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TRICHODERMA SP. AS ENDOPHYTIC FUNGI ONE CANDIDATE FOR POD ROT DISEASE COTROL OF COCOA

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ABSTRACT

Black pod rot of cocoa is one of the most dangerous diseases causing crop losses in cocoa plants around the world. The disease is caused by the fungus *Phytophthora palmivora* (Butler) Butler, the disease needs to be controlled through biocontrol with the *Trichoderma* sp. Is an endophytic fungus found in cocoa plant stems. The results showed that the isolate of endophytic fungi found both from the stem, leaf and skin of healthy cocoa fruit was 15 isolates, from 15 isolates having in vitro inhibitory ability were collected five types of isolates such as stem endophytic fungi 3 (*Trichoderma* sp.) of 95.92 ± 3.90%, followed by leaf endophytic 3 (*Oidium* sp.) of 93.33%, stem endophytic 5 (*Botryoderma* sp.) of 91.11%, stem endophytes 1 (Micelia strerilia) equal to 89,62 ± 1,29%, and last end of skin of fruit 1 (*Septocylindrium* sp.) equal to 73,33%. Test results *in vivoTrichoderma* sp. best in pressing with the lowest attack percentage of 36.25 ± 2.89%, and the field test showed that *Trichoderma* sp. with all dilution treatments having a very significant effect on control with disease incidence that can be saved of 83.33%.

KEYWORDS:

Endophytic fungus, *Trichoderma* sp. power inhibition, pod rot of cocoa, *Phytophthora palmivora.*

INTRODUCTION

Cocoa fruit rot disease is one of the most dangerous diseases causing crop losses in cocoa plants around the world. The disease is caused by the fungus *Phytophthora palmivora* (Butler) Butler, which is a major problem of cocoa cultivation in Southeast Asia. The pathogen also infects more than 200 other plant species in the tropics and sub tropic (1). There are more than 80 species of *Phytophthora* causing the disease, some of which include *P. palmivora, P. megakarya, P. citrophora,* and *P. capsici* responsible for cacao fruit rot disease. *Phytophthora* spp. Responsible for fruit rot disease and may result in a yield loss of 20% to 30% of total cacao annually (2).

The pathogen attacks some parts of the plant, but the most severe occurs in the fruit, especially in the fruit before it matures. The disease produces a brown pedicab with a regular edge that can spread rapidly, covering the fruit within a few days. The inside of the fruit causes chubby decay (3). The pathogen may survive in the soil or on the leaf and be dispersed from the diseased to the underside. Soil containing fungi can be moved by ants like building a house on fruit through a mealybug colony. Sporangia is washed by rain from shoots and infected leaves can diffuse into fruit in the canopy of trees. Fungus can also grow from cancer into the flower bearing, and from here into the fruit through the stalk. Spores can also be transported to new plants through pruning equipment, and through rats and bats by first chewing on sick fruit and then healthy fruit (4)

One useful endophytic fungus in the control of witches' broom disease in cocoa is *Trichoderma* (5). *Trichoderma* fungus has a high biocontrol activity in cocoa fruits that have been tried and showed a decrease in the intensity of the disease in relation to positive control (6). The *Trichoderma* strain colonizes the end of the trichome and forms an apresoria-like swell. The hyphae were observed arising from trichome bubbles on the sterilized stem surfaces of the inoculated cocoa seedlings each with the *Trichoderma* strain. Strains of four Trichoderma species are able to enter trichome tricot during coconut stem colonization where the fungus can survive on sterilized surfaces and can be re-isolated. The penetration of cocoa trichome can provide an entry point for *Trichoderma* spp. Into the cocoa rod that enables systemic colonization of this tissue (7).

MATERILAS AND METHODS

Place and time of research

The experiment was conducted in two places: 1) field test using farmer's cocoa plant in Perean Village, Tabanan, and 2) in vitro and in vivo test was conducted at Biotechnology Laboratory of Agricultural Technology Faculty of Udayana University. The study was conducted from June of August 2022 to February 2022. The use of pathogen isolates was obtained from the Lab. Plant Disease Science, Faculty of Agriculture Udayana University.

