Edubiotik : Jurnal Pendidikan, Biologi dan Terapan
ISSN 2528-679X (print), ISSN 2597-9833 (online)
Vol. 10, No. 02, November 2025, pp. 529 – 539

Available online at:
https://ejurnal.uibu.ac.id/index.php/edubiotik

Research Article

OPEN ACCESS

Characterization of soil pathogenic fungi in the rhizosphere of siwalan palm (*Borassus flabellifer* L.)

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Article Information

Article History:
Submitted: 2025-08-29
Revised: 2026-01-01
Accepted: 2026-01-09
Published: 2026-01-26

Keywords:
Aspergillus; identification; morphological; pathogenic fungi; siwalan

ABSTRACT

Soil-borne fungal pathogens represent a persistent threat to plant health due to their ability to colonize the rhizosphere and infect root systems, yet information on their diversity in non-industrial palm species remains limited. Siwalan (*Borassus flabellifer* L.) is a drought-tolerant palm of ecological and socio-economic importance, but its rhizospheric fungal communities have not been systematically characterized. This study aimed to (1) isolate soil-borne fungi from the rhizosphere of siwalan and (2) identify potential pathogenic fungi based on macroscopic and microscopic morphological characteristics. Rhizosphere soil samples were collected at depths of 5–20 cm and processed using the soil dilution plate method. Fungal isolates were cultured on potato dextrose agar (PDA), purified through subculturing, and identified using colony morphology and microscopic structures observed through slide culture and lactophenol cotton blue staining. Data were analyzed descriptively by comparing fungal traits with standard taxonomic determination keys. Five soil-borne fungal taxa were successfully isolated and identified, namely *Aspergillus flavus*, *Trichoderma longibrachiatum*, *Aspergillus oryzae*, *Aspergillus caelatus*, and *Aspergillus niger*. The isolates exhibited distinct differences in colony pigmentation, growth rate, hyphal structure, vesicle morphology, and conidial characteristics. Among them, *A. niger* showed the fastest radial growth and highest sporulation intensity, whereas *T. longibrachiatum* and *A. oryzae* exhibited relatively slower growth. These morphological variations indicate functional diversity and differing adaptive strategies among fungi inhabiting the siwalan rhizosphere. This study provides the first systematic morphological baseline of soil-borne fungi associated with the rhizosphere of *B. flabellifer*. The findings contribute novel information on fungal diversity in an underexplored palm species and highlight the importance of early fungal characterization as a foundation for future molecular identification, pathogenicity testing, and the development of sustainable disease management strategies for siwalan cultivation systems.

Publisher
Biology Education Department Universitas Insan Budi Utomo, Malang, Indonesia

How to Cite
Nurtjahyani, S. D., Sulistyaningrum, H., Sulistiono, Amin, M., Tegar Kurnia, D., Dhiaulhaq, M. R., & Mustofa, A. (2026). Characterization of soil pathogenic fungi in the rhizosphere of siwalan palm (*Borassus flabellifer* L.). *Edubiotik : Jurnal Pendidikan, Biologi Dan Terapan*, *10*(02), 529–539. https://doi.org/10.33503/ebio.v10i02.2156

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INTRODUCTION

Soil represents a complex and dynamic ecosystem that serves as a reservoir for soil-borne fungi inhabiting the rhizosphere, where they directly influence plant health, disease occurrence, and agricultural sustainability. Among these microorganisms, soil-borne pathogenic fungi represent a major phytopathological concern because they infect plant root systems and cause destructive diseases, including root rot and vascular wilts (Mustofa & Hastuti, 2024). These pathogens persist in soil ecosystems and interact intensively with the rhizosphere, making them difficult to detect and control (Alfaro-García et al., 2026). Numerous studies have shown that soil-borne fungal diseases significantly reduce crop productivity and threaten food security, especially in agricultural systems that rely on continuous cultivation (Qu et al., 2025; Song et al., 2022; Zhang et al., 2024). Consequently, understanding soil-borne pathogenic fungi remains a key priority in phytopathological research.

