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Exploration and Identification of Endophytic Molds on Leaves and Stem of the Mango's Mistletoe (*Dendrophthoe Pentandra* (L.) Miq)

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ABSTRACT

Mango's mistletoe is one of the herbal plants' rich in bioactivity. Secondary metabolites are not only produced by plants but also by microorganisms that live in tissues. One of these microorganisms is endophytic mold. The ability of endophytic molds to synthesize secondary metabolites is an opportunity for large-scale production in a short time without causing the exploitation of natural materials. This research aimed to explore and identify endophytic molds from the leaves and stem of the mango mistletoe to obtain the genus of molds. The stages of this research consisted of isolation by direct planting with sterilization, purification, growth rate measurement, characterization, and identification carried out macroscopically and microscopically. DBM 1 and DBM 2 belong to *Aspergillus* sp., DBM 3 belongs to *Cladosporium* sp., DBM 4 belongs to *Neurospora* sp., TDBM 1, TDBM 2, and TDBM 3 belong to *Hormiscium* sp. The growth rate of *Aspergillus* sp. relatively fast, with the increase in diameter of *Aspergillus* sp.1 colony from 2.45 cm to 5.05 cm and that of *Aspergillus* sp.2 from 2.73 cm to 5.35 cm. In the *Cladosporium* sp., there was an exponential phase with an increase in diameter from 2.15 cm to 4.65 cm. In *Neurospora* sp., there was an exponential phase with an increase in diameter from 0.63 cm to 3.65 cm. The growth rate of *Hormiscium* sp. is quite fast, with an exponential phase with an increase in the diameter of the colonies of *Hormiscium* sp.1 from 2.63 cm to 7.21 cm, *Hormiscium* sp.2 from 2.45 cm to 6.94 cm and *Hormiscium* sp.3 from 2.85 cm to 7.85 cm.

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INTRODUCTION

Plants are very important natural resources, especially in term of the potential of plants that can be used as herbal plants so that they can be used as a form of effort to maintain human health. Until now, it has been known that many herbs used by humans around the world come from active compounds isolated and developed from plants (Radji, 2005). One plant that has potential as an herb is parasite plants, which belongs to the Loranthaceae family. One type of plant from the Loranthaceae family is the mango's mistletoe (*Dendrophthoe pentandra* (L.) Miq) which has been known to be used as a traditional herb because it contains active compounds such as flavonoids, tannins, amino acids, carbohydrates, alkaloids, and saponins. Mango's mistletoe has antiplasmodial activity, which has the potential to be an anti-cancer agent (Wicaksono, 2013). Mango's mistletoes which have the potential as anti-cancer, have the main content, namely flavonoids that are quercetin compounds, so that they can be developed into phytopharmaca products (Oktaviana et al., 2021).

Every plant has one or more microorganisms, such as fungi and bacteria, that live in it (Rante et al., 2013). The fungi that live in plant tissues intracellularly are endophytes. These fungi can induce their hosts to produce secondary metabolites (Sia et al., 2013). Derived from a combination of microorganisms in plant tissues, namely endophytic fungi, plants have pharmacological potential (Murdiyah., 2017). Endophytic fungi can synthesize

secondary metabolites. Secondary metabolites that can be produced from endophytic fungi such as alkaloids, flavonoids, steroids, terpenoids, and others (Molina et al., 2012). The existence of endophytic fungi that can produce secondary metabolites has an interrelated relationship, so there are opportunities for secondary metabolites to be developed and produced in large quantities without damaging the ecological environment.

Isolation of bioactive compounds that are carried out directly requires a lot of biomasses from these plants. So that it can result in a reduced presence of plants if taken and used continuously. Regarding natural resources, especially plants that have limited potential as herbal ingredients, they will run out if they are not preserved or used in a sustainable manner. Therefore, efforts are needed to keep plants, one of which is the mango's mistletoe (*Dendrophthoe pentandra* (L.) Miq) which is preserved even though it is used or will be produced as herbal ingredients. Exploration and identification of endophytic molds derived from the mango's mistletoe plant (*Dendrophthoe pentandra* (L.) Miq) are urgently needed. With the discovery of endophytic molds, it will be easier to determine the potential for bioactive compounds from secondary metabolites contained in endophytic molds. The research aimed to obtain endophytic molds from the leaves and stem of the mango's mistletoe by isolating, characterizing, and identifying them, which it is hoped will allow it to determine the genus of endophytic molds.

MATERIALS AND METHODS

Identification of mango's mistletoe endophytic molds is a type of exploratory research. The research was conducted by isolating and characterization endophytic molds from leaf and stem samples of the mango's mistletoe (*Dendropthoe petandra* (L.) Miq). The research was carried out at the University of Islam Malang Microbiology Laboratory and from October 2021 until April 2022.

