E-2795-3248
P-2795-3222

10.5281/zenodo.6983034 Vol. 05 Issue 08 Aug - 2022 Manuscript ID: #0682

UTILIZATION OF EXOPHYTIC AND ENDOPHYTIC MICROBES IN CONTROLLING ALTERNARIA FRUIT ROT OF APPLE (*MALUS DOMESTICA BORKH*)

I Made Sudarma*, Ni Wayan Suniti* and Ni Nengah Darmiati*
**Staff Lecturer in Agroecotechnology Study Program Faculty of Agriculture, Udayana University Jl. PB. Sudirman Denpasar-Bali,*

Corresponding author: *****I Made Sudarma
Email: sudarma_made@ymail.com

ABSTRACT

Apple rot disease caused by *Alternaria* sp. is a postharvest disease that is often found when fruit is marketed, transported and stored. The results showed that there were 45 isolates of exophytic microbes and 27 isolates of endophytes. The microbial diversity index of healthyapple exophytes is 1.249, this means that the condition of the commodity structure is quite stable with a medium category and a scale of 3, while the dominance index is 0.896, meaningthat there are 10 isolates of the dominating species, namely *Lasiodiplodia theobromae*. With aevenness index of 0.885. The endophytic microbial diversity index in healthy apples was 2.793, which means the condition of the commodity structure was more stable with a good category and a scale of 4. The Simpson dominance index was 0.739, meaning that there was a dominantspecies, namely *Rhizopus* sp. as many as 23 isolates, with a evenness index value of
0.458. Theinhibition of exophytic and endophytic microbes against pathogens *in vitro* showed that the highest inhibition of exophytic microbes was *Actinomyces bovis* (Actinomycetes), *A. niger*, *Colletotrichum* sp., and *Rhizopus* sp. each of 83.33±00%, while the highest endophyte was shown by *L. theobromae* at 83.33±00%, followed by the fungus *Rhizopus* sp. by 80.55±5.89%and the smallest by the fungus *A. niger* by 78.89±4.19%. *In vivo* inhibition of exophytic and endophytic microbes against pathogens showed that their effect was very significant in suppressing pathogen growth in fresh fruit. The effect of treatment C (*A. niger*) was very goodin suppressing the growth of pathogens, so that the apples still looked smooth and fresh, different from treatments A (*L. theobromae*) and K+P (treatment with pathogens).

KEYWORDS

Alternaria sp., exophytes and endophytes, inhibition, diversity index and dominance

Introduction

Postharvest disease of apples (*Malus domestica* Burkh), is often found in traditional markets and supermarkets. The fruit undergoes decay starting with an accidental impact or scratch on the skin of the fruit so that the fruit is infected by pathogenic fungi. There are many types of apple rot, including: fruit rot caused by the fungus *Phacidiopycnis washingtonensis*, bitter rot (*Colletotrichum acutatum* complex, *C. gloeosporioides* complex), black rot (*Botryosphaeria obtusa*), white rot (*Botyrosphaeria dothidea*), and other fruit diseases. Such as apple scabies (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), rust (*Gymnosporangium juniperi-virginianae, G. clavipes, G. globosum*) and Sooty Blotch and Flyspeck (*Geastrumia polystigmatis, Zygophiala jamaicensis*) (Gautheir, 2019).

Apples with ALFS (Alternaria leaf and fruit spot) symptoms caused by *Alternaria* speciesbelonging to Phylum Ascomycota, Subphylum Pezizomycotina, Class Dothideomycetes, Order Pleosporales, family Pleosporaceae, and Genus *Alternaria*. The genus Alternaria is distinguished from other fungal genera by the morphology of the conidia which are large and dark, multi-celled, catenate or single, ovoid or obclavate, often beaked, brown, with transverse and longitudinal septa. *Alternaria mali*, the most reported and serious pathogen of apples and currently identified from America (US, Canada, Chile), Europe (Netherlands, France, Turkey, Serbia, Slovenia)Asia (India, China, Japan, Taiwan Korea), and Australia. Apart from *A. mali*, several other *Alternaria* species are associated with ALFS namely, *A. alternata, A. arborescens, A. longipes* and *A. tenuissima*. Different isolatesshowed varying pathogenicity and virulence (both within and between species complexes) (Madhu *et al.*, 2020).

Endophytic fungi originate from the environment of the host plant in which they live, including microorganisms, fungal spores, and the eating habits and processes of insects and animals around them. Endophytic fungi usually follow two transmission patterns, vertical transmission and horizontal transmission. In vertical transmission, endophytes are transmitted from the mother plant to the offspring, this method is a method of spreading the infection to the offspring. Under favourable conditions, the seed germinates and the endophytic fungi present in the seed enter the newly formed plant, thereby establishing the transmission of the endophytic fungus from the parent plant to the newly formed offspring. Horizontal transmission is the preferred way for above ground tissues, these fungal endophytes are transmitted through spores, biotic factors such as herbivores or insects and abiotic agents such as wind or rain from one plant to another, thus establishing the transmission of endophytic fungi between different host plants (Swamy and Sandhu, 2021).