A wide range of fungal genera have been reported as important soil-borne plant pathogens, including *Fusarium, Rhizoctonia, Sclerotium*, and *Phytophthora*, all of which contribute to root-associated diseases across diverse cropping systems. *Fusarium* species such as *F. solani, F. oxysporum, and F. graminearum* are frequently cited due to their association with severe yield losses in economically important crops, including cereals, legumes, and tubers. However, soil-borne plant diseases are rarely caused by a single pathogen. *Rhizoctonia solani* is widely recognized as the causal agent of root rot and damping-off in soybean and other crops (Paliwal et al., 2023), while *Sclerotium* spp. and *Phytophthora* spp. are associated with basal stem rot, collar rot, and root decay in various agricultural systems (Legrifi et al., 2023; Sonavane et al., 2023). Current phytopathological perspectives increasingly emphasize that soil-borne diseases arise from complex interactions among multiple pathogenic fungi coexisting within the rhizosphere rather than the dominance of a single genus.

The epidemiological significance of soil-borne pathogenic fungi is closely linked to their ability to survive in soil through resistant propagules such as chlamydospores and sclerotia, which enable long-term persistence under unfavorable environmental conditions (Gyeltshen et al., 2021; Qi et al., 2024). These structures serve as reservoirs of primary inoculum and facilitate recurrent disease outbreaks. For instance, chlamydospores produced by *Fusarium oxysporum* can remain viable in soil for extended periods, increasing the risk of repeated infection cycles (Ding et al., 2019; Vieira et al., 2022). Similar survival strategies are also reported in other soil-borne pathogens, underscoring the ecological resilience and complexity of pathogenic fungal communities in the rhizosphere.

Despite extensive research on soil-borne fungal pathogens affecting major agricultural crops, phytopathological studies focusing on palm species remain comparatively limited. Siwalan (*Borassus flabellifer* L.) is a drought-tolerant palm species of ecological and socio-economic importance, particularly in tropical and semi-arid regions, where it is widely utilized for its fruit, sap, and leaves. While above-ground utilization of siwalan has been well documented, its below-ground interactions with soil microorganisms, particularly pathogenic fungi in the rhizosphere, have received far less scientific attention. Previous studies have shown that soil-borne pathogens such as *Fusarium, Rhizoctonia, Sclerotium*, and *Phytophthora* possess broad host ranges and are capable of infecting various palm species (Katan, 2017; Mageshwaran et al., 2022). However, most existing research has concentrated on economically dominant palms such as oil palm (*Elaeis guineensis*) and coconut (*Cocos nucifera*) (Wong et al., 2021), leaving soil-borne fungal communities associated with siwalan largely unexplored.

To date, comprehensive studies that isolate and morphologically characterize soil-borne fungi from the rhizosphere of siwalan are notably absent. Available investigations on soil-borne pathogens generally lack host-specific information for *B. flabellifer* and rarely document the diversity of rhizospheric fungi using

standardized macroscopic and microscopic identification approaches. Consequently, critical baseline data regarding the composition and potential pathogenicity of soil-borne fungal communities associated with siwalan remain unavailable. This lack of host-specific and rhizosphere-focused information represents a clear research gap, particularly with respect to the diversity of soil-borne fungi colonizing siwalan roots and their morphological characteristics.

The novelty of the present study lies in providing the first systematic isolation and morphological characterization of soil-borne pathogenic fungi inhabiting the rhizosphere of siwalan (*Borassus flabellifer* L.), thereby generating baseline data for an underutilized and poorly studied palm species. By addressing this gap, the study offers new insights into the composition of rhizospheric fungal communities associated with siwalan and establishes a foundation for future investigations on plant–pathogen interactions and disease management strategies in non-industrial palm cultivation systems. In line with this objective, the present study aims to isolate soil-borne fungi from the rhizosphere of siwalan (*Borassus flabellifer* L.) and identify potential pathogenic fungi through macroscopic and microscopic morphological characterization using standard taxonomic determination keys. By elucidating the diversity of soil-borne fungi associated with siwalan, this research contributes to the broader understanding of soil-borne phytopathology and supports the development of sustainable management strategies for siwalan cultivation.

