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Isolation and Characterization of Endophytic Molds on Leaves and Stems of Tea Mistletoe (Scrrula atropurpurea (Bl.) Dans)

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Abstract

Mistletoe tea is a parasitic plant that lives on its host, and this plant has potential as an herb. The tea plant parasite has several metabolites, namely alkaloids, flavonoids, terpenoids, glycosides, triterpenes, saponins, and tannins. The metabolite compounds produced by endophytic fungi have potential as herbs. Metabolite compounds are not only produced by mistletoe tea but are also produced by endophytic molds. This research aimed to isolate and characterize endophytic molds macroscopically and microscopically and to determine the growth rate of colony diameter. This research uses an exploratory method with data analysis using descriptive methods. Endophytic fungi that have been isolated were characterized by macroscopic and microscopic characterization. The results of the macroscopic and microscopic characterization research showed that 7 isolates of mold were successfully isolated and characterized. Microscopic characterization found 5 different genus among the molds *Alternaria* sp., *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp. and *Fusarium* sp. The increase in diameter is different every day. Mistletoe tea (DBT) leaf isolate from the mold *Altenaria* sp. relatively faster growing than mold *Penicillium* sp. In the stems isolate of mistletoe tea (TDBT) the mold *Aspergillus* sp. relatively faster to grow than mold *Fusarium* sp.

Key words: mistletoe tea, endophytic mold, metabolite compounds

1. Introduction

Mistletoe tea (Scrulla atropurpurea (Bl.) Dans) is a parasitic plant that lives on its host, but this plant has potential as an herbal plant. This plant has several metabolites in it. Metabolite compounds are found in the leaves and stems of tea parasites such as alkaloids, flavonoids, terpenoids, glycosides, triterpenes, saponins, and tannins (Nasution, 2012). Pharmacologically metabolite compounds are not only produced from plants but are also produced by microorganisms that grow in plants. Based on research Simlai, et al (2014) proved that the isolation of antibiotic-producing microorganism species from various plant organs such as leaves, roots, and stems.

Endophytic molds are molds that live in plant tissue and are able to form colonies in the tissue and do not harm the host itself. In plants there are one or more endophytic microorganisms consisting of molds or bacteria (Rante et al, 2013). Healthy plant tissue is found in endophytic fungi that live intracellularly and can induce the host and can produce secondary metabolites.

The metabolite compounds produced by endophytic fungi have potential as herbs. This is because endophytic molds are easy to grow and have a short cycle and can produce large amounts of bioactive compounds. Especially in the content of the mistletoe, tea has active compounds that can be used as herbs. However, the existence of the mistletoe tea has a very limited stock and must be preserved. The mistletoe tea needs to be isolated and the endophytic mold taken to find out the genus name, so that the metabolite compounds from the endophytic mold can be used as herbs.

Based on the description that has been described, it is important for this research to isolate and characterize endophytic molds in mistletoe tea macroscopically and microscopically and to measure diameter growth. So that later the data obtained is able to provide information on the character of microorganisms, especially endophytic molds contained in the mistletoe tea and the discovery of the genus name of endophytic molds and determine the growth rate of colony diameter.

2. Materials and Methods

This research was conducted for 6 months. Starting from October 2021 to April 2022. Sampling of the leaves and stalks of the mistletoe tea was obtained in Ketindan Village, Lawang District, Malang Regency. Identification of tea parasites (conducted at the Laboratory of Balai Materia Medica, Batu, East Java. While the isolation and characterization of endophytic mold on the leaves and stalks of mistletoe tea were carried out at the Microbiology Laboratory of the Halal Center of the University of Islamic Malang. This research used an exploratory method with descriptive data analysis.

2.1. Sampling

Samples of mistletoe tea were obtained in Ketindan Village, Lawang District, Malang Regency, East Java. Samples of tea parasites were identified and determined at the Balai Materia Medica, Batu City, East Java. The parasitic mistletoe tea organs used as samples were leaves and stems stalks. Leaf and stems organs are taken from whole and healthy parts. Samples were stored in the refrigerator (freezer) at a temperature of ± 5°C.

