Research Article

Paper ID #352

THE POTENTIAL OF EXOPHYTIC MICROBIALS IN CONTROL OF BLACK ROT DISEASE OF MANGOES (*Aspergillus niger*)

I Made Sudarma*, Ni Wayan Suniti* and Ni Nengah Darmiati*

*Lecturer staff at the Agroecotechnology Study Programe, Faculty of Agriculture, Udayana University, Jl. PB. Sudirman Denpasar, Bali, Indonesia.

Corresponding author: *I Made Sudarma
Tel.: +62---------- Email: sudarma_made@ymail.com

ABSTRACT:
Postharvest black rot disease of mangoes is the most important disease in Bali, especially fruit that has been used for religious ceremonies cannot last long if stored, and therefore there is a desire to research efforts to prevent fruit rot with antaginist microbes. Postharvest fruit rot disease in mangoes is *Aspergillus niger*. In our study we looked for the exophytic fungi, and tested their inhibition of pathogen with exophytic microbes both *in vitro* and *in vivo*. The results showed that exophytic microbes found in healthy mangoes include: *Rhizopus* sp. the number of colonies was 24 x 10^2 cfu, while *Nucordia* sp., *A. flavus*, and *A. niger* with 18 x 10^2 cfu colonies, and Streptomyces sp. with a colony of 12 x 10^2 cfu. The highest microbial inhibition test results of exophyte against *A. niger* were obtained from *Rhizopus* sp. 1 and *Rhizopus* sp. 4 at 3 and 7 after inoculation. The results of the in vivo antagonistic inhibition test against pathogens (*A. niger*) were the highest C treatment (*Rhizopus* sp. 2) and very significantly different from K + P treatment (control with pathogens).

KEYWORDS: Antagonist, Black Rot, Exophytic, Inhibition, and Postharvest.

INTRODUCTION

Post-harvest black rot disease caused by *A. niger* is a major post-harvest disease in Bali, especially in fruit after being used during religious ceremonies. Mangoes cannot be stored for long, even they rot very quickly. Disease caused by the fungus [*Aspergillus niger* V. Tiegh, *A. variecolor* (Berk & Br.) Thorn. &Raper, *A. nidulans* (Eidorn) Wint, *A. fumigatus*Fres., *A. flavus* Link, *A. chevalieri* (Mang.) Thorn. &Chruch)] (Prakash *et al.*, 2011).

Infection occurs during and after harvest, when the fungus penetrates through cuts or stems. The infection usually starts at the malignant part of the fruit or the base of the stem and progresses to a grayish to pale brown tricycle mainly from the base of the stem, the lesions coalesce into dark brown to black and then cover with a brown / black spore mass. The spots are soft and settle quickly to spread. In the later stages of development there is an overgrowth of the development of fungal colonies. Disease progresses faster under high temperatures of around 30-36^{o}C (Prakash *et al.*, 2011).

Efforts to control that are environmentally friendly are by utilizing microbial exposures which are antagonistic to pathogens. The results of research by Sudarma*et al.* (2018) found that the exotic fungi originating from the leaves, fruit and twigs of the sugar apple plant were *Aspergillus* sp. *A. niger, Fusarium* sp., Mycelia sterilia, *Neurospora* sp., and *Rhizopus* sp. in controlling *Lasiodiplodia Theobromae Sevara* in vitro. Meanwhile, Sudarma*et al.* (2019) found that microbial exposures in overcoming post-harvest diseases of red grapes include *Neurospora* sp.1, *Neurospora* sp.2 and *Sterptomycesantagonensis*, respectively with in vitro inhibition of 88.89%, 77.78% and 83.33. %.

MATERIALS AND METHODS

Place and time of research

The study was conducted in two places: 1) looking for specimens of sick and healthy fruit from the Supermarkets and Batubulanmarkets. 2) Plant Disease Science Laboratory and Agricultural Biotechnology Laboratory. The research was carried out from January to March 2020.

Isolation of exophytic fungi

Isolation of exophytic fungi can be done by dipping the mango fruit into 250 ml of water, then shaking it and rinsing it evenly. This washing water of 250 ml was used as a dilution of the microbial population found. Then 1 ml was taken and poured into a Petri dish, which was first filled with PDA media and added with anti-bacterial livoploxasin at a dose of 0.1% (w/v).

