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Aspergillus niger, Neurospora sp. AND *Rhizopus* sp. AS AN ANTAGONISM FUNGI IN INHIBITING THE GROWTH OF *LASIODIPLODIA THEOBROMAE* (SRIKAYA FRUIT ROT PATHOGEN)

By

I Made Sudarma* & Ni Nengah Darmiati*

***Lecturer in Agroecotechnology Study Program, Faculty of Agriculture, Udayana University, Jalan PB. Sudirman Denpasar**

Corresponding author: madesudarma@unud.ac.id

Abstract

Srikaya fruit rot disease (*Annona squamosa* L.) is the main problem that affects the fruit when it is on the tree and after harvest. Therefore, it is necessary to carry out environmentally friendly alternative controls. Exophytic fungi such as *Aspergillus niger* and *Rhizopus* sp. provided high inhibition against *Lasiodiplodia theobromae* (srikaya fruit rot pathogen) of 80.71 ± 1.07% and 82.92 ± 0.50%, respectively. Meanwhile, the best endophytic fungus that is able to inhibit the growth of pathogens is *Neurospora* sp. and *Aspergillus* sp. with respective inhibition of 86.67 ± 3.14% and 88.35 ± 0.46% in vitro. In vivo test results showed that the fungi *A, niger* and *Aspergillus* sp. the best inhibition of each is 100%.

Keywords

***Lasiodiplodia theobromae*, exophytic and endophytic fungi, inhibited ability, *in vitro* and *in vivo*.**

INTRODUCTION

The srikaya plant (Annona squamosa L.) besides its edible fruit, is now widely used to prevent disease in the human body, such as the leaves can be used to treat as a vermicide, to treat tumors or cancer, insect bites, and other skin disease problems (1). Srikaya fruit extract can be used to inhibit the growth of several microbes such as *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Esherichia coli, Salmonella typhi, Streptococcus pyogene* and Aspergillus niger. The three extracts (acetone, ethanol, and aqueous) from srikaya show a positive effect on all of the above microorganisms (2).

Many diseases have been found to disturb sugar apple plants, these diseases include: Pseudocercospora fruit spot disease, Diplodia fruit rot, purple stripe (caused by *Phytophthora palmivora*), *Cylindrocladium colhounii* and *Cylindrocladium scoparium* fruit rot, and X disease (not yet identified) (3).

Lasiodiplodia theobromae is a fungus that has a fairly wide range of hosts and can cause diseases, such as mango fruit (4) and citrus stem rot (5). Pathogen that causes canker leaf blight and fruit rot in cocoa (6). As a cause of banana rot disease (7), as well as a cause of gummosis, branch death and mummification of citrus fruit (8).

Exophytic and endophytic microbes can now be used to control *L. theobromae. Bacillus velezensis* as endophytic bacteria is able to suppress the growth of *L. theobromae* on grapes (9). Endophytic fungi are wide spread and reside within plant cells for at least part of their lives without causing any symptoms of infection. Different host plants may have different levels of fungal endophytes and community composition. However, the association of fungal endophytes with host plants and their hostile behavior remains unknown (10). According to Maubasher et al. (2022) (11) stated that endophytic fungi are 1). Microfungi that live in host plant tissues intercellularly and/or intracellularly without clear pathological symptoms. 2). In order to maintain a stable symbiosis, endophytes secrete chemicals to help plants adapt to harsh environments. 3). As a treasure trove of bioactive metabolites, endophytic fungi are a sustainable source of various natural products namely: quinones, saponins, alkaloids, steroids, phenolic acids, terpenoids and tannins that exhibit antimicrobial and anti cancer properties.

Phylloplane fungi are mycotic fungi that grow on plant surfaces (12). There are groups of phyloplane fungi: resident (staying still) and casual (accidental). Residents can reproduce on healthy leaf surfaces without affecting the host while casuals land on leaf surfaces but cannot grow (13).

MATERIALS AND METHODS

Place and time of research

The research was carried out in two places: 1) looking for diseased and healthy plant specimens from cocoa plants planted in the Jimbaran Hill area. 2) Plant Disease Laboratory and Agricultural Biotechnology Laboratory. The research was carried out from April to August 2022.

