International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

Vol. 35 No. 2 November 2022, pp. 260-268

Isolation And Identification Of Endophytic Fungi In Mangrove Plant Nypa Fruticans From Tanjung Jabung Timur, Jambi

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Abstract – *Nypa fruticans* is a very important mangrove plant especially for coastal communities. It can be used as an alternative medicine. The research was conducted in several steps, there is isolation, identification, and activity test of endophytic fungi. Isolation of endophytic fungi includes medium preparation, plant sampling, isolation of endophytic fungi from some parts of *N. fruticans* organs. The identification stage includes purification, characterization, and identification of endophytic fungi. Endophytic isolation obtained 7 isolates are *Fusarium,* 3 isolates are *Aspergillus*, 2 isolates are *Acremonium*, 2 isolates are *Colletotrichum*, 2 isolates are *Scopulariopsis,* 2 isolates are *Gliocladium,* 1 isolate is *Trichocladium*, and 1 isolate is a *Geotrichum.*

Keywords – Macroscopic; Microscopic; Environmental factors; *Fusarium; Aspergillus; Scopulariopsis.*

I. INTRODUCTION

Nipah or Nypa fruticans is a member of the Palmae tribe that grows along tidal rivers, and the plant is also densely populated in mangrove forest ecosystems. Tanjung Jabung Timur, Jambi has a large mangrove forest, it is about 563 ha [1]. In some coastal areas in Indonesia, *N. fruticans* has been used as traditional medicine such as toothache [3], canker sores [2], cough [2], coral reefs [2], liver disease [4], tuberculosis [4], sore throat [4] and others.

Based on previous research, it is known that *N. fruticans* plant has bioactive abilities, there are antidiabetic [5], antioxidant [6], and antibacterial [7]. This shows that the *N. fruticans* plant has certain bioactive compounds, these bioactive compounds are known as secondary metabolites [8]. It is abundantly produced not only in plants, but also in microbial communities especially fungi. Endophytic fungi are live in plant tissue and able to form a colony in plant tissue without giving negative effects to the host. utilization of endophytes is more potential because endophytic microbes can produce bioactive compounds that are almost similar or even greater than their host. It means endophytic organisms have enormous potential to be exploited and produce new natural products that are useful as medicine, agriculture and industry.

The presence of endophytic fungal populations varies in each plant with the same or different species. Cruz *et al.*. [9] reported that 12 isolates of endophytes are isolated in *N. fruticans* from Bulacan, Phillipines. Pilantanapak *et al.*. [10] collected 81 species on Nypa in Thailand, while Hyde and Sarma [11] documented 46 species from the Tutong River in Brunei. But, isolation and

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identification endophytic fungi from *Nypa fruticans* in Indonesia doesn't done yet. Based on this background, the research will be conducted on Isolation and Identification of Endophytic Fungi in Mangrove *Nypa fruticans* from Tanjung Jabung Timur, Jambi.

II. RESEARCH METHODS

2.1. Sample Collection

Leaf, fruit, root and stem samples of Nypa fruticans were collected from Nipah Panjang, Tanjung Jabung Timur, Jambi. Stem and roots were collected from healthy parts. Meanwhile, leaf collection were collected from middle leaflets [12] and fruits were collected from ripe fruit [13]

2.2. Isolation of N. *fruticans* Endophytic Fungi

Surface sterilization were used before isolation. Samples of leaves, stems, fruits, and roots were washed in running water. Then the surface was sterilized by putting it in 70% alcohol for 1 minute, 5.25% sodium hypochlorite for 5 minutes, 70% alcohol for 30 seconds, and rinsing with sterile distilled water 3 times. Then the sample was cut to a size of 1 × 1 cm2 using a sterile knife [14]. The sterile sample were grown on Potato Dextrose Agar (PDA) media and incubated at 27 °C-29 °C for ± 7 days until fungal hyphae grew on the media. then, Growing mushrooms were purified on new PDA. Colonies that has a different shapes are characterized and identified.

2.3. Characterization and Identification of *N. fruticans* Endophytic Fungi

Endophytic fungus *N. fruticans* which has antibacterial activity was characterized macroscopically by observing colony shape, colony color, upper and lower surface color, and colony size. Microscopic characterization using a binocular microscope to observe fungal morphology based on hyphae, mycelium, conidia shape, spore shape, and spore color. Microscopic identification can be done using the Slide Culture method.

