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Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. *Biodiversitas* 7: 154-158.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) *Tropical Forest Community Ecology*. Wiley-Blackwell, New York.

Abstract:

Assaed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) *Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island*. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. *Mol Syst Biol* 4: 187. www.molecularsystemsbiology.com

BIODIVERSITAS

Journal of Biological Diversity
Volume 22 - Number 3 - March 2021

- Morphological, molecular and resistance responses to soft-rot disease variability among plantlets of *Phalaenopsis amabilis* regenerated from irradiated protocorms** 1077-1090
HALIDA ADISTYA PUTRI, AGUS PURWITO, SUDARSONO, DEWI SUKMA
- Agronomic characters, genetic and phenotypic diversity coefficients, and heritability of 12 genotypes of rice** 1091-1097
JAENUDIN KARTAHADIMAJA, SETYO DWI UTOMO, ERWIN YULIADI, ABDUL KADIR SALAM, WARSONO, ANUNG WAHYUDI
- A comparison of stand structure, species diversity and aboveground biomass between natural and planted mangroves in Sikka, East Nusa Tenggara, Indonesia** 1098-1103
JERIELS MATATULA, AHMAD YUSUF AFANDI, PANDU YUDHA ADI PUTRA WIRABUANA
- The potency of actinomycetes extracts isolated from Pramuka Island, Jakarta, Indonesia as antimicrobial agents** 1104-1111
SETIAWATI SETIAWATI, TITIK NURYASTUTI, ETI NURWENING SHOLIKHAH, PUSPITA LISDIYANTI, SYILVIA UTAMI TUNJUNG PRATIWI, TRI RATNA SULISTIYANI, SHANTI RATNAKOMALA, JUMINA, MUSTOFA
- Breed and age effects on concentration of adiponectin and reproductive performance in Anglo Nubian, Etawah grade and its crossbred bucks** 1112-1119
HAFIZUDDIN, NI WAYAN KURNIANI KARJA, LISA PRAHARANI, MOHAMAD AGUS SETIADI
- The genus *Dolichandrone* (Fenzl.) Seem. (Bignoniaceae) in Thailand** 1120-1128
WEEREESA BOONTHASAK, CHATCHAI NGERNSAENG SARUJAY
- Diversity of endophytic fungi in *Syzygium aqueum*** 1129-1137
UMMI H. HABISUKAN, ELFITA, HARY WIDJAJANTI, ARUM SETIAWAN, ALFIA R. KURNIAWATI
- Genetic diversity in seedling populations of *Dipterocarpus gracilis* in Kecubung Uluanang Nature Conservation Reserve, Indonesia** 1138-1145
NESTY PRATIWI ROMADINI, SAPTO INDRIOKO, WIDIYATNO, ENY FARIDAH, YENI W.N RATNANINGRUM
- Correlation and path analysis for agronomic traits contributing to yield in 30 genotypes of soybean** 1146-1151
ANNA SATYANA KARYAWATI, ENO SINTHIA VINKY PUSPITANINGRUM
- The biological prospective of red-pigmented bacteria cultured from contaminated agar media** 1152-1159
ACHMAD ARIFIYANTO, HAPIN AFRIANI, MEISHY HANDERLIN PUTRI, BERLIANA DAMAYANTI, CINDY LUKYTA RATIH RIYANTO
- Normalized Difference Vegetation Index (NDVI) analysis for vegetation cover in Leuser Ecosystem area, Sumatra, Indonesia** 1160-1171
ADISTI PERMATASARI PUTRI HARTOYO, ARZYANA SUNKAR, RAHMATULLOH RAMADANI, SYAHLAN FALUTHI, SYAFITRI HIDAYATI
- Profile of cryptobrachytone C accumulation in *Cryptocarya pulchrinervia* leaves using MALDI-MSI** 1172-1178
JUJUN RATNASARI, RIZKITA RACHMI ESYANTI, MARSELINA IRASONIA TAN, LIA DEWI JULIAWATY, SHUICHI SHIMMA

