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Biodiversity of *Costus speciosus* Phylloplane Fungi in Baturaden Botanical Gardens and Antagonist Testing against *Fusarium oxysporum*

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ABSTRACT

Indonesia has an abundant biodiversity of natural resources, one of which is phylloplane fungi. This abundant phylloplane mold needs further investigation to utilize its potential as an antagonistic agent. This study aims to prove the existence and to determine the types of phylloplane fungi species found on the leaf surface of *pacing tawar* (sweet ginger or crepe-ginger) plants, to analyze and determine the level of diversity and dominance of phylloplane, and to determine the types of phylloplane fungi species that have the antagonistic ability of the phytopathogenic mold *Fusarium oxysporum*. This research method includes isolating crepe-ginger phylloplane fungi using a contact plate, isolates purification, macromorphological and micromorphological identification, diversity and dominance index calculation, and antagonist test using a dual culture method. This study used a descriptive analysis research design. The isolation results obtained 57 species of crepe-ginger plants leaf phylloplane molds consisting of eight genera (*Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium*, and *Trichoderma*). The results of the diversity index calculation with a value of 2.02 showed that the diversity of phylloplane fungi was moderate. The result of the dominance index calculation, with a value of 0.1521, shows no tendency for phylloplane fungi species to dominate. The results of the antagonist test showed that 15 isolates could antagonize the *F. oxysporum* with an inhibitory proportion of up to 82% obtained from *T. viride* isolates with code FD5B3.2

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INTRODUCTION

Leaf epiphytic or phylloplane fungi grow and thrive on the surface of plant leaves. These fungi are divided into two groups: resident and casual. The resident group consists of fungi that can grow and reproduce by completing most or all of their life cycle on the surface of healthy leaves. In contrast, the casual group only resides on the leaf surface but cannot reproduce. Leaf epiphytic fungi utilize organic substrates on the leaf surface to grow. These fungi found in nature have different potentials and benefits due to their unique properties and characteristics. The potential of leaf epiphytic fungi has yet to be explored compared to endophytes, saprobes, and pathogens (Prabakaran et al., 2011). Haelewaters et al. (2021) studied mycobiota on lettuce plants and found various genera of fungi, including *Alternaria*, *Aureubasidium*, *Cladosporium*, *Filobasidium*, *Naganishia*, *Papiliotrema*, *Rhodotorula*, *Sampaiozyma*, *Sporobolomyces*, *Symmetrospora*, *and Vishniacozyma*. Not only fungi, but the diversity of yeast on corn leaves is also highly variable, originating from genera such as *Ustilago*, *Candida*, *Hannaella*, *Pichia*, *Rhodotorula*, and others (Into et al., 2020). Phylloplane fungi have the potential to act as antagonistic agents. Research by Sukmawati et al. (2021) mentioned that *Candida orthopsilosis* and *Aureubasidium pullulans* yeasts isolated from teak leaf surfaces can inhibit *Aspergillus*, the cause of fruit rot in oranges.

Baturaden Botanical Garden is administratively located in the Kemutug Lor Village, Baturaden

Subdistrict, Banyumas Regency, Central Java. The Baturaden Botanical Garden covers an area of 143.5 hectares and is the most extensive botanical garden on Java Island (Mandiriati et al., 2016). The flora diversity in Baturaden Botanical Garden is extensive, with around 250 species of flora from various plant groups, including medicinal plants (Kalima, 2007, Bidang Pengembangan Kawasan Konservasi Tumbuhan, 2016; Nofrianti et al. 2021). Medicinal plants in Indonesia have been utilized as traditional medicines since ancient times because they have specific parts that can be used, including roots, rhizomes, stems, leaves, fruits, and seeds. One such medicinal plant in Indonesia is 'pacing tawar'. Pacing tawar is often used as a diuretic, diaphoretic, antidote for snake venom, and anti-itch medication (Kinho et al., 2011). In 2015 and 2018, leaf blight and leaf spot diseases were reported on pacing tawar (sweet ginger or leaves in India and China, caused by the phytopathogenic fungi *Drechslera bicolor* and *Nigrospora oryzae* (Jadon et al., 2015, and Sun et al., 2020). Based on this, biological control using antagonistic agents is needed to prevent pacing tawar plants from being affected by plant diseases.

