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Diversity of Endophytic Fungi in Noni Plants (*Morinda citrifolia* L.)

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ABSTRACT

The exploration of endophytic fungi presents an alternative approach to producing a variety of plant-derived medicines. These fungi offer significant advantages to the health industry by enabling large-scale drug production. Extracts from the noni plant (Morinda citrifolia L.), a fruit-bearing plant native to Indonesia, have been traditionally used in various medicines for generations. The aim of this study was to identify and explore the diversity of endophytic fungi associated with noni plants. The research was conducted in the integrated laboratories of Raden Fatah State Islamic University, Palembang, specifically in the microbiology room E303 and genetics room E302. Using exploratory and survey methods, the study isolated 9 fungal species from 12 different tissue isolates, including leaves, fruits, stems, bark, and roots. The fungi identified from noni plants were classified into the following genera: *Acremonium* (isolate code AMC1), *Aspergillus* (isolate code AMC2), *Candida* (isolate code FMC3), *Fumago* (isolate codes FMC1 and FMC2), *Fusarium* (isolate codes DMC1 and DMC2), *Mucor* (isolate code KBMC2), *Pythium* (isolate codes BMC1 and BMC2), *Trichoderma* (isolate code KBMC1), and *Verticillium* (isolate code FMC4). The findings suggest that further research should investigate the bioactive compounds and secondary metabolites of these isolates for potential pharmaceutical applications.

INTRODUCTION

Morinda citrifolia L. (Rubiaceae), commonly known as noni, is a medicinal plant that has been utilized for thousands of years. Native to Southeast Asia but pantropical in distribution, noni has gained cross-cultural significance as both a food supplement and an alternative medicine for conditions such as cancer, inflammation, and diabetes. Its popularity in the herbal market is partly due to claims of its efficacy in treating a range of chronic conditions, including arthritis, diabetes, and hypertension. The fruit, flowers, bark, and roots of noni have all been used for various medicinal purposes, with the leaves being particularly noted for their traditional use as topical treatments for wounds and inflammation (Almeida et al., 2019).

Noni has been traditionally used as a medicine by Polynesian communities, where it is believed that every part of the plant can be used therapeutically. The genus Morinda comprises around 40 species, among which Morinda citrifolia stands out for its antioxidant properties (Pinheiro et al., 2019). The noni plant contains a variety of secondary metabolites, including structurally diverse active compounds such as anthraquinones, alizarin, aucubin, pyrazine, and L-asperuloside (Sogandi & Nilasari, 2019).

Technically, noni fruit contains numerous active compounds, but harvesting these compounds requires a large biomass. Recently, this issue has been addressed by utilizing endophytic fungi within plant tissues. These fungi are microorganisms that inhabit the internal

tissues of living plants without causing any direct negative effects on the host. The mutualistic symbiosis between endophytic fungi and their host plants offers several advantages. The use of biological products derived from these fungi can simplify technical processes, reduce production costs, and result in more affordable products (Rabima et al., 2020).

This study is a preliminary step in exploring the potential of endophytic fungi as an alternative strategy for the production of natural antimicrobial agents. Given the lack of available literature on fungal communities associated with Morinda citrifolia L., the objectives of this research were: (i) to identify endophytic fungi found in Morinda citrifolia L., and (ii) to investigate the diversity of fungi associated with the leaves, fruits, stems, bark, and roots of Morinda citrifolia L.

MATERIALS AND METHODS

Sample Collection

Samples of *Morinda citrifolia L.* were collected from the area of Jl. KH. Balqi, Seberang Ulu II District, Palembang City, with coordinates 2°99'75.35"S, 104°78'35.85"E. Fresh and healthy samples of leaves, fruits, stems, bark, and roots were immediately taken to the laboratory. The study employed exploratory and survey methods on the samples.

Sterilization

Glassware was washed with soap, rinsed thoroughly with tap water, and dried. Once dry, Petri dishes were wrapped in paper, and test tubes and Erlenmeyer flasks were sealed with cotton and gauze. The autoclave was set to sterilize the items for 20 minutes at 121℃ and 1 atm pressure. After sterilization, the equipment was allowed to cool for approximately 2 hours before being used in a clean environment.