Endophytic Molds Isolation

The isolation method-based citations of Kasi (2015) and Suhartina (2018) with modification is usedthe direct planting method. First, the sample was washed using distilled water, then placed on a tissue until the water content was reduced, then put into a 70% alcohol solution for 1 minute, then put into a 10% NaOCl (Sodium hypochlorite) for 3 minutes, then put into a 70% alcohol solution for 1 minute. After that, it was rinsed by dipping it into sterile distilled water three times for 30 seconds each. The plant samples were dried on the sterile tissue on the petri dish, then cut into pieces of about 1 cm. Samples of leaves and stems of mango's mistletoe that had been cut were placed on the surface of PDA (Potato Dextrose Agar) media that had been added with antibacterial in a Petri dish. The isolation process was carried out in an aseptic LAF (Laminar Air Flow). After the isolation process, incubated for 2-14 days in an incubator at 30°C.

Endophytic Molds Purification

The endophytic molds grown on PDA (Potato Dextrose Agar) media were gradually purified based on Kasi (2015) and Suhartina (2018). Each fungal colony that had grown and was considered a different isolate based on its macroscopic morphological appearance was then purified by cutting the fungal colony section to a size of about 1 cm using a sterile round tip ose needle and then placing it on new PDA media. The purified mold was then incubated in an incubator for 2-14 days at 30°C. Observations were made every day during the incubation process to see the growth of molds.

Growth Rate of Endophytic Molds Diameter

The diameter measurement is done by drawing two perpendicular lines at the bottom of the petri dish (Figure 1). The point of intersection is right at the center of the growth of the molds. The growth rate was measured using a caliper and recorded daily from the edge of the initial inoculum to the area of the growth margin of the molds. The value of the colony diameter was obtained from the average measurement of the increase in the development of the mold area from the horizontal and vertical sides. An Observation of the rate of increase in the diameter of the mold can be used with the equation (Sitanggang, 2016).

Characterization and Identification of Endophytic Molds

The characterization of endophytic mold isolates was carried out macroscopically and microscopically. Macroscopic characterization was carried out directly, including the color of the surface of colony, the color of the reverse of colony, surface texture of the colony, drops of exudate on the colony, zone growth, zoning, radial furrows, concentric circles on the Petri dish and the diameter

of the colony. Microscopic characterization was carried out using the slide culture method. Microscopic observations were carried out using lactophenol cotton blue and observed using a microscope.

$$D = \frac{d1 + d2}{2}$$

Note:
d1: horizontal diameter of the endophytic mold
d2: vertical diameter of the endophytic mold

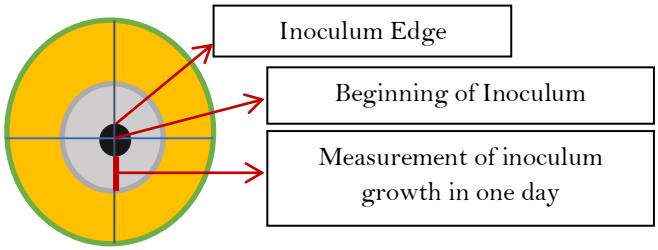

Figure 1. Mold Diameter Measurement Technique (Miyashira, 2010)

Endophytic mold characterization was carried out macroscopically and microscopically. The macroscopic characterization observed the morphology of the mold on the surface side and reserve side, including texture, zoning, growing area, radial furrows, drops of exudate, color and the lower surface of the colony called the reserve of colony, as well as the color and the upper surface of the colony which is usually called the surface of the colony. colony. Microscopic characterization of endophytic fungi isolates was carried out using the slide culture method. The microscopic characteristics of endophytic fungi observed were the shape of the conidial head, hyphae and other special structures. Microscopic characteristics were carried out based on the monograph of Pengenalan Kapang Tropik Umum (Gandjar et al., 1999) and Illustrated Genera of Imperfect Fungi (Barnett & Hunter, 1998). The number of isolates of leaf and petiole endophytic fungi of mango's mistletoe that were successfully isolated were seven isolates consisting of DBM 1, DBM 2, DBM 3, DBM 4, TDBM 1, TDBM 2, and TDBM 3.

Macroscopic characteristics of colonies of endophytic mold growing on PDA media (Potato Dextrose Agar): the upper surface of the colony was black while the color of the lower surface of the colony was black, the surface texture of the colony was like velvet, had zone growth and zonation. Does not have a radial furrow or drops of exudate colonies. Microscopic characteristics of colonies were characterized by septate hyphae. There are vesicles, conidia heads are round, conidiophores are long and have foot cells. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum, isolate DBM1 belongs to the genus *Aspergillus* (Figure 2).

