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Utilization of Rhizosphere Microbes to Control Empty Panicle Disease in Rice Plants (*Oryza sativa* L.) *invitro*

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A B S T R A C T

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 rhizosphere fungus which was successfully isolated and dominated was *Penicillium* sp. the rhizosphere microbial diversity index was 0.5517 with a dominance index dominance of 0.8264.The inhibition of rhizosphere fungi against pathogens (*Fusarium* sp.) *A. flavus* has the highest inhibition of 88.89 ± 0.09%, followed by *Penicillium* sp.2 at 88.89 ± 0.08%, *Penicillium* sp. 4 was 88.89 ± 0.05, *Penicillium* sp. 3 was the same as *Penicillium* sp. 5 each with inhibition of 88.89 ± 0.04% and the lowest *Streptomyces* sp. 1 of 77.78 ± 0.01% at 10 dai (days after inoculation).

Introduction

Fusarium disease is common in wet climates in Asia. This disease is known as "Fusarium blight" or “Gibberella blight "which can be interpreted as "Fusarium blight". In Japan, this disease is known as "bakanae" because plant growth deviates from normal (Semangun, 1991).

This disease has resulted in a rice yield loss of up to 20% when the disease explodes. For example in Japan, yield losses of up to 20-50% were observed. 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 the Untung rice type which was resistant to "mentek" was severely attacked by the fungus Fusarium and Dreschlera (Semangun, 1991).

Bekanae disease comes from Japanese which means "Foolish seedling". Bakanae disease in rice is known to occur in almost all rice plantations in the world. This disease was first recognized in 1828 but in 1898 it was certified

by Shotaro Hori that the disease was caused by a fungus. In the United States it was observed in 1999. Until 1890 it was believed that this disease was caused by *Fusarium moniliformis*, while later that the disease was caused by other species of Fusraium fungi, among them *Fusarium fujikori* which was responsible for these symptoms.

It is interesting to note that some Fusarium species are associated with diseased plants but do not contribute to the development of disease symptoms. *Fusarium fujikori* has a number of hosts scattered throughout the world (Naeem *et al.*, 2016).

The causative agent of bakanae "*Fusarium fujikuroi*" produced secondary metabolite fusaric acid and gibberellic acid (GA) which produced higher plantlets in the field with empty panicles.

For the management of this disease a variety of physical, chemical and biological methods are used, chemical methods are preferred over other methods due to the complexity of the disease and the wider range of pathogenic hosts.

In recent years, the incidence of bakanae has increased from season to season in Punjab and Haryana. The development and application of appropriate management techniques will be a strong challenge in the future in crop-based cropping systems in various places in Punjab and Haryana (Katoch *et al.*, 2019).

Materilas and Methods

Place and time of research

The research was carried out in two places: 1) looking for specimens of sick and healthy plants from rice plants grown on Jalan Siulan, East Denpasar. 2) Plant Disease Science Laboratory and Agricultural Biotechnology Laboratory. The research was conducted from January to March 2020.

Rhizosphere Fungi Isolation

Isoilation of rhizosphere fungi, take 1 gram of soil from the rhizosphere of healthy rice plants, then do the dilution level up to 10-3.

After that the colony is calculated by using the cfu (colony forming unit) unit. After 2 days, the colonies were separated into Petri dishes which had previously been filled with anti-bacterial antibiotics, namely livoploxasin with a concentration of 0.1% (w/v).

After 3 days the fungus is ready to be identified by micrococopic which previously tested the inhibition of rhizosphere fungi with pathogens and looking for the best fungi to be used as antagonists.

Rhizosphere Microbial Identification

The stored rhizosphere fungi were then grown on a Petri dish containing PDA and repeated 5 times. The cultures were 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 shape and sporangiophores.

Identification of fungi using the reference book Samson *et al.*, 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; and Indrawati *et al.*, 1999.

Determine the Diversity Index and the Domination Index

The diversity and dominance of rhizosphere microbes can be determined by calculating the Shannon-Wiener diversity index (Odum, 1971) and the dominance of rhizosphere

microbes is calculated by calculating the Simpson index (Pirzan and Pong-Masak, 2008).

