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THE ROLE OF EXOPHYTIC AND ENDOPHYTIC MICROBES IN CONTROLLING FRUIT ROT DISEASE ON PINEAPPLE (*ANANAS COMOSUS* (L.) MERR.

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ABSTRACT

Pineapple rot disease is one of the most significant postharvest diseases caused by *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, which can lead to substantial crop losses. There is a complex of microbes that may influence the growth and development of pineapple rot disease under natural conditions. This research was conducted to determine the role of exophytic and endophytic microbes in preventing pineapple rot. In healthy fruit, 45 isolates of exophytic fungi and 24 isolates of endophytic fungi were isolated from exophytes and endophytes, respectively. The dominant fruit exophytes of *Thermoplasma compositi* (Actinomycetes) were 15 isolates, while the dominant healthy fruit endophytes were as many as 12 isolates of *Colletotrichum* sp. Exophytes and endophytes were also found on healthy leaves, where the dominant exophytes were 45 isolates and the dominant endophytes were 24 isolates. *Rhizopus* sp. 36 and *Rhizopus* sp. 12 are both exophytes and endophytes. The diversity and dominance indices of fruit exophytic fungi were 1,767 and 0.800, respectively, while the diversity and dominance indices of fruit endophytic fungi were 1,386 and 0.6875, respectively. The diversity index for healthy leaf exophytes was 0,7201, with a dominance index of 0.3467, while the diversity index for endophytic microbes was 1.386, with a dominance index of 0.688. The inhibition of pathogens by fruit exophytic microbes ranged from 66.67 to 88.89 percent, with *A. flavus*1 achieving the highest level of 88.89 percent. *Rhizopus* sp. and *Neurospora* sp., both found in healthy leaf habitats, inhibit the growth of pathogens *in vitro* by 88.89%. *A. flavus* exhibited the most effective *in vivo* inhibition, with an percentage of inhibition of 3.8±1.39%.

KEYWORDS

Neoscytalidium dimidiatum, exophyte, endophyte, diversity index, dominance Index.



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INTRODUCTION

Pineapple fruit rot disease is a dangerous disease that has a negative impact on both the quality and quantity of pineapples. Pineapple stored in the open does not last long, as pathogens enter through the eyes of the fruit and cause it to quickly rot. Rohrbach and Phillips (1990) state that pineapple post-harvest disease symptoms can manifest either externally or internally. Pineapple fruit diseases can be categorized as follows: 1) pre-flowering infection by *Penicillium funiculosum*, which begins in the floret before the flower opens and causes inter-fruit cork, baggy, and fruit core rot; 2) post-flowering infection by *Phytophthora infestans*, which causes inter-fruit cork, baggy, and fruit core. After the flower blooms, a complex of bacteria, including pink and marbling disease, causes flower infection. The most common physiological disturbance is internal browning or black heart caused by chilling injury. Currently, *Ceratocystis* fruit rot is controlled by fungicide application and post-harvest wax dipping to prevent internal browning. Postharvest pineapples harbor numerous diseases, including *Phytophthora* heart (Top) The *Phytophthora nicotinae* fungus causes *Phytophthora nicotinae* rot. *Fusarium guttiforme* and *Penicillium funiculosum* are responsible for fruitlet core rot (green eye) and fusariosis, respectively.

Fruit rot caused by *Phytophthora cinnamomic*, water blister (*Chalara paradoxa*), yeast and *Candida* species (*Yeast Saccharomycetes* spp. and *Candida* spp.), bacteria and phytoplasma (*Pantoea ananatis* and *Acetobacter* spp.), and pink disease (*Pantoea citrea*, *Gluconobacter oxydans* or *Acetobacter acei*) (Joy and Sindhu, 2012). Endophytic fungi are fungi that grow within plant tissues, whereas exophytic fungi are surface fungi that can live saprophytically but do not cause plant disease. The Phylloplan fungus is a mycotic fungus that grows on the surfaces of plants (Langvard, 1980). There are two distinct types of phylloplan fungi: resident (remain silent) and casual (coincidentally). Residents are able to reproduce on healthy leaf surfaces without affecting the host, whereas casuals cannot grow on leaf surfaces. According to the findings of Sudarma et al. (2019), exophytic and endophytic fungi can inhibit the pathogenic potential of red wine both in vitro and in vivo. According to the findings of the most recent study by Sudarma et al. (2020), exophytic fungi such as *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus* sp. can suppress mango rot disease caused by *Lasiodiplodia theobromae* in vitro and in vivo.

