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Research Article

Exploring the Diversity of Arbuscular Mycorrhizal Fungi in Zingiberaceae Family Plants at the Tukung Gede Mountain Natural Reserve

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ABSTRACT
Arbuscular mycorrhizal fungi (AMF) are essential in improving soil quality and facilitating plant nutrient and water uptake through mutualistic associations. However, limited research exists on the diversity and distribution of AMF associated with plants in the Zingiberaceae family, especially in unique ecological habitats such as the Tukung Gede Mountain Natural Reserve. This study aims to assess and document the diversity of AMF linked to Zingiberaceae plants in this reserve. Sampling was performed at three locations with distinct plant compositions to explore the diversity of AMF genera. Soil samples were processed using a wet sieve technique, while root samples were chemically stained to evaluate AMF colonization. Key parameters studied included diversity indices, spore density, genus-level identification, and root colonization rates. The findings revealed the presence of eight AMF genera: *Sclerocystis*, *Septoglomus*, *Acaulospora*, *Gigaspora*, *Glomus*, *Scutellospora*, *Racocetra*, and *Rhizophagus*, identified based on spore morphology. Root staining revealed structural AMF infections, including vesicles, internal hyphae, and arbuscules. *Zingiber officinale* exhibited the highest AMF colonization rate (88 %) among the Zingiberaceae plants studied, whereas *Zingiber zerumbet* had the lowest (56 %). *Etlingera Solaris* and *Zingiber officinale* showed the highest spore densities at 172 and 254 spores per 100 g of soil, respectively. AMF diversity indices varied across locations, with values of 0.78 (Station I), 0.95 (Station II), and 0.84 (Station III). This research emphasizes the extensive AMF diversity within Zingiberaceae plants and its potential importance for conservation and ecological sustainability.

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INTRODUCTION

Protecting biodiversity has developed a universal importance in the twenty-first century, especially concerning agriculture. Global issues are severe because of the destabilizing consequences of climate change, economic instability, and biodiversity loss (FAO 2020; Kuila & Ghosh 2022). The Zingiberaceae family of plants is very adaptive, so protecting agricultural variety in this complex environment is imperative. Liang and Chen (2021) assert that these plants are essential for promoting a healthy and ecologically sustainable lifestyle. They are highly valued for their nutritional content and therapeutic potential because of their abundance of essential oils and polyphenols (Guerrini et al. 2023). Zingiberaceae are vital crops utilized in traditional medicine, the culinary arts, and cosmetic formulations; their significance extends beyond one's health.

The Zingiberaceae plant development and health are intimately connected to the activity of mycorrhiza, particularly arbuscular mycorrhiza. Mycorrhiza refers to the symbiotic association between fungi and plant roots, which is a common and critical component of natural ecosystems particularly arbuscular mycorrhiza. This relationship plays a vital role in plant health and soil fertility, with mycorrhizal fungi colonizing root systems and forming structures such as arbuscules, vesicles, and hyphal networks. In natural environments, mycorrhizae enhance nutrient cycling (Han et al. 2023) by increasing plant access to essential nutrients like phosphorus (Chiu & Paszkowski 2019), aid in water absorption (Qiao et al 2023) through extensive hyphal networks, improve soil structure by binding soil particles, and bolster plant health by forming protective barriers against pathogens (Kaur & Suseela 2020).

The characteristic growth of arbuscular, tree-like structures at the fungal hyphae terminals sets AMF apart. This fungus is surprisingly linked to about 80 % of terrestrial plants; in higher plant species that can grow in a wide range of environments, this percentage increases to 90 % (Alrejhei et al. 2021; Suharno et al. 2022). The mycorrhiza found in this habitat belongs to the Zingiberaceae family, which is found in tropical rainforests (Peng et al. 2022).

Specifically, one of these tropical rainforests is the Gunung Tukung Gede Nature Reserve in Banten Province. Indonesia is a global hotspot for biodiversity (Pironon et al. 2020). This verdant region spans 1519.50 hectares and is part of the A-type climate, distinguished by heavy annual rainfall with an average of 2151 mm (Banten Provincial Environmental and Forestry Service 2018). This climate causes this area to have a high level of biodiversity. Research related to AMF symbiosis in plants of the Zingiberaceae family has been conducted by Santos et al (2010) for a herbs spesies of *Zingiber officinale* ROSCOE. Additionaly Pandey et al. (2020) conducted a study for mycorrhiza in India on two types of ginger plants, *Zingiber montanum* and *Zingiber officinale* species. However, information on the diversity of arbuscular mycorrhizal fungi in plants of the Zingiberaceae family is still minimal. There has yet to be research related to AMF in the Mount Tukung Gede Nature Reserve.

Because these plants maintain the delicate balance of ecosystems, there is a solid connection between environmental protection and the Zingiberaceae family. Zingiberaceae plants are essential to biodiversity conservation methods because of their adaptability, particularly in natural reserves. Within these areas, sustainable agriculture methods that put the cultivation and protection of the Zingiberaceae family first can help create a harmonious relationship between human activity and the environment, which is in line with the overarching goal of responsible land management. Therefore, by emphasizing the relationship between biodiversity, agriculture, and environmental sustainability we can promote practices that support ecological balance and

the well-being of both natural ecosystems and human communities. The study's findings can serve as a scientific foundation for future investigations into various arbuscular mycorrhizal fungi that affect Zingiberaceae plants, which are more prevalent in the Mount Tukung Gede Nature Reserve. Understanding the diversity of Arbuscular Mycorrhizal Fungi (AMF) in this specific habitat is essential to enhancing our comprehension of the intricate relationships affecting ecological dynamics and plant health. Moreover, the increasing threats to biodiversity and global ecosystems emphasize the pressing need for these investigations. Examining the nuances of AMF in Zingiberaceae plants may offer fresh perspectives on long-term conservation tactics and essential data to the broader scientific community. Consequently, this expands our understanding of plant-fungal interactions and their impact on ecosystem resilience

MATERIALS AND METHODS

Materials

The materials and tools used in this study are as follows: digital camera (Canon EOS 1100 D), essential GPS, soil meter, thermometer, lux meter, Binocular Compound Microscope (Leica DM500), Stereo Microscope (Meiji Techno), preparation glass, petri dish, root samples of Zingiberaceae family from Gunung Tukung Gede Nature Reserve, shallots (Allium cepa), large plastic clips, 10 % KOH, 2 % HCl, glycerol, immersion oil, Trypan Blue, zeolite, hyponex 25-20-5 and distilled water.

