



Original Research Paper | Vol. 04 Issue 10 Oct - 2021 | Manuscript ID: #0503

# MOLECULAR IDENTIFICATION OF ANTHRACHNOSE PATHOGEN *(COLLETOTRICHUM MUSAE)* IN BANANA FRUITS AND THE USE OF EXOPHITIC AND ENDOPHYTICFUNGI TO CONTROL THE PATHOGEN

**I Made Sudarma**
*Professor of Plant Pathology, Agroecotecnology Study Program, Faculty of Agriculture, Udayana University.*
**Ni Wayan Suniti**
*Lecturer staff of the Agricultural Technology Study Program, Faculty of Agriculture, Udayana University*
**Ni Nengah Darmiati**
*Lecturer staff of the Agricultural Technology Study Program, Faculty of Agriculture, Udayana University*

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

## ABSTRACT

*Banana rot disease is often found in Bali, especially bananas are the main means for completing religious ceremonies in Bali. Banana rot often spoils during storage or post-harvest. The results showed that the cause of banana rot disease was Colletrotrichummusae with DNA fragments measuring 650 bp successfully amplified using universal primers ITS1/ITS4. The highest in vitro inhibition of exophytic fungi against pathogens was achieved by A. niger, Neurospora sp. and Rhizopus sp. each with an inhibitory power of 100%, the lowest was achieved by Neurospora sp. at the time of days after inoculation (DAI) by 50%. While the inhibition of endophytic fungi against pathogens was Rhizopus sp. with inhibition of 100% from 1 DAI to 4 DAI followed by A. niger at 100% at 3 and 4 DAI. The results of the in vivo inhibition of selected exophytic and endophytic fungi against pathogens were as follows: the highest inhibitory power was C (Rhizopus sp.1) of 94±5.48%, followed by treatment A (A. niger 1) of 92±8.37%, treatment B (A. niger 2) was 64±5.48% %, treatment E (Rhizopus sp. 2) was 54±5.48%, and the least inhibition was treatment D (Neurospora sp. 1) by 44±5.48%.*

## KEYWORDS

*Molecular identification, in vitro and in vivo inhibition, exophytes and endophytes, Colletorchummusae*

## INTRODUCTION

Banana (*Musa paradisiaca* L.) is one of the most popular fruits in Bali. Bananas are usually harvested before ripening and stored in cold temperatures during transportation and marketing processes. Long-distance transportation and long-term storage in the market make bananas sensitive to disease (Thompson and Burden, 1995). Moreover, it is often found in traditional markets, many bananas are consumed for offerings.

Anthracnose caused by *Colletotrichum musae* (Berk. & Brief.) Arx. is the most important pathogen in injured ripe and green bananas (Meredith, 1960;Stover and Simmonds, 1987). Sometimes the fungus attacks the neck of the green radius when it is damaged by bending (Wardlaw, 1995). The lesions are sunken and covered with salmon-colored acervuli (Sutton and Waterston, 1970). Infection stimulates fruit ripening and lesions lengthen with ripening. On ripe fruit, sunken brown spots develop with orange acervuli (Stover and Simmonds, 1987).

Fruit quality can be decreased due to anthracnose disease, this is due to lack of plant care, and poor handling in the garden and during transportation which causes mechanical damage and provides the opportunity for infection for larger banana fruit rot microbes. Anthracnose attacks can occur in addition to pathogens entering through wounds as well as fruit being penetrated from the start of fruit on the tree (Faizal *et al*., 2011). Some of the important consequences of the disease are (i) decreased nutritional value [2] (ii) contamination of foodstuffs by mycotoxins produced by pathogens (iii) toxic metabolites produced by diseased plant tissues in response to fungal attack (iv) bad taste. unacceptable associated with diseased material. Among the various factors responsible for banana postharvest losses, especially anthracnose caused by *C. musae*, is one of the main factors aimed at local as well as remote markets (Jeffries *et al*., 1990).

## I. MATERIALS AND METHODS

### I.1. Place and time of research

The research was carried out in two places: 1) looking for sick and healthy fruit specimens from the Batubulan market and supermarkets. 2) Laboratory of Plant Diseases and Agricultural Biotechnology Laboratory. The research was carried out from April to August 2021.

### I.2. Moleculer identification

#### a. DNA Extraction

DNA extraction followed the procedure of Doyle and Doyle (1987), 0.2 g of pathogenic fungal mycelium samples were ground with liquid nitrogen and powdered fungal pathogens were put into Eppendorf tubes. Then 500 L of CTAB buffer and 50 L -mercaptoethanol were added, then mixed until homogeneous using a vortex. To lyse the cell wall, heating at a temperature of 70ºC for 60 minutes where every 10 minutes is back and forth to speed up the lysis process.