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 to determine the color of the colony and growth rate, and identified microscopically to determine the septa on the hyphae, the shape of the spores/conidia and sporangiophores. Identification of fungi using reference books (14, 15,16, 17).

Test of the Inhibited Ability of Endophytic and Exophytic Fungi Against Pathogens

The endophytic and exophytic fungi found were each tested for their inhibitory power against the growth of pathogenic fungi using the dual culture technique (in one Petri dish each pathogenic fungus was grown flanked by two endophytic fungi). The resistance can be calculated as follows (18, 19): Inhibited ability (%) = A-B/A x 100%. Where: A = diameter of *L. theobromae* colonies in single culture (mm), and B = diameter of *L. theobromae* colonies indual culture (mm).

Prevalence of Endophytic and Exophytic Fungi

Determining the prevalence of endophytic and exophytic fungi is based on the frequency of endophytic and exophytic fungal isolates found (leaves, stems, flowers and fruit) per Petri dish, divided by all isolates found times 100%. The prevalence of isolates will determine the dominance of endophytic fungi in healthy sugar apple plant parts.

In Vivo Antagonist Test

The in vivo antagonistic test for endophytic and exophytic fungi was found by pricking fresh fruit with a spelden needle 20 times, then smearing it with antagonistic fungal spores (spores from one Petri dish in 250 ml of sterile distilled water), then dipping them in the fungalspore suspension. pathogen. Endophytic and exophytic fungi found include:
K-P = Control without pathogen

A = control (without being treated with antagonist) B = antagonist treatment 1 (spore suspension $5x10^7$) C = antagonist treatment 2 (spore suspension $5x10^7$) D = antagonist treatment 3 (spore suspension $5x10^7$) E = antagonist treatment 4 (spore suspension $5x10^7$) F = antagonist treatment 5 (spore suspension $5x10^7$)K+P = control with pathogen

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

RESULT AND DISSCUSSION

Exophytic and Endophytic Fungi

Exophytic and endophytic fungi originating from fruit, leaves and twigs were isolated using 1 g of material. The types of fungi found were *Aspergillus* sp., *Aspergillus niger*, *Neurospora* sp., *Fusarium* sp., *Rhizopus* sp., *Penicillium* sp., and Mycelia sterilia (Table 1)

Table 1. Types of exophytic and endophytic fungi originating from fruit, leaves and twigs

No.	Exophytic fungi	Number of isolate	Endophytic fungi	Number of isolate
Fruit			Fruit	
1	*Aspergillus* sp.	3 (20%)*	*Fusarium* sp.	6 (40%)
2	*Aspergillus niger*	9 (60%)	*Penicillium* sp.	3 (20%)
3	Miselia sterilia	3 (20%)	*Neurospora* sp.	3 (20%)
4			Miselia sterilia	3 (20%)

	Total	15		15
Leaf			Leaf	
1	*Aspergillus* sp.	6 (40%)	*Fusarium* sp.	9 (60%)
2	*Aspergillus niger*	6 (40%)	*Neurospora* sp.	3 (20%)
3	*Neurospora* sp.	3 (40%)	*Aspergillus* sp.	3 (20%)
	Total	15		15
Twig			Twig	
1	*Aspergillus niger*	3 (20%)	*Fusarium* sp.	6 (40%)
2	*Fusarium* sp.	3 (20%0	Miselia sterilia	9 (60%)
3	*Rhizopus* sp	9 (60%)		
	Total	15		15

***** Prevalence of fungi found during the study

The fungi that dominate (highest prevalence) exophyte types are *A. niger* and *Rhizopus* sp. with 9 isolates (60%), while the dominant fungal endophyte was *Fusarium* sp. and mecilia sterilia with 9 isolates (60%). The diversity of endophytic fungi in the phylloplane is on the surface of plant parts, and endophytes are in the inner tissues. Endophytes are known to be microbes that live in plants that are neutral or beneficial to the host plant. Specifically bacteria or fungi, and there may be 3 types: 1) other host pathogens that are not pathogenic in their endophytic relationships, 2) non-pathogenic microbes, and 3) pathogens that are not pathogenic but are still able to colonize through selection methods or genetic change (20). Plant endophytic fungi are important and useful as sources of natural bioactive compounds with potential applications in agriculture, medicine and the food industry. Many useful bioactive compounds with antimicrobial, insecticidal, cytotoxic and anti-cancer properties have been successfully investigated from endophytic fungi. Over long periods of coevolution, friendly relationships have been established between each endophyte and its host.