Index of rhizosphere microbial diversity

The rhizosphere microbial diversity index is determined by the Shannon-Wiener diversity index, namely the formula (Odum, 1971):

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

Where:

H '= Shannon-Wiener diversity index

S = Number of genera

Pi = ni/N as the proportion of species i (ni = total number of individuals microbial species i,

N = number of all individuals in total n)

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

Dominance index

The rhizosphere microbial dominance index is calculated by calculating the 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, namely the proportion of individuals of type i and all individuals (ni = total number of individuals of type i,

N = number of individuals in total n)

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

The criteria used to interpret the dominance of soil rhizosphere microbial types are: close to 0 = lower index or lower dominance by one rhizosphere microbial species or there are no species that dominate other species, close to 1 = large index or tend to be dominated by several rhizosphere microbial species (Pirzan and Pong-Masak, 2008).

Prevalence

Prevalence can be calculated by dividing the number of a given rhizosphere microbial population divided by the entire population times 100%.

Inhibition Test against Pathogens

Each of the rhizosphere microbes found were tested for their inhibitory power against the growth of pathogenic fungi using a dual culture technique (in one Petri dish, each one of the pathogenic fungi was grown flanked by two rhizosphere fungi).

The inhibition power can be calculated as follows (Dollar, 2001; Mojica-Marin *et al.*, 2008):

Inhibition ability = A-B/A x 100%. (A = Colony diameter of patogen in single culture (mm) and B = colony diameter of pathogen in dual culture (mm).

Results and Discussion

Diseases Study

Symptom

Diseases that are seen in growing panicles from flag leaves show emptiness, panicles appear to grow straight with empty grains (not filled). The empty panicle (hollow) is a sign that there is a clogged food channel from the root to the stem, and from the stem to the panicle. Upon close observation, it appears that there is a brownish colour at the base of the panicle attached to the stem (Figure 1). The base of the sliced stems (Figure 1B; left) was sliced and then cultured in a Petri dish filled with PDA, after 2 days white mycelium grew (Figure 2).

Figure 1. Symptoms of disease with empty panicles (arrows) (A), and (B) brown panicles (arrows) at the base of the panicles (left) and healthy panicles (right) (personal source)

Cause of disease

Disease caused by *Fusarium* sp. based on microscopic observations found crescent-shaped macroconidia with a size of 5-10 x 20-40 µm (Figure 2B). Mycelium is white with a little orange colour in the middle, it is a sign that the mycelium is Fusarium (Figure 2A).

Rhizosphere Microbial Colonies and Prevalence

The rhizosphere fungi that were isolated were *Penicillium* sp., 3 x 10^3cfu, followed by *Streptomyces* sp. (Actinimycetes) and A. flavus each as much as $2x10^3$cfu, and *Aspergillus* sp., *A. nidulan*, *A. niger*, and *Nicordia* sp., (Actinomycetes) each as much as $1x10^3$cfu. The highest prevalence was obtained from *Penicillium* sp. with a value of 27.27% (Table 2; Figure 3).

Diversity Index and Domination Index

The rhizosphere microbial diversity index was 0.5517 with a dominance index of 0.8264 (Table 3). The diversity index obtained according to the criteria of Table 1, it was found that the condition of the community structure was unstable, with a very bad category with a scale of 1 (Table 1). While the index of dominance is close to one, this means that there are species that dominate, namely *Penicillium* sp. amounted to 27.27% (Table 2).

Inhibition Ability of Rhizosphere against Pathogen

Inhibition of rhizosphere fungi against pathogens (*Fusarium* sp.) All have inhibition against pathogens except *Nucordia* sp. at 10 hsi*A. flavus* had the highest inhibitory power of 88.89 ± 0.09%, followed by *Penicillium* sp. 2 of 88.89 ± 0.08%, *Penicillium* sp. 4 of 88.89 ± 0.05%, and *Penicillium* sp. 3 of 88.89 ± 0.04% (Table 4).

The disease caused by Fusarium in rice is bekanae, this disease was studied in 1828 and named in 1898 which is believed to be caused by *Fusarium moniliformin* (its perfect stage is called *Gibberella fujikuroi*) but other Fusariums were also found to cause this disease (Naeem *et al.,* 2016).

Bekanae disease, also known as "Foolish seedling disease", appears as an important disease that causes significant disease in rice plants in the world (Katoch *et al.,* 2019).

Bekanae disease can reduce the yield from 3.0 to 95.4% and the percentage of disease varies according to the cultivar planted in the region in Asia. One of the problems with rice cultivation, especially in India for several years now and is a more serious threat to sustainable rice production in other parts of the world rice cultivation (Gupta *et al.,* 2015).