The phyllosphere refers to the entire aerial habitat of the plant while the phylloplane describes the entire leaf surface. Phylloplane provide niches for diverse microbial communities and are therefore an ecologically and economically important ecosystem. For many years, phylloplane dwellers have been studied as bioprotective and growth enhancers in host plants. Plants and phylloplane-microbial interactions result in increased fitness and productivity of agricultural crops. In this study, an attempt was made to compile previous studies to better understand the role of the phylloplane microbiota in influencing plant physiology. It is proposed the possibility of further studies to explore the molecular aspects of the signalling mechanisms created by the phylloplane microbial community with the host influencing the physiology of the latter (Goswami *et a*l., 2021).

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, steri lized 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 PDA media was initially administered an antibacterial antibiotic, specifically livopl oxacin 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.

Inhibitory Test of Endophytic and Exophytic Microbes Against Pathogens

The endophytic and exophytic microbes that were found were tested for their inhibition against the growth of pathogenic fungi using the dual culture technique (in one Petri dish, one pathogenic fungus was grown each flanked with two endophytic fungi). The inhibition can be calculated as follows (Lee et al., 2014; Verma et al., 2017):

Inhibitory activity (%) = (A – B)/A x 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.

Determining Diversity and Dominance Index

Diversity and dominance of contaminant fungi can be determined by calculating the Shannon -Wiener diversity index (Odum, 1971) and microbial dominance is calculated by calculating the Simpson index (Wilson and Gownaris, 2022):

(1) Microbial diversity index

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

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

where:

H’ = Shannon-Wiener diversity index S = Number of genera

Pi = ni/N is the proportion of individuals of type i and all individuals (ni = 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

(2) Evenness Index

The evenness index (E) describes the number of individuals between species in a fish community. The more evenly distributed individuals between species, the more balanced the ecosystem will be. The formula used is: E = H'/H max. Where E = Evenness index, H' = Diversity index, H max = ln S, S = Number of species found.
The evenness index value ranges from 0-1. Furthermore, the evenness index is: categorized as follows:

$0 < E \leq 0.5$ = Depressed community
$0.5 < E \leq 0.75$ = Unstable community
$0.75 < E \leq 1$ = Stable community

The smaller the evenness index, the population uniformity smaller as well. It shows the distribution of the number of individuals of each species is not similar so there is a tendency for one species to dominate. The greater the uniformity value describes the number of microbes in each species the same or not much different.

(3) Dominance index

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

$$C = \sum_{i=1}^{s} Pi2$$

Where:
C = Simpson index
S = Number of genera
Pi = ni/N is the proportion of individuals of type i and all individuals (ni = 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 exophyti c microbes found include:
A = antagonist treatment 1 (spore suspension $5x10^7$) B = antagonist treatment 2 (spore suspension $5x10^7$) C = antagonist treatment 3 (spore suspension $5x10^7$) D = antagonist treatment 4 (spore suspension $5x10^7$) E = antagonist treatment 5 (spore suspension $5x10^7$) 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 Duncan multiple range test

(DMRT) at the 5% and 1% level. Parameters measured by the formulation: sick fruit that was given a stab divided by all the punctures (20 stabs) times 100%.

RESULTS AND DISCUSSION

Disease Incidence

The rot disease found in apple fruit was brown with curved markings (Figure 1B) compared to healthy fruit (Figure 1A). Mycelium growing on Petri dishes was white in clusters (Figure 1B). After observing the pathogen under a microscope, it turned out that the conidia were club-shaped (Fig. 1C). Based on existing references, it turns out that the cause is *Alternaria* sp.

Diseases have occurred in apple orchards in China where apple plants are susceptible and highly susceptible to two pathogens, namely *Marssonina coronaria* and *Alternaria alternata* (Li *et al*., 2012). This disease is an important disease that also affects apples in Israel and is widespread worldwide, affecting the cultivars of Golden Delicious, Starking Delicious, Gala and Pink Lady (Gur *et al*., 2021). Pathogens persist on apple tree branches evaluated during the dormant phase and at the onset of vegetative growth (Gelain, *et al*., 2020).