MATERIALS AND METHODS

Location and time of study

The research was conducted in two locations: Sampling of diseased and healthy pineapple fruits was done at the Batubulan market and grocery stores. Isolation and identification of microbes was conducted at the Plant Diseases Laboratory and Agricultural Biotechnology Laboratory Faculty of Agriculture Udayana University, Bali. The study was conducted between April and August 2021.

Endophytic and Exophytic Fungi Isolation

Fruit parts with isolated endophytic fungi were washed with sterile running water, sterilized with 0.525% sodium hypochlorite for 3 minutes and 70% alcohol for 2 minutes, rinsed with sterile water for 1 minute, and then placed on the media. PDA was initially administered an antibacterial antibiotic, specifically livoploxacin at a concentration of 0.1% (w/v). The fungus that emerged from the leaf fragments was transferred to a test tube containing PDA for storage and morphospecies classification. While exophytic fungi can be sprayed onto plant parts (fruits and leaves), the washing water was collected in a tube, then 1 ml of growth was transferred to a PDA that had been previously filled with livoploxacin at a concentration of 0.1% (w/v).

Endophytic, Exophytic, and Actinomycetes Microbe Identification

The stored endophytic and exophytic fungi were subsequently grown in PDA-containing Petri dishes with five times replication. The cultures were incubated at room temperature (27°C) in the dark. After 3 days, isolates were identified macroscopically to determine colony color and growth rate, and microscopically to determine septa on hyphae, spore/conidia shape, and sporangiophores. Identification of fungi was done according to method developed by Samson et al., 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; and Indrawati et al., 1999. Identifying Actinomycetes using the cited source Miyadoh, 1997.

Evaluation of Endophytic and Exophytic Microbes' Inhibitory Capacity Against Pathogens

Using the dual culture technique, the endophytic and exophytic microbes discovered were evaluated for their ability to inhibit the growth of pathogenic fungi (in one Petri dish, one pathogenic fungus was grown each flanked with two endophytic fungi)(Dollar, 2001; Mojica-Marin et al., 2008). The inhibitory activity was calculated as follows:

$$\text{Inhibitory activity (\%)} = \frac{A - B}{A} \times 100$$

where:

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

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

Prevalence of Endophytic and Exophytic microbes

Determination of the prevalence of endophytic and exophytic microbes was done based on the frequency of endophytic and exophytic microbes isolates found in healthy fruit per Petri dish, divided by all isolates found multiplied by 100%. The prevalence of isolates will determine the dominance of endophytic and exophytic microbes present in healthy pineapple fruit.

(1) Microbial diversity index

The microbial diversity index in pineapple was determined based on the Shannon-Wiener diversity index, according to the following formula (Odum, 1971):

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

where:

H' = Shannon-Wiener diversity index

S = Number of genera

P_i = n_i/N is the proportion of individuals of type i and all individuals (n_i = the total number of individuals of type i,

N = the number of all individuals in the total n).

Table 1. Criteria for weighting environmental quality (Tauruslina et al., 2015)

Diversity index	Community structure conditions	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

(1) Dominance index

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

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

Where:

C = Simpson index

S = Number of genera

$P_i = n_i/N$ is the proportion of individuals of type i and all individuals (n_i = the total number of individuals of type i , N = the number of all individuals in the total n).

Furthermore, the species dominance index (D) can be calculated using 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 dominance by one microbial species or there is no species that extremely dominates other species, close to 1 = large index or tends to be dominated by several microbial species (Pirzan and Pong-Cook, 2008).