Methods

Study Area

The research was conducted between July 2022 and February 2023. Zingiberaceae plant roots and soil samples have been collected from the Gunung Tukung Gede Nature Reserve in the Mancak District of Serang Regency, Banten Province (Figure 1). This region has a type of mountain forest, with June-August being the dry months and September-May being the wet months. The exploration method was employed in this study.

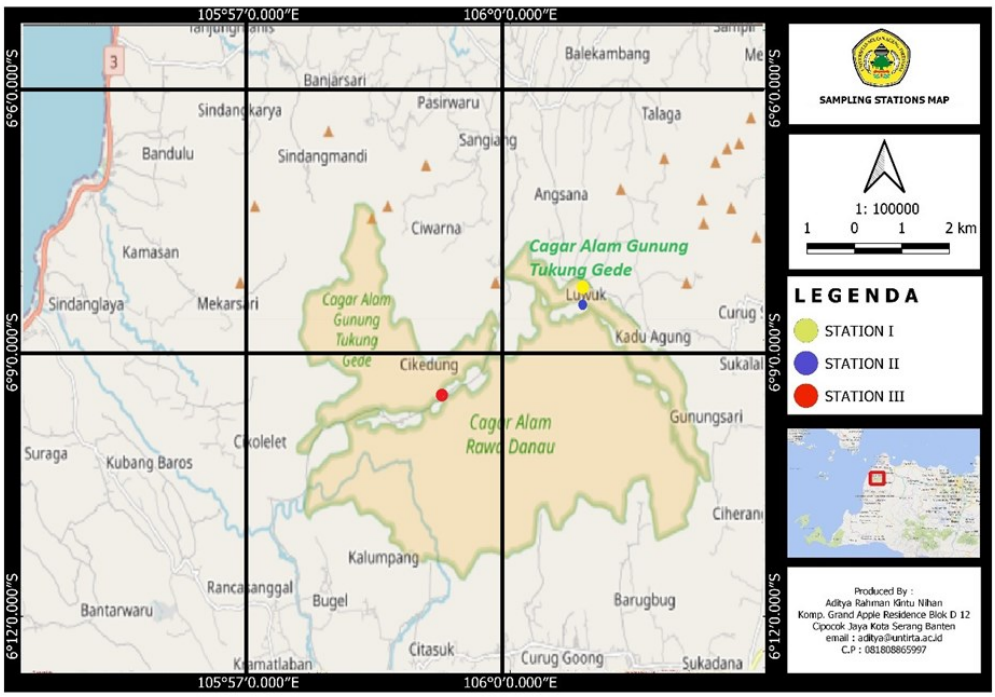

Figure 1. Sampling Stations in Tukung Gede Mountain Nature Reserves.

About three stations were established along the exploration path, each measured 100 m in length and 20 m in width. The selection of research station was based on the abudance of plant species of Zingiberaceae family. Sampling site condition can be seen in Figure 2. The environmental vegetation of Gunung Tukung Gede Nature Reserve was considered when separating the stations. Three stations were established along the exploration path, each measuring 100 meters in length and 20 meters in width, with five plots per station. The selection of research locations was based on the abundance of plant species of the Zingiberaceae family. The environmental vegetation of Tukung Gede Mountain Nature Reserve was considered when separating the stations. Station I and II are located in the eastern part of Mount Tukung Gede Nature Reserve. Station I with closed vegetation conditions at coordinates 6°8.1370'S 106°0.2460'E, while station II with open vegetation conditions at coordinates 6°8.2590'S 106°0.2460'E. Station III is located in the western part of Tukung Gede Mountain Nature Reserve with conditions near residential areas at coordinates 6°8.5220'S 105°59.0700'E. Environmental parameters measured were soil moisture, air temperature, soil pH, soil type, and light intensity.

Figure 2. Sampling site condition: a. Station I, b. Station II, c. Station III.

Sampling Methods

In the location where Zingiberaceae plants were found, designated sample points were established. Five sampling points were taken diagonally across a 5x10 meter plot. Soil and plant roots from the Zingiberaceae family were carefully gathered in samples ranging from 0 to 20 cm deep. With the help of a shovel, around 1 kg of soil was collected, and root samples were put in big plastic clips with openings for air and labeled separately. After that, these samples were sent to the lab for additional processing and analysis. The soil and root samples were stored at room temperature (25 °C) until the isolation began. While root samples were closely examined, soil samples were essential for setting up trapping cultures and assessing spore density.

Mycorrhiza bioassay methods

The root staining approach used in this study follows Brundrett et al. (1966) methodology. Root segments that are between one and two centimeters in length are removed in order to make staining and observation processes easier. Plant root samples are carefully washed under running water to remove any remaining dirt particles and put into test tubes. Immersion of the roots in a 10 % potassium hydroxide (KOH) solution lasts for around 24 hours or until the roots take on a pale or transparent yellow color. After being treated with KOH, roots are rinsed twice or three times with distilled water and then submerged in a 2 % hydrochloric acid (HCl) solution for a further twenty-four hours. This acid treatment is necessary to improve Trypan Blue's absorption capability during the ensuing staining procedure. After staining, the roots are put through a destaining step in which the color is lightened with a 50 % glycerol solution. Arbuscular Mycorrhizal Fungi (AMF)-infected roots are identified and designated for further examination. Brundrett et al. (1996) developed the colonized root length technique to calculate the proportion of col-

onized roots. One or more AMF structures, including hyphae, vesicles, and arbuscules, are markers of colonization. The thorough staining and analytical procedure offered crucial new information on the kind and degree of AMF root colonization in the plant samples under investigation.

$$Root\ Colonization\ Percentage\ (\%) = \frac{\sum marked\ root\ preparations}{\sum number\ of\ root\ preparations}\ x\ 100$$