Then cooled down to room temperature. Then 500 L of chloroform isoamylalcohol (24:1) was added to the tube and mixed until homogeneous by vortex and centrifuged at 12,000 rpm for 15 minutes. The supernatant obtained was transferred to a new Eppendorf tube by adding 500 L of sodium acetate, mixed until homogeneous by vortex and centrifuged again at 12,000 rpm for 10 minutes. The supernatant was transferred to an eppendorf tube and then 500 L of sodium acetate and isopropanol were added, mixed until homogeneous by vortex and

centrifuged again at 12,000 rpm for 10 minutes. The tube was shaken gently to bind DNA and incubated at -20° C for 30 minutes. The DNA threads obtained were precipitated by centrifugation for 10 minutes. The supernatant was discarded, the pellet was washed with ethanol (70%) then centrifuged at 8,000 rpm for 5 minutes. The ethanol was removed and the pellets were dried. The pellet was resuspended with 50 L of TE buffer and stored at -20ºC for further use in the DNA amplification process.

### b. DNA amplification

DNA amplification was carried out on a Thermo Cycle PCR machine. Amplification was carried out using universal primers to detect the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA), namely forward primer ITS1 (5'- CTTGGTCATTTAGAGGAAGTAA-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with target size results. amplification was 490 bp (Doyle & Doyle, 1987). The DNA amplification reaction was carried out with a total volume of 25 L consisting of 1 L of DNA, 2.5 L of 10 x buffer and Mg2+, 0.5 L of 10 mM dNTP, 1 L of each primer, 12.5 L of Taq DNA (10 units/ L), and 9.5 L H2O. The amplification conditions were divided into several stages, namely predenaturation at 94 C for 3 minutes, followed by 30 cycles of amplification, each cycle consisting of strand separation/denaturation of DNA at 94 C for 1 minute, primer attachment/annealing at 45 C for 1 minute, DNA synthesis at 72 C. for 2 minutes. Especially for the last cycle, the synthesis step is added for 10 minutes, then the cycle will end with a temperature of 4 C.

### c. DNA electrophoresis

The amplified product was analyzed using Blued electrophoresis with 1% agarose gel (0.5xTris-Borate EDTA/TBE). Electrophoresis was carried out at 100 volts for 28 minutes and then the agarose gel was incubated in dye containing ethidium bromide (1%) for 15 minutes, then washed with H2O for 10 minutes. The results of the electrophoresis were visualized with an ultraviolet transilluminator. The DNA bands formed on the electrophoresis results were documented with a digital camera.

### d. DNA Sequence Analysis

The amplification product was sent to Macrogen (Singapore) for nucleotide sequencing. The tracing results were then analyzed using the basic local alignment search tool (BLAST) program with an optimization program to obtain DNA base sequences that have homology with DNA sequences found on the National Center for Biotechnology Information (NCBI) website. The nucleotide sequences obtained were then analyzed using ClustalW multiple alignment on Bioedit sequence alignment editor software version 7.0.5. Homology results close to 100% similarity categorized as the same species as the sample species.

### I.3.Isolation of Endophytic and Exophytic Fungi

Isolation of endophytic fungi, plant parts such as fruit, leaves and stems were washed with sterile running water, then the plant parts were sterilized with 0.525% sodium hypochlorite for 3 minutes, and 70% alcohol for 2 minutes, then rinsed with sterile water for 1 minute. and then placed on PDA media (which was first given an anti-bacterial antibiotic, namely livoploxacin with a concentration of 0.1% (w/v). The fungus that emerged from the leaf pieces was transferred to a test tube containing PDA to be stored and classified by morphospecies.

Exophytes can be carried out by spraying plant parts (fruit, leaves and stems).The washing water is collected, then in a tube, then taken, from a 1 ml tube it is grown into a PDA which has previously been filled with livoploxacin with a concentration of 0.1% (w/v). ).

### I.4. Identification of Endophytic and Exophytic Fungi

The stored endophytic and exophytic fungi were then grown in Petri dishes containing PDA and repeated 5 times. Cultures were incubated in the dark at room temperature (±27oC). Isolates were identified macroscopically after 3 days of age to determine colony color and growth rate, and microscopic identification to identify septa on hyphae, spore/conidia shape and sporangiophores. Fungal identification using reference book Samson *et al.*, 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; and Indrawati *et al.*, 1999 and identification of actiomyceytesMiyadoh(1997).

