (eat repellent) or reduce the appetite of larvae resulting in weight loss (Gustianingtyas et al. 2021).

Based on the mortality data (Table 1), it is known that there were differences in the mortality of *T. molitor* larvae related to the differences in the applied endophytic fungus isolates. Three isolates, namely PIR1.3, PIR 6.2, and PIR 6.3 showed the highest (100%) larval mortality, whereas PIR 2.2 and PIR 3.2 showed 97.5% mortality, which was not different from the three isolates statistically. In contrast, PIR 2.1 and PC 2.1 isolates exhibited the lowest (7.5%) larval mortality, which was not different from PIR 5.3 significantly. Differences in mortality may be due to differences in physiological characteristics between isolates, such as conidial germination, amount of toxins and enzymes produced, and strain of fungus. Endophytic fungi can produce diverse toxins, such as alkaloids, terpenoids, steroids, quinones and flavonoids, phenylpropanoids and lignans, peptides, phenols, phenolic acids, and aliphatic compounds (Tan and Zou 2001). Endophytic fungi can produce metabolites similar to their host plants (Hapida et al. 2021). Furthermore, Ramos et al. (2020) stated that pathogenicity of endophytic fungi in insects depends on the strain of fungus. Gustianingtyas et al. (2021) reported that eight endophytic fungal isolates from corn plants were pathogenic to *Spodoptera frugiperda* J.E.Smith, 1797, with larval mortality ranging from 14.67-29.33%. The entomopathogenic fungus species and conidial concentration significantly influenced the mortality rate of *Galleria mellonella* (Linnaeus, 1758) larvae (Yakubu et al. 2022). Shariari et al. (2021) found that differences in conidial germination, sporulation rates, and extracellular enzyme activity showed of variations in *Chilo suppressalis* Walker, 1863 larval mortality. According to Chang et al. (2021), enzyme levels affect cuticle penetration among isolates from the same species or different species of entomopathogenic fungi showing a positive correlation with the pathogenicity of entomopathogenic fungi.

The growth of entomopathogenic endophytic fungal colonies from rice stems and symptoms of *T. molitor* larvae infection after the application of endophytic fungi can be seen in Figures 2 and 3.

