



Comparative Analysis of Soil Fungal Profiles in Healthy and Diseased Duku (*Lansium domesticum* Corr.) Plantations at Different Altitudes in Jambi Province, Indonesia

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10.18805/ag.DF-732

ABSTRACT

Background: Soil fungal communities significantly contribute to the health and productivity of plants, with their compositions influenced by plant disease presence at various altitudes. This study compares the variations in soil fungal diversity between healthy and diseased duku (*Lansium domesticum* Corr.) plantations in Jambi, Indonesia.

Methods: This study examines the potential fungal indicators on soil health, concentrated on four sites representing various altitudes: Kumpeh (10,32 m asl), Rano (5,80 m asl), Koto Rami (623,46 m asl) and a location of the Diseased site (194,01 m asl). The fungal communities were analyzed using DNA sequencing and the NCBI ITS RefSeq database.

Result: The dominant fungal phyla across all sites were Ascomycota, most abundant in the low-altitude from Kumpeh and Basidiomycota, primarily present in the high-altitude from Koto Rami. Mucoromycota and smaller groups like Zoopagomycota were also identified. The high-altitude site at Koto Rami showed high water content and organic matter, which may have supported a unique soil microbial community. The low-altitude site at Rano exhibited a neutral pH and well-balanced Phosphorus levels, both of which encourage fungal activity. In contrast, the low-altitude samples from Kumpeh displayed higher levels of Calcium and Magnesium, low organic matter and a sandy texture, which may have restricted the fungal community. In the Diseased site, stress was detected as indicated by reduced moisture levels and heightened microbial activity, suggesting that certain microbes might adversely affect plant health. Overall, increased moisture and good organic matter in the soil can enhance fungal growth, while a neutral pH, adequate phosphorus and suitable soil texture can facilitate fungal diversity.

Key words: Abundance, Alpha, Beta diversity, Core microbes, Kumpeh, Taxonomy.

INTRODUCTION

Fungal communities are important in the soil ecosystem because they facilitate plant growth, pathogen resistance and stabilize soil. Their interactions foster an environment conducive to ecological equilibrium. However, this equilibrium is susceptible to disruption from various environmental factors, such as altitude and plant health, which can significantly influence the composition and diversity of soil fungal populations. In Jambi, Indonesia, the emergence of diseases leading to sudden death (SD) (Fig 1) in Duku (*Lansium domesticum* Corr.) plantations poses a considerable challenge, especially in regions characterized by heterogeneous soil properties. This condition has been particularly alarming as the incidence of sudden death disease (SD) has contributed to a decline in Duku populations.

The Jambi region is known for its unique premium Duku varieties, particularly the *Kumpeh* and *Muaro Panco*. Sudden death disease, aging existing plants and inappropriate cultivation techniques have been causing a significant production decline. This phenomenon affected the economic livelihoods of local farmers and fruit industry traders. Consequently, there is an urgent need to ensure the continued conservation of the Duku plants in Jambi, especially through soil health indicators.

The characteristics of healthy soil include good structure, nutrient richness, optimal pH, organic matter content, active

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How to cite this article: Hayati, I., Rainiyati, Marwan, H., Wilia, W. and Antony, D. (2025). Comparative Analysis of Soil Fungal Profiles in Healthy and Diseased Duku (*Lansium domesticum* Corr.) Plantations at Different Altitudes in Jambi Province, Indonesia. *Agricultural Science Digest*. 1-10. doi: 10.18805/ag.DF-732.

Submitted: 06-03-2025 **Accepted:** 30-09-2025 **Online:** 18-10-2025

microbial life, adequate drainage and water retention, minimal soil erosion and absence of contaminants (Lehmann *et al.*, 2020; Vincze *et al.*, 2024). Despite an increasing understanding of soil biodiversity, soil health assessments rely heavily on chemical indicators (Alwazzan and Ati, 2024).

