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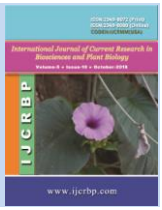

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The Diversity of Exophytic Fungus in Sugar-Apple Plant and their Inhibition Ability on *Lasiodiplodia theobromae* (Cause of Srikaya Fruit Rot) *In Vitro*

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ABSTRACT

Exophytic fungus diversity plays an important role in controlling pathogens that cause diseases, especially sugar-apple fruit rot. The greater the diversity, the more stable it is in the ecosystem, and the more opportunities that inhibit pathogens. The results showed that the exophytic fungi found in leaves, fruit and twigs were *Aspergillus* sp. *A. niger*, *Fusarium* sp., Mycelia sterilia, *Neurospora* sp., and *Rhizopus* sp. The diversity index of exophytic fungi was 2.374 with a dominance index of 0.8667. It indicates that the condition of the community structure is more stable with good categories. The dominance index approaches 1 means that there was a dominating fungus, namely *A. niger* fungus as much as 18 × 10^3 cfu. The inhibitory effect of exophytic fungi on *Lasiodiplodia theobromae in vitro* was found to be lower in *Aspergillus* sp. amounting to 65.68 ± 0.82 and it was higher in *Rhizopus* sp. amounting to 82.92 ± 0.50%.

Introduction

Exophytic fungi such as phylloplane fungus are the fungi in the surface which have been tested for fungitoxicity ability against *Alternaria brassicae*, a causative agent of leaf spot on cabbage plants. Colony interaction testing showed that *Trichoderma viridae* and *Aspergillus flavus* had maximum inhibition ability against *A. brassicae* (Yadav et al., 2011). Saha et al. (2011) stated that there are 17 species of fungi found on the surface of *Psidium guineense* leaves, including fungi that can be grown in March and January. Dominating fungi include *Cladosporium cladosporioides*, *Glicladium viridae*, *Mucor recemosus*, *Penicillium chrysogenum* and mycelia sterilia.

Diversity of fungi on the leaf surface of castor plants (*Ricinus communis* L.) grown in Northeastern India showed a total of 11 species. Fungi that can be

identified by five species include *Alternaria ricini*, *Aspergillus fumigatus*, *Cercospora ricinela*, *Curvularia clavata*, and *Fusarium* sp. and limited to the rainy season is the fungi *Emericella nidulans*, *Leveillula taurica*, and *Melampsora ricini* (Borgohain et al., 2014). Prabakaran et al. (2011) stated that the survey results found leaf surface fungi in medicinal plants such as *Phyllanthus amarus*, *Azadirachta indica* and *Ocimum sanctum* were 10 fungal spices with five genera, including *Aspergillus flavus, Penicillium expansum, Fusarium semitectum* and *Fusarium oxysporum* in *Ocimum sanctum*, whereas *Scopulariopsis* sp. was isolated from the phylloplane of *Phyllanthus amarus* and *Penicillium janthinellum, Aspergillus fumiculosis, Aspergillus* sp., *Curvularia lunata* and *Fusarium moniliforme* were successfully isolated from the *Azadirachta indica* plant. In the present study exophytic fungi sugar-apple tree were isolated and tested for their inhibition activity against *Lasiodiplodia theobromae* (cause of Srikaya fruit rot) *in vitro*.

Materials and methods

Place and time of research

The research was conducted in two places: 1) looking for sick, healthy plant specimens from cocoa planted in Bukit Jimbaran area. 2) Laboratory of Plant Disease Science and Agricultural Biotechnology Laboratory. The study was conducted from April to August 2018.

Isolation of exophytic fungus

Isolation of exophytic fungus can be done by spraying parts of plants (fruit, leaves and stems). The washing water was collected, then in a tube taken, 1 ml and grown into a PDA which had previously been filled with livoploxasin with a concentration of 0.1% (w / v). The water sprayed is then accommodated as much as 50 ml, so that it can be known how many colonies are growing (cfu).