GC-MS analysis of rhizome ethanol extracts from <i>Curcuma aeruginosa</i> accessions and their efficiency activities as anticancer agent WARAS NURCHOLIS, NURUL KHUMAIDA, MARIA BINTANG, MUHAMAD SYUKUR	1179-1186
Inhibition of α-amylase and α-glucosidase activities in vitro by extracts of selected medicinal plants DEEPAK RAJ PANT, BIVA ARYAL, DAMA PUN, SUSHILA SHARMA, GIRI PRASAD JOSHI	1187-1193
Combination on endophytic fungal as the Plant Growth-Promoting Fungi (PGPF) on cucumber (<i>Cucumis sativus</i>) SYAMSIA SYAMSIA, ABUBAKAR IDHAN, AMANDA PATAPPARI FIRMANSYAH, NOERFITRYANI NOERFITRYANI, IRADHATULLAH RAHIM, HENRY KESAULYA, RAKHMAD ARMUS	1194-1202
Salinity tolerance of larvae <i>Aedes aegypti</i> inland and coastal habitats in Pasangkayu, West Sulawesi, Indonesia ARINI RATNASARI, ARIF RAHMAN JABAL, SYAHRIBULAN, IRFAN IDRIS, NUR RAHMA, SRI NUR RAHMI NUR RUSTAM, MILA KARMILA, HAJAR HASAN, ISRAWAHID	1203-1210
Morpho-physiological changes of four tropical tree seedlings under aluminum stress CHARTINA PIDJATH, DIDY SOPANDIE , MAMAN TURJAMAN , SRI WILARSO BUDI	1211-1220
The association of follicle stimulating hormone receptor (<i>FSHR</i>) gene polymorphism of on egg productivity in hybrid chicken (<i>Gallus gallus gallus</i>, Linnaeus 1758) RIKHA RISKI KURNIA, INDRA LESMANA, ADITYA RAHMAN ERNANTO, AYUDHA BAHANA ILHAM PERDAMAIAN, TRIJOKO, BUDI SETIADI DARYONO	1221-1226
Exploring anticancer activity of wild and polyploid mutant of <i>Artemisia cina</i> SRI KASMIYATI, ELIZABETH B.E. KRISTIANI, MARIA M. HERAWATI, FERDY S. RONDONUWU	1227-1234
Forest cover changes in Indonesia's terrestrial national parks between 2012 and 2017 ASRI A. DWIYAHRENI,, HABIBURRACHMAN A.H. FUAD, SUNARYO, TRI EDHI BUDHI SOESILO, CHRIS MARGULES, JATNA SUPRIATNA	1235-1242
Characterization of banana bunchy top virus on Sumatra (Indonesia) wild banana RUTH FETI RAHAYUNIATI, SEDYO HARTONO, SUSAMTO SOMOWIYARJO, SITI SUBANDIYAH, JOHN E. THOMAS	1243-1249
Culturable endophytic fungal diversity in cassava tubers of Indonesia ANASTASIA TATIK HARTANTI, FELICIA NIKE SUSANTI, VIVITRI DEWI PRASASTY, NANI RADIASTUTI	1250-1260
Application of inorganic and liquid organic bio-fertilizers affects the vegetative growth and rhizobacteria populations of eight banana cultivars TRI HANDAYANI, TIRTA KUMALA DEWI, DIYAH MARTANTI, YUYU SURYASARI POERBA, SARJIYA ANTONIUS, WITJAKSONO	1261-1271
Short Communication: <i>Cremanthodium indica</i> (Asteraceae, Senecioneae), a new species from the Eastern Himalaya, India DIPANKAR BORAH, RAJEEV KUMAR SINGH, LOBSANGTASHI THUNGON	1272-1276
Molecular identification of five species of family Chaetomiaceae (Sordariomycetes, Ascomycota) from Iraqi soil MUSTAFA A. AL-DOSSARY, SHAYMAA S. RAHEEM, MAYSOON K. ALMYAH	1277-1284
Short Communication: A small-scale coprological survey of the endoparasites in the Himalayan goral <i>Naemorhedus goral</i> (Hardwick, 1825) in Nepal JAGAN NATH ADHIKARI, ROSHAN BABU ADHIKARI, BISHNU PRASAD BHATTARAI, TEJ BAHADUR THAPA, TIRTH RAJ GHIMIRE	1285-1290
Extracellular protease from <i>Bacillus subtilis</i> B315 with antagonistic activity against bacterial wilt pathogen (<i>Ralstonia solanacearum</i>) of chili NUR PRIHATININGSIH, ARI ASNANI, HERU ADI DJATMIKO	1291-1295

