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Isolation, identification, and charerization: Entomopathogenic fungi to *Blattella germanica,* Linnaeus (Orthoptera: Blattida

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Abstract

Isolation, identification and pathogenicity test entomopathogenic fungi against *B. Germanica* have been conducted. This study aims is to gain entomopathogenic fungal isolates derived from *B. germanica*, identify entomopathogenic fungal isolates obtained from *B. germanica*, and tested the pathogenicity of isolates of entomopathogenic fungi against *B. germanica*. The study was conducted by isolating the fungus derived from *B. germanica* were planted on the ground, then cultured and purified in an artificial medium Potato Dextrose Agar (PDA). Identification is done macroscopically by looking at pure culture of fungal isolates in Petri disc and microscopically by making culture slides were observed using a microscope. Pathogenicity test is done by applying a spore suspension on the entire body apart *B. germanica* and placed individually in sterile vial bottle. The results of isolation and identification of fungal isolates obtained five different types consisting of two genera, namely Mortierella and Aspergillus. Based on the results of pathogenicity test, all isolates of fungi were isolated have the ability to turn off *B. germanica* so that it can act as entomopathogenic fungus. The percentage value mortality fungal isolates 1, 2, and 3 is 40%. Value fungal isolates 4 percentage mortality is 60%. Isolates have value 5 percentage mortality by 20%.

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Introduction

Blatella germanica also known as German cockroach is vector-borne microorganisms such as pathogenic bacteria in the body and feces that can contaminate food (Miller & Koehler, 1993). They can found in homes, apartments, restaurants, supermarkets, hospitals, and other buildings (Lyon, 1991; Valles, 1996). Thus the popuation of cocroach would growing rapidly, and they cause human healty problem such as allergies, asthma, and other respiratory diseases.

In the past, people used an excessive insecticide to overcome cocroach's. Lately it was known it has a negative impact on human health and environment (Kaaya and Hasan, 2000). One of the alternative of control method is using biological agents, such as fungi and bacteria that are pathogenic, to insects including *Blatella germanica* (She and Feng, 2004). Currently, Integrated Vector Control attempted to use entomopathogenic fungi (barker and Barker, 2008).

Entomopathogenic fungi that commonly used are *Beauveria bassiana, Verticillium lecanii* and *Metarhizium anisopliae* (Boucias and Pendland, 2008) . *M. anisopliae* can infect several insects of the Order Coleoptera, Lepidoptera, Hemiptera and Isoptera (Ahmed *et al*., 2009). There still numerous number of potential local entomopathogenic which is come from soil that can infect to insect. The aim is to conduct a research about isolation, identification and pathogenicity test entomopathogenic fungi against *B. germanica*.

Material and method

Insect samples used in this study is *B. germanica* obtained from Vector and Reservoir Research Center of Disease Salatiga, Central Java Indonesia. Maintenance is carried out in the Department of Biology Botanical Gardens FPMIPA Universitas Pendidikan Indonesia Bandung.

B. germanica kept in an aquarium with a size of 50x30x30 cm, with a cover by the hole and covered with gauze and wire for ventilation. This enclosure is placed in a sheltered from sunlight at room temperature. During maintenance *B. germanica*fed pellets for chicken feed and water to drink is placed in a small container. As the nesting and habitat use wood pieces are nested. The resulting egg would develop into the adult stage. The adult stage that will be used in this stu.

Isolation of entomopathogenic fungus

Entomopathogenic fungus obtained by using insect bait technique (Zimmermann, 1986 in Barker & Barker, 1998). The insects are used as bait is dead cocroach. *B. germanica* was trapped and then put into the vial bottle, and then closed. The next bottle vial containing the *B. germanica* put in the refrigerator and allowed to die. After *B. germanica* die, and then sterilized by soaking *B. germanica* in 70% alcohol for 2-5 minutes, then *B. germanica* rinsed with distilled water and dried using filter paper. *B. germanica* are then placed on the ground in order to get the vial bottle entomopathogenic fungus spores. Vial bottles and then sealed using aluminum foil. Observed was coducted every day to see the fungal spores to grow.

