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ENVIRONMENTALLY FRIENDLY CONTROL OF FALSE SMUT DISEASE [USTILAGINOIDEA VIRENS (COOKE) TAKAHASHI] IN RICE

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ABSTRACT

False smut disease in rice plants is caused by the fungus *Ustilagoideia virens* (Cooke) Takahashi, with symptoms of yellow fungal mycelium clustering around the panicles and over time the powder turns black. Exophytic microbes found on leaves have the highest prevalence by *Streptomyces* sp. and *Aspergillus* sp. each with 6 isolates (20%), the highest prevalence of exophytic microbes in bauh was *Phytophthora* sp. as many as 9 isolates (33%) and exophytic microbes on stems with the highest prevalence of *Aspergillus flavus* as many as 15 isolates (33%). The microbial diversity indices of leaf exophytes, fruit exophytes and stem exophytes were 1.887, 1.369 and 1.617, respectively. Evenness uniformity indices for leaf, fruit and stem exophytes were 0.134, 0.244 and 0.136, respectively. The domination indices of leaf, fruit and stem exophytes were 0.84, 0.74 and 0.76, respectively. This means that the diversity is moderate with small evenness between species and high dominance, there are species that dominate as the highest prevalence mentioned above. Exophytic microbial inhibition *in vitro* was obtained on leaf exophytes of only *Aspergillus* sp. by $80 \pm 2\%$ and *Neurospora* sp. by $60 \pm 0.7\%$. In fruit exophytes only *Aspergillus* sp. can inhibit by $70 \pm 0.8\%$, and the highest stem exophytes by *A. flavus* by $80 \pm 0.9\%$, followed by *Rhizopus* sp. by $80 \pm 0.4\%$.

KEYWORDS

False smut disease, prevalence, exophytes, inhibition, diversity index, evenness uniformity index and dominance index.



INTRODUCTION

False smut disease in rice caused by *Ustilaginoidea virens* (Cooke) Takahashi (teleomorph form: *Villosiclava virens*) is an ascomycetes pathogenic fungus. This false smut disease occurs sporadically in rice planting areas (1). Symptoms produced by *U. viren* are seen after flowering only, when the fungus turns individual panicle grains into yellowish balls, which change to yellowish-orange, green, and olive green finally to greenish-black. *U. virens* produces both sexual (ascospores) and asexual (chlamydospore) stages in its life cycle (2). Recently, *Villosiclava virens* has been proposed as a new name for the false scorched mushroom teleomorph (3). Chlamydospores have conspicuously decorated ejaculates and spines. Overall, the results of the investigation indicated that the isolates collected from the false smut were confirmed based on morphological characteristics (4).

Environmentally friendly control of false scorch disease in rice plants is by utilizing exophytic microbes, namely microbes that are on the surface of the plant, either staying temporarily or remaining in symbiosis with the host without causing harm to the host plant (5). Phylloplane fungi are less studied than endophytes, saprobes, and pathogenic fungi. In recent years phylloplane studies have shown interactions with plants, herbivores and pathogens living on leaves, possibly related to the immune system, reabsorption of organic and mineral matter from leachates, redistribution of primary nutrients until leaf fall and participation in primary degradation of plant tissues (6). He found that growing phylloplane fungi such as *Trichoderma viride* and *Aspergillus flavus* could maximum suppress *Alternaria brassicae* on cabbage leaves (7).

Exophytic fungi can control sugar-apple fruit rot (*Annona squamosa* L.) isolated from leaves, fruit and twigs of healthy sugar-apple plants. The exophytic fungi found included *A. niger*, *Fusarium* sp., mycelia sterilia, *Neurospora* sp., and *Rhizopus* sp. with diversity and dominance indices of 2.3742 and 0.8667, respectively. The highest inhibition by exophytic fungi against sugar-apple fruit rot pathogen (*Lasioidiplodia theobromae*) was *Aspergillus* sp. by 100% (8).

MATERIALS AND METHODS

Place and time of research

The research was carried out in two places: 1) looking for sick and healthy panicle specimens from rice fields in Penatih Dangin Puri Village, East Denpasar District, Denpasar City. 2) Laboratory of Plant Diseases and Agricultural Biotechnology Laboratory. The research was conducted from January to May 2023.

Macroscopic Identification of Exophytic Microbes and Pathogens

Symptoms of the disease are observed to be known macroscopically, then followed by looking at the pathogen under a microscope. The optilab tool is used to help see under a microscope with a magnification of 400 x. Likewise to see exophytic microbes.

