Inventory of fungi from termite nests at Gunung Leuser National Park, northern Sumatra

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Abstract. Fungi and termite coexisted each other and have diverse symbiotic relationship in tropical forest. This research aims to determine the fungus found on termite nests in Suaq Balimbing Research Station so it can be used that as initial information in developing environmentally friendly termite control. The method used for collecting samples of fungus is a Standardized Sampling Protocol and for identification used slide culture method. The results showed that 5 of 12 termites nest are invaded by fungi. Based on macroscopic and microscopic observation, fungus species that obtained from termite nests are 2 species, namely: *Penicillium* sp.1, *Trichoderma* sp. 1

1. Introduction
Termites are social insects that build nests to live in. According to Nandika et al., [1] nest is the result of collective activity of individuals in a colony. Several types of termites make their nests in the form of passages in wood such as the Kalotermitidae family. However, there are several types of termites from the Termitidae family that can make hill-shaped nests with very strong and sturdy construction.

Termite nests are formed from organic material obtained from their surroundings, such as soil particles, dirt, and wood. These organic materials are generally digested and excreted through feces [1]. Termites digest organic matter not only to make nests but also to create mushroom gardens. These mushroom gardens are used as a food source in the nest [2].

Fungi can produce enzymes that help break down the structure of cellulose in plant tissues, so that the wood particles can be digested as food [3]. So far, research on fungi in termite nests has been carried out in other places. The dominant fungi found in termite nests are from the genus *Termitomyces*[4]. The types of fungi from the term *Termitomyces* found in termite nests in Tanzania are *Termitomycesumkowani*, *T. aurantiacus*, *T. cylpeus*, *T. striatus*, *T. saggitiformis*, *T. mammiformis*, *T. titanicus*, and *T. Lestestui*[5]. Other types of fungi apart from the genus *Termitomyces*, other types were found, namely *Pseudoxylaria*, *Trichoderma*, and *Coriolopsis* found in Mookgophong, South Africa [6]. The fungus found in termite nests is thought to have an antagonistic relationship to termites, so that the fungus produced can potentially be a pathogen for termites. The absence of information on the types of fungi in termite nests in Aceh is the reason for this study. Therefore, this study is expected to obtain data on the types of fungi found in termite nests at the SuaqBalimbing Research Station, GunungLeuser National Park.
2. Materials and Methods
This research was conducted using the Standardized Sampling Protocol method [7] by making a plot of 50 m x 50 m which was then divided into 5 m x 5 m. Collecting mushroom samples in the field by finding nests in each plot. The nests overgrown with mushrooms are then cut. The termite nest pieces are put in a plastic container that has been sterilized using 70% alcohol and given a label. Sampling is also carried out on termites. A total of 15-20 individual termites were taken and put in sample bottles filled with 70% alcohol. Then given information on the label paper and then put it in a storage container.

Isolation of fungi is done by cutting the collected termite nests into smaller sizes ± 1cm. The termite nest pieces were then planted in PDA media 4 pieces in one plate and incubated for 24-72 hours at 35° C. The growing fungal isolates were isolated back into new media to obtain pure culture. Fungi identification is done macroscopically by observing colony color, colony surface shape, and distribution pattern. Microscopic identification by observing the shape of the fungal conidia using a slide culture technique. The reference book used to help identify fungi is the "Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species" [8], A Laboratory Guide to The Common Aspergillus Species and Their Teleomorphs [9], A Laboratory Guide to Common Penicillium Species [10], and the journal Identification Key for Aspergillus Species from Maize and Soil of Nandi County, Kenya [11]. Termite identification was carried out by referring to termites available in the termite collection room of the Zoology Laboratory, Syiah Kuala University and the Termites of Peninsular Malaysia reference book [12].

3. Result and Discussion
The results of the research at the SuaqBalimbing Research Station found 12 termite nests which were collected from 28 subplots. Of these, only 5 termite nests are overgrown by fungi. Only two types of nests were found, namely: wooden nests and arboreal nests. There were 4 wood nests obtained, while only one arboreal nest was found. The wooden nests that were found came from rotten wood and dead tree stumps. The weathered wood that is used as a nest generally comes from tree branches. These termite nests are found on the ground and surrounded by litter (Figure 1.a). According to Horwood and Eldridge[13] subterranean termites build nests from stumps or near dead tree stumps. According to Bignell and Eggleton[14], the termites of the Rhinotermitidae family are termites that build nests in wood.

Arboreal nests were found under a lime tree (Dryobalonopsaromatica) with a height of about 30 cm and a diameter of 70 cm. According to Kuswanto and Pratama[15] Nasutitermes termites prefer to form nests in trees that have many branches, because the nests will be stronger if there are branches or trunks. According to Ningsih et al.,[16] Nasutitermes is a subterranean termite that has arboreal nests which are formed from a mixture of soil, wood litter and feces.