Macroscopic characteristics of colonies of endophytic mold growing on PDA media (*Potato Dextrose Agar*): the upper surface of the colony was black while the color of the lower surface of the colony was black, the surface texture of the colony was similar to velvet, had a growth zone, zonation, radial furrow, and drops of colony exudate. Microscopic characteristics of colonies were characterized by septate hyphae. There are vesicles, conidia heads are round, and conidiophores are long and have foot cells. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum isolate DBM2 belongs to the genus *Aspergillus* (Figure 3).

Macroscopic characteristics of colonies of endophytic mold that grow on PDA media (Potato Dextrose Agar) are that the upper surface of the colony is black, while the color of the lower surface of the colony is black, the surface texture of the colony is similar to a carpet, has a growth zone, does not have zonation, radial furrow, and colony exudate drops. Microscopic characteristics of colonies was characterized by septate hyphae and branched hyphae growth. There are conidia, which are oval and elongated and have branches. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum isolate DBM3 belongs to the genus *Cladosporium* (Figure 4).

Macroscopic characteristics of colonies of endophytic mold that grow on PDA media (*Potato Dextrose Agar*) include the upper surface of the colony which is blackish green while the color of the lower surface of the colony is black, the surface texture of the colony is similar to velvet, has a zone growth, zonation, radial stripes (radial). furrow), and drops of colony exudate. Microscopic characteristics of colonies was characterized by insulated hyphae and branched hyphae growth. There are conidia, with the shape of conidia being round to semi-round, elongated and having branches. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum isolate DBM4 belongs to the genus *Neurospora* (Figure 5).

Macroscopic characteristics of endophytic mold colonies growing on PDA media (Potato Dextrose Agar) were that the upper surface of the colony was black while the color of the lower surface of the colony was black, the surface texture of the colony was similar to cotton, it had zone growth, zonation, radial furrow, and drops of colony exudate. Microscopic characteristics of colonies were characterized by septate hyphae. There are conidia, with the shape of conidia being round and merging into several spheres. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum isolates of TDBM1 belong to the genus *Hormiscium* (Figure 6).

Macroscopic characteristics of endophytic mold colonies growing on PDA media (Potato Dextrose Agar): the upper surface of the colony was white, while the color of the lower surface of the colony was white with yellowish in the middle, the surface texture of the colony was similar to wool, did not have zone growth and radial furrow, has zonation, and drops of exudate colonies. Microscopic characteristics of colonies was characterized by septate hyphae. There are conidia, with the shape of conidia being round and merging into several spheres. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum isolates of TDBM2, which belong to the genus *Hormiscium* (Figure 7).

Macroscopic characteristics of endophytic mold colonies that grow on PDA (Potato Dextrose Agar) media are that the upper surface of the colony is white, while the color of the lower surface of the colony is yellowish white, the surface texture of the colony is similar to cotton, has zones growth, does not have zonation, radial furrow, and colony exudate drops. Microscopic characteristics of colonies were characterized by septate hyphae. There are conidia, with the shape of conidia being round and merging into several spheres. Microscopic characterization refers to the monograph of Pengenalan Kapang Tropik Umum, where isolates of TDBM3 belong to the genus *Hormiscium* (Figure 8).