Isolation of Endophytic Fungus

Endophytic fungi used in this study from a collection of groups of cacao plants grown in the Tabanan area. The picked leaf was washed with running water by the process as follows: 32 sheets with an area of 4 mm2 of leaves from the center of each leaf, the surface sterilized in 0.525% sodium hypochlorite for 3 minutes, and 70% ethanol for 2 minutes; Rinsed with sterile water for 1 minute; And subsequently placed on PDA media (firstly given antibiotic anti-bacterial livoploxasin with a concentration of 0.1% (w/v). The fungus emerging from the leaf piece is transferred to a test tube containing the PDA to be stored and classified by morphospesies. Endophytes of cocoa bean and fruit, washed with running water and then divided into 8 parts For sixteen pieces of cube shape (8 mm3) taken from each section: eight of the exocarp and eight more of the mesocarp.The surface is sterilized, and stored With the same procedure as isolating on leaves.

Identification of Endophytic Fungus

Endophytic fungus that is stored in its jut is grown on a Petri dish containing PDA and repeated 5 times. The culture is cubed in a dark room at room temperature (± 27°C). Isolates were identified macroscopically after 3 days to determine colony color and growth rate, and microscopic identification to determine septa in hyphae, spore/conidia and sporangiophore. Fungal identification using reference book (8; 9; 10; 11).

Test *in vitro*

The endophytic fungi found respectively tested their inhibitory power to the growth of pathogenic fungus (*P. palmivora*) with dual culture technique (in one Petri dish was grown each one of the pathogenic fungi flanked by two endophytic fungi). The inhibitory power can be calculated as follows (12; 13):

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

Where:
A = Colony diameter of *P. palmivora* in single culture (mm)
B = Colony diameter of *P. palmivora* in dual culture (mm)

Test *in Vivo*

Test antagonistic in vivo endophyte fungi were found by poking fresh fruit with needle spelden 20 times, then smeared with fungal spores antagonist (spores of the Petri dish in 250 ml of distilled sterile), then dipped in a spore suspension of pathogenic fungi (*P. palmivora*). Endophyte fungi are found, among others:
A = control (without being smeared with anything)
B = Control (given pathogens)
C = treatment of fruit endophyte antagonists 1 and pathogens (spore suspension $5x10^7$)
D = treatment endophyte antagonist rod 5 and pathogens ($5x10^7$ spore suspension)
E = leaf endophyte antagonist treatment 3 and pathogen (spore suspension $5x10^7$)
F = treatment of stem endophyte antagonists 1 and pathogens (spore suspension $5x10^7$)
G = treatment of stem endophyte antagonists 3 and pathogens (spore suspension $5x10^7$)
All treatments were repeated 5 times. The experiment was designed with a randomized block design (RBD), and after analysis of variance (ANOVA) followed by the least significant difference test (LSD) at 5% level. Parameters measured by the formulation attack: how punctures are attacked by fungi shared with the entire puncture (20 x) times 100%.

Test in the field

The best antagonists are tested airy by spraying onto the fruit that is still attached to the tree. The suspension is made by mixing the best endophyte fungi colonies into a 10% sugar solution. The treatments tested to the plot (cocoa tree) are as follows:
A = Control (without enforcement)
B = Treatment with spore suspension of one Petri dish/ 50 ml of sugar solution (10%)
C = Treatment with spore suspension of one Petri dish/200 ml of sugar solution (10%)
D = Treatment with spore suspension of one Petri dish/250 ml of sugar solution (10%)
E = Treatment with spore suspension of one Petri dish/500 ml of sugar solution (10%)
F = Treat with a spore suspension of one cup Petri/1000 ml of sugar solution (10%)
All treatments were repeated six times. The experiments were designed with randomized block design

(RAK), and after variance analysis (ANOVA) followed by the smallest real difference test (BNT) at 5%

RESULTS

Isolation of Endophytic Fungus

Based on observations, there were 15 isolates (5 isolates each came from the stem, 5 isolates were from leaves, and 5 isolates were from fruit peel). All isolates were white except B3 (stem 3) of green colonies, white colonies (front) and black backs were isolate colonies B1, and D5. While the other colonies are white both front and rear. Colonic diameter at 5 days (days after inoculation) ranged from 3.3 - 9 cm (Table 1).