A pilot study into ecological burning in forests as part of a species conservation plan MATTHEW W. PEARSON	1296-1303
Traditional knowledge of medicinal plants in the Kampung Orang Asli Donglai Baru, Hulu Langat, Malaysia MOHD RAZNAN RAMLI, SORAYYA MALEK, POZI MILOW, NURUL JANNAH AZIZ	1304-1309
Morphological diversity and heritability of nine local sorghum (<i>Sorghum bicolor</i>) genotypes in East Java MAFTUCHAH, LIA FEBRIANA, SULISTYAWATI, HELVI ARDANA RESWARI, ERFAN DANI SEPTIA	1310-1316
Evaluation of Indigenous <i>Pichia kudriavzevii</i> from cocoa fermentation for a probiotic candidate RAHAYU WULAN, RIKA INDRI ASTUTI, YAYA RUKAYADI, ANJA MERYANDINI	1317-1325
Short Communication: Identification of culturable marine fungi and bacteria from coastal region in Brunei Darussalam HUSSEIN TAHA, POOJA SHIVANAND, MOHAMAD ABDUL AZIM ZAINUDIN, NURUL AZIRAH HADANAN	1326-1331
Evaluation of the ambrosia beetles traps on <i>Pterocarpus indicus</i> in Indonesia HAGUS TARNO, YOGO SETIAWAN, BAMBANG TRI RAHARDJO, JIANGUO WANG	1332-1339
Short Communication: Taxonomy and the first report for pollen and leaf surfaces anatomy of the genus <i>Enkleia</i> (Thymelaeaceae) in Thailand PIYAPORN SAENSOUK, SURAPON SAENSOUK	1340-1344
Variations of grain physical properties, amylose and anthocyanin of upland red rice cultivars from East Nusa Tenggara, Indonesia JENNY E.R. MARKUS, A.S.S. NDIWA, SHIRLY S. OEMATAN, YOSEP S. MAU	1345-1353
Assessment of forest degradation condition in Natmataung National Park Watershed, Myanmar YADANAR OO, WATHINEE SUANPAGA, PONTHEP MEUNPONG	1354-1362
Prevalence, diversity and antimicrobial resistance of <i>Salmonella enterica</i> and <i>Pseudomonas aeruginosa</i> isolates from spring water in a rural area of Northwestern Morocco ILHAM NASSRI, LATIFA TAHRI, AMAL SAIDI, NAJIA AMEUR, MOHAMMED FEKHAOUI	1363-1370
Phenetic analysis and habitat preferences of wild orchids in Gunung Gajah, Purworejo, Indonesia THERESIA HENNY PRIMASIWI PURBA, ABDUL RAZAQ CHASANI	1371-1377
The occurrence of <i>Anisakis</i> larvae on hairtail, <i>Trichiurus lepturus</i> caught from the Pangandaran Waters, West Java, Indonesia NISA QUROTA AYUN, LUTFI SEPTIANA DEWI, MURWANTOKO, EKO SETYOBUDI	1378-1384
Involvement of purple acid phosphatase gene into nitrogen uptake of oil palm (<i>Elaeis guineensis</i>) SIGIT DWI MARYANTO, ZULFIKAR ACHMAD TANJUNG, ROBERDI, WIDYARTINI MADE SUDANIA, PUJIANTO, HAIRINSYAH, CONDRO UTOMO, TONY LIWANG	1385-1390
Short Communication: Prediction of body weight based on body measurements in female Saburai goat AKHMAD DAKHLAN, MUHAMMAD DIMA IQBAL HAMDANI, DELSI RUSITAIMI PUTRI, SULASTRI, ARIF QISTHON	1391-1396
Natural dye plants used by Dayak Iban in Sungai Utik, Kapuas Hulu, West Kalimantan, Indonesia WAHDINA, DEDE SETIADI, Y. PURWANTO, IBNUL QAYIM	1397-1404
Phytophagous insects and predatory arthropods in soybean and zinnia ERISE ANGGRAINI, WIDA NUR ANISA, SITI HERLINDA, CHANDRA IRSAN, SUPARMAN, SUWANDI, MUHAMMAD UMAR HARUN4	1405-1414

Cross shelf gradients of scleractinian corals in the Spermonde Islands, South Sulawesi, Indonesia	1415-1423
SYAFYUDIN YUSUF, MARIA BEGER, ASMI CITRA MALINA A. R. TASSAKKA, MAARTEN DE BRAUWER, AMANDA PRICELLA, RAHMI, WIDYASTUTI UMAR, GINO V. LIMMON, ABIGAIL MARY MOORE, JAMALUDDIN JOMPA	
Short Communication: Plant diversity utilization and land cover composition in the Subak Jatiluwih, Bali, Indonesia	1424-1432
SUTOMO, RAJIF IRYADI, I DEWA DARMA, I PUTU AGUS HENDRA WIBAWA, AYYU RAHAYU, SITI FATIMAH HANUM, SYAMSUL RIZAL, LEDYA NOVAMIZANTI, JANGKUNG RAHARJO	
Diversity of fungi associated with rhizosphere of healthy and diseased garlic crop	1433-1440
BAIQ NURUL HIDAYAH, NANI HERAWATI, AI ROSAH AISAH, NITA RIZKY UTAMI	
Morphological characteristics of Indonesian <i>Rubus</i> flowers	1441-1447
SULUH NORMASIWI, ANDI SALAMAH, MUHAMMAD IMAM SURYA	
Short Communication: Zooplankton as indicator of trophic status of lakes in Ilmen State Reserve, Russia	1448-1455
IRINA V. MASHKOVA, ANASTASIYA M. KOSTRYUKOVA, ELENA E. SHCHELKANOVA, VIKTOR V. TROFIMENKO	
Molecular characteristics and taxonomic status of morphologically similar barnacles (<i>Amphibalanus</i>) assessed using the cytochrome c oxidase 1 gene	1456-1466
SRI RIANI, ROMANUS EDY PRABOWO, AGUS NURYANTO	
Screening of endophytic fungi from oil palm (<i>Elaeis guineensis</i> Jacq.) in producing exopolysaccharides	1467-1473
YURNALIZA YURNALIZA, IT JAMILAH, ADRIAN HARTANTO, ANISA LUTFIA	
Identification of bioactive compounds in gambier (<i>Uncaria gambir</i>) liquid by-product in West Sumatra, Indonesia	1474-1480
AKHIS SOLEH ISMAIL, YOSE RIZAL, ARMENIA, ANWAR KASIM	
Identification of marine sponges-symbiotic bacteria and their application in degrading polycyclic aromatic hydrocarbons	1481-1488
ISMAIL MARZUKI, MUDYAWATI KAMARUDDIN, RASHEED AHMAD	
Mangrove ecosystem in North Sumatran (Indonesia) forests serves as a suitable habitat for mud crabs (<i>Scylla serrata</i> and <i>S. olivacea</i>)	1489-1496
RIKA KARNIATI, NURDIN SULISTIYONO, RIZKA AMELIA, BEJO SLAMET, YUNTHA BIMANTARA, MOHAMMAD BASYUNI	
Estimation Above Ground Biomass (AGB) and carbon stocks of <i>Tectona grandis</i> and <i>Gmelina arborea</i> stands in Gorontalo Province, Indonesia	1497-1508
YOSEP RUSLIM, DAUD SANDALAYUK, ROCHADI KRISTININGRUM, ANDI SAHRI ALAM	
Antifungal activities of the rhizome extract of five member Zingiberaceae against <i>Candida albicans</i> and <i>Trichophyton rubrum</i>	1509-1513
MUHAMMAD EVY PRASTIYANTO, NI'MATUR ROHMAH, LESITA EFENDI, RAHMATIA ARIFIN, FANDHI ADI WARDOYO, WILDIANI WILSON, ANA HIDAYATI MUKAROMAH, SRI SINTO DEWI, SRI DARMAWATI	
Surface ultrastructure of <i>Blastocystis</i> sp. isolated from cattle	1514-1518
NI KOMANG APRILINA WIDISUPUTRI, NUNUK DYAH RETNO LASTUTI, ENDANG SUPRIHATI, POEDJI HASTUTIEK, HANI PLUMERIASTUTI, MUFASIRIN, HENI PUSPITASARI, LUCIA TRI SUWANTI	
Indigenous <i>Streptomyces</i> spp. isolated from <i>Cyperus rotundus</i> rhizosphere indicate high mercuric reductase activity as a potential bioremediation agent	1519-1526
HANUM MUKTI RAHAYU,2 , WAHYU ARISTYANING PUTRI, ANIS USWATUN KHASANAH,6 , LANGKAH SEMBIRING, YEKTI ASIH PURWESTRI	
Fucoxanthin identification and purification of brown algae commonly found in Lombok Island, Indonesia	1527-1534
YENI SULISTIYANI, AGUS SABDONO, NORMA AFIATI, HAERUDDIN	