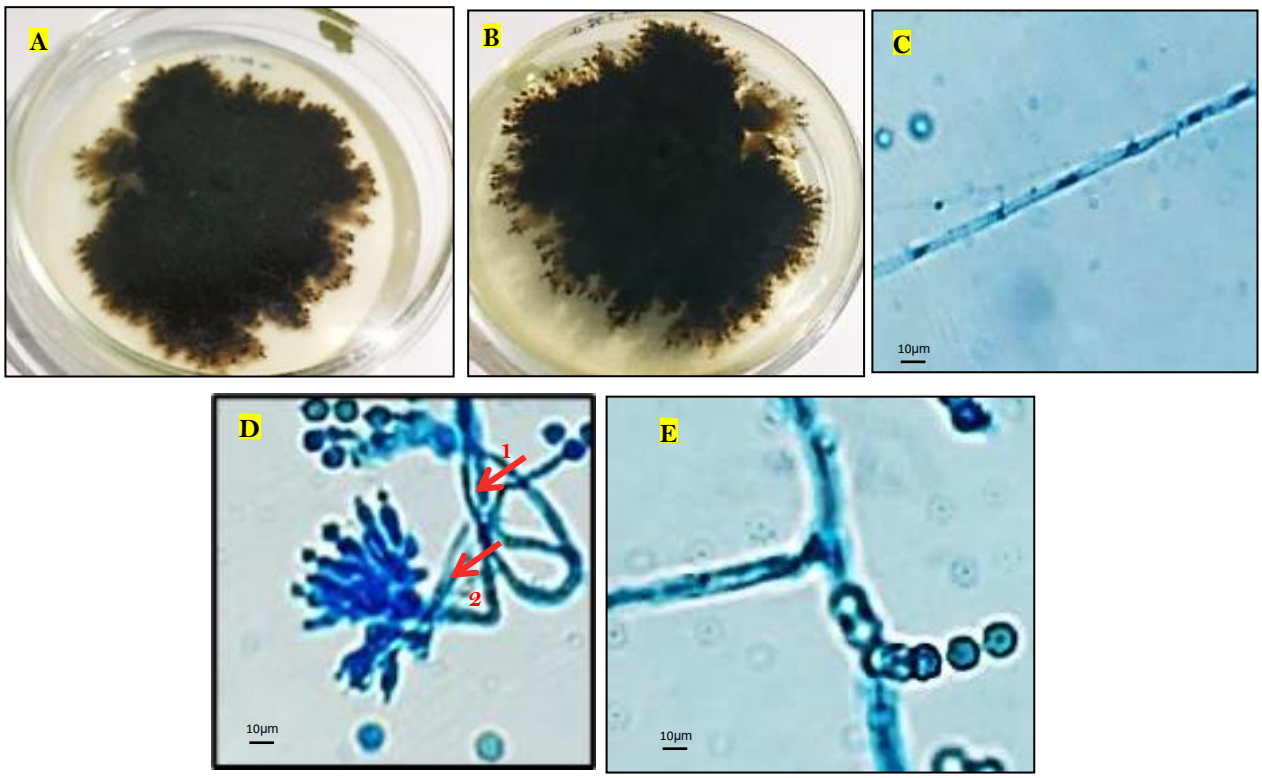

Figure 2. Macroscopic and microscopic description of DBM 1 isolates. (A. Fungal colonies on the surface side; B. Molds colonies on the reverse side; C. Septate hyphae; D. Conidial head (1) conidiophores (2) vesicles; E. Foot cells)