In Vivo Antagonistic Test

In vivo antagonistic test of endophytic and exophytic fungi found by pricking fresh fruit with a spelden needle 10 times, then smeared with antagonistic fungal spores (spores of one Petri dish in 250 ml of sterile distilled water), then immersed in fungal spore suspension of pathogens. Endophytic and exophytic microbes found include:

A = antagonist treatment 1 (spore suspension 5×10^7)

B = antagonist treatment 2 (spore suspension 5×10^7)

C = antagonist treatment 3 (spore suspension 5×10^7)

D = antagonist treatment 4 (spore suspension 5×10^7)

E = antagonist treatment 5 (spore suspension 5×10^7)

K-P = control without pathogen

K+P = control with pathogen

All treatments were repeated 5 times. The experiment was designed with a randomized block design (RBD), and after the analysis of variance (ANOVA) was carried out, it was continued with the least significant difference test (LSD) at the 5% level. Infection parameters were measured by the formulation: the percentage of fruit slices that were infected.

RESULTS AND DISCUSSION

Disease Incidence

Pineapple fruit diseases that were found showed symptoms starting from the tip of the fruit. When touched, the fruit feels soft but does not show black symptoms, but the smell is different from fresh fruit (Figure 1A), and the symptoms of diseased fruit are shown in Figure 1B. After being isolated in a Petri dish, it was found that the mycelium growing in the middle was black and followed by a white colour at the edges and with very fast growth at the age of 3 days already filled the Petri dish (Figure 1C). Observations under a light microscope showed that the conidia were spherical in shape with large conidia measuring 20-40 μm , and small ones 10-20 μm (Fig. 1E). After being matched with the existing literature, it turned out that the disease was caused by the fungus *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers (Kuruppu *et al.*, 2020).

Exophytic and Endophytic Microbes

Fruit exophytic microbes found in the study were *Actinoplanes missouriensis* (Actinomycetes) as many as 3 isolates, *Aspergillus flavus* as many as 6 isolates, *Colletotrichum* sp. *Frankia* sp. (Actinomycetes) as many as 3 isolates, *Nocardiodes hungaricus* (Actinomycetes) as many as 3 isolates, *Rhizopus* sp. as many as 6 isolates and *Thermasporomyces composti* (Actinomycetes) as many as 15 isolates (Table 2; Figure 2), while endophytic microbes were found, among others: *Actinoplanes missouriensis* (Actinomycetes) as many as 3 isolates, *Colletotrichum* sp. 12 isolates, *Neurospora* sp. 3 isolates, *Nocardiodes hungaricus* (Actinomycetes) 3 isolates, and *Micromonospora jinlongensis* (Actinomycetes) 3 isolates (Table 2; Figure 3).

