

Exploration of Endophytic Fungal Diversity from Red Betel Medicinal Plants (*Piper crocatum* Ruiz & Pav) in Sidomulyo Village Batu

Yogaswara*, Lisa Lisdiana

Study Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya
Kampus Unesa 1, Jln. Ketintang Surabaya 60321 Indonesia

*e-mail: Yogaswara.20059@mhs.unesa.ac.id

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Abstract

Information regarding endophytic fungi in red betel plants is still very limited, especially in plants that live in the highlands. Previous research states that the environment can be a factor in the diversity of the number and type of endophytic fungi, so it does not rule out the possibility that this phenomenon also applies to red betel plants. This research aims to explore the diversity of endophytic fungi in red betel plants that live in the highlands, especially in Sidomulyo Village, Batu. The methods used in this research were isolation, identification, and analysis of diversity index (H') values. The results showed that there were a total of 37 isolates from the roots, stems, and leaves of red betel plants with the highest number of isolates in the leaves (21 isolates) and the lowest in the roots (3 isolates). These isolates were grouped into 16 morphotypes. 12 morphotypes were identified including *Purpureocillium lilacinum*, *Corynespora cassicola*, *Penicillium* sp., *Curvularia lunata*, *Phyllosticta hostae*, *Cladosporium cladosporioides*, *Colletotrichum orientalis*, *Paecilomyces variotii*, *Colletotrichum nymphaeae*, *Colletotrichum nupharicola*, *Fusarium oxysporum*, and *Hypoxyton pulicidum*, four morphotypes namely MFE3, MFE10, MFE14, and MFE15 have not yet been identified. The diversity index in the roots, stems, and leaves is categorized as medium diversity with the highest in the leaves ($H' = 1.818$) and the lowest in the roots (1.099).

Keywords: Microorganisms; plants; biodiversity.

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INTRODUCTION

Endophytic fungi have been associated with plants for more than 400 million years (Krings *et al.*, 2007) and have been studied extensively across different geographies and climate zones (Sun and Guo, 2012). According to Li *et al.*, (2025), endophytic fungi play a role in increasing plant growth through the secretion of phytohormones, enhance host plant adaptability to biotic and abiotic stresses, and facilitate nutrient absorption. Endophytic fungi can be isolated from stems, flowers, fruits, and seeds (Roze *et al.*, 2011). Previous research shows that many endophytic fungi are isolated from plants that are known to have medicinal properties. Such as Hafsari and Asterina (2013) who isolated and identified endophytic fungi from surian medicinal plants (*Toona sinensis*) and Widowati *et al.*, (2016) who isolated and identified endophytic fungi from turmeric plants (*Curcuma longa* L.). Endophytic fungi that grow in medicinal plant tissues can produce compounds that have almost the same characteristics as their host plants (Prihatiningtyas *et al.*, 2005). In addition, endophytic fungi are able to produce bioactive compounds and have the potential as a source of new drugs to treat a disease (Kuncoro and Sugijanto, 2011).

Java Island has a variety of medicinal plants, one of which is the red betel plant. Red betel (*Piper crocatum* Ruiz and Pav) is one of the potential medicinal plants that is theoretically known to have properties to cure various diseases (Rachmawaty *et al.*, 2009). Red betel is a shrub plant, stems with tendrils and brushes, the upper leaves are dark green, with a silvery area around the leaves bones, and the lower ones are purplish red. Red betel generally lives at an altitude of 0 - 2,500 masl, but there are also some species that grow at altitudes above 3,000 masl (Quijano-Abril *et al.*, 2006). The habitat of red betel is a shady, cool place with sunlight intensity between 60 - 75%, and can grow well in mountainous areas. However, red betel plants can also grow in hot areas, but will experience morphological changes due to the influence of environmental temperature (Fadlilah, 2015).

Endophytic fungi on red betel plants have been studied in the Summersari Jember area at an altitude of 83 masl which is still categorized as lowland (0 - 200 masl). In this study, 11 types of endophytic fungi were successfully isolated and identified. However, there is no information on the diversity of endophytic fungi in red betel plants that live in highland areas. Red betel can thrive in highland areas such as in Sidomulyo Village, Batu Subdistrict. Sidomulyo Village, Batu Subdistrict, located at the foot of Mount Panderman with an altitude of 850 meters above sea level, is a place for the cultivation of many plants, one of which is red betel. Isolation and identification of endophytic fungi from red betel plants that live in highland areas, especially in Sidomulyo Village, Batu District has never been done, so there is still no information about the number and diversity.

Isolation of endophytic fungi on the same plant species but living at different altitudes has been done by previous researchers. Tambunan *et al.*, (2018) have examined the diversity of endophytic fungi in cocoa plants that live at different altitudes in the Bali area. In this study, there were no differences in the diversity of endophytic fungi isolated from cocoa plants living in lowlands and highlands, but there were differences in the number of colonies of endophytic fungi where more colonies were found in cocoa plants living in lowlands compared to cocoa plants living in highlands. The difference in the number of colonies is caused by temperature and humidity. Based on this research, it is possible that there are differences in the types and numbers of endophytic fungi isolated from red betel plants living in the Sidomulyo Batu area with an altitude of 850 meters above sea level with endophytic fungi isolated from the Summersari Jember area. Therefore, it is necessary to explore the diversity of types and numbers of endophytic fungi on red betel plants living in Sidomulyo Village.

This research is expected to enrich information about endophytic fungi from red betel plants that live in the highlands, especially in Sidomulyo Village Batu Subdistrict with an altitude of 850 meters above sea level in order to obtain information on whether there are differences in the diversity and number of endophytic fungi from red betel plants living in lowlands and highlands.

MATERIALS AND METHODS

This research was conducted from September to December. Samples of roots, stems, and leaves of red betel plants were collected from Sidomulyo Village, Batu District, Batu City. Isolation and identification of endophytic fungi were carried out at the Microbiology Laboratory, Building C10, Faculty of Mathematics and Natural Sciences, Surabaya State University. The tools used in this research were autoclave (*Tomy Kogyo* ES-215), incubator (*Lab-Line Instruments* 302-1) petri dish, pipette, inoculation needle, scissors, drigalsky rod, zip lock plastic, PP plastic, digital balance, glass beaker, test tube, light microscope, label paper, object glass, cover glass, light microscope, label paper, object glass, cover glass, test tube rack, Laminar Air Flow (LAF) (*ESCO*), tweezers, erlenmayer, label paper, marker, scissors, knife/cutter, scalpel, cool box, hot plate, refrigerator (*Sanyo Electric* MPR-31 1D (H)), magnetic stirrer, aluminum foil, and mobile phone camera. The materials used in this study were samples of roots, stems, and leaves of red betel nut plants from Batu District, Batu City, 70% alcohol, sterile distilled water, 2% sodium hypochlorite (NaOCl) solution, *Potato Dextrose Agar* (PDA; Merck Cat#1101300500) medium, tissue, chloramphenicol antibiotics, and *Lactophenol Cotton Blue* (LPCB) dye; (Merck Cat#1137410100).

