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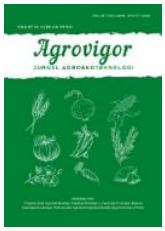

Article

Morphological Identification of Fungi Associated with Leaf Spot Symptoms on Pineapple (Ananas comosus L. Merr) in Rimbo Panjang, Riau, Indonesia

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ABSTRACT

Leaf spot is one of the major foliar diseases affecting pineapple production in tropical regions, yet information regarding the fungal pathogens associated with this disease in Riau Province, Indonesia, remains limited. This study aimed to isolate and morphologically identify fungi associated with leaf spot symptoms on pineapple cultivated in Rimbo Panjang Village, Kampar Regency, Riau. Symptomatic leaf samples were collected from pineapple plantations, and fungal isolation was carried out using Potato Dextrose Agar. Pure cultures were subsequently characterized based on colony and microscopic morphological features, including colony appearance, hyphal characteristics, conidial morphology, pigmentation, septation, and reproductive structures. Three morphologically distinct fungal isolates were successfully recovered from diseased leaf tissues. Based on morphological observations, isolate BNS-1 was provisionally identified as *Lasiodiplodia* sp., characterized by pigmented septate hyphae, immature hyaline conidia, mature dark brown one-septate conidia, and pycnidium-like structures. Isolate BNS-2 was assigned to the genus *Nigrospora* because it produced solitary, spherical, black aseptate conidia on hyaline septate hyphae. Isolate BNS-3 produced only sterile hyaline mycelia with chlamydospore-like structures and therefore remained unidentified at the genus level. The presence of multiple fungal genera associated with pineapple leaf spot symptoms indicates that the disease may involve a diverse fungal community rather than a single pathogen. This research provides baseline information on fungi associated with pineapple leaf spot in Rimbo Panjang and establishes a foundation for future pathogenicity studies and molecular identification to support effective disease management strategies.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is one of the most economically important horticultural crops cultivated in Indonesia, contributing substantially to farmers' income and regional agricultural development. Sustainable pineapple production largely depends on healthy foliage, as leaves are the primary photosynthetic organs supporting plant growth, fruit development, and overall productivity. Consequently, foliar diseases can significantly reduce photosynthetic efficiency, impair plant vigor, and ultimately decrease both fruit yield and quality (Román et al., 2026). Among these diseases, leaf spot has become an important constraint in pineapple cultivation due to its adverse effects on leaf function and crop performance (Cheaib & Killiny, 2025). Disease development is particularly favored in warm, humid tropical conditions, where prolonged leaf wetness and high rainfall create an environment conducive to fungal infection and pathogen dissemination (Thaochan et al., 2025).

Riau Province is recognized as one of Indonesia's major pineapple-producing regions, with Rimbo Panjang Village, Kampar Regency, representing an important production center (Badan Pusat Statistik, 2025). Pineapple cultivation in this area plays a significant role in local agricultural production; however, information regarding diseases affecting this crop remains limited. In particular, no published study has specifically investigated the fungal pathogens associated with leaf spot symptoms in pineapple plantations within Rimbo Panjang. Consequently, baseline information on the fungal communities associated with this disease under local agroecological conditions is still unavailable.

Leaf spot symptoms on pineapple can be associated with different fungal taxa, and similar necrotic symptoms may be produced by different pathogens. Previous studies have reported fungal pathogens associated with pineapple diseases in different production regions, including *Curvularia* associated with pineapple leaf spot and *Lasiodiplodia theobromae* associated with pineapple leaf blight (Zhong et al., 2016; Ganesan et al., 2022). However, information on fungi associated with pineapple leaf spot in Riau Province, particularly in the Rimbo Panjang production area, remains limited. Because pathogen composition may vary according to host, environment, and production conditions, information from other geographical regions cannot necessarily be directly extrapolated to local pineapple plantations. Therefore, morphological characterization of fungal isolates obtained from symptomatic leaves is needed as an initial step to establish baseline information on the fungal taxa associated with pineapple leaf spot in this area.