Farming practices can alter soil structure, harm microbial communities, cause erosion, reduce fertility and increase greenhouse gas emissions (Singh and Kumar, 2021). Pesticide use and intensive land management can reduce overall microbial diversity, affect crucial symbiotic fungi and

increase pathogenic fungi, which disrupt ecosystem functions (Mohapatra *et al.*, 2021; Chou *et al.*, 2024). Additionally, chemical disturbances like excessive nitrogen and herbicide use can inhibit important mutualistic fungi, leading to ecological imbalances (Brochado *et al.*, 2023).

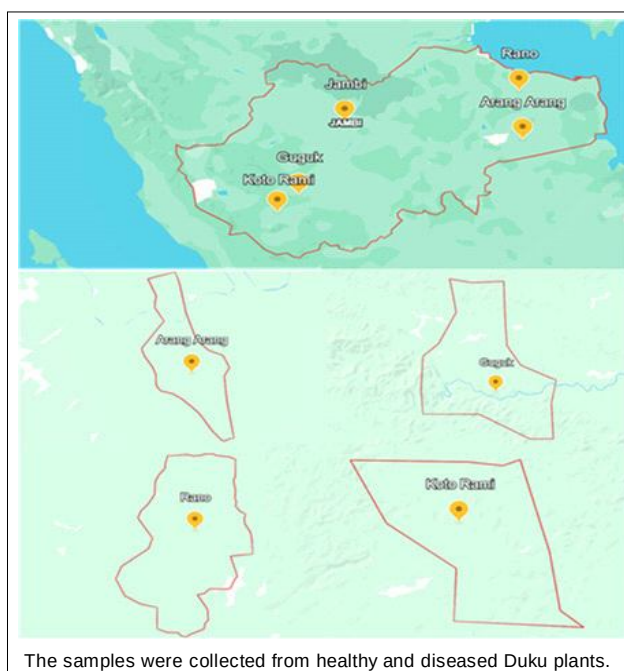
Microbial communities significantly influence plant metabolism by regulating nutrient uptake and overall health through interactions with plant roots, forming the plant-rhizosphere community (Dastogeer *et al.*, 2022; Petrushin *et al.*, 2024; Solomon *et al.*, 2024). Various studies have examined soil bacterial communities, for example, as studied by Fasusi *et al.* (2023). However, the diversity of fungal, ecological and soil character roles remains underexplored. Investigating variations in fungal communities

between healthy and disease-affected soils at different altitudes is essential to understanding the relationship between soil fungi and plant health. Understanding the dynamics of soil fungi concerning these environmental variables is crucial for developing effective management strategies in affected areas and is critical to assessing soil health, as this is the basis for healthy growth and thriving plant life.

This study investigated the fungal communities in soils of diseased and healthy Duku (*Lansium domesticum*) plants across different sites in Jambi Province, Indonesia. By comparing these communities, this study aimed to identify fungal patterns associated with plant health and provide baseline data to support future disease management



Fig 1: Performance of Duku explaining healthy and diseased plants (above) and flesh and fruit.



The samples were collected from healthy and diseased Duku plants.

Fig 2: Location of Sampling in several villages in Jambi Province.

strategies in tropical fruit cultivation. for mitigating the impacts of disease.

MATERIALS AND METHODS

Sampling site and procedure

Soil samples were gathered from 4 locations, representing healthy and diseased Duku from various altitudes. The locations were BPP Arang-Arang (Kumpeh H), 48 366090 E 9823483 S, elevation 10,32 m above sea level, Desa Guguk Merangin (soil of Diseased plant site), 48 175230 E 9759370 S, 194,01 m asl, Desa Rano Tanjabtim (Rano H), 48 363839 E 9866389 S, 5,80 m asl and Desa Rami (Koto Rami H), 47 823898 E 9740820 S, 623,46 m asl (Fig 2). Soil samples were collected from 4 points representing the North, South, West and East directions within 30-40 cm of the base of each stem. A soil drill was used to excavate the soil to a depth of 20 cm. At each location, 5-8 plants were sampled. The soils were then combined to create a composite for each location. Physical characteristics of the soils were analyzed and are shown in Table 1. These characteristics are invaluable for assessing the health, fertility and suitability of soils that support a particular agriculture or ecology.

