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Diversity of Entomophatogenic Fungi from Gunung Tukung Gede Nature Reserve

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A B S T R A C T

Background: Entomopathogenic fungi are one of the potential biodiversity assets to be used as biological control agents. However, information about the diversity of entomopathogenic fungi, especially in the Gunung Tukung Gede (GTG) nature reserve, is very limited. This study aimed to provide information on the biodiversity of entomopathogenic fungi in the GTG nature reserve. **Methods:** The entomopathogenic fungi were explored from 3 different stations: primary forest, secondary forest, and disturbed forest. Entomopathogenic fungi were isolated identified both macroscopically and microscopically. **Results:** A total of 15 specimens of entomopathogenic fungi consisting of 5 families and 8 types of fungi were successfully isolated. The entomopathogen fungi were *Basidiobolus haptosporus, Beauveria bassiana., Metarhizium aniesophalie, Paecilomyces sp., Aschersonia sp., Aspergillus sp. 1, Aspergillus sp.2, and Septobasidium sp*. These fungi infect insect hosts from the orders Lepidoptera, Hemiptera and Homoptera. The index of the diversity of entomopathogenic fungi at 3 consecutive stations is 1.5495; 1.3322; and 0.6365 (medium category). **Conclusions:** The GTG Nature Reserve has a diversity of entomopathogenic fungi. Therefore, further research is needed to determine the diversity and potential utilization of the existing entomopathogenic fungi.

Keanekaragaman Fungi Entomopatogen Lokal Asal Cagar Alam Gunung Tukung Gede

Kata kunci:
Cagar Alam;
Gunung Tukung Gede;
Cendawan Entomopatogen;
Keanekaragaman;

A B S T R A K

Background: Fungi entomopatogen merupakan salah satu aset keanekaragaman hayati yang potensial untuk dimanfaatkan sebagai agen pengendali hayati. Akan tetapi, informasi mengenai keragaman fungi entomopatogen terutama di cagar alam Gunung Tukung Gede (GTG) sangat terbatas. Tujuan dari penelitian ini adalah untuk menyediakan informasi mengenai keanekaragaman hayati fungi entomopatogen di cagar alam GTG**. Metode:** Cendawan entomopatogen dieksplorasi dari 3 stasiun berbeda yaitu hutan primer, hutan sekunder dan hutan perambahan. Fungi entomopatogen ditumbuhkan dalam medium buatan dan diidentifikasi secara makroskopis dan mikroskopis. **Hasil:** Berdasarkan hasil eksplorasi diperoleh 15 spesimen fungi entomopatogen yang terdiri dari 5 famili, dan 8 jenis fungi yaitu *Basidiobolus haptosporus, Beauveria bassiana., Metarhizium aniesophalie, Paecilomyces sp., Aschersonia sp., Aspergillus sp.1, Aspergillus sp.2, dan Septobasidium sp*. Fungi entomopatogen ini menginfeksi inang serangga yang berasal dari ordo Lepidoptera, Hemiptera dan Homoptera Indeks keanekaragaman jenis cendawan entomopatogen pada 3 stasiun berturut-turut adalah 1.5495; 1.3322; dan 0.6365 (kategori sedang). **Kesimpulan:** Cagar alam GTG memiliki kekayaan fungi entomopatogen. Oleh karena itu, penelitian lebih lanjut diperlukan pada berbagai wilayah GTG lainnya yang belum tereksplorasi guna mengetahui keragaman dan potensi pemanfaaatan fungi entomopatogen yang ada.

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Introduction

The nature reserve is a natural protected area that has distinctive flora and fauna. Gunung Tukung Gede Nature Reserve (GTG) is a conservation area located in the Serang Regency of Banten Province. Gunung Tukung Gede (GTG) nature reserve covering an area of 1,700 ha was designated as a nature reserve based on the Decree of the Minister of Agriculture No.395/Kpts/Um/6/1979 dated June 23, 1979 and updated through the Decree of the Minister of Forestry No. SK.3622/Menhut-VII/KUH/2014 dated May 6, 2014 Nature Reserve covering an area of 1,519.50 Ha m (BKSDA, 2016).