The centrifugation method (Brundrett et al. 1996) will be employed in conjunction with the wet filter pour technique (Pacioni 1992) as the spore isolation method to extract AMF spores. About 100 g of soil are weighed for each plant sample as the first step in the soil sample separation procedure. Then the soil sample is put in a beaker and 500 ml of distilled water is added, then soaked for ± 5 minutes. Then filtered in a set of stratified mesh sieves with sizes 0.2 mm, 0.15 mm, and 0.075 mm with running water. The soil remaining on the 0.15 mm and 0.75 mm sieves was transferred into a centrifuge tube. Add 60 % glucose solution into the centrifuge tube and start centrifuge Hitachi (Himac CT15RE) at 1200 g rpm for 5 minutes. The centrifuge produced supernatant solution which was transferred to a petri dish. The density of spores was observed on a stereo microscope (Meiji Techno) and a Binocular Coumpound Microscope (Leica DM500) to clearly see the morphology of the spores. After the process of isolating the spores, spore preparations were made and placed on the preparation glass and carefully broken by pressing the glass cover of the preparation using a pen needle. AMF identification is based on the morphological characteristics of its spores (spore arrangement, hyphal shape, spore color, spore shape and spore ornamentation). Microscopic characteristics of spores found were then matched with identification guidelines used by the International Culture Collection of Vesicular Asbuscular Mycorrhizal-INVAM (2022), Brundrett et al. (1996), and other reliable journals.

$$Spore\ Density = \frac{\text{Spore count}}{\text{100 g soil}}$$

Trapping spora AMF

This study also used a trapping culture technique. Trapping culture is a method of capturing Arbuscular Mycorrhizal Fungi originating from the rhizosphere of plants in culture pots to obtain the number and other types of spores and then identified based on the genus level and density (Sefrila et al. 2021). This trapping culture uses the method of Brundrett et al. (1966). The trapping culture used *Allium cepa* host maintained for three months with zeolite media. Plastic pots contain 50 g of zeolite at the bottom, then the middle part contains 100g of soil and put a host of 2-week-old shallot plants, and then the top contains 50 g of zeolite again. Maintain the spores for 2 months with regular fertilizer watering (Hyponex Red nutrient solution 0.05 %) diluted in water. The nutrient solution was applied every 2 weeks, approximately 20 ml per pot after which a stressing process was carried out to stimulate the spores due to the grip of drought.

Diversity Indices

The results of the diversity index data analysis were obtained from the type of AMF genus successfully identified in the soil samples of each plant of the Zingiberaceae family using quantitative analysis. The data obtained from identification is then calculated using the Shannon Weiner formulation, using the following formula (Parwi et al. 2018):

$$H' = -\Sigma Pi\ (Ln.Pi)$$

Description:
H' : Diversity index
Pi : ni/N

(the ratio of the number of individuals of the i-th species to the total number)
ni : Number of individuals of the i-th species
N : Total number of individuals
The results obtained are seen based on the criteria:
H' < 1 = Low Diversity
1< H' < 3 = Medium Diversity
H'> 3 = High Diversity

RESULTS AND DISCUSSION

Environmental condition of Study Site

Observational findings reveal different AMF infections are present in Zingiberaceae plant family root samples. About nine plants belonging to the Zingiberaceae family were found in three different locations. Station I, with closed vegetation, found plant species are *Amomum hochreutineri*, *Etlingera solaris*, and *Zingiber zerumbet*. Dusty, loamy soil characterizes the soil texture at station I, and the distance between plants scattered along the cruising path is not close together. These plants grow wild and fertile in the Tukung Gede Mountain Nature Reserve area. Plants at station I are mostly under tall trees so that little sunlight enters. Station II with open vegetation found plant species such as *Curcuma longa*, *Alpinia galanga*, *Amomum hochreutineri*, and *Coctus speciosus*. The soil texture at station II is relatively coarse soil with fine sand. Each plant species was found not close to each other and scattered along the observation path, which is 0-100 m. With vegetation near residential areas, Station III found plant species such as *Zingiber officinale*, *Curcuma longa*, *Curcuma xanthorrhiza*, *Alpinia galanga*, and *Etlingera elatior*. The soil texture at station III is fine sandy loam soil. Plants found at station III grow close to other plants maintained by residents around the Tukung Gede Mountain Nature Reserve area, such as *Musa paradisiaca*, *Manihot esculenta*, etc. Even some plants from the Zingiberaceae family are cultivated by the surrounding community to meet their daily needs.

The average values for every site were combined to assess the environmental factors (Table 1). Arbuscular mycorrhizal fungi (AMF) grow best at a temperature range between 29 °C and 30 °C, as shown by the reported temperatures at each station. Interestingly, there were notable differences in the soil pH at each station: Station I was 4.8, station II was 6, and station III was 5.7, indicating acidic soil conditions. Spore density was found to be greater in somewhat acidic soil. Station I recorded the maximum soil moisture level at 68 %, while stations II and III recorded 40 %. The station values varied. It is essential in dry soil conditions to reduce soil moisture since it might promote AMF spore formation. Station I showed lower levels (211) of light intensity than Stations II (461.5) and III (547). The varying kinds of host plants found at each site were the reason for the discrepancy in light intensity readings. The investigation results showed that stations II and III had more significant concentrations of AMF spores, explained by their slightly acidic soil pH and lower soil moisture levels. The pH and moisture content of the soil were measured with a Soil meter, and the amount of light was measured with a Lux meter. The study focused on the effects of soil properties on spore density, colonization rate, and the overall capacity of AMF to operate in the ecosystems studied.

AMF Infections in Zingiberaceae Plant Family Root Samples

Roots were classified as colonized or infected based on the observation of certain characteristics, such as vesicles, internal hyphae, spores, external hyphae, and arbuscular structures associated with arbuscular mycorrhizal fungus (AMF). Hyphae vesicles, and arbuscules were seen in the root samples. AMF hyphae have a variety of morphologies, including branching, straight,

Table 1. Measurement Results of Environmental Parameters at each station

Environmental Parameters	Station I	Station II	Station III
Coordinates	6°8.1370'S 106°0.2460'E	6°8.2590'S 106°0.2460'E	6°9.295'S 105°58.232'E
Temperature	29 °C	30 °C	30 °C
Soil pH	4.8	6	5.7
Soil Type	Regosol	Regosol	Latosol
Soil Moisture	68 %	40 %	40 %
Light Intensity	211	461.5	547

and H-shaped forms. Determining root colonization or AMF infection required identifying vesicles, arbuscular structures, internal hyphae, spores, and external hyphae in the examined root preparations. An indication of a possible AMF infection in the host plant was the presence of one or more AMF formations in root preparations. Hyphae were the most common AMF structures found in Zingiberaceae plant root preparations. Conversely, vesicles considered uncommon AMF structures were seldom seen in some root preparations and only in particular kinds of roots. Figure 3 presents in-depth observations of root samples.