RESEARCH METHODS

This study employed an exploratory–descriptive research design aimed at isolating and identifying soil-borne fungi from the rhizosphere of siwalan (*Borassus flabellifer* L.) based on macroscopic and microscopic morphological characteristics. A descriptive approach was applied to characterize the fungal isolates obtained without conducting inferential statistical analyses. The research was conducted from May to June 2025 at the Microbiology Laboratory of Universitas PGRI Ronggolawe, Tuban, Indonesia. The research design was implemented in sequential stages, including rhizosphere soil sampling, fungal isolation using the soil dilution method, isolate purification, morphological identification, and descriptive data analysis through comparison with standard taxonomic determination keys.

The research population comprised all fungi present in the rhizosphere soil of siwalan plants. The research samples consisted of rhizosphere soil collected from *Borassus flabellifer* L. plants with specific characteristics, namely mature and healthy trees with a height of approximately 10–15 m and a trunk diameter of 40–60 cm, showing no visible disease symptoms. The plants grew naturally in lowland areas at an elevation of approximately 5–15 m above sea level. Sample selection was conducted using purposive sampling to ensure sample representativeness and habitat homogeneity. Rhizosphere soil was collected at depths of 5–20 cm around the active root zone using sterile tools.

The research instruments included sterile sampling tools (hand trowel and spatula), an analytical balance, vortex mixer, micropipettes, test tubes, measuring cylinders, Petri dishes, an incubator, a compound light microscope, and a digital camera for documentation. Potato Dextrose Agar (PDA) supplemented with chloramphenicol was used as the culture medium to inhibit bacterial growth. All instruments were employed throughout the sampling, isolation, purification, observation, and data recording processes.

Data collection was carried out through fungal isolation using the soil dilution plate method. A total of 10 g of homogenized soil was suspended in 90 mL of sterile distilled water and homogenized using a vortex mixer. Serial dilutions were prepared up to 10^{-3}, and 1 mL of each dilution was inoculated onto PDA plates. All inoculated Petri dishes were incubated at 25–28°C for 5–7 days. Emerging fungal colonies were observed macroscopically based on colony color, shape, texture, margin, and growth pattern.

Morphologically distinct colonies were subsequently subcultured onto fresh PDA plates to obtain pure isolates.

Fungal identification was performed through macroscopic and microscopic observations. Microscopic examination was conducted using the slide culture method with lactophenol cotton blue staining to observe fungal structures, including hyphae, conidia, and conidiophores, under a compound light microscope. Identification was achieved by matching the morphological characteristics of the isolates with standard fungal taxonomic references and determination keys, following the procedures described by Mustofa et al. (2024). Diagnostic structures were documented using a digital camera to support the identification process.

Data analysis was conducted using a qualitative descriptive approach, in which macroscopic and microscopic observations were presented in narrative descriptions and morphological characterization tables. The determination of fungal genera or species was based on the conformity of isolate morphological traits with the applied taxonomic keys. This analysis aimed to provide an overview of soil-borne fungal species successfully isolated from the rhizosphere of siwalan plants, without employing diversity indices or quantitative statistical analyses.

FINDING AND DISCUSSION

Based on isolation and morphological identification, five soil fungal taxa were recovered from the rhizosphere of siwalan roots. These isolates were initially grouped based on colony morphology and subsequently examined using standard taxonomic determination keys (Campbell et al., 2013). The fungi identified at the genus or species-complex level included *Aspergillus flavus* (Figure 1), *Trichoderma longibrachiatum* (Figure 2), *Aspergillus oryzae* (Figure 3), *Aspergillus caelatus* (Figure 4), and *Aspergillus niger* (Figure 5). All isolates were able to grow on potato dextrose agar (PDA) and displayed cottony colony textures, indicating active mycelial development. However, marked differences were observed in colony color, basal pigmentation, growth rate, and sporulation intensity, suggesting morphological divergence among the isolates. These variations provide an important preliminary basis for distinguishing fungal taxa and for assessing their potential ecological roles within the siwalan rhizosphere.