The research was carried out in 8 stages, namely making medium, sterilizing tools and materials, adding chloramphenicol antibiotics to PDA medium, taking samples of red betel plants, isolating endophytic fungi, purifying endophytic fungi, characterizing endophytic fungi macroscopically and microscopically, and identifying endophytic fungal isolates with books and literature. Medium preparation was carried out by dissolving 39 grams of PDA medium in 1 L of distilled water, homogenized with a magnetic stirrer while heating to boiling on a hot plate. Sterilization of medium and tools and materials was carried out using an autoclave at 121°C and 1 atm pressure for 15 minutes. PDA medium after sterilization was added with 100 mg/L chloramphenicol antibiotic and then homogenized.

Sampling of red betel plants was carried out in Sidomulyo Village Batu. A total of 3 red betel plants were taken from 3 different locations. The plant organs taken were root samples (2 cm from the tip/root cap), stems that do not touch the ground (10 cm from the top of the soil), and leaves in position number 5 from the top of the red betel plant from each plant. Samples were taken using scissors and a knife. Samples that have been taken were then wrapped separately using zip lock plastic and labeled, then all samples were put into a cool box. The samples were then taken to the Microbiology Laboratory of FMIPA, State University of Surabaya.

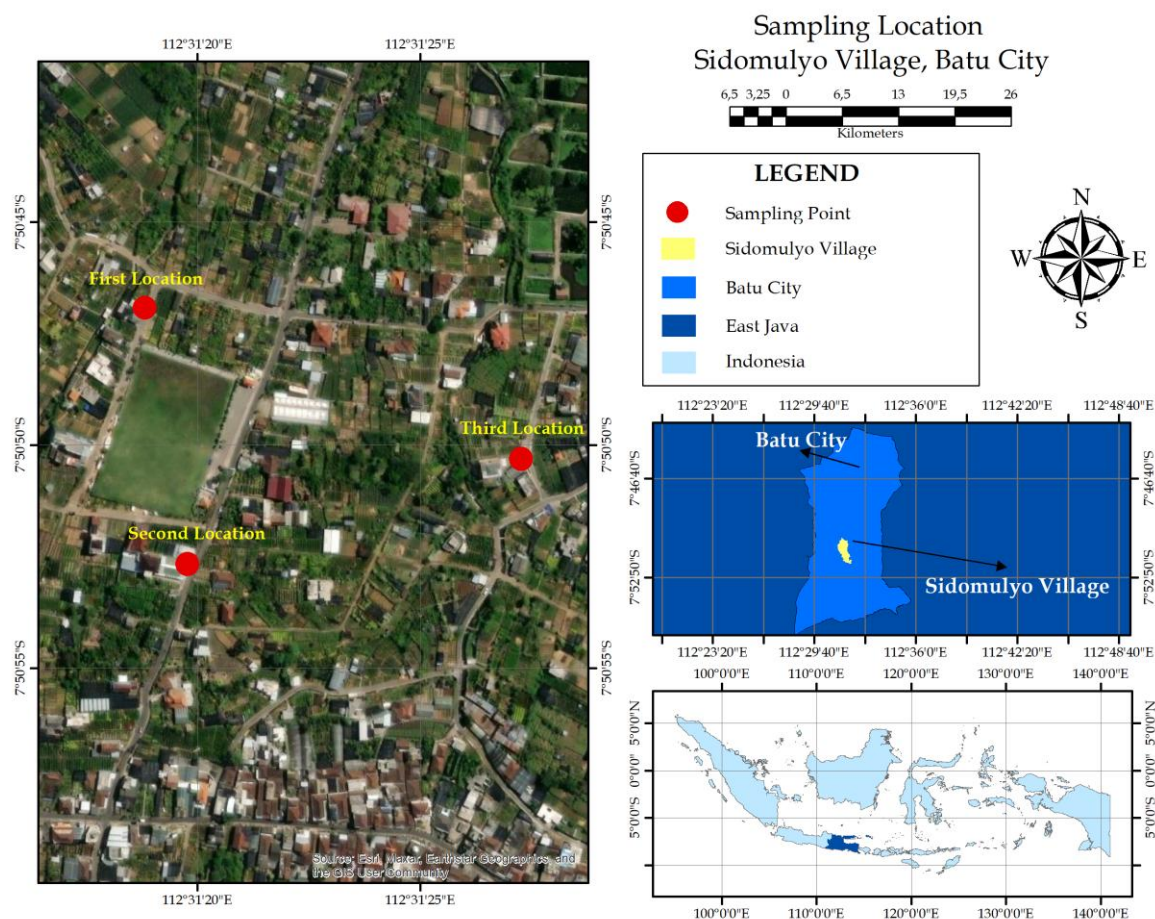


Figure 1. Map of sampling location

Isolation of endophytic fungi was performed using the method described by Hallman *et al.*, (2007) with modifications. All sample pieces were soaked in 70% alcohol solution for 1 minute. The samples were then transferred to 2% sodium hypochlorite (NaOCl) solution for 5 minutes. The samples were further immersed in 70% alcohol solution for 30 seconds. The samples were then rinsed with sterile distilled water 3 times. The last rinse of 1 ml of distilled water was spread over the surface of the PDA medium with a Drigalsky rod. The samples were placed in steril tissue paper inside the LAF (Tandon *et al.*, 2025). After the samples were dry, then each sample of red betel roots, stems, and leaves was cut into 1 cm segments for roots and stems, and 1 x 1 cm square pieces for leaves using a sterile scalpel. The cut samples were placed on the surface of a Petri dish containing PDA + 10% chloramphenicol medium with a total of 3 segments/cup. All isolation procedures were repeated 3 times (triplo) for each sample (a total of 81 segments). Culture incubation was carried out in an incubator at 27-29 °C for 3-14 days. Observations were made daily for 3-14 days to observe the growth of fungal isolates.

Purification of endophytic fungal isolates was carried out by aseptically transferring parts of the endophytic fungal mycelium that grew on the initial PDA medium into new PDA medium using the hyphal tip isolation method, transferring single mycelium to new medium for purification of endophytic fungi (Gupta and Chaturvedi, 2017). The purification results were again incubated in a room incubator for 7 days. Purified fungi were stored in test tubes containing PDA medium for working cultures in a refrigerator at 4°C.

Macroscopic characterization was carried out by observing characters such as color of surface and reverse colonies, surface texture (granular, like flour, mounting, slippery), zonation, growth area, lines of radial, concentric circle, and exudate drop (Demeni *et al.*, 2025). Isolates that have the same characters were grouped into one morphotype group and one is taken as a representative of the species to be identified. Isolates were documented using a mobile phone camera with a black or white background (Barnett and Hunter, 1998). Microscopic characterization was performed by observing endophytic fungal isolates under a light microscope with a magnification of 400x. Mycelium was taken using a sterile inoculation needle, placed on a sterile object glass and dripped with LPCB dye as much as one drop using a pipette and covered with cover glass and observed under a light microscope.

