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Diversity of endophytic fungi in *Syzygium aqueum*

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Abstract. *Habisukan UH, Elfita, Widjajanti H, Setiawan A, Kurniawati AR. 2021. Diversity of endophytic fungi in Syzygium aqueum. Biodiversitas 22: 1129-1137.* Exploring endophytic fungi may provide alternative, plant-based ethnomedicines. The discovery of endophytic fungi can produce many plant-derived drugs that give new horizons to the pharmaceutical industry for the availability and production such medicines on a large scale. Extracts from *Syzygium aqueum* Alston (jambu air), a fruit-bearing plant native to Indonesia, have long been used in traditional medicine. Therefore, the purpose of this study was to identify, and investigate, the antimicrobial activity of the endophytic fungi found in *S. aqueum*. Results revealed that total16 fungi were isolated from different tissues (stem bark, root bark, and leaves) of the plant. Fungal endophytes were identified through morphological characterization and subsequently compared with key fungal identification books. The ethyl acetate extracts of the isolated endophytic fungi were screened for their antimicrobial activity through paper disc diffusion assay fungi isolated from *S. aqueum* were identified as: *Aspergillus niger* (Isolate code R21, R41, T72), *Cylindrocarpon* sp. (Isolate code R11, R31), *Trichoderma aureoviridae* (Isolate code T11), *Trichoderma harzianum* (Isolate code T21), *Trichoderma* sp. (Isolate code T31), *Pestalotia* sp. (Isolate code T41and T62), *Beltrania* sp. (Isolate code T52), *Chaetomium* sp. (Isolate code L11), *Cochliobolus* sp. (Isolate code L22), *Penicillium* sp. (Isolate code L32), *Cylindrocladium* sp. (Isolate code L42), and an unidentified Ascomycota (T51). The values of Shannon-Weiner and Simpson diversity indexes for the overall fungal community were H′=2.133 and 1-D=0.859 respectively. All isolates showed potential antimicrobial activity against *Escherichia coli*, *Salmonella Typhi*, and *Staphylococcus aureus*, but the lowest activity against *Candida albicans*. The results indicate that the bioactive compounds and secondary metabolites of these isolates should be further investigated for pharmaceutical applications.

Keywords: Antimicrobial activity, diversity, endophytic fungi, fungal identification, *Syzygium aqueum*

INTRODUCTION

Syzygium aqueum Alston, which belongs to the Myrtaceae family, is a fruit-bearing plant native to Indonesia and Malaysia. In Indonesia, it is well known as jambu air. The leaves, bark, roots, and other tissues of *S. aqueum* have long been used in traditional medicine (Cock and Cheesman 2018). It has antibiotic, anti-inflammatory, antioxidant, and anti-tyrosinase properties (Palanisamy et al. 2011; Cock and Chessman 2018). Of these properties, the current researchers have chosen to analyze the antimicrobial activity of *S. aqueum*, which has also been highlighted by Mapatac and Mapaoag (2014) and Chua et al. (2019). Microbial infections are serious challenges to human health because of microbial resistance to antibiotics (Dos Santoz et al. 2015). Plant-based ethnomedicines are derived from compounds occurring in plant extracts. Investigating the properties of endophytic fungi could be an alternative prospect for producing plant-derived compounds, which will benefit the pharmaceutical industry (Nicoletti and Florentino 2015).

Endophytic fungi are polyphyletic microorganisms, which inhabit plant tissues without inciting disease symptoms and eventually establish mutualistic associations with their host plants. Numerous studies have been conducted on the diversity and isolation of endophytic fungi, which have revealed that fungi in plants are important and quantitative components of biodiversity, which have an impact on the diversity and composition of the plant community (Porras-Alfaro and Bayman 2011; Potshangbam et al. 2017). Exploration of the biosynthetic potential of endophytes has gained attention due to the ongoing discovery of strains that can synthesize plant compounds—a property that may reflect the adaptive functional role of endophytes in biocenosis (Nicoletti and Florentino 2015). Secondary metabolites serve multiple physiological functions, and, in a way, it is intuitive that similar compounds may be produced by ecologically associated entities (Jumpathong et al. 2010; Elfita et al. 2013; Budiono et al. 2019). Medicinal plants usually contain endophytic fungi, which produce natural compounds, similar to those found in the host plant such as saponins, alkaloids, flavonoids, tannins, phenolics, glycosides, and terpenoids (Budiono et al. 2019; Madhavi Ram 2015).