### I.5. Inhibitory Test of Endophytic and Exophytic Fungi Against Pathogens

The endophytic and exophytic fungi 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 inhibitory ability can be calculated as follows (Dollar, 2001; Mojica-Marin et al., 2008):

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

Where:
A = Pathogen colony diameter in single culture (mm)
B = Pathogen colony diameter in dual culture (mm)

### 1.6. Endophytic and Exophytic Fungal Prevalence

Determining the prevalence of endophytic and exophytic fungi was based on the frequency of endophytic and exophytic fungal isolates found in healthy fruit per Petri dish, divided by all isolates found times 100%. The prevalence of isolates will determine the dominance of endophytic fungi present in healthy mango fruit.

### I.7. In Vivo Antagonist 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. pathogens. Endophytic and exophytic fungi 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 smallest significant difference test (SDT) at the 5% level. Attack parameters measured by the formulation: the number of stabs attacked by the fungus divided by the total number of punctures (20 x) times 100%.

## II. RESULTS

### II.1. Microscopic Identification of Pathogens

Symptoms at the end of the fruit appear small spots (anthracnose) which over time can coalesce to form larger spots (Figure 1A), then after being isolated and transferred to a Petri dish, white mycelium appears with a pink color in the middle.(Figure 1B), colonies growing on the fruit skin were transferred to a new Petri dish, the

mycelium appeared pink (Figure 1C), and when viewed under a microscope the conidia were small round (Figure 1D).

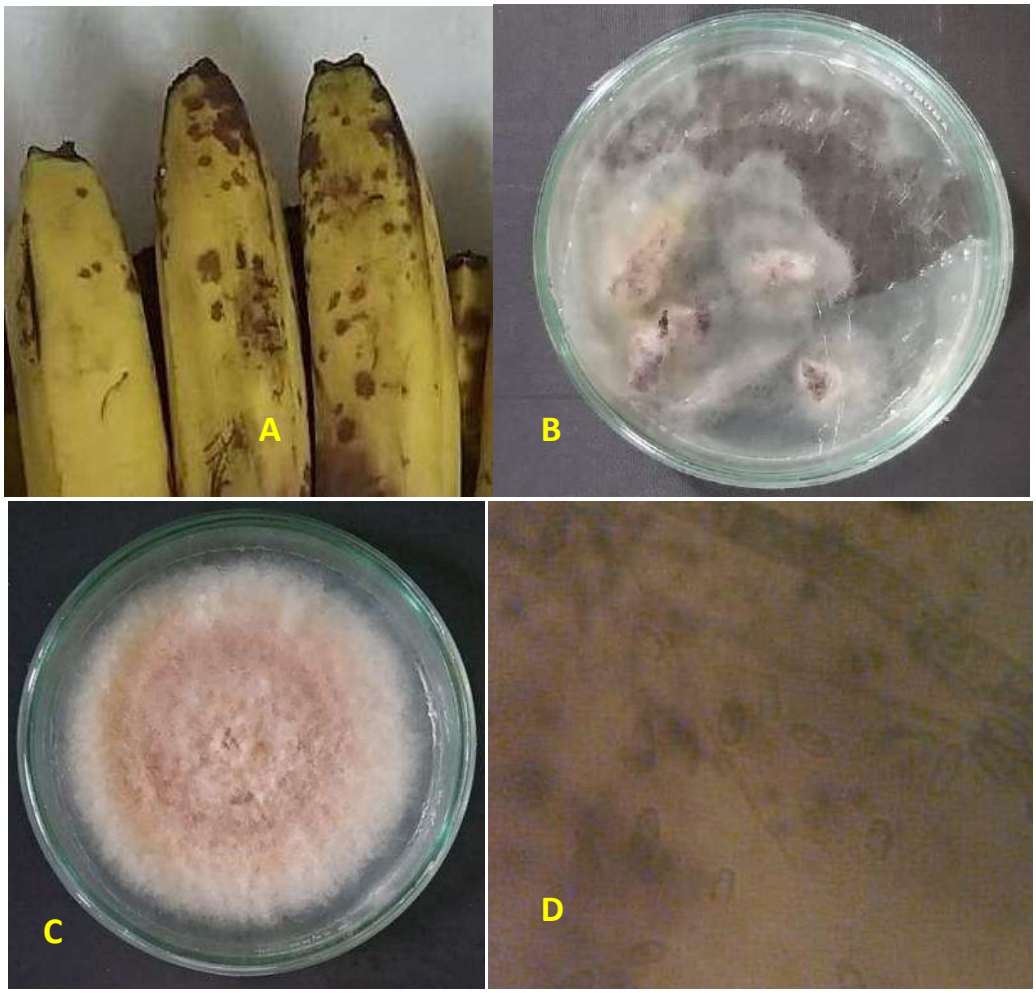


**Figure 1.** Symptoms of anthracnose (A), isolated pathogen (B), mycelium in Petri dishes (C, and conidia under a microscope (D), (s = symptom)