2.4. Data Analysis

The species of endophytic fungi were analyzed using a fungal identification book. Morphological characterization (macroscopic and microscopic) of endophytic fungi isolated from *N. fruticans* was compared with fungal morphology in the corresponding literature.

III. RESULTS AND DISCUSSION

3.1. Isolation of *N. fruticans* Endophytic Fungi

The endophytic fungi in this study were isolated from leaves, fruits, stems, and roots of the plant *N. fruticans*. An amount of 20 isolates of endophytic fungi have been obtained. The leaves had 7 isolates, followed by 6 isolates from fruit, 4 isolates from roots, and 3 isolates from the stem (Fig. 1).

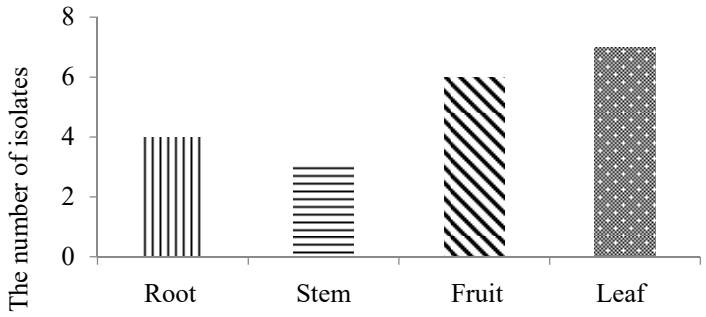

Fig. 1. The number of isolates found in various plant organs of *N. fruticans*

In this study, leave is part of the plant that has the highest number of isolates compared to fruit, roots, and stems. This is caused by differences in the amount of nutrient content and secondary metabolites found in these plant organs. Leaves have a higher amount of nutrients and secondary metabolites because they are the center of chemical reactions known as metabolism. Photosynthesis is a primary metabolism that only occurs in leaves. Primary metabolism will produce primary metabolites

(carbohydrates, proteins, fats, and nucleic acids) which have a very important role as nutrients in plant growth, and endophytic microbes also as natural ingredients for the formation of secondary metabolite compounds from secondary metabolic reactions [15]. So a large number of primary metabolites allows the leaves to have a higher number of secondary metabolites. This is evident in the phytochemical tests conducted by Lovly and Teresa [16], the results showed that nipah leaves contain many secondary metabolites including alkaloids, flavonoids, tannins, sterols, and triterpenoids, glycosides, and saponins.

The fruit is the part of the plant that has the highest number of isolates after the leaves. This is because nipah fruit is rich in nutrients contained in the sap or liquid sugar contained in the fruit. Previous research said that nipah fruit, especially ripe fruit contains high carbohydrates, protein, fat, and phenolics [17]. These compounds are known to be nutrients that are needed for endophytic fungi. In contrast to leaves and fruit, roots and stems had the lowest number of isolates. This is because these parts are the parts closest to the soil surface so that they are easily invaded by rhizosphere microorganisms. In addition, it can be caused by nutrients or primary metabolites that are not produced as much as other organs.

Based on the data in Fig. 1, the number of endophytes obtained in this study was less than the previous study, which was 38 isolates of endophytic fungi [18]. This difference is caused by edaphic and climatic factors. The edaphic factor or soil acts as a source of nutrients for mangrove plants and endophytic fungi in their tissues. This is following to Schulz *et al.*. [19] that the diversity of endophytic fungi in plants is influenced by several factors including geographical location, climate or season, and edaphic. Climate or seasons affect the physical and chemical factors of the environment.

Table I. Measurement Of Physical And Chemical Environmental Factors

Environmental Factors	Value
Soil Temperature	31°C
Air Temperature	31.2°C
Soil pH	6.8
Air Humidity	42%
Salinity	1 ppm

The air temperature and soil temperature (TABLE I) were obtained when sampling was relatively high and the air humidity was relatively low. Higher ambient temperatures and lower humidity make it more difficult for fungi to sporulate. From the measurement results, the degree of acidity value is 6.8, which indicates that it is still possible to colonize endophytic fungi. Based on Adejumo and Orole [20], explained that the optimum fungal colonization is in the pH range 7-8. Mangroves are influenced by salinity and from the measurements obtained a salinity value of 1 ppm. Salinity is a stress that makes endophytes need the ability to overcome stress so that it will affect the number of endophytes that can survive. In the research of Pirhadi *et al.*. [21], it was found that lower endophytic diversity was found in sugarcane plants that live in saline soils.