This is an initial step in exploring the potential of endophytic fungi as alternative strategies for the production of natural antimicrobial agents, and since there is no literature available for fungal communities associated with *S. aqueum*, the objectives of this study were: (i) to identify the endophytic fungi present in *S. aqueum*, (ii) to investigate the diversity of fungi associated with the stem bark, root bark, and leaves of *S. aqueum*, and (iii) to examine the antimicrobial activity of endophytic fungal extract.

MATERIALS AND METHODS

Sample collection

Syzygium aqueum plant samples were obtained from Ogan Ilir Regency, South Sumatra, Indonesia. Plant identification was confirmed at the Biosystematics Laboratory, Biology Department, Sriwijaya University, Ogan Ilir, Indonesia with certificate no. 329//UN9.1.7/4/EP/2020. Fresh and healthy samples of *S. aqueum* stem bark, root bark, and leaves were immediately transported to the laboratory.

Isolation of endophytic fungi

The *S. aqueum* stem bark, root bark, and leaf samples were washed with running tap water for 10 min and then air-dried. Sample fragments were successively surface sterilized by immersing each sample in 70% alcohol for ±1min, followed by immersion in 3% (w/v) sodium hypochlorite (NaOCl) for 1min. After being rinsed in sterile distilled water for ±1min, the outer tissue of sample was removed with a sterile scalpel. Small pieces of stem bark, root bark, and leaves were placed in Petri dishes containing potato dextrose agar (PDA) media (200 g potato, 20 g dextrose, and 15 g agar in 1 L of H_2O) supplemented with chloramphenicol (0.2g/L). Petri dishes were then incubated at 30 ± 2°C for seven days in the dark. All experiments were performed in triplicates. For the fungal growth from the leaf segments, the plates were monitored every day. Individual hyphal tips were transferred into fresh PDA and incubated at 30°C for seven days. The pure cultures were numbered, maintained in PDA slants, and kept at 4°C (Muharni et al. 2014; Elfita et al. 2019).

Identification of endophytic fungi

Macroscopic and microscopic features were used to characterize the morphology of the endophytic fungal isolates. Macroscopic characterization was based on the colony growth pattern, texture, margins, color, and other features. The microscopic characterization employed the slide culture method. Endophytic fungal cultures were placed on slides and mixed with one drop of lactophenol blue reagent. These slides were examined under a light microscope. The morphological characteristics data were then compared with key fungi identification books (Barnett 1969; Gandjar 1999; Watanabe 2002; Pitt and Hocking 2009).

Fungal diversity and ecological associations

Fungal diversity was estimated for each tissue sample and for the total sample population using the Shannon-Weiner (Shannon and Weiner 1949) and the Simpson (Simpson 1949) diversity indexes. Principal Component Analysis (PCA) was used to study the ecological interrelationships between the fungal species and different plant tissue types (PAST software, Hammer et al. 2001).

Cultivation and extraction

For the cultivation inoculum of fungi (±10^6 spores/mL) was inoculated as much as 5% (v/v) in 300ml of a potato dextrose broth (PDB) medium (20g dextrose monohydrate, 200g potato, and 1,000 mL aquadest) in 1 L bottles. They were then incubated at room temperature for four to eight weeks. Color change in the samples indicated that secondary metabolite compounds had been formed. As a comparison, 300ml of the PDB medium was placed into a separate 1L bottle along with secondary metabolites, which had been partitioned in ethyl acetate and extracted via evaporation (Elfita et al. 2019; Gustianingtyas et al. 2020). The concentrated extracts were used for antimicrobial assay.

Antimicrobial activity

Antimicrobial activity was carried out via paper disc diffusion assay. Each sample was dissolved in dimethyl sulfoxide 10% (DMSO; Merck, Germany), and the antibacterial activity was evaluated against one gram-positive bacterium (*S. aureus*), two gram-negative bacteria (*S. typhi* and *E. coli*), and one yeast (*C. albicans*). These organisms were maintained on Muller-Hinton agar (MHA). The resulting suspension turbidity was diluted with sterile aquadest to match a 0.5 McFarland turbidity standard (1 MacFarland is equivalent to approximately 3.0 x 10^8CFU/mL) (Singh et al. 2015). The extract concentration used for this assay was 1,000µg/mL. Absorbent disc (Whatman, 6.0mm in diameter) was impregnated with 10µL of the solution and then placed on the surface of inoculated plates (90mm). Positive control discs of ciprofloxacin 10 µL/mL were excluded from the assay. Diameters of the growth inhibition zones were measured after incubation at 37°C for 24h. All experiments were performed in triplicates (Talukdar et al. 2020).