Exploration for fungi

To characterize the fungal community present in the soil, a metagenomic approach was employed using DNA sequencing technologies. Soil samples were processed at the Genetica Science Laboratory, where total soil DNA was extracted and quantified using both NanoDrop spectrophotometers and Qubit fluorometers to ensure purity and accurate concentration measurements. Library preparations were conducted using Kits from Oxford Nanopore Technology. Nanopore sequencing was operated by MinKNOW software version 24.02.16. Basecalling was performed using Dorado version 7.3.11 with a high-accuracy model (Wick *et al.*, 2019). The quality of FASTQ files was visualized using NanoPlot and quality filtering was performed using NanoFilter (de Coster *et al.*, 2018; Nygaard *et al.*, 2020). Filtered reads were classified using the Centrifuge classifier (Kim *et al.*, 2016). The Fungi index was built using the NCBI ITS RefSeq database. The workflow is shown in Fig 3.

Data analysis

Data collected was presented descriptively. Extensive downstream analysis and detailed visualizations were

Table 1: Physicochemical values of Duku root soils in Koto Rami, Rano, Kumpeh and Diseased site.

Parameter	Methods	Unit	Locations			
			Koto rami healthy	Rano healthy	Kumpeh healthy	Diseased
pH	H ₂ O	Extract 1:5	4.6	5.5	5.8	5.5
	KCl	(Potentiometry)	4.3	4.6	4.6	4.7
Water content	Gravimetry	-	52.64	16.00	19.46	6.75
C-Organic	Spectrophotometry	%	4.36	2.06	1.11	2.26
N-Total	Kjeldahl	%	0.35	0.32	0.17	0.25
P ₂ O ₅ Potential	Spectrophotometry	mg/100 g	51.50	179.39	63.80	80.39
K ₂ O Potential	AAS	mg/100 g	21.26	75.35	39.68	42.28
Cation exchange capacity	Titrimetry	cmol(+)/kg	21.18	15.32	16.15	21.39
Exchangeable acidity	Al ³⁺ H ⁺	cmol(+)/kg	<0.04	<0.04	<0.04	<0.04
		cmol(+)/kg	2.46	0.41	0.40	0.46
Three Particle-size fraction	Sand	%	30	26	30	28
	Dust	%	19	27	36	28
	Clay	%	51	47	34	44
Calcium	HClO ₄ HNO ₃ - AAS	mg/Kg	292.60	868.54	1507.52	1279.77
Magnesium	HClO ₄ HNO ₃ - AAS	mg/Kg	819.29	1179.50	2654.09	1640.73
Iron	HClO ₄ HNO ₃ - AAS	mg/Kg	58520.54	54149.85	39811.39	49768.67
Zinc	HClO ₄ HNO ₃ - AAS	mg/Kg	222.38	139.40	92.36	164.07
Sulfur	HClO ₄ HNO ₃ -	%	<0.10	<0.10	<0,10	<0.10
	Spectrophotometry					
Boron	Spectrophotometry	mg/Kg	18.08	17.84	18.26	10.39
Molybdenum	HClO ₄ HNO ₃ - AAS	mg/Kg	8.02	7.94	7.71	4.25
Al saturation	Calculations	%	0	0	0	0
Salinity	Conductometry	PPT	0.04	0.25	0.05	0.05
Soil respiration	Determination of	mg/Kg C-CO ₂	5.91	3.76	5.47	7.20
	CO ₂ - Titrimetry	/day				

performed using several tools and visualizations were performed using Pavian (<https://github.com/fbreitwieser/pavian>) and Krona Tools (<https://github.com/marbl/Krona>). In addition, RStudio using R version 4.2.3 (<https://www.R-project.org/>) was utilized to improve the analytical capabilities and create insightful graphical representations of the data.

RESULTS AND DISCUSSION

Taxonomy group

Fig 4 illustrates the main fungal groups in healthy and Diseased sites across all sites. In Koto Rami Healthy (H) soil, the dominant fungi were Ascomycota and Basidiomycota and smaller groups like Mucoromycota and Zoopagomycota.