3.2. Characterization and Identification of *N. fruticans* Endophytic Fungi

Based on the results of the study, endophytic fungi isolates showed quite diverse macroscopic morphological characteristics based on the color of the fungal colonies on the upper and lower surfaces, margins, patterns, and colony textures (TABLE II and fig. 2).

Table II. Macroscopic Characterization Of Endophytic Fungi Isolates From *Nypa Fruticans*

Isolate Code	Characterization				
	Upper Color	Lower Color	Texture	Margine	Pattern
AR01	White to yellow	White to yellow	Cotton	Plain	Spread
AR02	White	white	Cotton	Plain	Spread
AR11	White	white	Cotton	Plain	Spread
AR12	White to grey	dark	Cotton	Plain	Spread
BH05	White to grey	White to grey	Cotton	Plain	Spread

BH06	White	white	Cotton	Plain	Spread
BH07	White	white	Cotton	Plain	Spread
BH11	White	white	Cotton	Plain	Spread
BH12	White	white	Cotton	Irregular	Like Root
BH13	Green	Green	Velvety	Plain	Spread
BT01	White	white	Cotton	Plain	Spread
BT02	Green	Green	Cotton	Plain	Spread
BT03	White	white	Cotton	Plain	Spread
DN11	Dark Green	Dark Green	Cotton	Plain	Spread
DN12	White	white	Cotton	Plain	Spread
DN13	White to grey	Dark	Cotton	Plain	Spread
DN14	White to yellow	White to yellow	Cotton	Plain	Spread
DN15	White to yellow	White to yellow	Cotton	Plain	Spread
DN16	Dark Green	Dark Green	Velvety	Plain	Spread
DN17	White to Brown	Dark	Cotton	Plain	Spread