Besides its floral diversity, another invaluable potential of Baturaden Botanical Garden is the diversity of microorganisms, especially leaf phylloplane fungi. This research aims to prove the existence, determine the species, and determine the level of diversity and dominance of filoplan mold found on the surface of the leaves of the freshwater pacing plant and to determine the type of filoplan mold that can be antagonistic to the phytopathogenic mold *Fusarium oxysporum*. There is no research on the diversity of phylloplane fungi in Baturaden Botanical Garden and their antagonistic abilities against phytopathogenic fungi. Therefore, further exploration of these phylloplane fungi is needed to uncover their potential and benefits as antagonistic agents.

MATERIALS AND METHODS

Sampling activity of the phylloplane molds on the leaves of sweet ginger (*Costus speciosus*) was carried out in the Baturaden Botanical Garden area, Kemutug Lor Village, Baturaden District, Banyumas Regency, Central Java. This research was conducted in November – December 2019 and January – May 2020 at the Biotechnology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang. Samples were taken in the form of mold spores on the upper and lower surfaces of the leaves with contact plates filled with PDA media. We use this method to get various and diverse phylloplane mold spores directly from the leaf surface in the field without cutting leaf samples. This makes sampling easier, and the phylloplane mold spores are obtained more accurately. This research uses a random sampling method. Three leaves (upper, middle, and lower stems) were selected from five randomly chosen sweet ginger plants. The contact plate was pressed onto the leaf surface for 30-60 seconds, then closed. The samples taken with the contact plate were then stored in a container box and taken to the laboratory for incubation for 2-3 days at a temperature of 25°C. Representative mold colonies were then purified on PDA media.

The grown mold was observed for colony morphology both macroscopically and microscopically. Pure mold colonies were transferred to slanted agar media to be preserved as culture stock. The mold was inoculated into Petri dishes, each containing PDA, MEA, CDA, and CMA media, and then incubated at room temperature (25°C) for seven days and observed both macroscopically and microscopically.

Macroscopic observations on the phylloplane mold included colony surface, front and reverse colony color, colony texture, radial lines, exudate drops, and the presence or absence of a growing zone. Microscopic observations of the mold included spore shape, spore size, the presence of septa in hyphae, conidia shape, and conidia size. The results obtained were then identified based on literature: Fungal Biodiversity (Crous et al., 2010), Identification of Common *Aspergillus* (Klich, 2002), and Illustrated Genera of Imperfect Fungi (Barnett and Hunter, 1998).

Each identified species of mold was then used to calculate the diversity index and its dominance to determine the diversity of phylloplane molds on the surface of sweet ginger leaves. The Shannon-Wiener diversity index, according to Ludwig and Reynold (1998), can be calculated using the formula:

$$H' = \sum_{i=1}^{s} \left(\frac{ni}{N}\right) \ln \left(\frac{ni}{N}\right) \qquad Pi = \left(\frac{ni}{N}\right)$$

Note:
H' = Shannon-Wiener Diversity Index
s = Total species
ni = The proportion of the number of individuals in a species
N = The total number of individuals of all species

According to Odum (1993), the Simpson dominance index can be calculated using the formula:

$$D = \sum \left(\frac{ni}{N} \right)^2$$

Note:
D = Simpson dominance index
ni = Proportion of the number of individuals in a species
N = Total number of individuals of all species

The antagonistic activity test of phylloplane mold against pathogenic mold (*F. oxysporum*) was conducted in vitro using the dual-culture method. The pathogenic mold *F. oxysporum* used for this test was obtained from Diponegoro University Culture Collection (DUCC), which causes wilt disease in chilies and used as a standard phytopathogenic molds for antagonist tests. Colonies of phylloplane molds and phytopathogenic mold growing on each Petri dish were cut using a cork borer with a diameter of 4 mm, then inoculated facing each other at a distance of 3 cm on a 9 cm diameter petri dish containing PDA media. Petri dishes inoculated with phylloplane and phytopathogenic mold were then incubated in the dark at 25°C.