Identification exophytic fungus

The endophytic fungus are exfused then grown on a Petri dish containing the PDA and repeated 5 times. The culture was incubated in a dark room at room temperature (± 27^{o}C). Isolates were identified macroscopically after 3 days to determine colony color and growth rate, and microscopic identification to determine septa in hyphae, spore/conidia and sporangiophore. Fungal identification using reference books (Samson et al., 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; Indrawati et al., 1999).

Inhibitory test of exophytic fungus against pathogens

The exophytic fungi found respectively were tested for their inhibitory resistance to the growth of pathogenic fungi with dual culture techniques (in one Petri dish grown each of a single pathogenic fungus flanked by two exophytic fungi). The inhibitory power can be calculated as follows (Dollar, 2001; Mojica-Marin et al., 2008):

$$\text{Inhibition ability (\%)} = \frac{A - B}{A} \times 100$$

Where:

A = Diameter of *L. theobromae* colony in single culture (mm)
B = Diameter of *L. theobromae* colony in dual culture (mm)

Prevalence exophytic fungus

Determining the prevalence of exophytic fungus was based on the frequency of exophytic fungal isolates found (leaves, stems, flowers and fruit) per Petri dish, divided by all isolates found 100% times. The magnitude of the prevalence of isolates will determine the dominance of exophytic fungi present in healthy sugar-apple plant parts.

Determining diversity and domination indices

The diversity and dominance of contaminant fungi can be determined by calculating the Shannon-

Wiener diversity index (Odum, 1971) and soil microbial dominance calculated by calculating the Simpson index (Pirzan and Pong-Masak, 2008).

(1) Index of fungus diversity

The soil micoflora diversity index is determined by the Shannon-Wiener diversity index by the formula (Odum, 1971):

$$H' = -\sum_{i=1}^{S} Pi \ ln \ Pi.$$

Where:

H' = Diversity index of Shannon-Wiener
S = Number of genera
Pi = ni/N as the proportion of species to i
(ni = total number of individuals total fungus type i, N = total number of individuals in total n)

The criteria used to interpret the diversity of Shannon-Wiener (Ferianita-Fachrul et al., 2005) are: H' value <1, meaning low diversity, H' value 1 - 3 means diversity is moderate and H' value> 3 means diversity pertained high.

(2) Dominance index

The soil microflora dominance index was calculated by calculating Simpson index (Pirzan and Pong-Masak, 2008), with the following formula:

$$C = \sum_{i=1}^{S} Pi^2$$

Where:

C = Simpson index
S = Number of genera
Pi = ni/N as the proportion of species to i
(ni = total number of individuals total fungus type i, N = total number of individuals in total n)

Furthermore, the species dominance index (D) can be calculated by a 1- C formulation (Rad et al., 2009). The criteria used to interpret the dominance of the soil microflora type are: close to 0 = low index or lower domination by one fungi species or no species that extreme dominates other species, close to 1 = large index or tends to be dominated by some fungi species (Pirzan and Pong-Masak, 2008).

Results and discussion

Exophytic fungi

Exophytic fungi derived from fruit, leaves and twigs that were isolated using 1 g of material. The types of fungi found were *Aspergillus* sp., *Aspergillus niger*, *Neurospora* sp., *Fusarium* sp., *Rhizopus* sp., and Mycelia sterilia (Table 1; Fig. 1).

Phylloplane fungus which is on the leaf surface is selected among the fungi to be tested for antifungals facing *Alternaria brassicae* causing leaf spot on cabbage. Colony interaction was demonstrated by *Trichoderma viride* and *Aspergillus flavus* with maximum inhibition of *A. brassicae* (Yadav et al., 2011). According to Borgohain et al. (2014) stated that there were 11 fungi found and 5 fungus species that dominated one of the fungi found in this study were *Aspergillus fumigatus* and *Fusarium* sp.

Table 1. The type of exophytic fungus that origin from fruit, leaves and twig.