RESULTS

The negative control showed no bacterial or fungal growth, meaning that the sterilization process of the sample surface was successful. Research related to the isolation and identification of endophytic fungi from red betel plants found as many as 37 isolates of endophytic fungi. Endophytic fungal isolates were successfully isolated from all parts of the red betel plant with the number of isolates from each part as presented in Figure 2.

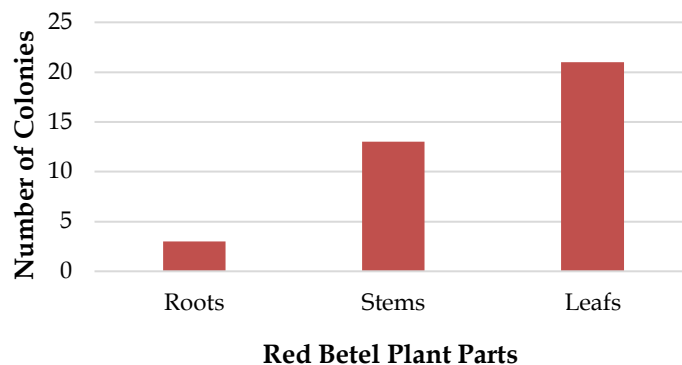


Figure 2. Number of fungal isolates in the root, stem, and leaves parts of red betel plants

Endophytic fungal isolates were grouped into 16 morphotype groups based on culture characters after the purification process and incubation for 3-14 days. Observations were made assuming the endophytic fungal isolates had reached maximum growth on PDA medium (Gandjar *et al.*, 2006). Morphotype data of endophytic fungal isolates based on morphological characteristics are shown in Table 1.

Table 1. Fungal Isolate with Morphotype Group

No	Isolate Code	Morphotype
1	AII2(2), BII2(2), BII3(2), DI1(1), DI3(1), DIII3(1), DII2(2)a, DII2(3), DIII3(3), BII1(1)	MFE1
2	BI1(1), BI2(1), BI2(2), BI3(1)	MFE2
3	AII2(1)	MFE3
4	BIII1(1), BIII1(2)	MFE4
5	DII2(2)	MFE5
6	DIII1(1)	MFE6
7	DIII3(2)	MFE7
8	DII1(2), DII1(2)b, DII1(1)a, DII3(1), DII1(1)b, DII2(1), DII3(3)b, BII2(1)	MFE8
9	AI1(1)	MFE9
10	BIII3(2)	MFE10
11	DII1(3), DII1(1)c	MFE11
12	DII3(2)	MFE12
13	BIII3(1)	MFE13
14	BII3(1)	MFE14
15	DII3(3)	MFE15
16	DI2(1)	MFE16

Note: MFE: Morphotype; A: Root; B: Stem; D: Leaves; I, II, III: Plant Number -; 1, 2, 3: Segmen Number -; (1, 2, 3): Repeat Number -; a, b, c: Isolate Number - in One Segmen

The characteristic observed in each endophytic fungal morphotype were macroscopic morphological data and microscopic data. The macroscopic characters of isolate MFE1 are purplish white upper surface color, yellowish white lower surface color, powdery surface texture, circular shape, raised elevation, filamentous edges, dense density, and there are concentric circles which can be seen in the picture a in Figure 2, Figure 3 and Table 2.

Macroscopically isolate MFE2 has a grayish white upper surface color, black lower surface, cottony surface texture, circular shape, umbonate elevation, filamentous edges, dense density and concentric circles which can be seen in picture b in Figure 2, Figure 3, and Table 2. The macroscopic characters of isolate MFE3 are white in the middle and yellowish at the edges color upper surface, yellowish in the middle and yellowish white at the edges lower surface color, the surface texture is powdery, circular shape, umbonate elevation, filamentous, dense density edges and there are radial lines as seen in picture c in Figure 2, Figure 3 and Table 2.

Macroscopic characters of isolate MFE4 are dark gray upper surface color, black lower surface color, cottony surface texture, circular shape, raised elevation, filamentous edges and dense density as seen in picture d in Figure 2, Figure 3 and Table 2. The macroscopic character of isolate MFE5 are brownish green upper and lower surface color, cottony surface texture, circular shape, raised elevation, filamentous edges, dense density and there are radial lines as seen in picture e in Figure 2, Figure 3 and Table 2.

The macroscopic characters of isolate MFE6 are black with transparent white edges upper and lower surface color, woolly surface texture, irregular shape, flat elevation, lobate edges and medium density as seen in picture f in Figure 2, Figure 3 and Table 2. The macroscopic characters of isolate MFE7 are grayish green with white edges upper surface color, blackish green with white edges lower surface color, powdery surface texture, irregular shape, umbonate elevation, filamentous edges, dense density and there are concentric circles as seen in the picture g in Figure 2, Figure 3 and Table 3.

The macroscopic characters of isolate MFE8 are white upper and surface color, yellowish white lower surface, velvety surface texture, circular to irregular shape, raised elevation, filamentous edges, dense density and concentric circles as seen in picture h in Figure 2, Figure 3 and Table 3. The macroscopic characters of isolate MFE9 are brownish yellow upper and lower surface color, powdery surface texture, irregular shape, raised elevation, filamentous edges and dense density as seen in picture i in Figure 2, Figure 3, and Table 3.

The macroscopic characters of MFE10 are white upper surface color, brownish white lower surface color surface, cottony surface texture, irregular shape, umbonate elevation, filamentous edges, dense density and there is green exudate as seen in picture j in Figure 2, Figure 3 and Table 3. The macroscopic characters of MFE11 are olive in the middle and white at the edges upper and lower surface color, cottony surface texture, circular shape, raised elevation, filamentous edges, dense density and there are concentric circles as seen in picture k in Figure 2, Figure 3, and Table 3.

The macroscopic characters of MFE12 are transparent white with orange exudate upper and lower surface color, cottony surface texture, circular shape, raised elevation, filamentous edges, dense density and concentric circles as seen in picture l in Figure 2, Figure 3 and Table 3. The macroscopic characters of MFE13 are white upper surface color, yellowish in the middle and white at the edges lower surface color, cottony surface texture, irregular shape, raised elevation, filamentous edges and there is orange exudate as seen in the picture m in Figure 2, Figure 3 and Table 4.

The macroscopic characters of MFE14 are grayish white upper surface color, brownish white to brown lower surface color, cottony surface texture, irregular shape, raised elevation, filamentous edges, dense density and there are concentric circles as seen in the picture n in Figure 2, Figure 3 and Table 4. The macroscopic characters of MFE15 are grayish white upper surface color, white with greenish white lines lower surface color, woolly surface texture, irregular shape, elevation raised, filamentous edges, dense density and there are concentric circles as seen in the picture o in Figure 2, Figure 3 and Table 4.

The macroscopic characters of MFE16 are grayish white upper surface color, brown lower surface color, velvety surface texture, circular shape, umbonate elevation, filamentous edges, dense density and concentric circles as seen in the picture p in Figure 2, Figure 3 and Table 4. Documentation of macroscopic and microscopic data of all endophytic fungal morphotypes is shown in Figures 2, 3, and 4.