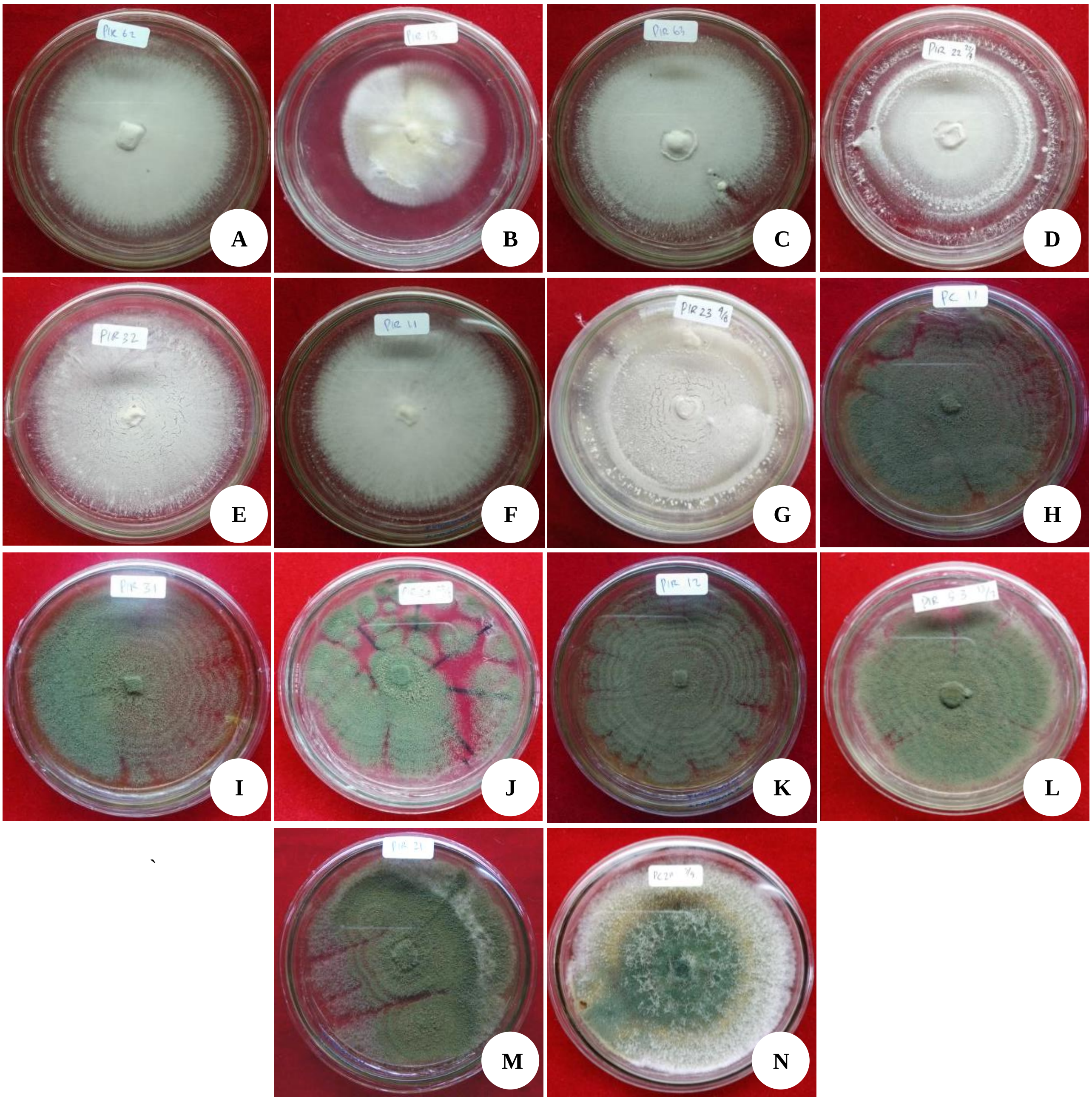

Figure 2. Morphology of entomopathogenic endophytic fungi from rice stems cultured on PDA media: A. PIR 6.2, B. PIR 1.3, C. PIR 6.3, D. PIR 2.2, E. PIR 3.2, F. PIR 1.1, G. PIR 2.3, H. PC 1.1, I. PIR 3.1, J. PIR 2.4, K. PIR 1.2, L. PIR 5.3, M. PIR 2.1, N. PC 2.1

Figure 3. Comparison between healthy *Tenebrio molitor* larvae and those infected with endophytic fungi from rice stems: A. healthy larvae, B and C. infected larvae by different fungi

Entomopathogenic endophytic fungi

Based on the macro and microscopic identification, only three genera of endophytic fungi were found as entomopathogenic on rice stems, namely *Aspergillus, Beauveria*, and *Trichoderma*. This finding is different from some previous studies. Ariyanto et al. (2013) found ten endophytic fungi in rice using Integrated Pest Management (IPM) system, namely *Aspergillus*, *Penicillium, Nigrospora, Trichoderma, Alternaria, Curvularia, Fusarium, Mucor, Mastigosporium*, and *Monosporium*, whereas in rice plants with conventional cropping systems found seven endophytic fungi *Aspergillus, Nigrospora, Curvularia, Trichoderma, Penicillium, Verticillium,* and *Acremonium*. The diversity of endophytic fungi in rice plants is also influenced by different varieties (Roy et al. 2020). The morphological characteristics of each entomopathogenic rice endophytic fungus are described as follows:

Aspergillus sp.

Six isolates, namely PC 1.1, PIR 3.1, PIR 2.4, PIR 1.2, PIR 5.3 and PIR 2.1 from rice stems belonged to genus *Aspergillus*. Macroscopically, the color of colony was grayish green. Microscopically, vesicles were round to oval in shape, insulated hyphae, conidia were rounded with elongated conidiophores (Figure 4).

Aspergillus spp. are primarily saprophytic fungi, found commonly in soils, other organic and inorganic substrates. *Aspergillus* spp. are opportunistic pathogen that requires immunocompromised hosts to establish infection. *Aspergillus* can infect Apis *Mellifera carnica* (Foley et al. 2014). Members of *Aspergillus* was endophytic in chili plants and can kill larvae of *T. molitor* and *Spodotera litura* (Trizelia et al. 2017). The mortality of *S. litura* larvae ranged from 23.34-64.99% depending on the conidial concentration. It can also live endophytes on shallot plants and kill larvae (Trizelia et al. 2021). The genus *Aspergillus* is found associated with *Syzygium aqueum* (Habisukan et al. 2021), and *Syzygium paniculatum* (Ploetz et al. 2009).

Beauveria sp.

A total of seven isolates of endophytic fungi, namely PIR 1.3, PIR 6.2, PIR 6.3, PIR 2.2, PIR 3.2, PIR 1.1 and PIR 2.3 were found to be pathogenic to insects belonging to the genus *Beauveria*. Macroscopic observation showed that fungus had a white colony color. Furthermore, microscopic observations showed that hyphae were hyaline and septate; conidiophores had a zigzag pattern; conidia were round or oval, hyaline, and produced from each branching end of the conidiophores (Figure 5).

Beauveria is an entomopathogenic fungus with the largest number of host species. The primary hosts are insects from the orders Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera, while the soil is the primary habitat (Tanada and Kaya 1993). Besides living in the rhizosphere, *Beauveria* is also reported to live as an endophyte in various types of plants such as corn (Gustianingtyas et al. 2021), cocoa (Trizelia and Winarto 2016), cauliflower (Gautam et al. 2016), coffee (Posada et al. 2007), and wheat (Trizelia et al. 2017).