2.2. PDA Media Making

PDA media were weighed as much as 10 grams and 0.13 grams of antibiotics were added. PDA media and antibiotics were dissolved with 250 mL of distilled water. All of these materials were heated to boiling using a hot plate at a temperature of 100°C and stirred using a magnetic stirrer until homogeneous. The media was sterilized using an autoclave at a temperature of 121°C, a pressure of 1 atm for 15 minutes.

2.3. Endophytic Mold Isolation

Samples from the leaf and stem organs of the mistletoe tea were selected and selected healthy ones to be used as research samples. The leaves and stems that have gone through the selection process are washed under running water for 10 minutes. Furthermore, the sterilization process of planting materials such as samples, 70% alcohol, and distilled water was carried out in Laminar Air Flow (LAF) for 30 minutes. After the sterilization process in the LAF the sample was put into 70% alcohol solution for 1 minute, then put into 5% NaOCl (Sodium Hypochlorite) solution for 4 minutes, then put into 70% alcohol solution for 1 minute, then rinsed using sterile distilled water 3 times each for 30 seconds. Furthermore, the dried samples were cut into small pieces with a size of 1cm x 1cm using a sterile scalpel.

Samples of leaves and stems of mistletoe tea that have been cut are placed on the surface of PDA (Potato Dextrose Agar) media that has been added with antibiotics. Isolation of endophytic molds was carried out by direct seed planting technique. Inoculation was carried out on the media and each petri dish contained two sample pieces (duplo). The isolation process was carried out aseptically in the LAF. The isolation process was incubated for 2-14 days in an incubator at room temperature of 30°C.

2.4. Endophytic Mold Purification

Endophytic molds growing on PDA media were purified in stages. Each mold colony that had grown on isolation media was considered a different isolate based on macroscopic observations and purification was carried out. The purification process (Purification) is to re-grow colonies on a petridish that already contains PDA media. Purification was carried out by cutting mold hyphae with a size of 1 cm x 1 cm using a round tip sterile ose needle, then implanted in a cup containing PDA media. The results

of the purification of the mold were incubated in an incubator for 2-14 days at room temperature of 30°C.

2.5. Endophytic Mold Characterization

The characterization of endophytic mold isolates was carried out macroscopically. Characterization was carried out directly including the color of the upper surface of the colony (surface of colony), the color of the lower surface (reserve of colony), the surface texture of the colony, drops of exudate on the colony, growth zone (zone growth), zoning, radial lines from the center. colonies towards the edge of the colony (radial furrow) and concentric circles on a petridish, as well as the colony growth rate (cm/7 days).

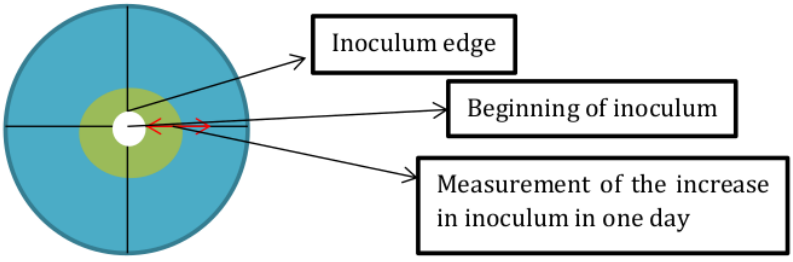
Microscopic characterization of endophytic fungi was carried out using the slide culture method. The slide culture method is a mold observation method by growing a mold culture which is considered better than the simple preparation method. The slide culture method can show the microscopic structure of the mold more fully and completely. The slide culture results were incubated in an incubator at room temperature 30°C for 3-7 days. Cultures of endophytic molds that had grown were observed microscopically using a binocular microscope. Microscopic observations using lactophenol cotton blue were dropped on a new object glass, then the cover glass used as a culture cover on the slide culture was placed on top of the droplets. Microscopic observations using a microscope were carried out using the smallest magnification of 40 times to 1000 times. Microscopic characterization includes hyphal septum (insulated or non-insulated), hyphal growth, presence or absence of conidia, and conidia shape (round, oval, or irregular).