Aspergillus is a phylloplane fungus found on healthy leaves of Okra [*Abelmoschus esculentus* L. (Moench] (21). The phyllosphere refers to the plant's entire aerial habitat, while the phylloplane describes the entire surface of the leaf. The phylloplane provides a home for a diversified microbial community and is thus a ecosystems are important both ecologically and economically. For many years, phylloplane inhabitants have been studied as bioprotectors and growth enhancers of host plants. Plant and phylloplane-microbe interactions result in increased fitness and productivity of agricultural plants (22). Study of phylloplane microflora in various plants Research has been carried out, it was found that many fungi *A. flavus, A. niger* and *Trichoderma* sp. Their abundance contributed 6.97% and 3.84% respectively (23). There are three types of fungi that inhabit the phylloplane of safflower (sunflower) plants. including *A. niger, Penicillium* sp. and *T. viride* (24).

Inhibited Ability of Exophytic and Endophytic Fungi

The inhibited ability of exophytic fungi against pathogens (*L. theobromae*) ranged from 60.32 ± 1.50% to 82.92 ± 0.50% (highest by the fungus *Rhizopus* sp.). Meanwhile, the inhibitory power of endophytic fungi ranged from 68.20 ± 1.49% to 88.35 ± 0.46% (the highest was *Aspergillus* sp.) (Table 2). All exophytic and endophytic fungi show inhibition of pathogens through competition for space and nutrients. It is proven that antagonistic fungi always grow faster and fill the petri dish until the pathogen cannot get food and then dies.

The endophytic fungus that dominates in suppressing pineapple fruit rot caused by *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, is the fungus *Rhizopus* sp., and the highest inhibitory power is achieved by *Neurospora* sp., *Rizopus* sp. and *A. flavus* (25). Next, the ability to produce phytase and amylase enzymes *by A. niger, R. oryzae* and *N. sitophila* was tested using sterilized tofu dregs. Phytase and amylase enzymes are enzymes that are widely used in the food, feed and health industry sectors (26).

The in vivo inhibition test showed that all treatments were significantly different from the control plus pathogen. The highest inhibitory power was achieved by treating *Aspergillus* sp. and *A. niger* each reached 100 ± 0%, and the lowest was achieved by *Rhizopus* sp. amounted to 70 ± 1.5%, and was significantly different from controls without pathogens and controls with pathogens (Table 3).

Table 2. Test the inhibited ability of exophytic and endophytic fungi

Fungi origin	Name of fungi	Inhibited ability (%)
1. Eksofit daun 1	*Neurospora* sp.	60,32±1,50
2. Eksofit daun 2	*Aspergillus flavus*	65,21±2,50
3. Eksofit daun 3	*Aspergillus niger*	68,64±1,59
4. Eksofit daun 4	*Aspergillus niger*	75,15±2,24
5. Eksofit daun 5	*Neurospora* sp.	74,69±0,72
6. Eksofit buah 1	*Aspergillus* sp.	65,68±0,82
7. Eksofit buah 2	*Neurospora* sp.	65,21±1,50
8. Eksofit buah 3	*Neurospora* sp.	70,32±2,30
9. Eksofit buah 4	*Aspergillus niger*	72,00±0,31
10. Eksofit buah 5	*Aspergillus niger*	80,71±1,07*
11. Eksofit ranting 1	*Neurospora* sp.	75, 14±1,50
12. Eksofit ranting 2	*Aspergillus niger*	71,31±0,68
13. Eksofit ranting 3	*Rhizopus* sp.	82,92±0,50*
14. Eksofit ranting 4	*Rhizopus* sp.	76,67±3,27
15. Eksofit ranting 5	*Rhizopus* sp.	82,22±3,27*
16. Endofit daun 1	*Fusarium* sp.	81,85±0,52*
17. Endofit daun 2	*Neurospora* sp.	86,67±3,14*
18. Endofit daun 3	*Fusarium* sp.	78,15±4,19
19. Endofit daun 4	*Aspergillus* sp.	88,35±0,46*
20. Endofit daun 5	*Fusarium* sp.	78,26±1,22
21. Endofit ranting 1	Miselia sterilia	68,20±1,49
22. Endofit ranting 2	Miselia sterilia	75,92±2,62
23. Endofit ranting 3	Neurospora sp.	70,23±2,10
24. Endofit ranting 4	Miselia sterilia	71,85±0,52
25. Endofit ranting 5	*Neurospora* sp.	75,25±1,20
26. Endofit buah 1	*Neurospora* sp.	70,05±2,20
27. Endofit buah 2	*Neurospora* sp.	70,55±1,30
28. Endofit buah 3	*Neurospppra* sp.	70,32±2,10
29. Endofit buah 4	*Neurospora* sp.	70,23±1,30
30. Endofit buah 5	*Neurospora* sp.	70,15±0,80