Identification of exophytic fungi

The stored exophytic fungi were then grown in a Petri dish containing PDA and repeated 5 times. The cultures were incubated in a dark room at room temperature (± 27^{o}C). Isolates were identified macroscopically after 3 days of age to determine colony color and growth rate, and microscopic identification to determine septa in hyphae, spore / conidia shape and sporangiophores. Fungal identification using the reference book Samson *et al.,* 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; and Indrawati*et al.,* 1999, and identification of Actinomycetes using references Miyadoh*et al.*, 2002.

Test of inhibition of exophytic microbes against pathogens

Each of the microbes found for their inhibition was tested for their inhibition against the growth of pathogenic fungi using a dual culture technique (each one of the pathogenic fungi was grown in one Petri dish, sandwiched with two exophytic fungi). The inhibition power can be calculated as follows (Dollar, 2001; Mojica-Marin et al., 2008):

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

Where:

A = Diameter of pathogenic colonies in a single culture (mm)

B = Pathogenic colony diameter in dual culture (mm)

Prevalence of exophytic microbes

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

***In Vivo*antagonistic test**

In vivo antagonistic test of the microbial exophytic was found by stabbing fresh fruit with a spore needle 20 times, then smearing it with antagonistic fungal spores (spores of one Petri dish in 250 ml of sterile distilled water), then immersed in a suspension of pathogenic fungal spores. Exophytic microbes found include:

A = antagonist treatment 1 (spore suspension 5x107)

B = antagonist treatment 1 (spore suspension 5x107)

C = antagonist treatment 2 (spore suspension 5x107)

D = antagonist treatment 3 (spore suspension 5x107)

E = antagonist treatment 4 (spore suspension 5x107)

K-P = control without pathogens

K + P = control with pathogens

All treatments were repeated 5 times. The experiment was designed with a randomized block design (RBD), and after analysis of variance (ANOVA) was carried out, it was followed by the least significant difference test (LSD) at the 5% level. Attack parameters measured by formulation: how many punctures were attacked by the fungus divided by all punctures (20 x) times 100%.

RESULTS AND DISCUSSION

Pathogens identification

Based on the results of post-harvest rotten fruit isolation, symptoms of disease were obtained, including black symptoms at the base of the fruit (Figure 1). The results of the identification of the pathogen that causes fruit rot at the end was *Aspergillis niger*. According to Prakash *et al.* (2011) diseases that interfere with black fruit are [*Aspergillus niger* V. Tiegh, *A. variecolor* (Berk & Br.) Thorn. &Raper, *A. nidulans* (Eidorn) Wint, *A. fumigatus*Fres., *A. flavus* Link, *A. chevalieri* (Mang.) Thorn. &Chruch].