The results of the isolation of the pathogen obtained pink fungal mycelium colonies and very slow growth of 7 days to fill the Petri dish (Figure 1C) and the observations under the microscope showed conidia in large numbers of one-celled oval round shape (Figure 1D). After being investigated, it turned out that the pathogen was *C.musae*. Outcomes were identified based on reference pathogens according to Balendres*et al* (2020).

## II. 2. Moleculer Identification

### *Colletotrichum musae*

DNA fragments measuring 650 bp were successfully amplified from 4 fungal samples using the universal primer ITS1/ITS4 (Figure 2). The amplified DNA sample was then used for the sequencing stage to determine the fungal species. Sequencing analysis confirmed that the fungus sampled was Colletotrichum musae with 98-100% homology to several Colletotrichum musae isolates in the genebank (Table 1). The phylogenetic tree of the pathogen (Colletotrichum musae) is shown in Figure 3).

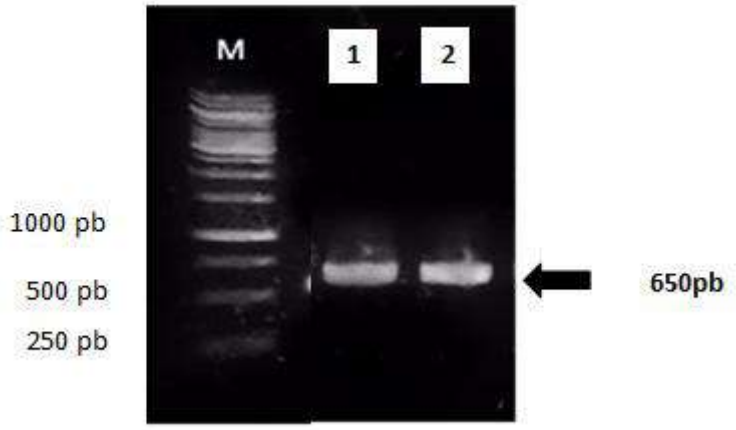


**Figure 2.** Visualization of amplified fungal pathogenic DNA using universal primers ITS1/ITS4 on 1% agarose gel. M: DNA marker (1kb ladder); Sample no. 1, and 2, (pathogen, *C. musae*)

### II.3.Exophytic and Endophytic Fungus Populations

Exophytic fungi found in healthy fruit were *Aspergillus niger* as many as 2 isolates, *A. flavus* as many as 4 isolates, *Oidium sp.*a total of 4 isolates, *Nocardia asteroids* (Actinomycetes) as many as 2 isolates, *Nocardia* sp. (Actinomycetes) as many as 2 isolates, *Neurospora* sp. a total of 12 isolates and *Rhizopus* sp. A total of 18 isolates (Table 2). While the endophytic fungi found in healthy banana fruit were 6 isolates of *A. niger*, *Colletotrichum* sp. as many as 2 isolates, *A. flavus* as many as 2 isolates and *Rhizopus* sp. a total of 6 isolates (Table 2).

**Table 1. Homology (%) of nucleotide sequences of *Colletotrichum musae* isolates with several isolates that have been reported in GenBank**

| Sikuen | C_musae | JX163232_MYS | MT351114_CHN | MK298312_AUS | MK087092_BRA | MH863550_CZH | MG386643_MYS | KX069828_AUS |
|---|---|---|---|---|---|---|---|---|
| Colletotrichum_musae | ID | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| JX163232_C_musae_MYS | 100.0% | ID | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| MT351114_C_musae_CHN | 100.0% | 100.0% | ID | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| MK298312_C_musae_AUS | 100.0% | 100.0% | 100.0% | ID | 100.0% | 100.0% | 100.0% | 100.0% |
| MK087092_C_musae_BRA | 100.0% | 100.0% | 100.0% | 100.0% | ID | 100.0% | 100.0% | 100.0% |
| MH863550_musae_CZH | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | ID | 100.0% | 100.0% |
| MG386643_C_musae_MYS | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | ID | 100.0% |
| KX069828_C_musae_AUS | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | ID |
| KT582190_C_musae_SRI | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| KM233233_C_musae_COL | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| KC790969_C_musae_IND | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| GQ369594_C_capsici | 91.3% | 91.3% | 91.3% | 91.3% | 91.3% | 91.3% | 91.3% | 91.3% |

Further phylogenetic analysis showed that Colletotrichum musae isolates formed one group with other isolates that had been registered in the genebank. As an out group isolate, *Colletotrichum capsisi* (GQ369594) isolate was used (Figure 3).