Trichoderma sp.

Based on macroscopic and microscopic identification, one isolate of endophytic fungi from rice stems belonging to the genus *Trichoderma*. Its colonies were green color. The hyphae were septate; conidiophores were branched and resembled a pyramid at the bottom; conidia were hemispherical and hyaline (Figure 6).

Trichoderma is a fungus often found in soil because it can act as a decomposer, producing organic matter that can absorb plant nutrients. Besides the soil, *Trichoderma* can also live endophytes in plants (Trizelia et al. 2020). *Trichoderma* can also infect *Aedes aegypti* (Linnaeus, 1762) mosquito larvae. The endophytic fungi *Trichoderma* (isolate SZR4 and SZR5) associated with plants can benefit their hosts in controlling pathogens and increasing plant growth (Syarifah et al. 2021). *Trichoderma*, as endophytic fungi, can be associated with the roots of several tropical plants (Cummings et al. 2016).

Mortality of BPH nymphs and adults

The isolates used to test the virulence of entomopathogenic fungi against brown planthopper killed more than 75% of *T. molitor* larvae (Table 1). Seven isolates were isolated from the IR 42 and belonging to the genus *Beauveria*. The virulence test results showed that all isolates of endophytic fungus could kill BPH nymphs and adults (Table 2). Statistical analysis showed that there was a significant difference in the mortality of nymphs ($F=9.32$; $df=7.31$; $P<0.0000$) and adults ($F=10.3$; $df=7.31$; $P<0.0000$) treated with endophytic fungal conidia suspension.

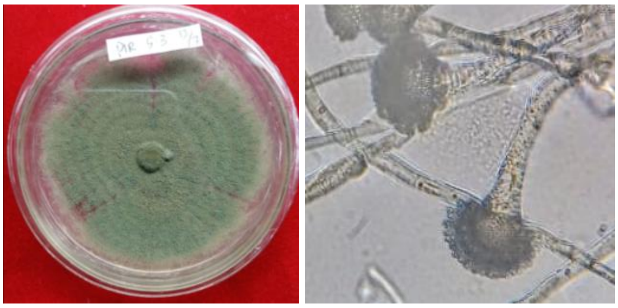

Figure 4. Morphology of *Aspergillus* sp.

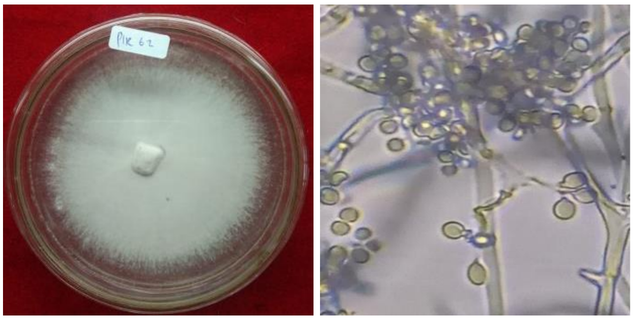

Figure 5. Morphology of *Beauveria* sp.

Figure 6. Morphology of *Trichoderma* sp.

Based on Table 2, PIR 1.3 was the most virulent isolate, with the highest nymph mortality rate of 53.33% during the seven days after fungus application, while PIR 1.1 isolate showed a very low virulence with mortality of 21.67%. Endophytic fungi can also infect BPH adults, but adult mortality was lower than that of nymphs. Adult BPH mortality after inoculating endophytic fungal isolates ranged from 21.67-34.99%. Nymphs of *N. lugens* were more susceptible to endophytic fungal infections than adults, although mortality was lower. Differences in virulence between isolates, both between species and within one species, were common in entomopathogenic fungi. The difference in virulence of 7 *Beauveri*a sp. isolates is assumed due to differences in genetic and physiological characteristics between isolates such as the excretion of extracellular enzymes. Virulent isolates have higher enzyme activity than avirulent (Umaru and Simarani 2022). The virulence of entomopathogenic fungi against insects can be correlated with the activity of extracellular chitinase enzymes (Dhawan and Joshi 2017).

Extracellular enzyme activity is considered an important factor for fungal virulence. Chitinase is an important enzyme for degrading the cuticle polymer of insect chitin. Isolate *B. bassiana* MTCC 4495 had the highest average chitinase activity, resulting in maximum mortality of 3rd instar *Pieris brassicae* (Linnaeus, 1758) larvae. The first stage of fungal infection in insects is the attachment of asexual spores to the surface of host, followed by penetration into living tissue and proliferation within the body cavity. The fungus must evade the animal's immune system for the insect to parasitize successfully and when established, the fungus rapidly kills insects (Vega et al. 2012; Branine et al. 2019).