Microscopic characters of MFE1 are septate hyphae, hyaline colored, smooth-walled and straight conidiophores, hyaline in color, elliptical conidia shape and hyaline in color which can be seen in the picture a in Figure 4 and Table 2. MFE1 identified as *Purpureocillium lilacinum*. The microscopic characters of MFE2 are septate hyphae, hyaline in color, straight conidiophores, smooth-walled, hyaline in color, straight cylindrical conidia shape and hyaline color as in picture b in Figure 4 and Table 2. MFE2 identified as *Corynespora cassicola*.

The microscopic characters of MFE3 are non-septated hyphae, blue in color, straight conidiophores, smooth-walled, blue in color, round conidia shape and hyaline/blue in color as shown in picture c in Figure 4 and Table 2. MFE3 can't be identified. Microscopic characters of MFE4 are septated hyphae, hyaline color, straight conidiophores, smooth walled, dark blue in color, bottle-like phialids, elliptical conidia shape and dark blue in color as in the picture d in Figure 4 and Table 2. MFE4 identified as *Penicillium sp.*

Microscopically MFE5 has the character of septated hyphae, hyaline color, straight conidiophores, smooth walled, hyaline in color, elliptical conidia shape with 3 septate, the 3rd cell of the conidia is wider and browner than the other cells and hyaline to brown color as in the picture e in Figure 4 and Table 2. MFE5 identified as *Curvularia lunata*. Microscopic characters of MFE6 are non-

septate hyphae and hyaline to gray color, branched conidiophores, smooth-walled, dark blue in color, round to elliptical conidia shape and hyaline in color as in picture f in Figure 4 and Table 2. MFE6 identified as *Phyllosticta hostae*.

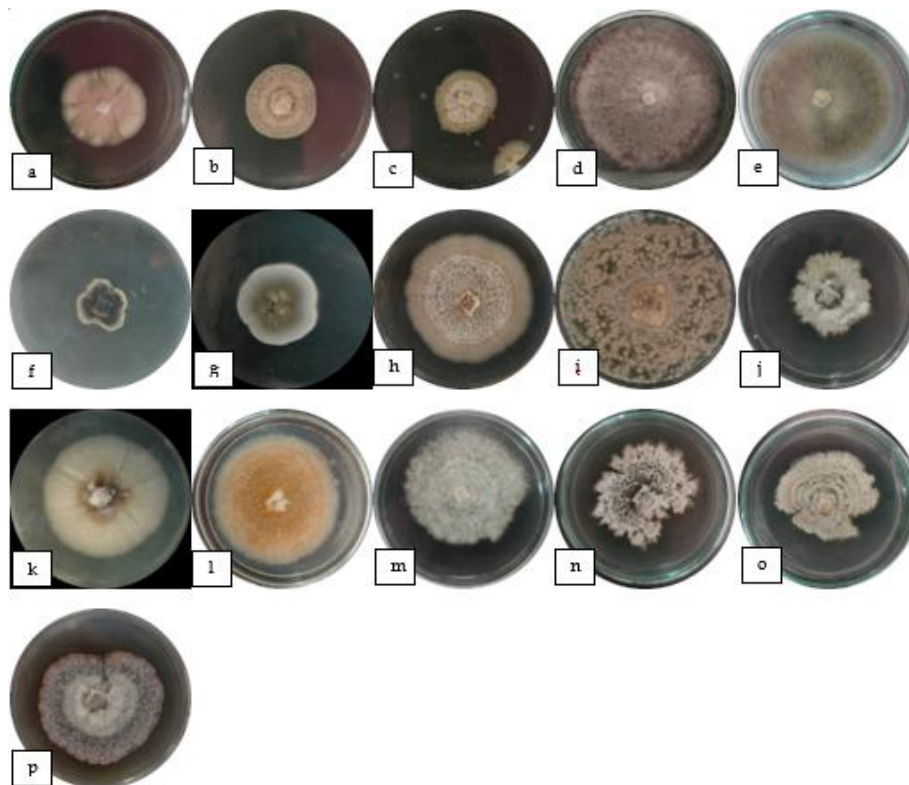


Figure 2. Upper surface of endophytic fungal isolates. a. MFE1; b. MFE2; c. MFE3; d. MFE4; e. MFE5; f. MFE6; g. MFE7; h. MFE8; i. MFE9; j. MFE10; k. MFE11; l. MFE12; m. MFE13; n. MFE14; o. MFE15; p. MFE16

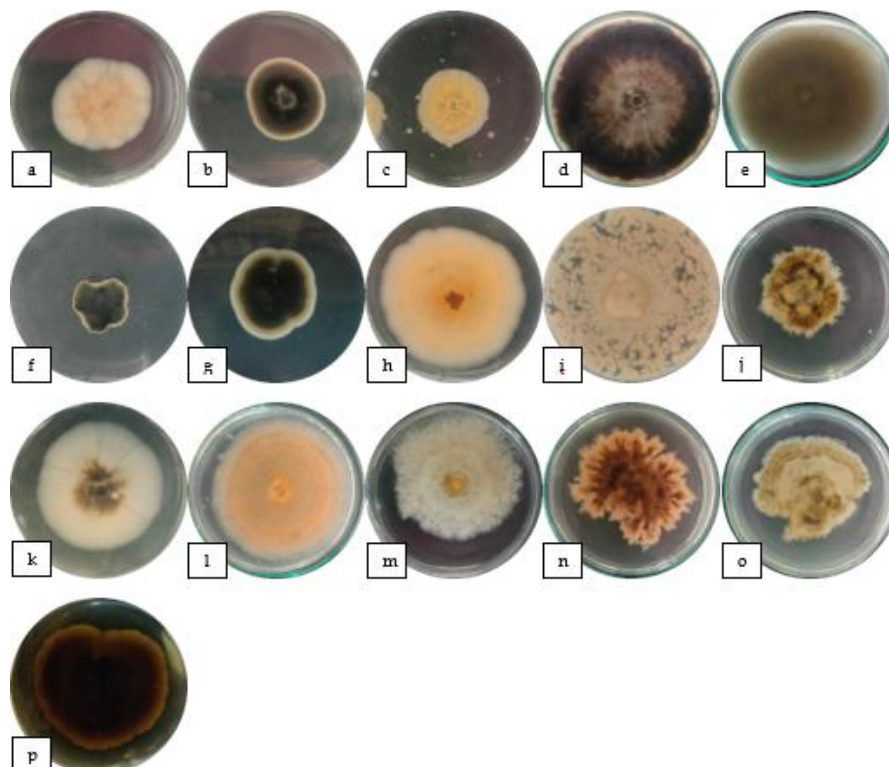


Figure 3. Lower surface of endophytic fungal isolates. a. MFE1; b. MFE2; c. MFE3; d. MFE4; e. MFE5; f. MFE6; g. MFE7; h. MFE8; i. MFE9; j. MFE10; k. MFE11; l. MFE12; m. MFE13; n. MFE14; o. MFE15; p. MFE16