Media Preparation

First, 1404 grams of PDA media were placed into an Erlenmeyer flask and dissolved in 360 mL of sterile distilled water. Chloramphenicol was added, and the flask was covered with cotton and gauze. The mixture was heated to boiling on a hot plate and stirred with a magnetic stirrer until homogeneous. The medium was then allowed to cool to 36-37℃ before being sterilized in an autoclave at 121℃ for 15 minutes at 1 atm pressure.

Isolation of Endophytic Fungi

The leaves, fruits, stems, bark, and roots of *Morinda citrifolia L.* were washed under running tap water for 10 minutes and then dried with tissue. The outer tissue of the samples was cut using sterile surgical scissors into 1x1 cm pieces. The sample surfaces were sterilized by immersing them in 70% alcohol for 1 minute, followed by immersion in 3% NaOCL for 1 minute. The samples were then rinsed with distilled water for 1 minute with two repetitions. Small pieces of leaves, fruits, stems, bark, and roots were placed on Potato Dextrose Agar (PDA) medium containing chloramphenicol (0.2g/L) in Petri dishes. The dishes were then incubated at room temperature for seven days, with all experiments performed in duplicate (Elfita et al., 2019).

Purification of Endophytic Fungi

Fungal growth from the wounded plant tissues was monitored daily. Once the endophytic fungi exhibited distinct morphological characteristics, they were transferred to fresh PDA medium to obtain pure isolates. The microorganisms were transferred using a flamed inoculation needle to Petri dishes containing PDA medium (Elfita et al., 2019).

Identification of Endophytic Fungi

The morphological characteristics were then compared with identification books on fungi (Habisukan et al., 2021). Identification of endophytic fungi was conducted through the observation of colony and morphological characteristics, both microscopically and macroscopically. Macroscopic identification involved observing colony characteristics, including colony color, shape (concentric or non-concentric), colony texture, topography, concentric rings, and colony growth rate (cm/day) (Sulistiyono & Mahyuni, 2019). Microscopic observation was conducted using a microscope to examine spore types, spore shapes, and hyphae. Microscopic features such as hyphal growth (branched or unbranched), the presence or absence of septa in hyphae, hyphal color, and conidia (dark or hyaline) were also observed. Microscopic identification was conducted using the slide culture method.

RESULTS AND DISCUSSION

Identification of Endophytic Fungi

A total of 12 endophytic fungi were isolated from various tissues (leaves, fruits, stems, bark, and roots) of *Morinda citrifolia L.* The growth of endophytic fungal isolates was marked by the appearance of hyphae around the wounded plant tissues (Figure 1). The fungal colonies exhibited various physical appearances, with hyphal growth still visible on samples dominated by white colonies and yellow pigmentation on leaf, stem, and bark samples, while greenish coloration was observed on fruit and root samples.

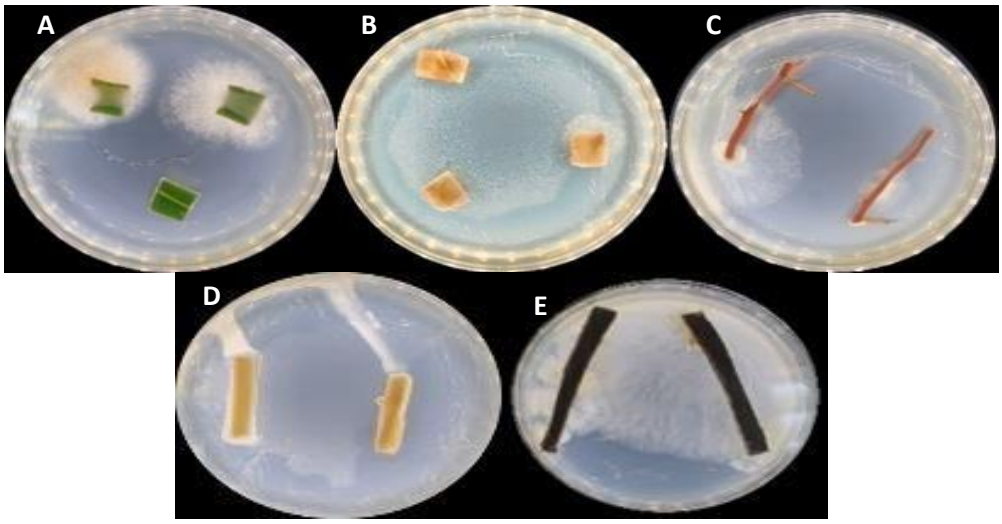

Figure 1. Hyphae of endophytic fungi that began to appear after the third day around the organs of *Morinda citrifolia L.*; A) leaves; B) fruit; C) roots; D) stem; E) bark