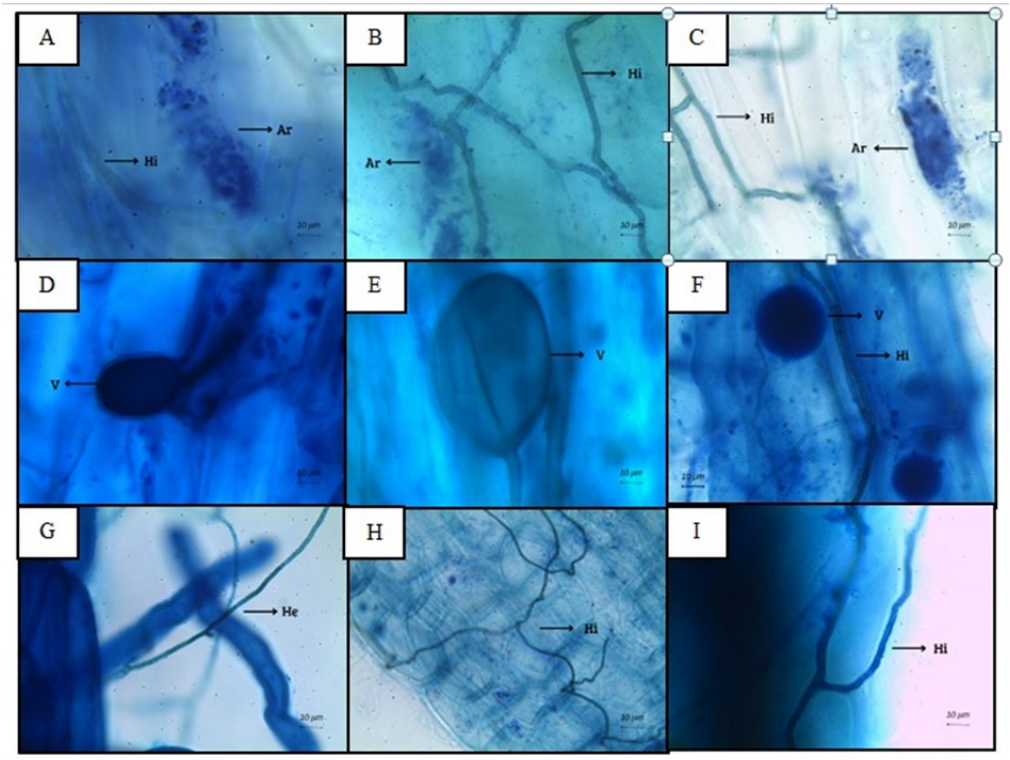

Figure 3. Structure of the Arbuscular Mycorrhizal Fungus; type: V: Vesicle, Hi: Internal hyphae, He: External hyphae, Ar: Arbuscular. A: *Zingiber officinale* roots, B & E*: Etlingera solaris* roots, C, D & H: Roots *of Amomum hochreutineri*, D: F root: *Alpinia galanga* root, G: *Curcuma longa* root, I: *Etlingera elatior* root.

The figure 4 presents a collection of various species zingiberace plant in Mount tukung Gede Nature reserves. Each of these species is not only significant within the Zingiberaceae family but also shows a distinct relationship with AMF, contributing to the understanding of plant-fungi interactions in this group.

As shown in Table 2, the examination of spore densities and AMF (arbuscular mycorrhizal fungi) colonization percentages among various stations and plant species provides important new information on the symbiotic interactions found in the Tukung Gede Mountain Natural Reserve. At Station I, we find that AMF root colonization percentages range from 56 to 66

Figure 4. Collection of Zingiberaceae Plants Associated with AMF: a. *Curcuma longa*, b. *Amomum hochreutineri*, c. *Zingiber officinale*, d. *Etlingera solaris*, e. *Etlingera elatior*, f. *Curcuma zanthorrhiza*, g. *Alpinia galanga*, h. *Zingiber zerumbet*, i. *Costus speciosus*.

%, percentage of colonization showed range 56-66 % and spore numbers from trapping methods range from 172 to 184 spores/100g of soil. In comparison to *Etlingera solaris* and *Zingiber zerumbet* at this station, *Amomum hochreutineri* exhibits the highest colonization rate, suggesting a possibly greater symbiotic interaction with AMF. This shows a somewhat steady but variable AMF presence. Spore densities in the nature (210–242 spores/100g of soil) and col-

onization percentages (70–80 %) are much higher at Station II, where the highest rates of colonization are seen in Alpinia *galanga* and *Amomum hochreutineri*. Information on AMF colonization percentage and spore densities also available in Station III with high AMF root colonization percentage of 88 % and 254 spores/100g in *Zingiber officinale*. *Etlingera solaris* showed the lowes spores (172/100g).

Identification of AMF Spore Genus

Based on the study's results, eight genera were identified in all soil samples. The genus identified were *Glomus* sp., *Septoglomus* sp., *Acaulospora* sp., *Gigaspor*a sp., *Scutellospora* sp., *Sclerocystis* sp., *Rhizophagus* sp., and *Racocetra* sp. Each type of soil sample of Zingiberaceae Family plants had different spore genus results and numbers. The types of genus *Glomus* sp. and *Acaulospor*a sp. were found in all soil samples of plants of the Zingiberaceae family, while the other six types of spores were not all found in each plant of the Zingiberaceae family The type of spores most commonly found in all soil samples of Zigiberaceae plants is the genus *Glomus* sp. The genus *Racocetra* sp. was mostly found in *Curcuma zanthorrhiza* plants. Figure 5 shows the genus data found at each station. Station I, II and III have the same genus of spores obtained are *Glomus* and *Acaulospora*. Station I, II and III have one type of spore in common which is *Gigaspora*. In contrast, stations II and III have five types in common which are *Gigaspora*, *Septoglomus*, *Scutellospora*, *Rhizophagus*, and *Sclerocystis*. *Racocetra* spores were only found at station III.