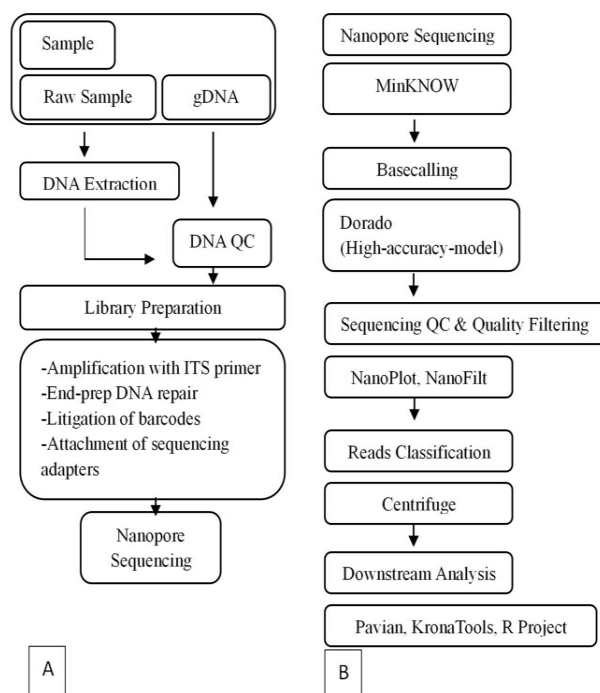


Fig 3: Experimental stages of DNA preparation and sequencing (A), Flow of Metagenomic Data Analysis using Nanopore Technology (B).

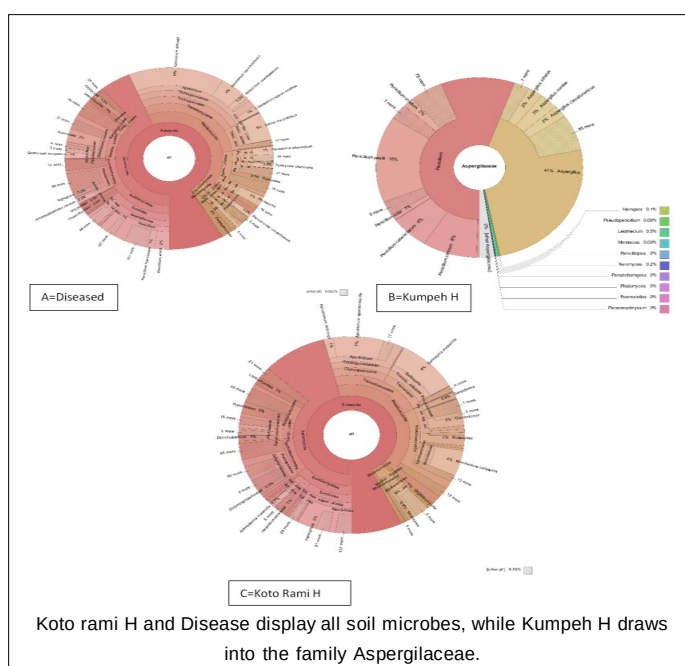


Fig 4: The Taxonomy group of Diseased site, Kumpeh H (above) and Koto Rami H (below).

The Ascomycota phylum includes Eurotiomycetes. When drawn into the family Aspergillaceae (Fig 4C), it was found in classes such as Tremellomycetes and notable genera like *Aspergillus* and *Penicillium*.

The quantitative distribution of *Aspergillus* and *Penicillium* is illustrated as follows (Fig 5). In Kumpeh H, out of 45.200 observed microbes, 22.400 were from the Didymosphaeriaceae family. The Trichocomaceae family had 4.110 members and the Aspergillaceae family included *Aspergillus* (2.620), *Penicillium* (3.530) and *Talaromyces* (4.080). In Koto Rami H, Clavariaceae led with 6.520 entities, followed by *Aspergillus* (3.690), *Penicillium* (2.510) and *Ganoderma* (741). Key species included *Penicillium flaviroseum* (414) and *Aspergillus fumigatus* (523). In Rano H, *Aspergillus* comprised 12.400 entities, while *Penicillium* had 31.500 entities, including *Aspergillus nomiae* (2.950) and *Penicillium citrinum* (5.900). In the Diseased site, *Aspergillus* counted 3.310 and *Ganoderma* 3.470, with various species identified.