The evaluation was carried out by calculating the percentage inhibition of phylloplane molds against phytopathogenic mold (*F. oxysporum*) from the first day after mold inoculation until the seventh day. The percentage inhibition categories in the antagonist test are based on Zivkovic et al. (2010) (Table 1). The percentage inhibition of the growth of phytopathogenic mold is calculated using the following formula:

$$P = \frac{R1 - R2}{R1} \; x \; 100 \; \%$$

Note:
P = The percentage of inhibition of phylloplane molds that have antagonistic properties
R1 = The radius of the colony of phytopathogenic molds growing away/in the opposite direction from antagonistic phylloplane molds
R2 = The radius of the colony of phytopathogenic molds growing close to/in the direction of the antagonistic phylloplane molds
KP = Pathogenic fungi
KF = Phylloplane fungi

Table 1. Growth Inhibition Category (GIC) percentage

Scale	Growth Inhibition Category (%)
0	No Inhibition
1	1-25
2	26-50
3	51-75
4	76-100

RESULTS AND DISCUSSION

Identification of Phylloplane Molds on the Leaves of Sweet Ginger Plants (Costus speciosus)

The total number of phylloplane molds isolates obtained from the sweet ginger plant is 57 isolates (Figure 1), with the highest number found on the sweet ginger plant number 5, and the total was 20 isolates. Morphological identification results revealed eight genera, including *Alternaria*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Curvularia*, and *Mucor* (Table 2).

Phylloplane molds are molds that live on the surface of leaves. The diversity of phylloplane molds, including their potential, has yet to be extensively studied. The sweet ginger plant in Baturaden Botanical Garden is one plant whose phylloplane diversity remains unexplored. Based on the obtained results, eight genera of molds were found and identified. The most prevalent were *Aspergillus*, *Fusarium*, and *Penicillium* genera molds. This is likely because these genera are cosmopolitan molds capable of living on various substrates and are commonly found. The presence of these mold on leaves is likely due to airborne spores that float and attach to leaf trichomes, as is the case with molds from other genera. Mold grows and spread through spores from one substrate to another. Research by Kansara et al. (2022) indicates that phylloplane molds isolated from tomatoes include *Aspergillus*, *Trichoderma*, *Penicillium*, and *Alternaria*.

Similarly, phylloplane molds found in the mangrove *Avicennia marina* include *Trichoderma*, *Aspergillus*, *Penicillium*, *Curvularia*, *Paecilomyces*, *Talaromyces*, and *Syncephalastrum* (Mahardhika et al., 2021). Varpe (2020) isolated various mycoflora from the *Sapindus mukorossi* plant, such as *A. niger*, *Candida* sp., *Cladosporium herbarum*, *Colletotrichum orbiculare*, *F. oxysporum*, *Fusarium* sp., *Epicoccum nigrum*, *P. expansum*, *Penicillium* sp., *A. alternata*, and *Torulla herbarium*. Some of the species identified from *S. mukorossi* were also found in the sweet ginger plant, including *A. niger*, *Cladosporium*, *Penicillium*, *A. alternata*, and *Fusarium* sp.

Table 2. Sweet Ginger's Phylloplane fungi isolate

Kode isolate	Species
FD5A1.1, FD5B1.1, FD4A3.2, FD4B3.2, FD3A3.1	*Alternaria alternata*
FD5A2.5	*Trichoderma harzianum*
FD5B2.3, FD5B3.2	*Trichoderma viride*
FD5A1.3, FD5A2.2, FD5A2.4, FD5B1.3, FD5B3.1, FD4A3.4, FD4B3.6, FD3A3.2, FD3B2.1	*Aspergillus* sp.
FD5B1.3	*Aspergillus flavus*
FD5B3.1	*Aspergillus niger*
FD4B3.6	*Aspergillus terreus*
FD5B1.4, FD5B1.5, FD5B2.1, FD5B2.2, FD4A2.4, FD4A3.3, FD4B3.4, FD4B3.5, FD3A2.3, FD3A2.4, FD3A3.3, FD3B2.2, FD2A2.1, FD2A3.2	*Fusarium* sp.
FD4A2.3, FD4A3.1, FD3A2.2, FD2A3.1	*Curvularia*
FD4A2.3, FD2A3.1	*Curvularia geniculata*
FD4A3.1, FD3A2.2	*Curvularia lunata*
FD5A2.1, FD5A2.6, FD5B2.4, FD4A2.2, FD4B2.1, FD3A2.1, FD3B2.3, FD3B3.2, FD2B1.1	*Penicillium* sp.
FD5A2.6	*Penicillium corylophilum*
FD4B2.1	*Penicillium oxalicum*
FD3A2.1	*Penicillium carneum*
FD2B1.1	*Penicillium roqueforti*
FD5A1.2, FD5A2.3, FD5B1.2, FD4A2.1, FD4B2.2, FD4B2.3, FD4B3.3	*Cladosporium* sp.
FD5A1.2	*Cladosporium sphaerospermum*
FD5A2.3, FD5B1.2, FD4A2.1, FD4B2.2, FD4B2.3, dan FD4B3.3	*Cladosporium macrocarpum*
FD2B3.1	*Mucor circinelloides*
FD4B1.1, FD4B3.1, FD3A3.4, FD3B1.1, dan FD3B3.1	Unidentified