Despite the occurrence of pineapple cultivation in Rimbo Panjang, Kampar Regency, no published study has specifically documented the fungal taxa associated with leaf spot symptoms in this production area. This represents an important information gap for disease diagnosis and subsequent pathogen confirmation. Therefore, this study aimed to isolate and morphologically characterize fungi associated with leaf spot symptoms on pineapple cultivated in Rimbo Panjang Village, Kampar Regency, Riau. The findings are expected to provide baseline information for subsequent pathogenicity testing and molecular identification of the associated fungi.

MATERIALS AND METHODS

Study Area and Sample Collection

This study was conducted using pineapple leaves exhibiting leaf spot symptoms collected from commercial pineapple plantations in Rimbo Panjang Village, Kampar Regency, Riau Province, Indonesia. The sampling locations were geographically recorded using a Global Positioning System (GPS) with latitude 0°27′03.3″ N and Longitude 101°18′49.3″ E. Field sampling was performed between November 2025 and February 2026. Symptomatic leaves were selected using purposive sampling to ensure that only tissues displaying typical leaf spot symptoms were included in the study. A total of 15 symptomatic leaves were collected from five sampling locations distributed across the plantation area. Immediately after collection, the samples were individually placed in sterile plastic bags, labeled, and transported to the Plant Disease Laboratory, Faculty of Agriculture, Universitas Riau for fungal isolation and morphological characterization.

Isolation and Purification of Fungal Isolates

Leaves exhibiting characteristic leaf spot symptoms were first rinsed thoroughly under running tap water to remove adhering soil particles and surface debris. Small tissue segments (approximately 1 × 1 cm) were excised from

the interface between healthy and diseased tissues, as this region is generally considered to contain actively growing pathogens. The excised tissues were surface-sterilized by immersion in 70% ethanol for 30 s, followed by 1% sodium hypochlorite (NaOCl) for 1 min, and finally rinsed three times with sterile distilled water. The sterilized tissues were dried on sterile filter paper under aseptic conditions before being transferred onto Potato Dextrose Agar (PDA) medium. Culture plates were incubated at 25–28°C for 5–7 days until fungal colonies emerged. Hyphal tips from morphologically distinct colonies were aseptically transferred onto fresh PDA plates to obtain pure cultures. Purification was repeated whenever necessary until each isolate exhibited uniform colony morphology. For isolates requiring further morphological characterization, purification and subsequent incubation were also performed on water agar, a low-nutrient medium, to facilitate the expression of morphological or reproductive structures that were not observed on PDA.

Morphological Characterization

Morphological identification was performed using both macroscopic and microscopic observations of purified fungal isolates. Colony morphology was evaluated after 7 days of incubation on PDA. The following characteristics were recorded: colony colour (upper and reverse surfaces), colony texture, colony margin, colony shape, radial growth pattern, and presence of zonation or pigmentation. These characteristics were documented photographically for each isolate.

Microscopic observations were performed using a compound light microscope equipped with a 10× ocular lens. Fungal structures were examined using 40× objective lenses, yielding a total magnification of 400×. A small amount of fungal mycelium was mounted on a clean glass slide using lactophenol cotton blue, covered with a coverslip, and examined immediately. The following taxonomic characteristics were observed: hyphal pigmentation, hyphal septation, branching pattern, conidial morphology, conidial pigmentation, conidial septation, and reproductive structures (pycnidia, conidiophores, chlamydospores, or other specialized structures). Measurements were made using the microscope calibration system whenever applicable.

Fungal Identification

Identification was performed by comparing the observed morphological characteristics with descriptions provided in standard fungal taxonomic references and published literature relevant to plant pathogenic fungi. The identification was based on established morphological identification keys, particularly Dugan (2017), and was further cross-checked against modern taxonomic treatments of the respective genera. Because this study relied exclusively on morphological characteristics, isolates were conservatively identified only to the genus level whenever diagnostic structures were available. Isolates that failed to produce reproductive structures under laboratory conditions were classified as mycelia sterilia, following current taxonomic practice, and their identity was discussed based on observable vegetative characteristics. The present study was designed as a preliminary morphological investigation; therefore, pathogenicity assays and molecular analyses were intentionally excluded and are proposed as the focus of subsequent studies.