Isolation of entomopathogenic Fungi

Isolation of entomopathogenic fungi pathogenic microorganisms aims to separate from its host, to obtain pure isolates. *B. germanica* which have been dense by fungal spores, the spores were then taken using an osse and inoculated in a Petri dish that already contains a sterile PDA medium. Then incubated at room temperature for 3-7 days to stimulate the growth of fungus.

Purification and Culture Propagation entomopathogenic fungus

Each of the fungal isolates are purified by taking isolates fungus growing on a petri dish with an osse and inoculated in a Petri dish that already contains other PDA medium. Purified back several times with the same technique in order to obtain pure cultures fungal isolates. After getting fungal isolates pure, pure

cultures are then identified

Identification of Isolates entomopathogenic fungus

Identification of fungal isolates were divided into two stages. The first phase of macroscopic observations conducted on pure cultures PDA a sterile Petri dish, which includes observation of color, shape, size, and growth rate of the colony. The second stage of microscopic observation made by making a culture slide (Malloch, 1997) which includes the observation of the structure of asexual spores, spore shape, and form hyphae. Identification of each isolate refer by determination key mushroom bouquet Malloch (1997) and Onions *et al*., (1981).

Pathogenicity Test (Koch's postulates)

Pathogenicity testing is done by applying a spore suspension on the entire body of *B. germanica* to ensure the spores attached to the body Blattella germanica. *B. germanica* then placed separately in a sterile vial bottle individually as much as 5 individuals against any fungal isolates. Then the bottles vials are placed in a plastic container and was observed for 10 days for mortality and growth of fungus on *B. germanica*. *B. germanica* control is dipped in a solution of 0.85% physiological saline. During treatment, *B. germanica* given a drink and meal in the form of pellets for chicken feed.

The percentage of the number of dead *B. germanica* (mortality) was calculated using the formula Abbot (Miller, 1997):

Notes:

M = mortality test insects during the observation

X = Number of *B. germanica* which died during the observation

Y = Number of *B. germanica* used

A fungus that grows on the body of *B. germanica* were isolated and inoculated back as the early stages. Then made observations on each of the fungal isolates and the results were compared with the initial isolates have been obtained. As supporting data, carried out measurements during treatment of climatic factors, namely light intensity, humidity and temperature at the site of treatment.

Results and discussion

Soil Sampling and Isolation of Entomopathogenic Fungi

Soil sample was done randomly at three stations, by taking three points at each station. Station 1 is determined in the Cikole-Lembang, station 2 specified in the Plumbing-Campus UPI, station 3 is determined in Arcamanik-Antapani Housing area.

The environment each station where the soil sampling different. Station 1 is conducted in the area around the planting of bananas and cassava. Station 2 do in the area behind the stadium UPI, and station 3 carried on bare ground around the residential area Arcamanik-Antapani.

Soil sampling was conducted to obtain entomopathogenic fungus from the soil by using insect bait techniques. Entomopathogenic fungi can be obtained from the soil, especially in the upper part (top soil) 5-15 cm of the soil surface, because the horizon is estimated there are many entomopathogenic fungus spore inoculum (Hashim *et al*., 2005). Many ecological studies of the fungus using soil as a substrate for growth (Onions *et al*., 1981).

Tabel 1. Measurement of colony diameter fungi isolate (cm).

Colony	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
1	5,5	3,5	3,5	3,5	2
2	4	3,3	2	2,5	2,5
3	4	3	3	1,7	1
Diameter	4,50	3,27	2,83	2,56	1,83

After purification of all types of fungal colonies on 2 Petri dishes containing PDA medium, obtains 8 colonies fungal isolates. Five colonies of fungal isolates in Petri dishes 1 and 3 fungal isolates colonies on a Petri dish 2. Once identified macroscopically, turns 3 colonies of fungal isolates that were in the same Petri dish 2 morphological characters with a colony isolates were in a Petri disc 1 (Figure 1). Therefore , colonies isloat fungus that found only 5 types of different fungal isolates colonies.

Tabel 2. Fungal characteristic from three site of samples.