Exophytic, Endophytic Microbes and Prevalence

Exophytic microbial populations found in healthy apples were 18 species, of which the most were *Lasiodiplodia theobromae* with 10 isolates, followed by *Actinomycetospora succina* (Actinomycetes) with 6 isolates and *Rhizopus* sp. as many as 5 isolates (Table 1; Figure 2), while the endophytic fungi found 3 species, including *Rhizopus* sp. the most with 23 isolates, followed by *A. niger* with 3 isolates and finally *L. theobromae* with 1 isolate (Table 1; Figure 3). The highest prevalence was in exophytes of 22% and endophytes of 85%, respectively, from *L. theobromae* and *Rhizopus* sp. (Table 1).

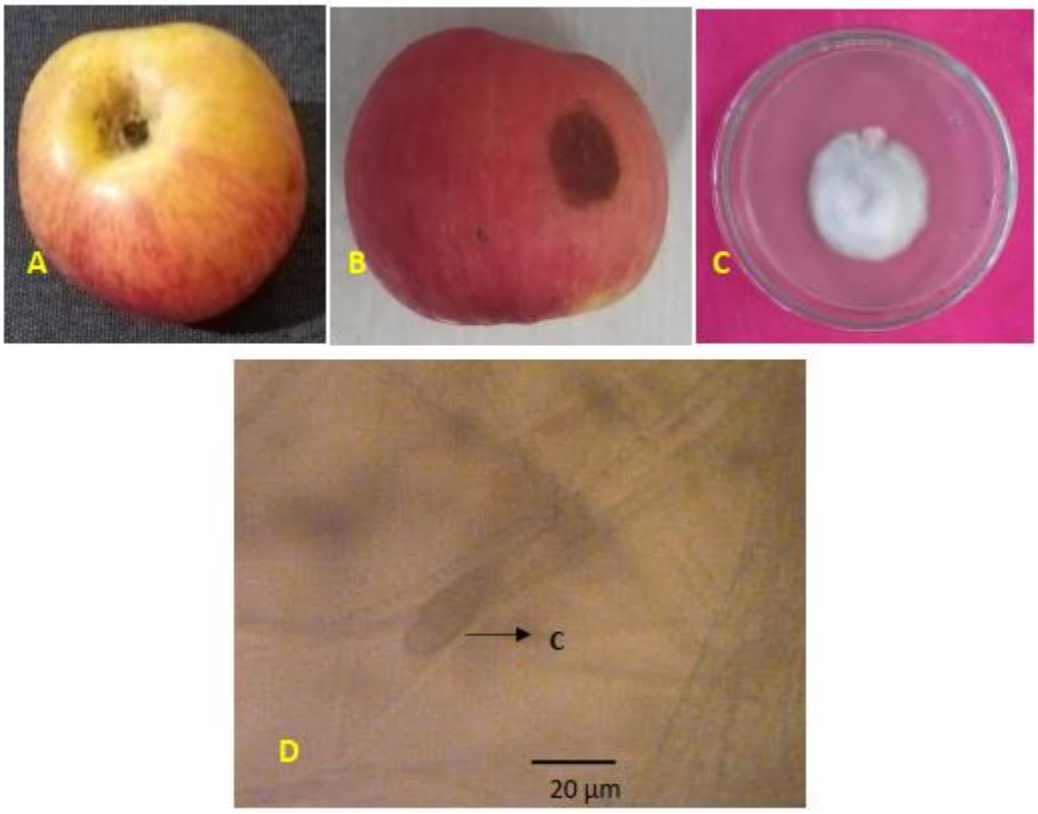


Figure 1. Study of disease, (A) healthy apples, (B) sick apples with rot symptom, (C) fungal mycelium and (D) club-shaped pathogenic conidia (C = conidium)

Sudarma *et al*. (2021a) stated in the results of his research that 7 species of exophytic microbes were found, dominated by the fungus *Rhizopus* sp. as many as 18 isolates, while endophytic fungi were found as many as 4 species which were dominated by *Rhizopus* sp. And *A. niger* with 6 isolates each. *Rhizopus* sp. dominates in

endophytes, this is due to the fact that cosmopolitan conidia are easily spread and infect fruit tissues, while in exophytes the fungus *L. theobromae* dominates because this fungus is a pathogen in several fruit commodities (Sudarma *et al*, 2022).