Fungal relative abundance

Table 2 presents fungal relative abundance (%) at the phylum level under Diseased site conditions and three healthy locations: Koto Rami, Kumpeh and Rano. Ascomycota and Basidiomycota were the dominant phyla across all conditions, with Ascomycota abundance ranging from 0,4482 to 0,5972% and Basidiomycota from 0,3359 to 0,4849%. Kumpeh (H) had the highest Ascomycota abundance (0,5972%), while Koto Rami (H) had the highest Basidiomycota (0,4849%). Other phyla, such as Blastocladiomycota, Chytridiomycota, Cryptomycota, Olpidiomycomata and Zoopagomycota, were less abundant, each below 0.05%. Blastocladiomycota was absent in Rano (H), while Chytridiomycota was highest at Kumpeh (H) (0.0122%). Mucoromycota increased at Rano (H) (0,1616%) and Zoopagomycota was most abundant at Kumpeh (H) (0,0161%). The diseased site soil, Ascomycota (0.455%) and Basidiomycota (0.4776%), displays a more balanced ratio than the Healthy sites.

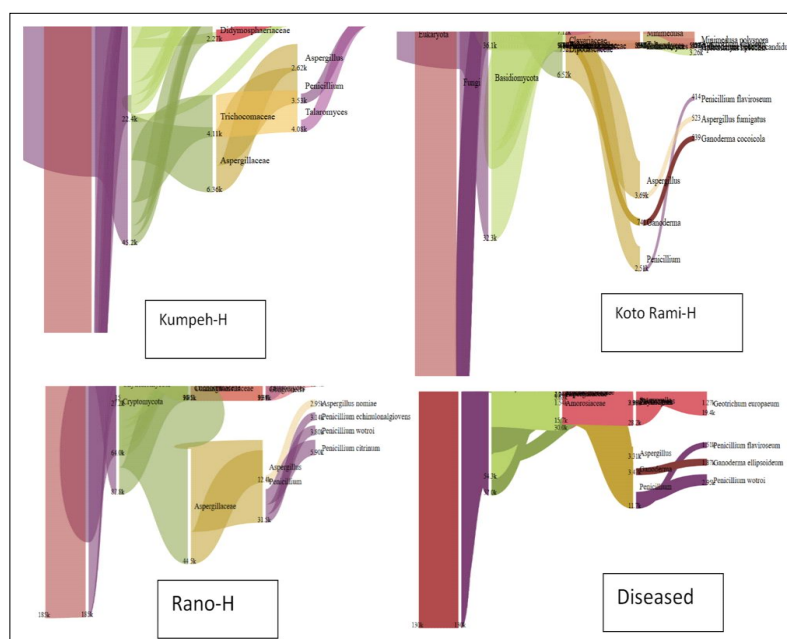


Fig 5: Quantitative distribution between the genus *Aspergillus* and *Penicillium* of all sites; Kumpeh H, Koto Rami H, Rano H and Diseased.

Table 2: The relative abundance of Phylum generated from soil samples of healthy at Rano H, Koto Rami Healthy, Kumpeh Healthy and Disease soil (%).

Phylum	Diseased	Koto rami H	Kumpeh H	Rano H
Ascomycota	0.455	0.4482	0.5972	0.4828
Basidiomycota	0.4776	0.4849	0.3359	0.3487
Blastocladiomycota	0.0005	0.0004	0.0006	0
Chytridiomycota	0.024	0.0099	0.0122	0.0051
Cryptomycota	0.0005	0.0007	0.0021	0.00021
Mucoromycota	0.0223	0.0486	0.035	0.1616
Olpidiomycomata	0.001	0.0004	0.001	0.00011
Zoopagomycota	0.0198	0.007	0.0161	0.0016

Fungal Alpha diversity

The soils sampled from Diseased sites demonstrated the highest observed species richness, quantified at 3,636. This significant level of diversity is further supported by high estimates of potential richness, with Chao1 calculated at 5,370.40 and ACE at 5,361.10. The Shannon Index for these samples was recorded at 39.60, marking it the highest among all sites. Simpson's Index was measured at 5.01, indicating moderate species-level dominance within these communities. Additionally, the InvSimpson's Index, at 0.96, suggests a less equitable species distribution compared to Kumpeh_H. Despite the Diseased site showcasing the highest observed species richness, it remains evident that its potential richness is lower relative to other studied areas.