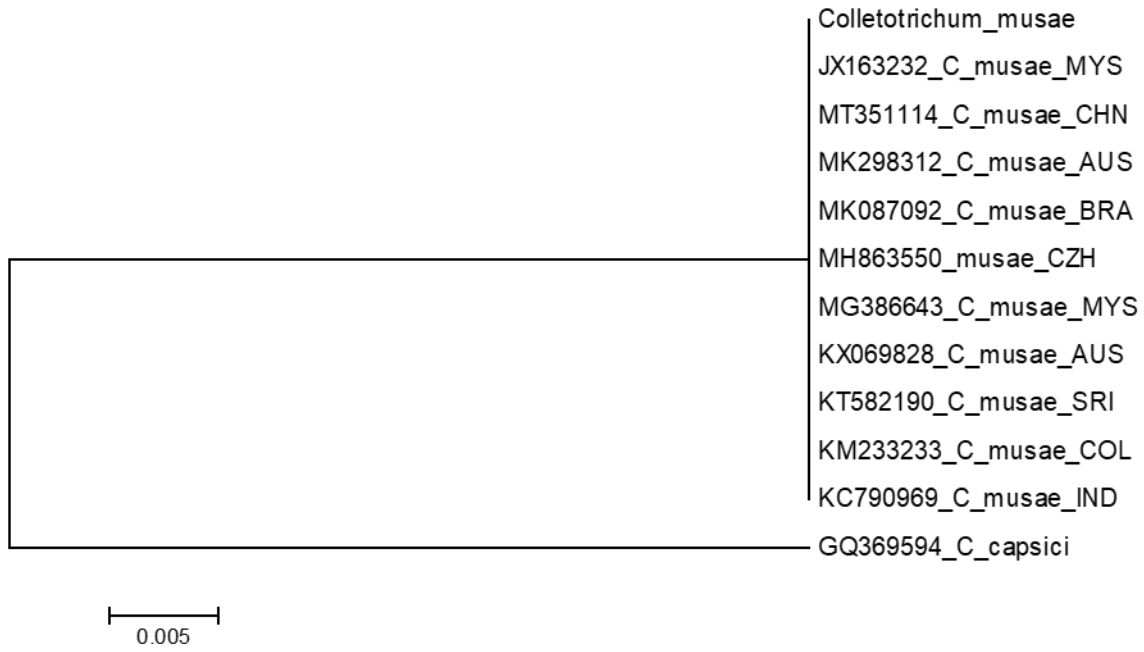


**Figure 3.** Phylogenetic analysis of Colletotrichum musae isolate based on partial nucleotide sequence alignment of DNA-A using Mega 6.06 (Neighbor Joining Algorithm with 1,000 bootstraps replicates)

### II.4. In Vitro Inhibitory Test

The highest inhibitory power of exophytic fungi against pathogens (*C.musae*) was achieved by *A. niger*, *Neurospora* sp. and *Rhizopus* sp. each with an inhibitory power of 100%, the lowest was achieved by *Neurospora* sp. at the time of DAI by 50% (Table 3). While the inhibition of endophytic fungi against pathogens is *Rhizopus* sp. with an inhibition of 100% from 1 DAI to 4 DAI followed by *A. niger* at 100% at 3 and 4 DAI (Table 4).