Table.1 Environmental quality weighted assessment criteria (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	Moderate	3
0,61 – 1,2	Less stable	Bad	2
<0,6	Unstable	Very bad	1

Table.2 Diversity index and dominance index of rhizosphere microbial

Name of microbials	Population	pi/P	Ln (pi)	pi/P x ln (pi/P)	$(pi/P)^2$
***Penicillium* sp.**	3	0.2727273	1.09861229	0.299621533	0.07438017
***Aspergillus* sp.**	1	0.0909091	0	0	0.00826446
A. nidulans	1	0.0909091	0	0	0.00826446
***Streptomyces* sp. (Actinomycetes)**	2	0.1818182	0.69314718	0.12602676	0.03305785
A. niger	1	0.0909091	0	0	0.00826446
A. flavus	2	0.1818182	0.69314718	0.12602676	0.03305785
***Nucordia*sp. (Actinomycetes)**	1	0.0909091	0	0	0.00826446
	11			0.551675053	0.17355372

H = diversity index 0.55168 and dominance index 1-0.1736 = 0.8264

Table.3 Inhibition ability of rhizosphere microbial against pathogen

No	Name of rhizospehere microbials	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	*Penicillium* sp.1	40±0.12	50±0.08	66.67±0.09	77.78±0.09
2	*Nocardia* sp.	-	-	-	-
3	*Penicillium* sp.2	40±0.08	50±0.03	83.33±0.08	88.89±0.08
4	*A. flavus* 1	60±0.11	75±0.04	83.33±0.08	88.89±0.09
5	*Streptomyces* sp. 1	40±0.09	50±0.05	66.67±0.07	77.78±0.01
6	*Penicillium* sp. 3	60±0.03	75±0.06	83.33±0.03	88.89±0.04
7	*Penicillium* sp. 4	40±0.05	63±0.12	83.33±0.04	88.89±0.05
8	*Streptomyces* sp. 2	40±0.08	63±0.13	75±0.05	83.33±0.06
9	*Aspergillus* sp. 1	40±0.12	63±0.14	75±0.12	83.33±0.05
10	*Aspergillus* sp. 2	60±0.09	75±0.11	83.33±0.11	88.89±0.02
11	*A. flavus* 2	60±0.11	63±0.15	75±0.12	83.33±0.01
12	*Penicillium* sp.5	40±0.13	50±0.08	83.33±0.18	88.89±0.04

dai = days after inoculation

Fig.1 Symptoms of disease with empty panicles (arrows) (A), and (B) brown panicles (arrows) at the base of the panicles (left) and healthy panicles (right) (personal source)

Fig.2 Brown panicle stalks were sliced and then placed in a Petri dish containing PDA (A) media, and (B) the fungal macromonidia *Fusarium* sp.