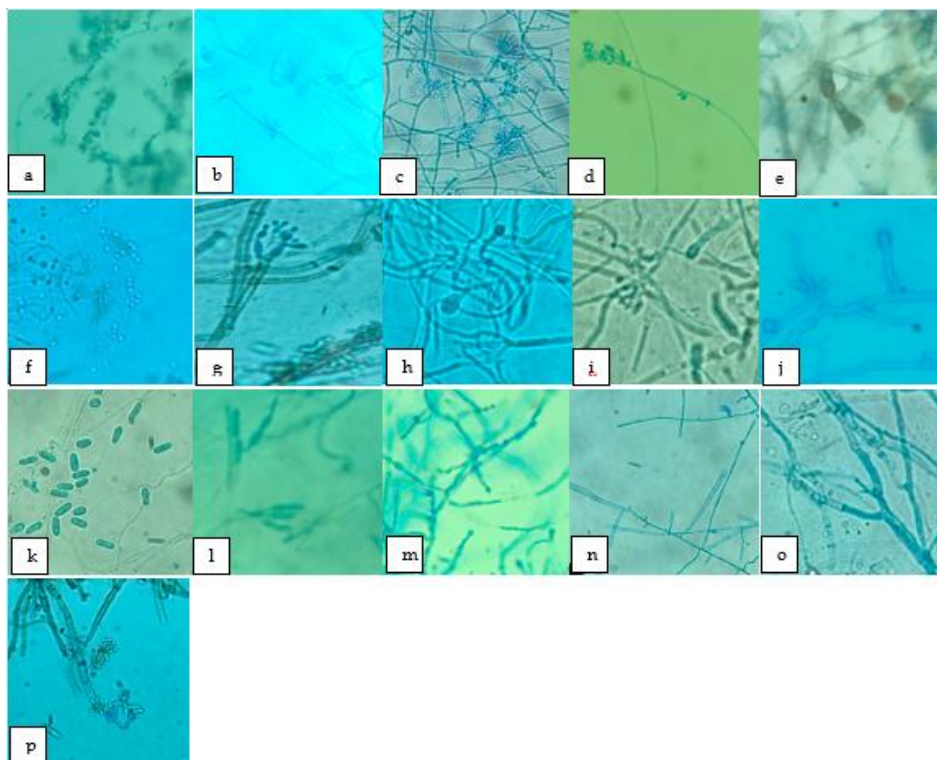


Figure 4. Microscopic morphology under light microscope. a. MFE1; b. MFE2; c. MFE3; d. MFE4; e. MFE5; f. MFE6; g. MFE7; h. MFE8; i. MFE9; j. MFE10; k. MFE11; l. MFE12; m. MFE13; n. MFE14; o. MFE15; p. MFE16

Microscopic characters of MFE7 in the form of hyphae are septate, hyaline color, branched conidiophores, smooth-walled, hyaline color, elliptical to cylindrical conidia, dark blue color and arranged like a chain as in the picture g in Figure 4 and Table 3. MFE7 identified as *Cladosporium cladosporioides*. Microscopically, MFE8 has the character of hyphae that are septated, hyaline color, straight or branched conidiophores, smooth-walled, dark blue color, fusiform or cylindrical conidia shape and hyaline color as in the picture h in Figure 4 and Table 3. MFE8 identified as *Colletotrichum orientale*.

Microscopic characters of MFE9 are septate hyphae, hyaline in color, branched conidiophores, smooth conidiophorous walls, dark blue conidiophores, semi circular to elliptical conidia shape, dark blue conidia color. The phialid is cylindric, narrowed at the end and forms like a neck as shown in picture i in Figure 4 and Table 3. MFE9 identified as *Paecilomyces variotii*. The microscopic characters of MFE10 are septate hyphae, hyaline in color, straight conidiophores, smooth walled as in the picture j in Figure 4 and Table 3. MFE10 can't be identified.

The microscopic characters of MFE11 are septate hyphae, blue in color, straight conidiophores, smooth-walled, hyaline color, cylindrical conidia shape and blue until green color as in picture k in Figure 4 and Table 3. MFE11 identified as *Colletotrichum nymphaeae*. The microscopic characters of MFE12 are non-septate hyphae, blue in color, cells scattered throughout the hyphae, straight conidiophores, smooth walls, blue in color, cylindrical conidia shape and blue color as in picture l in Figure 4 and Table 3. MFE12 identified as *Colletotrichum nuparichola*.

The microscopic characters of MFE13 are septate hyphae, blue in color, straight conidiophores, smooth-walled, blue in color, fusiform conidia shape and blue in color as in the picture m in Figure 4 and Table 4. MFE13 identified as *Fusarium oxysporum*. Microscopically characters of MFE14 are non-septate hyphae, hyaline in color, straight conidiophores, smooth walled, round conidia shape, hyaline in color as in the picture n in Figure 4 and Table 4. MFE14 can't be identified.

The microscopic characters of MFE15 are septate hyphae, blue in color, branched conidiophores, smooth walled, round conidia shape and hyaline color as in the picture o in Figure 4 and Table 4. MFE15 can't be identified. The microscopic characters of MFE16 are non-septate hyphae, hyaline in color, branched conidiophores, rather thick-walled, hyaline conidiophores and round shape as in the picture p in Figure 4 and Table 4. MFE16 identified as *Hypoxylon pulicicidum*.

Each morphotype has different macroscopic and microscopic characters. Macroscopic and microscopic character data of each morphotype of endophytic fungi are presented in Tables 2, 3, and 4.

Table 2. Macroscopic and microscopic characteristics of MFE1-MFE6

Character	Morphotype					
	MFE1	MFE2	MFE3	MFE4	MFE5	MFE6
Macroscopic Characteristics						
Growth rate	Slow	Slow	Slow	Fast	Fast	Slow
Top color	Purplish white	Grayish white	Yellowish white	Dark grey	Brownish green	Black, transparent edge
Bottom color	Yellowish white	Black	Yellowish white	Black	Brownish green	Black, white edge
Surface texture	Powdery	Cottony	Powdery	Cottony	Cottony	Wolly
Form	Circular	Circular	Circular	Circular	Circular	Irregular
Elevation	Raised	Umbonate	Umbonate	Raised	Raised	Flat
Edge	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Lobate
Density	Dense	Dense	Dense	Dense	Dense	Medium
Concentric circles/radia	+	+	-	-	+	+
1 lines						
Medium change	-	-	+	-	-	-
Microscopic Characteristics						
Hyphae	Septate	Septate	Septate	Septate	Septate	Aseptate
Hyphae color	Hyaline	Hyaline	Biru	Hyaline	Hyaline	Hyaline, grey
Conidiophore	Straight	Straight	Straight	Straight	Straight	Branching
Conidiophore wall	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Conidiophore color	Hyaline	Hyaline	Blue	Blue	Hyaline	Blue
Phialidss	-	-	-	Bottle-like	-	-
Conidia shape	Elliptic	Cylinder	Round	Elliptic	Elliptic	Round/ Elliptic
Conidia color	Hyaline	Hyaline	Blue	Blue	White to brown	Hyaline
Identified species	<i>Purpureocillium lilacinum</i>	<i>Corynespora cassiicola</i>	-	<i>Penicillium</i> sp.	<i>Curvularia lunata</i>	<i>Phyllosticta hostae</i>

