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The Ability of Entomopathogenic Fungus as Biocontrol of Periplaneta americana

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Abstract

Periplaneta americana (cockroach), are well-known pests that not only contaminate food with feces and germs that can cause food poisoning, but also transfer bacteria, fungus, and other dangerous microbes. Chemical pesticides are harmful to both the environment and individuals when used in excess. The natural balance has been disrupted, resulting in resistant pests, predatory threats, parasitic threats, fish, birds, and other animals. The objective of this research is to see the ability of an entomopathogenic fungus as a cockroach biocontrol agent. This research conducted with experimental research with ten treatments and three repetition. The variation are, K(-) only distilled water, K(+) synthetic pesticides, Botrytis sp., Mucors sp., Acremoniums sp., Rhizopus sp., Moniliella sp., Chaetomium sp., Nigospora sp., and Aspergillus sp. A one-way Completely Randomized Design was used to examine them (CRD). With a cell count of 2.35 x 108 CFU/ml, Rhizopus sp. was able to achieve a mortality rate of cockroach by 66.6 percent.

1. INTRODUCTION

Periplaneta americana (cockroach) is one of the insects that plays a key function in the ecological cycle. However, they , can be pests that can be a vector for spreading bacteria, fungi and pathogenic microorganisms to humans [1]. Cockroach habitat is in garbage and dirty places, active at night. Cockroaches transmit disease from various parts of their bodies (body, appendages, mouth) and secretions (feces) [2], [3].

Chemical control can be used to decrease the cockroach like baseboard sprays, aerosols, foggers, and bait. However, the baits contain phosphorus, boric acid, chlorpyrifos, sulfonamide, abamectin, boric acid, hydramethylenon, and other chemical baits [4].

Chemical pesticides used in excess can harm both the environment and individuals.

The natural equilibrium is disrupted, resulting in pests that are resistant to treatment, posing a hazard to predators, fish, birds, and other creatures[5]. The presence of residues in the soil poisons non-target creatures, carries them to water sources, and poisons the surrounding ecosystem, which is one of the causes of insecticides' detrimental environmental effect [2], [6].

Biological agents can be used as an alternative to pesticides in order to reduce cockroach populations without causing harm to the environment[7]. Entomopathogens are parasitic microorganisms that may infect and kill arthropods as they have insecticidal effects on cockroaches[8]. *Metarhizium, Beauveria*, *Isaria, Ophiocordyceps*, *Cordyceps*, Torubiella, Pochonia, Hirsutella, Paecilomyces, and Lecanicillium are the most investigated and extensively utilized species in crop production[9].

Because there has been little study comparing numerous entomopathogenic fungi as biocontrol of cockroaches in Indonesia, despite the fact that the population of these pests is relatively great due to Indonesia's tropical climate, further research is needed.

2. MATERIALS AND METHODS

The entomopathogenic fungus carried out from Laboratory of Genetic and Molecular UIN Sunan Gunung Djati Bandung were *Botrytis* sp., *Mucors* sp., *Acremoniums* sp., *Rhizopus* sp., *Moniliella* sp., *Chaetomium* sp., *Nigospora* sp., and *Aspergillus* sp.

PDA Preparation

First, the media's components are weighed, such as 300 grams of potatoes, 10 grams of sugar, 1 liter of distilled water, and 7/5 grams of agar. Potatoes that have been cut are boiled in distilled water for 1-3 hours or until soft then the extract is taken by filtering and squeezing it using filter paper and then accommodated in a new glass beaker, added sugar and agar into the potato extract then cook again over the fire while stirring until boiling and homogeneous. The media is poured into Erlenmeyer and then sterilized [10].

Cockroach Specimen Collection and Maintenance

Cockroaches were obtained from Pawidean Village, Jatibarang District, Indramayu Regency. Then put in a cylindrical plastic container and tightly closed and then brought to the laboratory to be separated and the length and width of the body measured to be homogeneous. Cockroach maintenance carried out in plastic containers or drink cups with a diameter of 3.5 cm and a height of 4 cm. The plastic container covered and made a hole in the top for ventilation. The cockroach pet care was carried out on thirty-one plastic containers containing one cockroach each. Cockroach food is placed in a plastic container, namely chicken feed, and moist cotton as a drink and moisture, used cardboard rolled up and tightened as a shelter.