2.6. Growth rate

Molds growth can be done by measuring the growth of endophytic mold diameter. Diameter measurements were carried out at several points, the value obtained from the colony diameter was the average of these measurements. Measuring the growth rate of mold diameter begins by making two perpendicular lines drawn from the bottom of each petri dish. Growth was measured and recorded every day from the edge of the initial inoculum to the edge of the mold area.

In observing the rate of increase in the diameter of the mold, the equation can be used (Sitanggang et al, 2016).

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



3. Results and Discussion

3.1. Results

Isolation of endophytic molds was obtained from the leaves and stems of mistletoe tea taken from Ketindan, Lawang, Malang Regency. Each isolate had different macroscopic and microscopic characteristics. In this research, 7 isolates were successfully isolated and characterized macroscopically and microscopically. While the genus found amounted to 5 genus.

Isolate DBT 1

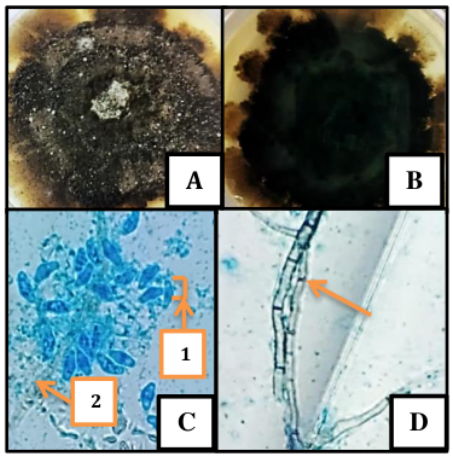
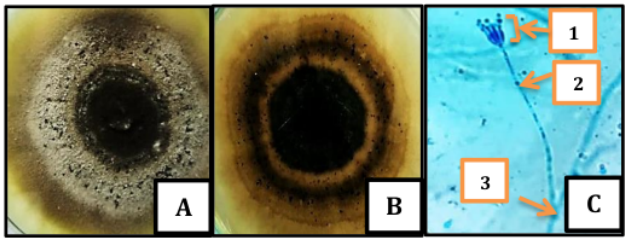


Figure 1. Macroscopic and microscopic description of DBT 1 isolate (A. Endophytic mold colony on the surface side; B. Endophytic mold colony on reverse side; C. Complete structure including (1) conidia, (2) Phalophora); D. Insulated hyphae.

Macroscopic characteristics of endophytic mold colonies growing on PDA media (Potato Dextrose Agar) have a black upper surface of the colony, black reverse side of the colony, the surface texture of the colony is similar to velvet, there is a growth zone. there is zoning, there are no radial forrows, and there are drops of colony exudate.

Microscopic characteristics of endophytic mold colonies growing on PDA (Potato Dextrose Agar) media using the slide culture method. There are conidia, and the shape of the conidia is oval with the tip resembling a septate beak of a duck, the conidiophores are bent. Microscopic characterization refers to the Introduction to General Tropical Molds book, DBT 1 isolate belongs to the genus *Altenaria* sp.

Isolate DBT 2



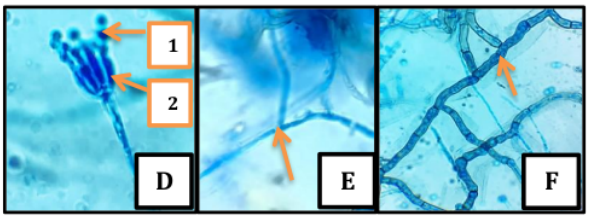


Figure 2. Macroscopic and microscopic description of DBT 2 isolate (A. Endophytic mold colony on the surface side; B. Endophytic mold colony on the reverse side; C. Complete structure including (1) conidial head, (2) conidiophores, (3) foot cell; D. Conidial head includes (1) conidia, (2) sterigma; E. Foot cell; F. septate hyphae)

Macroscopic characteristics of colonies of endophytic molds growing on PDA media (Potato Dextrose Agar) have a black top surface (surface side), black reverse side of the colony, a velvety-like colony surface texture, and a growth zone. there is zonation, has a radial forrow, and has drops of colony exudate.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (insulated), hyphal growth (branching), hyphal color like hyaline. There are conidial heads that are round, conidiophores are branched, sterigma is metula, conidia are round to semi-round and there are foot cells. Microscopic characterization refers to the Introduction to General Tropical Molds book, isolates DBT 2 belongs to the genus *Penicillium* sp.1.