Comparative analysis of colony morphology revealed distinct growth patterns among the isolates, which are relevant for preliminary species differentiation. *A. niger* exhibited the fastest radial growth and the highest sporulation intensity, reaching a colony diameter of 72.75 mm by day five, indicating strong saprophytic competitiveness. In contrast, *T. longibrachiatum* and the unidentified *Aspergillus* sp. showed slower growth rates, suggesting different ecological strategies or physiological constraints. Differences in colony pigmentation, particularly basal coloration, are taxonomically informative within the genus *Aspergillus* and may reflect variations in secondary metabolite production. For instance, the dark pigmentation observed in *A. niger* is associated with melanin accumulation, which is known to enhance resistance to environmental stress. Thus, colony-level characteristics not only support morphological identification but also provide insights into the biological adaptability of each isolate.

Detailed macroscopic characterization further revealed species-specific colony traits among the identified fungi. *A. flavus* formed yellowish-green colonies with white margins and a fine cotton-like texture, reaching 48.82 mm in diameter by day five, indicative of relatively rapid growth (Guo & Ji, 2021). *T. longibrachiatum* produced white to cream-colored colonies with a bright yellow center and irregular margins, exhibiting slower growth (27.78 mm) consistent with previous descriptions (Prameeladevi et al., 2018). *A. caelatus* showed moderate growth (38.08 mm) with yellowish-green centers and white edges, while *A. niger* was readily distinguished by its black conidial mass and vigorous radial expansion (Balajee

& Brandt, 2022). These differences reinforce the usefulness of colony morphology as an initial diagnostic tool for distinguishing soil-borne fungi. Microscopic examination of the five isolates revealed clear structural differences in hyphae, conidiophores, vesicles, phialides, and conidia (Table 1). All isolates possessed hyaline hyphae, characteristic of hyalohyphomycetes, indicating the absence of melanin pigmentation (Lin et al., 2025). Septate hyphae were observed in *A. flavus*, *T. longibrachiatum*, and *A. caelatus*, whereas *A. niger* exhibited narrower hyphae with smaller diameters. Conidiophore length and branching patterns varied substantially, with branched conidiophores uniquely observed in *T. longibrachiatum*, reflecting its distinct reproductive strategy. Variations in vesicle shape and size further supported taxonomic differentiation among isolates.

Phialide morphology and conidial characteristics provided additional distinguishing features. The presence of metulae in *A. flavus*, *A. caelatus*, and *A. niger* indicates biseriate conidiophore structures, whereas *T. longibrachiatum* lacked vesicles entirely. Conidial pigmentation also varied, with black conidia in *A. niger* attributed to melanin deposition, a trait associated with enhanced environmental resilience (Segers et al., 2018). Although all isolates produced globose conidia in chains, differences in phialide arrangement and vesicle morphology were sufficient to discriminate among taxa. Collectively, these microscopic traits strengthen the reliability of morphological identification at the genus and species-complex levels. Overall, the integration of macroscopic and microscopic observations allowed for the differentiation of soil fungal isolates associated with siwalan roots and the identification of taxa with known pathogenic potential. While morphological characterization alone cannot fully resolve species identity, particularly within morphologically conserved genera such as *Aspergillus*, it provides critical baseline information for understanding fungal diversity in the siwalan rhizosphere. These findings establish a foundation for future molecular studies and contribute to the early detection of soil-borne fungi that may pose risks to siwalan cultivation.