GTG nature reserve has two types of vegetation, namely mountain rainforest vegetation types and plant forests that strongly support animals, plants, and microorganisms to grow and develop, so it can be ascertained that this region has a high level of biodiversity. Biodiversity can be a very useful asset. However, there are still various obstacles such as lack of information data related to microorganism biodiversity, especially entomopathogen mushrooms. This causes exploration, identification and inventory of biodiversity activities carried out to be still limited (Purwati, 2013). Therefore biodiversity loss is the most common environmental challenge faced in developing countries such as Indonesia (Adenle et al., 2015). Information related to the diversity of microorganisms, one of which is the group of entomopathogen fungi in gtg nature reserves, is still minimal and has not been widely studied. Meanwhile, entomopathogen fungi have a lot of potentials to be utilized in human life, ranging from antibacterial in the world of medicine (Lee et al., 2005) to as a biological controlling agent in the agricultural world (Evans et al., 2018).

As a bio-controlling agent, entomopathogen fungi become the primary regulator of insect populations in integrated pest management strategies and become effective in their application compared to chemical insecticides because they do not cause resistance in controlling pests (Jiang et al., 2020). Entomopathogen fungi have mycelium that overgrows colonizing insects as their host. The fungi can also produce toxins (Davari et al., 2015) specific to certain target insects, and the side effects for non-target organisms are so low that they do not kill useful insects. In the infection process, entomopathogen fungi can also produce secondary metabolites or production of extracellular enzymes. The production of secondary metabolite compounds and extracellular enzymes by entomopathogen fungi (Ismail et al., 2020) can suppress the population of plant-damaging pest insects (Chandrasekaran, R. et al., 2012).

Based on the results of several studies, there are more than 700 types of entomopathogen fungi represented by 90 genera (Goettel et al., 2010) that have been widely studied in the Asia Pacific region. The types of entomopathogens mostly found in insects are Beauveria and Metarhizium. Two kinds of mushrooms infect insects from the groups, often Lepidoptera, Homoptera, Coleoptera and Diptera (Khastini et al., 2019).

The purpose of this study is to provide information on the biodiversity of entomopathogen fungi in gtg nature reserves that are the basis for further studies of the potential use of these fungi in the future.

Methods

This entomopatogen mushroom diversity research was conducted from March 2019 to October 2020. The sampling