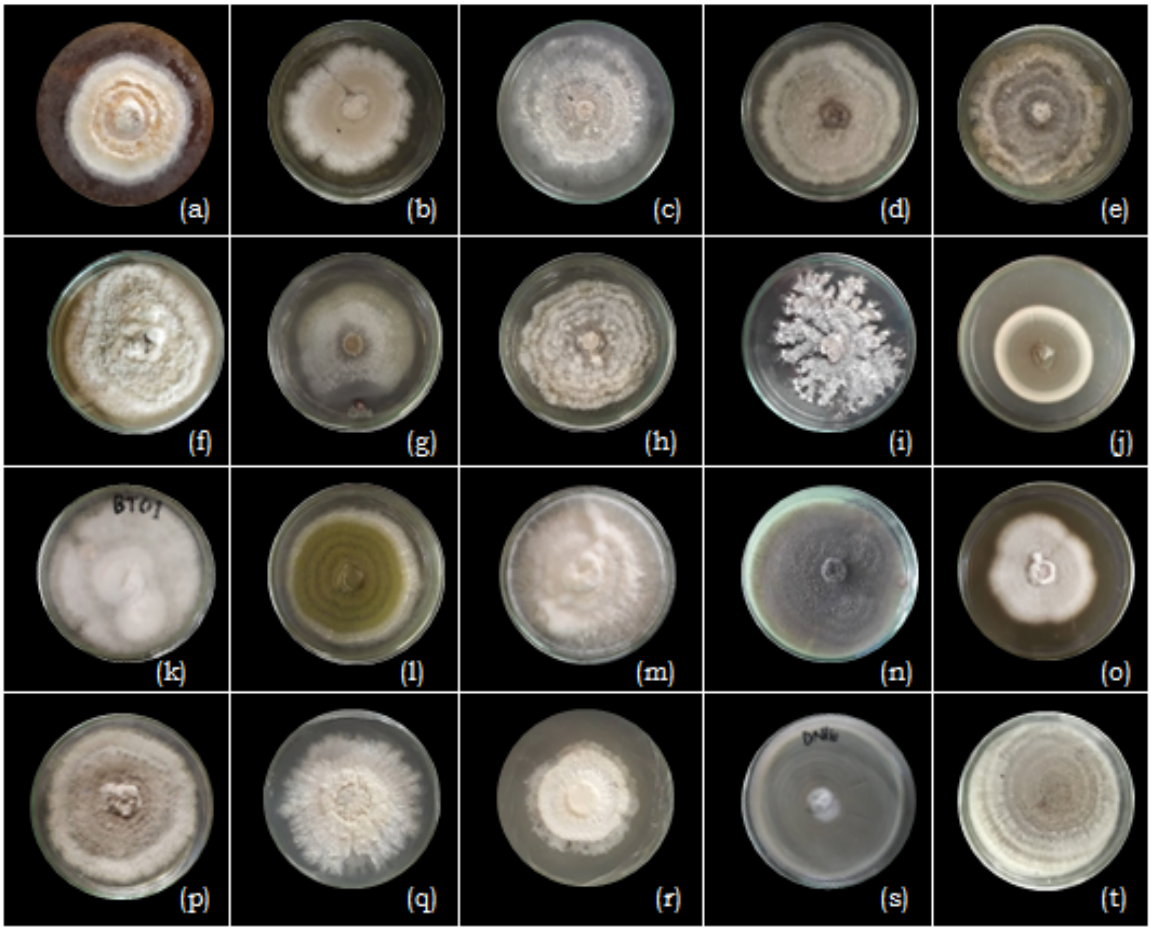

Fig. 2. Endophytic Fungi Colony

Notes: a.) AR01, b). AR02, c).AR11, d). AR12, e). BH05, f).BH06, g).BH07, h).BH11, i).BH12, j). BH13, k).BT01, l).BT02, m).BT03, n).DN11, o).DN12, p).DN13, q).DN14, r).DN15, s).DN16, t).DN17 (Personal Documentation, 2018).

Isolates AR02, AR11, BH06, BH07, BT01, BT03, and DN12 indicated that these seven isolates were *Fusarium* sp. It is characterized by the presence of 3 types of spores observed including macroconidia, microconidia, and chlamydospores (Fig. 3). According to Nelson *et al.* [22], these three types of spores are specific characteristics of *Fusarium*. *Fusarium* also has a false head that functions to produce microconidia. Microconidia are single cells or spores with single septae that are ovoid. While macroconidia are long spores with curved ends like sickles and have 3-5 septa [23].

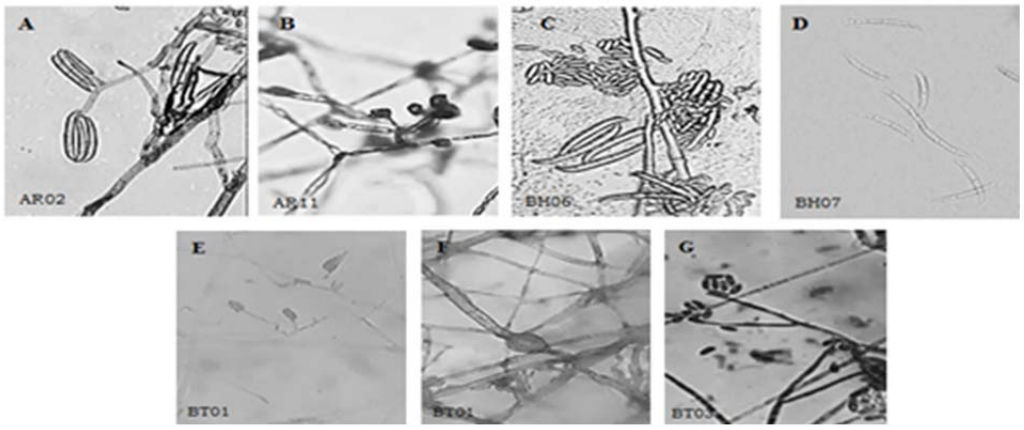

Fig. 3. Microscopic Observation of *Fusarium*

Notes*:* A. Macroconida, B. Falsehead, C. Macroconidia and Microconidia, D. Macroconidia, E. Falsehead, F. Chlamydospore, and E. Conidiospore

Isolates BT02, DN11, and DN17 indicated that these three isolates were *Aspergillus* sp. On macroscopic observation, BT02 colonies were light green, DN11 was dark green, while DN17 isolates were brownish white. According to Gautam and Bhadauria [24], the colony color of *Aspergillus* sp. can be dark brown to black, greenish-yellow, green to dark green, and reddish which will turn black with an increasing incubation period. On microscopic observation, these three isolates had insulated and branched hyphae did not have sexual carpus (fruiting bodies), but had asexual carpus, namely conidia in their hyphae. The type of conidia observed was Phialospore-type (Fig. 4). Phialospore-type is a type where at the end of the conidiophores there are expanding vesicles and phyalids that form conidia [25].