Inhibition of α-glucosidase activity, metals content, and phytochemical profiling of <i>Andrographis paniculata</i> from different geographical origins based on FTIR and UHPLC-Q-Orbitrap HRMS metabolomics	1535-1542
MOHAMAD RAFI,, DEWI ANGGRAINI SEPTANINGSIH, ALFI HUDATUL KAROMAH, LUKMAN, BAMBANG PRAJOGO, MUHAMMAD BACHRI AMRAN, ABDUL ROHMAN	
Valuing ecotourism in Bukit Merah Orang Utan Island, Malaysia based on visitors' experience	1543-1549
NABILA FARHANA MOHD-ASRI, SITI NORSYUHADA KAMALUDDIN, SABAPATHY DHARMALINGAM, WAN MOHD RAZI IDRIS, BADRUL MUNIR MD-ZAIN	
Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia	1550-1557
ARIS MUMPUNI, ADI AMURWANTO, DANIEL JOKO WAHYONO	
Effect of fertilizer application on winter wheat productivity under precision agriculture in Kazakhstan	1558-1563
ALIBEK BAIMURATOV, SHOLPAN BASTAUBAYEVA, MEHMET ARSLAN, ZHANAR YERALIYEVA	
Molecular identification of a new isolate of actinobacteria ATIS61 and characterization of the protease activities	1564-1569
FADHLIAH AMFAR, LENNI FITRI, SUHARTONO	
Morphological evaluation and determination keys of 21 citrus genotypes at seedling stage	1570-1579
RAHMAT BUDIARTO, ROEDHY POERWANTO, EDI SANTOSA, DARDA EFENDI	
Diversity, conservation, potential uses, and alien species of lowland plants of Batang Toru, Sumatra, Indonesia	1580-1591
HIRMAS F. PUTRA, WENDY A. MUSTAQIM	

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 of 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 total 16 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 T41 and 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 Mapaog (2014) and Chua et al. (2019). Microbial infections are serious challenges to human health because of microbial resistance to antibiotics (Dos Santos 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 (Porrás-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 ± 1 min, followed by immersion in 3% (w/v) sodium hypochlorite (NaOCl) for 1 min. After being rinsed in sterile distilled water for ± 1 min, 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 1L of H₂O) supplemented with chloramphenicol (0.2g/L). Petri dishes were then incubated at $30 \pm 2^\circ\text{C}$ 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 ($\pm 10^6$ 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 3.0×10^8 CFU/mL) (Singh et al. 2015). The extract concentration used for this assay was $1,000\mu\text{g/mL}$. Absorbent disc (Whatman, 6.0mm in diameter) was impregnated with $10\mu\text{L}$ of the solution and then placed on the surface of inoculated plates (90mm). Positive control discs of ciprofloxacin $10\mu\text{L/mL}$ 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 L11, 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, filial circling vesicles, and round, black conidia.

