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Isolation and Identification of Fungi from *Bactrocera dorsalis* as Candidate Entomopathogenic Fungi

(Isolasi dan Identifikasi Cendawan asal *Bactrocera dorsalis* sebagai Kandidat Cendawan Entomopatogen)

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Abstrak

Pengendalian *Bactrocera* telah dilakukan dengan berbagai cara, mulai dari melindungi buah dengan pembungkus sampai dengan penggunaan insektisida sintetis yang menimbulkan banyak kerugian. Cendawan entomopatogen merupakan kandidat yang berpotensi dijadikan bahan dasar bioinsektisida. Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi cendawan entomopatogen dari *Bactrocera dorsalis.* Penelitian ini dilaksanakan pada bulan Februari – Juli 2022 dengan pengambilan hewan uji di Kebun Percobaan Natar Balai Pengkajian Teknologi Pertanian (BPTP) Lampung sedangkan isolasi dan identifikasi dilakukan lebih lanjut di Laboratorium Mikrobiologi. Metode isolasi cendawan entomopatogen menggunakan metode *moist chamber*. Isolat cendawan yang diperoleh akan diidentifikasi secara makroskopis dan mikroskopis sampai tingkat genus. Hasil yang diperoleh akan dianalisis secara deskriptif dan disajikan dalam bentuk gambar dan tabel. Hasil isolasi menunjukkan terdapat lima jenis cendawan yang teridentifikasi dari tubuh *Bactrocera dorsalis* yaitu, *Nigrospora* sp., *Penicillium* sp. 1, *Phytopthora* sp., *Gliocladium* sp., and *Penicillium* sp. 2. Kelima cendawan tersebut dapat dijadikan sebagai kandidat cendawan entomopatogen.

Kata kunci: Cendawa entomopatoen, Isolasi, Identifikasi, *Bactrocera dorsalis*, Bioinsektisida

Abstract

Bactrocera controlling activities have been done in various ways, ranging from protecting the fruit with wrappers to the use of synthetic insecticides that cause many losses. Overcoming this requires new innovations using biological control. Entomopathogenic fungi are candidates that have the potential to be used as a bioinsecticide base material. This study aims to isolate and identify entomopathogenic fungi from Bactrocera dorsalis. This research was conducted from February to July 2022 with the collection of test animals at the Natar Experimental Farm of the Lampung Agricultural Technology Assessment Center (BPTP) while isolation and identification were further carried out at the Microbiology Laboratory. The entomopathogenic fungus isolation method uses the moist chamber method. The fungal isolates obtained were identified macroscopically and microscopically to the genus level. The results obtained were analyzed descriptively and presented in the form of figures and tables. The isolation results showed that there were five types of fungi identified from the body of Bactrocera dorsalis namely Nigrospora sp., Penicillium sp. 1, Phytopthora sp., Gliocladium sp., and Penicillium sp. 2. The five fungi can be used as candidates for entomopathogenic fungi.

Keywords: *Entomopathogenic fungi, Isolation, Identification, Bactrocera dorsalis, Bioinsecticide*

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INTRODUCTION

Citrus plants are a significant horticultural commodity in Indonesia, known for their high diversity of varieties [1]. Originally from Asia, citrus plants have been cultivated in Indonesia for a long time [2]. Today, citrus fruits are a valuable commodity [3], enjoyed both fresh and processed, making them highly attractive to the public [4]. Lampung is one of the provinces in Indonesia with high citrus production, highlighting the potential for further development in the region [5]. However, increasing citrus production often faces challenges, particularly from plant pest organisms [6]. One of the major pests is the fruit fly of the genus *Bactrocera sp.* [7].

Fruit flies (*Bactrocera sp.*) are notorious worldwide for their ability to attack over a hundred horticultural crops, causing significant losses [8]. Initially, plants attacked by fruit flies display small black dots on the fruit's skin, making the damage hard to detect [9]. Female fruit flies lay eggs in the fruit by making holes with their ovipositor. The eggs then develop into larvae, which damage the fruit flesh, causing it to rot and fall off, leading to crop failure [10].

Various methods have been employed to control fruit fly populations. Farmers often use traditional methods such as wrapping fruits and setting traps [11]. Many also resort to chemical pest control due to its perceived practicality and speed. However, the use of synthetic insecticides has negative impacts, including pest resistance, environmental pollution, and contamination of agricultural products [12]. To address these issues, there is a growing interest in biological control methods using biological agents.