Note: (+) = available, (-) = not available

Table 3. Macroscopic and Microscopic Characteristics of MFE7-MFE12

Character	Morphotype					
	MFE7	MFE8	MFE9	MFE10	MFE11	MFE12
Macroscopic Characteristics						
Growth rate	Slow	Fast	Slow	Slow	Slow	Fast
Top color	Grayish green, white edge	White	Yellowish brown	White	Olive center white edge	Transparent with orange dot point
Bottom color	Blackish green, white edge	Yellowish white	Brownish yellow	Brownish white	Olive center white edge	Transparent
Surface texture	Powdery	Velvety	Powdery	Cottony	Cottony	Cottony
Shape	Irregular	Circular/irregular	Irregular	Irregular	Circular	Circular
Elevation	Umbonate	Raised	Raised	Umbonate	Raised	Raised
Edge	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous
Density	Dense	Dense	Dense	Dense	Dense	Dense
Concentric circles/radia	+	+	-	-	+	+
1 lines						
Medium change	-	-	-	-	-	-
Microscopic Characteristics						
Hyphae	Septate	Septate	Septate	Septate	Septate	Aseptate
Hyphae color	Hyaline	Hyaline	Hyaline	Hyaline	Blue	Blue
Conidiophore	Straight/branching	Branching	Branching	Straight	Straight	Straight

Character	Morphotype					
	MFE7	MFE8	MFE9	MFE10	MFE11	MFE12
Macroscopic Characteristics						
Conidiophore wall	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Conidiophore color	Hyaline	Blue	Blue	-	Blue	Blue
Phialids	-	-	Cylinder like neck	-	-	-
Conidia shape	Elliptic to cylinder	Fusiform/cylinder	Semi round to round	-	Cylinder	Fusiform
Conidia color	Blue	Hyaline	Blue	-	Blue	Hyaline
Identified species	<i>Cladosporium cladosporioides</i>	<i>Colletotrichum orientale</i>	<i>Paecilomyces variotii</i>	-	<i>Colletotrichum nymphaeae</i>	<i>Colletotrichum nupharicola</i>

Note: (+) = available, (-) = not available

Table 4. Macroscopic and Microscopic Characteristics of MFE13-MFE16

Character	Morphotype			
	MFE13	MFE14	MFE15	MFE16
Macroscopic Characteristics				
Growth rate	Slow	Slow	Slow	Slow
Top color	White	Greyish white	Greyish white	Greyish white
Bottom color	Yellowish center, white edge	Brownies white to brown	White with green lines	Brown
Surface texture	Cottony	Cottony	Wolly	Velvety
Shape	Irregular	Irregular	Irregular	Circular
Elevation	Raised	Raised	Raised	Umbonate
Edge	Filamentous	Filamentous	Filamentous	Filamentous
Density	Dense	Dense	Dense	Dense
Concentric circles/radial lines	-	+	+	+
Medium change	-	-	-	+
Microscopic Characteristics				
Hyphae	Septate	Aseptate	Aseptate	Septate
Hyphae color	Blue	Hyaline	Blue	Hyaline
Conidiophore	Straight	Straight	Branching	Branching
Conidiophore wall	Smooth	Smooth	Smooth	Slightly thick
Phialids	Like bottle	-	-	-
Conidia shape	Cylinder	Round	Round	Round
Conidia color	Blue	Hyaline	Hyaline	Hyaline
Identified species	<i>Fusarium oxysporum</i>	-	-	<i>Hypoxyylon pulicidum</i>

Note: (+) = available, (-) = not available

The diversity index of endophytic fungi in red betel plants can be known using the Shannon-Wiener diversity index formula. Based on the results of the calculation, it shows that in the roots, stems, and leaves, the diversity index value is 1.099; 1.778; 1.818, respectively. Diversity index value data can be seen in Figure 5.

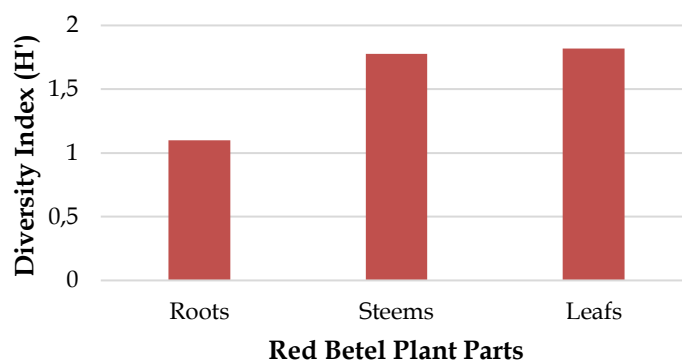


Figure 5. Diversity index of red betel plants endophytic fungi

DISCUSSION

Endophytic fungi that were successfully isolated from various parts of the red betel plant varied in number. Figure 4 shows the highest number of endophytic fungi isolates on the leaves with 21 isolates. The second highest number of isolates was in the stem (13 isolates) and the least in the root (3 isolates). Endophytic fungal isolates were then purified and identified using identification books and other literature sources. The identification results show there were 16 morphotypes where each morphotype is a different species. The following are the results of species identification for each morphotype.

Ten isolates belonging to the group of endophytic fungal isolates with the code MFE1 were identified as *Purpureocillium lilacinum*. This is in accordance with the characteristics mentioned by Sun *et al.*, (2021) and Gandjar *et al.*, (1999), namely purple *P. lilacinum* colonies, dense colony texture, round to elliptical conidia. Microscopically, *P. lilacinum* has the characteristics of concentrated hyphae, hyaline hyphal color, straight conidiophores, smooth conidiophore walls, hyaline conidiophore color, elliptical conidia shape and hyaline conidia color. *P. lilacinum* is commonly found in soil (Gandjar *et al.*, 1999) and according to Lopez *et al.*, (2014) this species is widely detected in plant root tissue. However, the isolation and identification data show that *P. lilacinum* isolates are mostly found in the leaves of plants. According to Christian *et al.*, (2016), this can occur because the colonization of endophytic fungi can be influenced by the characteristics of the host plant and the habitat where the host plant grows. *P. lilacinum* is known for its entomopathogenic properties, which act as a biocontrol agent against a variety of insect pests both living in and above the soil (Goffré and Folgarait, 2015). Lopez *et al.*, (2014) reported in their study that *P. lilacinum* as an endophytic fungus causes adverse effects on the performance of herbivorous insects and is parasitic on some nematodes.

