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Identification of Endophytic Fungi on Healthy and Disease Stem of Vanilla Plants (*Vanilla planifolia*) Caused by Stem Rot Disease

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ABSTRACT

One of the plantation crops with high economic value is vanilla. The biggest constraint in vanilla cultivation is stem rot disease. Endophytic fungi are fungi that live inside healthy plant tissues without causing symptoms or damage to the host plant. This study was aimed at determining the percentage of vanilla stem rot disease caused by the fungus Fusarium oxysporum f.sp. vanilla, identifying and selecting the type and amount of endophytic fungi found in healthy vanilla plants and vanilla plants with mild, moderate, and severe symptoms of stem rot. The research was carried out in a vanilla plantation belonging to the residents of Sinogo Village, West Plono Village, and West Ngalian Samigaluh Village, Kulon Progo, Yogyakarta from January to March 2021. The research method used was a descriptive method for symptoms of the disease. A survey and collection of healthy stems and stem rot disease were then conducted. In addition, environmental conditions such as air temperature, humidity, sunlight intensity, and soil pH were also recorded. Identification of endophytic fungi was carried out in the laboratory. The data were analyzed both quantitatively (percentage of disease attack) and qualitatively (morphological characteristics of fungi macroscopically and microscopically). The results showed that the percentage of vanilla stem rot disease from the largest to the smallest was 30% in West Plono Village, 25.3% in West Ngalian Village, and 22.8% in Sinogo Village. In healthy vanilla plants, there are 4 types of endophytic fungi: Aspergillus niger, Cladosporium sp., Penicillium sp., and Trichoderma sp. In vanilla stem rot disease, 2 types of endophytic fungi were found, Aspergillus niger and Cladosporium sp.

Keywords*: stem rot, Fusarium oxysporum f.sp. vanilla, endophytic fungus, vanilla*

1. INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is one of the important commodity crops because the fruit has high economic value and can be exported to increase the country's foreign exchange. The fruit can be used as a mixture of drinks and food. Vanilla is cultivated in various countries, one of which is Indonesia (Kartikawati & Rosman, 2018).

In Indonesia, vanilla plantations are cultivated by farmers. The area of vanilla plantations in Indonesia in 2019 was 9,532 ha with a production of 1,461 tons and a productivity of 376 tons/ha. In 2019, the volume of vanilla exported was 261 tons, with a value of $69,610 (Ditjenbun, 2020).

In Indonesia, vanilla plantations are found in Sumatra, Java, Nusa Tenggara, Bali, Sulawesi, and Papua (Ditjenbun, 2020). In Java, one of the locations for vanilla plantations is in Samigaluh District, Kulon Progo Regency, Special Region of Yogyakarta. Local farmers believe that growing vanilla is economically profitable because the price of wet vanilla is Rp. 300,000–Rp. 500,000 per kg and dry vanilla is Rp. 500,000–Rp. 2,000,000 per kg.

One of the main diseases that become obstacles in vanilla cultivation is stem rot, which is caused by the fungus Fusarium oxysporum f.sp. vanillae (Astuti et al., 2021).

The current control of stem rot is the use of chemical fungicides, as well as control with antagonistic agents, namely the non-pathogenic Fusarium oxysporum and Pseudomonas fluorescens (Pinaria, 2020). One alternative is biological control using endophytic fungi which are antagonistic. Endophytic fungi are fungi that live in healthy plant tissues without causing symptoms or damage to the host plant (Wen et al., 2022).

Isolation of fungi can also be taken from the rhizosphere samples of shallot plants. The fungi found were the genus Fusarium, Aspergillus, Trichoderma, Penicillium, and Gliocladium (Hikmahwati et al., 2022).

In addition to endophytic fungi, plants also contain endophytic bacteria, which can suppress the growth of pathogenic fungi (Andriani & Oktafiyanto, 2019).

Research on the identification of endophytic fungi in vanilla plants and the mechanism of their antagonism against the fungus F. oxysporum f. sp. vanillae has been carried out by (Sudantha & Abadi, 2007). The vanilla plant samples used were from East Lombok, Central Lombok, and West Lombok. There has never been a report on the intensity of vanilla stem rot disease and endophytic fungus on healthy plants and stem rot disease caused by Fusarium oxysporum f.sp. vanilla in Samigaluh District, Kulon Progo Regency, Yogyakarta Special Region. The purpose of this study was to determine the percentage of vanilla stem rot disease caused by the fungus Fusarium oxysporum f.sp. vanillae in the garden, to identify morphologically the endophytic fungi found in healthy and diseased vanilla plants, and to determine the number and types of endophytic fungi found in healthy and diseased vanilla plants. It is hoped that this research will provide an explanation for this.