Examining each species of spores under a microscope reveals distinctive characteristics which can be seen in Figure 6. The germinal walls and spore decorations of *Acaulospora* sp. are characteristic of the genus. Subtending hyphae are a kind of hyphal holder seen in *Glomus* sp. Except for the germinal shield within, the bulbous suspensor of the genus *Gigaspora* sp. is found at the base of the hyphae and shares traits with *Scuttelospora* sp. The genus *Sclerocystis* sp. is characterized by its hyphal plexus and is found in clusters. The *Rhizophagus* genus is distinguished by its spores, which range in color from pale yellow to light brown and have broad subtending hyphae. The spores are round to subglobose and feature a thick, three-layered wall, a pattern consistent with other *Glomus* species (INVAM 2022). In this study we observed 2 species of *Rhizophagus*. *Rhizophagus* sp.1 has very thick, pale yellow-brown spores with cylindrical subtending hyphae, while *Rhizophagus* sp.2 has darker yellow-brown spores and broader subtending hyphae. Both species are found solitarily at Stations II and III, with distinct structural features (INVAM 2022). Among arbuscular mycorrhizal fungi (AMF),

Table 2. Calculation of AMF Colonization Percentage and Spore Density.

Station	Plant Spesies	Vernacular name	Sampel Code	Spores/100g soil		AMF root colonization (%)
				Nature	Trapping Methods	
I	*Amomum hochreutineri*	Kapulaga	KP_T	120	184	66 ± 2.38
	Etlingera solaris	Tepus	TP_T	109	172	62 ± 1.63
	Zingiber zerumbet	Lempunyang	LE_T	143	178	56 ± 1.63
II	*Curcuma longa*	Kunyit	KN_B	189	224	70 ± 1.63
	Alpinia galanga	Lengkuas	LA_B	178	236	78 ± 5.89
	Amomum hochreutineri	Kapulaga	KP_B	147	210	80 ± 2.83
	Coctus speciosus	Pacing	PA_B	224	242	72 ± 2.83
III	*Zingiber officinale*	Jahe	JH_W	153	254	88 ± 1.63
	Curcuma longa	Kunyit	KN_W	160	206	78 ± 1.63
	Alpinia galanga	Lengkuas	LA_W	138	210	82 ± 4.90
	Etlingera elatior	Honje	HJ_W	215	230	74 ± 2.83
	Curcuma zanthorrhiza	Temulawak	TM_W	262	212	70 ± 3.27

Septoglomus is a distinct genus that stands out for having characteristics that set it apart from other genera. In contrast to the clustered spores of many AMF taxa, its spores are usually colored and develop solitarily in the soil, making them easier to recognize. Unlike the more homogeneous architecture of taxa such as *Glomus* or *Rhizophagus*, the spores have a unique multi-layered wall with a hyaline, semi-flexible outer covering that peels with maturity. Moreover, *Septoglomus* spores have cylindrical or funnel-shaped bases with subtending hyphae that complement the color of the spore wall, in contrast to the less uniform hyphal characteristics of taxa such as *Acaulospora*. In contrast to other AMF taxa, the spores are often subglobose and have a more hard inner coating. The *Racocetra* genus is characterized by spores that range from pale yellow to light cream, with a round to subglobose shape and sporogenous cells. These spores possess two adherent layers, providing uniform thickness, with the second layer thickening as the spore grows and the wall differentiates (INVAM 2022). *Racocetra* spp. from research and literature, revealing that immature spores are pale cream to brownish. This genus was found exclusively on Temulawak plants at Station III, making it quite rare. *Racocetra* sp.1 exhibits slightly darker cream-colored, subglobose spores with two thin wall layers and sporogenous cell walls. The sporogenous cell walls consist of two hyaline layers, matching the spore's surface color, and are oval-shaped at the top. These spores are observed in solitary conditions.

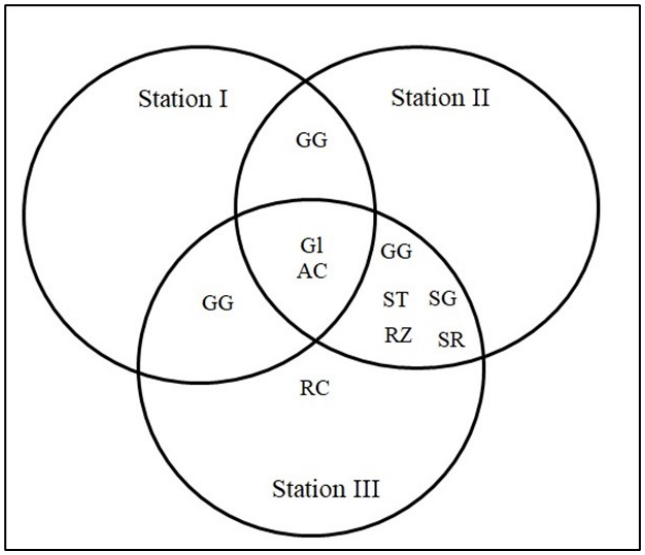

Figure 5. Types of spores found at each station. Description; Gl: *Glomus*, AC: *Acaulospora*, GG: *Gigaspora*, SG: *Septoglomus*, ST: *Scutellospora*, RZ: *Rhizophagus*, SR: *Sclerocystis*, RC: *Racocetra*.

There are differences in the number of spore genera across stations. Three genera were on display at Station I, seven genera at Station II, and eight genera were spotted at Station III. These differences in spore diversity and features draw attention to the unique qualities connected to each species in the studied settings.