RESULTS AND DISCUSSION

A total of 16 endophytic fungi were isolated from different tissues (stem bark, root bark, and leaves) of *S. aqueum*. The growth of endophytic fungi isolates was indicated by the appearance of hyphae around the plant tissue segments (Figure 1). The colonies of fungal isolates exhibited various physical appearances. Massive hyphae growth appeared in the stem bark samples, with white colony dominance and yellow pigmentation. White filament colonization appeared around the root bark segments, and small, dark green and white colonies appeared on the leaf segments.

Identification of endophytic fungi

The 16 fungal colonies exhibited diversity in colors (white, black, green, and yellow) and texture (velvety, powdery, cottony, and granularly). Only isolates T11 and T21 evidenced exudates droplets and emitted yellow pigment. The macroscopic features of the isolated fungi are presented in Table 1.

Microscopic analysis showed that 16 isolates belong to ten different genera. Nine identified genera were (*Aspergillus*, *Cylindrocarpon*, *Trichoderma*, *Pestalotia*, *Beltrania*, *Chaetomium*, *Penicillium*, *Cochliobolus*, and *Cylindrocladium*) and one unidentified genus from the Ascomycota phylum (Table 2). The endophytic fungi isolated from *S. aqueum* showed diversity in their spore shapes (cylindrical, globose, subglobose, spindle, and ellipsoidal). Most of the isolates had septate hyphae, except for isolates R11, R31, T51, and T21, which had coenocytic hyphae.

Four endophytic fungi were isolated from the root bark of *S. aqueum* (Figure 2), with the codes R11, R21, R31, and R41. Isolates R21 and R41 were spreading black colonies with white margins (Figure 2), and the microscopic characteristics showed that they had hyaline straight conidiophores, round vesicles, fialid circling vesicles, and round, black conidia.

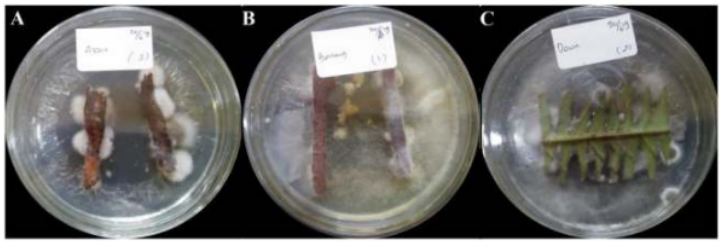

Figure 1. Mycelium appearance of endophytic fungi around *Syzygium aqueum* segments; A. Root bark; B. Stem bark; C. Leaves

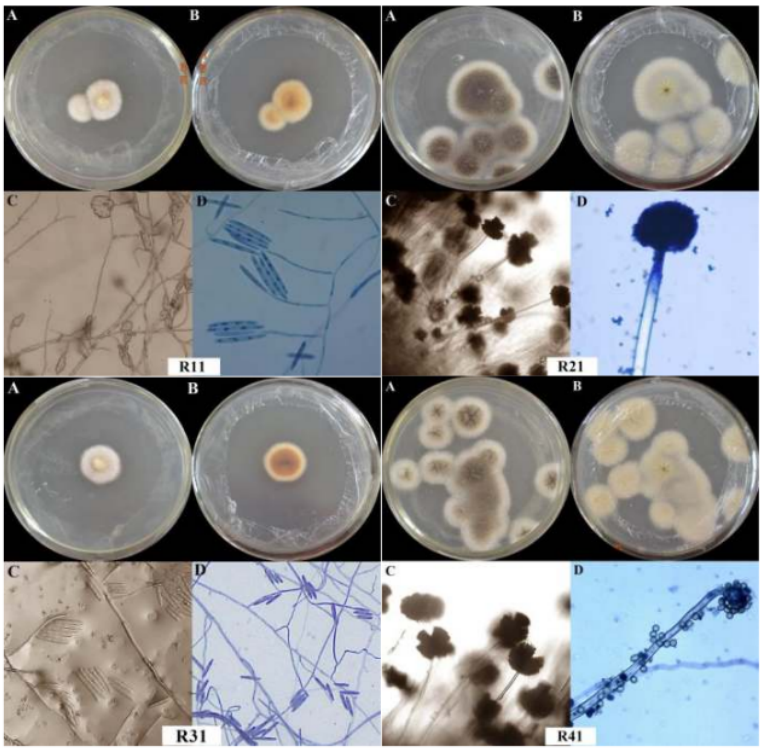

Figure 2. Morphology of endophytic fungi isolated from *Syzygium aqueum* root bark. R11. *Cylindrocarpon* sp., R21. *A. niger*, R31. *Cylindrocarpon* sp., R41. *A. niger*; A. Front view colony; B. Reverse colony; C. Conidia without staining; D. Conidia with lactophenol blue dye