Isolate DBT 3

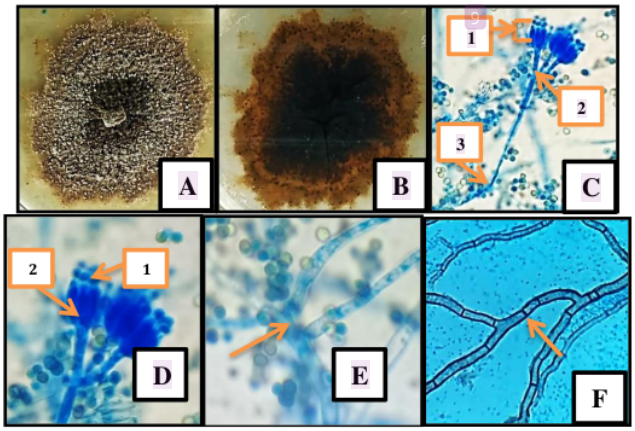


Figure 3. Macroscopic and microscopic description of DBT 3 isolate (A. Endophytic mold colony on the surface side; B. Endophytic mold colony on the reverse side; C. Complete structure including (1) conidial head, (2) conidiophores, (3) foot cell; D. Conidial head includes (1) conidia, (2) sterigma; E. Foot cell; F. septate hyphae)

Macroscopic characteristics of colonies of endophytic molds growing on PDA media (Potato Dextrose Agar) have a black top surface (surface side), black reverse side of the colony, a velvety-like colony surface texture, and a growth zone. , there is zonation, there are radial forrows, and has drops of colony exudate.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (insulated), hyphal growth (branching), hyphal color like hyaline. There are conidial heads that are round, conidiophores are branched, sterigma is metula, conidia are round to semi-round and there are foot cells. Microscopic characterization refers to the Introduction to General Tropical Molds book, isolates DBT 2 belongs to the genus *Penicillium* sp.2.

Isolate TDBT 1

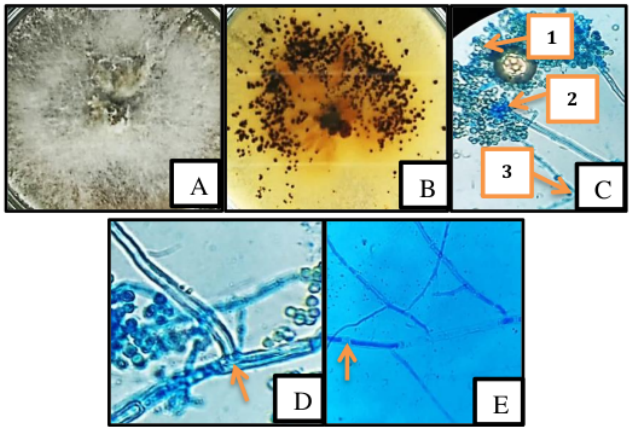


Figure 4. Macroscopic and microscopic description of TDBT 1 isolate (A. Endophytic mold on the surface side; B. Endophytic mold on the reverse side; C. Conidial head includes (1) conidia, (2) vesicles, (3) conidiophores; D. Foot cell; E Hyphae septate)

Macroscopic characteristics of endophytic mold colonies growing on PDA media (Potato Dextrose Agar) have a white upper surface of the colony (surface side), the lower surface of the colony (reverse side) is yellow-black in the middle, the surface texture of the colony is similar to cotton, there is a growth zone, there is zonation, there are radial forrows, and there are drops of colony exudate.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (insulated), hyphal growth (branching), hyphal color like hyaline. There are round conidial heads, relatively long conidiophores, round vesicles, metula-shaped phyalids, round conidia and foot cells. Microscopic characterization refers to the Introduction to Tropical Molds, the TDBT 1 isolate belongs to the genus *Aspergillus* sp.