No.	Exophytic fungus	Number found (10^3 cfu)
Fruit		
1	*Aspergillus* sp.	3
2	*Aspergillus niger*	9
3	Mycelia sterilia	3
Leaves		
1	*Aspergillus* sp.	6
2	*Aspergillus niger*	6
3	*Neurospora* sp.	3
Twig		
1	*Aspergillus niger*	3
2	*Fusarium* sp.	3
3	*Rhizopus* sp	9

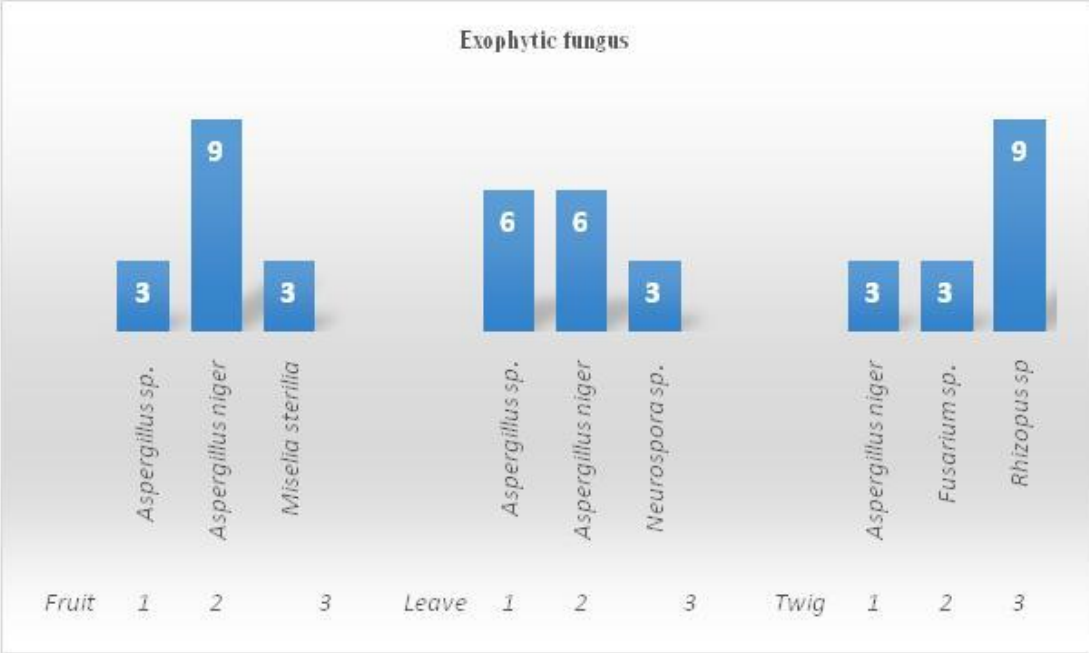

Fig. 1: Exophytic fungus that found on fruit, leave and twig of sugar-apple.

Index of diversity, domination and prevalence

The index of diversity and dominance in exophytic fungi are 2.374 and 0.8667, respectively (Table 2). According to Table 3 which states that the diversity index with a value of <2.4 means that the fungus population is more stable with a good category, and the condition of the community structure is more stable with a scale of 4. According to Table 2, the dominance index approaches 1, meaning that the fungus *A. niger* is dominance with a prevalence of 18% namely fungus *A. niger*.

Table 2. Index of diversity, dominance and prevalence of exophytic fungus (10^3cfu).

No.	Name of fungi	pi	pi/P	LN pi	(pi/P) x ln(pi)	(pi/P)2
1	*Aspergillus* sp.	9	0.2	2.197224577	0.439444915	0.04
2	*Aspergillus niger*	18	0.4	2.890371758	1.156148703	0.16
3	Mycelia sterilia	3	0.066667	2.890371758	0.192691451	0.004444444
4	Neurospora sp.	3	0.066667	1.098612289	0.073240819	0.004444444
5	*Fusarium* sp.	3	0.066667	1.098612289	0.073240819	0.004444444
6	*Rhizopus* sp	9	0.2	2.197224577	0.439444915	0.04
		45		H'=	2.374211623	0.253333333
				D = 1-0.2533 = 0.8667		

Tabl 3. Criteria for assessing environmental quality weighting (Tauruslina et al., 2015).