Table 1. Macroscopic Characteristics of Endophytic Fungi Isolated from *Morinda citrifolia L.*

Isolat	Color of front colony	Color of rear colony	Texture	Topography	Concentric Circles	Genus
AMC1	White with greenish-yellow	White with yellowish tint	Cottony	Zonate	-	*Acremonium*
AMC2	White with greenish-yellow	White with greenish-yellow	Powdery	Spread	-	*Aspergillus*
BMC1	White with brownish-black	White with brownish-black	Cottony	Zonate	-	*Pythium*
BMC2	White with blackish tint	White with brownish-black	Cottony	Zonate	-	*Pythium*

Isolat	Color of front colony	Color of rear colony	Texture	Topography	Concentric Circles	Genus
DMC1	White with grayish tint	White with blackish tint	Cottony	Zonate	√	*Fusarium*
DMC2	White with blackish tint	White with grayish tint	Cottony	Zonate	-	*Fusarium*
FMC1	White with greenish tint	White with greenish tint	Velvety	Zonate	-	*Fumago*
FMC2	White with yellowish-brown	White with yellowish-brown	Velvety	Zonate	-	*Fumago*
FMC3	White with brown tint	White with brown tint	Cottony	Zonate	-	*Candida*
FMC4	White with green tint	White with green tint	Cottony	Zonate	-	*Verticillium*
KBMC1	White with greenish-yellow	White with yellowish tint	Cottony	Flowery	√	*Trichoderma*
KBMC2	White with greenish tint	White with greenish tint	Cottony	Zonate	-	*Mucor*

note: (-) = Characteristics do not appear; (√) = Characteristics appear

The 12 fungal colonies exhibited diversity in color (white, black, green, brown, cream, and yellow) and texture (cottony, powdery, and velvety). The macroscopic features of the isolated fungi are presented in Table 1. Microscopic analysis revealed that the 12 isolates belong to nine different genera. The identified genera were *Acremonium*, *Aspergillus*, *Candida*, *Fumago*, *Fusarium*, *Mucor*, *Pythium*, *Trichoderma*, and *Verticillium* (Table 2). The endophytic fungi isolated from *Morinda citrifolia L.* displayed diversity in spore shapes (round, nearly round, and oval). Most isolates had septate hyphae, except for isolates AMC1 and KBMC2, which had non-septate hyphae.

All endophytic fungal isolates were obtained from leaves, fruits, stems, bark, and roots (Figure 2). Isolate AMC1 was identified as belonging to the genus *Acremonium* based on its macroscopic characteristics, which included a white colony surface with a yellow center and a green spot at the core. The colony had a cottony texture with zonate topography. Microscopically, this fungus displayed short, oval spores, hyaline septate hyphae, and branched conidiophores forming grape-like clusters (Riadi et al., 2021). Isolate AMC2 was identified as belonging to the genus *Aspergillus*, based on its macroscopic characteristics, which included a white colony edge with a yellow-green center and a brown spot at the core. The colony had a powdery texture with spread topography. Microscopically, this fungus exhibited hyaline, septate hyphae with a conidiophore at the top and rounded spores at the tips (Sopialena et al., 2019).

Isolates BMC1 and BMC2 were identified as belonging to the genus *Pythium*, based on their macroscopic characteristics, which included white-brown colony edges with black centers. The colonies had a cottony texture with zonate topography. Microscopically, these fungi displayed hyaline, septate somatic hyphae with slender mycelium and nearly round chlamydospores (Akhsan et al., 2022).

Isolates DMC1 and DMC2 were identified as belonging to the genus *Fusarium*. Their macroscopic characteristics included white colony edges with gray centers and black spots at the core. The colonies had a cottony texture with zonate topography. Microscopically, these fungi exhibited curved macroconidia with 2 or 3 cells, slender conidiophores, and irregularly branched hyaline septate hyphae (Jamilatun & Shufiyani, 2019).