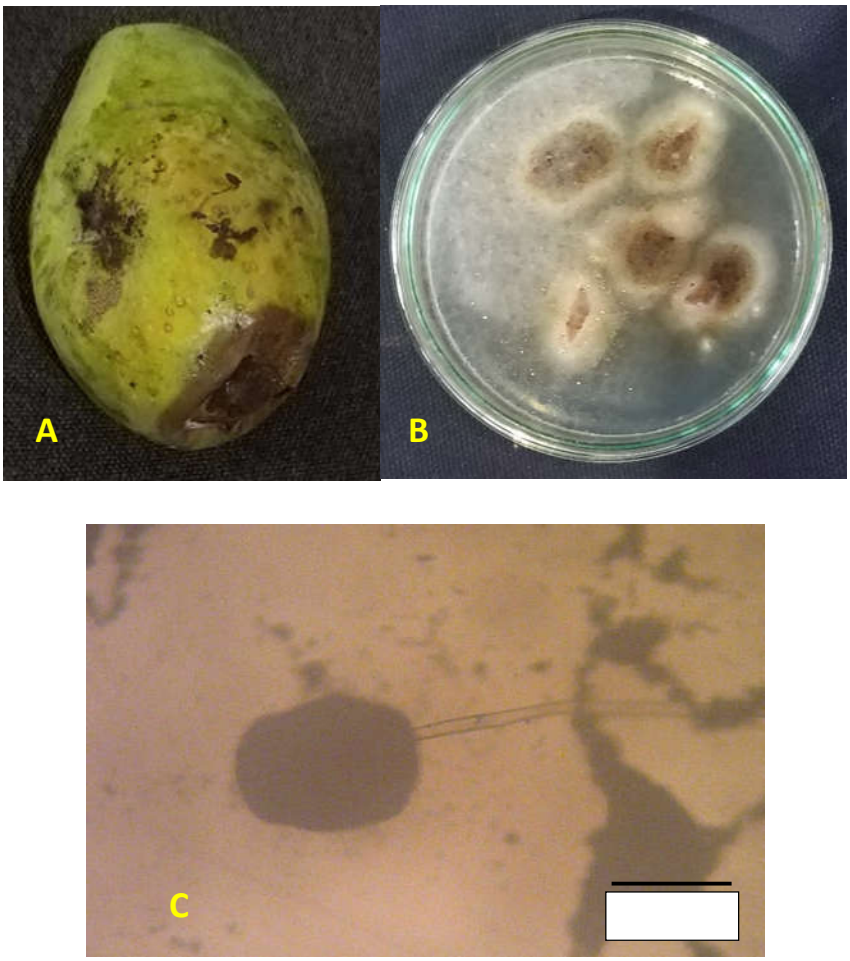

Figure 1. Symptoms of mango fruit rot seen at the tip of the fruit (A), mycelium growth in Petri dishes, and (C) the form of conidia and conidiophores of pathogenic fungi (*A. niger*)

Exophytic Microbes and Prevalence

The most abundant microbes found in mangoes were *Rhizopus* sp. with a total population of 24 x10^2cfu, followed by *Aspergillus flavus, A. niger* and *Nucordia* sp. (Actinomycetes) each with a population of 18 x 10^2cfu, and the last is at least *Streptomyces* sp. (Actinomycetes) as much as 12 x 10^2cfu, (Table 2; Figure 3). Mean that the highest prevalence is held by *Rhizopus* sp. with a value of 26.67%, followed by *Nucordia* sp., *A. flavus* and *A. niger* each 20% and the lowest was held by *Streptomyces* sp. with a value of 13.33% (Table 1).

Table 1. Population and prevalence of exophytic microbe that found in fruit mangoes healthy

No.	Name of microbes	Population x 10^2cfu	Prevalence (%)
1	*Rhizopus* sp.	24	26,67
2	*Nucordia* sp. (Actinomycetes)	18	20
3	*Streptomyces* sp. (Actinomycetes)	12	13,33
4	*Aspergillus flavus*	18	20
5	*Aspergillus niger*	18	20
Total		90	100

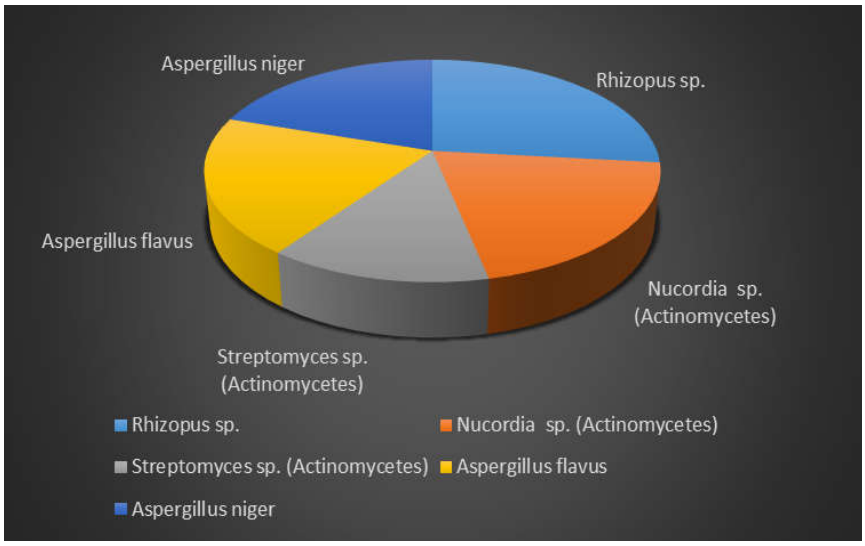

Figure 2. Types of exophytic microbes in healthy mangoes

According to Sudarma*et al.* (2019) found in healthy sugar apple plants, among others, *Aspergillus* sp. population as much as 9 x10^3cfu, *A. niger* population as much as 18 x 10^3cfu, *Fusarium* sp. as much as 3 x 10^3cfu, Mycelia sterilia 3 x 10^3cfu, *Neurospora* sp. as much as 3 x 10^3cfu, and *Rhizopus* sp. as much as 3 x 10^3 cfu. According to Stone *et al.* (2014) *Aspergillus* spp. has been recorded as widespread and polyphyletic endophytic *Penicillia*.