Isolate TDBT 2

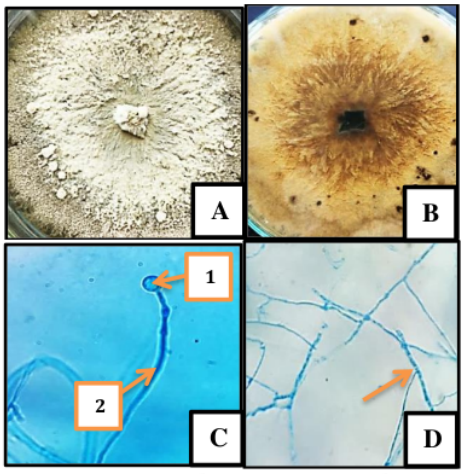
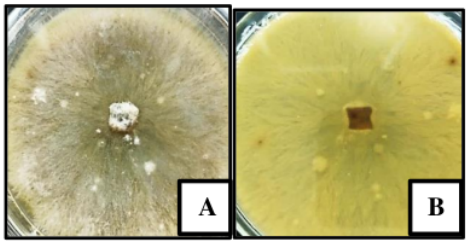


Figure 5. Macroscopic and microscopic description of TDBT 1 isolate (A. Endophytic mold on the surface side; B. Endophytic mold on the reverse side; C. Complete structure including (1) conidia, (2) conidiophores; D. Non-septate hyphae)

Macroscopic characteristics of endophytic mold colonies growing on PDA media (Potato Dextrose Agar) have a white colony surface side, a brownish yellow reverse side of the colony, the surface texture of the colony is similar to wool, there is a growth zone. There is zonation, there were no radial forrows, and there were no drops of colony exudate.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (not insulated), hyphal growth (branching), hyphae color like hyaline. There are conidia, round conidia, and elongated conidiophores. Microscopic characterization refers to the Introduction to General Tropical Molds book, isolate TDBT 2 belongs to the genus *Cladosporium* sp.1.

Isolate TDBT 3



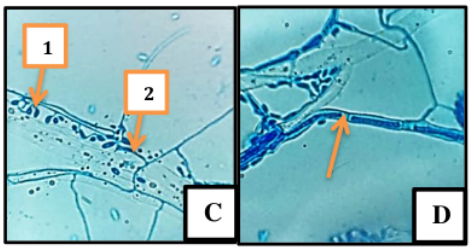


Figure 6. Macroscopic and microscopic description of TDBT 1 isolate (A. Endophytic mold on the surface side; B. Endophytic mold on the reverse side; C. Complete structure including (1) conidia, (2) conidiophores; D. Septate hyphae)

Macroscopic characteristics of endophytic mold colonies growing on PDA (Potato Dextrose Agar) media have white colony surface side, white colony reverse side surface, colony surface texture similar to cotton, there is a growth zone. There is zonation, no radial forrows, and no drops of colony exudate.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (insulated), hyphal growth (branching), hyphal color like hyaline. There are conidia, and the shape of the conidia is oval, the conidiophores are elongated. Microscopic characterization refers to the Introduction to General Tropical Molds book, isolates of TDBT 3 belong to the genus *Cladosporium* sp.2.

Isolate TDBT 4

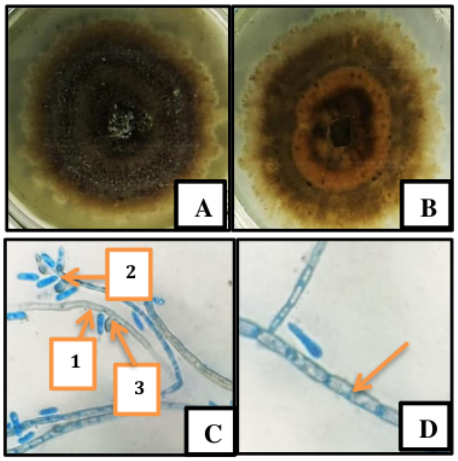


Figure 7. Macroscopic and microscopic description of TDBT 4 isolate (A. Endophytic mold on the surface side; B. Endophytic mold on the reverse side; C. Complete structure includes: (1) conidiophores, (2) microconidium, (3) macroconidium, (4) conidia; D. Hyphae septate)