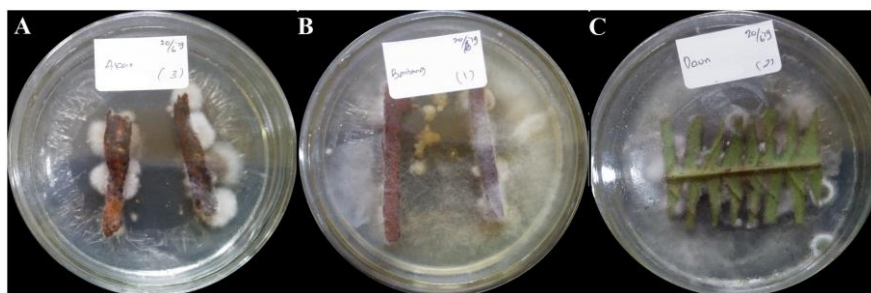


Figure 1. Mycelium appearance of endophytic fungi around *Syzygium aqueum* segments; A. Root bark; B. Stem bark; C. Leaves

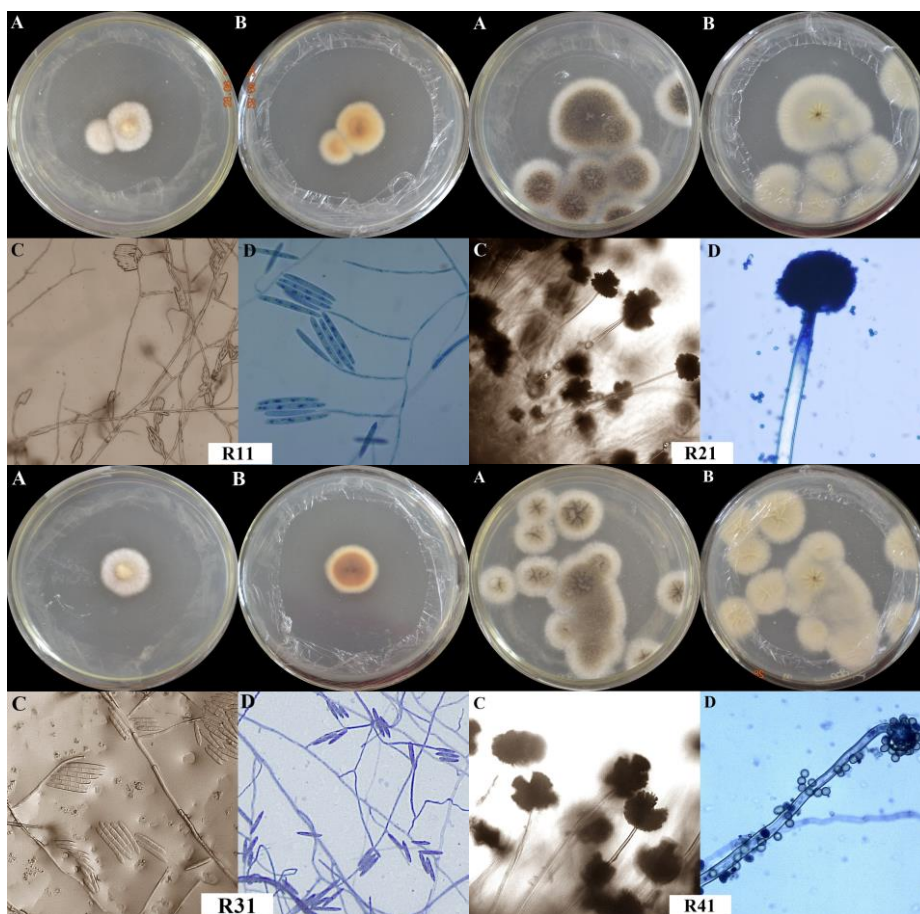


Figure 2. Morphology of endophytic fungi isolated from *Syzygium aqueum* root bark. R11. *Cylindrocarpon* sp., R21. *A. niger*, R31. *Cylindrocarpon* sp., R41. *A. niger*; A. Front view colony; B. Reverse colony; C. Conidia without staining; D. Conidia with lactophenol blue dye