Data Interpretation

The study employed a descriptive approach. Morphological data obtained from colony and microscopic observations were compiled and compared among isolates to determine similarities and distinguishing characteristics. The observed morphological features were subsequently matched with published taxonomic descriptions to establish the most probable genus for each isolate. Since molecular analyses and pathogenicity assays were beyond the scope of the present study, the taxonomic identification presented herein should be regarded as a preliminary morphological assessment.

RESULTS AND DISCUSSION

Field Symptoms of Pineapple Leaf Spot

Field observations revealed that disease symptoms were predominantly confined to the leaves. Affected plants exhibited irregular leaf discoloration accompanied by circular to oval lesions distributed across the leaf surface. The

lesions were characterized by a light gray to yellowish-gray center surrounded by a dark brown margin. Pale yellow chlorotic zones were also observed extending along the leaf blade, while some infected tissues had progressed to dark brown or black necrotic areas (Figure 1). Based on these symptom characteristics, the disease observed in the study area was classified as pineapple leaf spot. Comparable symptoms, including grayish-yellow necrotic lesions bordered by dark brown margins, have previously been described in pineapple infected by fungal pathogens (Zhong et al., 2016).



Figure 1. Representative symptoms of leaf spot observed on pineapple leaves collected from Rimbo Panjang Village, Kampar Regency, Riau Province. (A) Leaf spot symptoms in pineapple leaves, (B) Leaf with dark brown or black necrotic areas indicated by arrowheads.

Fungal Isolation and Morphological Characteristics

Isolation of symptomatic leaf tissues followed by purification procedures resulted in three morphologically distinct fungal isolates, designated **BNS-1**, **BNS-2**, and **BNS-3**. Each isolate exhibited unique colony morphology and microscopic characteristics, indicating the presence of different fungal taxa (Tables 1 and 2).

Table 1. Colony characteristics of fungal isolates associated with pineapple leaf spot symptoms

Characteristic	BNS-1	BNS-2	BNS-3
Colony color			
a. Upper surface	Initially grayish-white, becoming dark black with age	Grayish-white margins with a dark central region	White with a pale yellow tint
b. Reverse surface	Initially grayish-white, later dark black	Grayish-white with dark brown patches	White with a slight pale-yellow coloration
Colony texture	Dense, cottony	Floccose with abundant aerial mycelia	Cottony with compact white mycelial aggregates
Colony shape	Circular with slightly irregular margins	Irregular circular colony with uneven margins	Circular with smooth margins
Growth pattern	Symmetrical radial growth with faint concentric zones	Irregular concentric growth	Irregular concentric growth

Note: Colonies were evaluated after seven days of incubation on PDA at room temperature

The present study successfully recovered three fungal isolates from pineapple leaves exhibiting leaf spot symptoms. Based on morphological characteristics, two isolates were assigned to the genera *Lasiodiplodia* (BNS-1) and *Nigrospora* (BNS-2), whereas the third isolate (BNS-3) remained unidentified because no reproductive structures were produced during culture. These findings indicate that pineapple leaf spot in Rimbo Panjang is associated with multiple fungal taxa rather than a single pathogen, suggesting that the disease may involve a complex of fungi under local environmental conditions.

