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The Effectiveness of *Nigrospora* sp. and *Penicillium* sp. as Entomopathogenic Fungi Against *Bactrocera* sp.

(Efektivitas *Nigrospora* sp. dan *Penicillium* sp. Sebagai Pembasmi Entomopatogen Pada *Bactrocera* sp.)

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Abstrak

Serangan hama akibat lalat buah (*Bactrocera* sp.) dapat menimbulkan kerusakan pada buah. Kerusakan dapat menjadi lebih parah karena adanya infeksi sekunder oleh bakteri ataupun jamur yang dapat mengakibatkan busuk pada buah. Tujuan penelitian ini adalah untuk mengetahui efektivitas dan perubahan morfologi yang diakibatkan oleh cendawan *Nigrospora* sp. dan *Penicillium* sp. terhadap mortalitas *Bactrocera* sp. Penelitian ini menggunakan Rancangan Acak Kelompok (RAK) dengan dua faktor yaitu jenis cendawan entomopatogen dan kepadatan spora. Isolat cendawan entomopatogen diperoleh dari penelitian sebelumnya isolasi cendawan asal lalat buah (*Bactrocera* sp.). Kepadatan spora yang digunakan dari masing-masing cendawan adalah 10^5, 10^6, dan 10^7 spora/mL. Setiap unit perlakuan menggunakan serangga uji 5 ekor *Bactrocera* sp. dewasa dengan 3 kali pengulangan. Pada isolat *Nigrospora* sp. menghasilkan mortalitas pada kepadatan spora 10^5 spora/mL sebesar 86%, kepadatan 10^6 spora/mL sebesar 93%, dan kepadatan 10^7 spora/mL sebesar 100%. Pada isolat *Penicillium* sp. seluruh perlakuan menyebabkan mortalitas sebesar 100%.

Kata kunci: *Bactrocera* sp., Entomopatogen, Fungi, *Nigrospora* sp., *Penicillium* sp.

Abstract

Pest attacks caused by fruit flies (Bactrocera sp.) can cause damage to fruit quality. Damage can become more severe due to secondary infection by bacteria or fungi, which can cause fruit to rot. This research was conducted to determine the effectiveness and morphological changes caused by the fungi Nigrospora sp. and Penicillium sp. on the mortality of Bactrocera sp. This research was conducted using a Randomized Block Design (RBD) with two factors: the type of entomopathogenic fungi and the density of spores. Entomopathogenic fungi isolates were obtained from the isolation of entomopathogenic fungi from fruit flies (Bactrocera sp.) from previous studies. The spore density used for each fungus was 10^5*,* 10^6*, and* 10^7 *spores/mL. Each treatment unit used 5 Bactrocera sp. adults with 3 repetitions. In the isolates of Nigrospora sp., mortality has resulted at a spore density of* 10^5 *spores/mL of 86%, a density of* 10^6 *spores/mL of 93%, and a density of* 10^7 *spores/mL of 100%. In the isolates of Penicillium sp., all treatments resulted in 100% mortality.*

Keywords: *Bactrocera sp., Entomopathogenic Fungi, Nigrospora sp., Penicillium sp.*

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INTRODUCTION

Repeated attacks by fruit flies can significantly reduce both the quality and quantity of fruit [1], thereby hampering fruit production. Fruit flies (*Bactrocera sp.*) are prevalent pests known for targeting fruit plants, particularly in cool climates with high humidity and moderate wind conditions. Factors such as temperature, humidity, wind speed, and rainfall influence the intensity of these attacks [2].

Infestation by fruit flies (*Bactrocera sp.*) can lead to fruit damage, which can escalate if secondary infections by bacteria or fungi occur, causing the fruit to rot [3]. Infested fruit typically shows small puncture marks and dark spots or stains from the female fly's ovipositor during egg laying. Female fruit flies can penetrate the skin of fruits like oranges up to 6 mm deep to lay eggs. Pest activity within the fruit flesh, along with secondary infections, accelerates fruit decay and premature dropping. Fruit flies preferentially target nearly ripe fruits due to their high sugar content, which serves as a food source for the flies [4].