Isolate	Colony			Hiphae	Asexual spore	Genus
	Shape	Color	diameter			
1	Colony with a loose texture like cotton, form colonies spread, long hyphae	White Green light to the edge of the	4,50 cm	unsegmented	Sporangiospore	*Mortierella*
2	Colony with a loose texture, colony surface is convex, form colonies round	white colonies, after the old dark green	3,27 cm	Segmented	Conidiospore	*Aspergillus*
3	Colony with a dense texture, colony surface flat, form colonies spread	Dark green	2,83 cm	segmented	Conidiospore	*Aspergillus*
4	Colony with a loose texture, colony surface flat, form round colonies with the sides spread	White yellowish	2,56 cm	segmented	Conidiospore	*Aspergillus*
5	Colony with a loose texture, colony surface is convex, form colonies round	White with brownish-colored edges, dark colored green after green	1,83 cm	Segmented	Conidiospore	*Aspergillus*

Identification of Fungi Isolates

Measuring the diameter of each colony fungal isolates, Based on the result of the isolation of *B. Germanica* entomopathogenic fungus grown on artificial medium PDA, obtained five different isolates.

Tabel 3. An average mortality of German Cocoroach.

Isolate	Day										Total	Percentage
	1	2	3	4	5	6	7	8	9	10		
1	0	0	0	0	0	0	1	1	2	2	40	40 %
2	0	0	0	0	0	0	1	1	2	2	40	40 %
3	0	0	0	0	0	0	1	1	2	2	40	40 %
4	0	0	0	0	0	0	0	1	2	3	60	60 %
5	0	0	0	0	0	0	0	1	1	1	20	20 %
Control	0	0	0	0	0	0	0	0	0	0	0	0

Fifth fungal isolates have an average diameter different colonies. Colony diameter was calculated when the culture was seven days. Data on average colony diameter can be seen in Table 1.

Large average diameter of the largest colonies are colonies isolates 1, while the average diameter of the smallest colonies are colonies isolates isolates 5.

The identification of each fungal isolates

An overview of each microscopic fungal isolates were observed in the microscope can be seen in Figure 2. Observations on the whole of the characteristics of each isolates obtained are presented in Table 2.

Based on the data in Table 2, it can be seen that the fungal isolates were grouped into two groups of fungal isolates based on whether there is a bulkhead on hyphae. According Pelczar & Chan (1981), the characteristics of the fungus class divided by the presence or absence bulkhead on the mycelium. Fungi that do not have insulation on miseliumnya put

into the class Zygomycetes, while a fungus that has insulation on miseliumnya inserted into one of the classes Ascomycetes, Basidiomycetes or Deuteromycetes. Isolates 1 is including fungal isolates that have not insulated hyphae, while isolates 2, 3, 4, and 5 were incorporated into the group of fungal isolates that have insulated hyphae.

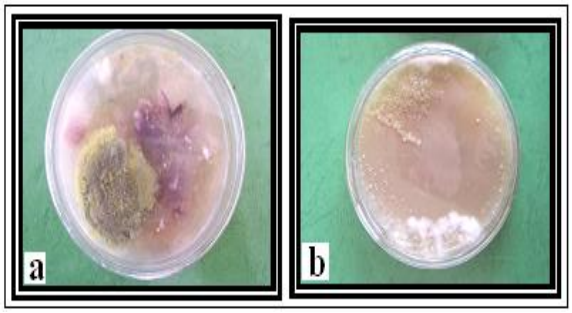

Fig. 1. Entomopathogenic Isolate in PDA (a) A petri disc composed of 5 isolat colony; (b) A petri disc composed of 3 isolat colony.

Isolates 1 belong to a class Zygomycetes, because it has characteristics include: hyphae are not insulated, resulting in a form of asexual spores sporangiofor rounded at the ends, colonies are white with loose texture and delicate as cotton and form colonies spread. Malloch (1997) states that the genus Mortierella colonies have a relatively fast-growing and often overlapping, white hyphae and length, and usually produces spore that are round and lightly browned. According to Ellis (2005), the genus Mortierella belonging to the family Mortierellaceae, the characteristic color somewhat grayish to gray slightly yellowish, colony growth very quickly by producing sporangia were small and very little columella, spore usually without columella (Onions *et al.*, 1981), sporangiospores simple or branched. Sporangiafor consists of one or many spores.