Table 2. Microscopic characteristics of fungal isolates associated with pineapple leaf spot symptoms

Characteristics	BNS-1	BNS-2	BNS-3
Hyphal pigmentation	Light to dark brown (dematiaceous)	Hyaline	Hyaline
Hyphal characteristics	Septate, branched	Septate, branched	Septate with irregular branching
Conidial morphology	Young conidia ellipsoid to oblong, aseptate; mature conidia ellipsoid with one septum	Spherical, aseptate	Conidia absent
Conidial pigmentation	Hyaline when immature, dark brown when mature	Black	Not observed
Distinctive structures	Dark brown sac-like structures resembling pycnidia	None observed	Thick-walled spherical structures resembling intercalary chlamydospores
Presumptive identification	*Lasiodiplodia* sp.	*Nigrospora* sp.	Unidentified (*Mycelia sterilia*)

Note: Microscopic observations were performed using a light microscope at 400× magnification

Isolate BNS-1

Isolate BNS-1 displayed morphological characteristics consistent with members of the genus Lasiodiplodia. The BNS-1 isolate initially produced grayish-white colonies that gradually became dark black after seven days of incubation on PDA. This color transition was evident on both the upper and reverse surfaces of the colony. Colonies exhibited a dense cottony texture with symmetrical radial expansion and faint concentric zonation (Figure 2A). Microscopically, the isolate possessed septate and branched dematiaceous hyphae, while immature conidia were hyaline, aseptate, and ellipsoid to oblong in shape, whereas mature conidia became dark brown, remained ellipsoid to oblong, and developed a single transverse septum (Figure 2B). Dark brown sac-like reproductive structures resembling pycnidia were also observed after incubation on water agar (Figure 2C). These morphological characteristics, particularly the transition from hyaline aseptate to pigmented one-septate conidia and the presence of pycnidium-like structures, were the primary basis for the provisional assignment of BNS-1 to *Lasiodiplodia*.



Figure 2. Morphological characteristics of isolate BNS-1. (A) Colony morphology on PDA; (B1) immature hyaline and (B2) mature septate conidia with arrowheads indicating the one-septate mature conidia; (C) septate hyphae with pycnidium-like structures indicated by arrowheads. Scale based on 400× magnification

BNS-1 displayed morphological characteristics that closely correspond to those reported for members of the genus *Lasiodiplodia*. Colonies expanded rapidly on PDA, initially appearing grayish-white before gradually becoming dark black as the culture matured. The colony texture was distinctly cottony, with symmetrical radial growth and faint concentric zonation (Huda-Shakirah et al., 2022).

Reports of Lasiodiplodia from other plant hosts were used only as comparative morphological references and were not considered evidence of host association or pathogenicity on pineapple. Comparable colony characteristics have been described for *Lasiodiplodia theobromae*, whose colonies typically change from white or pale gray during early growth to dark gray or black following sporulation (Atallah et al., 2024). Similar observations have also been reported for *Lasiodiplodia* isolates recovered from rubber trees in Thailand, where colonies exhibited circular growth with smooth margins and progressive dark pigmentation during maturation (Pornsuriya et al., 2023).

Microscopic examination provided additional evidence supporting the identification of isolate BNS-1 as *Lasiodiplodia*. Young conidia were hyaline, aseptate, and ellipsoid to oblong, whereas mature conidia became dark brown and developed a single transverse septum. This developmental transition from hyaline, single-celled conidia to pigmented septate conidia is regarded as one of the most diagnostic features of the genus (Atallah et al., 2024; He et al., 2025). Similar conidial morphology has been documented for *Lasiodiplodia* isolates obtained from rubber plantations in China, where immature conidia were hyaline and aseptate before maturing into dark brown, one-septate spores (He et al., 2025).

Another important diagnostic characteristic observed in isolate BNS-1 was the presence of dark brown pycnidium-like structures. These structures were observed after 14 days of incubation on water agar, as pycnidium-like structures were not yet visible after 7 days of incubation on PDA. Members of *Lasiodiplodia* typically produce dark, globose to subglobose pycnidia either within infected host tissues or on artificial media (Bhunjun et al., 2021). Similar pycnidial development has been reported in *L. theobromae* isolated from longan fruit, further supporting the taxonomic placement of isolate BNS-1 within this genus (Serrato-Díaz et al., 2014).