Tabel 1. Population of exophytic and endophytic microbes in healthy apple fruit

No.	Name of exophytic microbes	Number of isolate	Name of endophytic microbes	Number of isolate
1	*Actinomyces bovis* (Actinomycetes)	2 (4%*)	*Aspergillus niger*	3 (11%)
2	*Actinomycetospora succina* (Actinomycetes)	6 (13%)	*Lasiodiplodia theobromae*	1 (4%)
3	*Actinomyces israelii* (Actinomycetes)	1(2%)	*Rhizopus* sp.	23 (85%)
4	*Arthrinium* sp.	1 (2%)		
5	*Asanoa irimotensis (Actinomycetes)*	1 (2%)		
6	*Aspergillus niger*	2 (4%)		
7	*Catenulispora subtropic* (Actinomycetes)	1 (2%)		
8	*Colletotrichum* sp.	1 (2%)		
9	*Lasiodiplodia theobromae*	10 (22%)		
10	*Micromonospora* sp. (Actinomycetes)	2 (4%)		
11	*Micromonospora jinlongensis* (Actinomycetes)	1 (2%)		
12	*Nocardia asteroides* (Actinomycetes)	1 (2%)		
13	*Nonomuraea monospora* (Actinomycetes)	4 (9%)		
14	*Rhizopus sp.*	5 (11%)		
15	*Sacharomonospora* sp. (Actinomycetes)	2 (4%)		
16	*Streptomyces eurocidicus* (Actinomycetes)	1 (2%)		
17	*Streptomyces roseoverticillatus (*Actinomycetes)	3 (7%)		
18	*Streptosporagium* sp. (Actinomycetes)	1 (2%)		
	Total	45		27

***** The numbers in brackets indicate the prevalence in each microbe

Diversity, Dominance and Evenness Index of Exophytic and Endophytic Microbes

The microbial diversity index of healthy apple exophytes is 1.249, this means that the condition of the commodity structure is quite stable with a medium category and a scale of 3 (Table 2), while the dominance index is 0.896, meaning that there are species that dominate, namely *L. theobromae* as many as 10 isolates (Table 3). The endophytic microbial diversity index in healthy apples is 0.503, which means the condition of the commodity structure is unstable with a very poor category and a scale of 1. Simpson's dominance index is 0.739, meaning that there is a dominating species, namely *Rhizopus* sp. a total of 23 isolates. The evenness index of exophytic microbes is 0.885 (stable community) and evenness index of endophytic is 0.458 in mean depressed community (Table 3).

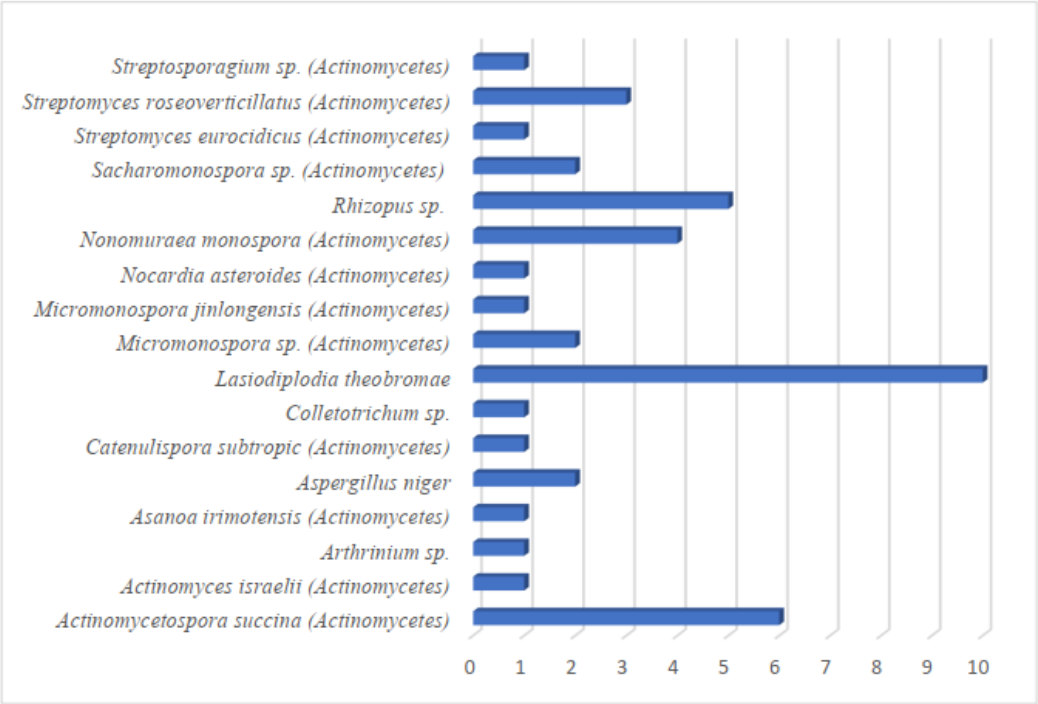
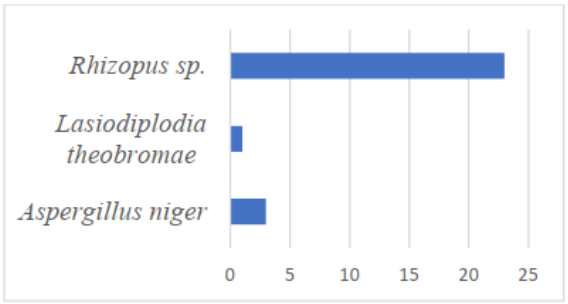