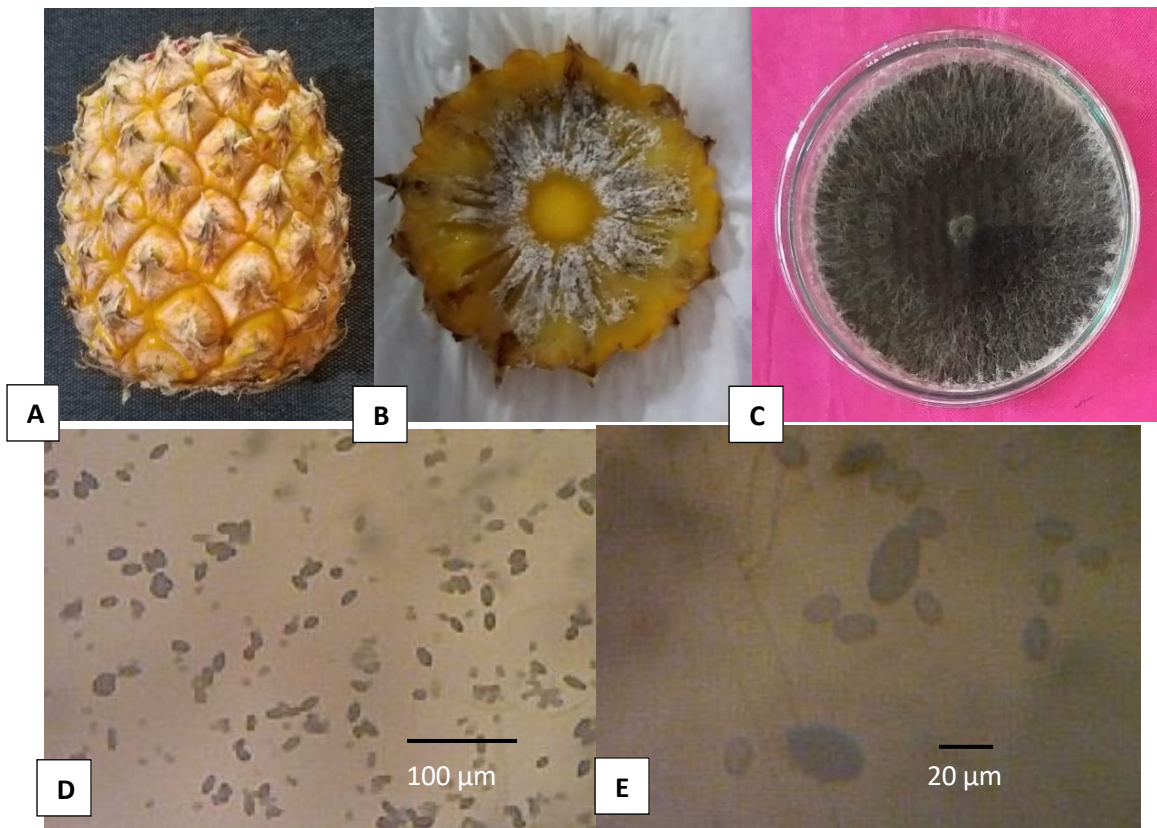


Figure 1. Diseased and healthy pineapple fruits, (A) healthy pineapple fruit, (B) diseased pineapple fruit after slicing, (C) mycelium growth on Petri dishes, (D) fungal conidia observed under a 100x magnification with light microscope, and (E) fungal conidia which was observed under a light microscope with magnification of 450x

Table 2. Exophytic and endophytic microbes from healthy pineapple

No.	Name of exiphytic microbes	Number of isolates	Name of endophytic microbes	Number of isolates
1	<i>Actinoplanes messourinsis</i> (Actinomycetes)	3	<i>Actinoplanes missouriensis</i> (Actinomycetes)	3
2	<i>Aspergillus flavus</i>	6	<i>Colletotrichum</i> sp.	12
3	<i>Colletotrichum</i> sp.	9	<i>Neurospora</i> sp.	3
4	<i>Frankia</i> sp. (Actinomycetes)	3	<i>Nocardiodes hungaricus</i> (Actinomycetes)	3
5	<i>Nocardiodes hungaricus</i> (Actinomycetes)	3	<i>Micromonospora jinlongensis</i> (Actinomycetes)	3
6	<i>Rhizopus</i> sp.	6		
7	<i>Thermasporomyces composti</i> (Actinomycetes)	15		
	Jumlah	45		24

The results of the study by Sudarma *et al.* (2021a) found that exophytic microbes on bananas included *A. niger*, *A. flavus*, *Oidium* sp. *Nocardi asteroid* (Actinomycetes), *Nocardia* sp. (Actinomycetes), *Neurospora* sp. and *Rhizopus* sp., and endophytic microbes found in healthy bananas include: *A. niger*, *Colletotrichum* sp., *A. flavus*, *Rhizopus* sp. Likewise with the research results of Sudarma *et al.* (2021b) found exophytic microbes in papaya, including *Rhizopus* sp., *A. niger*, *Actinomycetes israelii* (Actinimycetes), *Actinomadura cremea* (Actinomycetes), *Streptomyces* sp. (Actinmycetes), and *Micromonospora* sp. (Actinomycetes). Meanwhile,

endophytic microbes found from healthy papaya fruit include: *A. niger*, *Rhizopus* sp. *Agromyces romosus*, and *Trichoderma* sp.