The use of biological agents for integrated pest control is rapidly advancing in Indonesia, especially the use of entomopathogenic fungi as bioinsecticides [13]. These fungi infect insect pests, causing death through toxins and extracellular enzymes they produce [14]. Entomopathogenic fungi are crucial in controlling insect pests, as almost all insect orders are susceptible to fungal diseases [15]. They offer advantages such as natural availability, selectivity, short life cycles, and environmental safety [16]. This study aims to explore and identify entomopathogenic fungi isolated from *Bactrocera dorsalis* through macroscopic and microscopic examination, with the goal of finding suitable candidates for biological pest control.

METHODS

Bactrocera dorsalis Sampling

Bactrocera dorsalis flies used in this study were collected from citrus plants (*Citrus sp.*) at the Natar Experimental Farm of the Lampung Agricultural Technology Assessment Center (BPTP). The flies were captured using attractant traps (Figure 1). The traps were constructed using 1.5-liter mineral water bottles painted yellow, with perforated bottle caps and attached with approximately 50 cm ropes. A hole or window was placed in the center of the bottle for the flies to enter. At the lower end of each rope, a cotton swab infused with methyl eugenol attractant was attached. Each bottle was filled with approximately 200 mL of water and hung or tilted on branches of orange trees at a height of 1.5 meters from the ground [17]. Three such traps were deployed in different trees for replication in this study.

Figure 1. *Bactrocera dorsalis* Imago Trap

Isolation of Entomopathogenic Fungi

Fungi isolation was conducted using the moist chamber method. Dead *Bactrocera dorsalis* fruit flies were placed in a Petri dish with a sterile tissue soaked in sterile distilled water to maintain moisture. The Petri dishes were then incubated at room temperature for 10-14 days [18].

Purification of Entomopathogenic Fungi

Fungal spores growing on the bodies of *Bactrocera dorsalis* insects were collected using a needle and then inoculated by making a single point on the surface of a fresh PDA (Potato Dextrose Agar) medium supplemented with chloramphenicol antibiotics to prevent bacterial contamination. The plates were subsequently incubated for 7-14 days [19].

Identification of Entomopathogenic Fungi

Single isolates of purified fungi will be identified following the method described by Barnett and Hunter [20]. Fungal identification involves observing macroscopic morphological characteristics such as colony color and shape [19]. Additionally, microscopic observations will be conducted using the slide culture method to examine hyphal shape, conidiophores, conidial structure, phialid shape, and the presence or absence of foot cells and vesicles [21].

The results of macroscopic and microscopic morphological identification of fungi obtained will be analyzed descriptively and presented in the form of figures and tables.

RESULT AND DISCUSSION

Isolation of Entomopathogenic Fungi

The installation of fly traps on citrus plants (*Citrus sp.*) at the Natar Experimental Garden of the Lampung Agricultural Technology Assessment Center demonstrated successful trapping of *Bactrocera dorsalis* (Figure 2). The first trap captured two flies, the second trap captured three flies, and the third trap captured two flies. *Bactrocera dorsalis* exhibits distinctive morphological characteristics, including predominantly yellow to brown abdominal segments, a transverse black band on tergites 2 and 3 with black spots on both sides. The thorax features a black scutum, and the wings are colorless with brown to black bands along the costa and a narrow anal line that is short and widens downward [22].

Figure 2. *Bactrocera dorsalis*

The isolation results of entomopathogenic fungi growing on the bodies of *Bactrocera dorsalis* flies (Figure 3) in this study revealed the presence of five entomopathogenic fungus candidates labeled as IB1, IB2, IB3, IB4, and IB5. The IB1 isolate was obtained from the first fruit fly, while IB3 and IB4 were isolated from the same fly (second fruit fly). IB2 and IB5 were isolated from the third fly.

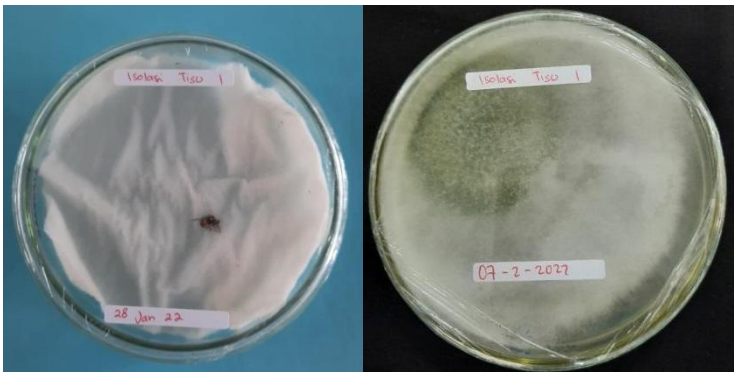

Figure 3. Isolation by Moist Chamber Method

Identification of Entomopathogenic Fungi

Each isolated entomopathogenic fungus was observed for macroscopic characteristics, which are presented in Table 1. These characteristics include colony diameter, colony color, and colony shape.