Figure 2. Population of exophytic microbes in healthy apple fruit



Gambar 3. Population of endofitic microbes in healthy apple fruit

Table 3. Diversity, dominance and evenness index of exophytic and endophytic healthy apple fruit

Index	Exophytic microbes		Endophytic microbes
H' (diversity index)	1.249		2.793
D (dominance index)	0.896	Simpson (C)	0.739
E (evenness index)	0.885		0.458

The research results of Sudarma *et al.* (2019) found that the index of diversity and dominance of exophytic fungi in healthy sugar-apple fruit was 2.3742 and 0.8667, respectively, while for endophytic fungi the index of diversity and dominance was 2.6356 and 0.6489, respectively. Likewise, the research results of Sudarma *et al*. (2021b) found that the diversity and dominance index for exophytic microbes was 2.450 and 0.4078, respectively, while for endophytic microbes the diversity and dominance index was 1.876 and 0.580, respectively. Almost all the dominant fungi are *Rhizopus* sp. this indicates that the fungus Rhizopussp. Many postharvest fruits were found both exophytic and endophytic. The more species found, the greater the opportunity to inhibit pathogens from attacking plants, so that apples can be saved from pathogen attacks.

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

In vitro inhibition of exophytic and endophytic microbes against pathogens showed that the best exophytes were *Actinomyces bovis* (Actinomycetes), *A.niger*, *Colletotrichum* sp. and *Rhizopus* sp. each of 83.33 ± 00%, while the best endophytic microbes were *L. theobromae* of 83.33 ± 00% (Table 4).

Tabel 4. Inhibition of exophytic and endophytic microbes against patogens *In Vitro*

No.	Name of exophytic microbes	Inhibition (%)	Name of endophytic microbes	Inhibition (%)
1	*Actinomyces bovis* (Actinomycetes)	83.33±00	*Aspergillus niger*	78.89±4,19
2	*Actinomycetospora succina* (Actinomycetes)	73.34±2,36	*Lasiodiplodia theobromae*	83.33±00
3	*Actinomyces israelii* (Actinomycetes)	-	*Rhizopus* sp.	80.55±5,89
4	*Arthrinium* sp.	-		
5	*Asanoa irimotensis (Actinomycetes)*	-		
6	*Aspergillus niger*	83.33±00		
7	*Catenulispora subtropic* (Actinomycetes)	-		
8	*Colletotrichum* sp.	83.33±00		
9	*Lasiodiplodia theobromae*	77.5±8,24		
10	*Micromonospora* sp. (Actinomycetes)	-		
11	*Micromonospora jinlongensis* (Actinomycetes)	75±00		
12	*Nocardia asteroides* (Actinomycetes)	66.67±00		
13	*Nonomuraea monospora* (Actinomycetes)	-		
14	*Rhizopus sp.*	83.33±00		
15	*Sacharomonospora* sp. (Actinomycetes)	70±00		
16	*Streptomyces eurocidicus* (Actinomycetes)	-		
17	*Streptomyces roseoverticillatus* (Actinomycetes)	-		
18	*Streptosporagium* sp. (Actinomycetes)	-		

Aspergillus niger and *Rhizopus* sp. still showed the highest inhibition against pathogen
B. The inhibition was competitive because no seed zone was found as a sign of antibiotic inhibition. According to Wahdania et al. (2016) found the effect of *A. nger* against cocoa pod rot pathogen (*Phytophthora palmivora*). *Aspergillus niger* produces a-amylase and glucoamylase enzymes that allow starch to be broken down into simple glucose and then fermented into ethanol. *A. niger* also produces enzymes such as amylase, amyloglucosidase, pectinase, cellulose, glycosides that can break down amino acids and CO2 (Wulandari et al., 2016).

Vigianti (2015) succeeded in isolating Indonesian tempeh mushroom, which is a food product made from soybeans fermented by *Rhizopus oligosporus*. The role of *R. oligosporus* as the main fungus in soybeans is very important, thus changing its composition from soybeansubstrate to a more nutritious food and containing many enzymes and bioactive compounds, including antibacterial compounds. Furthermore, the results of research by Endrawati and Kusumaningtyas (2017) stated that Rhizopus sp is a fungus that easily grows in soil, vegetables and fruits as well as fermented processed products. *Rhizopus* sp. can increase the nutritional value of feed ingredients. *Rhizopus* sp. has long been known in Indonesia, especially for the manufacture of tempe. Several

studies on *Rhizopus* sp. have opened up opportunities forthe use of *Rhizopus* sp. for other functions. The fungus *Rhizopus* sp. can suppress the growth of the toxigenic fungus Aspergillus flavus and degrade aflatoxins. *Rhizopus* sp. can also producecompounds that can inhibit pathogenic bacteria and function as antioxidants. *Rhizopus* sp. absorbs some mineral elements and converts them into organic minerals so that they can improve mineral absorption in the body better. The use of fermented feed ingredients by *Rhizopus* sp. in livestock showed better results than without fermentation. *Rhizopus* sp. Sudarma *et al*. (2018) stated that the diversity of exophytic fungi plays an important role in controlling disease-causing pathogens, especially sugar apple fruit rot. The greater the diversity, the more stable the ecosystem, and the more opportunities there are to inhibit pathogens.