Although morphological characterization in this study provides essential baseline information on the diversity of soil-borne fungi inhabiting the siwalan rhizosphere, this approach remains limited in its ability to achieve high taxonomic resolution, particularly within genera that exhibit cryptic diversity such as *Aspergillus*. Future studies should therefore incorporate molecular markers, including the ITS rDNA region and protein-coding genes such as β-tubulin, to validate species-level identification and clarify phylogenetic relationships among isolates. The integration of molecular tools is expected to reduce the risk of misidentification arising from phenotypic plasticity and overlapping morphological traits (Lücking et al., 2020; Zulkifli & Zakaria, 2017). Furthermore, combining molecular identification with functional assays and pathogenicity testing would enable clearer differentiation among pathogenic, opportunistic, and potentially beneficial fungi associated with the siwalan rhizosphere. In addition, the application of metabarcoding-based community profiling may reveal unculturable or low-abundance fungal taxa that remain undetected by culture-dependent methods, thereby offering a more comprehensive perspective on soil fungal ecology (Yusufu et al., 2025). Overall, the integration of morphological, molecular, and ecological approaches in future research will strengthen phytopathological interpretations and support the development of more accurate and sustainable disease management strategies for this underexplored siwalan cultivation system.

Table 1. Microscopic Characteristics of Each Isolate

No	Characteristic	*A. flavus*	*T. longibrachiatum*	*A. oryzae*	*A. caelatus*	*A. niger*
1	Hypha color	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
2	Septa on hypha	Present	Present	Absent	Present	Absent
3	Hypha diameter	2–3 µm	3 µm	3 µm	3 µm	2 µm

No	Characteristic	*A. flavus*	*T. longibrachiatum*	*A. oryzae*	*A. caelatus*	*A. niger*
4	Conidiophore color	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
5	Conidiophore diameter	10 µm	3 µm	6 µm	9 µm	3 µm
6	Sporangiophore length	120–530 µm	11 µm	270 µm	360 µm	165 µm
7	Branched conidiophore	Absent	Branched	Absent	Absent	Branched
8	Conidiophore wall (smooth/rough)	Smooth	Smooth	Smooth	Smooth	Smooth
9	Vesicle shape	Ampulliform	-	Ampulliform	Ampulliform	Pyriform
10	Vesicle diameter	30 µm	-	10–20 µm	20 µm	5 µm
11	Metula present/absent	Present	-	Absent	Present	Present
12	Metula size	5 µm	-	5 µm	3 µm	4 µm
13	Phialide color	Green	-	Green	Green	Black
14	Phialide position on vesicle	Attached	-	Attached	Attached	Attached
15	Type of conidiophore branching	Absent	Absent	Absent	Absent	Branched
16	Phialide size	3 µm	5–8 µm	3 µm	7 µm	3 µm
17	Phialide shape	Ellipsoidal	Pyriform	Clavate	Clavate	Ellipsoidal
18	Conidia shape	Globose	Globose	Globose	Globose	Globose
19	Conidia wall (smooth/rough)	Smooth	Smooth	Smooth	Smooth	Smooth
20	Conidia diameter	4 µm	2 µm	3 µm	3 µm	3 µm
21	Conidia color	Green	Green	Green	Green	Black
22	Conidia growth type	Chain formation	Chain formation	Chain formation	Chain formation	Chain formation

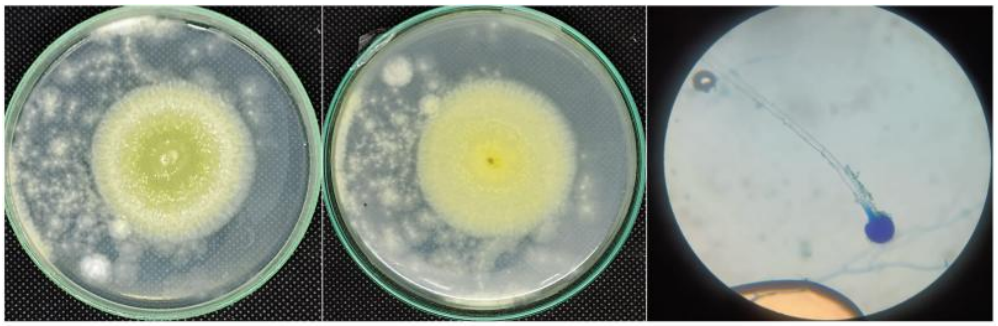


Figure 1. Morphology of *Aspergillus flavus*
(Colonies on PDA are yellowish-green with white margins and a cotton-like texture, while microscopic features include radiate conidial heads, globose vesicles, and globose to subglobose conidia, supporting its identification as a potential soil-borne pathogen)