Effective management of fruit fly infestations (*Bactrocera sp.*) is crucial. Currently, chemical pesticides are widely employed due to their accessibility, ease of use, and rapid efficacy. However, excessive pesticide use can lead to adverse effects on consumers and farmers, as well as harm natural predators and contribute to pesticide resistance among pests [5].

Biological control using entomopathogenic fungi represents a promising alternative for managing fruit fly populations (*Bactrocera sp.*) [6]. These fungi produce toxins and enzymes such as chitinase, protease, and lipase, which invade and disrupt the physiology of target insects [7]. This study aims to determine the optimal spore concentration for controlling *Bactrocera sp.* flies and to investigate the morphological changes in infected flies caused by entomopathogenic fungi.

METHODS

This research was conducted from February to August 2022 at the Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Lampung. The test insects used were fruit flies (*Bactrocera sp.*) sourced from citrus plants (*Citrus sp.*) at Balai Penelitian Teknologi Pertanian (BPTP) Lampung. *Nigrospora sp.* and *Penicillium sp.* were obtained from previous research results. The entomopathogenic fungi selected for the study exhibit high enzymatic activity. The experimental design employed a Randomized Block Design (RBD) with two factors: type of entomopathogenic fungus and spore density (105, 106, and 107 spores/mL) [8].

Preparation and Maintenance of Test Insects

Adult fruit flies (*Bactrocera sp.*) of similar size were collected from BPTP and housed in 24 rearing jars. They were provided with fruit pieces for food and water via moistened cotton wool for hydration. After acclimatizing for 2 days, each jar contained 5 mature *Bactrocera sp*. individuals.

Fungal Re-Culture

Isolates of the fruit fly entomopathogenic fungus (*Bactrocera sp.*) were cultured on PDA medium in petri dishes and incubated for 14 days. Pure isolates were then multiplied by inoculating fungal spores onto PDA medium in large test tubes, followed by another 14-day incubation periode.

Preparation of Fungal Suspension

Fourteen-day-old fungal spores were transferred into test tubes containing 10 mL of sterile distilled water supplemented with 1% molasses and 0.1% Tween 80. The suspension was homogenized using a vortex mixer until uniform. Spore density in the suspension was determined by counting spores using a hemocytometer and a light microscope, calculated using the formula. The spore density of the fungus suspension was calculated using the following formula:

$$C = \frac{\mathrm{t}}{(\mathrm{n\ x\ 0{,}25})}\, x10^6$$

C : Spore density
t : The total number of spores in the sample observed
n : Number of sample used
0,25 : Small scale sample correction factor

After determining the desired spore density, dilution is performed to achieve suspensions of 10^5, 10^6, and 10^7 spores/mL. According to research conducted by [7], the dilution of the spore suspension can be calculated using the following formula:

$$\mathrm{N1.V1 = N2.V2}$$

N1 : Initial spore suspension density
V1 : Initial volume of spore suspension
N2 : Target spore suspension density
V2 : Total volume (10 mL)

Application of Fungal Spores

To assess the efficacy of entomopathogenic fungi on fruit fly (*Bactrocera sp.*) mortality, a suspension of fungal spores was sprayed onto *Bactrocera* sp. test insects. The treated insects were then placed in a dark environment.

Observations Post-Application

Observations were conducted three days after the application of entomopathogenic fungal isolates, as outlined in [7]. This assessment consisted of two stages:

a. Evaluation of the mortality rate among fruit flies (*Bactrocera* sp.) exposed to the entomopathogenic fungus spore suspension.

b. Examination of morphological changes in fruit flies (*Bactrocera* sp.) following treatment, including alterations in texture, body shape, and color.