Isolate MFE2 was identified as the species *Corynespora cassiicola*. *C. cassiicola* is characterized by very abundant cotton-like aerial mycelium and a gray upper surface (Toulet *et al.*, 2021). Microscopically, *C. cassiicola* has the characteristics of concentrated hyphae, hyaline hyphal color, straight conidiophores, smooth conidiophore walls, hyaline conidiophore color, straight cylindrical conidia shape, and hyaline conidia color. A total of 4 isolates including MFE2 (*C. cassiicola*) were all found on red betel plant parts. *C. cassiicola* has been found on the roots, stems, leaves, and fruits of more than 300 plants in tropical to subtropical regions (Farr and Rosman, 2024). *C. cassiicola* is a pathogenic fungus to the leaves, stems, roots, fruits and roots of about 300 plant species living in the tropics to subtropics that cause leaves spot disease (Zou *et al.*, 2022). However, there are reports that *C. cassiicola* produces bioactive compounds as an endophytic fungus. According to Toghueo *et al.*, (2017), *C. cassiicola* is able to produce enzymes for industrial purposes such as amylase, cellulase, lipase, and laccase.

MFE4 was identified as *Penicillium* sp. Macroscopically *Penicillium* sp. has the characteristics of rapid growth, dense aerial mycelium, and velvety surface texture Naeimi *et al.*, (2021). Microscopically *Penicillium* sp. has the characteristics of skeletal and hyaline hyphae, branched conidiophores, smooth conidiophore walls, smooth conidia walls, there are Phialidss and metula (Ristiari *et al.*, 2018). There are 2 isolates that include MFE4 and both are found in the stem of the plant. According to Abastabar *et al.*, (2016) and Gandjar *et al.*, (1999), *Penicillium* sp. has been isolated from soil, air, vegetables, and foodstuffs such as flour and fruits. According to Toghueo & Boyom (2019), *Penicillium* species are able to protect plant species against biotic stress, and also able to promote plant growth.

MFE5 was identified as the species *Curvularia lunata*. According to Gandjar *et al.*, (1999), *C. lunata* has the characteristics of dark green colony top surface, olive to white edges, olive to dark green colony back, and cottony surface texture. Microscopically, *C. lunata* is characterized by septate hyphae, elliptical conidia, 3-septate, 3rd cell of conidia is wider and browner than other cells (Gandjar *et al.*, 1999). The isolate identified as *C. lunata* species was only one isolate and was found on the leaves of the plant. *C. lunata* is generally found in the tropics and has been isolated from plants, soil, and air (Gandjar *et al.*, 1999). Research by Nwobodo *et al.*, (2022) reported that *C. lunata* was successfully isolated and identified from healthy leaves of *Elaeis guineensis* plants. *C. lunata* was reported to cause brown spot disease on the leaves of rice plants in Cambodia (Tann and Soyong, 2017). However, although it can cause disease (pathogen), *C. lunata* can also act as an endophyte in healthy plant tissues (Nwobodo *et al.*, 2022). According to Mehta *et al.*, (2022), *C. lunata* extract as an endophytic fungus in *E. guineensis* plants has strong antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. *C. lunata* isolated from marine red algae (*Acanthophora spicifera*) is reported to produce the macrolide secondary metabolite apralactone A which has cytotoxic activity against tumor cells (Mehta *et al.*, (2022). In fact, according to Khan *et al.*, (2023), *C. lunata* strain AR11 is able to increase rice plant growth and reduce stress due to salinity and drought.

MFE6 was identified as *Phyllosticta hostae* species. According to Yi *et al.*, (2015), *P. hostae* has morphological characteristics on PDA medium the color of the upper and reverse surfaces (lower surface) of black colonies, and irregular shape. Microscopically, *P. hostae* has the characteristics of colorless to opaque shiny conidia, subcylindrical to doliform conidiophores, solitary conidia, hyaline in color, septate, smooth-walled, elliptical to obovoid, tapering towards the base (Yi *et al.*, 2015). One isolate identified as *P. hostae* species was found on the leaves of the plant.

One isolate with code MFE7 was identified as *Cladosporium cladosporioides*. According to Gandjar *et al.*, (1999), colonies of *C. cladosporioides* are initially velvety then after a few days become powdery due to dense conidia, the upper surface of the colony is grayish green, the reverse (lower surface) of the colony is blackish green, conidiophores are lateral/terminal in hyphae, conidia shape like a chain. *C. cladosporioides* is commonly found in soil and plant parts (Gandjar *et al.*, 1999). Microscopically, *C. cladosporioides* has the characteristics of elliptical conidia (like lemons), greenish brown in color, chain-like arrangement, and lateral conidiophores on hyphae (Gandjar *et al.*, 1999). *C. cladosporioides* is found on the leavess plant. This species is commonly distributed in soil and on various plants and has been widely isolated from soil and air (Gandjar *et al.*, 1999). *C. cladosporioides* is a pathogenic fungus that causes scab disease on papaya, sooty mold on persimmon, and flower blight on strawberries (Nam *et al.*, 2015). However, in another study, *C. cladosporioides* was reported to be able to increase the resistance of thistle plants to insects by producing toxic chemicals (Tripathi *et al.*, 2022).

MFE8 was identified as *Colletotrichum orientale* species. Macroscopic characteristics of *C. orientale* include dense colony density, cottony surface texture, white upper surface of colonies and aerial mycelium, brownish orange in the middle and white at the edges (Chen *et al.*, 2022). Microscopically, *C. orientale* has the characteristics of hyaline-colored conidiophores, smooth-walled, single or branched, septate, hyaline-colored conidia, smooth-walled, septate, fusiform or cylindrical (Chen *et al.*, 2022). A total of eight isolates were identified as *C. orientale* with details of one isolate found in the stem and seven isolates in the leaves of the plant.

MFE9 was identified as *Paecilomyces variotii*. According to Gandjar *et al.* (1999), *P. variotii* has morphological characteristics in the form of slow colony growth, the color of the upper and reverse surfaces (lower surface) is brownish yellow, and the surface texture is like powder/dry flour (powdery). Microscopically, *P. variotii* has the characteristics of irregularly branched conidiophores, semicircular to elliptical conidia, dark blue conidia color. The Phialids is cylindrical, narrowed at the end and forms like a neck (Rezakhani *et al.*, 2019). One isolate identified as *P. variotii* was found in the roots plant. *P. variotii* is cosmopolite and has been isolated from various substrates such as air, various types of soil, to uncommon substrates such as chemicals (Gandjar *et al.*, 1999). According to Moreno-Gavira *et al.*, (2021), the bioactive metabolites of *P. variotii* have the potential to control diseases caused by pathogenic fungi by reducing their mycelial development. *P. variotii* is also reported to have been widely utilized in China by using the substance ZNC (ZhiNengCong) which is an extract of the endophytic fungus *P. variotii* as a biocontrol agent (Lu *et al.*, 2019). According to Lu *et al.*, (2019), ZNC plays a role in protecting plants from pathogens and also encourages the growth of host plants. ZNC plays a role in promoting plant growth by increasing the absorption of nitrogen (N) and phosphorus (P) and auxin biosynthesis at the root tip (Lu *et al.*, 2019).