* Continue to determine the inhibited ability *in vivo*

Table 3. The of the inhibited ability exophytic and endophytic fungi *in vivo*

Treatmenet.	Fungi origin	Name of fungi	Disease incidence (%)	Inhibited ability (%)
K-P	Control without pathogen		0±0.0 a*	100±0.0 a*
A	Leaf endophytic 4	*Aspergillus* sp.	0±0.0 a	100±0.0 a
B	Leaf exophytic 5	*Aspergillus niger*	0±0.0 a	100±0.0 a
C	Leaf endophytic 1	*Fusarium* sp.	3±0.8 ab	97±0.8 ab
D	Leaf endophytic 2	*Neurospora* sp.	7±1.2 ab	93±1.2 ab
E	Twig exophytic 5	*Rhizopus* sp.	15±1.6 ab	85±1.6 ab
F	Twig exophytic 3	*Rhizopus* sp.	30±0.9 b	70±0,9 b
K+P	Control with pathogen	*Lasiodiplodia theobromae*	70±2.0 c	30±2,0 c

* Least significant difference test (LSD) at the 5% level.

Endophytic fungi in particular are asexual, for example systemic endophytes in grasses, are generally seen as mutually beneficial to plants primarily through the action of mycotoxins, such as grass-infecting alkaloids, which protect the plant host from herbivores. Much of the evidence for the concept of mutual defence comes from agronomic studies of grass cultivars, especially some endophyte-host interactions (27).

Aspergillus flavus suppressed the maximum growth of *Alternaria brassicae*, the influence of volatile and non-volatile metabolite compounds released by phylloplane fungi was also observed (28). According to Thakur and Harsh (2016) (29) stated that the phylloplane fungus *A. niger* can suppress *Alternaria alternata* on Sarpgandha (*Rauwolfia serpentina*) plants by 50%. Borgohain and Chutia (2014) (30) stated that Aspergillus fumigatus and Fusarium sp. is a phylloplane fungus found on castor plants (Ricinus communis L.). Meanwhile, *A. fumiculoris*, *Aspergillus* sp. and *F. moniliforme* has been isolated from the phylloplane of the medicinal plant (*Azadirachta indica*). These medicinal plants release phytochemical compounds in the form of flavonoids, cardiac glycosides and terpenoids (31). *Rhizopus* sp. is a phylloplane fungus that dominates mature leaves on Muga host plants (32).

CONCLUSION

Srikaya fruit rot disease (*Annona squamosa* L.) is the main problem that affects the fruit when it is on the tree and after harvest. Therefore, it is necessary to carry out environmentally friendly alternative controls. Exophytic fungi such as *A. niger* and *Rhizopus* sp. provided high inhibition against *L. theobromae* (srikaya fruit rot pathogen) of 80.71 ± 1.07% and 82.92 ± 0.50%, respectively. Meanwhile, the best endophytic fungus that is able to inhibit the growth of pathogens is *Neurospora* sp. and *Aspergillus* sp. with respective inhibition of 86.67 ± 3.14% and 88.35 ± 0.46% in vitro. In vivo test results showed that the fungi *A*, *niger* and *Aspergillus* sp. the best inhibition of each is 100%.

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