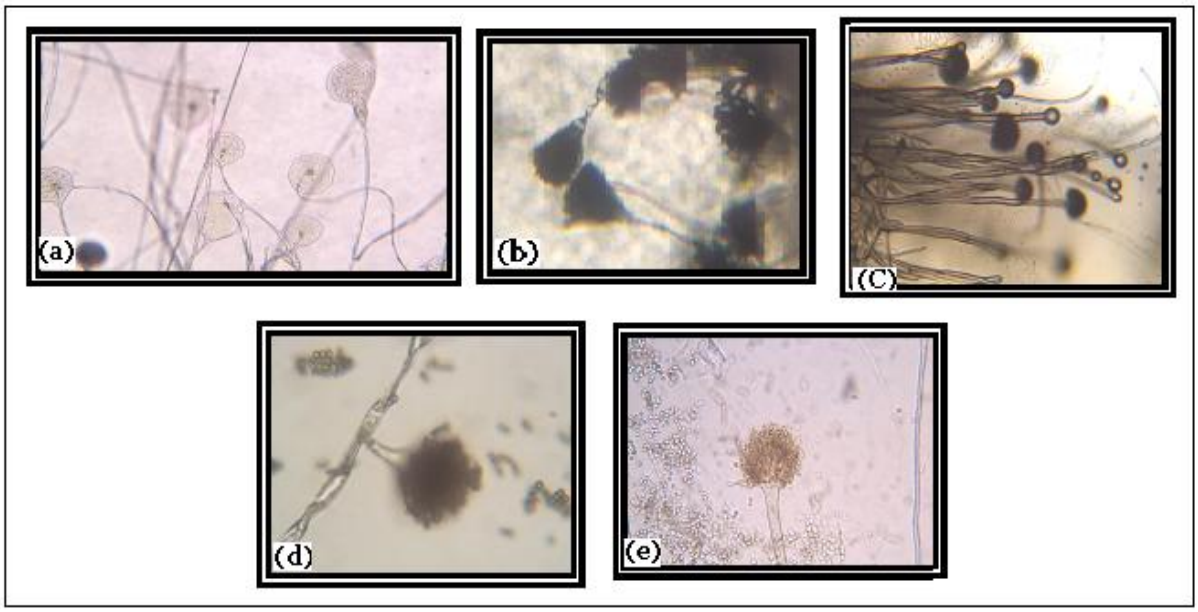

Fig. 2. Entomopathogenic on microscopis images (400X)(a) isolate 1, (b) isolate 2, (c) isolaet 3, (d) isolate 4, (e) isolate 5.

Based on these data, it can be seen that all fungal isolates obtained have potential as entomopathogenic fungus. 1 isolates can infect German Cockroach with a value of mortality by 40%. This is consistent with the statement Kang *et al.* (1998) which enter into the genus Mortierella microorganism produces chitinase enzyme that can degrade chitin with both compounds which is one of the elements forming the insect cuticle.

The German cockroach who have experienced death and then left a few days, the mycelium fungus began to appear cockroach body wrap. Hashim *et al.*, (2005)

states that the mushroom looks out of the insects infected and after a couple of days later the body surface of infected insects will be covered by a mass of white fungus. According to Zurek *et al*. (2002), fungal hyphae will continue to grow until the whole body is full of insects by fungal mycelia. When the internal network has been depleted to digest, will penetrate the cuticle and fungal sporulation, which makes it appear hairy. It is known also that a cockroach has been overgrown by fungi have a very fragile body. She and Feng (2004) suggested that the inclusion of fungus on the host's body is through the integument, digestive tract, spiracles and other openings.