Diversity Index

As shown in Figure 7, the AMF diversity index study shows that station II has the most significant diversity index value at 0.9571, while station I has the lowest value at 0.7823. H' < 1 indicates that the Shanon-Wiener AMF diversity index values at each station are in the low category. Notably, the reported genera are not evenly distributed among all soil samples. Few species were found during soil isolation, including *Gigaspora* sp., *Scutellospora* sp., *Sclerocystis* sp.,*Racocetra* sp., *Rhizophagus* sp., and *Septoglomus* sp. As

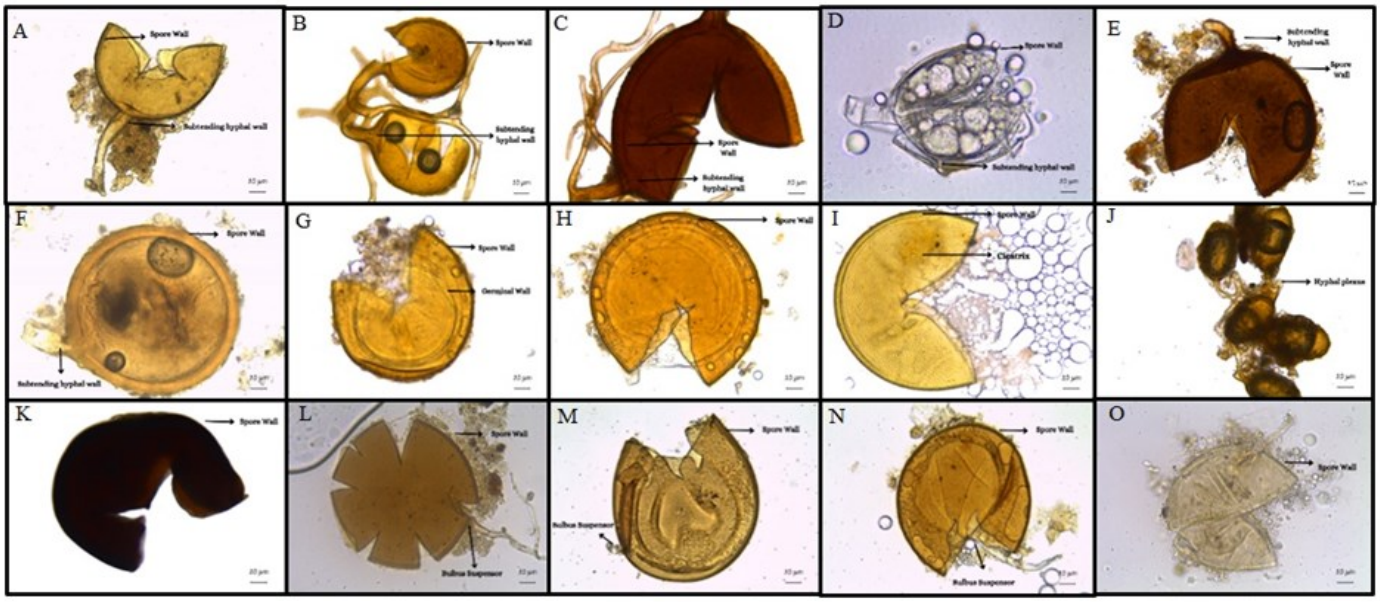

Figure 6. Microscopic images of AMF genus found in soil samples of Zingiberaceae family plants,, A: *Glomus* sp. 1, B: *Glomus* sp. 2, C: *Glomus* sp. 3, D: *Septoglomus* sp. 1, E: *Septoglomus* sp. 2, F: *Rhizophagus* sp., G: *Acaulospora* sp. 1, H: *Acaulospora* sp. 2, I: *Acaulospora* sp. 3, J: *Sclerocystis* sp., K: *Gigaspora* sp. 1, L: *Gigaspora* sp. 2, M: *Scutellospora* sp. 1, N: *Scutellospora* sp. 2 , O: *Racocetra* sp.

opposed to the other six recognized genera, *Glomus* sp. and *Acaulospora* sp. showed larger numbers. These results highlight the differential distribution patterns across various genera and offer insights into the varying AMF diversity throughout the tested stations.

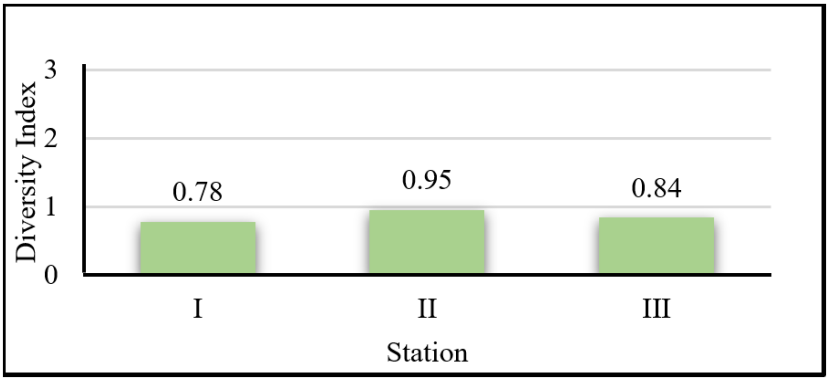

Figure 7. AMF diversity index at each station.

Discussion

The analysis reveals that the mycorrhiza colonization rate and spore density found at Station 3 indicate distinct ecological dynamics from those of Stations 1 and 2. Station 3 in the western half of Tukung Gede Mountain Nature Reserve has unique difficulties due to its proximity to residential areas. While Station 2 has open vegetation and Station 1 has closed vegetation, Station 3's environment encourages the growth of mycorrhizal fungi. The diversity index contradicts these findings, indicating that Stations 2 have greater values than Station 3. Numerous studies indicate that complex interactions between ecological parameters, such as soil type, moisture content, and temperature, may account for the observed changes in the diversity index (Sangwan & Prasanna 2021; Chikoti et al. 2022). Mycorrhizal colonization, spore density, and diversity index have a complex connection that demonstrates the complexity of the ecosystem and the need for a complete understanding of the linked elements impacting fungal symbiosis under different environmental conditions.

In this study, soil pH measurements at each station ranged from 4.8-6, this pH value indicates acidic soil. Not only soil pH, soil moisture and temperature also affect the growth of AMF spores. This study demonstrates that spore abundance is highly dependent on the characteristics of the soil. According to Alayya and Prasetya (2022), soils with a pH of 3.8–8 are suitable for the growth of AMF spores. Because AMF spores are arcidophylis, or comfortable in acidic soil conditions, they can exploit soil with an acidic pH to adapt to their surroundings and encourage the development of additional AMF spores (Mathurin et al. 2022). Environmental temperature in this study showed an optimal temperature ranging from 29-30 °C for AMF growth. This temperature measurement is in line with research conducted by Nugroho and Prasetya (2023), the best temperature for AMF development is 30 °C and the best mycelia development is at 28-34 °C. Soil moisture in this study ranged from 40-68 %. The number on the Soil meter shows that the more acidic the soil pH value is, the greater the soil moisture.