Species belonging to *Lasiodiplodia* possess an exceptionally broad host range and are recognized as important pathogens of numerous tropical and subtropical crops. *L. theobromae* alone has been reported to infect more than 500 plant species (Atallah et al., 2024). On pineapple, *Lasiodiplodia* has previously been identified as the causal agent of leaf blight, producing irregular brown lesions that gradually expand and coalesce into extensive necrotic areas (Ganesan et al., 2022). The environmental conditions prevailing in Riau, characterized by consistently high humidity and abundant rainfall, are highly favorable for disease development because warm and moist environments promote infection, colonization, and sporulation of *Lasiodiplodia* spp. (Atallah et al., 2024).

Despite the strong morphological evidence obtained in this study, isolate BNS-1 was conservatively identified only to the genus level. Species delimitation within *Lasiodiplodia* has become increasingly dependent on molecular phylogenetic analyses because many species exhibit overlapping morphological characteristics. Current taxonomic studies recommend multilocus sequencing using ITS, *tef1-α*, and *tub2* markers to achieve reliable species identification (Jose et al., 2023; Rathnayaka et al., 2023). Therefore, molecular confirmation remains necessary before assigning isolate BNS-1 to a particular species.

Although members of *Lasiodiplodia* have been reported as pathogens of several tropical crops, the present study did not determine whether BNS-1 was pathogenic to pineapple. Therefore, its role in pineapple leaf spot remains to be confirmed through pathogenicity testing.

Isolate BNS-2

Colonies of isolate BNS-2 were grayish-white with a darkened central region and exhibited a floccose texture accompanied by abundant aerial mycelia. Colony growth formed irregular concentric rings on PDA (Figure 3A). Microscopic examination revealed hyaline, septate, and branched hyphae. Conidia were solitary, spherical, aseptate, and intensely black, developing at the tips of conidiophores (Figures 3B and 3C). These conidial characteristics were the primary basis for the provisional assignment of BNS-2 to the genus *Nigrospora*.

Isolate BNS-2 exhibited morphological characteristics consistent with those commonly reported for the genus *Nigrospora*. Colonies were initially grayish-white but gradually developed a dark central region as sporulation progressed. The colonies possessed a floccose texture with abundant aerial mycelia, a pattern frequently described for *Nigrospora* species cultured on PDA (Han et al., 2021). The most distinctive feature of isolate BNS-2 was its conidial morphology. The fungus produced solitary, spherical, aseptate conidia that were intensely black and borne at the tips of conidiophores. These characteristics closely match published descriptions of *Nigrospora*, whose conidia are

typically black, smooth-walled, unicellular, and globose to subglobose, measuring approximately 10–22 µm in diameter (Deepika et al., 2020).

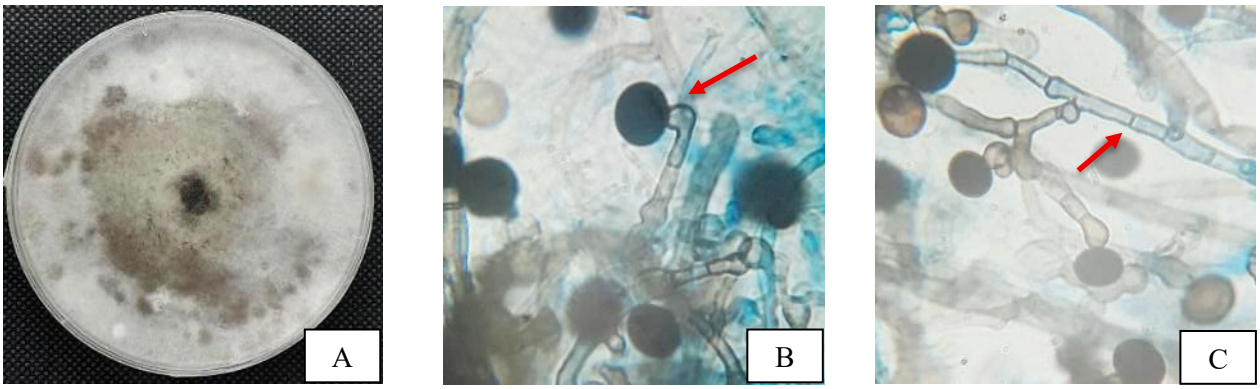

Figure 3. Morphological characteristics of isolate BNS-2. (A) Colony on PDA; (B) solitary conidia and conidiophore indicated by arrowheads; (C) hyaline septate hyphae indicated by arrowheads. Images captured at 400× magnification.