Macroscopic characteristics of endophytic mold colonies growing on PDA (Potato Dextrose Agar) media have black colony surface side, black colony reverse side is orange

in color, colony surface texture is similar to velvet, there is a growth zone, there is zonation, no radial forrows, and has exudate drops.

Microscopic characteristics of colonies growing on PDA media (Potato Dextrose Agar) using the slide culture method. This colony has a character with hyphae (insulated), hyphal growth (branching), hyphal color like hyaline. There are conidia which include crescent-shaped microconidia with rounded ends and macroconidia with crescent-shaped, canoe-shaped, or slightly turned and tapered ends, cano-shaped and oval conidia. Conidiophores are erect and insulated. Microscopic characterization refers to the Introduction to General Tropical Molds book, isolate TDBT 4 belongs to the genus *Fusarium* sp.

The growth rate of the diameter of the endophytic mold isolated from the endophytic mold obtained from the leaves and stalks of the tea parasite obtained a growth rate curve for 7 days and it can be seen that the diameter growth rate. Seen in Figure 8 and Figure 9.

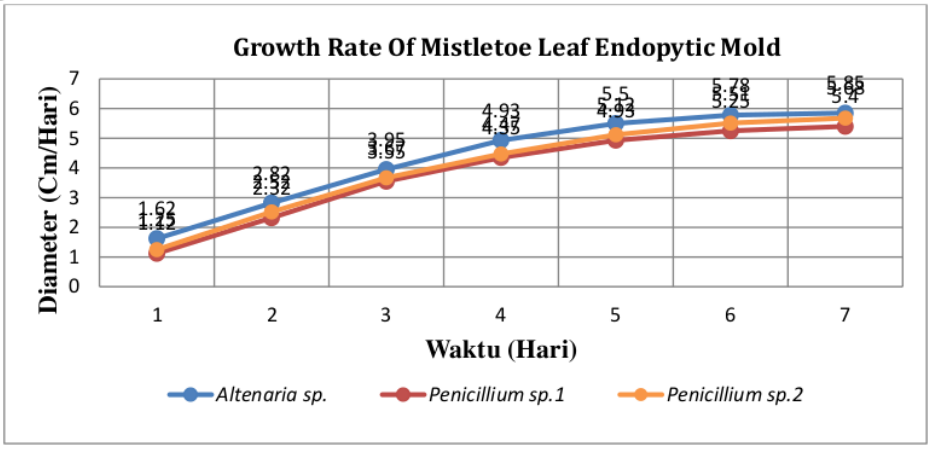


Figure 8. Growth Rate Curve of Tea Parasite Leaf Endophytic Mold

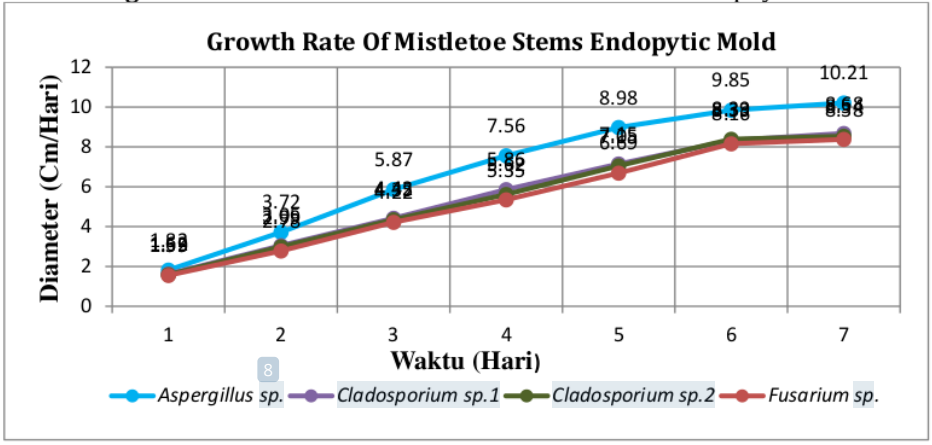


Figure 9. Growth Rate Curve of Endophytic Mold Leaf Stem parasite Tea

3.2. Discussion

Based on the results of the research, the mold isolates from the leaves and stalks of the mango parasite had different macroscopic and microscopic characteristics. The endophytic mold isolates were characterized macroscopically and microscopically to the genus level as shown in Figures 1 to 7. Based on the macroscopic and microscopic characterization, it could be seen that the isolates obtained from the isolation of the leaves and stalks of the tea parasite consisted of the genera Altenaria, Penicillium, Aspergillus, Cladosporium and Fusarium. The Altenaria genus was found in DBT 1 isolates, the Penicilluium genus consisted of DBT 2 and DBT 3 isolates, the Aspergillus genus was found in TDBT 1 isolates, the Cladosporium genus consisted of TDBT 2 and TDBT 3 isolates, and the TDBT4 isolate was the Fusarium genus.

Endophytic microorganisms such as fungi and bacteria are found in more than one plant that lives in it (Rante, 2013). In plant tissues that live intracellularly are called endophytic fungi. Plant tissues such as roots, stems, leaves and fruit contain many endophytic microorganisms in them, but these endophytic molds do not have a negative impact on the host (Sofiyani, 2014). These endophytic molds live in healthy plant tissues and can induce their host to produce secondary metabolites. Recombinant genetics or coevolution lead to the induction of endophytic molds in plants (Sia, 2013). The metabolites produced by endophytic molds produce bioactive secondary metabolites, both known compounds and new compounds (Alvin et al, 2014).