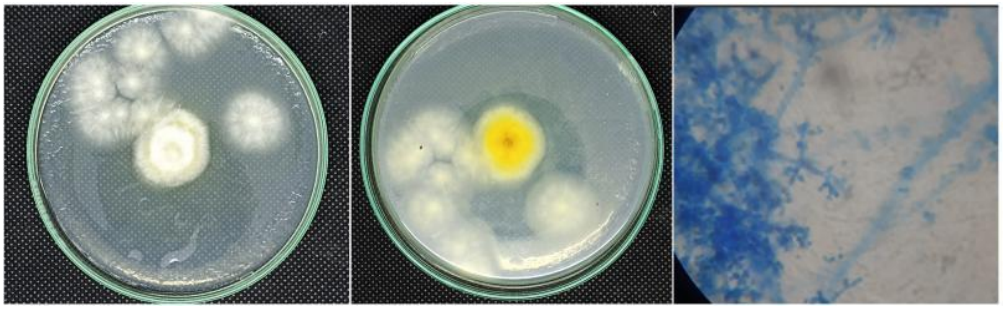


Figure 2. Morphology of *T. longibrachiatum*
([a-b] The colonies exhibit a cream-white coloration with a bright yellow central zone, irregular spreading, and fine hyphal textures resembling cotton fibers. [c] Microscopic examination shows slender and elongated phialides (longibrachiatum-type phialides) along with hyaline to greenish ellipsoid conidia, which represent key diagnostic traits of the genus *Trichoderma*)

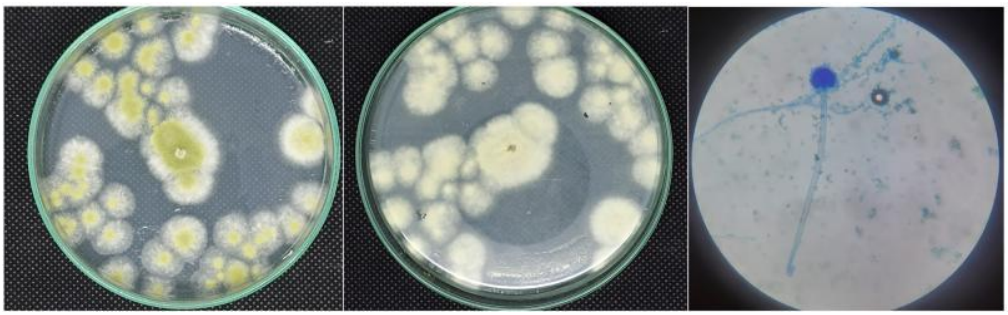


Figure 3. Morphology of *A. oryzae*
([a–b] The colonies display a cream-white coloration with a pale green center, irregular margins, and slow growth. [c] Microscopic structures show a smaller vesicle, slender conidiophores, and conidial heads lacking uniformity. These differences distinguish the isolate from *A. flavus*]

