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Diversity of Types of Arbuscular Mycorrhizae Fungi in Acid Sulfate Soil

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ABSTRACT

Background: Land with acid sulfate soil type is suboptimal land for agricultural cultivation. Identification of mycorrhizae fungi diversity in acid sulfate soil is one of the critical strategies needed to determine the quality of land and as a basis for determining land use for agriculture.

Methods: The research was conducted at the Faculty of Agriculture, Tanjungpura University, Pontianak City, Indonesia. The research period from December 15th 2023 to January 15th 2024. Analysis the number of mycorrhizae spores and colonization was conducted at the plant disease laboratory. Analysis of the chemical properties of acid sulfate soil was conducted at the Soil Chemistry and Fertility Laboratory. The physical properties of acid sulfate soil were conducted at the Soil Physics and Land Conversion Laboratory.

Result: Management of mycorrhizae fungi can be one strategy to improve plant growth and development in acid sulfate soil. The results of the study showed the types of mycorrhizae fungi in acid sulfate soil are *Glomus* sp., *Scutelospora* sp., *Gigaspora* sp. and *Acaulospora* sp., with the highest number of spores in the *Glomus* sp. type. The density of spores obtained in 50 g of acid sulfate soil ranges from 32 to 49 spores. Colonization of mycorrhizae fungi on various pioneer plants ranges from 25.00 to 95.48%, with the number of colonizations being in the *Limnocharis flava* type (95.48%).

Key words: Acid sulfate soil, Microorganisms, Spores density.

INTRODUCTION

Acid sulfate soil is one of the potential lands to be managed and utilized as agricultural land in West Kalimantan, Indonesia. This is related to the distribution area of tidal land in West Kalimantan, which reaches 1,904,100 hectares or 12.95% of the area of West Kalimantan (Badan Pusat Statistik, 2019). Acid sulfate soil is soil with pyrite (FeS_2) content caused of tidal inundation of seawater (Ratmini, 2019). Pyrite (FeS_2) contained in agricultural land can limit soil fertility in supporting plant growth and development (Nursanti and Defitri, 2024; Primayuda *et al.,* 2022; Thuy *et al.,* 2024). However, pioneer plants can grow on acid sulfate land, so it is suspected that there are microbes that help in the growth and development of these plants, one of which is mycorrhizae fungi.

Mycorrhizae fungi are microorganisms beneficial for plant growth and development in conditions of minimal soil fertility quality. Several research results reveal that mycorrhizae fungi that are symbiotic with plant roots can support increased plant growth and development even in infertile soil conditions, with the role of mycorrhizae in the process of absorbing water and nutrients in limited soil, namely expanding the root absorption area to the smallest soil particles (Fattahi *et al.,* 2020; Wartanto *et al.,* 2020; Balasubramanian *et al.,* 2021; Mahmudi *et al.,* 2023; Amina and Hamida 2024). Therefore, the isolation and identification of mycorrhizae fungi in acid sulfate soils are important parts that need to be done to support the productivity of acid-sulfate land for agricultural cultivation.

The diversity and distribution of mycorrhizae vary greatly, often due to different environmental conditions and compatible environments that support spore growth and development (Samsi *et al.,* 2017). Environmental factors that affect the distribution of mycorrhizae include soil structure, phosphorus and nitrogen levels in the soil, organic carbon content, water availability, pH and soil temperature (Hartoyo *et al.,* 2020). Variations in location and rhizosphere also cause differences in the diversity of mycorrhizae species and populations. In addition, not all mycorrhizae have the same morphological and physiological characteristics, so it is important to identify them accurately (Rasyid *et al.,* 2016).

In acidic sulfate soils, limited research has been conducted on mycorrhizae, especially regarding their presence, population, colonization and morphological characteristics of mycorrhizae in the rhizosphere of pioneer plant roots. One method to determine the population of

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mycorrhizae spores is isolation. Isolation is carried out to separate spores from soil samples, allowing for the assessment of the characteristics of mycorrhizae spores and populations. To examine the morphological characteristics of mycorrhizae spores, morphological identification can be made by observing the shape and color of the spores (Budi *et al.,* 2024; Budi and Dewi, 2016).