Table 1. Diameter and color of endophytic fungal colonies of the sample

No	Name and isolate code	Endophytic fungal						
		Diameter of fungus colony (cm)					Color of fungus colony	
		1 day	2 days	3 days	4 days	5 days	Front	Back
1	Micelia sterilia (B1)	2.9	8.1	9.0	9.0	9.0	White	Black
2	*Botryoderma* sp. (B2)	2.2	5.8	8.6	9.0	9.0	White	White
3	*Trichoderma* sp. (B3)	3.2	7.1	8.8	9.0	9.0	Green	Green
4	*Cylindrocarpon* sp. (B4)	1.2	1.8	3.9	5.2	6.5	White	White
5	*Botryoderma* sp. (B5)	3.1	8.8	9.0	9.0	9.0	White	White
6	*Dactylium* sp. (D1)	1.0	1.6	2.0	2.7	3.3	White	White
7	*Fusarium* sp. (D2)	1.4	4.8	6.9	7.8	8.9	White	White
8	*Oidium* sp. (D3)	2.2	7.3	9.0	9.0	9.0	White	White
9	*Mortierella* sp.(D4)	1.4	3.1	4.0	6.2	8.4	White	White
10	*Mucor* sp. (D5)	2.7	6.2	8.6	9.0	9.0	White	Black
11	*Septocylindrium* sp. (K1)	1.4	2.0	4.3	5.4	6.2	White	White
12	*Verticillium* sp. (K2)	1.5	3.2	5.3	7.0	7.8	White	White
13	Micelia sterilia (K3)	1.6	2.4	3.9	6.0	8.4	White	White
14	*Botryoderma* sp. (K4)	1.6	5.1	7.2	8.0	8.5	White	White
15	*Cylindrocarpon* sp (K5)	1.0	1.6	3.2	5.0	7.0	White	White

Description: B: isolates of stem, D: isolates of leaf, and K: isolates of fruit skin

Test *in Vitro*

Based on the observation found the best five that have the inhibition to pathogen is endophytic stem 3 (*Trichoderma* sp.) Get the highest value with inhibitory power of 95,92 ± 3,90%, followed by endofit of leaf 3 (*Oidium* sp.) Equal to 93, 33%, stem endophytes 5 (*Botryoderma* sp.) Of 91.11%, stem endophytes 1 (Micelia strerilia) of 89.62 ± 1.29%, and the final end of fruit shoot 1 (*Septocylindrium* sp.) of 73.33% (Table 2).

Table 2. Inhibitory ability of endophytic fungal on *Phytophthora palmivora*

No.	Name of endophyte isolate	Inhibitory ability (%)
1	Micelia sterilia (B1)	89.62±1.28 c
2	*Botryoderma* sp. (B2)	62.96±1.28 g
3	*Trichoderma* sp. (B3)	95.92±3.90 a
4	*Cylindrocarpon* sp. (B4)	68.14±1.28 f
5	*Botryoderma* sp. (B5)	91.11 g
6	*Dactylium* sp. (D1)	71.11 e
7	*Fusarium* sp. (D2)	66.66 f
8	*Oidium* sp. (D3)	93.33 b
9	*Mortierella* sp. (D4)	71.85±1.28 de
10	*Mucor* sp. (D5)	55.55 h
11	*Septocylindrium* sp. (K1)	73.33 d
12	*Verticillium* sp. (K2)	65.92±1.28 f
13	Micelia sterilia (K3)	65.92±1.28 f
14	*Botryoderma* sp. (K4)	66.66 f
15	*Cylindrocarpon* sp. (K5)	62.22 g

Test *in Vivo*

Based on observations of the five endophyte isolates tested in vivo showed that *Trichoderma* sp. Still produces the best inhibitory power compared to other endophytes with an attack percentage of 36.25 ± 2.89%, followed by Micelia sterilia of 67.5 ± 17.32%, *Septocylindrium* sp. equal to 71.25 ± 33.76%, *Botryoderma* sp. of 80 ± 33.67% and the last of *Oidium* sp. of 97.5 ± 2.89% (Table 3)

Table 3. Disease incidence as test results *in Vivo*

Treatment	Disease incidence (%)	Notation
Control	100	a
Pathogen	100	a
Micelia sterilia	67.5±17.32	ab
Trichoderma sp.	36.25±2.89	b
Botryoderma sp.	80±33.67	a
Oidium sp.	97.5±2.89	a
Septocylindrium sp.	71.25±33.76	ab

Test in the field

Suppressing the disease from a 150-ml dilution, then 200 ml, 250 ml, 500 ml and 1000 ml still give encouraging results (Table 4). The percentage of fruit attack that could be rescued from each treatment was by 1000 ml dilution of 66.67%, with a 500 ml dilution of 72.22%, with a dilution of 250 ml of 77.78%, with a dilution of 200 ml of 77.78 % And highest with 150 ml dilution can save fruit by 83.33% (Table 4).