Calculation of Fruit Fly Mortality Percentage (*Bactrocera sp.*)

The percentage of fruit fly (Bactrocera sp.) mortality was calculated using the formula:

$$P = \left({}^{x}\!/_{y}\right) 100\%$$

P : Percentage of deaths
x : Number of deceased fruit flies
y : Total number of fruit flies

RESULTS AND DISCUSSION

The research indicates that *Nigrospora sp.* and *Penicillium sp.* demonstrate significant potential in controlling *Bactrocera sp.* fly populations. Data on the mortality percentage of *Bactrocera sp.* flies can be found in Table 1.

Table 1. Mortality percentage of fruit flies (*Bactrocera sp.*) at various spore densities Entomopathogenic Fungi Post Application

Types of Fungi	Spore Density (spore/mL)	Initial Amount (hyphae)	Final Amount (hyphae)	Mortality Percentage (%) of *Bactrocera sp.*
Nigrospora sp.	10^5	15	2	86
	10^6	15	1	93
	10^7	15	0	100
Penicillium sp	10^5	15	0	100
	10^6	15	0	100
	10^7	15	0	100
Negative Control		15	15	0
Vehicle Control		15	15	0

The data demonstrate that the fungus *Nigrospora sp*. caused mortality with an average percentage of 93%. Among the different spore densities tested, *Nigrospora sp*. at 10^7 spores/mL was the most effective, achieving 100% mortality for *Bactrocera sp*. This was followed by 10^6 spores/mL, resulting in 93% mortality, and 10^5 spores/mL, resulting in 86% mortality. These findings are consistent with research [10] and [11], indicating that *Nigrospora sp*. and *Penicillium sp*. are highly virulent in killing *Bactrocera sp*. pupae at three days old, with concentrations ranging from 10^8 to a maximum of 10^{10} spores. According to the statement [12], higher spore densities increase the adhesion of spores to the insect's body, facilitating invasion and causing death.

Application of *Penicillium sp*. at all tested densities resulted in 100% mortality of *Bactrocera sp*. Therefore, using Penicillium sp. at 10^5 spores/mL is deemed the most effective density for controlling *Bactrocera sp*. populations, consistent with the statement [8] that lower spore concentrations can achieve comparable mortality rates to higher concentrations.

Both fungi used in this study exhibited high mortality rates due to their specific efficacy in infecting insects. The entomopathogenic fungi were isolated from fruit flies (*Bactrocera sp*.), highlighting their effectiveness in controlling *Bactrocera sp*. populations, as corroborated by the statement [13] indicating that optimal pathogenicity is achieved when using fungi isolated from the same host and ecosystem as the test insect.

To assess the impact of different fungi types and spore densities on *Bactrocera sp*. mortality, statistical analysis was conducted using Analysis of Variance (ANOVA), as detailed in Table 2.

Table 2. ANOVA test results on the influence of different fungus types and spore densities on the mortality of *Bactrocera sp.* flies

Tests of Between-Subjects Effects

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.
Types of mushrooms	Hypothesis	.500	2	.250	1.135	.349
	Error	3.083	14	.220[a]		
Density	Hypothesis	.333	2	.167	.757	.487
	Error	3.083	14	.220[a]		
Repetition	Hypothesis	.250	2	.125	.568	.579
	Error	3.083	14	.220[a]		
Types * density	Hypothesis	.333	2	.167	.757	.487
	Error	3.083	14	.220[a]		

Based on the results of the ANOVA analysis, it was found that there were no significant differences among the treatments conducted. Therefore, no further statistical tests were performed. However, despite the lack of significant differences in mortality rates, each treatment did result in distinct morphological changes in *Bactrocera sp.* mortality.