The research aimed to assess species diversity, expression and density of mycorrhizae fungi, mycorrhizae colonization and spore characteristics in the rhizosphere of pioneer plants in acidic sulfate soil.

MATERIALS AND METHODS

The research was conducted at the Faculty of Agriculture, Tanjungpura University, Pontianak City, Indonesia. The research implementation period was from December 15th 2023, to January 15th 2024. The research involved soil collection from land acid sulfate in Kalimas Village, Kubu Raya Regency, West Kalimantan, Indonesia. Mycorrhizae spore counting and colonization assessment were conducted at the Plant Disease Laboratory. Analysis of the chemical properties of acid sulfate soil was conducted at the Soil Chemistry and Fertility Laboratory. Analysis of the physical properties of acid sulfate soil was conducted at the Soil Physics and Land Conversion Laboratory.

The research method used an exploratory and descriptive approach with purposive sampling to collect soil samples in the field. The sampling location was selected based on the researcher's assessment, which was located in Kalimas Village, Kubu Raya Regency, West Kalimantan, Indonesia. The research was carried out through a field survey process and soil sampling. Determination of soil samples taken in the field was random for 5 sample plots. Furthermore, soil samples were collected from 5 observation points in each plot at a 0-20 cm depth, each taking 500 g of soil. Soil samples from each observation point in the plot were combined into a composite sample to represent the plot. After being mixed to become homogeneous, a 500 g soil sample was taken from each plot.

Intact soil samples were taken using the ring method at a 0-20 cm depth and their physical properties were analyzed in the Laboratory of Soil Physics and Land Conversion. The variables observed determined include observations of soil bulk density, permeability, water content in field conditions and soil porosity (Nurhartanto *et al.,* 2021). Furthermore, disturbed soil samples were taken for analysis in the Soil Chemistry and Fertility Laboratory, with the observation variables determined consisting of observations of soil pH (H_2O), C-organic (%), total nitrogen content, C/N ratio and phosphorus content (P_2O_5) (Darlita *et al.,* 2017).

Spore isolation using 50 g of soil sample was carried out using the wet filter pour method (Boyno *et al.,* 2023), followed by centrifugation (Brundrett *et al.,* 1999). Soil samples mixed with water are taken as much as 300 ml, poured into a chemical glass, then stirred until evenly distributed and allowed to settle. The liquid is poured into filters arranged from 200 μm to 53 μm in size. The soil is rinsed on each filter with running water from the tap, the rinsing process is carried out carefully so that the water does not directly hit the filter but uses hand media to prevent spores from breaking.

The rinsing process on the 120 μm and 53 μm filters was carried out using distilled water. The liquid from the 120 μm and 53 μm filters was poured into a glass beaker. Furthermore, 3 ml of 60% sucrose solution was added to the beaker and stirred. The mixture of the filtered solution with the sucrose solution was poured into a test tube and left for 24 hours. Next, refiltering was done using a 53 μm filter and put into a Petri dish. The shape and color of mycorrhizae spores were observed using a microscope with 40x magnification and the number of spores was counted. Melzer's solution was applied to the identified spores. The spores' characteristics were recorded and identified using a fungal identification book (Manual for Identification of Mycorrhizae Fungi) (Schenck and Perez-Collins, 1990). Mycorrhizae colonization was observed using the root staining technique (Kormanik and Mc Graw, 1982).

RESULTS AND DISCUSSION

Soil physics criteria

The results of the Soil Physics and Land Conversion Laboratory analysis showed the physical condition of the soil has a bulk density value of 1.62-1.80 g per cm^3 with a reasonably heavy criteria. The soil permeability condition is relatively slow, with a value between 1.23-1.44 cm per hour. The soil water content is very high, between 79.86% -90.23%. Soil porosity has a low of 2.24%-29.30% (Table 1).

Based on the assessment of the physical criteria of the soil above, it shows that soil compaction caused by finer soil particles causes a fairly heavy bulk density value, the condition of the soil pores formed will be smaller and the soil permeability will be slower (Killa *et al.,* 2024; Klimandang *et al.,* 2024). Porosity and soil water content are closely related to soil bulk density and permeability conditions. Soil porosity describes the condition of the soil pore space occupied by water and air, where macro pores will be occupied by water and micropores will be occupied by water, so that the slower the soil permeability indicates that the soil pores will be smaller and the water content in the soil will be higher (Haryati, 2014; Kusuma and Yulfiah, 2018).