In Vivo Inhibition of Exophytic and Endophytic Microbes

In vivo inhibition of exophytic and endophytic microbes against pathogens showed that their effect was very significant in suppressing pathogen growth in fresh fruit. The effect of treatment C (*A. niger*) was very good in suppressing the growth of pathogens, so that the apples still looked smooth and fresh, different from treatments A (*L. theobromae*) and K+P (Table 5; Figure 4). Fendiyanto and Satrio (2020) stated that *A. niger* has the ability, as a biological agent, to suppress food spoilage pathogens in many foods, including bread, however, there are still few reports of antagonistic tests on bread, particularly between *A. niger* as a biological control agent against rotting fungus. Therefore, to analyze the growth antagonist test of *A. niger* against food spoilage fungi, with the hope that the shelf life of bread can be extended and mycotoxin contamination can be avoided. Antagonist test is a test that utilizes the characteristics of microorganisms that grow faster than pathogens or produce antibiotic compounds. The fungus with the highest inhibition value was *Hyphopichia burtonii*, while the lowest was *Saccharomyces cerevisiae*. These findings suggest that *A niger* can be used asa biocontrol in extending bread storage in the future.

Table 5. Inhibition exophytic and endophytic microbes against pathogens *in vivo*

Treatment	Average Inhibition (%)	Notation	
		DMRT 5%*	DMRT 1%*
K-P tanpa pathogen)	5±0	a	a
K+P (dengan pathogen)	96±5.48	c	c
A (*L. theobromae*)	92±8.37	c	c
B (*Nocardia asteroides* (Actinomycetes)	12±4.47	bc	a
C (*A. niger*)	6±2.24	a	a
D (*Sacharomonospora* sp. (Actinomycetes)	44.48±5.48	b	b
E (*Rhizopus* sp.)	10±2.24	bc	a

* Note: the same letter in the same column means that the DMRT test level is significantly different at 5% and 1% is very significant.

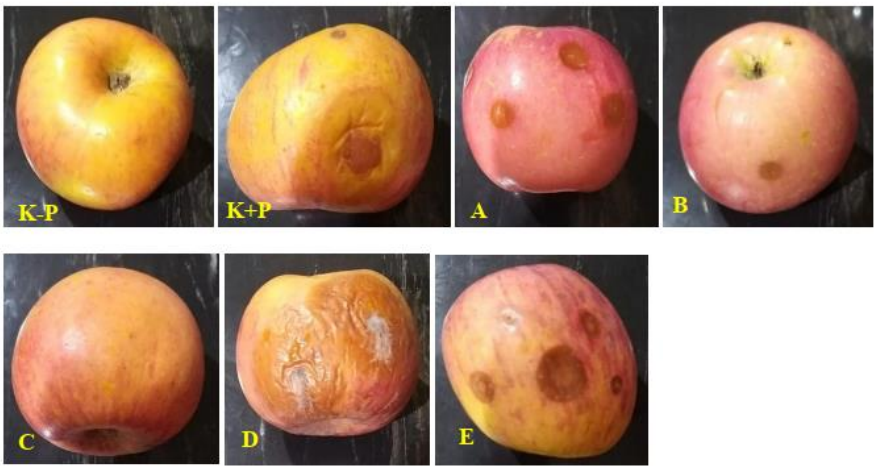


Figure 4. Effect of exophytic and endophytic microbial treatment on pathogens, (K-P = without pathogen, K+P

= with pathogen, A = *L. theobromae*, B = *Nocardia asteroides* (Actinomycetes., C = *A. niger*, D = *Sacharomonospora* sp. (Actinomycetes), and E = *Rhizopus* sp.) 3 days after inoculation

Sriherwanto et al. (2017) stated that fermentation using *Rhizopus* sp. has the potential to be further developed as a biofloating agent in the manufacture of floating fish feed. Moensaku *et al.* (2021) stated that based on the results of the study, it was stated that the mold in red bean tempeh was fermented by *Rhizopus oligosporus* and *Rhizopus* was antagonistic against pathogenic bacteria. The results of this study strengthen the benefits of tempeh as a functional food.