Table 3. The diversity and dominance indices of phylloplane molds on the leaves of sweet ginger plants

No.	Genus	Ni	Pi	ln(Pi)	Pi*ln(Pi)	Pi^2	D	s	N
1.	*Alternaria*	5	0.09	-2.43	-0.21	0.0081			
2.	*Trichoderma*	3	0.05	-2.94	-0.15	0.0025			
3.	*Aspergillus*	9	0.16	-1.85	-0.29	0.0256			
4.	*Fusarium*	14	0.25	-1.40	-0.34	0.0625			
5.	*Curvularia*	4	0.07	-2.66	-0.19	0.0049	0.1521	9	57
6.	*Penicillium*	9	0.16	-1.85	-0.29	0.0256			
7.	*Cladosporium*	7	0.12	-2.10	-0.26	0.0144			
8.	*Mucor*	1	0.02	-4.04	-0.07	0.0004			
9.	Unidentified	5	0.09	-2.43	-0.21	0.0081			


FD2A2.1
FD2A3.1
FD2A3.2
FD2B1.1
FD2B3.1
FD3A2.1
FD3A2.2
FD3A2.3
FD3A2.4
FD3A3.1
FD3A3.2
FD3A3.3
FD3A3.4
FD3B1.1
FD3B2.1
FD3B2.2
FD3B2.3
FD3B3.1
FD3B3.2
FD4A2.1
FD4A2.2
FD4A2.3
FD4A2.4
FD4A3.1
FD4A3.2
FD4A3.3
FD4A3.4
FD4B1.1
FD4B2.1
FD4B2.2
FD4B2.3
FD4B3.1
FD4B3.2
FD4B3.3
FD4B3.4

