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Article

Antifungal Activity of Secondary Metabolites From *Trichoderma* sp. Against *Fusarium Oxysporum* f.sp. *Cubense*

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Abstract: *Fusarium oxysporum* f.sp. *cubense (Foc)* is an important pathogenic fungus that causes Panama disease in banana. One approach to overcome this problem is to utilize secondary metabolites from *Trichoderma* sp. This research was conducted to determine the effect of administering secondary metabolites from *Trichoderma* sp. against pathogen *Foc.* These metabolites were applied at concentrations of 10%, 20%, and 30%, 40% alongside a fungicide active ingredient of phosphoric acid 400 SL. This study used a Completely Randomized Design with 6 treatments 4 repetition. The parameters observed included colony diameter and percentage of inhibitory. The extraction of secondary metabolite isolation could be obtained as much as 500 ml with reddish brown, thick and odorless characteristics. Secondary metabolites showed a significant effect in inhibiting the growth of the pathogen *Foc*. The results showed the 10% concentration proved to be the most effective to inhibit pathogen *Foc*, resulting in the smallest colony diameter of 0.58 cm and an in vitro inhibition rate of 90.73%.

Keywords: biological control; fusarium wilt, integrated pest management, plant disease management, organic agriculture

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1. Introduction

The low productivity of bananas in Indonesia is largely caused by OPT (Plant Pest Organism) disturbances. One of the diseases that is often found and is the main disease in horticulture is Panama disease. The cause of this disease is *Fusarium oxysporum* f. sp. *cubense (Foc),* which is a type of fungus that infects plants through the roots [1]. *Foc* is a soil-borne fungus with asexual reproduction, producing microconidia, macroconidia and chlamydospores as survival structures. This is very difficult to handle, because the fungus is able to survive in the soil or host tissue that has been colonized for a long period of time. Fusarium fungi have the ability to produce chlamydospores without requiring a host plant, so they are able to re-infect plants if they find a suitable host, by growing as hyphae in organic remains [2]. Fusarium spp. is a type of fungus that spreads easily, spreading through land and plants that have been infected with the fungus [1]. On banana plants *Foc* can infect at various stages of growth [3]. Symptoms of this disease seen on the roots, stems and leaves, then the plants will appear yellowish to brown and then die [4]. Not only on bananas, this pathogenic *F. oxysporum* also infects shallots and causes the problem of shallot plants wilting [5]–[9]

In controlling Panama disease, farmers often use chemical pesticides, the excessive use of which can kill beneficial organisms, decline of non-target species, affecting plant biodiversity, and damage the ecosystem in the environment [9]–[11]. Continuous chemical control with fungicides is not recommended because it will pollute the soil and

water environment. Chemical control is not economical because it requires a lot of fungicide. As an agricultural country, pesticide use in Indonesia is quite high. Excessive use of pesticides will increase control costs, increase the death of non-target organisms and can reduce environmental quality [12].

Biological control (biocontrol) offers an environmentally sustainable alternative to chemical pesticides in banana plantations, helping to manage pests and diseases while reducing negative impacts on human health and ecosystems. These include beneficial microbes such as *Trichoderma* spp., *Bacillus subtilis*, and *Pseudomonas fluorescens*, which can suppress pathogen growth through mechanisms like competition, parasitism, and induction of plant defenses [11]. For instance, *Trichoderma* species have shown strong antagonistic activity against *Foc*, significantly reducing disease incidence in field conditions [13]. Secondary metabolites produced by *Trichoderma* sp. can act as an inhibitor of pathogen development, because it produces volatile and nonvolatile compounds that are able to inhibit the growth of mycelia from various fungi. Secondary metabolites *Trichoderma* sp. Treatment with a concentration of 40% in vitro indicates growth inhibition *Phytopthora capsici* the best was 72.53% [14]. It is also one of the sources of important compounds for making antimicrobial compounds in sustainable agricultural programs *Trichoderma* sp. contains a variety of secondary metabolites, including anthraquinones, trichodermol, antibiotics, and various other compounds [15].