Biocontrol Test

In this biological test, there are two steps, the first step is the manufacture of fungus suspension and the second step is biological testing of cockroaches. One ose of fungus was inoculated into 15 streaks of inclined PDA media, then incubated for 48 hours at room temperature (25-27°C). From each isolate of the head, upper wings, lower wings, front legs, middle legs, hind legs, middle body parts, back body, 5 tubes were taken, each tube was added with 5 ml of sterile distilled water so that a cell suspension with a cell count equivalent to 10^8 CFU/ml was obtained. Cultures were scraped with sterile round planting needles and homogenized using a vortex [11].

The biological test procedure was carried out by dipping a cockroach into a fungus suspension for 30 seconds. For control (-) only 20 ml of sterile distilled water, for control (+), 5 ml of synthetic insecticide. Then the cockroaches are put into a container measuring 10 cm high and 5 cm wide in diameter. The cockroach feed is placed in a cage and given wet cotton, the feed given is a type of chicken feed, the cockroach container

is closed with a cup cover, then given a hole in the top for circulation air, and labelled with a description, observed for 10 days and recorded temperature and humidity.

Calculation of the Number of Fungus Colonies

The method to determine the number of colonies from a fungus suspension was carried out by counting the number of fungus colonies using the Total Plate Count (TPC) method. A total of one ose of two-week-old fungus culture was inoculated 15 streaks into inclined PDA medium and then incubated at room temperature of 28oC for two weeks. The fungus culture was then scraped using a loop and diluted with 5 ml of sterile distilled water, homogenized with a vortex to produce a cell suspension. Dilution was carried out by taking 1 ml of fungal cell suspension into a test tube containing 9 ml of sterile distilled water with dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. The suspension was shaken with a vortex for 1 minute.

A total of 1 ml of fungal spore suspension from each dilution was spread by micropipette onto the surface of the PDA medium in a petri dish with two repetitions. Then flatten with a Drygaslsky spatula. Petri dishes were then incubated for six days at room temperature, and the total number of colonies was counted at each dilution and repetition. The number of cells or spores per 1 ml sample can be calculated based on Fardiaz (1992), with the formula:

$$\text{CFU/mL} = \frac{\text{number of colonies x dilution factor}}{\text{Volume of culture plate}}$$

Data analysis

Data were analyzed using analysis of variance Completely Randomized Design (CRD) one-way ANOVA with a significance level (α = 0.05) With 10 treatments and 3 repetitions. The treatment consists of K (-): Only use sterile distilled water without treatment. K (+): Synthetic insecticide, *Botrytis* sp., *Mucors* sp., *Acremoniums* sp., *Rhizopus* sp., *Moniliella* sp., *Chaetomium* sp., *Nigospora* sp., *Aspergillus* sp

3. RESULTS and DISCUSSION

Biocontrol results

Table 1.1 displays the outcomes of the one-way ANOVA analysis of variance, which reveal that mould isolates provided to cockroaches have an impact, which is claimed since the significance threshold is 0.01 0.05. The Duncan's test, as indicated in table, was then used to determine which therapy was more influential among the other isolates.

Based on Duncan's further test (table 1.2), it can be seen that the treatment that was more influential than the eight isolates was in the fourth treatment (suspension isolates of the Rhizopus) with a cell count of 2.35 x 108 CFU/ml, which had been isolated from the forelegs of the cockroach. The second treatment (suspension isolates of the Mucor) with a cell count of 1.18 x 10^8 CFU/ml also experienced mortality but had no significant effect. Compared to the control, the control (+) given synthetic insecticide experienced 100% mortality, which means that the fungus had a similar effect on the synthetic insecticide in suppressing the cockroach population with a mortality percentage of 66.6%.

Based on observations of Rhizopus sp. with a cell number of 2.35 x 10^8 CFU/ml, it was more influential than the other fungus. During ten days of observation, cockroaches that had been treated died on the second and eighth days.

Rhizopus belongs to the phylum zygomycetes, which has filaments that are not septate. These organisms are also characterized by stolon, which connect rhizoids. Rhizopus has metabolites or antigens, one of which is the polyketide rhizoxin which is thought to cause cockroach mortality. Rhizoxin is a compound that has been isolated from Rhizopus Chinensis, which has antibiotic and antifungal activity. This substance has been shown to have antitumor activity[12].