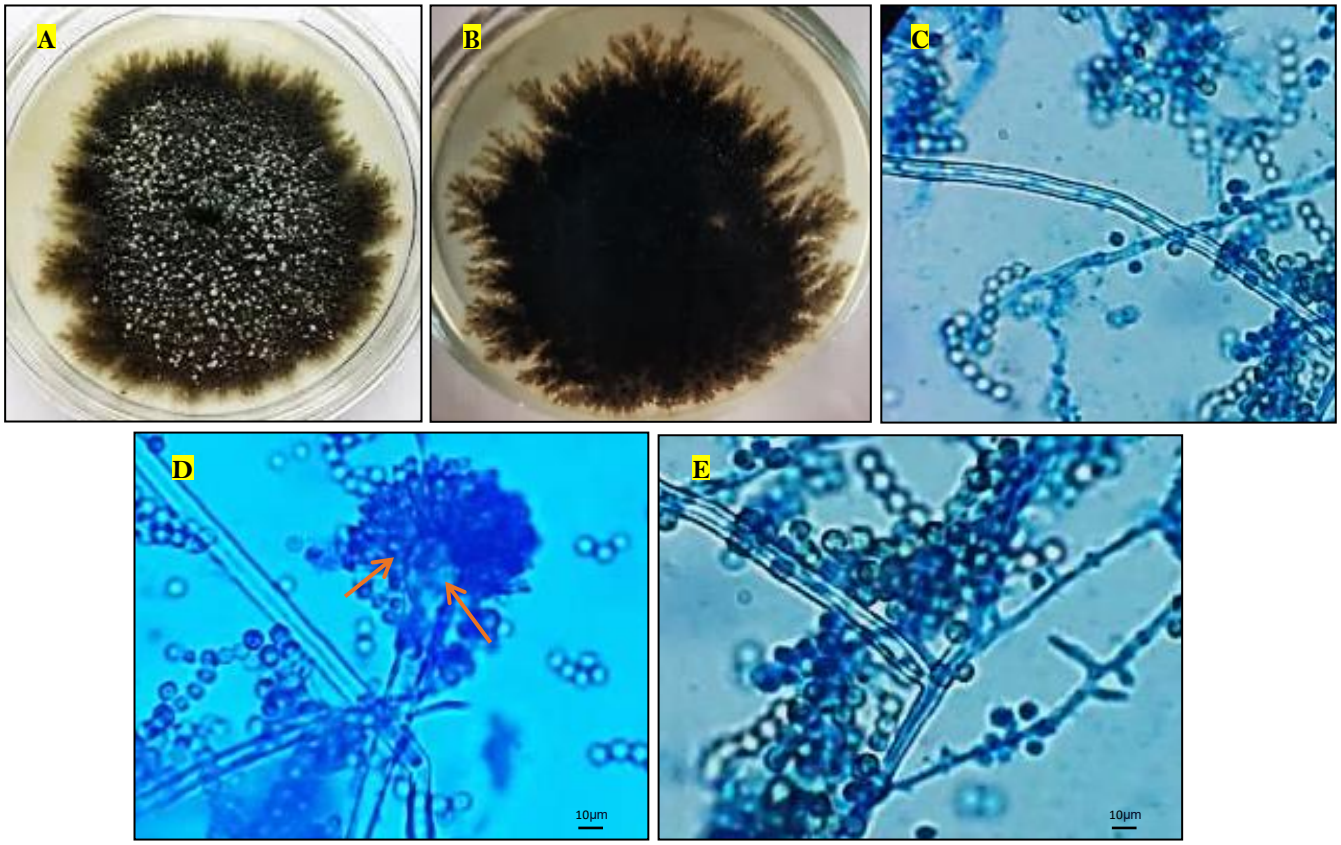

Figure 3. Macroscopic and microscopic picture of DBM 2 isolates. (A. Fungal colonies on the surface side; B. Molds colonies on the reverse side; C. Septate hyphae; D. Conidial head (1) conidiophores (2) vesicles; E. Foot cells)

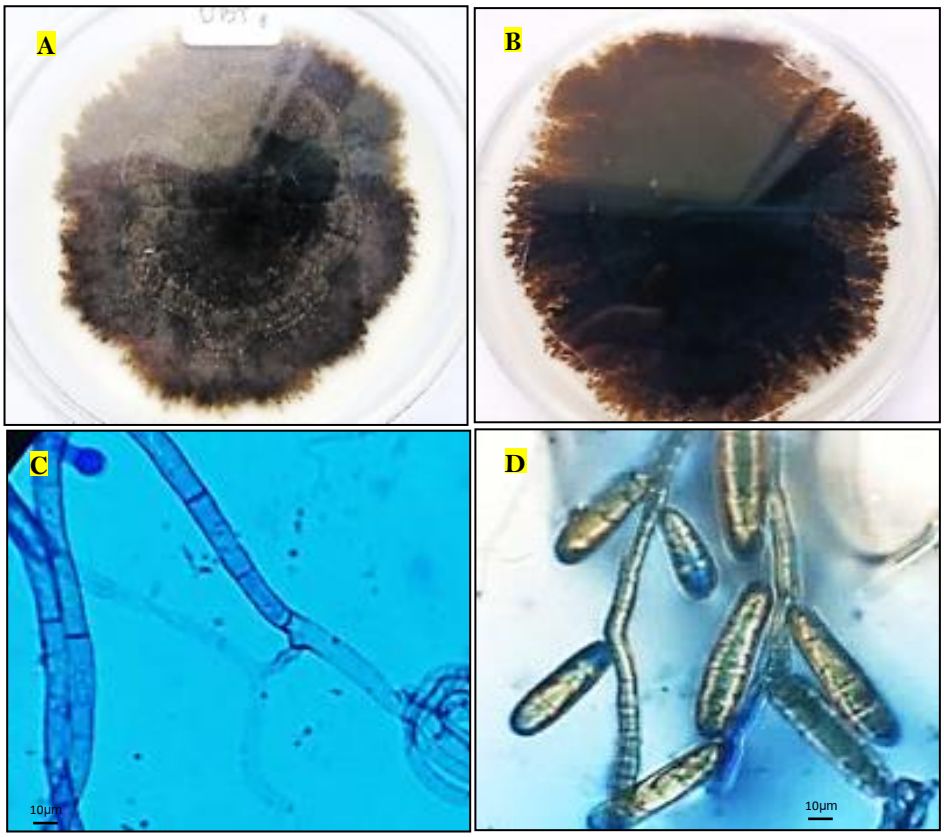

Figure 4. Macroscopic and microscopic description of DBM 3 isolates (A. Fungal colonies on the surface side; B. Mold colonies on the reverse side; C. Septate hyphae; D. Conidial head)