The objective of this research is to evaluate the antifungal activity of secondary metabolites produced by *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the causative agent of Panama disease in bananas. This study aims to assessing their efficacy in vitro, the research seeks to explore the potential of these metabolites as alternative biocontrol agents, contributing to the development of eco-friendly strategies for managing Panama disease in banana plantations and reducing dependency on chemical fungicides. Therefore, the research aims to determine the potential of secondary metabolites from fungi *Trichoderma* sp. to control *Foc* in vitro.

2. Materials and Methods

2.1. Preparation theAantagonistic Trichoderma sp.

Fungal isolate *Trichoderma* sp. that will be used in this research comes from Laboratory of Food Crop & Horticulture Pest and Disease Observation (LPHPTPH Tanggul, Jember). Fungal isolation *Trichoderma* sp. inoculated on sterilized PDA media. The isolate taken with a size of 0.5 – 1 cm was then placed in the middle of the PDA medium, then incubated for 7 days at room temperature [16].

2.2 Preparation the Pathogen Foc

Fungal isolation *Fusarium oxysporum* f.sp. *cubense (Foc)* used in this research came from the LPHPTPH Tanggul, Jember which was the result of research on Kepok banana plants. Isolation *Foc* was purified on PDA media and incubated for 7 days.

2.3. Preparation of Potato Sugar Extract (ECG) media

A total of 250 g of potatoes. peel, wash clean then cut into small squares. Potato pieces were boiled in 1000 ml distilled water for 20 minutes. The boiled liquid is filtered and then 10 g of sugar solution is added. Then the ECG media that has been prepared is sterilized by autoclaving at 121°C for 15 minutes and then cooled for 24 hours.

2.4. Making Potato Dextrose Agar (PDA) media

Weigh 19.5 g of instant PDA and mix with distilled water until it reaches a volume of 500 ml. The media is dissolved on the hotplate while stirring until it is completely dissolved. Next, PDA was sterilized using an autoclave at 121°C for 15 minutes

2.5 Production of secondary metabolites of Trichoderma sp.

Purification results *Trichoderma* sp. which had been incubated for 7 days, was inoculated into 150 ECG media. Erlenmeyer containing fungus isolates *Trichoderma* sp. shaken for 14 days using a rotary shaker at a speed of 90 rpm at room temperature 22°C. Next, the shaken secondary metabolites were transferred into a 50 ml test tube and then centrifuged at 10,000 rpm for 10 minutes. Next, it is filtered using white filter paper. Then the supernatant was filtered using a filter syringe. The supernatant obtained is a secondary metabolite *Trichoderma* sp.

2.6. Antifungal assay

The testing method carried out this time was by adding secondary metabolites *Trichoderma* Sp. on Potato Dextrose Agar (PDA) media according to the concentration level. The media which is still liquid is then shaken until it hardens. 20 ml of PDA liquid media was poured into a petri dish and waited until it solidified. Pure culture *F. oxysporum* f.sp. *cubense* measuring 7 mm is placed in the middle of the PDA media. Then the media was incubated at room temperature. Observation test for antifungal secondary metabolites of *Trichoderma* sp. done every day until *Foc* in the control treatment filled the entire petri dish.

2.7. Data analysis

Data were analyzed using SPSS 2.1 ANOVA to determine whether there was an effect of secondary metabolite treatment compared to control and chemical pesticides. If there was a significant effect on the variable, further testing was carried out using the Duncan Multiple Range Test (DMRT) at the 5% level.