Isolates FMC1 and FMC2 were identified as belonging to the genus *Fumago*, based on their macroscopic characteristics, which included white colony edges with green-brown centers. The colonies had a velvety texture with zonate topography. Microscopically, these fungi displayed oidiofor spores, round spore shapes, and hyaline septate hyphae (Walsh et al., 2018).

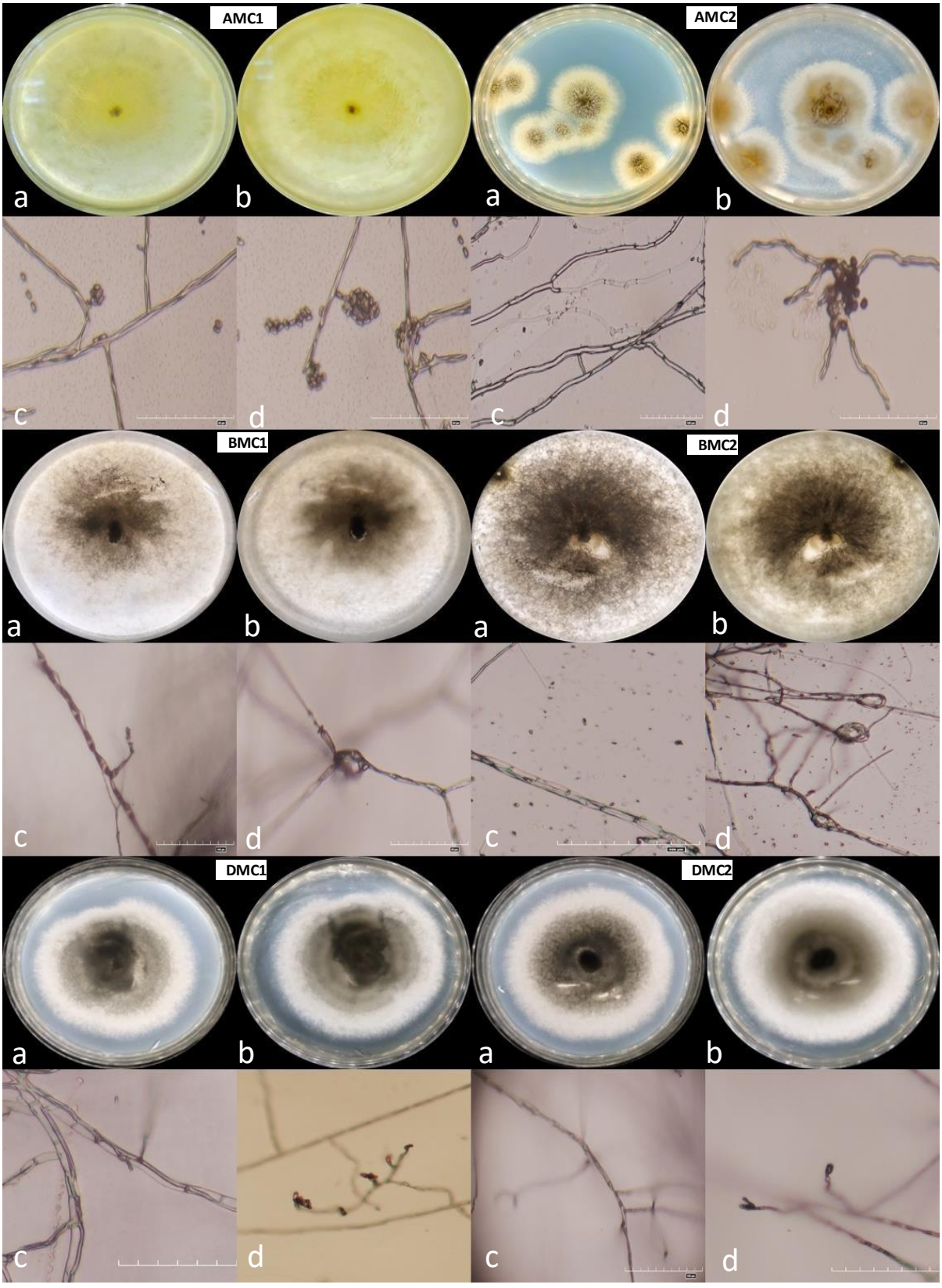

Figure 2. Morphology of endophytic fungi in noni plants (*Morinda citrifolia L.*), a. Front colony color, b. Back colony color, c. Hyphae, d. Spores.