Leaf exophytic microbes were found to consist of four species, namely *Rhizopus* sp. as many as 36 isolates, *Agococcus jenensis* (Actinomycetes) as many as 3 isolates, *Catenulispora yoronensis* (Actinomycetes) as many as 3 isolates and *Nocardioides hungaricus* (Actinomycetes) as many as 3 isolates (Table 3; Figure 4), while leaf endophytic microbes were found as many as 5 species, namely *Rhizopus* sp. as many as 12 isolates, *Neurospora* sp. as many as 3 isolates, *Amblyosporium* sp. as many as 3 isolates, *Microsmonospora* sp. as many as 3 isolates, and *Hyalodendron* sp. as many as 3 isolates (Table 3; Figure 5).

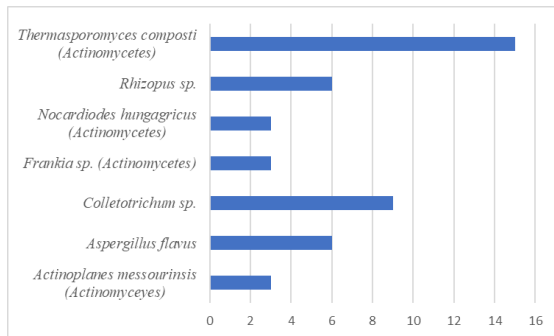


Figure 2. Population of exophytic microbes in healthy pineapples

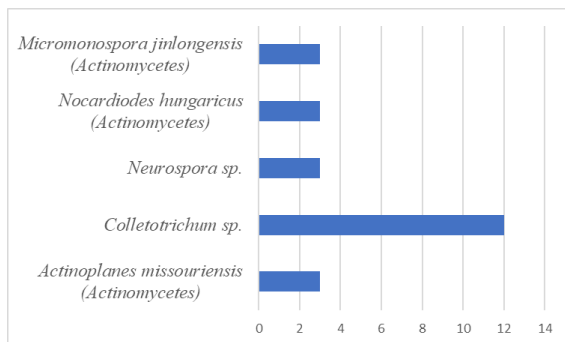


Figure 3. Population of endophytic microbes in healthy pineapples

Table 3. Exophytic and endophytic microbes from healthy pineapple leaves

No.	Exophytic microbes	Number of isolates	Endophytic microbes	Number of isolates
1	<i>Agococcus jenensis</i> (Actinomycetes)	3	<i>Amblyosporium</i> sp.	3
2	<i>Catenulispora yoronensis</i> (Actinomycetes)	3	<i>Hyalodendron</i> sp.	3
3	<i>Nocardioides hungaricus</i> (Actinomycetes)	3	<i>Microsmonospora</i> sp. (Actinomycetes)	3
4	<i>Rhizopus</i> sp.	36	<i>Neurospora</i> sp.	3
5			<i>Rhizopus</i> sp.	12
Total		45		24