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

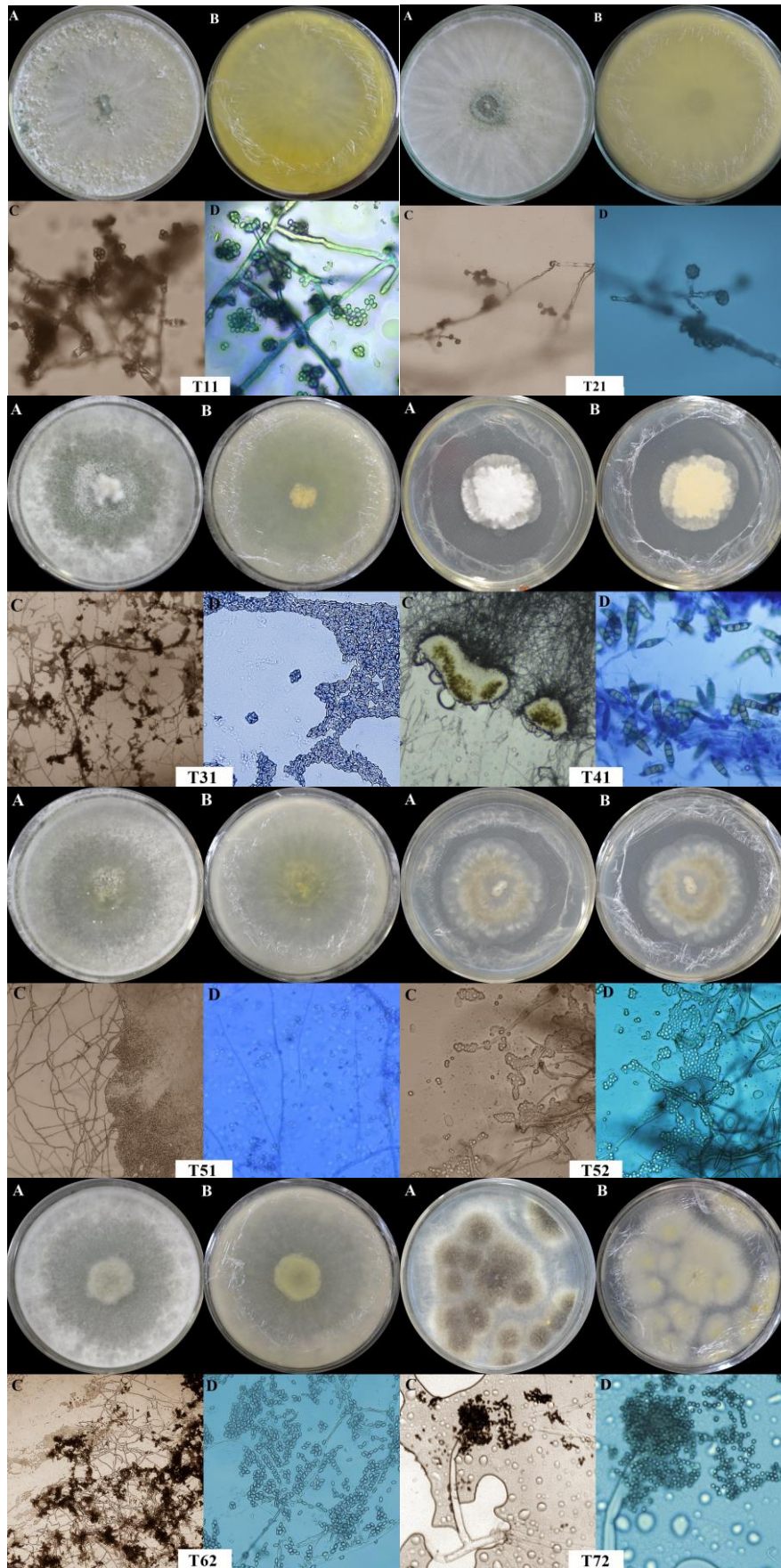


Figure 3. Morphology of endophytic fungi isolated from *Syzygium aqueum* stem bark; T11. *T. aureoviridae*, T21. *T. harzianum*, T31. *Trichoderma* sp., T41. *Pestalotia* sp., T51. Unidentified, T52. *Beltrania* sp., T62. *Trichoderma* sp., T72. *A. niger*; A. Front view of colony; B. Reverse colony;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