The presence of AMF spores is also influenced by soil type, some studies report that soil types with dusty loam fractions are predominantly abundant and good for the development of spores of the *Glomus* genus, while for sandy soil types, the spores found are *Gigaspora* and *Acaulospora* genus (Salim et al. 2019; Yuwati et al. 2020; Mahulette et al. 2021). This is in line with this study that station I has a loamy soil texture, at this station the types of genus found are fewer, only three genus were identified and dominated by the *Glomus* genus. Station I and II have a sandy soil texture, this study shows the number of spores at this station is more and the types of genus identified are also more varied. Station II has seven genus identified and for station III has eight genus identified.

Arbuscular Mycorrhizal Fungi (AMF) indicates a symbiotic relationship between AMF and plants in the root (Abdullahi et al. 2021). This study found AMF colonization in all root samples with varying percentage levels. Stations II and III showed higher AMF colonization than station I. According to Bernaola et al. (2018) AMF can be found in almost all soils and generally does not have a specific plant host. The level of colonization is influenced by soil fertility, soil type, and environmental conditions such as temperature and soil moisture. AMF is an obligate symbiont fungus, where AMF can work after infecting the host plant. AMF can infect plant roots quickly in dry land conditions that lack water and nutrients (Silva et al. 2022). The study results align with Pandey et al. (2020) that AMF spores and root colonization were found at slightly acidic soil pH. Soils with slightly acidic pH comparatively have a higher spore density than acidic soils. A high percentage of AMF colonies does not always indicate a high spore density, it is suspected that AMF infection in the roots has yet to reach the stage of spores. There is no close correlation between root infection and the number of spores produced, so a high number of spores does not necessarily mean that the percentage level of root infection is also high (Bohacz et al. 2022; Husein et al. 2022)

The different values of AMF colony percentage can be caused by environmental factors such as soil pH, soil moisture, and temperature and plant factors such as root length, root density and root infection (Rajpurohit & Jaiswal 2022) at each station. Soil depth can affect the number of spores studied by Samal et al. (2023). The results obtained are that the highest number of spores is at a depth of 10-20, this is due to the large number of roots that are still there. At a depth of 0-20 cm is also part of the top soil, which is part of the AMF spores. The deeper the soil layer, the fewer spores that will be found because it is further away from the root zone. INVAM (2022) stated that the genus *Glomus* Sp. has the most species and high frequency of presence compared to other genera. The abundance of the

Glomus sp. genus is because it is generally suitable for most of its natural habitats. *Glomus* sp. has a better resistance and adaptation level than other AMF spore types. *Glomus* sp. can adapt to nutrient-poor environments and environments polluted with hazardous waste and at acidic or neutral pH conditions.

Plant photosynthesis depends on light intensity, which also affects the availability of nutrients and energy needed for symbiotic partnerships with arbuscular mycorrhizal fungus (AMF). Increased photosynthesis from higher light levels boosts the synthesis of carbohydrates, which is beneficial to AMF colonization and function. On the other hand, low light levels decrease photosynthetic productivity, which restricts AMF's resources and could decrease the effectiveness of the symbiotic relationship (Guisande-Collazo et al 2022). Changes in light levels can impact the development of mycorrhizal structures and plant growth, which can impact nutrient intake and general health (de la Hoz et al. 2021). Knowing how light intensity functions contributes to the optimization of plant and AMF performance and provides information on how to enhance plant-fungal interactions (Graham & Eissenstat 1998).

The number of spores after trapping increased at each station. Differences in the number of spores can also be caused by many interrelated factors such as salinity, temperature, season and environment. This factor can affect the community structure of the genera obtained (Pace et al. 2019). Alrejhei et al. (2021) reported no correlation between the number of spores and the level of root colonization, the number of spores in natural habitats is not always related to the percentage of AMF colonies. It is explained that AMF spores in certain species sometimes take a long time to germinate. Soil salinity can also affect AMF spore germination, hyphal growth rate and colonization percentage (Danesh et al. 2022). Mukhongo et al. (2023) findings, which showed that mycorrhiza sporulation was higher in acidic soils, are consistent with the observation that mildly acidic soils had a higher spore density than acidic soils. The diversity index value determines the diversity of spore types or genus at each station. This study's spore diversity index value shows that it is classified as low. This is thought to be because not all genus of AMF spores are evenly distributed and the richness of the spore genus also has a different amount (Liu et al. 2021). In line with the research of Sefrila et al. (2021) if in a community it is composed of species with uneven abundance or certain types of AMF dominate, then the diversity is low. A community's high and low diversity index depends on the number of species and individuals of each species (species richness).

At station III the number of spores and root colonization was higher than at stations I and II. In contrast to the diversity index value, the highest value was found at station II. The factor of difference in the number of spores occurs because the number of plants found at station III is more, namely five samples found. The number of spores is also caused by differences in soil factors. At station III the soil pH shows a value of 5.8, meaning that the pH of this soil is slightly acidic with little soil moisture compared to station I. The type of host plant also affects the infection of the host plant. The type of host plant also affects spore infection.

In our current study, we identified eight genera of arbuscular mycorrhizal fungi (AMF)—*Sclerocystis*, *Septoglomus*, *Acaulospora*, *Gigaspora*, *Glomus*, *Scutellospora*, *Racocetra*, and *Rhizophagus*—based on morphological characteristics. While this method allowed us to categorize the AMF genera observed, we acknowledge that morphological identification has limitations and can be challenging due to overlapping features among genera. Morphological methods may not always provide the most precise differentiation, as many AMF genera share similar spore structures and characteristics that can complicate

accurate identification (Noreen et al. 2023). Although our study did not include molecular analysis, we recognize that molecular techniques, such as DNA sequencing and polymerase chain reaction (PCR), offer enhanced accuracy and resolution for AMF identification as classification and systematics of AM fungi have substantially changed in recent years (Stürmer 2012).