Figure 1. Types of termite nests found (a). wooden nest (red arrow); (b) fungi in the arboreal nest after the nest has been opened (yellow arrow).
The fungi isolated from the wood nest were obtained six isolates from the genus *Penicillium*, and four isolates from the *Trichoderma* clan. Fungi found in termite nests can be seen in Table 1. Fungi isolated from arboreal nests obtained one isolate from the genus *Trichoderma*, while in wood-type termite nests, both genera of fungi were obtained. Wójcik and Andres [17] found that the fungi found in the wood nests of *Reticulitermes lucifungus* (Rhinotermitidae) were from the genera *Aspergillus, Penicillium, Trichoderma, Fusarium, Alternaria, and Rhizopus*.

**Table 1.** The diversity of fungi found in termite nests in the Suaq Balimbing.

| No | Fungi     | Speceis     | Nest of termite | Nest type |
|----|-----------|-------------|-----------------|-----------|
| 1  | *Penicillium* | *Penicillium* sp. | *Hospitalitermes* | Wood      |
|    |           |             | *Odontotermes*   |           |
|    |           |             | *Schedorhinotermes* |           |
| 2  | *Trichoderma* | *Trichoderma* sp. | *Coptotermes*     | Wood      |
|    |           |             | *Odontotermes*   |           |
|    |           |             | *Schedorhinotermes* |           |
|    |           |             | *Nasutitermes*   | Aboreal   |

3.1. *Penicillium* sp.1

Macroscopic observations on PDA media obtained white colonies. Colony surface texture soft like cotton. The back of the colony is white (Figs. 2. a and b). The growth pattern is spreading. According to Pitt [10] several types of *Penicillium* produce a cotton-like layer on the surface of the agar which is called floccos. According to Sharma and Pandey [18] *Penicillium* sp. produce many conidia when the incubation period is longer than seven days.

![Figure 2](image_url)

**Figure 2.** Colony morphology of *Penicillium* sp. (a) the surface of the colony; (b) the back of the colony; Microscopic structure of *Penicillium* sp. (c) conidia with phialides in the form of Biverticillate: (1) conidiospores; (2) phialide; (3) metulae; (4) ramuli (branching); (5) conidiophores; (d) conidia with phialides in the form of terverticillate (magnification 10 x 20).
Microscopic observation showed that the fungus Penicillium sp. has 2 kinds of conidiophore branching patterns, namely biverticillate and terveticillate (Figure 6. c and d). Biverticillate is a form of phialide which has one branching stage, whereas terveticillate has two stages of branching. This fungus has conidiophores which are septa (insulated) and metulae before phialide. Several types of Penicillium produce a cylindrical phialide shape and a sopherical conidia [10].

3.2. *Trichoderma* sp.
Macroscopic observation on PDA media of fungi suspected of *Trichoderma* sp. experiencing colony color changes. The second day of incubation, the colony showed white color, and over time the colony turned green and had a floury texture (Figure 3a). However, some isolates remained white over seven days of incubation and had a cotton-like texture (Figure 3c). The back of the colony was grayish green, while the white colony had a white back (Figure 3b and d).

![Figure 3](image_url)

**Figure 3.** Colony morphology of Trichodermasp. (a) and (c) the surface of the colony; (b) and (d) the back of the colony. Microscopic structure of Trichoderma sp. (e) conidia colony forms green: (1) conidiospores; (2) phialide; (3) conidiophores; (f) white colony conidia (magnification 10 x 40).
Microscopic observation showed that the conidia of *Trichoderma* sp. branched like a creeper. This fungus has hyphae that are septa and there are phialides that branch off at the ends. The tip of the phialide contains conidiospores. According to Watanabe [8] *Trichoderma* has branched conidiophores and has conidiospores on each phialide. Phialides are generally vertical, short, and thick.

4. Conclusion
As many as 12 termite nests were examined; only 5 termite nests were found which were covered by fungi from the genera *Penicillium* and *Trichoderma*. The five genera of termite nests are *Odontotermes*, *Hospitalitermes*, *Schedorhinotermes*, *Coptotermes*, and *Nasutitermes*. Macroscopic and microscopic identification proved that the characters of the two fungal genera were *Penicillium* and *Trichoderma*. The two genera of fungi have the potential to be developed as biological agents for termite control in an environmentally friendly manner.

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Acknowledgments
We thank the Termite Research Group of Universitas Syiah Kuala for asistance in the field and laboratory. We also thanks for the Forestry Department in Tapak Tuan, Head and Staff of Suq Balimbing Field Station for various help. This work was partly supported by funds from Thesis Master 2020 Research Grant (Leader Syaukani) of DRPM RISTEKDIKTI.