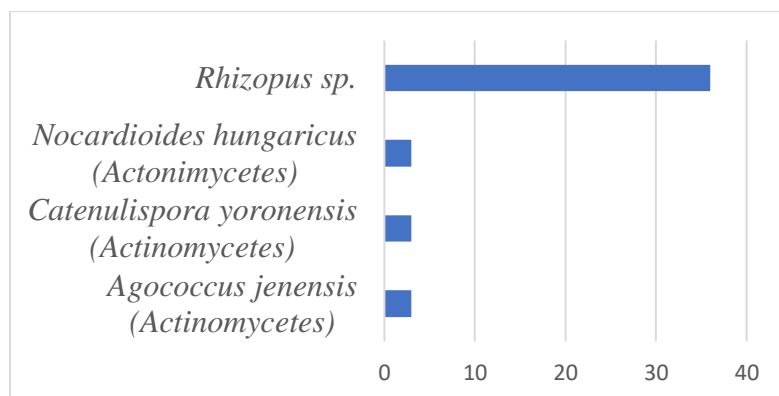


Figure 4. Population of exophytic microbes from healthy pineapple leaves

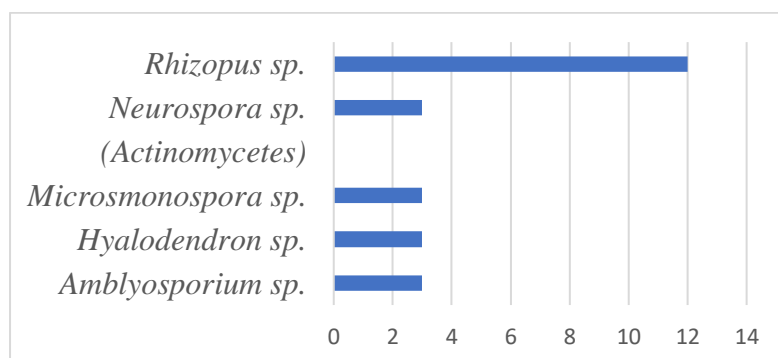


Figure 5. Population of endophytic microbes from healthy pineapple leaves

Diversity and Dominance Index

The results of this study showed that the fruit exophytic microbial diversity index was 1,767, this means that the condition of the community structure was stable enough with a currently category and a scale of 3 (Table 1) (Tauruslina *et al.*, 2015). Meanwhile, the dominance index was 0.800 (Table 5). The dominance index of fruit exophytic microbes, which was 0.8, (above 0.5), means that there were 15 isolates of the dominant microbe, *Thermasporomyces composti* (Actinomycetes). As for the fruit endophytic fungi found in healthy apples, the diversity index of 1,388 means that the condition of the community structure is stable enough, with a currently category and a scale of 3 (Table 1). The dominance index is still above 0.5, which was 0.688, this means that there was a dominant microbe, namely *Colletotrichum sp.* as many as 12 isolates.

The leaf exophyte diversity index was 0,720, meaning that the community structure less stable, with a bad category and a scale of 2 (Table 1), the dominance index was 0,347, means that there was not a dominant species, but namely *Rhizopus sp.* as many as 36 isolates. The endophytic diversity index of healthy leaves was 1.386, meaning that it was stable enough in the currently category, and a scale of 3, and the dominance index was 0.688, which means above 0.5, which means that there is a dominant species, namely *Rhizopus sp.* as many as 12 isolates (Table 5).

Table 5. Index of diversity and dominance of exophytic and endophytic microbes of pineapple fruit and leaves

Index	Exophytic microbes	Endophytic microbes
Fruit pineapple healthy		
H (diversity index)	1,767	1,3863
D (dominance index)	0,800	0,6875
Leaf pineapple healthy		
H (diversity index)	0,7201	1,3863

D (dominance index)

0,3467

0,6875

The research results of Sudarma *et al.* (2018) regarding exophytic and endophytic microbes in healthy grapes, the diversity and dominance index of exophytic microbes was 2.3742 and 0.8667, while the endophytic microbe diversity and dominance index was 2.6356 and 0.6489. This indicates that the diversity and dominance index is almost the same as that found in pineapples. The dominance was held by the fungus *A. niger* as many as 18 in the exophytic fungus and the endophytic fungus *Fusarium sp.* as many as 21 isolates.

Inhibition of Exophytic and Endophytic Microbes against Pathogens *In Vitro*

The inhibition of fruit exophytic microbes against pathogens ranged from 66.67% to 88.89%, the highest was achieved by *A. flavus*1 of 88.89% and the lowest was achieved by the fungus *Rhizopus sp.* by 66.67%, while in fruit endophytic microbes, namely *Neurospora sp.* by 66.70% and *Colletotrichum sp.*5 the inhibitory activity of 69.23% (Table 6).