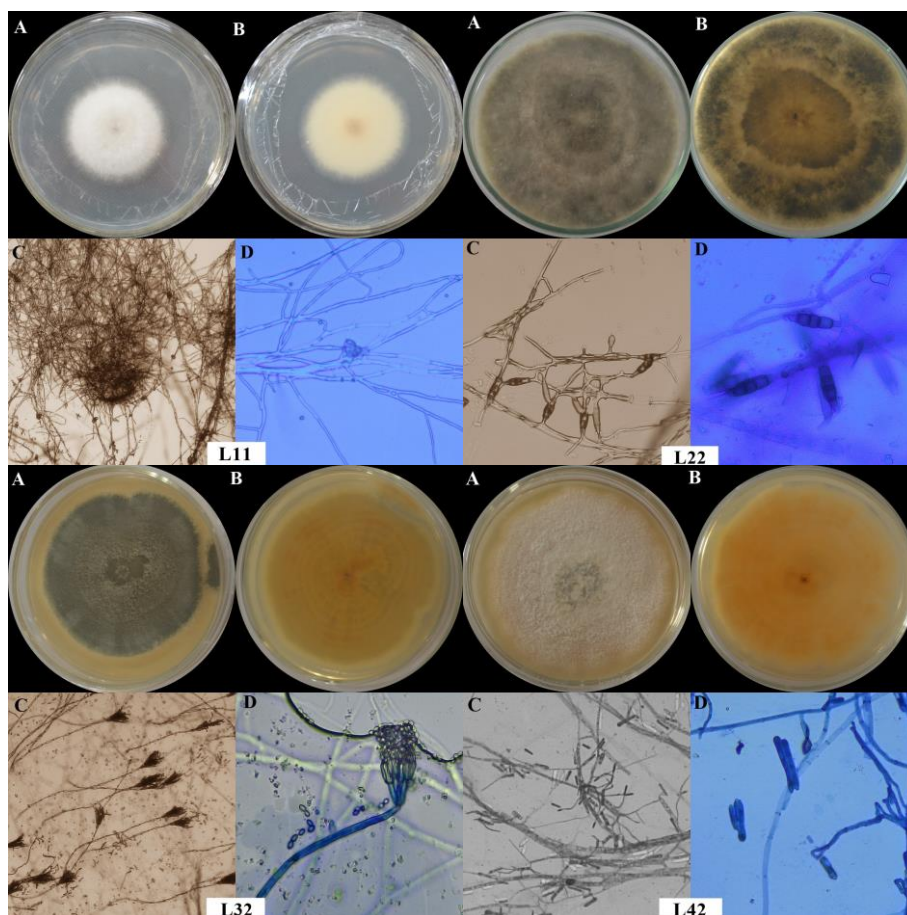


Figure 4. Morphology of endophytic fungi isolated from *Syzygium aequum* leaves; L11. *Chaetomium* sp., L22. *Cochliobolus* sp., L32. *Penicillium* sp., L42. *Cylindrocladium* sp.; A. Front view of colony; B. Reverse colony; C. Conidia without staining; D. Conidia with lactophenol blue dye

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

Taxon (Genera)	Tissues of <i>Syzygium aequum</i>			
	Leaves	Stem bark	Root bark	Total isolated
<i>Cylindrocarpon</i>	0	0	2	2
<i>Aspergillus</i>	0	1	2	3
<i>Trichoderma</i>	0	4	0	4
<i>Pestalotia</i>	0	1	0	1
Unidentified	0	1	0	1
<i>Beltrania</i>	0	1	0	1
<i>Chaetomium</i>	1	0	0	1
<i>Cochliobolus</i>	1	0	0	1
<i>Penicillium</i>	1	0	0	1
<i>Cylindrocladium</i>	1	0	0	1
No. 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

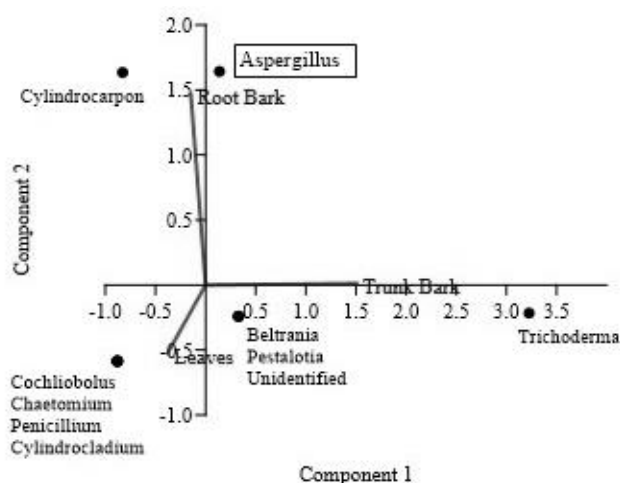


Figure 5. PCA of fungal endophytes isolated from different tissues of *Syzygium aequum*. Components 1 and 2 explained 68% of the total fungal variation