Morphologically similar conidial characteristics have been reported for *Nigrospora* isolates obtained from several plant hosts, including *Rhododendron simsii*, *Cyclocarya paliurus*, and cowpea. These reports were considered only as comparative taxonomic references and were not interpreted as evidence that the present isolate shares the same host association or pathogenicity. Comparable observations have been reported for *Ni sphaerica* isolated from *Rhododendron simsii*, in which solitary black conidia developed on hyaline vesicles located at the apex of conidiophores (Wang et al., 2024). Similar morphological features have also been described for *Nigrospora* associated with leaf spot disease of *Cyclocarya paliurus* (Zheng et al., 2020).

Although species of *Nigrospora* have been reported in association with leaf spot symptoms on several plant species, the present study did not determine whether BNS-2 is pathogenic to pineapple. Therefore, its association with pineapple leaf spot symptoms should be interpreted cautiously and requires confirmation through pathogenicity testing. Members of the genus *Nigrospora* have been isolated from a wide range of economically important crops, including tobacco, ornamental plants, kiwifruit, medicinal plants, grasses, and numerous horticultural species (Du et al., 2026). Similar findings were reported by Obisesan & Ojo (2023), who isolated *Nigrospora sphaerica* from cowpea leaves exhibiting leaf spot symptoms, where the fungus produced solitary, spherical, dark-colored conidia comparable to those observed in isolate BNS-2.

As with isolate BNS-1, identification of BNS-2 was restricted to the genus level because morphological differences among *Nigrospora* species are often subtle and insufficient for reliable species delimitation. Recent taxonomic studies emphasize that species identification within this genus should combine morphological observations with molecular phylogenetic analyses, particularly ITS sequence data, to ensure accurate classification (Qiu et al., 2022).

Isolate BNS-3

Isolate BNS-3 formed white colonies with a faint yellowish tint and a cottony appearance accompanied by compact white mycelial masses. Colony expansion was uniform and radial across the PDA medium (Figure 4A). Microscopically, the isolate consisted of hyaline, septate hyphae with irregular branching. Thick-walled spherical structures resembling intercalary chlamydospores were observed along the hyphae (Figures 4B and 4C). However, neither conidia nor other reproductive structures were produced throughout the incubation period. Because reproductive structures were absent, reliable identification based solely on morphology was not possible. Consequently, isolate BNS-3 could not be assigned to a fungal genus and was provisionally categorized as mycelia sterilia, pending confirmation through molecular characterization (Bhunjun et al., 2021).