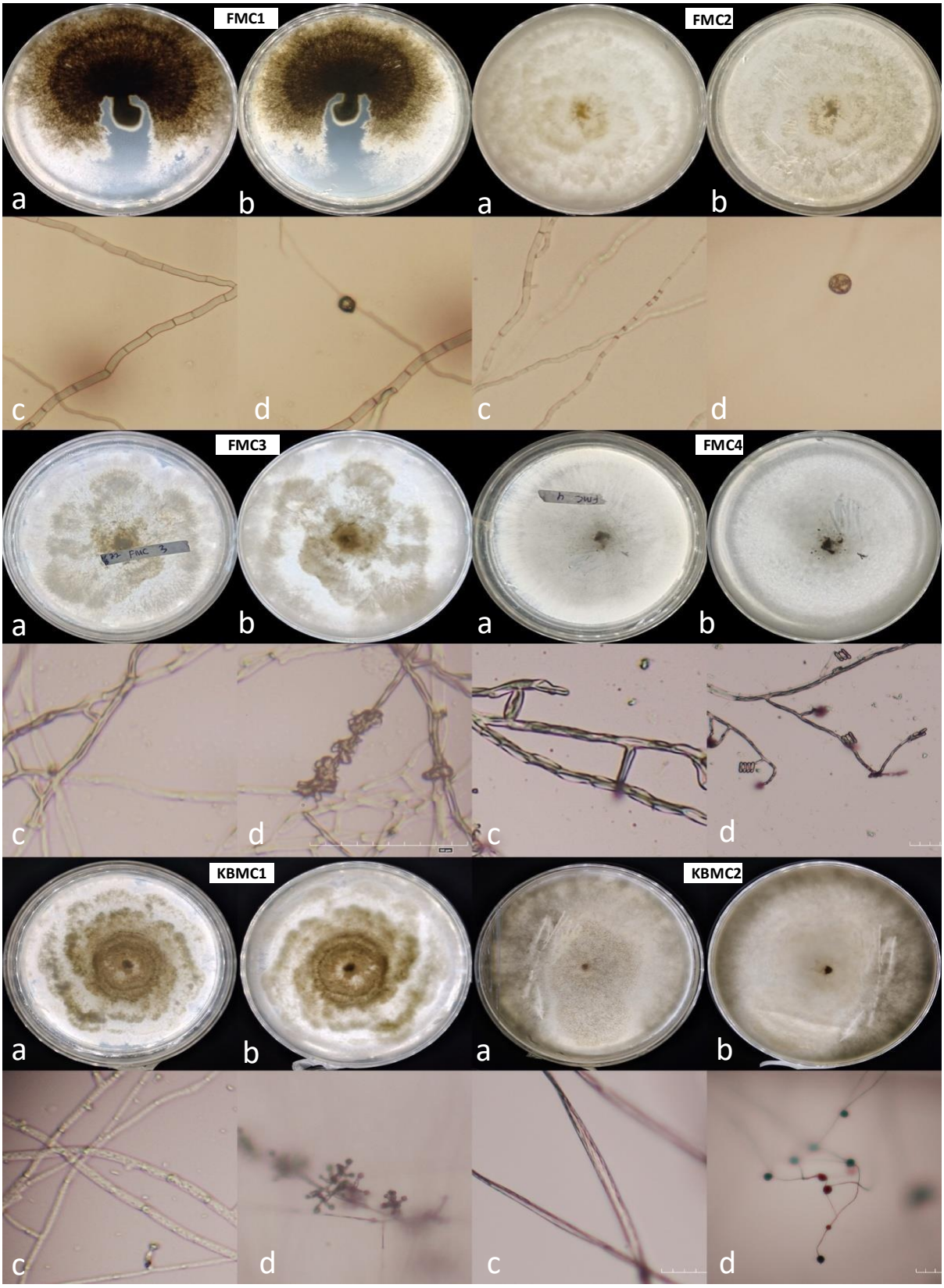

Figure 3. Morphology of endophytic fungi in noni plants (*Morinda citrifolia L.*), a. Front colony color, b. Back colony color, c. Hyphae, d. Spores.