3. Results and Discussion

The research results show in Figure 1, that the growth of *Foc* in treatments P1, P2, P3 and P4 for 7 days after inoculation there was no growth in diameter of the fungal colony. Meanwhile, in the negative control treatment or without secondary metabolites, growth in diameter of the fungal colony *Foc* approximately + 6.5 cm and results from administration fungicide in treatment P5, fungal mycelia *Foc* develops spreadingly above media and did not form a thick layer like the negative control treatment. Results shown This occurs because the fungicide given to the culture media contains the active ingredient phosphoric acid which effectively inhibits the growth of the pathogenic *Foc.*

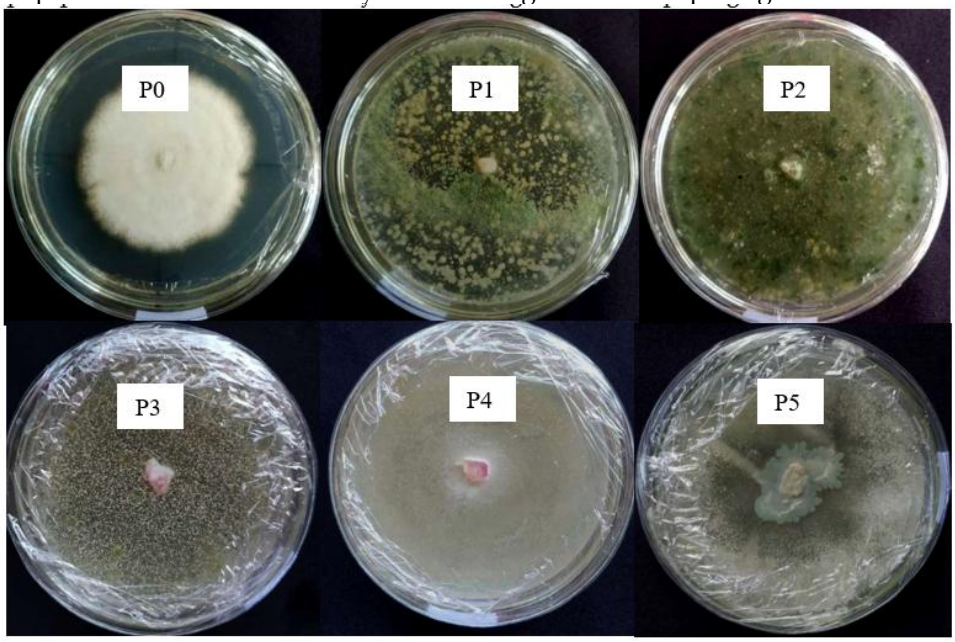

Figure 1. Antifungal test for secondary metabolites *of Trichoderma* sp. against *Foc* day 7 on PDA media, (P0): Control, (P1): concentration 10%, (P2): 20% (P3): 30%, (P4): 40%, (P5): Fungicide

The results of macroscopic observations also showed that several isolates had the ability forms colonies with different color variations. In the P0 or control treatment, the colonies spread above medium and white in color, in treatment P1 with a concentration of 10% and P2 with a concentration 20% of the colonies were white while in treatment P3 with a concentration of 30% and P4 with concentration of 40%, shows the colony changes color to pink with the distribution of the colonies thin white.

White mycelium can quickly turn reddish, in contrast to the research results of [17], which stated that at the beginning of growth the mycelium was white, this is supported by the statement that fusarium fungi are unstable so they are prone to change [18]. Meanwhile, the fungicide treatment showed a distribution of green colonies. This is in accordance by [19], the white to pale green coloration of *T. harzianum* colonies on growth media can be attributed to the specific environmental conditions and the type of culture medium used.

Table 1. Colony diameter of *Foc*

Treatment	Colony diameter (cm) (day after inoculation)						
	1	2	3	4	5	6	7
Control	0,75 a	1,66 a	2,71 a	3,69 a	4,35 a	5,18 a	5,73 a
10%	0,54 b	0,54 b	0,56 c	0,56 c	0,58 c	0,58 c	0,58 c
20%	0,63 ab	0,65 b	0,68 c	0,68 c	0,81 c	0,71 c	0,71 c
30%	0,75 a	0,76 b	0,76 c	0,73 c	0,76 c	0,76 c	0,76 c
40%	0,64 ab	0,64 b	0,64 c	0,64 c	0,64 c	0,64 c	0,64 c
Fungicide	0,75 a	0,98 b	1,48 b	2,41 b	2,50 b	2,50 b	2,50 b

Note: Numbers followed by the same letter in the same column showed no significant difference at 1% DMRT.