Exophytic Microbe Isolation

Isolation of exophytic microbes can be done by spraying plant parts (fruits, leaves and stems). The washing water is collected, then in a tube, then taken, from a 1 ml tube grow it into a PDA which has previously been filled with livoploxacin with a concentration of 0.1% (w/v).

Identification of Pathogenic Fungi and Exophytes Microbes

The stored exophytic microbes were then grown in Petri dishes containing PDA and repeated 5 times. The cultures were incubated in the dark at room temperature ($\pm 27^{\circ}\text{C}$). The isolates were identified macroscopically after 3 days of age to determine colony colour and growth rate, and microscopically to identify septa on hyphae, spore/conidia forms and sporangiophores. Identification of fungi using the reference book (9, 10, 11, 12, and 13).

Test of Inhibition of Exophytic Microbes against Pathogen

The endophytic and exophytic fungi found were each tested for their inhibition on the growth of pathogenic fungi using the dual culture technique (one pathogenic fungus was grown in one Petri dish each flanked by two endophytic fungi). The inhibition can be calculated as follows (14 and 15):

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

Where:

A = Diameter of pathogen colony in single culture (mm)

B = Diameter of pathogen colony in dual culture (mm)

Determine the Diversity and Dominance Index

The diversity and dominance of contaminating fungi can be determined by calculating the Shannon-Wiener diversity index (16) and the dominance of soil microbes is calculated by calculating the Simpson index (17).

Relative abundance/prevalence

Relative abundance according to (16) is the percentage of the number of individuals of a species to the total number of individuals present in a certain area in a community and is formulated as follows:

$$\text{KR} = \frac{n_i}{N} \times 100\% \quad \text{Where:}$$

KR = relative abundance

n_i = number of individuals of each i-th species

N = total number of individuals

Microbial diversity index

The diversity index of soil microbes is determined by the Shannon-Wiener diversity index, namely by the formula (16):

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

Where:
 H' = Shannon-Wiener diversity index
 S = Number of genera
 $P_i = n_i/N$ as the proportion of species i (n_i = total number of individuals of total microbial type i , N = number of all individuals in total n)

The criteria for assessing environmental quality can be seen in Table 1.

Table 1. Criteria for assessing environmental quality (18)

Diversity index	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	Currently	3
0,61 – 1,2	Less stable	Bad	2
<0,6	Unstable	Very bad	1

Evenness uniformity index (E)

To find out the balance of the community, the uniformity index is used, which is a measure of the similarity in the number of individuals between species in a community. The more similar the number of individuals between species (the more evenly distributed), the greater the degree of balance. The uniformity index formula (E) is obtained from (19):

$$E = H' / \ln S$$

Where:

H' = diversity index

S = Number of species

E = Evenness uniformity index

The smaller the value of the diversity index (H'), the smaller the uniformity index (E), which indicates the dominance of one species over another.

Here's the range:

E < 0.4: small population uniformity

0.4 < E < 0.6: moderate population uniformity

E > 0.6: high population uniformity

Dominance index

The soil microbial dominance index is calculated by calculating the Simpson index (17):

s

Where:

C = Simpson's 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).

$$C = \sum_{i=1} P_i^2$$

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

The criteria used to interpret the dominance of soil microbial species are: close to 0 = low index or lower domination by one microbial species or no species dominates other species extreme, close to 1 = large index or tends to be dominated by several microbial species (Pirzan and Pong-Cook, 2008).

RESULTS AND DISCUSSION

Population of Exophytic Microbes

The exophytic microbial population on leaves was 7.2×10^5 cfu/ml, fruit exophytic population was 3.8×10^5 cfu/ml, and stem exophytic population was 3.1×10^5 cfu/ml. The number of isolates that could be isolated from leaf exophytes were *Streptomyces* sp., *Aspergillus* sp. and *Nucordia* sp. 6 isolates each, while *Micromonospora* sp., *Miselia sterilia*, *Varicosporium* sp. and *Neurospora* sp., each with 3 isolates. In fruit exophytic microbes, *Phytophthora* sp. was found to be the highest with 9 isolates, *Aspergillus* sp., *Fusarium* sp., and *Miselia sterilia* with 6 isolates each. Stem exophytes found the highest *A. flavus* with 15 isolates, *A. niger* with 12 isolates, *Neurospora* sp., and *Penicillium* sp., with 6 isolates each, *Nucordia* sp. and *Rhizopus* sp. each of 3 isolates (Table 1; Figure 1).