Table 6. Inhibition of exophytic and endophytic microbes of fruit against pathogen

No.	Exophytic microbes	Inhibited ability (%)	No.	Endophytic microbes	Inhibited ability (%)
1	<i>Colletotrichum sp. 1</i>	-	1	<i>Neurospora sp.</i>	66,70
2	<i>Frankia sp.</i> (Actinomycetes)	-	2	<i>Colletotrichum sp. 1</i>	-
3	<i>Nocardiodes hungaricus</i> (Actinomycetes)	-	3	<i>Colletotrichum sp. 2</i>	-
4	<i>Colletotrichum sp. 2</i>	-	4	<i>Colletotrichum sp.4</i>	-
5	<i>Aspergillus flavus 1</i>	88,89	5	<i>Micromonospora jinlongensis</i> (Actinomycetes)	-
6	<i>Actinoplanes messourinsis</i> (Actinomyceyes)	-	6	<i>Colletotrichum sp.5</i>	69,23
7	<i>Thermasporomyces composti</i> (Actinomycetes) 1	-	7	<i>Actinoplanes missouriensis</i> (Actinomycetes)	-
8	<i>Rhizopus sp. 1</i>	85,56	8	<i>Nocardiodes hungaricus</i> (Actinomycetes)	-
9	<i>Thermasporomyces composti</i> (Actinomycetes)2	-			
10	<i>Thermasporomyces composti</i> (Actinomycetes)3	-			
11	<i>Rhizopus sp.2</i>	66,67			
12	<i>Colletotrichum sp. 3</i>	-			
13	<i>Thermasporomyces composti</i> (Actinomycetes)4	-			
14	<i>Thermasporomyces composti</i> (Actinomycetes)5	-			
15	<i>A. flavus 2</i>	-			

The inhibition of pineapple leaf exophytic microbes showed that the fungus *Rhizopus sp.* on average, it was able to inhibit pathogens by 88.89%, while the pineapple leaf endophytic fungus that was able to inhibit pathogens was *Rhizopus sp.* and *Neurospora sp.* each of 88.89% (Table 7).

The fungus *Rhizopus sp.* has the ability to suppress the growth of pathogens *in vitro* both exopitis and endophytic (Sudarma *et al.* 2021c). Similarly, the results of research by Sudarma *et al.* (2020) showed that *Rhizopus sp.* is a fungus with the best inhibition against pathogens (*Lesiodiplodia theobromae*) that infect mangoes both *in vitro* and *in vivo*. Inhibition of *Rhizopus sp.* against pathogens, the way it works is by

competition, which covers the entire surface of the Petri dish so that the growth of the pathogen was inhibited. Besides, *Rhizopus* sp. has a very fast growth covering the entire surface of the Petri dish.

Table7. Inhibition of exophytic and endophytic of leaf against pathogen

No.	Exophytic microbes	Inhibited ability (%)	No.	Endophytic microbes	Inhibited ability (%)
1	<i>Rhizopus</i> sp. 1	88,89	1	<i>Rhizopus</i> sp.	88,89
2	<i>Rhizopus</i> sp. 2	88,89	2	<i>Rhizopus</i> sp.	88,89
3	<i>Rhizopus</i> sp. 3	88,89	3	<i>Neurospora</i> sp.	88,89
4	<i>Agococcus jenensis</i> (Actinomycetes)	88,89	4	<i>Amblyosporium</i> sp. (Actinomycetes)	-
5	<i>Rhizopus</i> sp. 4	88,89	5	<i>Microsmonospora</i> sp. (Actinomycetes)	-
6	<i>Rhizopus</i> sp. 5	88,89	6	<i>Rhizopus</i> sp.	-
7	<i>Rhizopus</i> sp. 6	-	7	<i>Hyalodendron</i> sp.	-
8	<i>Catenulispora yoronensis</i> (Actinomycetes)	-	8	<i>Rhizopus</i> sp.	-
9	<i>Rhizopus</i> sp. 7	-			
10	<i>Rhizopus</i> sp. 8	88,89			
11	<i>Rhizopus</i> sp. 9	-			
12	<i>Nocardioides hungaricus</i> (Actonimycetes)	-			
13	<i>Rhizopus</i> sp. 10	88,89			
14	<i>Rhizopus</i> sp. 11	-			
15	<i>Rhizopus</i> sp. 12	-			