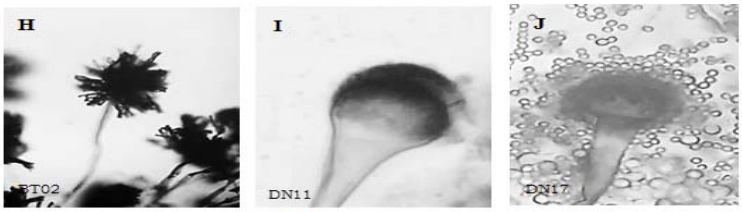

Fig. 4. Microscopic Observation of *Aspergillus*

Notes: H. Conidiospore, I-J. Vesicle.

The isolates AR01 and BH11 showed that these isolates were *Acremonium* sp. The characteristics of the isolated colonies were yellowish-white and white. This is by following per under research conducted by Agustina [26], that *Acremonium* has white colonies. Microscopically isolates AR01 and BH11 had insulated hyphae, erect conidiophores, some branched, and some single (Fig. 5). The cylindrical conidia are arranged in a circle and are colorless. According to Watanabe [25], these characteristics have similarities with the characteristics of *Acremonium* sp. i.e. hyaline conidiophores, erect, simple, or branched, gradually tapering from base to apex, with a mass of terminal spores. Hyaline conidia can be cylindrical, elongated elliptical, and round.

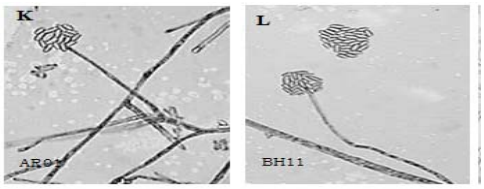

Fig. 5. Microscopic Observation of *Acremonium*

Notes: K-L. Conidiospore

The isolates AR12 and DN13 were *Colletotrichum* sp. Both of these isolates were grayish-white. According to Rangkuti *et al.* [27], Colletotrichum has cream, gray, blackish gray to dark green colonies. On microscopic observation, it was known that the hyphae in this isolate were insulated and had asexual carpus with Sporodochium type. Sporodochium is asexual carpus in the form of thick pads [23]. These isolates have an appresorium (Fig.6) that is used to attach to other organisms and is produced from the development of hyphal branch tips [28].

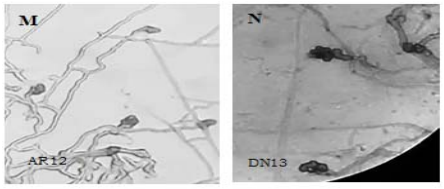

Fig. 6. Microscopic Observation of *Colletotrichum*
Notes: M-N. Appresorium

BH13 and DN16 isolates were *Scopulariopsis* sp. These isolates had green colonies with white margins. by following Woudenberg *et al.* [29], that *Scopulariopsis* has white to green colonies with white edges. Microscopically this isolate was characterized by an annellospore type of spore (Fig. 7), namely conidiophores carrying conidia that grew on Annelid annelids in *Scopulariopsis* shaped like bowling pins [30]. Based on Watanabe [25], it has branched conidiophores, conidia in isolates are orange-brown, round, and spiny.

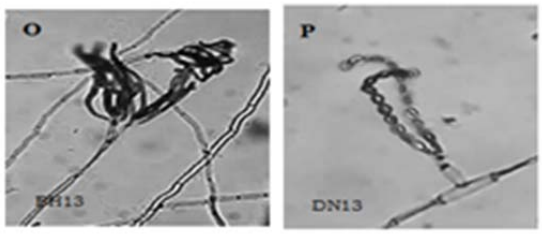

Fig. 7. Microscopic Observation of *Scopulariopsis*

Notes: O-P. Conidiospore

The isolates DN14 and DN15 were *Gliocladium* sp. These isolates had yellowish-white colonies. Following to Watanabe [25] that *Gliocladium* has a pale yellow colony color, grayish-green with pink to dark green color. Microscopically this isolate has metula and phialides which at the ends hold the spore mass or conidia collection (Fig. 8). In addition, Gliocladium has conidiophores that are hyaline, single metula or branched 1 to 3 vertically or opposite to elliptical and hyaline or green conidia [25].

BH05 isolate is *Trichocladium* sp. This isolate had grayish-white colonies. This isolate had insulated hyphae, straight conidiophores, and had aleurospora conidia type. This is following to Park *et al.*, [31] that the characteristics of the genus *Trichocladium* are colonies like Cotton, the color of the colony is initially white then becomes gray, conidia are solitary and dry. The type of conidia of the genus Trichocladium is Aleurospora (Fig. 8), which is conidia in the terminal part, the spores are formed by the top of the conidiophores and are limited by the septum in the early stages.