Table 1. Macroscopic Characteristics of Entomopathogenic Fungi Isolates

Isolate code	Average colony diameter on day 7 (cm)	Colony Color	Colony Shape
IB1	6,6	White	Round
IB2	2,3	Green	Round
IB3	9	White	filled the petri dish
IB4	9	greenish white	filled the petri dish
IB5	3,6	Green	Round

Description : IB = Fungi isolate from *Bactrocera dorsalis*

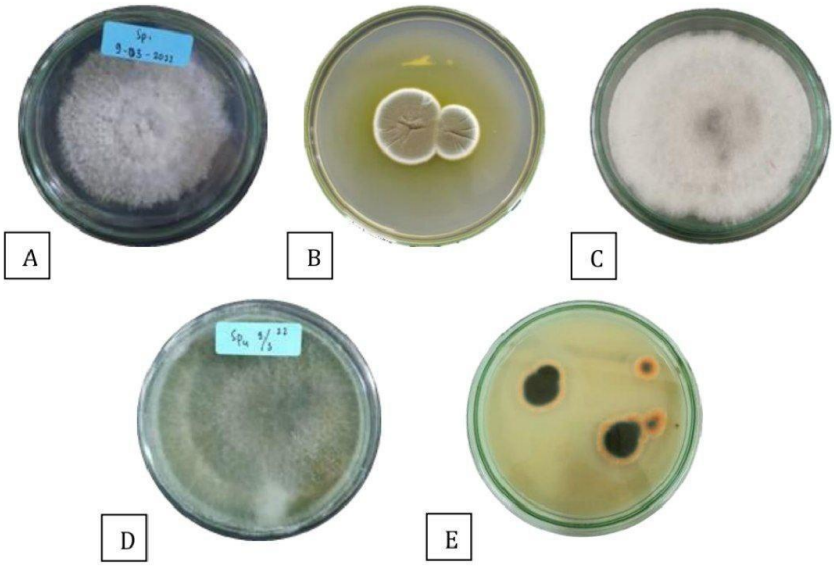

Figure 4. Colonies of Entomopathogenic Fungi Isolates on PDA Media Day 7.
(A) IB1 (B) IB2 (C) IB3 (D) IB4 (E) IB5

The macroscopic observations of each isolate colony revealed round shapes with distinct characteristics. Isolate IB1 appeared white with a powdery surface texture, while IB2 exhibited green colonies with white edges. Similarly, IB3 was white but had a cotton-like surface texture, and IB4 displayed white colonies with a slight green hue. IB5 appeared green with orange edges. Among the isolates, IB3 and IB4 showed rapid growth on PDA media, completely filling the Petri dish within 7 days of incubation with an average colony diameter of 9 cm. In contrast, IB1 had an average diameter of 6.6 cm, IB5 of 3.6 cm, and IB2 of 2.3 cm.

The differences in isolate characteristics may be attributed to both external and internal factors. External factors include pH, temperature, humidity, environment, growth media, and nutrients, while internal factors stem from fungal genetics [23]. Although external factors were controlled on a laboratory scale in this study, variations such as colony color persisted among the isolates. This observation aligns with Apriliani [24], indicating that fungal isolates grown under controlled conditions can still exhibit diverse characteristics.

Microscopic observations of isolates aged 7-10 days revealed distinct differences in fungal genera. Microscopic characteristics observed included hyphal shape, conidiophores, foot cells, vesicles, phialids, and conidial size, as detailed in Table 2. According to Barnett and Hunter [20], four fungal genera were successfully isolated: *Nigrospora sp.* (IB1), *Penicillium sp.* (IB2 and IB5), *Phytophthora sp.* (IB3), and *Gliocladium sp.* (IB4).

Table 2. Microscopic Characteristics of Entomopathogenic Fungus Isolates

Isolate code	**Hypha**	**Conidiophores/sporangiophores**	**Leg Cells**	**Vesicles**	**Fialid**	**Conclusion (Genus)**
IB1	Septate	Simple	Not available	Not available	Not available	*Nigrospora* sp.
IB2	Septate	Branched	Not available	Not available	Single	*Penicillium* sp. 1
IB3	Aseptate	Simple	Not available	Not available	Not available	*Phytopthora* sp.
IB4	Septate	Branched	Not available	Not available	Group	*Gliocladium* sp.
IB5	Septate	Branched	Not available	Not available	Single	*Penicillium* sp. 2

