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The Potential of Exophytic Microbes in Control Empty Panicle Disease of Rice Plant (*Oryza sativa* L.)

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ABSTRACT
Empty panicle disease found in the study was caused by Fusarium sp. which according to reference is F. moniliformin (the perfect stage is called Gibberella fujikuroi). The microbial colonies of healthy rice leaves found in the predominant fungus were Streptomycetes sp., Aspergillus sp. and Nucordia sp. of 2 x 10^3 cfu, while the dominant fruit exophyte was Aspergillus sp. of 4 x 10^3 cfu, as well as for stem exophytes. Then followed by Miselia sterilia for 3 x 10^3 cfu and Phytophthora sp., Nucordia sp., Fusarium sp. 2 x 10^3 cfu each. The diversity index obtained from the analysis of exophytic microbes was 2.1622 with a dominance index of 0.9230.The highest inhibitory yield of fruit exophytes was Meselia setrilia at 4 dai (days after inoculation), 6, 8 and 10 dai the inhibitory power was 80 ± 0.58% and 96 ± 0.58% at 6 dai but for 8 and 10 dai each inhibitory power is 100%, while for the highest leaf exophytes achieved by Aspergillus sp. at 6, 8 and 10 dai respectively at 100%, but for exophytes in the stem of Penicillium sp. has the highest inhibitory power at 6 dai, while for 8 and 10 dai obtained from microbes Streptomycetes sp., Penicillium sp., and Aspergillus sp. each with a 100% inhibitory power. In vitro inhibition of leaf endophytic microbes against pathogens was only Stigmina sp. and Aspergillus sp. has the highest inhibitory power when 10 dai, respectively 88.89 ± 0.13% and 88.89 ± 0.12%.
KEYWORDS: *empty panicle, exophyte microbes, diversity and dominance index, and inhibition.*

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I. INTRODUCTION

Fusarium disease is common in wet climate rice planting areas in Asia. This disease is known as "Fusarium blight" or Gibberella blight "which can be interpreted as" Fusarium blight ". In Japan the disease is known as "bakanae" because plant growth deviates from normal (Semangun, 1991).This disease has resulted in 20% loss of rice crops when the disease explodes. For example in Japan observed yield loss of 20-50%. In India it reaches 15% and in Thailand it reaches 3.7% (IRRI, 1983). Fusarium disease in Indonesia has been reported since 1938, in that year in Cirebon Untung type rice that was resistant to the disease "Mentek" received severe attacks by Fusarium and Dreschlera fungi (Semangun, 1991).

Plants infected with bakanae show abnormal growth, extension of plants in abnormal nurseries and plants in the field show yellowish green leaf color. The disease can reduce saplings and leaf drying in late infections, and dry seedlings in the early formation of tillers. Further development, partial filling of seeds, empty, or empty seeds until the plants mature (IRRI, 1983).Infected plants are higher than normal in nurseries and in the field, thin plants with yellowish green leaves and pale green flag leaves. Seedlings dry at the beginning of the formation of tillers, then the tillers slightly or decreases and the leaves dry out on subsequent infections. Partial or empty filling or empty grain for survival of the plant until cooking. Infection during nursery in beds found spotting on dead roots that may die before or after transplanting to rice fields (IRRI, 1983).

According to Semangun (1991) Fusarium fungus can attack flowers and seeds, especially young ones. The seeds are light brown or dark and hollow. The fungus can also attack deadly seedlings and pata. In Japan, diseased plants have long, thin and pale stems. These symptoms of bakanae are also found in Thailand. In Indonesia the existence of symptoms like this has never been reported. In 1989 the symptoms of bakanae were widespread in South Kalimantan in the Tajum species. More sick plants; pale and have a length of 1.5-2 times that of healthy plants.

The disease is caused by the fungus *Fusarium moniliforme* (Sheldon) anamorf stage, the synonyms are*: F. fujikuroi* (Sawada), and *F. verticillioides* (Saccardo) Wineland, while *Gibberella fujikuroi* (Sawada) Wollenworth (teleomorphic stadium) which has synonyms, namely: *G. moniliforme* (Winchil). (Sheldon) Wineland, and *Lisea fujikuroi* Sawada (Seneviratne *et al.*, 2004). Pathogenic classification is as follows: class Sordariomycetes, order Hypocreales, Nectriaceae family, genus *Fusarium*, and species *Fusarium fujikuroi* (Sheldon) (Sharma, 2010).