CONCLUSION

Apple rot disease caused by *Alternaria* sp. The results showed that there were 45 isolates of exophytic microbes and 27 isolates of endophytes. The exophytic microbial diversity index of healthy apples was 1.249, the dominance index was 0.896, and the harmony index was 0.885. The endophytic microbial diversity index in healthy apples was 2.793, Simpson's dominance index was 0.739, and the concordance index was 0.458. The inhibition of exophytic and endophytic microbes against pathogens in vitro showed that the highest inhibition of exophytic microbes was *Actinomyces bovis* (Actinomycetes), *A. niger*, *Colletotrichum* sp., and *Rhizopus* sp. each of 83.33±00%, while the highest endophyte was shown by *L. theobromae* at 83.33±00%, followed by the fungus Rhizopus sp. by 80.55±5.89 % and the smallest by the fungus *A. niger* by 78.89±4.19%. *In vivo* inhibition of exophytic and endophytic microbes against pathogens showed that their effect was very significant in suppressing pathogen growth in fresh fruit. The effect of treatment C (*A. niger*) was very good in suppressing the growth of pathogens, so that the apples still looked smooth and fresh, different from treatments A (*L. theobromae*) and K+P (treatment with pathogens).

Acknowledgements

Authors wish to thank to the Rector of Udayana University for their assistance and the opportunity given so that research can be resolved, Dean of the Faculty of Agriculture, Udayana University, and Chairman of the Institute for Research and Community Service Udayana University, for their help and cooperation so that research can be funded to completion.

REFERENCE

Barnett, H.L. and B.B. Hunter. 1998. *Illustrated Genera of Imperfect Fungi*. APS Press. The American Phytopathological Sociey. St Paul, Minnesota.

Endrawati, D. dan E. Kusumaningtyas. 2017. Beberapa fungsi Rhizopus sp. dalam meningkatkan nilai nutrisi bahan pakan. *Jurnal Wartazoa* 27(2): 081-088 (Indonesian language)

Fendiyanto M.H, and R.D. Satrio. 2020. Identification, aflatoxin content, and antagonistic test of spoilage fungi in bread to *Aspergillus niger*. *Bioteknologi* 17: 60-66.

Gauther N.W. 2019. Fruit Diseases of Apple. College of Agriculture, Food and Environment. Cooperative Extension Service. University of Kentucky.

Gelain J., N.A. Hamada, and L.L. May de Mio. 2020. Survival of pathogens after dormancy inapple tree twigs indicates potential risk as source of inoculum. *Acta Scientiarum* 44: 1-11.

Godani K. 2022. Types of Diversity Indices of Biodiversity. https://www.biologydiscussion.com/biodiversity/types/2-types-of-diversity-indices-of-biodiversity/8388. Accessed 7/5/2022.

Goswami S., N. Goel1, and R.S. Majumdar. 2021. Phylloplane microbes impact hostphysiology: a review. *Journal of Plant Protection Research* 61(3): 213-221.

Gur L., M. Reuveni and Y. Cohen. 2021. B-Aminobutyric acid induced resistance against Alternaria fruit rot in apple fruits. Journal of Fungi 7: 1-13.

Indrawati. G., R.A. Samson, K. Van den Tweel-Vermeulen, A. Oetari dan I. Santoso. 1999.*Pengenalan Kapang Tropik Umum*. Yayasan Obor Indonesia. Universitas Indonesia (University of Indonsia Culture Collection) Depok, Indonsia dan Centraalbureau voorSchirmmelcultures, Baarn, The Netherlands.

Lee JM, WS Tan, and ASY Ting. 2014. Revealing the antimicrobial and enzymatic potentials of culturable fungal endophytes from tropical pitcher plants (*Nepenthes* spp.). *Mycosphere* 5(2): 364-377.

Li Y., P.M. Hirst, Y. Wan, Y. Liu, Q. Zhou, H. Gao, Y. Guo, Z. Zhao, L. Wang and M. Han. 2012. Resistance to *Marssonina coronaria* and *Alternaria alternata* Apple Pathotype in the Major Apple Cultivars and Rootstocks Used in China. *Hortscience* 47(9):1241–1244.

Madhu G.S., Sajad Un Nabi, J.I. Mir, W. H. Raja, M. A Sheikh, O.C. Sharma and D.B.Singh. 2020. Alternaria leaf and fruit spot in apple: Symptoms, cause and management. European Journal of Biotechnology and Bioscience 8(3): 24-26.

Miyadoh, S. 1997. Atlas of Actinomycetes. Asakura Publishing Co Ltd. Japan.

Moensaku, E., Y. Sine and L. Pardosi. 2021. Isolasi dan identifikasi kapang *Rhizopus* pada tempe kacang merh (*Phaseulus vulgaris* L.). *Jurnal Pendidikan Biologi Undiksha* 8(2):61-69 (Indonesian language).