Table 2. Microscopic Characteristics of Endophytic Fungi Isolated from *Morinda citrifolia L.*

Isolat	Spore Type	Spore Form	Hyphae	Genus
AMC1	Conidia	Oval	non septate	Acremonium
AMC2	Zoospore	Round	septate	Aspergillus
BMC1	Chlamidospore	Nearly Round	septate	Pythium
BMC2	Chlamidospore	Nearly Round	septate	Pythium
DMC1	Conidia	Oval	septate	Fusarium
DMC2	Conidia	Oval	septate	Fusarium
FMC1	Oidiofor	Round	septate	Fumago
FMC2	Oidiofor	Round	septate	Fumago
FMC3	Oidiofor	Oval	septate	Candida
FMC4	Conidia	Oval	septate	Verticillium
KBMC1	Conida	Round	septate	Trichoderma
KBMC2	Sporangiofor	Round	non septate	Mucor

Table 3. Diversity of Endophytic Fungi Isolated from Different Tissues of *Morinda citrifolia L.*

Genus	Tissues of *Morinda citrifolia L*					Total
	Fruit	Leaves	Stem	Stem Bark	Root	
Acremonium	0	0	0	0	1	1
Aspergillus	0	0	0	0	1	1
Pythium	0	0	2	0	0	2
Fusarium	0	2	0	0	0	2
Fumago	2	0	0	0	0	2
Candida	1	0	0	0	0	1
Verticillium	1	0	0	0	0	1
Trichoderma	0	0	0	1	0	1
Mucor	0	0	0	1	0	1

Isolate FMC3 was identified as belonging to the genus *Candida*, based on its macroscopic characteristics, which included white colony edges with brown centers. The colony had a cottony texture with zonate topography. Microscopically, this fungus displayed oidiofor spores, oval spore shapes around the septum, and thick-walled spores clustered at the tips and edges of each phialide. The conidiophores were branched, with pseudohyphae and hyaline septate hyphae (Walsh et al., 2018).

Isolate FMC4 was identified as belonging to the genus *Verticillium*, based on its macroscopic characteristics, which included white colony edges with green centers. The colony had a cottony texture with zonate topography. Microscopically, this fungus displayed conidia, oval spore shapes clustered at the top of conidiophores, and solitarily arranged spores. The conidiophores were thick and short, with hyaline septate hyphae (Walsh et al., 2018).

Isolate KBMC1 was identified as belonging to the genus *Trichoderma*, based on its macroscopic characteristics, which included white colony edges with green centers and brown spots at the core. The colony had a cottony texture with flowery topography. Microscopically, this fungus displayed round conidia, thick and short phialides, upright branched conidiophores, and hyaline septate hyphae (Habisukan et al., 2021).

Isolate KBMC2 was identified as belonging to the genus *Mucor*, based on its macroscopic characteristics, which included white-brown colony edges with green centers. The colony had a cottony texture with zonate topography. Microscopically, this fungus displayed sporangiofor spores, round spore shapes, and hyaline septate hyphae (Walsh et al., 2018).

CONCLUSION

Twelve endophytic fungi were successfully isolated from the leaves, roots, stems, bark, and fruits of *Morinda citrifolia L.*, belonging to eight genera: Acremonium, Aspergillus, Candida, Fumago, Fusarium, Mucor, Pythium, Trichoderma, and Verticillium. The total number of isolates was as follows: Fumago (2 isolates), Fusarium (2 isolates), Pythium (2 isolates), Acremonium (1 isolate), Aspergillus (1 isolate), Candida (1 isolate), Mucor (1 isolate), Trichoderma (1 isolate), and Verticillium (1 isolate). The diversity of fungal populations varied across the plant tissues, indicating that *Morinda citrifolia L.* provides a suitable habitat and nutrients for the survival of endophytic fungi. Further investigation into the secondary metabolites and bioactive compounds of *Morinda citrifolia L.* is recommended, particularly for developing antioxidant and antimicrobial agents.

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