In Vivo Inhibition of Exophytic and Endophytic Microbes

In vivo inhibition of exophytic and endophytic microbes can be shown by K+P treatment (treatment with pathogens) of 94±5.48% followed by treatment A (endophyte 2 from leaf), 84±5.48%, then treatment C (endophyte 1 from leaf, *Rhizopus* sp.) was 76±6.52%, and treatment E (endophyte 3 from leaf, *Neurospora* sp.) was 63±5.70%, treatment D (exophyte 4 from leaf, *Rhizopus* sp.) was 43±4.47%, the smallest in treatment B (exophyte 5 from fruit, *A. flavus*) was 3.8±1.30% and K-P treatment (without pathogens) was 5±0.7% (Table 8).

In vivo test results indicate that *A. flavus* has excellent inhibitory activity against pathogens, but in terms of the treatment recommended to the public, recommendations cannot be given because *A. flavus* secretes aflatoxins which are very dangerous for consumers. The only hope is that treatments A and B each came from *Rhizopus* sp. isolates, but *Neurospora* sp. also showed moderately severe attacks (Figure 6).



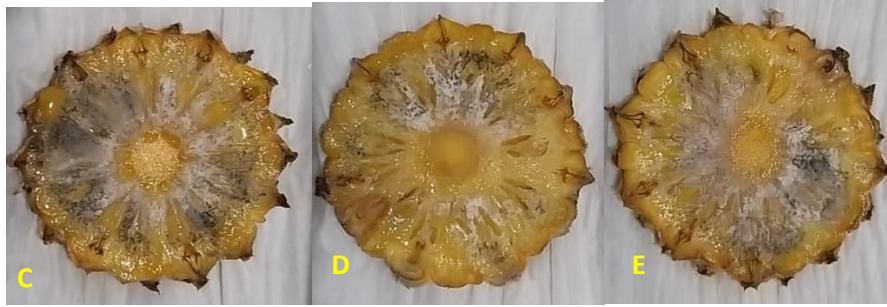


Figure 6. *In vivo* inhibition test of each treatment from the best isolate against pathogen infection (3 days after inoculation), (K+P = treatment only with pathogens, K-P = treatment without pathogens, A = endophytic 2 from leaves, *Rhizopus* sp., B = exophyte 5 from fruit, *A. flavus*, C = endophyte 1 from leaf, *Rhizopus* sp., D = exophyte 4 from leaf, *Rhizopus* sp. and E = endophyte 3 from leaf, *Neurospora* sp.)

CONCLUSION

Based on the results and discussion above, it can be concluded as follows: Pineapple rot disease was found to be caused by the fungus *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers. This is in accordance with the reference for comparison, namely Kuruppu *et al.*, 2020. Exophytic and endophytic fungi in healthy fruit were found to be 45 isolates on exophytes and 24 isolates on endophytes. The dominant fruit exophytes was *Thermasporomyces composti* (Actinomycetes) with the number of 15 isolates and the dominant healthy fruit endophytes were *Colletotrichum* sp. as many as 12 isolates, while exophytes and endophytes on healthy leaves were also found 45 isolates as dominant exophytes and 24 isolates of endophytes. Both exophytes and endophytes are *Rhizopus* sp. with 36 and 12 isolates respectively. The index of diversity and dominance of healthy fruit exophytic fungi was 1,767 and the dominance index was 0.800, while the endophytic diversity index of fruit was 1,386 and the endophytic dominance index was 0.6875. The index of diversity for healthy leaf exophytes was 0,7201 with a dominance index of 0.3467, and for endophytic microbes the diversity index was 1.386 with a dominance index of 0.688. The inhibition of fruit exophytic microbes against pathogens ranged from 66.67% to 88.89%, the highest was achieved by *A. flavus*1 of 88.89%. Meanwhile, in healthy leaf habitats, the antagonists against the of pathogens *in vitro* were *Rhizopus* sp. and *Neurospora* sp. each with an inhibitory activity of 88.89%. Under *in vivo* inhibition test was found that *A. flavus* had the best inhibition with an percentage of infection of $3.8 \pm 1.39\%$.

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