II. MATERIALS AND MATHODE

Place and Time of Research

The study was conducted in two places: 1) looking for specimens of sick and healthy plants from rice plants planted on Jalan Siulan, East Denpasar. 2) Plant Disease Laboratory and Agricultural Biotechnology Laboratory. The study was conducted in January to April 2020.

Eschophytic Microbial Isolation

Exophyte microbes can be isolated by spraying plant parts (fruits, leaves and stems) with sterile water or soaking them, shaking them with sterile water as much as 200 ml volumes. Then the water used as a washing agent can be used as dilution in determining the microbial colonies. The washing water is collected, then in a tube, then taken, from a 1 ml tube, it is grown into a PDA that has been previously filled with livoploxacin with a concentration of 0.1% (w / v).

Identification of Exophytic Microbes

The stored exophyte mushrooms were then grown on a Petri dish containing PDA and repeated 5 times. Culture is incubated in a dark room at room temperature (± 27^{o}C). Isolates were identified macroscopically after 3 days of age to determine colony color and growth rate, and microscopic identification to determine septa in hyphae, spore/conidia and sporangiophores. Fungal identification using the reference book Samson *et al*., 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; and Indrawati *et al*., 1999, and for the identification of Actinomycetes using references Miyadoh *et al*., 2002.

Inhibition Ability Test on Pathogen

The exophyte microbes found were each tested for their inhibition on the growth of pathogenic fungi by the dual culture technique (in one Petri dish each pathogenic fungus was flanked with two endophytic 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 = Pathogenic colonies diameter in a single culture (mm)
B = Pathogenic colony diameter in dual culture (mm)

Prevalence of Exophytic Microbes

Determining the prevalence of exophyte microbial is based on the frequency of exophyte microbial isolates found per Petri dish, divided by all isolates found 100 times. The amount of isolate prevalence will determine the dominance of the rhizosphere microbes.

Determining the Index of Diversity and Domination

The diversity and dominance of exophytic microbes can be determined by calculating the Shannon-Wiener diversity index (Odum, 1971) and the dominance of exophytic microbes is calculated by calculating the Simpson index (Pirzan and Pong-Cook, 2008).

(1) Index of microbial diversity

The index of exophyte microbial diversity was determined by the Shannon-Wiener diversity index by formula (Odum, 1971):

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

Where:
H '= Shannon-Wiener diversity index
S = number of genus
Pi = ni/N as proportion of species i (ni = total number of individuals of total microbial type i, N = total number of individuals in total n)

The criteria used to interpret Shannon-Wiener diversity (Ferianita-Fachrul *et al.,* 2005) namely: H'value <1, means low diversity, H' value 1 - 3 means diversity is classified as moderate and H'value> 3 means diversity is classified high.

(1) Index of dominance

The dominance index of exophyte microbes was calculated by calculating the Simpson index (Pirzan and Pong-Cook, 2008), with the following formula:

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

Where:
C = Simpson index
S = Number of genera
Pi = ni / N, namely the proportion of individuals of type i and all individuals (ni = total number of individuals of type i, N = total number of individuals in total n).

Furthermore, the species dominance index (D) can be calculated by formulation 1- C (Rad *et al.* 2009).

Creteria used to interpret the dominance of soil microbial types, namely: close to 0 = lower index or lower dominance by one microbial species or there are no species that extreme dominate other species, close to 1 = large index or tends to be dominated by several microbial species (Pirzan and Pong-Cook, 2008).

III. RESULTS AND DISCUSSION

Exophytic Microbial Colony

The microbial colonies of healthy rice leaves found in the predominant fungus were *Streptomycetes* sp., Aspergillus sp. and *Nucordia* sp. of 2 x 10^3 cfu, while the dominant fruit exophyte was *Aspergillus* sp. of 4 x 10^3 cfu, as well as for stem exophytes. Then followed by Miselia sterilia for 3 x 10^3 cfu and *Phytophthora* sp., *Nucordia* sp., *Fusarium* sp. each of them was 2 x 10^3 cfu (Table 1; Figure 1).