Zingiber officinale is known to be a host plant that supports a particularly high diversity of arbuscular mycorrhizal fungi (AMF) spores, exhibiting the largest variety of spore types, the highest number of spores, and significant root infections. This plant was found exclusively at Station III, which is characterized by dry, sandy soil. The dry and sandy texture of the soil at Station III may contribute to the increased AMF diversity and abundance observed. Research has shown that soil texture and moisture levels can significantly impact the distribution and diversity of AMF communities (Cardoso & Kuyper 2006; Smith & Smith 2011). Sandy soils, for instance, often exhibit different AMF community structures compared to clayey or loamy soils due to variations in nutrient availability and water-holding capacity (Becerra et al. 2014).

Furthermore, the diversity index of the AMF community at each station is significantly influenced by a range of environmental and ecological factors, such as soil texture, moisture, and nutrient content (Reynolds et al. 2003). These factors collectively shape the AMF colonization patterns and species diversity. Studies have indicated that variations in soil conditions, including pH and organic matter content, play crucial roles in determining AMF community composition and their effectiveness in symbiotic relationships with host plants (Brundrett et al. 1996; Treseder 2004).

The exploration of arbuscular mycorrhizal fungi (AMF) associations in Zingiberaceae plants is crucial for enhancing our understanding of plant-fungal interactions and their implications for example, in agricultural practices. Marsh et al. (2021) highlighted the synergistic effects of AMF in *Zingiber officinale* Rosc. significantly boost growth, nutrient uptake, and the quality of ginger rhizomes, demonstrating improvements in secondary metabolites such as phenolic compounds, flavonoids, and essential oils. These findings underscore the vital role of AMF symbiosis in promoting plant health and biochemical production. Complementing these insights, Azizah and Hariyono (2022) further confirm the positive influence of AMF on ginger, revealing that the application of AMF fertilizer leads to significant enhancements in growth metrics, including plant height, wet and dry weight, and secondary metabolite content, particularly essential oils.

Considering the ecological implications of mycorrhizal colonization, it is essential to recognize how various environmental and anthropogenic factors shape the distribution and diversity of AMF. These fungi are vital for maintaining soil health and facilitating nutrient exchanges between plants and soil. By studying AMF communities across different habitats, we gain valuable insights into these interactions' ecological dynamics. Previous research has demonstrated that habitat type, vegetation structure, and human activities are key determinants influencing the diversity and functionality of AMF communities in diverse ecosystems. Marinho et al. (2019) investigated arbuscular mycorrhizal fungi (AMF) in the tropical semi-arid region of Caatinga, Brazil, revealing that AMF diversity and distribution are significantly influenced by habitat type, vegetation, and anthropogenic activities. Their findings highlighted notable differences in AMF communities between natural and anthropized areas, with distinct habitat types playing a pivotal role in shaping the structure of AMF communities. Our observation aligns with the finding that mycorrhizal colonization rates and spore density vary between stations in the Gunung Tukung Gede Mountain Nature Reserve, where proximity to residential areas (Station 3) and differences in vegetation cover (Stations 1

and 2) likely contribute to the observed dynamics.

In a related study, an evaluation of AM fungal diversity in agricultural fields in Estonia (Martinez et al. 2024) demonstrated how the availability of natural habitat, both current and historical, influences the diversity of AM fungi. This study found that the richness of AM fungal species was positively correlated with the amount of natural habitat available, even extending back 130 years. This historical legacy effect of past land use on current soil biodiversity underscores the long-lasting impact of habitat availability on AM fungal communities, particularly in disturbed or anthropized areas (Sangwan & Prasanna 2021). Similarly, our study shows how proximity to anthropogenic activity (as seen at Station 3) alters fungal colonization and community structure in the Gunung Tukung Gede reserve. These studies together suggest that maintaining natural habitats, whether in nature reserves or surrounding agricultural fields, can have lasting benefits for soil biodiversity, including AM fungal diversity. The comparison of these ecosystems highlights how AM fungi mediate nutrient exchanges between plants and soil across a wide variety of habitats, both natural and disturbed. Integrating these insights into conservation and land management strategies may enhance the sustainability and ecological resilience of such environments.

Bioengineering, particularly in bioremediation, offers immense potential for restoring degraded environments and enhancing soil health. Our findings highlight the synergistic role that bioengineering, specifically through the application of Arbuscular Mycorrhizal Fungi (AMF), can play in bioremediation. AMF, known for enhancing nutrient uptake, improving plant tolerance to stress, and facilitating soil structure stability, could be pivotal in restoring contaminated soils by improving their nutrient cycling and resilience (Gou et al. 2023; Zhu et al. 2024). By integrating AMF into bioengineering frameworks, bioremediation efforts can benefit from enhanced soil fertility and structure, ultimately leading to sustainable agricultural practices. This integration supports the regeneration of ecosystems, particularly in soils with degraded structures or contamination, fostering crop growth and improving food security in impacted areas (Barea et al. 2011). Future research should optimize AMF strains to improve their efficiency in specific environmental conditions and explore their combined use with other bioremediation technologies to expand their application in sustainable agriculture.

CONCLUSION

In the Tukung Gede Mountain Natural Reserve, Zingiberaceae plants are associated with Arbuscular Mycorrhizal Fungi (AMF). This study identifies eight different AMF genera such as *Sclerocystis*, *Septoglomus*, *Acaulospora*, *Gigaspora*, *Glomus*, *Scutellospora*, *Racocetra*, and *Rhizophagus* that are critical to the growth and well-being of these plants. Among the zingiberaceae growth in the area, *Zingiber officinale* exhibiting the most significant colonization and spore density revealed notable differences in AMF colonization rates and spore densities between species. The impact of regional environmental conditions on fungal dispersal was reflected in the diversity index for AMF spores, which likewise varied by location. By addressing a significant knowledge gap about AMF diversity in this biodiverse region of Indonesia, this study provides valuable information for conservation initiatives and sustainable farming methods.

AUTHOR CONTRIBUTION

R.O.K. led the conceptualization of the study, provided guidance throughout the research process, and contributed to writing and revising the manuscript. I.D.L. coordinated the fieldwork, collected soil and root samples, and performed data analysis related to A.M.F. colonization rates and spore density.

I.J.S. was responsible for identifying the A.M.F. genera through spore morphology and root staining techniques and contributed to analyzing diversity indices. N.S. assisted in statistical analysis and contributed to writing the manuscript. All authors participated in the interpretation of results and provided critical feedback, approving the final manuscript for submission.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the research or the research funding.

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