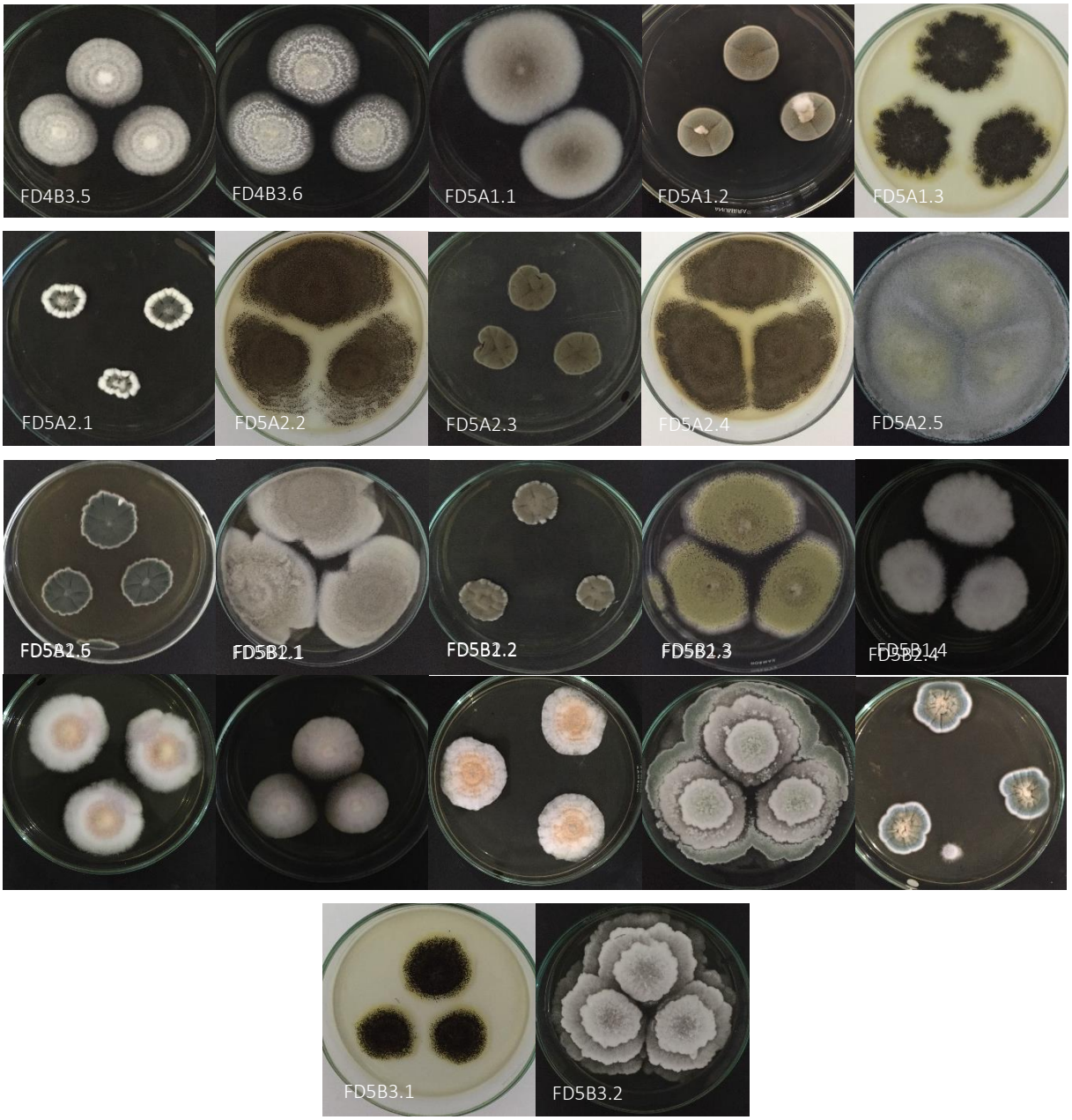

Figure 1. Phylloplane fungi from sweet ginger on PDA medium (7 days)

Diversity and Dominance Index of Phylloplane Molds on the Leaves of Sweet Ginger Plants (*Costus speciosus*)

The diversity and dominance index for phylloplane molds on the sweet ginger leaves indicate no dominant mold species (Table 3). This is evidenced by the value of 's' being 9, representing the nine identified genera of molds.

The value of N is 57. The ni values for each genus are as follows: ni for *Alternaria* is 5, ni for *Trichoderma* is 3, ni for *Aspergillus* is 9, ni for *Fusarium* is 14, ni for *Curvularia* is 4, ni for *Penicillium* is 9, ni for *Cladosporium* is 7, ni for *Mucor* is 1, and ni for unidentified genus isolates is 5. Then, using the Shannon-Wiener diversity index formula, the diversity index (H') is calculated to be 2.02. Therefore, the diversity of phylloplane molds on the leaves of sweet ginger plants falls into the moderate category. The dominance index for phylloplane molds is calculated using the Simpson dominance index formula, resulting in a value of D = 0.1521.