Table 1. Exophytic microbial colony of leaf, stem and fruit from healthy plant

No.	Leaf exophytic	Colony (cfu)*	Fruit exophytic	Colony (cfu)*	Stem exophytic	Colony (cfu)*
1	*Streptomyces* sp. (Actinmycetes)	2	*Phytophthora* sp.	2	*Aspergillus* sp.	4
2	*Aspergillus* sp.	2	*Aspergillus* sp.	4	Miselia sterilia	3
3	*Micromonospora* sp. (Actinmycetes)	1	*Fusarium* sp.	2	*Nucordia* sp. (Actinomycetes)	2
4	Miselia sterilia	1	*Phytophthora* sp.	1	*Penicillium* sp.	1
5	*Nucordia* sp. (Actinomycetes)	2	Miselia sterilia	2		
6	*Varicosporium* sp.	1				
7	*Neurospora* sp.	1				

*x10^3

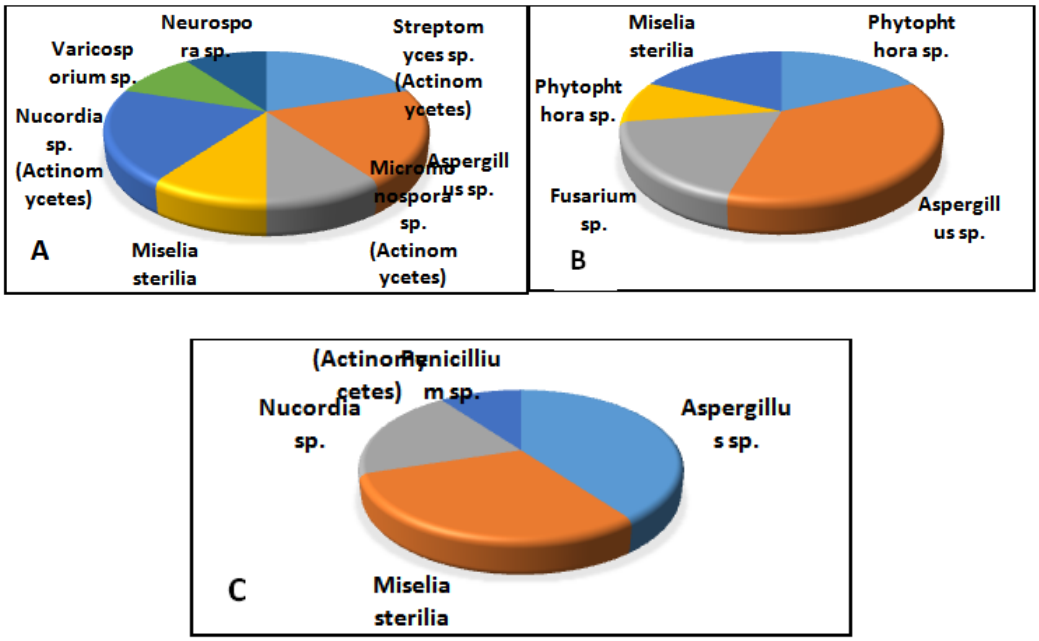

Figure 1.Number of exophytic microbes of leaf (a), (b) fruit exophyticand (c) stem exophytic

Exophyte fungus that dominates in the leaves of vanilla plants by as much as 90% is *Rhizopus* sp., *Aspergillus niger* is only 10%, but in the endophytic leaves and stems that dominate is *Neurospora* sp. respectively 90% and 100% (Suniti and Sudarma, 2016). Exophytic fungi found in leaves, healthy Sugar-apple fruit, which dominated was *A. niger* each as many as 9 isolates, while in healthy branches found that dominated was *Rhizopus* sp. 9 isolates. Endophytic fungi found in healthy fruits and leaves are *Fusarium* sp. each of 6 and 9 isolates and in healthy branches was Miselia sterilia with 9 isolates (Sudarma *et al*., 2019). Selected were A. niger each as many as 9 isolates, whereas in healthy twigs found conflicting was *Rhizopus* sp. A total of 9 isolates. Endophytic fungi found in healthy fruits and leaves are *Fusarium* sp. respectively 6 and 9 isolates and in healthy twigs are Miselia sterilia as many as 9 isolates (Sudarma *et al.*, 2019).

Diversity Index and Domination Index

The diversity index obtained from the analysis of exophytic microbes was 2.1622 with a dominance index of 0.9230. Based on the acquisition of diversity index related to criteria, Table 2 shows that the condition of community structure is more stable with good category and scale 4 (Table 3). While the dominance index is approaching number one, it means that there is one species that dominates, namely *Aspergillus* sp. 32.26% (Table 2).