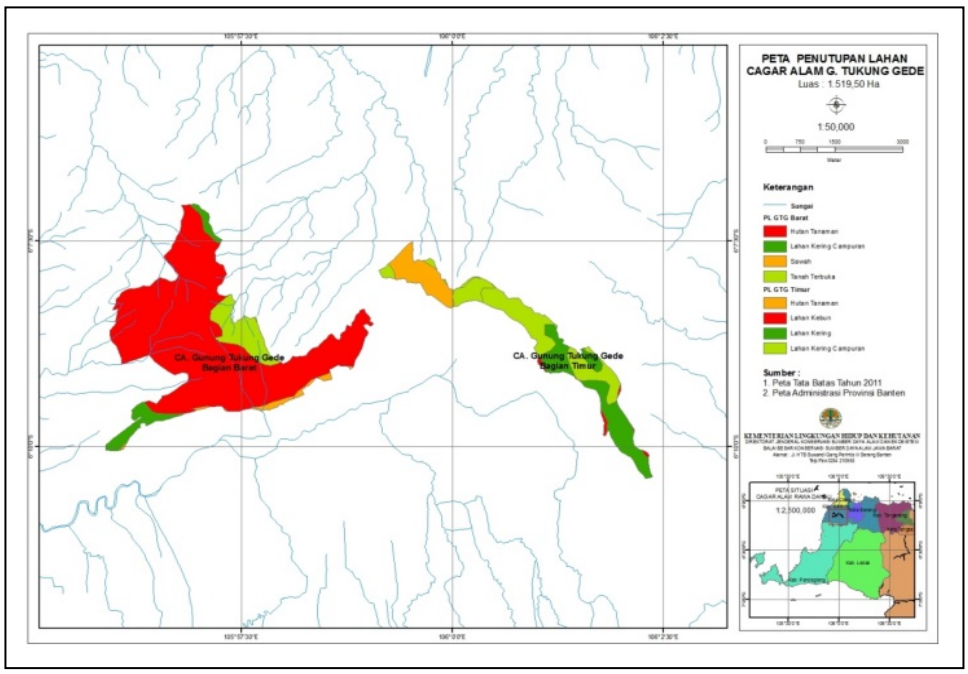

Figure 1. location of the GTG nature reserve

was conducted at GTG Nature Reserve, Serang Regency, Banten Province, which is geographically located at 6°14' - 6°20' LS and 105°52' - 105°57' BT (Figure 1).

The method for selecting observation areas is to use the cruising method at three different stations, namely primary and secondary forests and encroachment areas in gtg nature reserves. These three stations were selected as sampling locations considering that environmental conditions influence the diversity of entomopathogen mushrooms. Environmental parameters in the form of temperature, relative humidity and light intensity at the sampling site are measured and documented (Table 1).

Table 1. Conditions of the location of the sampling plot in the Tukung Gede Mountain nature reserve

Station	GPS	Temp (°C)	Relative humidity (%)	Light intensity	Vegetation character
HP	6°07'58.1"LS-106°00'23.1"BT	28	92	1323	The dominating plant at the site is the mahogany tree (*Swietenia macrophylla*), *Pulus (Laporta stimulant),* and *Burahol (Stelechocarpus burahol)*
HT	6°08'13.6"LS-106°00'15.0"BT	31	69	1441	The dominant plant in the location is the Bayur Tree *(Pterpspernum javanicum)* and durian (*Durio zibethinus*)
P	6°08'48.9"LS-106°01'45.8" BT	32	52	1570	The dominant plant in the location is plantation crops, namely Cocoa *(Theobroma cacao),* Melinjo *(Gnetum gnemon)* and Coffee *(Coffea canephora)*

Ket: HP: Primary forest, HS: Secondary forest, P: Encroachment forest

Sampling is carried out on entomopathogen fungi that live parasites on insects as hosts, found in parts of plant organs such as leaves, twigs and stems, and litter on the forest floor. Specimens that have been successfully found in research locations, then documented with their natural habitat, then stored in paper bags are then taken to a laboratory for identification.

Isolation and identification of entomopathogen fungi is carried out in the Laboratory of Biological Education, FKIP, UNTIRTA through direct isolation techniques that scratch the fungal spores that grow on the surface of the host's body in the growth medium Pottato Dextrose Agar (PDA) (Luangsa-ard et al., 2006). The cup containing the isolate is then stored in a closed box with a temperature of 25o C. Spores that have grown are then transferred to a new PDA medium to get pure breeding.

Identification is made through macroscopic observation and microscopy. Macroscopy is observation in plain sight, including the color, shape and size of the stroma, the color and size of the cinema and its host. Microscopic observations are made by making thin slices of specimens, then made preparations with methylene blue colouring to be observed using a microscope (Pratiwi, 2012). Microscopic observations include the shape and size of peritesium, ascetics and ascetics, the colour and shape of picnidium and dium. Identification refers to Luangsa-ard et al., (2008) based on the reproductive structure of fungi found in the bodies of insect animals.