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

No	Exophytic of leaf		Exophytic of fruit		Exophytic of stem	
	Name of microbes	Number of isolates	Name of microbes	Number of isolates	Name of microbes	Number of isolates
1	<i>Streptomyces</i> sp. (Actinomycetes)	6 (20%)*	<i>Phytophthora</i> sp.	9 (33%)	<i>Aspergillus flavus</i>	15 (33%)
2	<i>Aspergillus</i> sp.	6 (20%)	<i>Aspergillus</i> sp.	6 (22%)	<i>Aspergillus niger</i>	12 (27%)
3	<i>Micromonospora</i> sp. (Actinomycetes)	3 (10%)	<i>Fusarium</i> sp.	6 (22%)	<i>Neurospora</i> sp.	6 (13%)
4	<i>Miselia sterilia</i>	3 (10%)	<i>Miselia sterilia</i>	6 (22%)	<i>Nucordia</i> sp. (Actinomycetes)	3 (7%)
5	<i>Nucordia</i> sp. (Actinomycetes)	6 (20%)			<i>Penicillium</i> sp.	6 (13%)
6	<i>Varicosporium</i> sp.	3 (10%)			<i>Rhizopus</i> sp.	3 (7%)
7	<i>Neurospora</i> sp.	3 (10%)				
	Jumlah	30		27		45

*The numbers in brackets indicate the relative abundance (KR) or prevalence of each isolate found

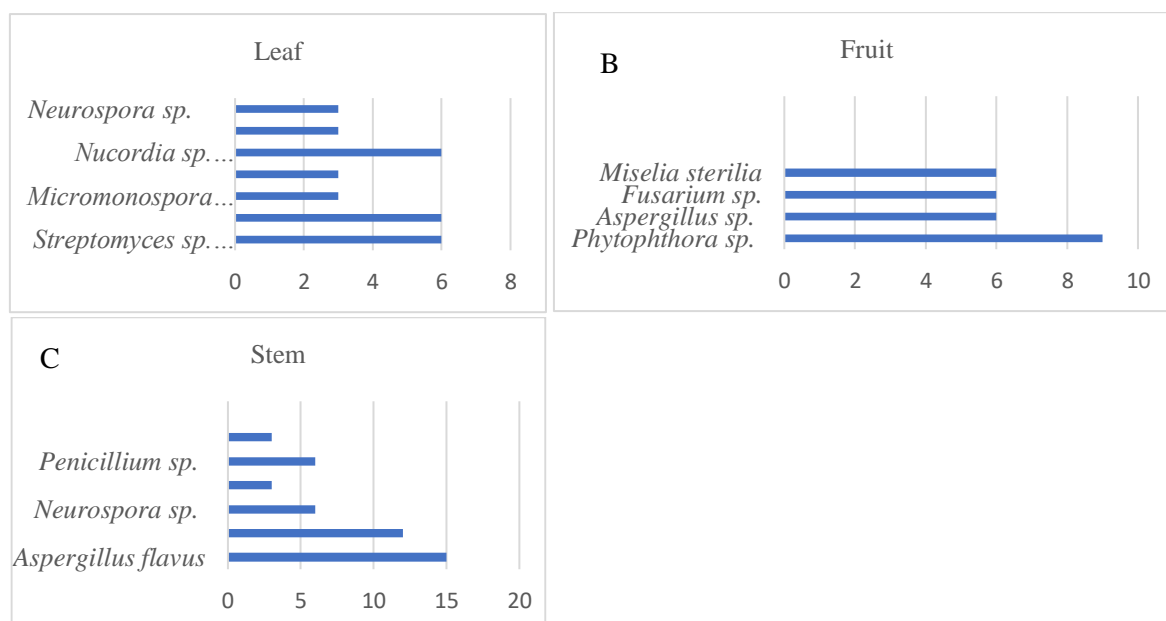


Figure 1. Number of leaves exophytic microbial isolates (A), (B) fruit and (C) stem

In fact, the highest KR leaf exophytes were achieved by *Streptomyces* sp. by 20%, the highest KR fruit exophytes were achieved by the fungus *Phytophthora* sp. by 33% and the highest KR stem exophytes were achieved by *Aspergillus flavus* by 33% (Table 1). It means that these microbes dominate in every occupied habitat.