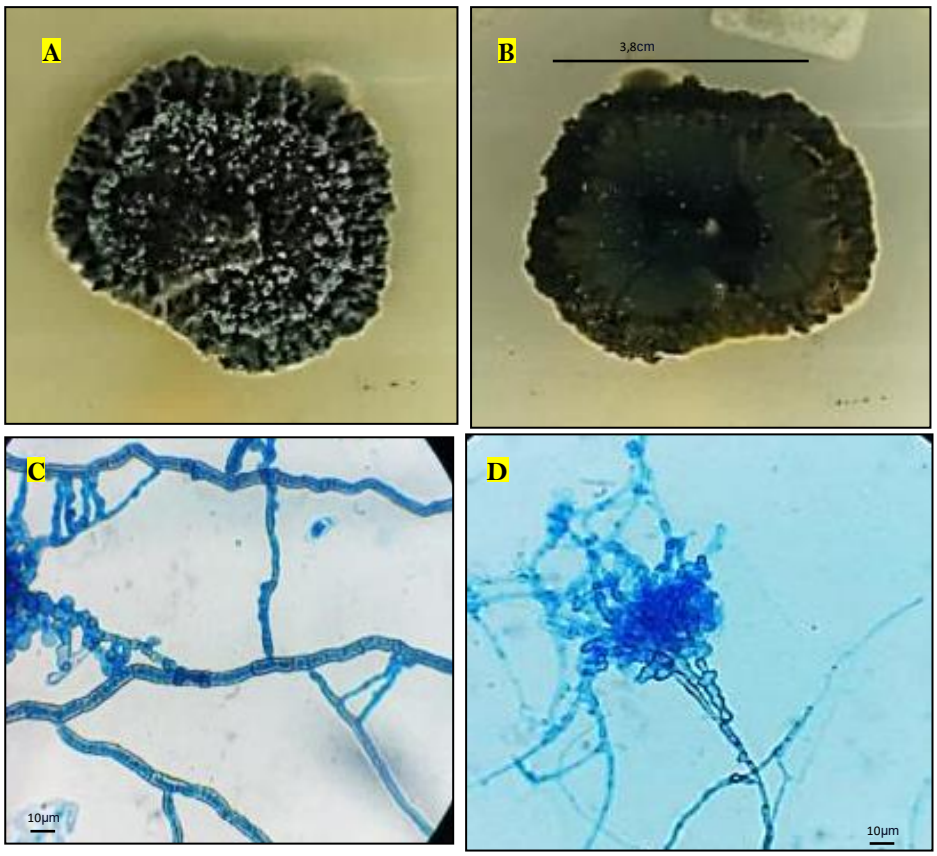

Figure 5. Macroscopic and microscopic description of DBM 4 isolates (A. Fungal colonies on the surface side; B. Mold colonies on the reverse side; C. Septate hyphae; D. Conidial head)

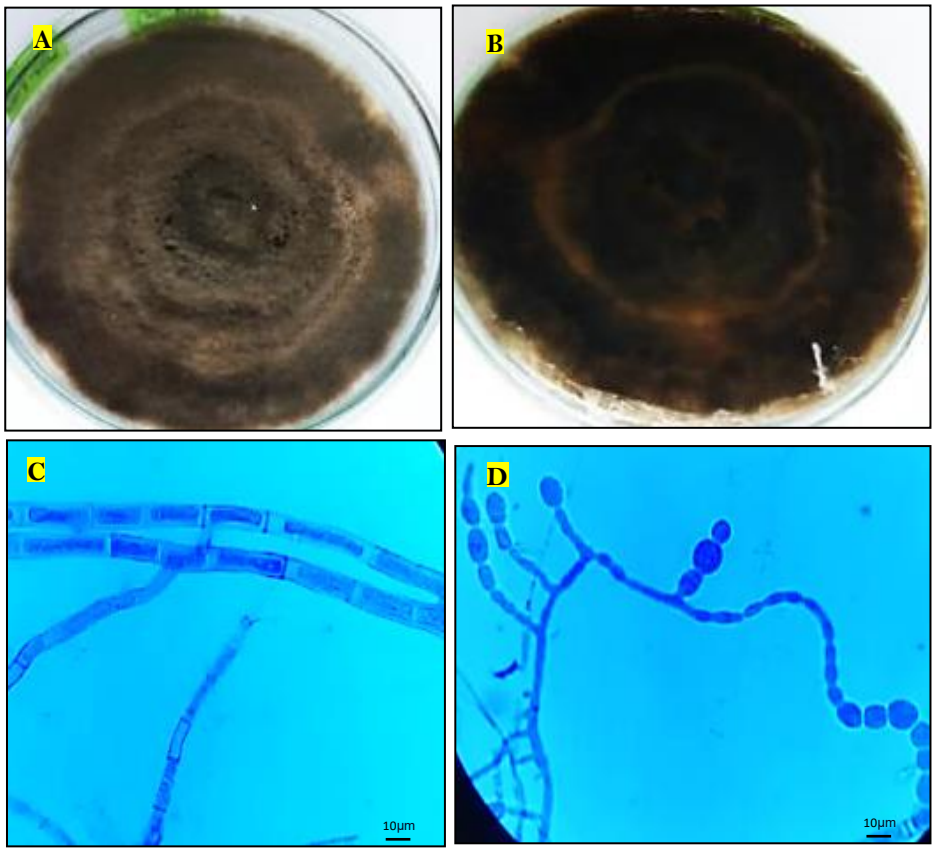

Figure 6. Macroscopic and microscopic description of TDBM 1 isolate (A. Fungal colonies on the surface side; B. Mold colonies on the reverse side; C. Septate hyphae; D. Conidial head)