BH12 isolate was *Geotrichum* sp. This isolate had white colonies and conidia with arthrospore type. Arthrospores are spores or conidia formed by the detachment of the compartments of hyphae from the mycelium into segments (Fig. 8) that function as

asexual spores [23]. Following to Watanabe [25] that *Geotrichum* has a white colony color. Conidia are cylindrical in shape and consist of 1 cell and hyaline

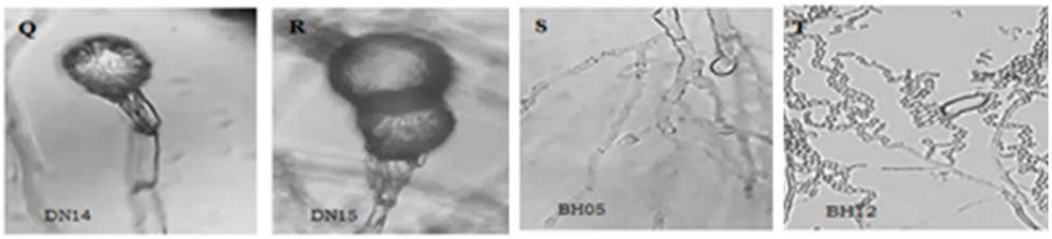

Fig. 8. Microscopic Observation of *Scopulariopsis*, *Trichocladium* and *Geotrichum*

Notes: O-P. Conidiospore *Scopulariopsis*, S. Aleurospore *Trichocladium*and T. Hiphae

In this study, from 20 isolates, there were 8 genera found. That are 7 isolates from *Fusarium*, 3 isolates from *Aspergillus*, 2 isolates from *Acremonium*, 2 isolates from *Colletotrichum*, 2 isolates from *Scopulariopsis*, 2 isolates from *Gliocladium*, 1 isolates *Trichocladium*, and 1 isolate from *Geotrichum* (TABLE III).

Table III. Identification Results Of Endophytic Fungus From *Nypa Fruticans*

Plant Organs	Isolate Code	Endophytic Fungi	Taxa
Root	AR01	*Acremonium* sp.	Ascomycota
	AR02	*Fusarium* sp.	Ascomycota
	AR11	*Fusarium* sp.	Ascomycota
	AR12	*Colletotrichum* sp	Ascomycota
Fruit	BH05	*Trichocladium* sp.	Ascomycota
	BH06	*Fusarium* sp.	Ascomycota
	BH07	*Fusarium* sp.	Ascomycota
	BH11	*Acremonium* sp.	Ascomycota
	BH12	*Geotrichum* sp.	Ascomycota
	BH13	*Scopulariopsis* sp.	Ascomycota
Stem	BT01	*Fusarium* sp.	Ascomycota
	BT02	*Aspergillus* sp.	Ascomycota
	BT03	*Fusarium* sp.	Ascomycota
Leave	DN11	*Aspergillus sp.*	Ascomycota
	DN12	*Fusarium* sp.	Ascomycota
	DN13	*Colletotrichum* sp.	Ascomycota
	DN14	*Gliocladium* sp.	Ascomycota
	DN15	*Gliocladium* sp.	Ascomycota
	DN16	*Scopulariopsis* sp.	Ascomycota
	DN17	*Aspergillus* sp.	Ascomycota

IV. CONCLUSIONS

Based on the research that has been done, the following conclusions can be drawn that a total of 20 isolates of endophytic fungi were obtained from the *Nypa fruticans* plant, There are 7 isolates of the genus *Fusarium*, 3 isolates of the genus *Aspergillus*, 2 isolates of the genus *Acremonium*, 2 isolates of the genus *Scopulariopsis*, 2 isolates of the genus *Gliocladium*, 2 isolates of the genus *Colletotrichum*, 2 isolates of the genus *Trichocladium*, and 1 isolate of the genus *Geotrichum*.

ACKNOWLEDGMENT

My deepest appreciation goes to my beloved parents for the endless love, pray, and support. Also this thesis would not have been possible without the help, support and patience of my first advisor, Dr. Madyawati Latief for her supervision, advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the past few years. Then to Hasnaul Maritsa, M.Sc. as my second advisor who has helped him patiently finishing this undergraduate thesis by giving suggestion, guidance, and correction until the completion of this research.

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