The number of exophytic microbial isolates found will determine the chance of inhibition of the pathogen. The more isolates will provide opportunities the greater the inhibition that occurs. Exophytic fungi will take advantage of space for growth and are able to compete with pathogens on the surface of leaves, fruit and stems (5). The research found leaf fall disease in rubber caused by the fungus *Corynespora cassiicola* significantly reduced rubber productivity. The number of exophytic microbial isolates found will determine the chance of inhibition of the pathogen. The more isolates will provide opportunities the greater the inhibition that occurs. *C. cassiicola* causes year-round leaf fall, delays in tapping of immature rubber trees, decreased yields of producing plants, and even death of susceptible clones. The research aimed at obtaining phylloplane and endophytic microbes that have the potential to inhibit the disease was carried out from January to December 2016. The study was to evaluate antagonistic fungi and phylloplane and endophytic bacteria against *C. cassiicola* in isolates obtained through exploration in West Java. and West Kalimantan. Pathogen isolates showed *Corynespora* sp to be pale brown in colour, single conidia slightly bent, rod-shaped which was swollen at the base, with 2–14 septa. Inhibition analysis found 42 fungal isolates and 19 bacterial isolates that had the potential to inhibit *C. cassiicola*(20).

Exophytic Microbial Diversity and Dominance Index

Based on the results of the study, it was found for leaf exophytes the diversity index (H') was 1.887, the evenness uniformity index (E) was 0.134 and the dominance index (D) was 0.84; fruit exophytes with an H' value of 1.369, an E value of 0.244 and a D value of 0.74; stem exophytes with H' reaching 1.617, E values of 0.136 and D values of 0.76 (Table 5.3). The diversity index in leaf exophytes shows fairly stable criteria with medium category and scale 3, while E is very small (< 0.4) indicating small population uniformity, so there are species that dominate with a D value close to 1 (> 0.5) meaning leaf exophytes, fruits and stems were dominated by *Streptomyces* sp. (Actinomycetes) by 20%, *Phytophthora* sp. by 33% and *A. flavus* by 33% (Table 2).

Table 2. Diversity index, evenness uniformity index and dominance index of leaf, fruit and stem exophytic microbes

Variable (index)	Exophytic of leaf	Exophytic of fruit	Exophytic of stem
Diversity index (H')	1,887	1,369	1,617
Evenness uniformity index (E)	0,134	0,244	0,136
Dominance index (D)	0,84	0,74	0,76

The smaller the value of diversity as well as the evenness uniformity value means that there are species dominating one over the other. Evidenced by the dominance index value greater than 0.5. The index of diversity in leaf exophytes, fruit exophytes, stem exophytes and leaf endophytes with relatively stable community structure conditions (1.21-1.8), with moderate categories and a scale of 3 (Table 2). The greater the number of species found, the greater the diversity index obtained, so that the loss of one species is still covered by other species, and vice versa.

Inhibition of Exophytic Microbes against Pathogens *in Vitro*

The inhibition of leaf exophytes was only *Aspergillus* sp. and the fungus *Neurospora* sp. can inhibit pathogens by $80 \pm 2\%$ and $60 \pm 0.7\%$, respectively, while fruit exophytic fungi are only *Aspergillus* sp. can inhibit by $70 \pm 0.8\%$. Stem exophytes capable of inhibiting pathogens were *A. flavus* by $80 \pm 0.9\%$, *A. niger* by $76 \pm 0.5\%$, *Neurospora* sp. can inhibit by $74 \pm 0.3\%$ and *Rhizopus* sp. can inhibit by $80 \pm 0.4\%$ (Table 3).

Table 3. Inhibition of leaf, fruit and stem exophytic microbes from healthy plants

No	Exophytic of leaves	Number of isolates	Exophytic fruit	Number of isolates	Exophytic of stems	Number of isolates
1	<i>Streptomyces</i> sp. (Actinomycetes)	-	<i>Phytophthora</i> sp.	-	<i>Aspergillus flavus</i>	$80 \pm 0,9\%$
2	<i>Aspergillus</i> sp.	$80 \pm 2\%$	<i>Aspergillus</i> sp.	$70 \pm 0,8\%$	<i>Aspergillus niger</i>	$76 \pm 0,5\%$
3	<i>Micromonospora</i> sp. (Actinomycetes)	-	<i>Fusarium</i> sp.	-	<i>Neurospora</i> sp.	$80 \pm 1,0\%$
4	<i>Miselia sterilia</i>	-	<i>Miselia sterilia</i>	-	<i>Nucordia</i> sp. (Actinomycetes)	-
5	<i>Nucordia</i> sp. (Actinomycetes)	-			<i>Penicillium</i> sp.	$74 \pm 0,3\%$
6	<i>Varicosporium</i> sp.	-			<i>Rhizopus</i> sp.	$80 \pm 0,4\%$
7	<i>Neurospora</i> sp.	$60 \pm 0,7\%$				