Biocontrol is a way to control an organism by another organism using its natural enemies with antagonistic abilities. One way to control the population of pathogenic organisms is by using entomopathogenic fungus. In this study, the fungus used resulted from isolation from the outer surface of the cockroach. The biocontrol mechanism using entomopathogenic fungi generally attacks the host insect by penetrating the insect integument through the intermediary hyphae, but some require injury to the integument first.

Entomopathogenic fungi can infect insects through the digestive tract of food. This can occur if the fungal conidia are ingested when the insect eat and carried into the digestive tract [9] after infection. The tip of the hyphae will penetrate the wall of the digestive tract, which causes digestive tract fluids to enter the hemocoel, resulting in changes in pH in the digestive tract and haemolymphs[13].

In this study, cockroaches experienced mortality, but there were no mycelia that wrapped the cockroach cuticles or mummified, maybe this is because the mechanism that occurs is that fungus infect insects through the digestive tract of food, at the time of immersion into suspension, conidia are carried into the digestive tract through the mouth of the cockroach with chewing and then swallowing it, therefore that the conidia are carried away.

The American cockroach has a common mouthpart, this insect is omnivorous, and the mouthparts are very suitable for chewing various foods. Each pair of mouthparts consists of two bilateral segment appendages, the labral, mandibular, maxillary and labial segments. Two segments have sensory palps as well as other functional parts [2].

With the help of the blood circulation system fragments, the fungus attacks other tissues such as adipose tissue, muscle, nervous system, glandular system, and others. In advanced infection, blood circulation slows down and even stops, which results in the death of the infected insect[6][13].

The success of fungi in infecting insect pests is influenced by several factors, including temperature and humidity. Fungi require high humidity to germinate conidia. At the time of observation, the average temperature during was 27°C and humidity 83 RH. At temperatures and humidity that do not support the occurrence of sporulation, the development of the fungus only takes place inside the insect's body without exiting through the integument. Fungi require high humidity and the optimum temperature for growth, pathogenicity, and survival of fungi is generally between 20-30°C [7].

The time of death of insects is influenced by the application dose and the virulence of each isolate. The level of pathogenicity of pathogenic fungi to be able to cause disease is determined by various factors, including the physiological properties of the host such as host defence mechanisms and physiological properties of the fungus such as viability, growth rate, sporulation ability and secondary metabolism produced in the form of the ability to produce enzymes and toxins. And environmental influences[14].

In control (+) who had been given synthetic insecticides, the cockroaches experienced 100% mortality on the second day. The synthetic insecticide used has the active ingredients Propoxurs and Deltamethrin, which can cause mortality in cockroaches[5].

Propoxur is a carbamate insecticide used to control ants, cockroaches, and bees in or around residential premises. Propoxurs is made through the alkylation process of dihydroxy phenol compounds, where the alkyl group usually replaces the H atom in the hydroxy group. So far, propoxurs has been used to eradicate mosquitoes or other insects. Deltamethrin is a synthetic pesticide pyrethroid that kills insects through skin contact and digestion. Deltamethrin is used in various commercial crops to control pests [15]. The mechanism of deltamethrin occurs through cuticle penetration or oral absorption. Insect susceptibility depends on various factors and may vary according to the

environment and conditions, as with many other insecticides [16].

Table 1.1 Analysis of variance of One-way ANOVA

Mortality	ANOVA				
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig**
Variation	3.467	9	.385	5.778	.001
Error	1.333	20	.067		
Total	4.800	29			

Table 1.2 Duncan distance test level = 0.05

Variation	Subset for α = 0,05	Sig (α = 0,05)
K (-)	0,00	a
K (+)	1,00	c
Botrytis sp.	0,00	a
Mucors sp.	0,33	ab
Acremoniums sp.	0,00	a
Rhizopus sp.	0,66	bc
Moniliella sp.	0,00	a
Chaetomium sp.	0,00	a
Nigospora sp.	0,00	a
Aspergillus sp	0,00	a

Note: A = Not significantly different, AB = Close to significantly different, BC = significantly different but less influential, C = significantly different and very influentia

4. CONCLUSION

The biological test results showed that from the eight species of entomopathogenic fungi, the most potential as a biocontrol was *Rhizopus* sp. P4 with a mortality of 66.6%.

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