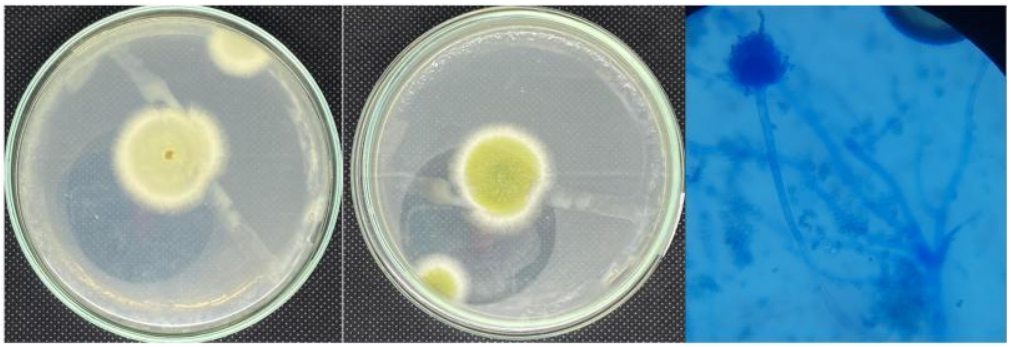


Figure 4. Morphology of *A. caelatus*
([a–b] The colonies exhibit yellowish-green pigmentation at the center with white margins, a smooth colony surface, and moderate growth. [c] Microscopy reveals a globose vesicle, biseriate phialides, and roughened greenish conidia, consistent with the diagnostic characteristics of *A. caelatus*)

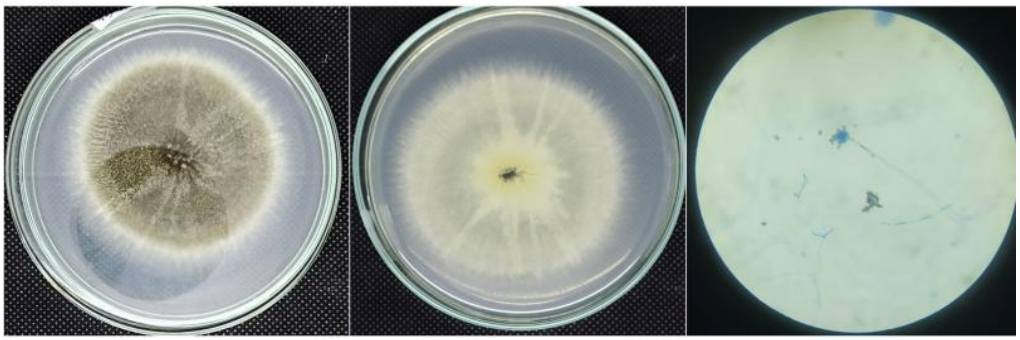


Figure 5. Morphology of *A. niger*
([a–b] The colonies are characterized by a dense black central region, white margins, and a cream-to-yellowish base, indicating heavy sporulation and rapid growth. [c] Microscopic observation shows thick, dark conidiophores, a large globose vesicle, and roughened black conidia, which are typical morphological features of *A. niger*]

The present study demonstrates that the rhizosphere of siwalan (*Borassus flabellifer* L.) harbors a diverse assemblage of soil-borne fungi with known or potential pathogenic relevance. Based on macroscopic and microscopic morphological characterization, five fungal taxa were identified, namely *A. flavus*, *T. longibrachiatum*, *A. oryzae*, *A. caelatus*, and *A. niger*. These findings align with previous reports indicating that members of the genera *Aspergillus* and *Trichoderma* are common inhabitants of soil and root-associated environments, where they may function as pathogens, saprophytes, or opportunistic colonizers depending on environmental conditions and host susceptibility (Ramzan et al., 2023; Teh & Latiffah, 2018).

Comparative analysis of colony morphology revealed clear interspecific variation in colony color, basal pigmentation, and radial growth rate on PDA medium, which are widely recognized as important diagnostic traits in fungal taxonomy. The rapid growth of *A. niger* (72.75 mm within five days) observed in this study is consistent with previous reports describing this species as highly competitive and ecologically resilient due to its strong saprophytic capacity and melanized conidia (Balajee & Brandt, 2022). In contrast, the slower growth rates recorded for *T. longibrachiatum* and *A. oryzae* suggest alternative ecological strategies, potentially reflecting differences in nutrient utilization, stress tolerance, or host interaction dynamics. These growth-related traits not only support preliminary species differentiation but may also provide indirect insights into the biological potential of each isolate within the siwalan rhizosphere.