He stated that the results of *in vitro* screening using multiple culture techniques were carried out to assess the potential of three species of *Aspergillus*, *A. fumigatus*, *A. repens* and *A. niger* as biological control agents against *Phytophthora palmivora*, a cacao pod disease pathogen. The test organism was isolated from the same cocoa farm where the disease occurred. The results revealed that all the test antagonists effectively inhibited the growth of the pathogen (20).

The test antagonist grew faster than the pathogen and produced an inhibition zone thereby limiting the growth of the pathogen. In solid media, *A. repens* was the most antagonistic organism under the conditions of this study. The test mushroom culture filtrate also inhibited the growth of *P. palmivora* with *A. niger* showing the highest percentage of inhibition (54%) and *A. repens* the least (44.5%) (20).

The inhibition that occurs is all competition for space and nutrition, bearing in mind that there is no inhibition zone in the form of a clear layer so that no antibiotic inhibition is found. The results revealed that in multiple cultures, the test antagonist effectively examined the growth of *P. palmivora*. Antagonists grow faster than pathogens and produce zones of inhibition. In the liquid media test, the test mushroom culture filtrate also inhibited the growth of *P. palmivora*. *Rhizopus stolonifera* is slightly more efficient (45%) than *Paecilomyces* sp. (43%) in inhibiting the growth of *P. palmivora* (20).

The false scorch pathogen in rice produces mycotoxins, including ustiloxin and ustilaginoidin. Ustiloxins contain 13-membered cyclic core structures with phenol ether linkages, including ustiloxins A, B, C, D, E, F and G in *U. virens*. Ustiloxin from *U. virens* inhibits microtubule assembly and the framework of eukaryotic cell formation. Ustilaginoidin is a class of bis-naphtho- γ -pyrones, which has cytotoxic activity on cancer cells and an inhibitory effect on radicle elongation of rice seeds. To date,

26 ustilaginoidins, have been identified in *U. virens*. Moreover, several analytical methods have been established, such as high performances liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), enzyme linked immunosorbent assay (ELISA), and lateral flow immunoassays (LFIA) to detect or measuring *U. virens* mycotoxins. Although many mycotoxins have been identified from *U. virens*, at least five aspects still require further exploration, including the activity and toxicity of each mycotoxin in humans or animals, the function of mycotoxins during infection from *U. virens*, differences in mycotoxins in *U. virens* isolates different, the molecular mechanism of mycotoxin synthesis in *U. virens* and a new type of mycotoxin besides ustiloxin and ustilaginoidin (1).

CONCLUSION

False smut disease is caused by *Ustilagoidea virens* (Cooke) Takahashi, with symptoms of yellow powder clustered covering the rice panicles which gradually turn black. There were 30, 27 and 45 isolates of exophytic fungi found on leaves, fruit and stems, with the highest prevalence on leaves, namely *Streptomyces* sp. and *Aspergillus* sp. 6 isolates each with a prevalence of 20% in *Phytophthora* sp. the prevalence was 9 isolates (33%) and in stems, namely *Aspergillus flavus*, 15 isolates (33%). In leaf exophytes the diversity index (H') was 1.887, the evenness uniformity index (E) was 0.134 and the dominance index (D) was 0.84; fruit exophytes with an H' value of 1.369, an E value of 0.244 and a D value of 0.74; stem exophytes with H' reached 1.617, E value of 0.136 and D value of 0.76. The inhibition of leaf exophytes was only *Aspergillus* sp. and the fungus *Neurospora* sp. can inhibit pathogens by $80 \pm 2\%$ and $60 \pm 0.7\%$, respectively, while fruit exophytic fungi are only *Aspergillus* sp. can inhibit by $70 \pm 0.8\%$. Stem exophytes capable of inhibiting pathogens were *A. flavus* by $80 \pm 0.9\%$, *A. niger* by $76 \pm 0.5\%$, *Neurospora* sp. can inhibit by $74 \pm 0.3\%$ and *Rhizopus* sp. can inhibit by $80 \pm 0.4\%$.

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