In Vitro Microbial Effectiveness against Pathogens

Inhibition of microbial exophytic against pathogens (*A. niger*) was the highest. 1 at 3 days (days after inoculation) was 98 ± 0.2%, followed by *Rhizopus* sp. 3 of 86.67 ± 0.4%, *Rhizopus* sp. 2 of 83.33 ± 0.7%, *Rhizopus* sp. 4 of 83.33 ± 0.3% and *Rhizopus* sp. 4 at 7 days was 99.0 ± 0.1%, followed by *Sterptomyces* sp. 1 of 88.89 ± 0.6%, *Streptomyces*sp, 2, *Nucordia* sp.1 and *Rhizopus* sp. 1, respectively 88.89 ± 0.5%, 88.89 ± 0.4% and 88.89 ± 0.3% (Table 2).

Basically *A. niger* is a non-ligninolytic fungus that is soil borne. Contamination can occur due to the presence of spores through the wind and their transportation to water, food, plants, humans and animals. The spores can be easily carried by humans and animals, while in plants spores exist and germinate through absorption of nutrients from the location of the infection, for example fruit, stems, leaves and flowers, so it can be said that fungi contaminate through water, air and soil (Sharma, 2012).

Table 2. Inhibition ability of exophytic microbe on *A. niger*

No	Name of fungi	*A. niger*	
		ai 3 hsi (%)	ai 7 hsi (%)
1	*Rhizopus* sp.1	98±0,2	88.89±0.3
2	Nucordi sp. (Actinomycetes) 1	77.78±0.5	88.89±0.4
3	*Streptomyces* sp. (Actinomycetes) 1	77.78±0.3	88.89±0.6
4	*Aspergillus flavus* 1	66.67±0.2	-
5	*A. niger* 1	77.78±0.4	77.78±0.5
6	*A.niger* 2	-	-
7	*Rhizopus* sp. 2	83.33±0.7	86.67±0.3
8	*Nucordia* sp. (Actinomycetes) 2	77.78±0.2	-
9	*A. flavus* 2	80±0.5	-
10	*Rhizopus* sp.3	86.67±0.4	-
11	*A.flavus* 3	77.78±0.3	72.22±0.4
12	*Streptomyces* sp. (Actinomycetes) 2	83.33±0.6	89±0.5
13	*Rhizopus* sp.4	83.33±0.3	99.0±0.1
14	*Nucordia*sp. (Actinomycetes) 3	77.78±0.6	-
15	*Rhizopus* sp. 5	-	-

ia = inhibition ability

Inhibition ability test of exophytic microbes on pathogen *in vivo*

The results of the observation 3 days after inoculation of the *in vivo* antagonist test, the best antagonist with the pathogen (*A. niger*) obtained treatment A (*Streptomyces* sp. 1) the percentage of attack reached 4.90 ± 2.83% the same as the K-P treatment, followed by treatment C (*Rhizopus* sp. 2) with an attack percentage of 14 ± 3.74% and D (*Rhizopus* sp. 4) with an attack percentage of 11.4 ± 1.96%, then treatment B (*Rhizopus* sp.1) the percentage of attacks 29 ± 2%, and treatment E (*Streptomeces* sp. 2) the

percentage of attack was 53 ± 4%, K-P treatment (control without pathogen) the percentage of attack was 92 ± 5.10%, and K + P (control with pathogen) was the same with K + P treatment the attack percentage was 100%. All treatments were significantly different except for treatment A and K-P (Table 3; Figure 3).

As many as 20 species of *A. niger* were found to be potentially used as biological agents against the pathogen (*Phytophthora palmivora*) pod rot pathogen in cocoa. *Aspergillus niger* is directly related to food ingredients in the media. *A. niger* also produces every enzyme such as enzyme amylase, amyloglucosidase, pectinase, cellulose, glucoside, which break down urea into amino acids and CO_2 (Wulandari*et al.,* 2016).