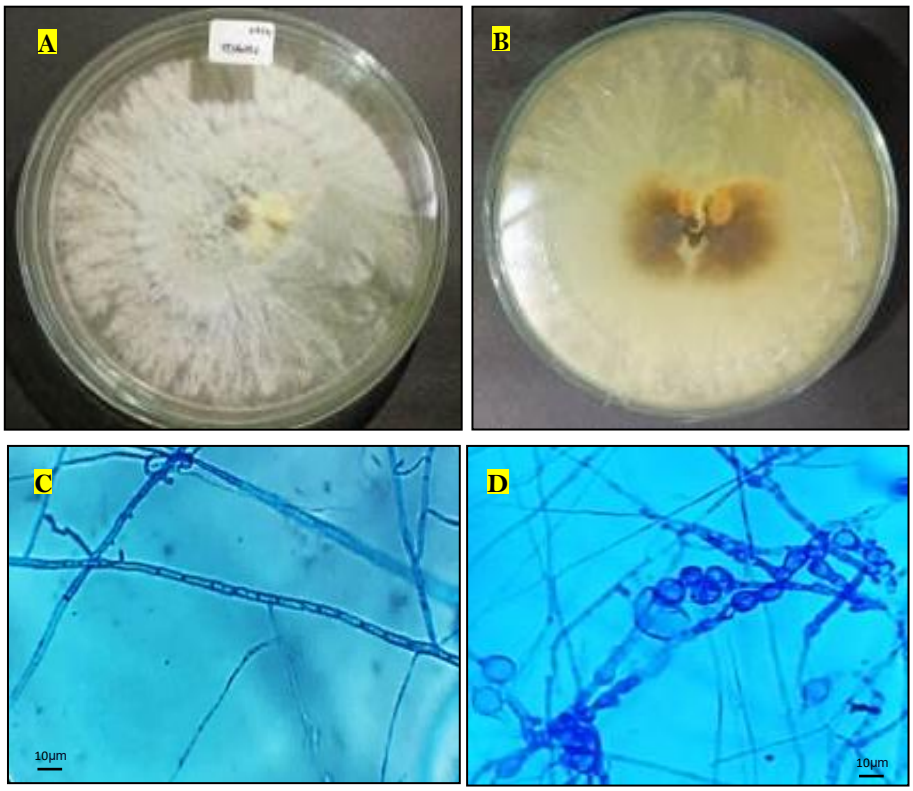

Figure 7. Macroscopic and microscopic description of TDBM 2 isolates. (A. Fungal colonies on the surface side; B. Mold colonies on the reverse side; C. Septate hyphae; D. Conidial head)

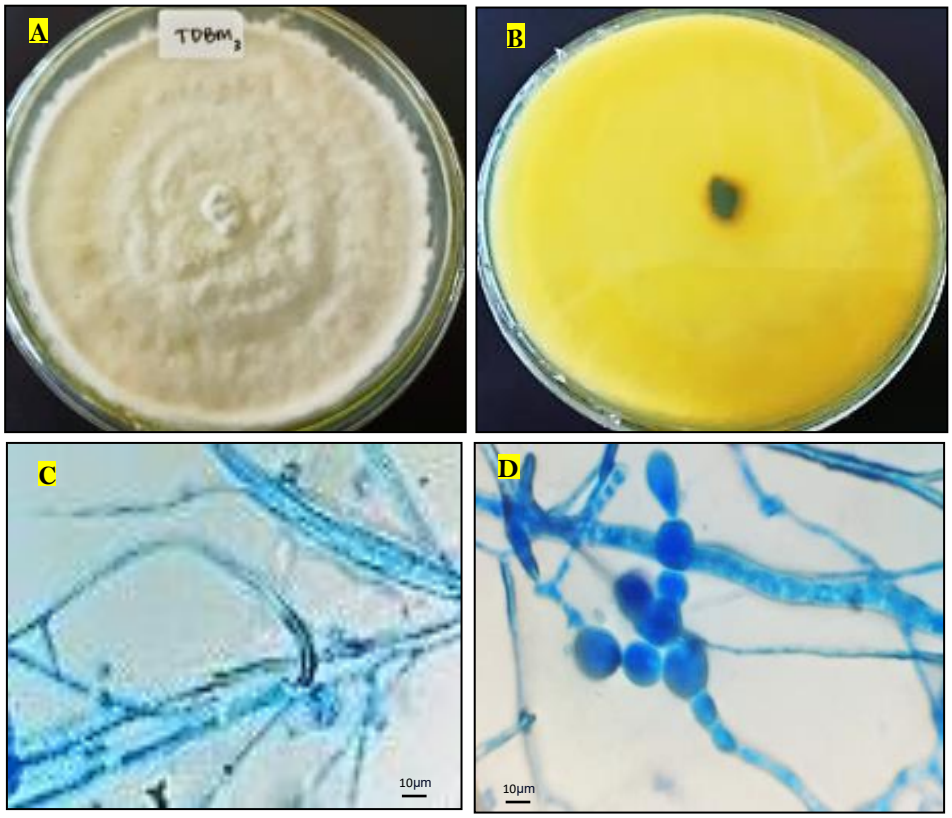

Figure 8. Macroscopic and microscopic description of TDBM 3 isolates (A. Fungal colonies on the surface side; B. Mold colonies on the reverse side; C. Septate hyphae; D. Conidial head)