Based on the results of diversity indices and dominance, no mold dominates the sweet ginger plants in Baturaden Botanical Garden. This aligns with the statement by Magurran (1988) that diversity categories are divided into three groups: low with an H' value less than 1.5, moderate with an H' value ranging from 1.5 to 3.5, and high with an H' value greater than 3.5. Odum (1993) states that the dominance index ranges from 0 to 1, where a

Table 4. The results of the antagonistic test of phylloplane molds on the leaves of sweet ginger plants against *F. oxysporum*

No.	Isolate	Percent Growth Inhibition (%)	No.	Isolate	Percent Growth Inhibition (%)
1.	FD5A1.1	49	30.	FD4B2.1	20
2.	FD5A1.2	4	31.	FD4B2.2	20
3.	FD5A1.3	56	32.	FD4B2.3	23
4.	FD5A2.1	7	33.	FD4B3.1	42
5.	FD5A2.2	55	34.	FD4B3.2	46
6.	FD5A2.3	21	35.	FD4B3.3	19
7.	FD5A2.4	56	36.	FD4B3.4	36
8.	FD5A2.5	62	37.	FD4B3.5	38
9.	FD5A2.6	40	38.	FD4B3.6	54
10.	FD5B1.1	28	39.	FD3A2.1	58
11.	FD5B1.2	10	40.	FD3A2.2	32
12.	FD5B1.3	72	41.	FD3A2.3	42
13.	FD5B1.4	42	42.	FD3A2.4	47
14.	FD5B1.5	38	43.	FD3A3.1	33
15.	FD5B2.1	33	44.	FD3A3.2	63
16.	FD5B2.2	36	45.	FD3A3.3	39
17.	FD5B2.3	75	46.	FD3A3.4	15
18	FD5B2.4	24	47.	FD3B1.1	31
19.	FD5B3.1	71	48.	FD3B2.1	58
20.	FD5B3.2	82	49.	FD3B2.2	19
21.	FD4A2.1	35	50.	FD3B2.3	24
22.	FD4A2.2	31	51.	FD3B3.1	35
23.	FD4A2.3	39	52.	FD3B3.2	41
24.	FD4A2.4	47	53.	FD2A2.1	35
25.	FD4A3.1	35	54.	FD2A3.1	48
26.	FD4A3.2	42	55.	FD2A3.2	48
27.	FD4A3.3	48	56.	FD2B1.1	56
28.	FD4A3.4	63	57.	FD2B3.1	76
29.	FD4B1.1	45			

smaller dominance index value indicates no dominating species. Conversely, an immense dominance index value indicates the dominance of certain species.

Antagonistic Test Sweet Ginger Phylloplane fungi

Based on the results of the antagonistic test of phylloplane molds against *F. oxysporum*, a total of 15 isolates has the potential to inhibit the growth of the test mold (Table 4). The inhibition percentages produced by 9 *Aspergillus* isolates range between 55-72%, indicating that they fall within scale 3 in the category of inhibition percentages. This scale suggests that *Aspergillus* has good antagonistic abilities to inhibit the growth of *F. oxysporum*. *Aspergillus* multiplies on the test medium. Clear zones not covered by *F. oxysporum* were observed on the test medium. This phenomenon indicates that the antagonistic mechanism exhibited by this genus and competition for space and nutrients also involve an antibiosis mechanism. This implies that mold from the *Aspergillus* genus produce secondary metabolites that inhibit phytopathogenic mold growth. Phylloplane molds obtained from sweet ginger plants can serve as antagonistic agents against phytopathogenic molds. Research by Izzatinnisa et al. (2020) states that *Aspergillus* sp. fungi can suppress the growth of *F. oxysporum*. *Aspergillus* sp. fungi exhibit antimicrobial activity by producing enzymes that inhibit the growth of other microbes, including amyloglucosidase, cellulase, lactase, invertase, pectinase, and acid protease. Afifi et al. (2017) add that the formation of inhibitory zones indicates the presence of secondary metabolites and antibiosis compounds secreted by *Aspergillus* sp. molds.