Table 2. Results of analysis diversity and dominance index in exophytic microbes

Microbes name	pi	pi/P	Ln (pi)	pi/P x Ln(pi/P)	(PI/P)2
Streptomyces sp.	2	0,0645161	0,69314718	0,742769385	0,00416233
Aspergillus sp.	10	0,3225807	2,30258509	0,742769385	0,00104058
Micromonospora sp.	1	0,0322581	0	0	0,00104058
Nucordia sp.	4	0,1290323	1,38629436	0,178876692	0,01664932
Varicosporium sp.	1	0,0322581	0	0	0,00104058
Neurospora sp.	1	0,0322581	0	0	0,00104058
Phytophthora sp.	3	0,0967742	1,09861229	0,106317318	0,00936525
Fusarium sp.	2	0,0645161	0,69314718	0,044719173	0,00416233
Miselia sterilia	6	0,1935484	1,79175947	0,346792155	0,03746098
Penicillium sp.	1	0,0322580	0	0	0,00104058
	31			2,162244108	0,07700312

H diversity index =2,1622, D dominance index = 1-C = 1-0,077003 = 0,9230

Tabel 3. Criteria for weighting environmental quality assessment(Tauruslina *et al*., 2015)

Diversity index	The condition of the community structure	Category	Scale
>2,41	Very stable	Very good	5
-2,4	More stable	good	4
1,21 – 1,8	Pretty stable	Middle	3
0,61 – 1,2	Not stable enough	Bad	2
<0,6	Unstable	Very bad	1

Inhibition Ability of Exophytic Microbes on Pathogen

The highest microbial inhibition obtained was Meselia setrilia at 4 dai (days after inoculation), 6 of them, 8 days after inoculation and 10 dai of inhibitory powers of 80 ± 0.58% and 96 ± 0.58% at 6, however, for 8 and 10 dai each inhibitory power was 100%, respectively (Table 4), whereas for the highest leaf microbes produced by *Aspergillus* sp. at 6, 8 and 10 dai respectively 100% (Table 5), but for microbes in the stem of *Penicillium* sp. has the highest inhibitory power at 6 dai while for 8 and 10 dai obtained from microbes *Streptomycetes* sp., *Penicillium* sp., and *Aspergillus* sp. each with a 100% inhibitory power (Table 6).

Table 4. Inhibition ability of fruit exophytic on pathogen (*A. niger*)

No.	Microbes name	4 dai (%) (control diameter = 3,5 cm)	6 dai (%) (control diameter 5 cm)	8 dai(%) (control diameter 5,5 cm)	10 dai (%) (control diameter 9 cm)
1	*Phytophthora* sp. 1	-	-	-	-
2	*Aspergillus* sp. 1	57.14 ± 0.48	60 ± 0.58	63.64 ± 0.03	77.78 ± 0.01
3	*Phytophthora* sp. 2	28.57 ± 0.02	40 ± 0.58	45.45 ± 0.03	66.67 ± 0.02
4	Miselia sterilia	71.43 ± 0.13	80 ± 0.58	100	100
5	*Fusarium* sp.	65.71 ± 0.06	76 ± 0.58	81.82 ± 0.01	88.89 ± 0.23

6	*Aspergillus* sp. 2	57.14 ± 0.02	60 ± 1.53	63.64 ± 0.02	77.78 ± 0.11
7	Miselia sterilia	71.43 ± 0.05	96 ± 0.58	100	100
8	*Aspergillus* sp. 3	28.57 ± 0.05	40 ± 1.04	45.54 ± 0.05	66.67 ± 0.07
9	*Aspergillus* sp. 4	42.86 ± 0.25	50 ± 0.58	63.64 ± 0.05	77.78 ± 0.02

dai = days after inoculation

Table 5. Inhibition ability of leaf exophytic on pathogen (*A. niger*)

No.	Microbes name	4 dai (%) (control diameter, 3,5 cm)	6 dai (%) (control diameter, 5 cm)	8 dai (%) (control diameter, 5,5 cm)	10 dai (%) (control diameter, 9 cm)
1	Miselia sterilia	71.43 ± 0.1	80 ± 1.0	81.82 ± 0.58	88.89 ± 0.63
2	*Aspergillus* sp. 1	71.43 ± 0.07	100	100	100
3	*Streptomyces* sp.	42.86 ± 0.05	60 ± 0.58	63.64 ± 0.45	77.78 ± 0.26
4	*Phytophthora* sp.	57.14 ± 0.02	70 ± 0.76	81.82 ± 0.05	100
5	*Trichoderma* sp.	71.43 ± 0.01	80 ± 0.61	81.82 ± 0.52	100
6	*Aspergillus* sp. 2	71.43 ± 0.06	70 ± 0.61	100	100
7	*Aspergillus* sp. 3	42.86 ± 0.05	60 ± 0.53	63.64 ± 0.43	77.78 ± 0.32
8	*Tiletiopsis* sp.	-	-	-	-
9	*Micromonospora* sp.	-	-	-	-
10	*Aspergillus* sp. 4	42.86 ± 0.08	100	100	100
11	*Aspergillus* sp. 5	28.57 ± 0.1	50 ± 0.32	54.55 ± 0.33	100
12	*Aspergillus* sp. 6	42.86 ± 0.3	80 ± 1.13	100	100