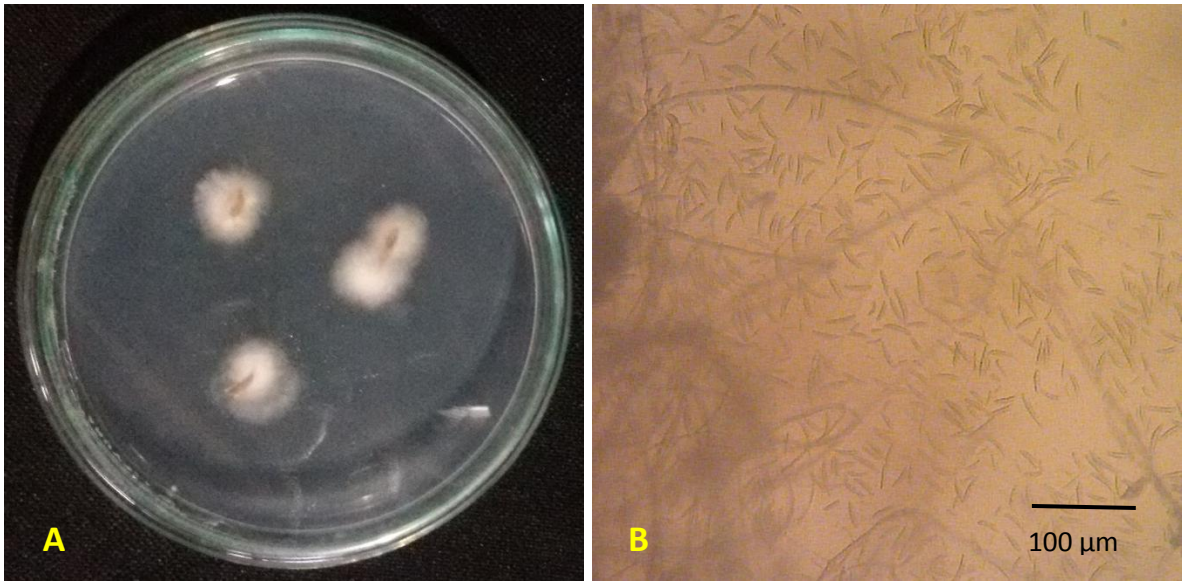
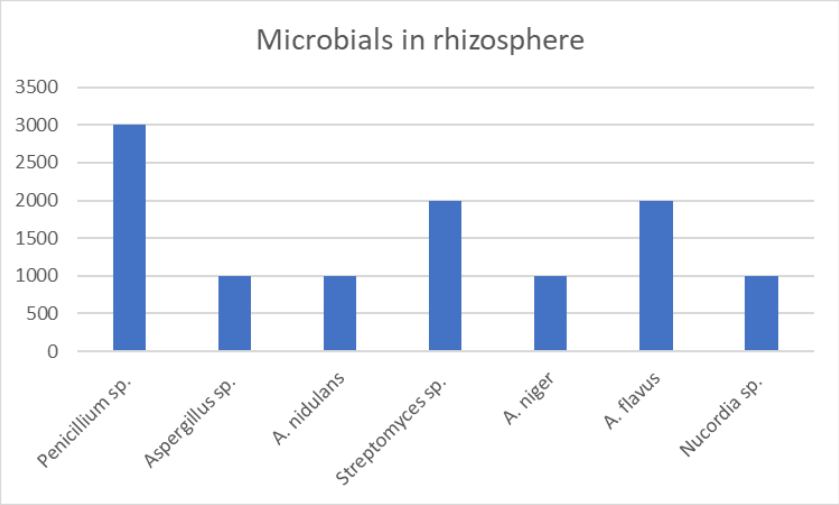


Fig.3 Number of microbial colonies in healthy rice plant rhizosphere



The rhizosphere fungi found in the roots of healthy chilies were *Penicillium digutatum* of 30 x 10^3cfu, followed by *Penicillium expansum* of 12 x 10^3cfu, 6 x 10^3cfu of *Trichoderma harzianum,* then *A. nidulan, A, niger*, *Penicillium* sp., And *T. virens* each as much as 3 x 10^3cfu (Sudarma*et al.*, 2015). *Penicillium* and *Aspergillus* are fungi that exist and spread throughout the world in the soil, and have always been there. This fungus produces a lot of spores so that it is easily carried away by wind and other vectors.

The highest inhibition of rhizosphere fungi against pathogens (*Phytophthora capsici*) was achieved by *Aspergillus* sp. amounting to 75.93 ± 2.62% (Sudarma *et al.*, 2019). The results of Vasanthakumari and Shivanna's (2011) research on rhizosphere fungi that were most commonly found were *Aspergillus, Chaetomium, Penicillium* and *Trichoderma* fungi on grass roots. The diversity of microbes and types in the rhizosphere largely determines the interactions between microbes that help plant health and quality, has implications for the success of suppressing disease (Garbeva *et al.*, 2004).

Based on the results and discussion above, it can be concluded as follows: Empty panicle disease found in the study was caused by *Fu*sarium sp. which according to reference is *F. moniliformin* (the perfect stage is called *Gibberella fujikuroi*). The rhizosphere fungi that were isolated were *Penicillium* sp., 3 x 10^3cfu, followed by *Streptomyces* sp. (Actinimycetes) and *A. flavus* each as much as 2x103 cfu, and *Aspergillus* sp., *A. nidulan, A. niger*, and *Nicordia* sp., (Actinomycetes) each as much as 1x10^3cfu. The rhizosphere microbial diversity index was 0.5517 with a dominance index of 0.8264. Inhibition of rhizosphere fungi against pathogens (*Fusarium* sp.)

All have inhibition against pathogens except *Nucordia* sp. at 10 dai *A. flavus* had the highest inhibitory power of 88.89 ± 0.09%, followed by *Penicillium* sp. 2 of 88.89 ± 0.08%, *Penicillium* sp. 4 of 88.89 ± 0.05%, and *Penicillium* sp. 3 amounted to 88.89 ± 0.04% at 10 days after inoculation.

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