MFE11 was identified as *Colletotrichum nymphaeae*. According to Chen *et al.*, (2022), *C. nymphaeae* has the characteristics of white to grayish colonies, cotton-like surface texture, behind (bottom surface) colonies are dark gray in the middle and white at the edges. Microscopically, *C. nymphaeae* has the characteristics of hyphae that are concentrated and branched, hyaline conidiophores, smooth-walled, septate. Conidia are generally cylindrical (Chen *et al.*, 2022). There are two isolates identified as *C. nymphaeae* found in the leaves of plants.

MFE12 was identified as *Colletotrichum nupharicola* species based on its macroscopic and microscopic characteristics. *C. nupharicola* has macroscopic characteristics in the form of transparent white upper and reverse surfaces (lower surface) of colonies with white edges, bright orange aerial mycelium spread in concentric circles (Weir *et al.*, 2012). Isolates identified as *C. nupharicola* were found on the leaves of plants.

Isolate with code MFE13 was identified as *Fusarium oxysporum* species. According to Gandjar *et al.*, (1999), *F. oxysporum* has morphological characteristics such as the upper surface of the colony is white, the reverse (lower surface) of the colony is yellowish in the middle and white at the edges, dense air mycelium, looks like cotton and then like velvet. Microscopically, *F. oxysporum* is characterized by branched/non-branched conidiophores, microconidia 0-2 intercepted, elliptical to cylindrical in shape. Macroconidia are 3-5-septed, fusiform, slightly bent, both ends pointed. Phialidss on short-branched

conidiophores, numerous, various shapes and sizes (Gandjar *et al.*, 1999). The isolate was identified as *F. oxysporum* species found on the stem of the plant. This species is a cosmopolite and soil saprophyte (Gandjar *et al.*, 1999). *F. oxysporum* is commonly known as the cause of fusarium wilt disease, even *F. oxysporum* is included in the top 10 pathogenic fungi in plants in the world (de Lamo and Takken, 2020). The initial symptoms of this disease are indicated by yellowing of the lower leaves of the plant, which then wilts as the plant matures (Ghufron *et al.*, 2017). However, according to de Lamo and Takken (2020), some strains of *F. oxysporum* such as Fo47 and CS-20 actually provide protection for host plants against root pathogens. *F. oxysporum* strain Fo47 has also been identified as a wilt disease suppressant (de Lamo and Takken, 2020).

MFE16 was identified as *Hypoxyton pulicidum* species. Ma *et al.*, (2022) reported that *H. pulicidum* species on PDA medium has the characteristics of white to yellowish colonies, velvety surface texture, entire edges, and dark brown colony color. Microscopically, *H. pulicidum* has the characteristics of conidiophores, 4-level branching, slightly thick conidiophore walls at the base, hyaline conidiophore color to olive brown or grayish brown. Conidia are round to elliptical in shape and white to shiny (Ma *et al.*, 2022). One isolate identified as *H. pulicidum* was found on the leaves of the plant. This species undergoes a life cycle by growing on dead wood and on living plants alternately (Bills *et al.*, 2012). *H. pulicidum* is reported to be a strong producer of antimicrobial VOCs (Volatile Organic Compounds) that actively inhibit several species of fungi (Fadji and Babalola, 2020), thus affecting fungal interactions in host plant tissues (Tomscheck *et al.*, 2010). In addition, the VOCs produced by *H. pulicidum* are utilized in industry, medicine, and energy production to improve agricultural practices (Fadji and Babalola, 2020).

MFE3, MFE10, MFE14 and MFE15 cannot be identified using the macroscopic and microscopic data obtained. There is only one isolate from each morphotype. MFE3 was found in the roots, MFE10 and MFE14 were found in the stems, and MFE15 was found in the leaves.

The value of the diversity index (H') in the roots, stems, and leaves of the plant respectively amounted to 1.099; 1.778; and 1.818. According to Brower and Zar (1997) the diversity index in endophytic fungi in all parts of the red betel plant is classified as a medium category because the value of H' is between $1 < H' < 3$. Based on Figure 5, the largest H' value is found in the leaves and the smallest in the roots of the plant. This is in accordance with what Huang *et al.*, reported (2008) that the tissue parts of the leaves and stems of plants are the most common locations for endophytic fungi. This can occur because it is related to the function of the leaves as the location of the photosynthesis process so that nutrients can support the growth and development of endophytic fungi (Afandhi *et al.*, 2018). According to Afandhi *et al.*, (2018), that in their research showed that mature apple leaves (not too old and too young) have a high number and diversity of endophytic fungi compared to old and young leaves. This is because adult leaves have a higher amount of chlorophyll than old and young leaves, causing a high photosynthesis process in adult leaves. The results of the photosynthesis process are useful for plant growth and these results are also used by endophytic fungi that live in plant tissue (Afandhi *et al.*, 2017). In quantity, the most number of individuals is found in the leaves, which is directly proportional to the value of the diversity index. The value of the diversity index itself is influenced by the number of species (Heri *et al.*, 2020).

Some of the identified endophytic fungi such as *C. lunata*, *C. cladosporioides*, and *F. oxysporum* are commonly known as pathogenic fungi. However, in other studies these fungi are actually endophytic fungi that are associated with their host plants. According to Chauhan *et al.*, (2019), pathogenic fungi will evolve over time to form mutualistic relationships with their host plants.

In the study that was conducted in Summersasi Jember, all endophytic fungi that were successfully isolated three genus including *Curvularia* sp., *Colletotrichum* sp., and *Penicillium* sp. There are similarities but also differences in the results of this study, namely the same three genus were found from all identified species including *Culvularia* sp. (*C. lunata*), *Colletotrichum* sp. (*C. orientalis*, *C. nymphaeae*, *C. nupharicola*), and *Penicillium* sp., while other species are different.

Other species successfully isolated and identified include *P. lilacinum*, *C. cassicola*, *P. hostae*, *C. cladosporioides*, *P. variotii*, *F. oxysporum*, and *H. pulicidum* have never been reported isolated from betel nut plants. This shows that there are differences in the types and numbers of endophytic fungi of red betel plants that live in lowlands and highlands. This difference can occur due to environmental influences such as rain, temperature, and humidity (Materatski *et al.*, 2019). In addition, the composition of the endophytic fungal community in host plants is influenced by biotic and abiotic factors such as plant species, plant tissue, plant chemical content, soil conditions, and plant habitat (Guevara-Araya *et al.*, 2020).

CONCLUSION

A total of 16 morphotypes of endophytic fungi in the roots, stems, and leaves of red betel plants living in the highlands (Sidomulyo Batu Village) with details of 12 morphotypes identified namely *P. lilacinum*, *C. cassicola*, *Penicillium* sp., *C. lunata*, *P. hostae*, *C. cladosporioides*, *C. orientalis*, *P. variotii*, *C. nymphaea*, *C. nupharicola*, *F. oxysporum*, *H. pulicicidum*, and also 4 morphotypes that were not identified were MFE3, MFE10, MFE14, and MFE15. This number is greater both in terms of total individuals and the level of diversity when compared to previous studies. Some of the identified species are also known to not only benefit the host plant related to their role as endophytic microorganisms, but also have benefits in the industrial, and medical fields.

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CONFLICT OF INTEREST

There is no conflict of interest.

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