2. RESEARCH METHOD

This research was conducted through several stages (figure 1) from January to March 2021.
2021.

Figure 1. Research Flow Chart

A survey of the area was undertaken and permission was requested before choosing the research location. The locations chosen were vanilla plantations in Sinogo village, West Plono Village, and West Ngalian village, Kec. Samigaluh, Kab. Kulon Progo, Special Region of Yogyakarta. The selection of observation locations was carried out by purposive sampling. Plant samples (stems) taken are 3-5 years old. At each location, 5 plants were selected that had been determined at the beginning. The stem pieces from each plant were put in a plastic bag, then taken to the laboratory at the Yogyakarta STIPER Agricultural Institute campus.

The medium used to grow endophytic fungi is PDA (Potato Dextrose Agar). PDA was made by mixing 39 g of instant PDA powder with 1,000 ml of sterile distilled water, then cooking in an electric bath until boiling while stirring slowly. The media that has been homogeneous, wait until it is warm to add chloramphenicol as an antibiotic and check the pH. The media was divided into test tubes. Each tube contains 15 ml. The test tubes were sterilized using an autoclave for 15 minutes at 121˚C. The medium in the sterilized test tube was then poured into a petri dish. Media pouring is done in a Laminar Air Flow Cabinet.

From both healthy and sick vanilla stems, endophytic fungi were identified. This work was conducted in a laminar airflow cabinet. Surface-sterilized the vanilla stems three times: once with sterile distilled water, once with Clorox for one minute, and once with 70% alcohol for 30 seconds. Each stem was surface cleaned, the bark was scraped to make the remaining stems clean, and the stems were then put on PDA medium in a petri. Label paper was also used to label the petri.

Observations were made on colony diameter, colony color, colony shape, hyphae shape, hyphal color, spore form, sporangium, conidia, and conidiophores. Identification was carried out by matching all morphological data to ensure that stem rot was caused by the fungus Fusarium oxysporum. From healthy stems and stem rot disease, endophytic fungi are expected to be found.

Observation parameters are

1. Percentage of stem rot disease.
2. Colony morphology of F. oxysporum and endophytic fungi both macroscopically and microscopically

Data on the percentage of stem rot disease was analyzed quantitatively. The morphology of endophytic fungi and F. oxysporum was analyzed qualitatively. Qualitative analysis of the results of macroscopic observations included the speed of colony growth, the color of the upper and lower surfaces of the colonies, the shape of the colonies, and the type of the surface of the colonies. Microscopic observations included the shape of the hyphae and the presence or absence of spores and conidia.

3. RESULTS AND DISCUSSION

Surveys of vanilla plants affected by stem rot were carried out in three different locations; Sinogo, West Plono, and West Ngalian villages, Samigaluh, Kulon Progo district, Special Region of Yogyakarta. Healthy vanilla plants have hard green stems with stem segments that measure between 10–15 cm with a diameter of 2 cm. In each segment of the stem, roots appear that function to attach to the enforcement plants and roots that hang in the air. The leaves are green, the plants are fresh and do not show brownish or wilted spots with the growth of the branches looking good. Vanilla plants that are attacked by stem rot have brown to blackish spots, a small stem diameter of ± 0.1-15 cm, wilted plants, yellowing leaves, and even some plants on the land are stunted (dwarf). Sick vanilla plants with mild symptoms have brownish spots with a diameter of ± 0.1 – 0.6 cm, moderate symptoms ± 0.6 – 3 cm, and severe symptoms ± 3 cm and above. Healthy and

sick vanilla plants are presented in Figure 2.