According to the key identification books, these isolates were identified as *A. niger*, which has specific features including black mass spores with globose vesicles. Isolate T72 had similar morphological characteristics to isolates R21 and R41, therefore isolate T72 was also identified as *A. niger*.

Isolates R11 and R31 were yellowish-white colonies with umbonate topography. Their microscopic features showed simple conidiospores (phialides). They were hyaline, erect, perceptic, and mostly 4-5-septate. They also evidenced ellipsoidal conidia and were apiculate at one end, slightly straight on one side, and curved on one side. According to their morphology, the closest species resembling R11 and R31 was *Cylindrocarpon* sp.

Isolate T41 was a white flowery colony with a cottony surface (Figure 3, T41 a-b). Microscopically, it showed short conidiospores, in spindle or ellipsoidal shapes, with four to five cells, two to three centrally pigmented cells, two to four appendages (setulae) in the apical cells, and one short appendage (pedicel) at the basal cell. Based on these characteristics, isolate T41 was identified as *Pestalotia* sp.

Isolate T52 (Figure 3, T52 a-b) was showing greenish brown with white margins and grew in a flowery pattern. Isolate T52 had pale brown conidiophores, had simple and occasionally proliferated forming nodes, and was inflated slightly at the apex. *Beltrania* sp. was the closest match to the fungus.

Four endophytic isolates i.e. L11, L22, L32, and L42 were isolated from the leaves of *S. aqueum* (Figure 4). Isolate L11 showed round, white colony with a cottony surface. Microscopically, it had dark perithecia and subglobose or ovate spores. It was covered with terminal hairs on the upper surface and was rhizoidal at its base. Thus, isolate L11 was thought to be closest to *Chaetomium* sp. Isolate L22 had a white cottony surface with a dark reverse color. Microscopically, it had septate hyphae, and its conidiophores were upright, brown, branched, straight or curved, porous, and subellipsoidal. It was mostly four-cell and was darker brown at the two-cell center, with a larger middle at the peripheral cells and indistinct hilar at the base. Thus, the characteristics of isolate L22 were considered to be closest to those of *Cochliobolus* sp.

Table 1. Macroscopic characteristics of endophytic fungi isolated from *Syzygium aqueum*

Isolate	Colony color	Reverse colony color	Texture	Topography	Pattern	Exudate drops	Radial line	Concentric circle
R11	Yellowish white	Dark brown	Velvety	Umbonate	Zonate	-	√	-
R21	Black	Broken white	Powdery	Rugose	Spread	-	-	-
R31	Yellowish white	Dark brown	Velvety	Umbonate	Zonate	-	√	-
R41	Black	Broken white	Powdery	Rugose	Spread	-	-	-
T11	White with green spots	Light yellow	Granular	Flat	Radiate	√	-	√
T21	White with green spots	Light yellow	Granular	Flat	Radiate	√	-	√
T31	Dark green and white	Greenish white	Cottony	Umbonate	Zonate	-	√	√
T41	White	Yellowish white	Cottony	Flat	Flowery	-	√	-
T51	Light green and white	Greenish white	Granular	Umbonate	Zonate	-	√	√
T52	Brown and greenish-white	Army green	Cottony	Flat	Flowery	-	√	√
T62	Dark green and white	Greenish white	Cottony	Umbonate	Zonate	-	√	√
T72	Black	Broken white	Powdery	Rugose	Spread	-	-	-
L11	White	Yellowish white	Cottony	Convex	Zonate	-	-	-
L22	Grey with marginal white	Black with marginal white	Cottony	Verrucose	Zonate	-	-	√
L32	Dark green and white	Broken white	Powdery	Rugose	Spread	-	-	-
L41	White	White	Cottony	Verrucose	Flowery	-	√	-

Note: -: characteristic does not appear; √: characteristic appear

Table 2. Microscopic characteristics and list of endophytic fungi isolated from *Syzygium aqueum*