Microscopic characteristics further strengthened taxonomic discrimination among the isolates. Variations in vesicle morphology, the presence or absence of metulae, phialide arrangement, and conidial size were consistent with established taxonomic descriptions for *Aspergillus* and *Trichoderma* species (Simes et al., 2013). For instance, the large vesicle diameter and well-developed metulae observed in *A. flavus* are characteristic features associated with this species, while the black-pigmented conidia and compact conidial chains of *A. niger* reflect melanin-mediated adaptations that enhance resistance to environmental stressors. These findings reinforce the notion that, although species within the same genus may share general morphological frameworks, fine-scale structural differences remain critical for species-level interpretation. An interesting outcome of this study is the isolation of *T. longibrachiatum* from rhizosfer of siwalan roots. Although *Trichoderma* species are commonly reported as biological control agents and antagonists of plant pathogens, several studies have highlighted their capacity to behave as opportunistic colonizers under specific ecological conditions (Ramzan et al., 2023).

The novelty of this study lies in providing the first systematic morphological characterization of soil-borne fungi associated with the rhizosphere of siwalan, an underexplored palm species that has received limited attention in phytopathological research. Unlike previous studies that primarily focus on major industrial crops or economically dominant palms, this work establishes baseline data on fungal diversity and potential pathogenic taxa in siwalan cultivation systems. By documenting both pathogenic and opportunistic fungi using standardized morphological criteria, this study fills a critical knowledge gap and offers a foundation for subsequent molecular identification, pathogenicity testing, and disease management research. The findings highlight the importance of localized soil microbiota surveys for non-industrial plant species such as siwalan, which, despite their ecological adaptability, remain vulnerable to soil-borne fungal interactions. Morphological characterization, as applied in this study, represents a crucial initial step in phytopathological investigation, enabling informed hypothesis generation prior to molecular confirmation. The integration of these results with future molecular and ecological studies will enhance understanding of plant–fungal interactions in siwalan and support the development of more targeted and sustainable disease management strategies.

CONCLUSION

This study successfully achieved its objectives by isolating and morphologically identifying soil-borne fungi from the rhizosphere of siwalan (*Borassus flabellifer* L.). Based on macroscopic and microscopic characterization, five fungal taxa were identified, namely *Aspergillus flavus*, *Trichoderma longibrachiatum*, *Aspergillus oryzae*, *Aspergillus caelatus*, and *Aspergillus niger*. Each isolate exhibited distinct colony morphology, growth rate, and microscopic structures, confirming the effectiveness of morphological approaches as an initial tool for fungal identification in the siwalan rhizosphere.Among the

identified isolates, *A. niger* demonstrated the fastest radial growth and highest sporulation intensity, while *T. longibrachiatum* and *A. oryzae* showed relatively slower growth, indicating interspecific variation in ecological adaptation within the soil environment. These findings highlight the diversity of soil-borne fungi associated with siwalan roots and suggest the presence of taxa with known or potential pathogenic relevance.This study provides the first baseline data on soil-borne fungal diversity in the rhizosphere of siwalan, an underexplored palm species. The morphological identification presented here serves as a foundational step for future studies incorporating molecular identification and pathogenicity assays to better elucidate the ecological roles and disease potential of these fungi in siwalan cultivation systems.

ACKNOWLEDGMENT

The authors have the same contribution to this article. Regular Fundamental Grant Funded by: Direktorat Penelitian dan Pengabdian kepada Masyarakat, Direktorat Jenderal Riset dan Pengembangan, Kementerian Pendidikan Tinggi, Sains, dan Teknologi. Number: SP DIPA-139.04.1.693320/2025 revision 04 dated April 30, 2025; Number: 128/C3/DT.05.00/PL/2025 dated May 28, 2025; and Number: 054/LL7/DT.05.00/PL/2025 dated May 28, 2025.

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