Soil chemical criteria

The Soil Chemistry and Fertility Laboratory analysis showed that the soil reaction is very acidic, with a pH of 4.1-4.4. The C-organic content of the soil is very low to medium (0.26%-1.99%). The total nitrogen content is very low to medium (0.02%-0.24%), the phosphorus content (P_2O_5) is very low (2.40-8.00 ppm) and the C/N ratio is low

to medium (8.26-13.50). The C-organic content of the soil is very low to medium (0.26%-1.99%) (Table 2).

The low pH value of the soil can be caused by iron sulfide minerals that have been oxidized to produce sulfuric acid (Department of Environment Regulation, 2015). The condition of internal problems in acid-sulfate soil is inherent, which is not beneficial for soil fertility. The formation of sulfuric acid causes the soil reaction to become very acidic, followed by a lack of nutrients in the soil (Soil Survey Staff, 2014). According to Bahri *et al.* (2023), phosphorus fixation by free Al- and Fe- in the soil causes changes in phosphorus elements, namely to Al-P or Fe-P, which are insoluble in the soil.

Density and quantity of spores

The average spore density in each sample showed that the number of spores found in the five acid sulfate soil samples was almost the same, with a range of 32-49 spores per 50 grams of soil (Table 3). According to Daniels and Skipper (1982), soil has a high mycorrhizae spore population if the spore density is 20 per gram (200 per 10 grams). Based on these criteria, the study's results showed that all soil samples from the root area of pioneer plants in acid sulfate soil had a relatively low number of spores.

The low number of spores in all soil samples at the research location may be due to the very high water and low phosphorus content in the soil. These conditions inhibit the development of mycorrhizae. According to Puspitasari *et al.* (2012), the low population of mycorrhizae spores can be attributed to the fact that soil samples from the root area of pioneer plants in acidic sulfuric soil had not sporulated at the time the mycorrhizae samples were collected, coupled with environmental factors such as soil conditions that were not favorable for the growth and development of mycorrhizae spores.

Each of the five acidic sulfuric soil samples contained different types and numbers of spores for each species. The results of spore extraction from these samples revealed that four species were consistently found in each sample. The species identified were *Glomus* sp., *Scutellospora* sp., *Gigaspora* sp. and *Acaulospora* sp. (Table 4).

The diversity of mycorrhizae spores in each sample did not differ significantly, although the number of each species varied. Sample 2 had the lowest number of spores found, with 12 *Glomus* sp. spores, 8 *Scutellospora* sp. spores, 9 *Gigaspora* sp. spores and 3 *Acaulospora* sp. spores. Sample 3 had 16 *Glomus* sp. spores, 6 *Scutellospora* sp. spores, 11 *Gigaspora* sp. spores and 4 *Acaulospora* sp. spores. Sample 5 contained 19 *Glomus* sp. spores, 7 *Scutellospora* sp. spores, 14 *Gigaspora* sp. spores and 2 *Acaulospora* sp. spores. Sample 1 had 17 *Glomus* sp. spores, 3 *Scutellospora* sp. spores, 12 *Gigaspora* sp. spores and 4 *Acaulospora* sp. spores. Sample 4 had 17 *Glomus* sp. spores, 11 *Scutellospora* sp. spores, 9 *Gigaspora* sp. spores and 3 *Acaulospora* sp. spores (Table 4).

The types of spores found in each sample varied per 50 g of soil. This is because not all mycorrhizae spores can grow simultaneously. According to Oehl *et al.* (2014), not all mycorrhizae are active during the same period, even if they are of the same type. The results showed that the genus *Glomus* had the highest density found in all plant samples at the study site. According to INVAM (2013), the

Table 1: The physical analysis of acid sulfate soil.