The endophytic molds isolated from the leaves and stem of the mango's mistletoe obtained seven isolates, namely DBM 1, DBM 2, DBM 3, DBM 4, TDBM 1, TDBM 2 and TDBM 3. From the results of characterization and identification, isolates DBM 1 and DBM 2 were included in *Aspergillus* sp. The genus *Apergillus* sp. isolated in this research has antagonistic properties against wilt disease in several plants (Suryanti, 2013). This group of molds includes cosmopolitan properties with a very wide distribution and can be found in various places (Agrios, 2005). *Aspergillus* sp. can also be used to inhibit pathogenic molds such as *Fusarium oxysporum* (Suniti & Sudarma, 2016). Isolate DBM 3 included in the *Cladosporium* sp. Some member of the genus *Cladosporium* have pathogenic properties that can produce aflatoxins and some are antagonistic which can be used as biological controllers by reducing the use of chemicals such as pesticides (Hasanuddin, 2003). From the results of the characterization of DBM 4 isolates included in the *Neurospora* sp. From the genus of *Neurospora*, it can produce carotenoid substances that can be used for making traditional foods in Indonesia such as oncom (Matitaputty & Nurfaizin, 2015). It can also be used as a fermented feed with a balanced nutrient content from the carotenoid-rich genus of *Neurospora* (Fenita, 2010).

From the characterization and identification of isolates TDBM 1, TDBM2, and TDBM 3 included in the *Hormiscium* sp. From the genus of *Hormiscium* has the potential to be a biocontrol agent for pathogenic bacteria in sugarcane plants with the ability to reduce pathogen disturbances, one of which is inhibiting *Xanthomonas albilineans* bacteria that causes vascular disease, so that plants can grow and develop healthily (Wahyuni, 2015). In this regard, endophytic molds have a symbiotic relationship with their host plants, besides helping in the growth rate, they can also increase the availability of nutrients for plants (Hidayati, 2010).

The growth rate of the endophytic mold diameter of the mango's mistletoe leaves carried out for seven days was found to be *Aspergillus* sp. relatively faster than the mold of *Cladosporium* sp. and *Neurospora* sp. The three molds experienced a lag phase on the first day. After that, it will experience a log phase or exponential phase with optimum growth and a rapid increase in the number of cells (Mukhlis et al., 2018). In the mold *Aspergillus* sp. experienced a log or exponential phase that occurred on the second to fourth day for three days, with an increase in the diameter of the *Aspergillus* sp.1 isolate colony from 2.45 cm to 5.05 cm and the *Aspergillus* sp.2 from 2.73 cm to 5.35 cm. In the mold *Cladosporium* sp. exponential phase occurred from the second day to the fifth day for four days, with an increase in colony diameter from 2.15 cm to 4.65 cm. In the mold *Neurospora* sp. there was a log or exponential phase from the second day to the sixth day for five days.

The end of the exponential phase is followed by a stationary phase which begins with the slowing down of cells, so that the number of living cells is almost the same as the number dead cells which are affected by the depletion of nutrients. In this phase, secondary metabolites can be produced from endophytic mold isolates (Mukhlis et al, 2018). The stationary phase in *Aspergillus* sp. occurred on the fifth day, in *Cladosporium* sp. on the sixth day andin *Neurospora* sp. on the seventh day. The growth rate of the endophytic mold diameter of the mango's mistletoe stem which was carried out for seven days was obtained by the *Hormiscium* sp. experienced a lag phase on the first day, then an exponential phase from the second to the sixth day for five days with an increase in colony diameter of *Hormiscium* sp 1 isolates from 2.63 cm to 7.21 cm, *Hormiscium* sp 2 isolates from 2.45 cm to 6.94 cm and isolates *Hormiscium* sp 3 from 2.85 cm to 7.85 cm. The stationary phase of endophytic mold isolated from the petiole of the mango's mistletoe, namely *Hormiscium* sp. happened on the seventh day.

CONCLUSION

Based on the research results on the exploration and identification of the leaf and stem endophytic molds of the mango's mistletoe (*Dendrophthoe pentandra* (L.) Miq), seven isolates were successfully isolated and four genus were successfully identified. From the results of macroscopic and microscopic characterization, it was found that the four endophytic molds isolated from the leaves of the mango's mistletoe were DBM 1 and DBM 2 belonging to *Aspergillus* sp, DBM 3 belonging to *Cladosporium* sp and DBM 4 belonging to *Neurospora* sp. From the results of macroscopic and microscopic characterization, it was found that the endophytic mold isolates from the stem of the mango's mistletoe were TDBM1, TDBM2 and TDBM3 belonging to the *Hormiscium* sp.

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