**Table 3. Observations of the inhibitory power of exophytes against pathogens (*Colletotrichum musae)***

| No. | Name of exophytic microbes | 2 DAI (%) | 3 DAI (%) | 3 DAI (%) | 5 DAI (%) |
|---|---|---|---|---|---|
| 1 | *A. niger* 1 | - | 62 | 100 | 100 |
| 2 | *A. niger* 2 | 100 | 100 | 100 | 100 |
| 3 | *Oidium* sp.1 | - | - | - | - |
| 4 | *Nocardia asteroids* 1 (Actinomycetes) | - | - | - | - |
| 5 | *A. flavus* 1 | - | - | - | - |
| 6 | *Nocardia* sp.1 (Actinomycetes) | - | - | - | - |
| 7 | *Neurospora* spp.1-6 | 100 | 100 | 100 | 100 |
| 8 | *A. flavus* 2 | - | - | - | - |
| 9 | *Oidium* sp. 2 | - | - | - | - |
| 10 | *Oidium* sp.3 | - | - | - | - |
| 11 | *Nocardia asteroids* 2 (Actinomycetes) | - | - | - | - |
| 12 | *A. flavus* 3 | - | - | - | - |
| 13 | *Nocardia* sp.2 (Actinomycetes) | - | - | - | - |
| 14 | *Neurospora* sp. | - | - | - | - |
| 15 | *Neurospora* sp. 8 | 50 | 67 | 75 | 83 |
| 16 | *Neurospora* spp. 9-12 | - | - | - | - |
| 17 | *A. flavus* 4 | - | - | - | - |
| 18 | *Oidium* sp. 4 | - | - | - | - |
| 19 | *Rhizopus* spp. 1-18 | 100 | 100 | 100 | 100 |

DAI = days after inoculation

**Table 4. Observations of endophytic inhibition against pathogen B (*Colletotrichum musae*)**

| No. | Name of eendophytic fungi | 1 DAI (%) | 2 DAI (%) | 3 DAI (%) | 4 DAI (%) |
|---|---|---|---|---|---|
| 1 | *A. niger*1 | 40 | 54 | 100 | 100 |
| 2 | *Colletotrichum* sp. 1 | - | - | - | - |
| 3 | *A. niger*2 | 10 | 38 | 100 | 100 |
| 4 | *A. flavus* 1 | - | - | - | - |
| 5 | *A. niger*3 | 40 | 54 | 63 | 70 |
| 6 | *A. niger*4 | - | - | - | - |
| 7 | *Colletotrichum* sp. 2 | - | - | - | - |
| 8 | *A. niger*5 | 50 | 71 | 78 | 83 |
| 9 | *A. flavus* 1 | - | - | - | - |
| 10 | *A. niger*6 | - | - | - | - |
| 11 | *Rhizopus* spp. 1-5 | 100 | 100 | 100 | 100 |

### II.5. In Vivo In Vivo Inhibition Test

In vivo inhibition between selected exophytic and endophytic fungi with pathogen B (*C. musae*) was as follows: C (*Rhizopus* sp.1) had the highest inhibitory power of 94±5.48%, followed by treatment A (*A. niger* 1) was 92±8.37%, treatment B (*A. niger* 2) was 64±5.48% %, treatment E (*Rhizopus* sp. 2) was 54±5.48%, and the smallest inhibitory power was treatment D (*Neurospora* sp. 1) was 44±5.48% (Table 5).

**Table 4. Inhibition of selected exophytic and endophytic fungi against pathogen B (*Colletotrichum musae) in vivo***

| Treatment | Replication | | | | | Average | Notation | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | | 5% | 1% |
| K-P | 100 | 100 | 100 | 100 | 100 | 100 | F | F |
| K+P | 0 | 0 | 0 | 0 | 0 | 0 | A | A |
| A | 100 | 100 | 90 | 80 | 90 | 92±8.37 | E | E |
| B | 70 | 60 | 70 | 60 | 60 | 64±5.48 | D | D |
| C | 100 | 90 | 100 | 90 | 90 | 94±5.48 | E | E |
| D | 50 | 40 | 50 | 40 | 40 | 44±5.48 | B | B |
| E | 60 | 50 | 60 | 50 | 50 | 54±5.48 | C | C |

**Information**:A = *Aspergillus niger* 1 (exophytic fungus), B = *A. niger* 2 (exophytic fungus), C = *Rhizopus* sp. 1 (endophytic fungi), D = *Neurospora* sp. 1 (exophytic fungi), and E = *Rhizopus* sp. 2 (endophytic fungi)

## III. DISCUSSION

Exophytic and endophytic fungi found in healthy grapes are exophytic fungi, including *Neurospora* sp. as many as 2 isolates, Actinomycetes as many as 12 isolates, and *A. flavus* as many as 15 isolates, while the endophytic fungi were A. flavus as many as 5 isolates and *Aspergillus* sp. as many as 2 isolates (Sudarma*et al*. 2019). Likewise, in healthy mangoes, 6 isolates of A. flavus exophytic herbs were found, 6 isolates of *A. niger, Nucordia* sp. (Actinomycetes) as many as 6 isolates, *Rhizopus* sp. as many as 8 silat and Streptomyces (Actinomycetes) as many as 4 isolates (Sudarma*et al.*, 2020).