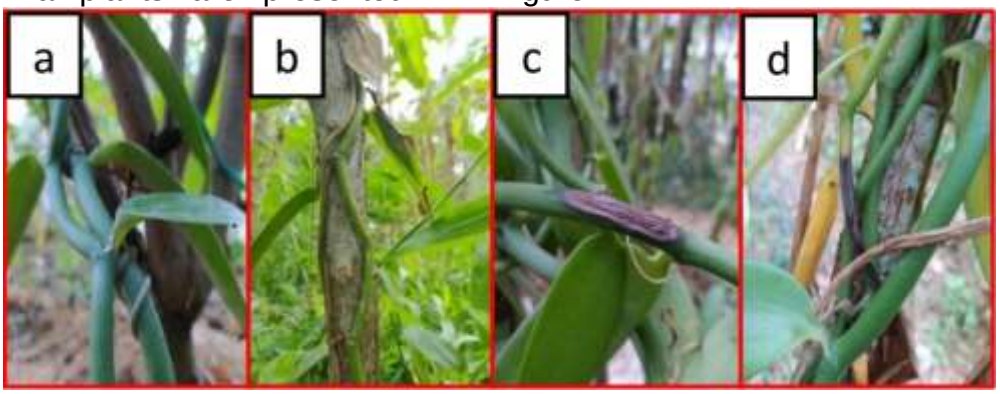

Figure 2. A healthy vanilla plant with stem rot disease. Description: a. healthy; b. mild diseased; c. moderate diseased; d. serious diseased

Data on the percentage of stem rot disease at three different locations are presented in table 1.

Table 1. Percentage of vanilla stem rot disease

Location	Healthy	Stem rot disease (%)		
		Diseased		
		Mild	Moderate	Serious
Sinogo Village	77,1	3,2	14,0	5,7
West Plono Village	69,3	6,0	9,6	15,0
West Ngalian Village	74,8	3,0	10,0	12,2

Table 1 shows that the intensity of vanilla stem rot attack was greatest in West Plono village, with 6% mild disease, 9.6% moderate disease, and 15% severe disease. In addition to observing the intensity of the attack, the environmental conditions of the vanilla plantation were also observed. The measurement results are presented in Table 2.

Table 2. Data on environmental conditions of vanilla plantation

Location	Altitude Place (m dpl)	Air Temperature (℃)	Humidity (%)	Sun Intensity (Lux)	Soil pH
Sinogo Village	394	29,5	63	76.400	5,5 - 7
West Plono Village	563	27,8	71	80.800	4,5 - 7
West Ngalian Village	557	29,5	76	18.650	6 - 7

The occurrence of disease in plants, including vanilla plants, usually follows the concept of the disease triangle. The components of the disease triangle are the host plant, the pathogen, and the environmental conditions. Plants can get sick if they are weak and vulnerable. Pathogens that attack plants are virulent or virulent (Sopialena, 2017).

Environmental conditions that support the growth and development of pathogens actually inhibit the growth and development of host plants, such as high humidity and acid soil pH. Table 2 shows that the environmental conditions in West

Plono Village were favorable for the growth of Fusarium fungus. This was evidenced by the pH of the soil under acidic conditions which was 4.5.

The research of (Supriadi et al., 2014) showed that Agro-climatic factors influenced the growth and yield components of vanilla plants significantly. Vanilla plants develop generative growth and have better yield components at an altitude of 825 m above sea level, whereas vegetative growth has the reverse effect. Microclimate conditions, especially the intensity of sunlight, air temperature, and soil nutrients, are thought to be the cause of these differences.

To ensure that the cause of vanilla stem rot disease is due to Fusarium fungus, it is identified macroscopically and microscopically. Based on (Sudantha & Abadi, 2007) and Pinaria (2020) It was proven that the fungus causing stem rot in this study was the fungus Fusarium oxysporum. The appearance of the F. oxysporum fungus colonies on the 3rd day was white, the 5th day was cotton white, and the 7th day was pink white. After being observed microscopically, the hyphae were septate, with the presence of microconidia and macroconidia.

Another study also stated that the cause of vanilla stem rot was the fungus Fusarium moniliforme. Plant samples were taken from the Namblong District, Jayapura Regency, Papua (Setame et al., 2020).

Endophytic fungi can be identified macroscopically and microscopically by referring to the research of Sudantha and Abadi (2007) and (Gamboa-Gaitan & Otero-Ospina, 2016). Macroscopic and microscopic appearance of the fungus is shown in Figures 3 – 6.

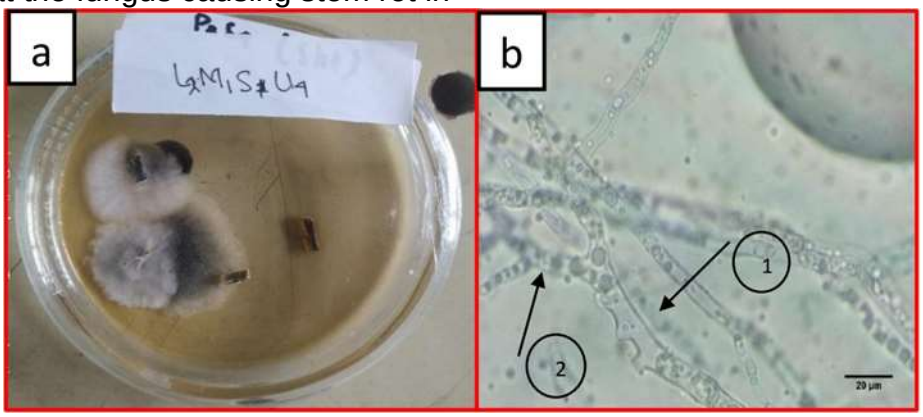

Figure 3. Endophytic fungus Aspergillus sp. macroscopically and microscopically.
Description: a. fungal colonies, b. fungal hyphae, hyphae (1), conidiophores (2)