Nymphs or adults of brown planthopper that died of *B. bassiana* infection were characterized by the presence of white mycelia or conidia on the surface of nymph's body. A day after the insect's death, the white mycelia penetrate the cuticle out of the insect's body, then expand and finally cover the entire body of the insect. The symptoms of endophytic fungal infection in brown planthopper can be seen in Figure 7.

Table 1. Mortality of *Tenebrio molitor* larvae after seven days of endophytic fungus application

Rice varieties	Isolates	Mortality (%)	Mycosis (%)
IR 42	PIR 6.2	100.0 a	100.0
IR 42	PIR 1.3	100.0 a	100.0
IR 42	PIR 6.3	100.0 a	100.0
IR 42	PIR 2.2	97.5 a	94.87
IR 42	PIR 3.2	97.5 a	100.0
IR 42	PIR 1.1	85.0 b	100.0
IR 42	PIR 2.3	77.5 b	100.0
IR 42	PIR 3.1	17.5 cd	100.0
IR 42	PIR 2.4	15.0 cd	100.0
IR 42	PIR 1.2	12.5 cd	100.0
IR 42	PIR 5.3	10.0 cde	100.0
IR 42	PIR 2.1	7.5 de	100.0
Cisokan	PC 1.1	20.0 c	87.5
Cisokan	PC 2.1	7.5 de	100.0
-	Control	0.0 e	0.0

Note: Values within a column followed by the same letter is not significantly different at $P < 0.05$ according to Tukey's HSD test

Table 2. Mortality of nymph and adult of BPH after 7 days of endophytic fungus application from rice stems

Isolates	Mortality (%) ± SE	
	Nymph	Adult
PIR 1.3	53.33 ± 2.72 a	34.99 ± 1.67 a
PIR 6.2	35.00 ± 4.19 b	23.33 ± 1.92 b
PIR 6.3	33.33 ± 2.72 bc	30.00 ± 1.92 ab
PIR 2.2	31.67 ± 3.19 bc	21.67 ± 3.19 b
PIR 2.3	30.00 ± 1.92 bc	28.33 ± 3.19 ab
PIR 3.2	26.67 ± 2.72 bcd	25.00 ± 1.67 ab
PIR 1.1	21.67 ± 1.67 cd	23.33 ± 3.33 b
Control	14.99 ± 1.67 d	8.33 ± 1.67 c

Note: Values within a column followed by the same letter is not significantly different at $P < 0.05$ according to Tukey's HSD test

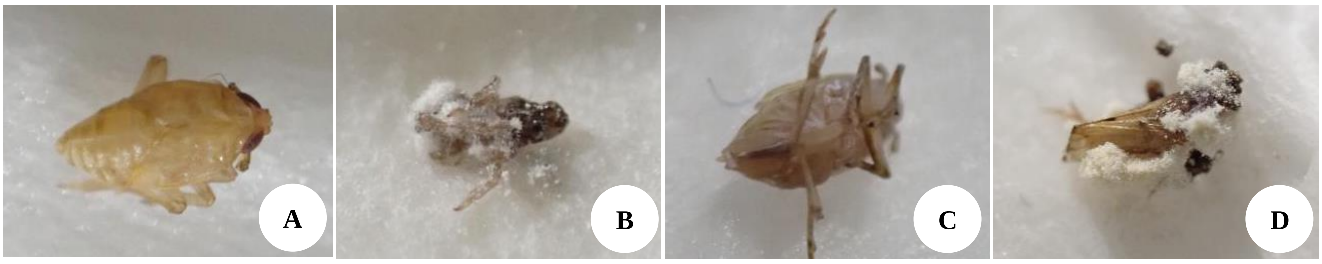

Figure 7. Comparison between brown planthopper (BPH) normal with BPH infected by *Beauveria* sp.: A. Normal nymph, B. Nymph infected by *Beauveria* sp., C. Normal adult, D. Adults infected by *Beauveria* sp.

Generally, the entomopathogenic fungi isolated from the IR 42 and Cisokan rice varieties were the members of genus *Aspergillus*, *Trichoderma*, and *Beauveria*. The entomopathogenic fungi of IR 42 were more diverse than Cisokan, and *Beauveria* was the most common genus found to have better ability to kill brown planthopper. The highest mortality was found in the PIR 1.3 isolate treatment, but the mortality was still relatively low (53.33% of nymphs and 34.99% of adults). For this reason, further research is necessary to enhance the effectiveness of these endophytic fungi against brown planthopper directly or indirectly by inducing resistance.

ACKNOWLEDGEMENTS

This research was financially supported by the research grant (T/78/UN.16.17/ PT.01.03 /PPS-PTMPangan/2022) of the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology, Indonesia, on behalf of Trizelia.

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