Index of diversity	Condition of community structure	Category	Scale
>2.41	Very stable	Very good	5
-2.4	More stable	Good	4
1.21 – 1.8	Stable enough	Medium	3
0.61 – 1.2	Less stable	Bad	2
<0.6	Unstable	Very bad	1

Inhibition ability of exophytic fungus *in vitro*

The observation results of *in vitro* inhibitory tests of exophytic fungi ranged from 65.68 ± 0.82% to 82.92 ± 0.50%. The inhibitory mechanism occurs competitively, wherein the pathogenic fungus is pressed for its micelle growth. The biggest inhibition is obtained from *Rhizopus* sp. that was 82.92 ± 0.50% and the lowest is obtained from the fungus *Aspergillus* sp. amounting to 65.68 ± 0.82%, which are derived from exophytic fungus origin from twig 3 and exophytic fungus origin from fruit 1 (Table 4). *Aspergillus niger, Rhizopus oryzae* and *Neurospora sitophila* are starters for the production of amylase and phytase enzymes in tofu pulp media (Kanti, 2017). *Aspergillus niger* is a member of the genus *Aspergillus* which is included in a set of fungi that are generally asexual, although a perfect form has been found. Geographically it is widespread and has been observed in a wide range as saprophyte on dead leaves, stored seeds, compost and decaying vegetation (Sharma, 2012).

Table 4. Inhibition ability of exophytic fungus *in vitro.*

Origin of fungus	Name of fungus	Average of inhibition ability (%)
1. Leaf 3	*Aspergillus niger*	68.64 ± 1.59
2. Leaf 4	*Aspergillus niger*	75.15 ± 2.24
3. Leaf 5	*Neurospora* sp.	74.69 ± 0.72
4. Fruit 1	*Aspergillus* sp.	65.68 ± 0.82
5. Fruit 3	*Aspergillus niger*	72.00 ± 0.31
6. Fruit 5	*Aspergillus niger*	80.71 ± 1.07
7. Twig 2	*Aspergillus niger*	71.31 ± 0.68
8. Twig 3	*Rhizopus* sp.	82.92 ± 0.50
9. Twig 4	*Rhizopus* sp.	76.67 ± 3.27
10. Twig 5	*Rhizopus* sp.	82.22 ± 3.27

Rhizopus sp. was a type that is easily grown on soil, fruit, vegetables and food (Endrawati and Kusumaningtyas, 2017). *Fusarium* was a large genus of filamentous fungi (Sirdariomycetes: Hypocreales: Nectriaceae) including several important plant-producing pathogens from agriculture. Collectively, *Fusarium* disease includes wilt, blight, rot and cancer in some horticultural plants, in fields, ornamental plants and forests. *Fusarium* also produces various secondary metabolites (mycotoxins), such as trichothecenes and fumonisins (Woloshuk and Shim, 2013). Mycelia sterilia has been isolated primarily as endophytes from the host plant range and to answer several questions. Mycelia sterilia has no taxonomic status and cannot be compared between hosts or locations (Naik, 2009).

Conclusion

Based on the results and the discussion above, it can be concluded that the exophytic fungi found in leaves, fruit and twigs are *Aspergillus* sp. *A. niger*, *Fusarium* sp., Mycelia sterilia, Neurospora sp., And Rhizopus sp. The index of diversity and dominance of exophytic fungi is 1.6575 and 0.8667. The antagonistic inhibitory test of the fungi against *Lesiotheobromae theobromae* in vitro, from exophytic fungi ranged from 65.68 ± 0.82% to 82.92 ± 0.50%. The biggest inhibition is obtained from *Rhizopus* sp. that is 82.92 ± 0.50% and the lowest is obtained from the fungus *Aspergillus* sp. amounting to 65.68 ± 0.82%, respectively from exophytic that origin from twig 3 and exophytic that origin from fruit 1 with a competitive inhibition mechanism.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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