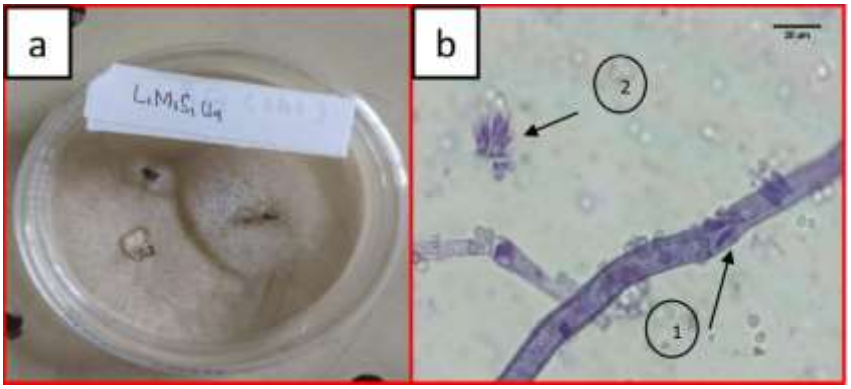

Figure 4. Endophytic fungus Cladosporium sp. macroscopically and microscopically.
Description: a. fungal colonies, b. fungal hyphae, hyphae (1), conidiophores (2)

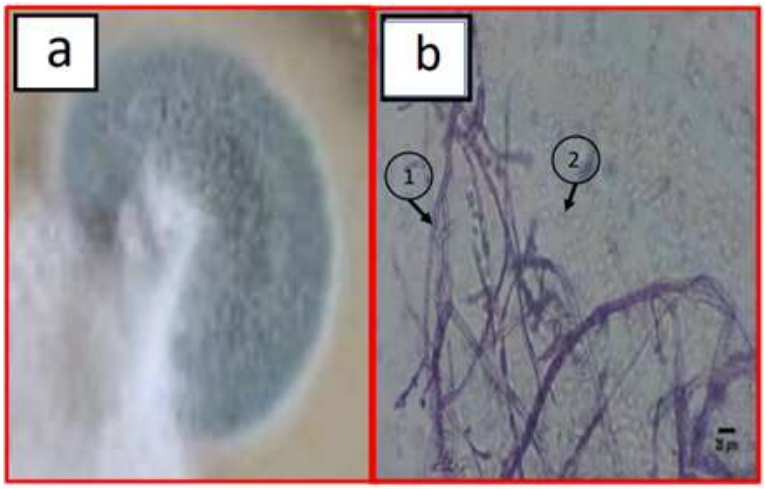

Figure 5. Endophytic fungus Penicillium sp. Macroscopically and microscopically.
Description: a. fungal colonies; b. fungal hyphae, hyphae (1), conidia (2)

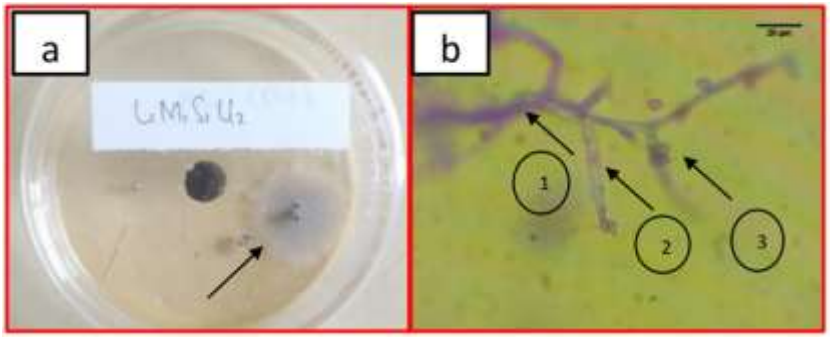

Figure 6. Endophytic fungus Trichoderma sp.
Description: a. fungal colonies; b. fungal hyphae, hyphae (1), conidiophores (2), conidia (3)

The names of endophytic fungi and their characteristics can be seen in table 3.