Isolate	Type of spore	Shape of spore	Hyphae	Specific characteristic	Genus / species
R11	Conidia	Cylindrical	Coenocytic	Macroconidia cylindrical, 4-5 septate	*Cylindrocarpon* sp.
R21	Conidia	Globose	Septate	Globose vesicle, conidia black in mass	*Aspergillus niger*
R31	Conidia	Cylindrical	Coenocytic	Macroconidia cylindrical, 4-5 septate	*Cylindrocarpon* sp.
R41	Conidia	Globose	Septate	Globose vesicle, conidia black in mass	*Aspergillus niger*
T11	Conidia	Subglobose	Septate	Phialide verticillate, chlamydospore subglobose	*Trichoderma aureoviridae*
T21	Conidia	Subglobose	Septate	Spore masses apically at verticillate phialides	*Trichoderma harzianum*
T31	Conidia	Globose	Septate	Phialide verticillate, chlamydospore subglobose	*Trichoderma* sp.
T41	Conidia	Spindle	Septate	Conidia spindle, pigmented cell, apparent appendages	*Pestalotia* spp.
T51	Sporangia	Globose	Coenocytic	Branched hyphae, apparent asci with granular spore	Unidentified
T52	Conidia	Globose	Septate	Apex bearing 3-4 conidia sympodially	*Beltrania* sp.
T62	Conidia	Ellipsoidal	Septate	Phialide verticillate, chlamydospore subglobose	*Trichoderma* sp.
T72	Conidia	Globose	Septate	Globose vesicle, conidia black in mass	*Aspergillus niger*
L11	Ascospore	Subglobose	Coenocytic	Ascocarps with well-developed hair, perithecia black	*Chaetomium* sp.
L22	Conidia	Ellipsoidal	Septate	Subellipsoidal, mostly four-celled, central dark brown	*Cochliobolus* sp.
L32	Conidia	Globose	Septate	Branch apex, 2-3 metulae, verticillate phialides	*Penicillium* sp.
L42	Conidia	Cylindrical	Septate	Terminal vesicle subglobose, clavate, lanceolate	*Cylindrocladium* sp.

Macroscopically, isolate L32 (Figure 4) was a spreading, dark green color colony with white edges. The reverse colony was yellowish white. Microscopically (Figure 1E), it had upright hyaline conidiophores, which branched into two to three metulae; each metula contained verticillate phialides, and each phialide contained dangling, round, green conidia. After comparison, isolate L22 was identified as *Penicillium* sp.

Isolate L42 identified as *Cylindrocladium* sp. The colony was white with a more yellowish reverse colony. Its surface was cottony, and it grew in a flowery pattern (Figure 4, L42 a-b). Its conidiophores were erect and branched, with elongated primary and secondary branches. The phialide branches carried spore masses with cylindrical stalk and terminal vesicles. The conidia were cylindrical and phialosporous (Figure 4, L42 c-d).

Endophytic fungal diversity and ecological association

Eight isolates were obtained from the stem bark of *S. aqueum*, and four were obtained from the root bark and leaves. The diversity index values (Shannon-Wiener and Simpson) of *S. aqueum* tissues are listed in Table 3. The α-diversity values were obtained via the PAST software (Shadegi et al. 2019). The Shannon-Weiner and Simpson diversity index values were highest in leaves (H′=1.386 and 1-D=0.750, respectively) and lowest in root bark (H′=0.693 and 1-D=0.500, respectively). In addition, the overall diversity values of the endophytic fungal populations were H′=2.133 and 1-D=0.859 (Table 3).

The two principal components or axes (1-2) obtained via PCA explained 68% of the total fungal variation (Figure 5). The analysis showed that some isolates had an affinity for a specific tissue. Host affinity could reflect functional differences in phenology, chemistry, or other traits, which vary among fungi and host plant taxa. Differences in diversity and species composition between stems and leaves may also reflect their functional similarities.

Antimicrobial activity of endophytic fungi

Antimicrobial activity of the endophytic fungi isolated from *S. aqueum* is presented in Table 4. Ten out of 16 isolates were used in the antimicrobial analysis. Overall, the endophytic fungal extracts inhibited the growth of the three bacterial species, but they had little effect on the yeast (*C. albicans*). Isolate R42 (*A. niger*) showed strong antimicrobial activity against *E. coli*, and isolate T31 (*T. harzianum*) showed strong antimicrobial activity against *S. aureus* and *S. typhi*. On average, the lowest level of antimicrobial activity was presented by isolate L22 (*Cochliobolus* sp.).