Diversity Index

The identified sample then calculated the value of diversity. Species diversity can be said to be the homogeneity of species and is a hallmark of community structure. The formula used to calculate species diversity is the formula of the Shannon-Wiener Diversity index (Batten, 1976).

$$H' = -\Sigma pi \ln pi; pi = ni/N$$

Note:
H' = Shannon-Wienner diversity index
Pi = Relative abundance of a species
ni = number of individuals of a species
Nt = total number of individuals of all species

Then the H' value obtained is matched with the criteria for the Shannon-Wienner diversity index

H'> 3: High diversity, uniform number of individuals, no dominant species.

1≤ H'≤ 3: Moderate diversity, the number of individuals is almost uniform, there are several dominant species.

H'< 1: Diversity is low, the number of individuals is not uniform, there is a dominant species.

Results

Entomopathogenic fungi are microorganisms that live parasitic on insects that are commonly found in the tropics. Figure 2 shows the morphological variations of fungi that colonize insect hosts, which can be seen from the shape of the mycelium of the fungus on the surface of the host.

Based on the calculation results, it is known that the index numbers for the first and second plots are H´= 1.5495 and H´= 1.3322. Diversity Index of Entomopathogenic Fungi in Mount Tukung Gede Nature Reserve. Note: Station 1=primary forest, Station 2=secondary forest and Station 3=encroached forest.

Figure 2. Morphology of entomopathogenic fungi in insects a. cicadas; b. Centipede; c. aphids; d. caterpillar; e. moth; f. spider scale bar = 1 cm.

Discussion

The entomopathogen fungi were found in three different host orders of insects. According to Ortiz-Urquiza & Keyhani, (2013), entomopathogen fungi can infect an extensive range of host insects. The mechanism of infection is carried out by entomopathogen fungi in the susceptible host through direct penetration in the cuticle as an initial interaction. Entomopathogen fungi have developed tools for adhesion and recognition of host surface cues that help immediate adaptive responses that include the production of: (a) hydrolytic enzymes, assimilation, and/or detoxification including lipase/esterase, catalase, cytochrome P450, protease, and chiinase; (b) special infection structures, e. g. appressoria or penetrant tubes; and (c) secondary metabolites and other metabolites that facilitate infection.

The conditions that support the growth of life's entomopathogen fungi are influenced by the abiotic factors that exist in the environment in which entomopathogen fungi grow. These abiotic factors include physical and chemical factors in the form of temperature, humidity, light intensity, and degree of acidity (pH). Environmental conditions at the 3 sampling stations can be seen in Table 1. Related to this, Purwowidodo, (2015), states that fungi have different conditions to grow especially against sun exposure. This leads to differences in the diversity of the types of fungi that grow at these locations. This varied canopy cover is also supported by a forest area that looks larger than the area at the third station. Koneril & Suroyo, (2012) state that habitat structures and diverse vegetation forms have a positive relationship with the diversity of a species including entomopathogen fungi.

The percentage of diversity of an organism in an ecosystem can be known from the value of H'. The value of species diversity can also be used to measure the stability of existing communities in the environment (Thibaut & Connolly, 2013). Based on the diversity index analysis (H') at the three stations where sampling fungi entomopatogen is medium, two of the three stations, namely station one and station two, have a higher index than the third station. The value of this index indicates the diversity of "moderate" levels. This moderate level of diversity shows that the environmental conditions at both stations are still quite good and support the life of microorganisms in it, especially entomopatogen mushrooms, but there need to be efforts to maintain the sustainability of the entomopatogen mushrooms. Good environmental conditions can support

Table 2. Identification of entomopathogenic fungi in Mount Tukung Gede Nature Reserve

Family	Spesies	Colony color		Hifa	Conidia	Host
		Upper	Button			
Basidiobolaceae	*Basidiobolus haptosporus*	ash-gray	white	Hialin, septum	sphere	Lepidoptera
Clavicipitaceae	*Beauveria bassiana*	white	white	Hialin, no septum	Oval	Lepidoptera
	Aschersonia sp.	white	white	Hialin, septum	sphere	Homoptera
Moniliaceae	*Metarhizium aniesophalie*	gray	gray	Hialin, septum	sphere	Hemiptera
	Paecilomyces sp.	brown	brown	Hialin, septum	sphere	Homoptera
Trichocomaceae	*Aspergillus* sp.1	gray	green	Hialin, no septum	Oval	Hemiptera
	Aspergillus sp.2	purple	purple	Hialin, septum	sphere	
Septobasidiaceae	*Septobasidium* sp.	black	black	Hialin, septum	Oval	Lepidoptera

entomopathogen mushroom spores to germinate, mycelia can grow and develop and the mushroom cansporulate (Lopez & Sword, 2015).