In addition to secondary metabolites produced by endophytic molds, endophytic fungi are widely known for their biological activity benefits. *Altenaria* sp. including types of parasites or saprophytes on plants. In Furi's research (2018), Altenaria mold can cause leaf spots to appear on strawberry plants. Meanwhile, according to research by Arisanti, et al (2012) that penicillium is a type of mold that is widely used as a penicillin antibiotic. Penicillium functions to stimulate plant growth by producing citrinins such as cellulase and endoglucanase enzymes (Rahayu, 2019). The genus Penicillium also has bioactivity as an antimicrobial and is cytotoxic (Yunianto et al, 2014). *Aspergillus* sp. It has been isolated as an endophyte capable of producing antimicrobial activity (Elfita et al, 2011). Endophytic fungi *Cladosporium* sp. And Collectrichum on grapefruit is able to inhibit the growth of Rhizoctonia solani (Suciatmih et al, 2011). *Fusarium* sp. Can produce antifungal pentaketides isolated from *Selaginella pallescens* (Suciatmih, 2010).

In this research, the measurement of the growth rate of endophytic mold diameter was used to determine the fast or slow increase in endophytic mold diameter. This measurement without special treatment for 7 days and depicted by curves in Figure 8 and Figure 9. According to Roosheroes et al (2014) the growth curve of endophytic molds has several phases, including the lag phase, acceleration phase or exponential phase, stationary phase and death phase. accelerated. Mistletoe tea (DBT) leaf isolate from the mold *Altenaria* sp. relatively faster growing than *Penicillium* sp. These three molds lag phase occurs on the first day. The log phase or exponential phase is the phase of optimum growth and a rapid increase in the number of cells. In DBT 1 isolate, *Altenaria* sp. an exponential phase occurred on the second day to the sixth day with an increase in colony diameter from 1.62 cm to 5.78 cm. Meanwhile, the exponential phase of DBT 2 isolates of *Penicillium* sp.1 occurred on the second day to the fifth day with an increase in colony diameter from 1.12 cm to 4.93 cm. Meanwhile, the exponential phase of DBT 3 isolates *Penicillium* sp.2 occurred on the second day to the fifth day with an increase in colony diameter from 1.25 cm to 5.12 cm.