Koto Rami documented 3105 species, surpassing Rano_H (2900) but still behind Diseased site (3636) and Kumpeh_H (3297), indicating a diverse ecosystem. Chao1 and ACE estimates suggested potential species richness of around 4693.01 and 4586.20, respectively. The Shannon index was 36.79, slightly lower than Kumpeh_H (38.35), while both the Simpson's Index (5.38) and InvSimpson Index (0.97) indicated an even distribution of species. Koto Rami's Fisher's Alpha of 34.81 suggested a high potential for species richness. In contrast, Rano_H had the lowest

species richness, 2900 species and potential richness estimated at 4482.63. Its Shannon Index of 35.74 and Simpson Dominance Index of 4.65 revealed an uneven distribution, dominated by certain species. Rano_H's Fisher's Alpha of 30.40 indicated limited diversity. Rano_H was the least diverse, with an imbalanced species distribution (Fig 6).

Site distinctive of fungal diversity

Each site had unique microbial communities, as shown in Fig 8. The Diseased site soil contains the highest number of exclusive fungi (805) (Fig 7 B) and has a similarity with Kumpeh_H (296). Overall, 1064 microbes were identified. Kumpeh_H had the highest variety with 1036 unique species, while Rano_H and Koto Rami_H had 741 and 980, respectively. Shared species included 409 between Rano_H and Koto Rami_H, 545 between Rano_H and Kumpeh_H and 511 between Koto Rami_H and Kumpeh_H, totaling 1205 common microbial phyla across all sites.

Beta diversity

Beta diversity describes the variation or differences between healthy and Diseased site of duku plantations. Fig 8 presents a principal coordinate analysis (PCoA) based on the Bray-Curtis distance, which evaluates the soil microbial community structure at Kumpeh_H, Koto Rami_H, Rano_H

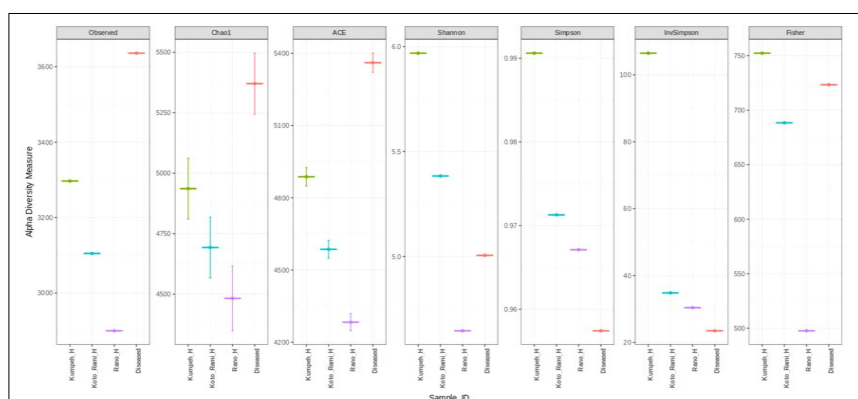


Fig 6: Comparison of alpha diversity index in fungal soil at various sites.