Discussion

The sampling of *S. aqueum* endophytes indicated that a diverse community of fungi live on the plant's various tissues. The purpose of surface sterilization during the isolation process was to remove microorganisms from the plant surface so that the fungi growing in the media are only endophytic fungi (Strobel and Daisy 2003). 3% NaOCl and 70% alcohol were used in chemical solutions for surface sterilization as both are widely employed as disinfectants. Hypochlorite ions regenerated from hypochlorous acid (HOCl) and dissolved in water are capable of damaging bacterial plasma membranes (Rahayu et al. 2019).

Results showed that all endophytic fungi isolated from the different tissues of *S. aqueum* were categorized into three classes of Ascomycota: Eurotiomycetes, Sordariomycetes, and Ascomycetes. To date, most fungi reported as endophytes have been identified as Ascomycetes and their anamorphs. Basidiomycetous endophytes have only been reported in a limited number of studies (Rivera-Orduña et al. 2011; Sridhar and Raviraja 1995; Wang et al. 2005). The most evenly distributed genus was *Trichoderma* isolated only from *S. aqueum* stem bark. *Trichoderma* species are typically soilborne organisms commonly associated with plant roots and stems. Some strains of *Trichoderma*, such as *T. harzianum*, *T. stromaticum*, and *T. asperellum*, have antagonistic effects on some fungal diseases (Bailey et al. 2008; Gazis and Chaverri 2010).

Some isolated endophytic taxa prefer specific tissue types. Some sapwood endophytes may invade tree hosts through their root systems, moving from the soil to their new niche inside the plant (Gazis and Chaverri 2010). In this research, PCA showed an increased occurrence and frequency of tissue specificity (Rodrigues 1994; Photita et al. 2001; Kumar and Hyde 2004). Certain endophytes were found only in one *S. aqueum* tissue (stem bark, root bark, or leaves), indicating that they may have an affinity for a particular tissue type (Rivera-Orduña et al. 2011). The fungal endophytes from *S. aqueum* had a greater affinity for the stem bark than for any other tissue.

Antimicrobial analysis results showed that endophytic fungal extracts have the ability to inhibit the growth of bacterial species. Hariyati et al. (2015) reported that *S. aqueum* leaf extract inhibited the growth of *Shigella dysentriae, Vibrio cholerae, S. aureus, E. Coli,* and *S. typhi*. He also reported that *S. aqueum* left extract showed strong activity against *E. Coli, S typhi*, and *S. aureus* at 50% concentration. Sobeh et al. (2016) have also reported that some bioactive compounds are found in the essential oils of *S. aqueum* leaves, which exhibit antimicrobial activity. Phenolic and flavonoid compounds are dominant in the *S. aqueum* plant, and both have strong antioxidant and antimicrobial properties (Tehrani et al. 2011; Palanisamy et al. 2011; Marinova 2005). The in vitro antibacterial activity of several classes of polyphenols can be due to direct action against bacteria, viruses, and fungi, as well as the suppression of microbial virulence factors (Daglia 2012). The most common constituents in *S. aqueum* with antimicrobial properties are α-selinene, β-caryophyllene, and β-selinene (Sobeh et al. 2016). Trichoderma species showed strong antimicrobial activity against S. aureus and S. typhi. Trichoderma species can protect their hosts from direct parasitism, antibiosis, and nutrient competition via enhanced plant growth or induced resistance (Gazis and Chaverri 2010).