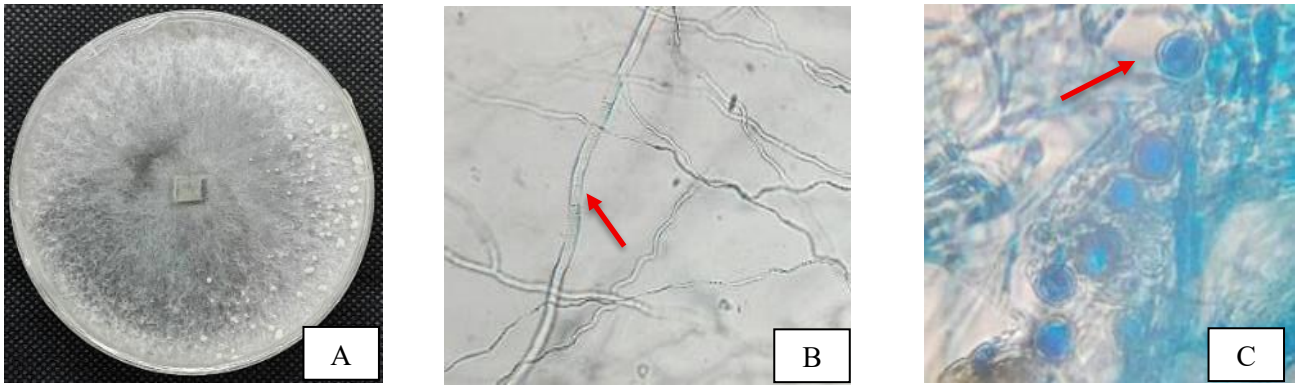

Figure 4. Morphological characteristics of isolate BNS-3. (A) Colony morphology on PDA; (B) hyaline septate hyphae indicated by arrowheads; (C) thick-walled chlamydospore-like structures indicated by arrowheads. Images captured at 400× magnification.

Unlike the other isolates, BNS-3 failed to produce conidia or any recognizable reproductive structures throughout the incubation period, preventing reliable identification using conventional morphological criteria. The isolate consisted primarily of hyaline, septate hyphae bearing thick-walled intercalary spherical structures resembling chlamydospores. Such structures generally function as survival propagules formed in response to unfavorable environmental conditions rather than as taxonomically diagnostic reproductive organs (Francisco et al., 2019).

The absence of sporulation suggests that isolate BNS-3 belongs to the group commonly referred to as *mycelia sterilia*. Sterile fungi represent a well-recognized challenge in fungal taxonomy because conventional identification relies heavily on reproductive morphology. Nevertheless, sterile isolates have frequently been associated with plant diseases, including leaf spot diseases affecting various crops (Deepika et al., 2020). For example, *Dactuliophora mysorensis*, originally recovered as a sterile mycelial isolate from cowpea leaf spot, was successfully identified as a novel species only after molecular characterization by Deepika et al. (2020).

The inability of isolate BNS-3 to produce reproductive structures under laboratory conditions does not necessarily indicate the absence of pathogenicity. Several plant pathogenic fungi remain sterile on artificial media but readily sporulate on host tissues or under specific environmental conditions. Failure to produce spores on PDA is not uncommon and may result from unsuitable nutritional composition, inadequate light exposure, or incubation conditions that do not stimulate sporulation (Su et al., 2012). Consequently, additional approaches should be considered before concluding that an isolate is morphologically sterile. Alternative culture media, host tissue substrates, nutrient-limited media, or environmental manipulations may successfully induce sporulation (Mattoo & Nonzom, 2022). When these approaches remain unsuccessful, molecular identification based on universal fungal barcode regions, particularly the ITS rDNA sequence, becomes the most reliable strategy for determining taxonomic identity (Carolus et al., 2021; Hariharan & Prasannath, 2021).

CONCLUSION

Three fungal isolates, designated BNS-1, BNS-2, and BNS-3, were recovered from pineapple leaves exhibiting leaf spot symptoms in Rimbo Panjang, Kampar Regency, Riau. Based on morphological characteristics, BNS-1 was provisionally assigned to the genus *Lasiodiplodia* sp., whereas BNS-2 was provisionally assigned to the genus *Nigrospora* sp. BNS-3 remained unidentified at the genus level and was classified as mycelia sterilia because reproductive structures were not observed under the culture conditions used. These findings indicate that pineapple leaf spot symptoms in the study area were associated with multiple fungal taxa. However, the present study did not establish the causal pathogenicity of the isolates. Further molecular characterization and pathogenicity testing are required to confirm their taxonomic identity and determine their roles in pineapple leaf spot development.

AUTHORS CONTRIBUTIONS

AHP and NM conceived and designed the research. AHP conducted the isolation and morphological identification of fungal associated with pineapple leaf spot symptoms, analyzed the data, and prepared the original manuscript draft. NM contributed to the identification of fungal isolates and critically revised the manuscript for important intellectual content. PH collected diseased leaf samples from the field, curated the samples, and contributed to data interpretation. All authors contributed to discussions regarding the research design, data analysis, interpretation of the findings, and manuscript development. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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