Sample	Fill weight (g per cm^3)	Criteria*	Total permeability (cm per hour)	Criteria*	Soil water content (%)	Criteria*	Porosity (%)	Criteria*
1	1.80	Reasonably heavy	1.35	Relatively slow	85.61	Very high	27.14	Low
2	1.74	Reasonably heavy	1.42	Relatively slow	90.23	Very high	22.11	Low
3	1.72	Reasonably heavy	1.23	Relatively slow	79.86	Very high	25.27	Low
4	1.62	Reasonably heavy	1.44	Relatively slow	83.80	Very high	20.24	Low
5	1.64	Reasonably heavy	1.37	Relatively slow	86.17	Very high	29.30	Low

Source: Physics and Soil Conservation Laboratory, Faculty of Agriculture, Tanjungpura University.
*Ramli *et al.* (2016).

Table 2: The chemical analysis of acid sulfate soil.

Types of analysis	Soil sample					Criteria *
	1	2	3	4	5	
pH (H_2O)	4.2	4.2	4.4	4.1	4.3	Very acidic
C-organic (%)	0.27	0.26	0.43	1.99	1.67	Very low-medium
N-total (%)	0.02	0.03	0.05	0.24	0.19	Very low-medium
C/N ratio	13.50	8.67	8.60	8.29	8.79	Low- medium
P_2O_5 (ppm)	8.0	2.40	5.60	3.60	3.60	Very low

Source: Chemistry and Soil Fertility Laboratory, Faculty of Agriculture, Tanjungpura University.
*Litbang Penelitian Tanah (1983).

genus *Glomus* has the most species and a higher frequency of occurrence than other genus.

Mycorrhizae colonization

The level of mycorrhizae colonization on the roots of pioneer plants growing on acid-sulfate soil at the research location varied from low to high (Table 5). It was indicated by hyphal and spore structures (Fig 1). The presence of mycorrhizae colonization on the roots of plants growing on acid sulfate land at the research location indicates the vital role of mycorrhizae in supporting the successful growth and formation of plant communities in the area. According to Prayudyaningsih *et al.* (2021), the relationship formed between mycorrhizae and plant roots shows its potential to influence processes in the ecosystem, including determining plant diversity in its natural community.

Table 3: Mycorrhizae spore density.

Sample	Spore density per 50 g of soil
1	36
2	32
3	37
4	49
5	42

Source: Plant Disease Laboratory, Faculty of Agriculture, Tanjungpura University.

Characteristics and identification of mycorrhizae

The results of characterization and identification of mycorrhizae carried out using the method of Schenck and Perez-Collins (1990), obtained four mycorrhizae species, namely *Glomus* sp., *Scutellospora* sp., *Gigaspora* sp. and *Acaulospora* sp. types and morphological characteristics of arbuscular mycorrhizae fungi spores found in acid sulfate land in Kalimas Village, Kubu Raya Regency (Table 6).

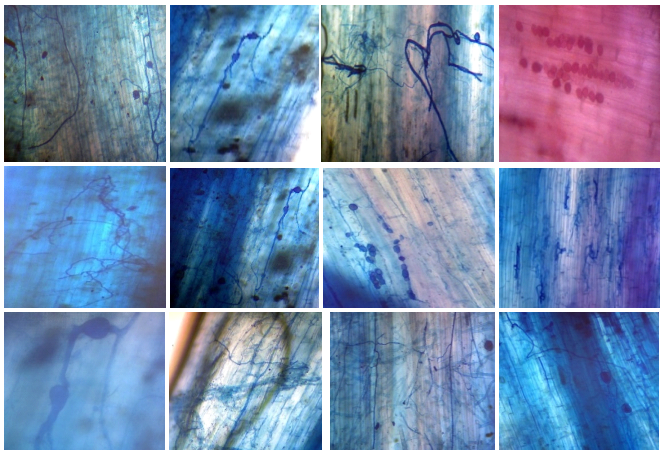
According to Brundrett and Tedersoo (2018), the presence of mycorrhizae species is greatly influenced by the composition of the community and plants, in line with the results of spore measurement observations, where the fifth sample showed a similar number of spores due to similarities in sample types. In the five samples, various spores were found in the soil. This is associated with similarities in soil and environmental conditions. Every spore is suitable for or has the same adaptation to this soil and ecological conditions. Among the mycorrhizae species in several soil samples, *Glomus* sp. is the most frequently found species in each rhizosphere sample of the pioneer plants observed.

