Preliminary study of entomopathogenic fungi from termite nests

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Abstract. The purpose of this study was to develop inventory system of potentially entomopathogenic fungi from the termites’ nests at Seulawah Ecosystem, Aceh, Indonesia. The identification and pathogenicity test of the entomopathogenic fungi were conducted at Zoology and Microbiology Laboratories, Syiah Kuala University. We found that a potential entomopathogenic fungus, Aspergilus sp., from nest of termite (Nasutitermes spp.). Laboratory studies have shown that this entomopathogenic fungus affected to control termite Coptotermes sp. Aspergilus fungus has a great potency to be applied as a biological control agent against the termite.

1. Introduction
Termites are a group of insects that have the potential to destroy wood in buildings in Indonesia. Coptotermes termites are destructive soil termites in damaging woody buildings, agricultural and forestry plants in the world, especially in Indonesia [1]. The losses caused by termite attacks can reach 224 – 238 billion rupiah each year in Indonesia [2]. Therefore, a control handling system is needed to reduce losses caused by termite pests.

Termites belong to eusocial insects capable of living in a population called colonies. Generally, nests are a major characteristic for most eusocial insects [3]. Each individual in the colony cooperate with each other to form a nest. Some nest functions for termites are as a shelter from the effects of...
extreme conditions, as a chamber for reproductive and non-reproductive castes, as well as substrates to grow fungi which are used as food source for termites in the Macrotermiteinae [4].

The humidity of nest is the determined factor that could allow fungi to grow. Then the interaction among them, such as trophallaxis or grooming, can allow spores of pathogenic fungi to infect healthy termites [5]. Previous entomopathogenic researches have focused mainly on the *Metarhiziumanisopliae* and *Beauveriabassiana*, while research on other potentially entomopathogenic fungi has been limited. In this research we investigated a potentially entomopathogenic fungi that isolated from termite nest in Seulawah Ecosystem, Aceh Province, Indonesia.

2. Materials and methods

2.1 Collecting samples of fungi in the field

Samples were collected by searching the termite nests in Seulawah ecosystem, Aceh Province, Indonesia. A Standardized Sampling Protocol [6] was applied to collect the nest of termites by directly examining rotted wood, litter, and trees that allowed the presence of nests (figure 1). A piece of the nest was cut and wrapped in plastic when fungi was found inside a nest. Furthermore, as many as 5-20 individual termites contained in the nest were put in a collection bottle containing 70% alcohol to identify the termites.

![Figure 1](image_url)

*Figure 1.* The location of research: a) the Sumatran map; b) The area of Seulawah ecosystem, Aceh (Google Earth)

2.2 Fungi isolation

Isolation of the fungi was conducted by cutting termite nest to 1 × 1 cm² section and it was inoculated on PDA media. Four cuts were inserted in a sterile Petri dish. Isolates were incubated using a memmert incubator at 37°C for 24-72 hours. The isolates of fungi were re-inoculated on new PDA media until a pure culture was obtained.

2.3 Identification of entomopathogenic fungi

The isolate of fungi was identified by morphological character. Macroscopic features included colony color, surface structure and growth pattern, and characteristic microscopic of isolates were analyzed based on the form of the spore using the Slide Culture Method. The reference used in assisting the
Identification of the growing entomopathogenic fungi species was "Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species 2nd Edition" [7].

Identification of the termite species was accomplished by refer to [8, 9], and a number of reference collections at Zoology Laboratory, Syiah Kuala University. Microscope digital camera SCMOS was used to get the picture of termites.

2.4 Fungal pathogenicity test
Pathogenicity test was performed by spraying 5 mL of fungi suspension to tested termite as bioindicator [10]. *Coptotermes curvignathus* termite was used in the pathogenicity test being placed on a cotton and filter paper-coated Petri dish. Observations were conducted daily to observe the signs of infection for 7 days. The entomopathogenic fungi found on the body of termites was photographed using Dino-Lite digital microscope to identify the fungus.

3. Results
The results of this research showed three termite nests overgrown with fungi. Two nests were found to be built by *Nasutitermes*. This genus is able to build arboreal nest and inside of fallen trees (figure 2).