In mistletoe tea stems isolates (TDBT) the growth rate of endophytic mold diameter was carried out for 7 days. The mold *Aspergillus* sp. from TDBT 1. isolates were relatively faster growing than Fusarium sp. from TDBT isolate 4. In TDBT 1 isolate the mold *Aspergillus* sp. exponential phase occurred on the second day to the sixth day with an increase in colony diameter from 3.72 cm to 9.85 cm. Meanwhile, the exponential phase of TDBT 2 isolate of Cladosporium sp.1 occurred on the second day to the sixth day with an increase in colony diameter from 3.05 cm to 8.33 cm. Meanwhile, the exponential phase of TDBT 3 isolates of *Cladosporium* sp.2 occurred on the second day to the sixth day with an increase in colony diameter from 2.99 cm to 8.54 cm. And the exponential phase of TDBT 4 isolates of *Fusarium* sp.

The end of this phase is followed by the death phase. In the stationary phase, the cells begin to experience a slowdown, so the number of cells almost dying due to the influence of the nutrients produced begins to run out. In the stationary phase produces secondary metabolites. This stationary phase isolates DBT 1 on *Altenaria* sp. occurred on the 7th day, DBT 2 isolates of Penicillium sp.1 mold on the 6th day, DBT 3 isolates of *Penicillium* sp.1 molds on the 6th day. TDBT isolate 1 mold *Aspergillus* sp, 7th day, TDBT isolate 2 mold *Cladosporium* sp.1 7th day, TDBT 3 isolates of *Cladosporium* sp.2 the 7th day and isolate TDBT 4 mold *Fusarium s*p. 7th day.

4. Conclusion and Suggestion

4.1. Conclusion

Based on the results obtained in this research, the isolates of endophytic molds obtained from isolation and macroscopic and microscopic characterization of the leaves and stems of the mistletoe tea were obtained as many as 7 isolates. Of the 7 isolates can be characterized and produce 5 different genera. Isolat DBT 1 belongs to the genus Alternaria, DBT 2 and DBT 3 belongs to the genus Penicillium, TDBT 1 belongs to the genus Aspergillus, TDBT 2 and TDBT 3 belongs to the genus Cladosporium and TDBT 4 belong to the genus Fusarium.

The diameter growth rate in the five genera of endophytic molds was known through the growth phase curve after observing for seven days. Mistletoe tea (DBT) leaf isolate from the mold *Altenaria* sp. relatively faster growing than *Penicillium* sp. In the petiole isolate of mistletoe tea (TDBT) the mold *Aspergillus* sp. relatively faster growing than *Fusarium* sp. Endophytic mold *Altenaria* sp. experienced the highest increase in diameter in the exponential phase of 5.50 cm/day. In the mold *Penicillium* sp.1 experienced the highest increase in diameter in the exponential phase of 4.93 cm/day. The mold *Penicillium* sp.2 experienced the highest increase in diameter in the exponential phase of 5.12 cm/day. The mold Aspergillus sp. experienced the highest increase in diameter in the exponential phase of 9.85 cm/day. The mold *Cladosporium* sp.1 experienced the highest increase in diameter in the exponential phase of 7.15 cm/day. The fungus Clasporium sp.2 experienced the highest increase in diameter in the exponential phase of 7.05 cm/day. And the mold *Fusarium* sp. experienced the highest increase in diameter in the exponential phase of 6.69 cm/day.

4.2. Suggestion

1. The mold isolates DBT 1, DBT 2, DBT 3, TDBT 1, TDBT 2, TDBT 3, and TDBT 4 were potential and used for further research with molecular identification.
2. The mold isolates DBT 1, DBT 2, DBT 3, TDBT 1, TDBT 2, TDBT 3, and TDBT 4 were potential and used for further investigation of secondary metabolites in molds.

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2. PUI-PT Research Center for Biological Molecular Engineering (BioMe) University Airlangga Surabaya, which has assisted in the process of identifying the genus of endophytic molds isolated from the leaves and stems of the mistletoe tea.

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