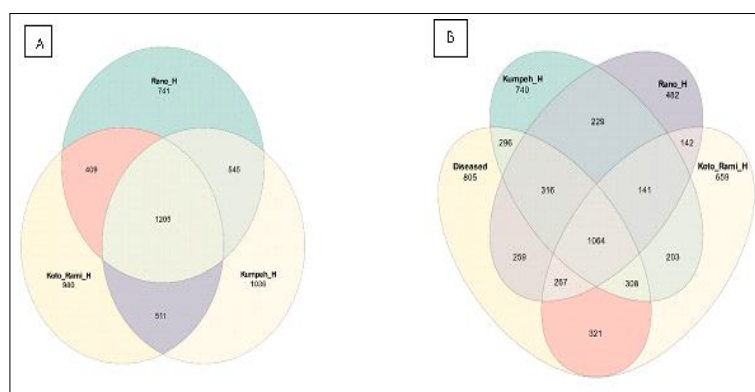


Fig 7: The distinctive soil fungal diversity across Rano_H, Koto_Rami_H and Kumpeh_H (A) and all across those plus Diseased site soil (B).

populations can stabilize microbial interactions, disruptions such as land use changes and agricultural practices may diminish their abundance, ultimately impacting soil health (Egidi *et al.*, 2019; Xiang *et al.*, 2024). Moreover, fungal activity significantly influences soil structure by producing microbial exudates that improve soil aggregation (Arias *et al.*, 2023; Sudheer *et al.*, 2024).

The metagenomic analysis conducted in this study successfully identified a diverse fungal community across all sampling sites, including healthy and diseased duku plantations. Dominant genera such as *Aspergillus*, *Penicillium*, *Ganoderma* and members of the *Didymosphaeriaceae* and *Clavariaceae* families were detected in varying abundance. However, it is notable that previously reported pathogens associated with duku diseases, such as *Phytophthora palmivora* (Handoko, 2014) and *Ceratocystis* sp (Firmanto, 2023), were not detected in the current dataset.

Several factors may account for this discrepancy. First, metagenomic sequencing is highly dependent on the DNA extraction method and sequencing depth. It is possible that the DNA of *Phytophthora* or *Ceratocystis* was present at very low concentrations, falling below the detection limit of the sequencing pipeline (Sharpton, 2014). Additionally, the reference database used for taxonomic classification (ITS RefSeq) may have limitations in resolving certain oomycete taxa like *Phytophthora*, which often require other markers such as *cox1* or *cox2* for accurate identification (Robideau *et al.*, 2011).

Second, the symptoms observed in the diseased site may not be caused solely by fungal or oomycete pathogens, but could be the result of complex biotic interactions, including latent infections, bacterial co-infections, or abiotic stressors that mimic pathogen-induced symptoms (Scholthof, 2007). Without pathogen isolation and pathogenicity testing (fulfilling Koch's postulates), it remains speculative to attribute symptoms to specific taxa based solely on sequencing data.

Finally, spatial and temporal variation in pathogen presence could have influenced the results. Pathogens like *P. palmivora* may not be uniformly distributed in soil or plant tissues and may only be detectable during specific stages of infection (Hardham and Blackman, 2018). The findings highlight the need to combine metagenomic approaches with traditional plant pathology methods like culturing, microscopy and pathogenicity tests for better disease diagnostics.

CONCLUSION

This study found that fungal communities differed between healthy and diseased duku plantations. Healthy soils, such as those from Koto Rami, Kumpeh and Rano, showed a more even distribution of fungi, with common genera like *Aspergillus* and *Penicillium*. These communities were supported by better soil conditions, such as higher organic matter or phosphorus levels. In contrast, the diseased site had the highest fungal richness but showed dominance

by certain taxa, such as *Ganoderma*, suggesting microbial imbalance. This imbalance may reflect stress conditions in the soil, which could suppress beneficial fungi and promote opportunistic or harmful species. Known pathogens like *Phytophthora palmivora* and *Ceratocystis* were not detected, possibly due to low DNA abundance or database limitations. These results highlight that fungal community structure can reflect soil health status. Maintaining balanced and diverse fungal communities is important for sustaining healthy duku plantations. Soil management practices that support microbial diversity are key to preventing disease outbreaks.

ACKNOWLEDGEMENT

The present study was supported by the Ministry of Higher Education, Science and Technology of the Republic of Indonesia, as part of the Fundamental Research 2024 program, Contract number: 1612/UN2.11/PT.01.05/SPK/2024.

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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