Isolate FD5A2.5 is identified as *Trichoderma harzianum* with an inhibition percentage of 62%. FD5B2.3 (inhibition percentage of 75%) and FD5B3.2 (inhibition percentage of 82%) are identified as *Trichoderma* viride species. The inhibition percentages produced by these three *Trichoderma* genus molds are very high, falling within scales 3 and 4 in the antagonist test inhibition percentage categories. This scale indicates that *Trichoderma* is an excellent antagonistic agent against phytopathogenic molds. *Trichoderma* molds proliferate, filling the space in the Petri dish within six days. In the Petri dish, the mycelium of *Trichoderma* genus molds is observed growing over the phytopathogenic mold *F. oxysporum*, with little space at the meeting point between *Trichoderma* and *F. oxysporum* mycelium. This condition suggests that the antagonistic mechanisms produced by these molds involve not only competition for space and food but also mycoparasitism and antibiosis mechanisms. *Trichoderma*, isolated from sweet ginger, demonstrates superior antagonistic abilities. Research by Mardani and Hadiwiyono (2018) indicates that *Trichoderma* grows faster than pathogens and produces zones free of both colonies. This suggests that these zones are caused by specific compounds produced by *Trichoderma*, such as antibiotics or secondary metabolites capable of inhibiting pathogen growth. Berlian et al. (2013) add that *Trichoderma* spp. produces various secondary metabolites, including enzymes like chitinase, glucanase, and protease for lysing cell walls, as well as antibiotics like alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, and sesquiterpenes to inhibit the growth of pathogenic fungal spores and mycelium.

Isolate FD3A2.1 is identified as *Penicillium carneum* with an inhibition percentage of 58%. FD2B1.1 is identified as *Penicillium roqueforti* with an inhibition percentage of 56%. Based on the inhibition percentage categories, both *Penicillium* species fall into scale 3, meaning they are excellent antagonistic agents. In the Petri dish, clear zones free of *Penicillium* and *F. oxysporum* mycelium are observed. These clear zones represent inhibitory zones consisting of secondary metabolites produced by the *Penicillium* genus molds to inhibit the growth of *F. oxysporum*. Therefore, the antagonistic mechanism of these molds is antibiosis.

Penicillium, originating from sweet ginger, can inhibit phytopathogenic molds, and this genus is indeed known for producing antimicrobial compounds. According to Khaerati et al. (2018), the ability of *Penicillium* mold to inhibit pathogen growth has been extensively reported. Up to 90% inhibition percentages were observed when tested as antagonists using dual-culture methods. The antagonistic mechanisms involve antibiosis and nutritional competition, demonstrated by clear zones on the culture medium. *Penicillium* sp. can compete by releasing several alkaloid compounds, such as agroclavine and ergometrine, which possess antifungal properties. Mardani and Hadiwiyono (2018) added that *Penicillium* sp. could inhibit the growth of *Phytophthora capsici*, a mold with parasitic mechanisms against pathogens and the production of antibiotics.

Isolate FD2B3.1 is identified as *Mucor circinelloides*, with an inhibition percentage of 76%.

Based on the inhibition percentage categories, this mold falls into scales 3 and 4, indicating that it is an outstanding antagonistic agent. *Mucor circinelloides* overgrows in the Petri dish, outcompeting the growth of *F. oxysporum*. This is because of competition for nutritional resources in the medium for the growth of *Mucor circinelloides*. The antagonistic mechanism produced by the *Mucor* genus mold involves competition for space and nutrition. *Mucor* molds also exhibit the ability to inhibit the growth of *F. oxysporum*, potentially due to their faster growth rate. According to Imu Rohayatun et al. (2017), the growth of *Mucor* sp. is rapid, filling the growth space by the seventh day. This is likely due to competition for space and nutrients. Izzatinnisa et al. (2020) added that some *Mucor* genera have high antagonistic mechanisms involving competition for space and nutrients, mycoparasitism, and antibiosis. *Mucor* sp. can produce hydrogen cyanide (HCN) to inhibit the growth of pathogens.

CONCLUSION

Based on the research findings, phylloplane molds on sweet ginger plants are pretty diverse, with a total species of 57 isolates were obtained, consisting of 8 known genera: *Alternaria*, *Aspergillus*, *Penicillium*, *Curvularia*, *Cladosporium*, *Fusarium*, *Trichoderma*, and *Mucor*. The calculation of the dominance index with a value of 0.1521 indicates no tendency for a specific mold species to dominate. Antagonistic tests show that there are 15 isolates with antagonistic abilities against the mold *F. oxysporum*, with the highest inhibition percentage reaching 82%, obtained from the *T. viride* isolate with the code FD5B3.2. These results are the first research report conducted on sweet ginger plants at the Baturaden Botanical Garden, and the use of phylloplane mold as a biological agent has yet to be widely done. We hope this research results become a reference to enrich scientific information regarding the diversity and ability of phylloplane molds as antagonistic agents.

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