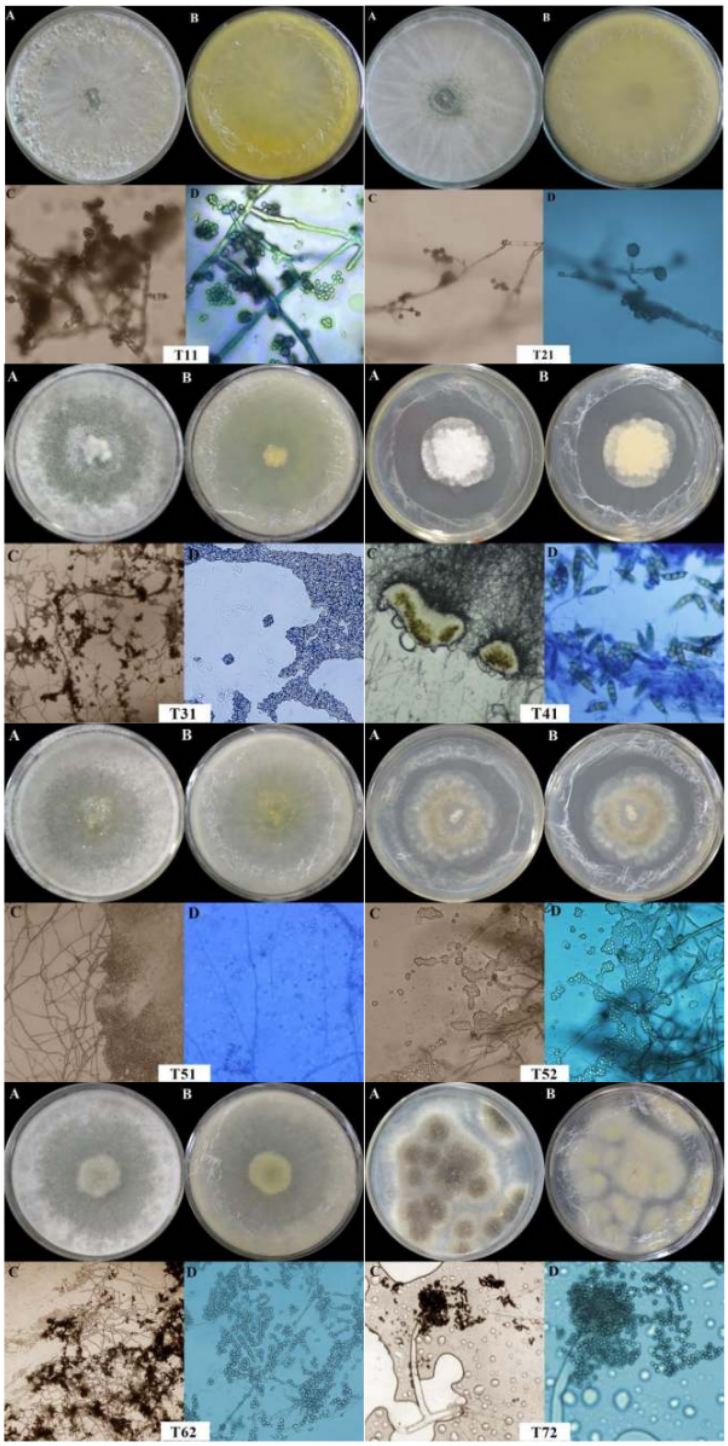

Figure 3. Morphology of endophytic fungi isolated from *Syzygium aqueum* stem bark; T11. *T. aureoviridae*, T21. *T. harzianum*, T31. *Trichoderma* sp., T41. *Pestalotia* sp., T51. Unidentified, T52. *Beltrania* sp., T62. *Trichoderma* sp., T72. *A. niger*; A. Front view of colony; B. Reverse colony; C. Conidia without staining; D. Conidia with lactophenol blue dye

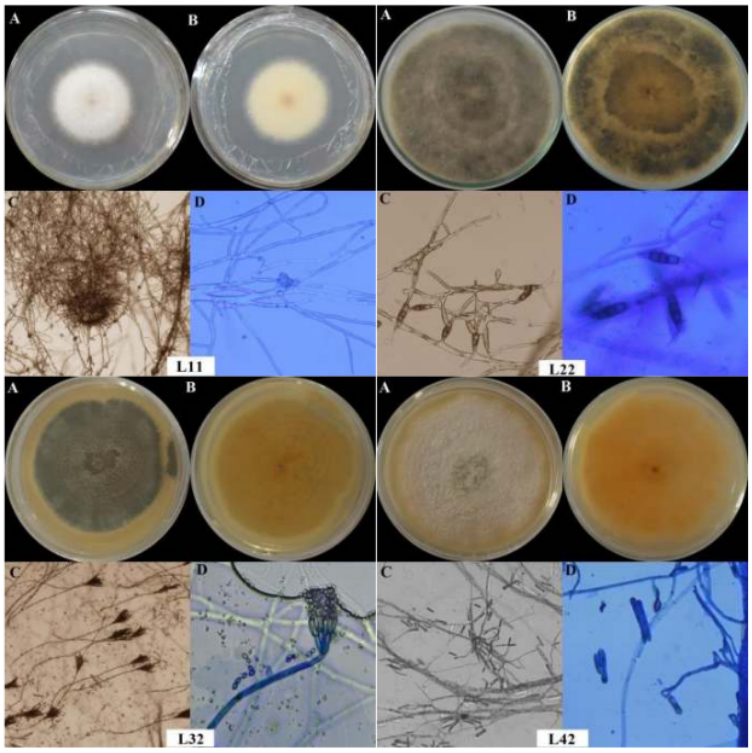

Figure 4. Morphology of endophytic fungi isolated from *Syzygium aqueum* leaves; L11. *Chaetomium* sp., L22. *Cochliobolus* sp., L32. *Penicillium* sp., L42. *Cylindrocladium* sp.; A. Front view of colony; B. Reverse colony; C. Conidia without staining; D. Conidia with lactophenol blue dye