![Figure 2. Nests of termite genera *Nasutitermes*: a) arboreal nests; b) the nest inside a fallen tree (*Shorea* sp.)](image)

The third termite nest found from the forest was constructed by *Coptotermes*. The termite nest was found in stump at 50 cm height from the ground (figure 3). The condition of this nest showed that it filled with fungi growth and the termites were not found.

![Figure 3. Termite nest of *Coptotermes* in dead wood](image)

There were 12 isolates of fungi being successfully cultured from the three nests (1 × 1 cm²). Based on pathogenicity tests of the 12 isolates against *Coptotermes* sp., only four isolates from the arboreal termite nest showed pathogenic potential, while the other isolates were not pathogens (table 1). According to the test, the four isolates fungi (I, II, III, and IV) were identified as *Aspergillus*.
Morphological characteristics of *Aspergillus* colonies on PDA media showed green-faded color with a velvety surface structure. It was suggested [11] that morphological characteristics of *Aspergillus* fungus on PDA media would produce a faded green-colony color with a wave-shaped mycelium growth pattern. The main characteristic of genus *Aspergillus* are the conidial forms. According to [12], the conidial type of *Aspergillus* is globe (round) for *Aspergillus niger* or half-round for *Aspergillus flavus* (figure 2).

Table 1. Data of potentially entomopathogenic fungi from the nest in Alur Mancang

| No. | Type of the Nest          | Nest of Termite | Isolates of Fungi | Pathogenicity |
|-----|---------------------------|-----------------|-------------------|---------------|
| 1   | Arboreal                  | Nasutitermes    | I                 | Pathogenic    |
|     |                           |                 | II                | Pathogenic    |
|     |                           |                 | III               | Pathogenic    |
|     |                           |                 | IV                | Pathogenic    |
|     |                           |                 | I                 | Non-pathogenic |
| 2   | Nest Inside Fallen Tree   | Nasutitermes    | II                | Non-pathogenic |
|     |                           |                 | III               | Non-pathogenic |
|     |                           |                 | IV                | Non-pathogenic |
|     |                           |                 | I                 | Non-pathogenic |
| 3   | Nest in Dead Wood         | Coptotermes     | II                | Non-pathogenic |
|     |                           |                 | III               | Non-pathogenic |
|     |                           |                 | IV                | Non-pathogenic |

Figure 4. Morphological colonies and conidia *Aspergillus*: (a) surface morphology of fungi colony on PDA; (b) morphology of the fungal conidia (enlargement microscope of Swift M10D microscope: 10x40)

Figure 5. The growth of fungus *Aspergillus* in the surface of termites *Coptotermes* sp. body: (a) termites *Coptotermes* sp. infected by *Aspergillus* fungi; (b) conidium (i) and mycelium (ii) fungi growing on the surface of termite body.
The pathogenicity of *Aspergillus* fungus was determined by testing against *Coptotermes curvignathus* and an infected termite was filled with fungal growth and conidium sprouting on the fifth day of observation. According to [13] the time required for fungus to develop and kill the host is 5-7 days. *Aspergillus* on the dead darker, termite body was yellowish-green which corresponds to the description of [14] (figure 3).

*Aspergillus* has great prospect as a biological control agent against insect pests. A study conducted by [15] showed that a species from the *Aspergillus* group, *A. flavus* has potential to be a biological control agent for red spiders (*Oligonychus coffeae* Nietner) attacking tea plants with a 62.22% mortality rate 96 hours after treatment. *Aspergillus* is easy to find in Alur Mancang Area because the temperature and the humidity in this tropical region is good for *Aspergillus* fungal growth 30°C and 86% RH.

4. Conclusion

The pathogenicity fungal isolates from three termite nests found in Alur Mancang area indicated that only isolates from an arboreal nest were potentially entomopathogenic. The characters of yellow-greenish fungal colonies on the body of *Coptotermes* sp. were recorded and characterized. According to morphological characteristics and colonies grown on PDA media as well as conidial morphology we determined that the fungus was classified into genus *Aspergillus*.

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