Mushroom Name	Characteristic	
	Macroscopis	Microscopis
Aspergilus niger	Colonies on day 3 are white. On day 5 it becomes light green. On the 7th day it becomes blackish green	Hyphae are septate and hyaline. conidia round
Cladosporium sp.	Colonies on day 3 are white. On the 5th day, it becomes brownish white. On the 7th day, it becomes light brown	Hyphae are septate, conidiophores are branched, conidia are round to oval
Penicillium sp.	Colonies on day 3 are white. On day 5 it becomes light blue. On the 7th day, it becomes turquoise	Septate hyphae, unbranched conidiophores, round conidia
Trichoderma sp.	Colonies on day 3 are white. On day 5 it becomes faintly creamy white. On the 7th day, it becomes creamy-white with white still dominant and there is a slight mixture of green, the texture is like cotton	Septate hyphae, branched conidiophores

Table 3. Macroscopic and microscopic characteristics of endophytic fungi

Details of data on various types of endophytic fungi on healthy and diseased vanilla plants from three different gardens can be seen in Table 4.

Tabel 4. Macam-macam jamur endofit di setiap lokasi kebun vanili

Lokasi	Jenis Jamur	Batang Tanaman Vanili			
		Sehat	Sakit		
			Ringan	Sedang	Berat
Dusun Sinogo	*Aspergillus niger*	+	+	-	-
	Cladosporium sp.	+	-	-	-
	Penicilium sp.	+	-	-	-
	Trikoderma sp.	+	-	-	-
Dusun Plono Barat	*Aspergillus niger*	+	-	-	-
	Cladosporium sp.	+	+	+	-
	Penicilium sp.	+	-	-	-
	Trikoderma sp.	-	-	-	-
Dusun Ngalian Barat	*Aspergillus Niger*	+	-	-	-
	Cladosporium sp.	+	-	-	-
	Penicilium sp.	-	-	-	-
	Trichoderma sp.	+	+	-	-

sign + = exists; Sign - = none

Table 4 shows that endophytic fungi found on vanilla stems in Sinogo Village obtained 4 types of endophytic fungal isolates: Aspergillus niger, Cladosporium sp., Penicillium sp., and Trichoderma sp. Jamur endofit dari batang vanili Dusun Plono Barat diperoleh 3 macam isolat yaitu *Aspergillus niger, Cladosporium* sp., *Penicillium* sp.. Three types of endophytic fungus, including Aspergillus niger, Cladosporium sp., and Penicillium sp., were recovered from the vanilla stems in West Plono Village. In West Ngalian Village, three isolates were obtained: Aspergillus niger, Cladosporium sp., and Trichoderma sp. Moreover, on healthy vanilla stems, 4 types of endophytic fungi were found. While on mildly diseased stems, one endophytic fungus was found and on the diseased stem, there was one endophytic fungus. Meanwhile, there were no endophytic fungi found on severely diseased stems.

There were several isolates of Trichoderma spp. which were potential to become a biological agent to suppress the development of vanilla shoot rot disease. The effectiveness of 6 isolates of Trichoderma spp. in suppressing the developing shoot rot disease in vanilla seedlings ranged from 66.67-68.00% (Taufiq, 2012).

Besides endophytic fungi, it was found that endophytic bacteria that could be the antagonists to the fungus Fusarium oxysporum f. sp. vanilla (Suniti, 2015).

These results were different from the results of research on endophytic fungi on healthy vanilla plants on Reunion Island, France. Fusarium proliferatum was the most common endophytic fungus, 37.6% of all isolates and found in all samples from 9 locations. Botryosphaeria ribis and Aspergillus fumigatus are the second most abundant endophytic fungi. Each of 5.8% of all isolates (Khoyratty et al., 2015).

4. Conclusion

The percentage of vanilla stem rot disease was greatest in West Plono were 6% mild symptoms, 9.6% moderate symptoms, and 15% severe symptoms. Furthermore, in West Ngalian village, there were 3% of mild symptoms, 10% of moderate symptoms, and 12.2% of severe symptoms. The lowest was in Sinogo village were mild symptoms at 3.14%, moderate symptoms were 14%, and severe symptoms was 5.71%. Four endophytic fungi were found in healthy vanilla plants: Aspergillus niger, Cladosporium sp., Penicillium sp., and Trichoderma sp., while on stem rot diseased vanilla, two endophytic fungi were found: Aspergillus niger and Cladosporium sp.

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