Table3. The results of the best *in vivo* antagonist inhibition test against pathogens (*A. niger*)

Treatment	Name of microbes	Diseases incidence (%)	Notation	
			5%	1%
A	*Streptomyces* sp. 1	4.90±2.83	F	F
B	*Rhizopus* sp. 1	29±2	C	C
C	*Rhizopus* sp. 2	14±3.74	D	D
D	*Rhizopus* sp. 4	11.4±1/96	E	E
E	*Strteptomyces* sp. 2	53±4	B	B
K-P	Control without pathogen	4.90±2.83	F	F
K+P	Control with pathogen	92±5.10	A	A

Rhizopus sp. can suppress the growth of *Aspergillus flavus* toxic fungi and degrade aflatoxins. *Rhizopus* sp. can also produce compounds that can inhibit pathogenic bacteria and function as antioxidants. *Rhizopus* sp. absorbs some mineral elements and converts them into organic minerals so as to increase the absorption of minerals in the body better. Utilization of fermented feed ingredients by *Rhizopus* sp. in livestock shows better results than without fermentation. *Rhizopus* sp. also has the potential to be applied as supplementary feed for livestock (Endrawati and Kusumaningtyas, 2017). Sixteen endophytic fungi have been identified, such as *Acremonium* sp., *Aspergillus* spp., *Cephalosporium* sp., *Fusarium* spp., *Helicocephalum* spp., *Penicillium* spp., *Rhizopus* sp., And 4 species were unable to be identified. The results of the anatgonistic test, the percentage of inhibition power ranged from 36.93% - 100%. Statistical analysis shows that endophytic fungi are able to control *P. infestans* (Wulandari*et al*., 2014).

Figure 3. Best in vivo antagonist test with pathogens (*A. niger*), (A = *Streptomyces*sp 1), B = *Rhizopus* sp. 2, C = *Rhizopus* sp. 4, D = *Rhizopus* sp.1, E = *Streptomyces* sp. 2, K-P = control without pathogens and K + P = control with pathogens) 3 days after inoculation.

CONCLUSION

Based on the results and discussion above, it can be concluded as follows: The pathogen found to cause fruit rot in mangoes is *Aspergillus niger*. Exophytic microbes found in healthy mangoes include: *Rhizopus* sp. the number of colonies was 24 x 10^2 cfu, while *Nucordia* sp., *A. flavus*, and *A. niger* with 18 x 10^2cfu colonies, and *Streptomyces* sp. with a colony of 12 x 10^2cfu. The highest microbial inhibition test results of exophytic against *A. niger* were obtained from *Rhizopus* sp. 1 and *Rhizopus* sp. 4 at 3 days and 7 days. The results of the in vivo antagonistic inhibition test against pathogens (*A. niger*) were the highest treatment C (*Rhizopus* sp. 2) and very significantly different from the K + P treatment (control 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.

REFFRENCES

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

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

Endrawati, D., dan E. Kusumaningtyas. 2017. BeberapaFungsi Rhizopus spdalamMeningkatkan Nilai NutrisiBahanPakan.*Wartazoa* 27(2): 081-088 (Indonesian language)

Haggag W. M. 2010. Mango Diseases in Egypt. *Agriculture and Biology Journal of North America* 1(3): 285-289.

Indrawati. G., R.A. Samson, K. Van den Tweel-Vermeulen, A. Oetari dan I. Santoso. 1999. PengenalanKapangTropikUmum. Yayasan Obor Indonesia. Universitas Indonesia (University of Indonsia Culture Collection) Depok, Indonsia dan CentraalbureauvoorSchirmmelcultures, Baarn, The Netherlands (Indonesian language)

Miyadoh, S.N., Tsuchizaki., J. Ishikawa and Hotta. 2002. *Digital Atlas of Actinomycetes*. The Society for Actinomycetes. Japan.

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.

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.

Prakash, O.M., A.K. Misraand and P.K. Shukla. 2011. Post-harvest diseases of mango and their management. Global Confrence on Augmenting Production and Utulization of Mango: Biotic and Abiotic Stress. P: 137-144.

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.

Sharma, R. 2012. Pathogenecity of Aspergillus Niger in Plants. *Cibtech Journal of Microbiology* 1(1): 2319-3867.

Stone E.K., J.D. Polishook and J.F. White. 2014. Endophytic Fungi. The State University of New Jersey. 241-270.

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

Wulandari, D., L. Sulistyawati, dan A. Muhibuddin. 2014. KeanekaragamanJamurEndofit pada TanamanTomat (*Lycopersicum Esculentum* Mill.) dan KemampuanAntagonisnyaTerhadap*Phytophthora Infestans*, *Jurnal HPT* 2(1): 110-118(Indonesian language).

Wulandari, D.E., Asrul, dan I. Lakani. 2016. SeleksiJamurAntagonis*Aspergillus Niger* Dari BeberapaLahan Perkebunan KakaoUntukMengendalikan*Phytophthora Palmivora. J. Agroland*23 (3): 233 – 242 (Indonesian language).