Table 4. Percentage of attacks and percentage of fruit that can be saved by *P. palmivora* attack

Treatment	Disease incidence (%)	Percentage of fruit can be saved (%)	Notation
Kontrol	90		a
Trichoderma sp. (1000 ml)	30	66.67	b
Trichoderma sp. (500 ml)	25	72.22	b
Trichoderma sp. (250 ml)	20	77.78	b

Trichoderma sp. (200 ml)	20	77.78	b
Trichoderma sp. (150 ml)	15	83.33	b

DISCUSSION

Many *Trichoderma* spsies as endophytic fungi are known to be natural antagonists of pathogenic vascular (tracheomycosis) caused by *Fusarium* spp. On the coffee plant. *Trichoderma* sp. This was successfully isolated from the coffee rhizosphere. Mushroom *T. gamsii* is a nonpatogenic endophytic fungus to the host, has the ability as an effective biocontrol, and can tolerate the concentration of chemical fertilizer to 20%. *T. gamsii* is displayed as an antagonistic activity against the pathogenic fungus *Panax notoginseng* through the production of VOC (volatile organic compound). On the basis of the identification of spectrometric mass gas chromatography, the VOC is identified as dimethyl disulfide, dibenzofuran, methalmethiol, ketone as an effective material for antagonistic activity (14).

Endophyte fungi isolated from garlic identified as *Trichoderma brevicompactum* produce bioactive calories from the culture extract as 4β-acetoxy-12, 13-epoxy-A-trichothecene (trichodermin) which has inhibitory activity to *Rhizoctonia solani*. Strong inhibition by trichodermin is also found for Botrytis cinerea. When compared with the positive Carbendazim rearrangement, trichodermin exhibits the antifungal activity of the phytopatogen as described above (15). *Trichoderma* sp. Can colonize the intercellular root space, and can induce plant resistance, may produce plant growth triggers and tolerance to abiotic stress (16).

The valuable technology in all agricultural areas has improved production, but some modern practices are renewing the environment. The challenge now is by the progress of farming to achieve higher yield and environmentally friendly. There is an immediate desire to find eco-friendly biological agents, including fungi and bacteria, the genus Trichoderma produces different enzymes that play a leading role in biological control activities such as cell wall degradation, tolerance of abiotic stress, hypha growth and so on. The notion of filamentous fungus belonging to the genus *Trichoderma* is continuously used in two decades, from the simple concept of biological control agents to its present role as symbionts with different beneficial effects on plants. It is now found from functional genomic structures and approaches which explain that there are useful additions to these microbes as models for study mechanisms including multiple role interactions, such as eco-microbial-crops (17).

Biological control is the use of genetically modified organisms, genes or gene products, to reduce the effects of organisms that are not beneficial to beneficial organisms useful to humans, such as plants, trees, animals and useful microorganisms. Trichoderma was low cost agents that can manifestly exist in different pathosystems, have moderate effects on soil balance and are not harmful to useful organisms that contribute towards pathogen control. The fungus of the genus *Trichoderma* is a tular soil, ascomycetes green spore that exist everywhere in nature. *Trichoderma* sp. As an effective chance of plant symbionts and micro-parasites, a number of species of this genus have the potential to become commercial biofungicides. This biocontrol agent is harmless affecting humans, wild life and useful organisms. Safe and effective control both naturally and controlling the environment is not accumulated in the food chain, has a characteristic of growing fast, the conidoid is bright green and the branched conidiophore structure. Trichoderma strains used as biocontrol agents may act as: 1) colonize the soil and / or plant part, 2) produce enzymes degrading cell walls against pathogens, 3) produce antibiotics that can kill pathogens, 4) trigger crop development (18), 5) triggered the mechanisms of plant resistance and phosphate solvents and produced phytohormones (19).

CONCLUSION

The results showed that the isolate of endophytic fungi found both from the stem, leaf and skin of healthy cocoa fruit was 15 isolates, from 15 isolates having in vitro inhibitory ability were collected five types of isolates such as stem endophytic fungi 3 (*Trichoderma* sp.) of 95.92 ± 3.90%, followed by leaf endophytic 3 (*Oidium* sp.) of 93.33%, stem endophytic 5 (*Botryoderma* sp.) of 91.11%, stem endophytes 1 (Micelia strerilia) equal to 89,62 ± 1,29%, and last end of skin of fruit 1 (*Septocylindrium* sp.) equal to 73,33%. Test results *in vivoTrichoderma* sp. best in pressing with the lowest attack percentage of 36.25 ± 2.89%, and the field test showed that *Trichoderma* sp. with all dilution treatments having a very significant effect on control with disease incidence that can be saved of 83.33%.

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