Various fungi have been present on the leaf surface, among these fungi have been tested for their fungitoxicity against *Aternariabrassicae* which causes leaf spot disease in cabbage. Colony interaction showed that *Trichodermviride* and Aspergillus flavus gave maximum suppression for the inhibition of the fungus *A. brassicae* (Yadav et al., 2011). The potential of the phylloplane fungus Sarpgandha (Rauwolfia serpentina) as a biocontrol agent was screened for antagonistic activity against *Alternaria alternata* in vitro, causing leaf spot on Rauwolfia serpentina. The antagonist culture filtrate was examined under “with cells” and “cell-free” conditions and showed that *T. piluliferum* (79.50) was equivalent to T. *harzianum* ISO-1(76.80) followed by *T. harzianum* ISO-2 (64.02), *P. sublateritium* (59.00) and *A.niger* (50.00). Meanwhile, *C. cladosporioides* showed minimum suppression (13.10) (Thkur and Harsh, 2016).

The phylloplane of the plant *Psidium guineense* was examined for fungal diversity in three months. A total of 17 fungal species were identified and one mycelium was sterile. Among them, most of the microorganisms grew in May and the least were found in March and January. *Cladosporium cladosporioides, Gliocladiumviride, Mucor racemosus, Penicillumchrysogenum* and sterile mycelium were found to be dominant among all species. The highest number of species was obtained from the month of May. The Shannon diversity index is maximum in May. Simpson high dominance index in May and high evenness in March. Thus the results showed that the monthly variation was not significant in fungal species in the leaves of *P. guineense* (Saha*et al*., 2013).

Potential of endophytic fungi isolated from healthy cocoa pods to control *Phytophthora palmivorain vitro* and *in vivo*. Endophytic fungal isolates were classified based on the morphological characteristics of their culture and reproductive structure. All isolates found were tested to inhibit *P. palmivora* by double culture method, and the 10 best isolates were continued for separate pod tests. Then, the five best isolates (*Aspergillus*4, *Aspergillus*5, *Aspergillus*6, *Fusarium*6, *Ramichloridium* sp.) were evaluated for their ability to reduce P. palmivora in cocoa seedlings and plants in the field. *Aspergillus, Fusarium*, and *Ramichloridium* showed maximum activity against *P. palmivora* in multiple culture assays, pods, and seedlings. However, when these five isolates were applied in the field, they did not suppress disease progression (Simamora*et al*., 2021).

Endophytes are asymptomatic fungal or bacterial microorganisms found in almost all reported living plant species. They are plant-associated microbes that form symbiotic associations with their host plants by colonizing internal tissues, which makes them valuable for agriculture as a tool in improving crop performance. Many endophytic fungi produce secondary metabolites like auxins, gibberellins etc which help in the growth and development of their host plants. Some of these compounds are antibiotics that have antifungal, antibacterial and insecticidal properties, which strongly inhibit the growth of other microorganisms, including plant pathogens (Dutta *et al.*, 2014).

## CONCLUSION

The results showed that the cause of banana rot disease was *Colletrotrichummusae* with DNA fragments measuring 650 bp successfully amplified using universal primers ITS1/ITS4. The highest in vitro inhibition of exophytic fungi against pathogens was achieved by *A. niger*, *Neurospora* sp. and *Rhizopus* sp. each with an inhibitory power of 100%, the lowest was achieved by Neurospora sp. at the time of days after inoculation (DAI) by 50%. While the inhibition of endophytic fungi against pathogens was *Rhizopus* sp. with an inhibition of 100% from 1 DAI to 4 DAI followed by *A. niger* at 100% at 3 and 4 DAI. The results of the in vivo inhibition of selected exophytic and endophytic fungi against pathogens were as follows: the highest inhibitory power was C (Rhizopus sp.1) of 94±5.48%, followed by treatment A (*A. niger* 1) of 92±8.37%, treatment B (*A. niger* 2) was 64±5.48% %, treatment E (*Rhizopus* sp. 2) was 54±5.48%, and the least inhibition was treatment D (*Neurospora* sp. 1) by 44±5.48%.

### 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.

## REFFRENCES

Dutta, D., K.C.Puzari, R.Gogoi and P. Dutta. 2014. Brazilian Archives Of Biology and Technology*An international journal*57(5): 621-629.

Simamora, A.V., M.V. Hahuly, and J. Bd. Henuk. 2021. Endophytic fungi as potential biocontrol agents of *Phytophthorapalmivora*in the cocoa plant. Biodevarsitas. 22(5): 2601-2609.