Figure 1. Morphology of *Bactrocera sp.* without treatment

A B C

Figure 2. Changes in the morphology of *Bactrocera sp.* infected with *Nigrospora sp.*: A. Density of 10^5 spores/mL, B. Density of 10^6 spores/mL, C. Density of 10^7 spores/mL; (1) and (2) Attachment of spores to wings

Morphological changes were observed in *Bactrocera sp.* following treatment with the fungus *Nigrospora sp.* at different spore densities. Bactrocera treated with a spore density of 10^5 spores/mL did not exhibit significant morphological changes. However, mortality occurred as fungal spores attacked the internal tissues of the insect. Observations after three days showed no visible fungal growth on the body surface, indicating that the spores had not yet reached the surface. The only visible morphological change was the presence of brownish spots on the wings, indicative of spores attached to them.

Specimens treated with a density of 10^6 spores/mL displayed morphological changes characterized by the growth of white fungus on the abdomen and brownish spots across the body. The body texture became hardened and stiffened. *Bactrocera dorsalis* exhibited specific thoracic characteristics such as a black and reddish-brown scutum with transverse bands on each side of the scutellum's tip, a narrow descending costal band at the end of the R vein, and a very narrow anal line [14]. The abdomen showed distinct features including black lines across terga II and III, and a T-shaped pattern formed by a black line across terga III to V [15].

Bactrocera dorsalis treated with a density of 10^7 spores/mL showed morphological changes including brownish spots on the wings, white fungus growth on the head and abdomen, and a darker or blackish-brown body color.

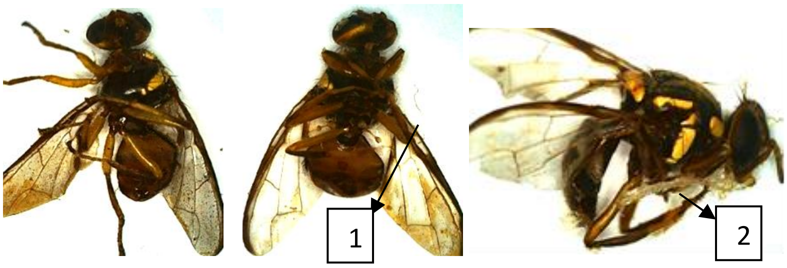

Figure 3. Changes in the morphology of *Bactrocera sp.* infected with *Penicillium sp.*: A. Density of 10^5 spores/mL, B. Density of 10^6 spores/mL, C. Density of 10^7 spores/mL

Bactrocera sp. treated with *Penicillium sp.* at a spore density of 10^5 spores/mL did not exhibit significant morphological changes. Only brownish spots were observed on the wings, indicating the presence of attached spores. Specimens treated with a density of 10^6 spores/mL showed morphological changes with brownish spots across the body and visible hyphae growth on the wings. The body texture became hardened and stiff.

At a density of 10^7 spores/mL, *Bactrocera sp.* showed similar morphological changes to those treated with 10^6 spores/mL. Additionally, mycelium growth was observed on the legs and abdomen.

Fruit flies (*Bactrocera sp.*) that succumbed to infection with entomopathogenic fungi appeared dry, stiff, and hardened. This occurs because the fungi utilize all fluids in the insect's body for their growth and development. As noted in statement [13], entomopathogenic fungi consume all tissues and body fluids of insects during this process, leading to the insect's demise with a hardened and stiffened body.

The body color of *Bactrocera* flies infected with entomopathogenic fungi darkened to a blackish hue. This change results from enzymatic degradation and chemical processes that lead to melanin formation, causing the insect's body to darken [16].

CONCLUSION

The use of *Nigrospora sp.* isolates was most effective in controlling *Bactrocera sp.* at a density of 10^7 spores/mL, achieving 100% mortality. Similarly, *Penicillium sp.* isolates were most effective at 10^5 spores/mL, also resulting in 100% mortality. Morphological changes observed in *Bactrocera sp.* treated with *Nigrospora sp.* and *Penicillium sp.* included visible fungal hyphae and mycelium growth on the insect's body at densities of 10^6 and 10^7 spores/mL.

Based on the findings of this study, it is recommended to further explore other potential fungal isolates and expand the study to include a larger population of test insects.

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