Dai = days after inoculation

Table 6. Inhibition ability of stem exophytic on pathogen (*A. niger*)

No.	Microbes name	4 dai (%) (control diameter, 3,5 cm)	6 dai (%) (control diameter 5 cm)	8 dai (%) (control diameter 5,5 cm)	10 dai (%) (control diameter 9 cm)
1	*Aspergillus* sp. 1	57.14 ± 0.61	60 ± 0.51	63.64 ± 0.12	77.78 ± 0.08
2	Miselia sterilia	-	-	-	-
3	Miselia sterilia	-	-	-	-
4	*Aspergillus* sp. 2	57.14 ± 0.14	60 ± 0.45	72.73 ± 0.23	83.33 ± 0.09
5	*Sterptomycetes* sp.	71.14 ± 0.17	80 ± 0.12	100	100
6	*Penicillium* sp.	57.14 ± 0.14	100	100	100
7	*Oerskovia turbata*	-	-	-	-
8	Miselia sterilia	-	-	-	-
9	*Aspergillus* sp. 3	65.71 ± 0.07	76 ± 0.14	100	100
10	*Aspergillus* sp. 4	65.71 ± 0.06	80 ± 0.23	81.82 ± 0.25	88.89 ± 0.12
11	*Aspergillus* sp. 5	57.14 ± 0.21	80 ± 0.21	81.82 ± 0.13	100
12	*Aspergillus* sp. 6	42.86 ± 0.49	60 ± 0.12	63.64 ± 0.05	83.33 ± 0.11

Dai = days after inoculation

Sudarma *et al*., (2018) the results of his study showed that the highest inhibitory power of exophytic fungi against pathogens (*Lesiodiplodia theobromae*) in sugar-apple plants showed that *Rhizopus* sp. the highest of 82.22 ± 3.27% was only followed by *A. niger* and *Neurospora* sp. respectively 80.71 ± 1.07% and 74.69 ± 0.72%. Jalander and Gachande (2012) research on phyloplane fungi in medicinal plants is dominated by *Aspergillus* and *Penicillium* fungus.

IV. CONCLUSION

Based on the results and discussion above, it can be concluded as follows: The empty panicle disease found in the study was caused by *Fusarium* sp. which according to reference is *F. moniliformin* (the perfect stage is called *Gibberella fujikuroi*). The microbial colonies of healthy rice leaves found in the predominant fungus were *Streptomycetes* sp., Aspergillus sp. and *Nucordia* sp. of 2 x 10^3 cfu, while the dominant fruit exophyte was *Aspergillus* sp. of 4 x 10^3 cfu, as well as for stem exophytes. Then followed by Miselia sterilia for 3 x 103 cfu and *Phytophthora* sp., *Nucordia* sp., *Fusarium* sp. 2 x 10^3 cfu each.The diversity index obtained from the analysis of exophytic microbes was 2.1622 with a dominance index of 0.9230. The highest inhibitory yield of fruit exophytes was Meselia setrilia at 4 dai (days after inoculation), 6, 8and 10 dai the inhibitory power was 80 ± 0.58% and 96 ± 0.58% at 6 dai but for 8 and 10 dai each inhibitory power is 100%, while for the highest leaf exophytes achieved by *Aspergillus* sp. at 6, 8 and 10 dai respectively at 100%, but for exophytes in the stem of *Penicillium* sp. has the highest inhibitory power at 6 hsi, while for 8 and 10 hsi obtained from microbes *Streptomycetes* sp., *Penicillium* sp., and Aspergillus sp. each with a 100% inhibitory power. In vitro inhibition of leaf endophytic microbes against pathogens was only *Stigmina* sp. and Aspergillus sp. has the highest inhibitory power when 10 dai respectively 88.89 ± 0.13% and 88.89 ± 0.12%.

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