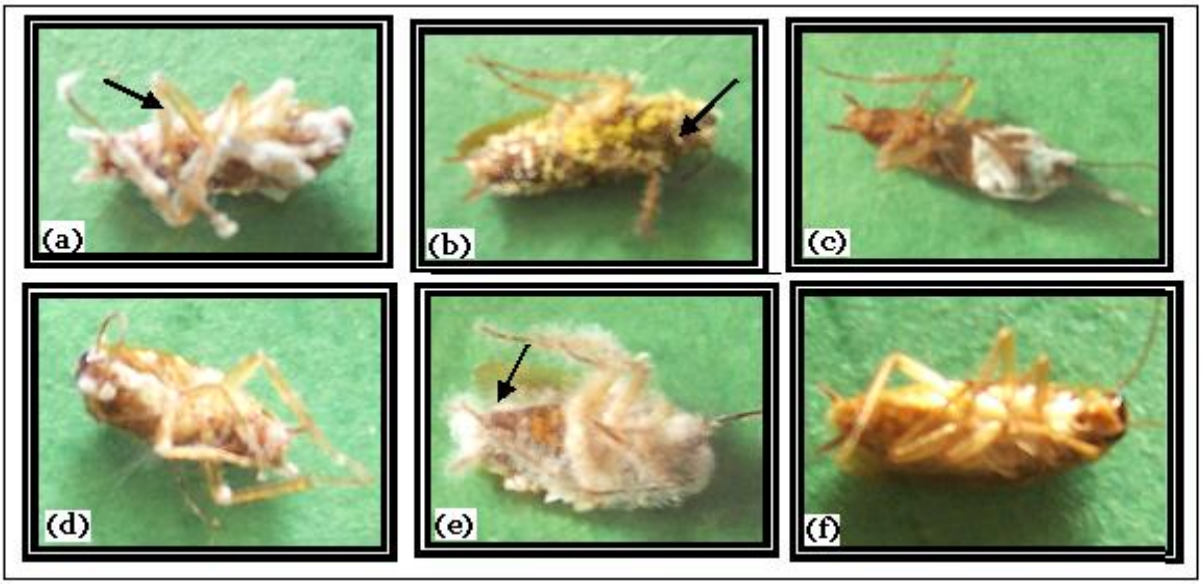

Fig. 3. German cocroach infection on different Isolat (a) isolate 1; (b) isolate 2; (c) isolate 3; (d) isolate 4; (e) isolate 5; (f) control.

According to Zurek *et al.* (2002), cockroaches as living things contain a variety of proteins, or lipids in the body that can be utilized by fungi as food. Therefore, the cockroach's body is used as a medium to grow new fungus. According to Samson *et al.* (1988), some members of the genus Aspergillus belong to the species of entomopathogenic fungi. Kang *et al.* (1998) also states that the genus Aspergillus belongs to the pathogenic microbes to insects as Aspergillus can produce chitinase enzymes that can degrade chitin compound fine.

The genus Aspergillus includes over 185 species. About 20 species so far reported are agents that are pathogenic, among others, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus clavatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus oryzae, Aspergillus terreus, Aspergillus Ustus, and Aspergillus versicolor* Onions *et al*., (1981) states that the genus Aspergillus is a group that produce mycotoxins such as aflatoxin. After inoculate of isolates 2, 3, 4 and 5, cockroach death and after standing for a few days began to appear in her white fungal mycelium (Figure 3).

Based on the results, it can be seen that there are five types of fungal isolates were isolated and identified from dead German cockroaches with insect bait methods are grown in soils derived from three stations in Bandung. The results show that isolation, a fungus that grows only on soil with higher humidity.

The results show that the identification of the isolates belong to the genus Mortierella because it has not insulated hyphae with sporangiosporea as reproduction aseksualnya. Isolates of two, three, four, and five belong to the genus Aspergillus hyphae as it

has insulated with conidiospore as asexual reproduction.

The test results showed that the pathogenicity of fungal isolates obtained have pathogenic properties of the German Cockroach. The concentration of spores that are given in this study amounted to 10^7 spores / ml. The average mortality varies each isolate. Isolates one has a value of mortality by 40%, two and three isolates had similar mortality value is 40%, isolates 4 has a value of mortality by 60%, and isolates five mortality has a value of 20%.

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