According to Masters-Clark (2021), *Glomus* sp. has a short dormancy period, shows good germination capacity and has the fastest germination time of around ±6 weeks. This indicates that *Glomus* has a relatively high level of adaptation to various environmental conditions. In addition,

Table 4: Number of mycorrhizae spores.

Species	Sample				
	1	2	3	4	5
Glomus sp.	17	12	16	26	19
Scutelospora sp.	3	8	6	11	7
Gigaspora sp.	12	9	11	9	14
Acaulospora sp.	4	3	4	3	2

Source: Plant Disease Laboratory, Faculty of Agriculture, Tanjungpura University.



Source: Plant Disease Laboratory, Faculty of Agriculture, Tanjungpura University.

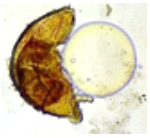
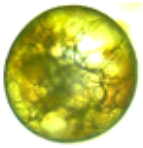
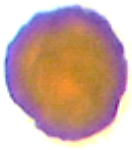
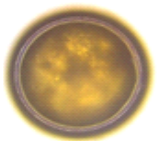
Fig 1: Structure of mycorrhizae hyphae and spores in various root cross-sections.

Table 5: Mycorrhizae colonization on roots of pioneer plants in acid sulfate soil.

Plant types	Habits	Mycorrhizae colonization (%)
Cyperus rotundus	Herb	62.35
Clerodendrum minhassae	Bush	73.99
Musa spp.	Herb	39.00
Limnocharis flava	Water Herbs	95.48
Solanum nigrum	Herb	26.22
Pteridium aquilirum	Ferns	27.46
Eichhornia crassipes	Water weed	42.76
Imperata cylindrica.	Perennial grass	82.91
Solenostema scutellarioides	Annual herbs	31.14
Dillenin suffruticosa	Bush	25.00
Mimosa pudica.	Perennial herbs	41.82
Colocasia esculenta	Perennial herbs	84.20

Source: Plant Disease Laboratory, Faculty of Agriculture, Tanjungpura University.

Table 6: Types and morphological characteristics of arbuscular mycorrhizae fungi spores.

Whole spore form	Spore form: Broken down	Characteristic
 *Glomus* sp.	 *Glomus* sp.	Spores are formed singly or in pairs at the terminal non-gametangium hyphae that are not differentiated in the sporocarp. Spores are globose, sub-globose, ovoid, or obovoid, with walls consisting of more than one layer. They are hyaline to yellow in color, brownish red, chocolate and black, measuring between 20 and 400 μm (INVAM, 2013).
 *Scutelospora* sp.	 *Scutelospora* sp.	Spores may be with or without decoration. They consist of flexible spore walls. The spore structure can be ovoid, obovoid, pyriform, or irregular, with sizes ranging from 72.5 to 170 μm. *Scutellospora* spores have the distinctive characteristic of possessing a germinal shield (GS), which differentiates them from the genus *Gigaspora*. The spores are hyaline in color, as well as yellow and chocolate (INVAM, 2013).
 *Gigaspora* sp.	 *Gigaspora* sp.	Spores are produced singly in the ground and do not have an inner spore wall layer. They have a bulbous suspensor and are globose or sub-globose in shape, cream to yellow in color, measuring 125 to 600 μm (INVAM, 2013).
 *Acaulosporas* sp.	 *Acaulospora* sp.	Spores have 2-3 walls and are globose to elliptical. They possess a saccule (S), the swollen remnant of the hypha. Spores are hyaline, yellow, or yellowish-red in color, measuring between 100 and 400 μm (Schenck and Perez-Collins, 1990).

Source: Plant Disease Laboratory, Faculty of Agriculture, Tanjungpura University.

the high clay fraction in the soil mass will affect the presence of mycorrhizae types.

CONCLUSION

The types of mycorrhizae fungi found in acid sulfate soil from Kalimas Village, Kubu Raya Regency, West Kalimantan, Indonesia are *Glomus* sp., *Scutelospora* sp., *Gigaspora* sp. and *Acaulospora* sp., with the highest number of spores in the *Glomus* sp. type. The density of spores obtained in 50 g of acid sulfate soil ranges from 32 to 49 spores. Colonization of mycorrhizae fungi on various pioneer plants ranges from 25.00 to 95.48%, with the number of colonizations being in the *Limnocharis flava* type (95.48%).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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