Table 4. Antimicrobial activity of endophytic fungi

Code isolate	Isolate name	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Candida albicans</i>
R31	<i>Cylindrocarpon</i> sp.	++	++	++	+
R41	<i>Aspergillus niger</i>	+++	++	+	+
T11	<i>Trichoderma aureoviride</i>	++	++	++	-
T31	<i>Trichoderma</i> sp.	++	+++	+++	+
T41	<i>Pestalotia</i> sp.	++	+	+	+
T52	<i>Beltrania</i> sp.	+	++	++	+
L11	<i>Chaetomium</i> sp.	++	++	+	+
L22	<i>Cochliobolus</i> sp.	+	+	+	+
L32	<i>Penicillium</i> sp.	++	+	++	+
L42	<i>Cylindrocladium</i> 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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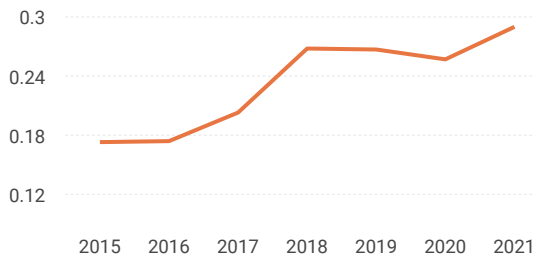
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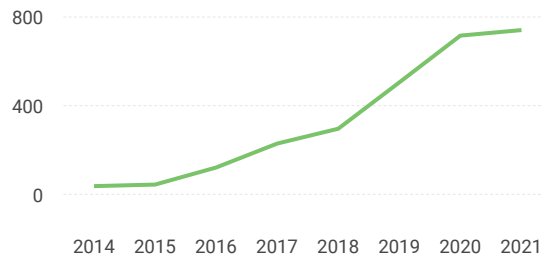
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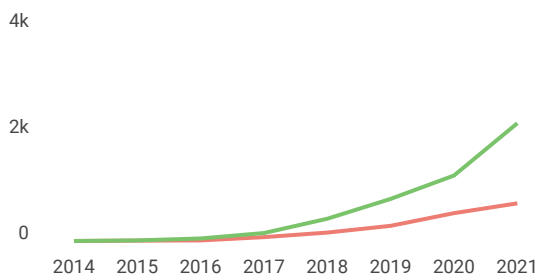
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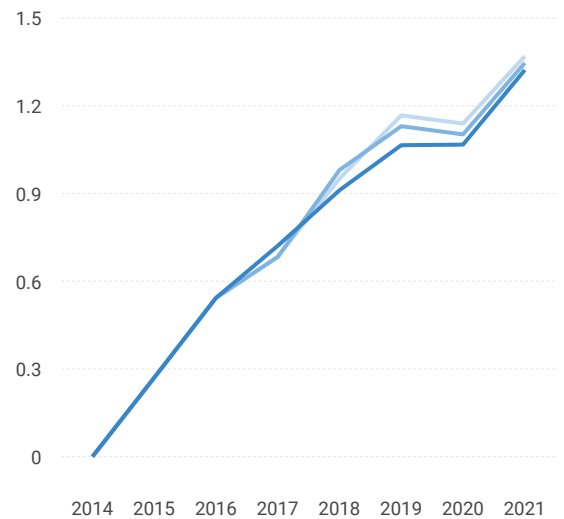
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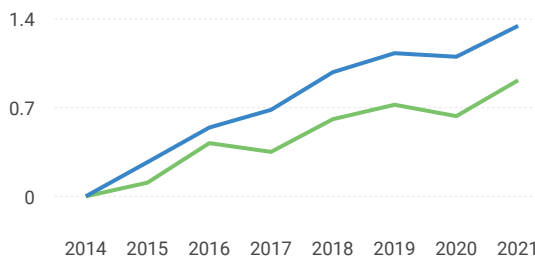
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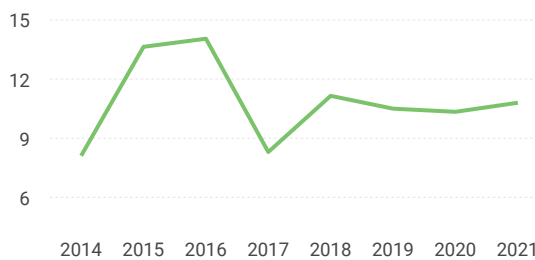
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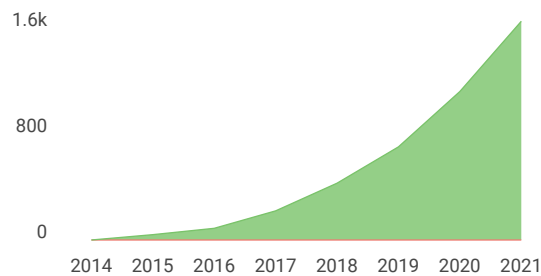
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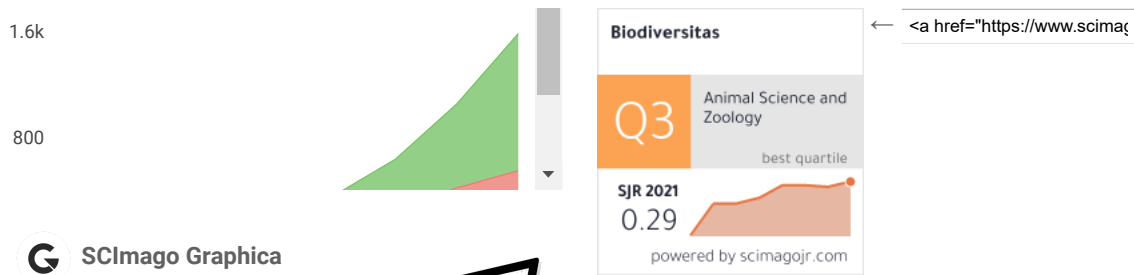
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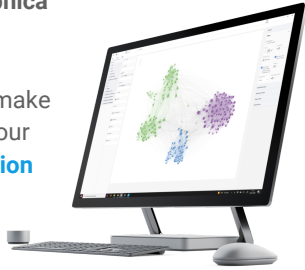
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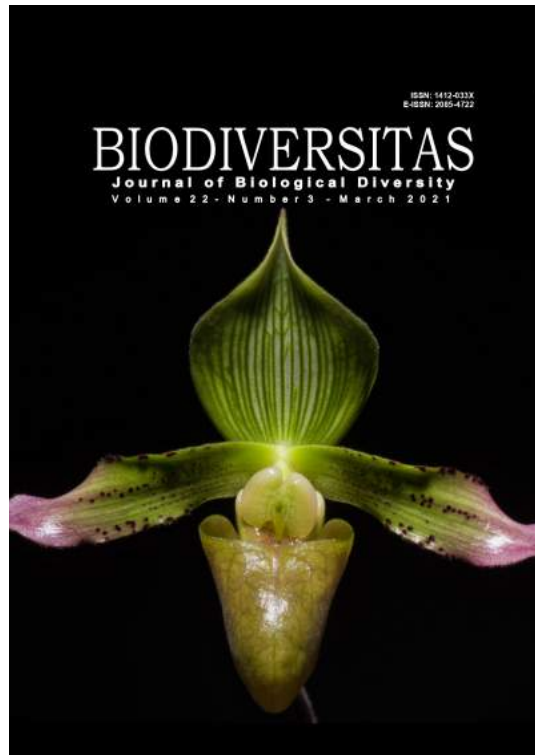
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