Table 3. Fungal diversity and fungal endophytes isolated from different *Syzygium aqueum* tissues

Taxon (Genera)	Tissues of *Syzygium aqueum*			
	Leaves	Stem bark	Root bark	Total isolated
Cylindrocarpon	0	0	2	2
Aspergillus	0	1	2	3
Trichoderma	0	4	0	4
Pestalotia	0	1	0	1
Unidentified	0	1	0	1
Beltrania	0	1	0	1
Chaetomium	1	0	0	1
Cochliobolus	1	0	0	1
Penicillium	1	0	0	1
Cylindrocladium	1	0	0	1
% of total fungal isolates	4	8	4	16
Simpson's index (D)	0.250	0.313	0.500	0.141
Simpson's index of diversity (1-D)	0.750	0.688	0.500	0.859
Shannon index of diversity (H′)	1.386	1.386	0.693	2.133

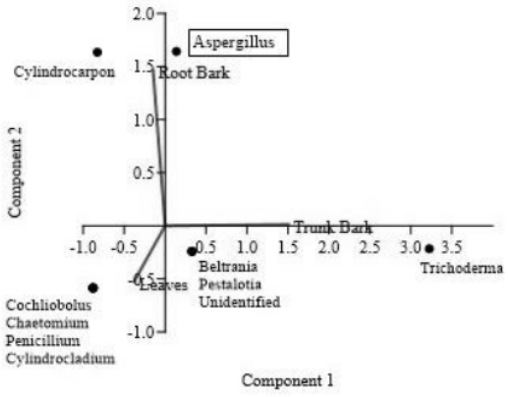

Figure 5. PCA of fungal endophytes isolated from different tissues of *Syzygium aqueum*. Components 1 and 2 explained 68% of the total fungal variation

Table 4. Antimicrobial activity of endophytic fungi

Code isolate	Isolate name	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Candida albicans*
R31	*Cylindrocarpon* sp.	++	++	++	+
R41	*Aspergillus niger*	+++	++	+	+
T11	*Trichoderma aureoviride*	++	++	++	-
T31	*Trichoderma* sp.	++	+++	+++	+
T41	*Pestalotia* sp.	++	+	+	+
T52	*Beltrania* sp.	+	++	++	+
L11	*Chaetomium* sp.	++	++	+	+
L22	*Cochliobolus* sp.	+	+	+	+
L32	*Penicillium* sp.	++	+	++	+
L42	*Cylindrocladium* sp.	++	++	+	+
Positive control [a]	-	+++	+++	+++	+

Note: [a]: Ciprofloxacin 30 µg/mL; +++: strong activity with an inhibition zone >20 mm; ++: moderate activity with an inhibition zone 10-20 mm; +: low activity with an inhibition zone 1-9 mm; -: negative activity with an inhibition zone <1 mm (Islamia et al. 2019).

In conclusion, 16 endophytic fungi were isolated from *S. aqueum* stem bark, root bark, and leaves. These endophytic fungi belonged to nine genera such as *Aspergillus*, *Cylindrocarpon*, *Trichoderma*, *Pestalotia*, *Beltrania*, *Chaetomium*, *Penicillium*, *Cochliobolus*, and *Cylindrocarpon*. The diversity values of the endophytic fungal populations were H′=2.133 according to the Shannon-Weiner index and 1-D=0.859 according to the Simpson index. Therefore, it shows that *S. aqueum* can supply a comfortable habitat and nutrients for fungal endophyte survival. Ten endophytic fungal extracts exhibited antimicrobial activity. The high antimicrobial potential was shown to be exclusively by *Trichoderma* sp. (T31), which acted strongly against *S. aureus* and *S. typhi* but had low antimicrobial activity against *C. albicans*. Therefore, *S. aqueum* is worth further investigating its bioactive secondary metabolites and isolating their bioactive compounds to produce antioxidant and antimicrobial agents.

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