Odum, E.P. 1971. *Fundamentals of Ecology*. Third Edition. W.B. Saunders Company.Philadelphia, Toronto, London. Toppan Company, Ltd. Tokyo, Japan.

Pitt, J.I. and A.D. Hocking. 1997. *Fungi and Food Spoilage*. Blackie Avademic and Professional. Second Edition. London-Weinhein-New York-Tokyo-Melboune-Madras.

Samson, R.A., E.S. Hoekstra, and C. A.N. Van Oorschot. 1981. *Introduction to Food-Borne Fungi*. Centraalbureau Voor-Schimmelcultures. Institute of The Royal Netherlands. Academic of Arts and Sciences.

Sriherwanto, C., I. Sujadi dan S. Soraya.2017. Pemanfaatan kapang *Rhizopus* sp. sebagai agenhayati pengapung pakan. *Jurnal Mikologi Indonesia* 1(2), 70-81 (Indonesian language).

Sudarma I M., N.W.Suniti, N.N. Darmiati and I G.N. Bagus. 2018. The Diversity of Exophytic Fungus in Sugar-Apple Plant and their Inhibition Ability on Lasiodiplodia theobromae(Cause of Srikaya Fruit Rot) In Vitro. *Int. J. Curr. Res. Biosci. Plant Biol.* 5(10), 32- 38.

Sudarma I M., N.W. Suniti and N.N. Darmiati.2019. Exophytic and Endophytic Fungus that Potential as Biocontrol Agents on *Lasiodiplodia theobromae* caused Fruit Rot at Sugar- Apple. *Int.J.Curr.Microbiol.App.Sci* 8(2): 131-142.

Sudarma I M., N. N, Darmiati and N. W. Suniti. 2021a, Utilization Exophytic and Endophytic Fungi to Control Banana Fruit Rot (*Lasiodiplodia theobromae*). *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 14(10): 18-25.

Sudarma I M., N.N. Darmiati and N.W. Suniti. 2021b. Control of Papaya Rot Disease by Using Exophytic and Endophytic Fungi Which is Environmentally Friendly. *Int.J.Curr.Microbiol.App.Sci* 10(09): 600-612.

Sudarma I M., K. Khalimi, N.W. Suniti and N.N. Darmiati. 2022. The first report: rotten fruit sugar-apple in Bali. *Int.J.Curr.Microbiol.App.Sci* 11(02): 256-263.

Swamy N., and S. S. Sandhu.2021. Fungal endophytes: Entry, establishment, diversity, future prospects in agriculture. In Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology. Vol. Fungal Diversity of Sustainable Agriculture. Pages 97-105.

Tauruslina ,E,Trizelia ,Yaherwandi dan Hasmiandy ,H. 2015. Analisis keanekaragaman hayati musuh alami pada eksosistem pada sawah di daerah endemik dan non - endemik WerengBatang Cokelat Nilaparvata lugens di Sumatera Barat. *Pros Sem Nas Masy Biodiv Indon*1(3): 581 – 589 (Indonesian language).

Ulfah,M. S.N. Fajri, M. Nasir, K. Hamsah and S. Purnawan. 2019. Diversity, evenness and dominance index reef fish in Krueng Raya Water, Aceh Besar. IOP Conf. Series: Earth and Environmental Science 348: 1-5.

Verma R., A. Dutta, A.K. Choudhary, and S. Maurya. 2017. Trichoderma asperellum, a potential fungal biocontrol agent against *Aspergillus niger. Journal of Advanced Laboratory Research in Biology* 8(4): 74-78.

Virgianti, D.P. 2015. Uji Antagonis Jamur Tempe (Rhizopus Sp) terhadap Bakteri PatogenEnterik. *Biosfera* 32(3): 162-163 (Indonesian language).

Wahdania, I. Asrul, dan Rosmini. 2016. Uji daya hambat *Aspergillus niger* pada bebrbagai bahan pembawa terhadap *Phytophthora palmivora* penyebab busuk buah kakao (*Theoborma cacao* L.). *e-j Agrotekbis* 4(5): 521-529 (Indonesian language).

Wilson, A. and N. Gownaris. 2022. Diversity Indeces. Biology. LibreTexts. Department of Education Open Textbook Pilot Project, the UC Davis Office of the Provost, the UC Davis Library, the California State University Affordable Learning Solutions Program, and Merlot.

Wulandari, D.E., Asrul dan I. Lakani. 2016. Seleksi jamur antagonis *Aspergillus niger* dari berbagai lahan perkebunan kakao untuk mengendalikan *Phytophthora palmivora. J. Agroland* 23(3): 233-242 (Indonesian language).