Ajay Krishna Saha, A.K. R.Aparajita and P. Das. 2013. Fungal Colonization of Phylloplane of *Psidium guineense*Sw. Growing in Suryamaninagar, Tripura, Northeast India. *International Journal of Basic and Applied Chemical Sciences* 3 (1): 62-67.

Thakur, S. and N.S.K. Harsh. 2016. Biocontrol Potential of Phylloplane Fungi Against Alternaria Alternata Causing LeafSpot in *Rauwolfia Serpentina in Vitro.*Research Paper. 5(2): 14-15.

Yadav, S.L., A.K Mishra, P.N. Dongre and Rashmi Singh. 2011. Assessment of fungitoxicity of phylloplane fungi against*Alternaria brassicae*causing leaf spot of mustard. *Journal of Agricultural Technology*7(6): 1823-1831.

Ferianita-Fachrul, M., H. Haeruman, dan L.C. Sitepu. 2005. *KomunitasFitoplanktonSebagai Bio-IndikatorKualitasPerairanTeluk Jakarta*. FMIPA-Universitas Indonesia Depok.

Dolar, F.S. 2001. Antagonistic effect of *Aspergillus melleus* Yukawa on soilborne pathogens of Chickpea. *TarimBilimleriDergisi*, 8(2): 167-170.

Mojica-Marin, V., H. A. Luna-Olvera, C. Fco, Sandoval-Coronado, B. Pereyra- Alférez, H. Lilia, Morales-Ramos, E. Carlos, Hernández-Luna and G. O. Alvarado-Gomez. 2008. Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani*of chili pepper. *African Journal of Biotechnology*, 7 (9): 1271-1276.

Pirzan, A.M., dan P. R. Pong-Masak. 2008. HubunganKeragamanFitoplanktondenganKualitas Air di PulauBauluang, KabupatenTakalar, Sulawesi Selatan. *Biodiversitas*, 9 (3) 217-221.

Rad, J.E., M. Manthey and A. Mataji. 2009. Comparison of Plant Species Diversity with Different Plant Communities in Deciduous Forests. *Int. J. Environ. Sci. Tech*, 6(3): 389-394.

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

Thompson, A. K. and O. J. Burden. 1995. Harvesting and fruit care. In: Bananas and plantains, S. Gowen, Chapman & Hall. 403-433 p.

Meredith, D. S. 1960. Studies on GloeosporiummusarumCke.& Mass. causing storage rots of Jamaican Banana. II. Somefactors influencing anthracnose development. Ann. Appl. Biol.48(3):518-528.

Stover, R. H. and N/W. Simmonds. 1987. Diseases and disorders. In: Bananas, Longman, 281-323 p.

Wardlaw, C. W. 1934. The nature and occurrence of pitting diseases and fruit spots. Tropical agriculture. 11: 8-13 p.

Sutton, B. C. and J.M. Waterston. 1970. Colletotrichum musae.CMI Description of pathogenic fungi and bacteria. CMI. No.222.

Faisal, P.M., K. Nagendran, T. P.Ranjitham,P.Kalaiselvi, R. Senthil, K. Gandhi, R.Thiruvengadam and K. Prabakar. 2011. Specific Detection of *Colletotrichum musae*Inciting

Anthracnose Disease in Banana. *Libyan Agriculture Research Center Journal International* 2 (6): 279-286.

Jeffries, P., J.C. Dodd, M.J. Jeger and R.A. Plumbley,1990. The biology and control of *Colletotrichum*species on tropical fruit crops. *J. Plant Pathol.*,39: 343-366.

Balendres, M.A., J.V. Mendoza and F.D. Cueva. 2020. Characteristics of *Colletotrichum musae* PHBN 0002 and the susceptibility of popular banana cultivars to postharvest anthracnose. Indian Phytipathology. 73: 57-64.

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

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 voor Schirmmelcultures, Baarn, The Netherlands.

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.

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

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

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*, **19:** 11-15.

Sudarma, I M., N. N. Darmiati and N.W. Suniti. 2019. Fungus and Actinomycetes Diversity of Exophytic and Endophytic in Red Grape and its Inhibition Ability to Pathogen *Aspergillus niger*(Caused Rot Fruit Grape). *Int.J.Curr.Microbiol.App.Sci* 8(10): 2442-2451.

Sudarma, I M., N. W. Suniti and N. N. Darmiati. 2020. Use of exophytic microbial on the controlof fruit rot diseases of mango (*Lasiodiplodiatheobromae*). *Int.J.Curr.Microbiol.App.Sci*9(4): 845-854.