Description : IB = Fungi isolate from *Bactrocera dorsalis*

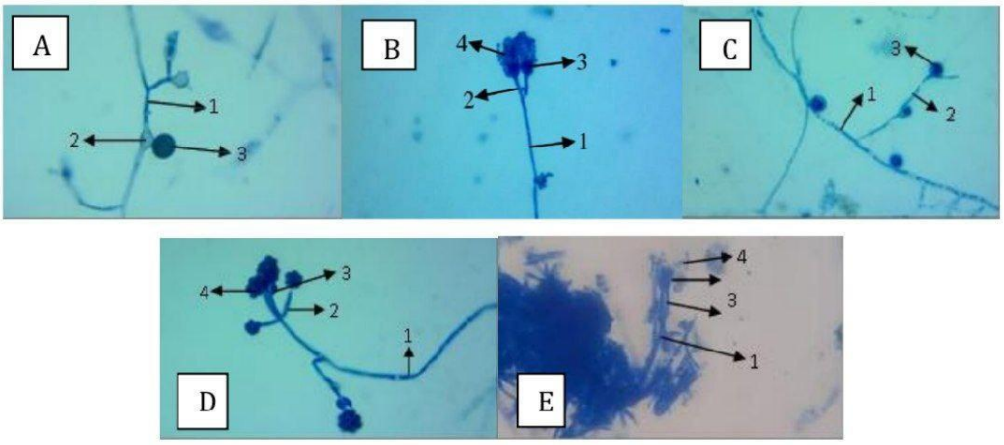

Figure 5. Isolates of Entomopathogenic Fungi 400x magnification (1. Hypha, 2. Conidiophores, 3. Fialid, 4. Conidia) (A) *Nigrospora* sp. (B) *Penicillium* sp. 1 (C) *Phytopthora* sp. (D) *Gliocladium* sp. (E) *Penicillium* sp. 2

A type of *Nigrospora sp.* was successfully isolated, exhibiting microscopic characteristics of densely packed hyphae, small erect and simple conidiophores without foot cells, vesicles, or phialids. The conidia of *Nigrospora sp.* isolates are single, black, and measure 17.5 µm (Figure 5A). *Nigrospora sp.* is known as an endophytic fungus in citrus plants, residing within plant tissues. Endophytic fungi like *Nigrospora sp.* are potential biological control agents due to their production of secondary metabolites [25]. Thakur et al. [26] demonstrated that *Nigrospora sp.* can alter the cell shape of Spodoptera litura larvae after application for 5 days. Additionally, *Nigrospora sp.* has been identified as an entomopathogen in *Nilaparvata lugens* [27], indicating its potential as an entomopathogenic fungus.

This study identified two types of *Penicillium sp.* isolated from *Bactrocera dorsalis* flies. Both *Penicillium sp.* 1 and *Penicillium sp.* 2 exhibit microscopic characteristics of concentrated hyphae, erect and branched conidiophores without foot cells or vesicles, and single bottle-shaped phialids with round conidia arranged in chains. However, these two isolates differ in conidia size: *Penicillium sp.* 1 conidia are 12.5 µm (Figure 5B), while *Penicillium sp.* 2 conidia are 17.5 µm (Figure 5E). *Penicillium sp.* fungi have been isolated from various insects, including *Periplaneta americana, Musca domestica* [28], *Aedes aegypti* [18], *Xystrocera festiva*, and *Coptotermes sp.* [29]. Known for its potential in biological control [30], *Penicillium sp.* was shown by Tambingsila [31] to reduce cocoa pod borer (*Conopomorpha cramerella*) infestation rates by 90%.

The isolated *Phytophthora sp.* exhibited microscopic characteristics of non-concentrated hyphae, erect and simple sporangiophores without foot cells, vesicles, or phialids. The spores measured 10 µm (Figure 5C). *Phytophthora sp.* is recognized as a pathogenic fungus causing root rot and stem base rot in citrus plants, as noted by Ningsih et al. [32]. In this study, *Phytophthora sp.* may have been isolated due to environmental conditions, as several trees sampled in the field showed symptoms resembling stem rot caused by *Phytophthora sp.* [33]. However, there is no existing research on *Phytophthora sp.* as an entomopathogenic fungus.

Isolated *Gliocladium sp.* isolates displayed microscopic characteristics of concentrated hyphae, erect and branched conidiophores without foot cells or vesicles, grouped phialids, and green, flower-like conidia measuring 20 µm (Figure 5D). *Gliocladium*

sp. has been reported to infect various insects, including *Xystrocera festiva* [29] and *Helicoverpa armigera* [34]. This entomopathogenic fungus is known to infect *Tenebrio molitor* with a mortality rate of 53.33% [35].

CONCLUSION

Five types of entomopathogenic fungi candidates isolated from Bactrocera dorsalis were identified as *Nigrospora sp., Penicillium sp.* 1, *Phytophthora sp., Gliocladium sp.,* and *Penicillium sp.* 2.

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