Based on the results of the analysis, it can be known that the difference in numbers is relatively very small, which is 0.2173. This relatively small difference is due to the similarity of the constituent vegetation structures found in primary forests and secondary forests. Both stations have habitat types that are dominated by tall tree types. However, the type of canopy is different, which impacts the intensity of incoming sunlight becomes different.

In contrast to the index values seen on the first and second stations, the diversity index value on the third station is classified as low-level diversity, with an index value of 0.6365. This is because the type of habitat at this third station is an encroachment forest whose constituent vegetation structure is different from primary and secondary forests. This area is dominated by the encroachment of residents around the nature reserve in the form of durian trees (*Durio zibethinus*) and melinjo (*Gnetum gnemon*). The area of this encroachment forest also appears smaller than the primary and secondary forests. This condition causes the types of plants and insects that live in it to be less, so that the host insects of entomopathogen mushrooms become fewer as well.

The difference in the diversity of entomopathogen mushrooms in these three types of habitats is closely related to abiotic factors. According to Wang et al., (2017), organisms and their abiotic environment are closely related and affect each other. In this case, the growth of entomopathogen mushrooms depends on the temperature of the environment, so entomopathogen mushrooms must be in the appropriate environment.

Research station 1 has the highest diversity index value among other research stations, which is 1.5495. According to Morris et al., (2014), the value of the diversity index is influenced by two components, namely the number and merity of species. The study's diversity index tends to be high because no species dominates at each station. It is also supported by adequate abiotic factors. Temperature and humidity in this area is also enough to support the growth of entomopathogen mushrooms. The second research station had a diversity index value that tended to be low, although it was still at a moderate criterion. The diversity index at this station is only 1.3322. The low diversity index at this station is due to the area of the station which is a secondary forest. Secondary forests in the tukung gede mountain nature reserve have canopy cover that tends to be less dense, so the temperature in the area is quite high.
This high temperature also produces low humidity, so the growth of entomopathogen mushrooms in the area is less than optimal. This is evidenced by only found as many as 4 types of entomopathogen mushrooms. Barlow, J. et al., (2007), stated that naturally regenerating secondary forests can provide conservation services like primary forests but cannot match their biodiversity value.

Station 3 has the lowest diversity index among other stations. This is because the area of station 3 is an encroachment area that residents around the GTG Nature Reserve often visit. This area is adjacent to the settlement of residents so often the surrounding residents who live near the nature reserve area pass around the area to disrupt the growth of entomopatogen mushrooms. In the forest, encroachment is also often given by motor vehicles from the surrounding residents, causing air conditions that are bad enough for the survival of entomopatogen mushrooms.

Conclusion

This research has provided essential data on the diversity of entomopathogen fungi whose diversity and distribution of mosses are strongly influenced by environmental conditions. The results of exploring the diversity of entomopatogen mushrooms in gtg nature reserve obtained 15 samples consisting of 8 types of mushrooms. These entomopathogen fungi infect insect hostes of the orders Lepidoptera, Hemiptera and Homoptera. The index of entomopathogen mushroom diversity at three stations in a row is 1.5295; 1.3322; and 0.6365 (medium category). The results of research on the diversity of entomopathogen mushrooms in GTG Nature Reserve can be used as a basis for information for research and development of potential entomopathogen mushrooms that are useful for human life.

Declaration statement

The authors reported no potential conflict of interest.

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