The application of *Trichoderma* sp. metabolites demonstrated a significant effect in inhibiting the growth of *Foc,* it was able to inhibit the growth of fungal mycelia in 1 - 8 days after inoculation. The different concentrations given have the same notation Treatments P1, P2, P3 and P4 showed that the results were not significantly different. Treatment P1 concentration 10% is a fairly good treatment in inhibiting the growth of the colony diameter of *Foc.* However, the comparison between treatment P0 (negative control) and treatment P5 (fungicide) shows different notations so that they indicate very real differences in results.

Based on the recapitulation of the results of various fungal colony diameter parameters on day 8, showed that administration of secondary metabolites *of Trichoderma* sp. in treatments P1, P2, P3 and P4 can inhibits the growth of the diameter of the fungal colony *Foc o*f the five treatments tested, treatments P1, P2, P3, and P4 had lower growth than treatment P5 which had a colony diameter growth of 2.50 cm on day 8. From the results of observations for 8 days, P1 treatment with a concentration of 10% was the best treatment in inhibiting the growth of *Foc.* because it has a colony diameter of only 0.59 cm. This matter indicated that secondary metabolites *of Trichoderma* sp. able to inhibit the growth of *Foc* is higher compared to fungicides.

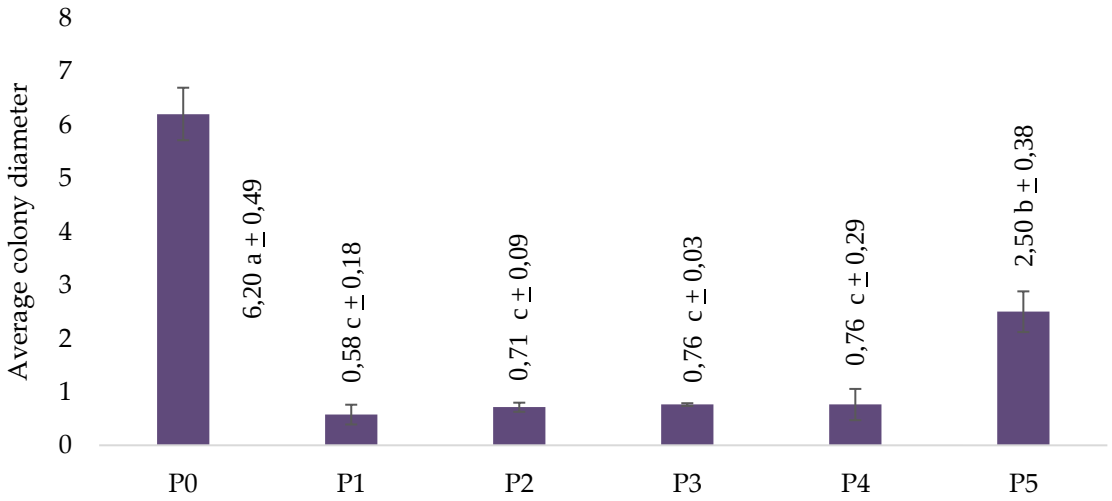

Figure 2. Average of colony diameter of *Foc* on the last day (8 day after inoculation) (P0): Control, (P1): concentration 10%, (P2): 20% (P3): 30% (P4): 40%), (P5): fungicide. The value of the value on the diagram standard deviation is the average of four repetitions. The same letters in the diagram indicate the results were not significantly different in the 1% DMRT test.

There is a significant difference between the results of treatments with concentrations of 10, 20, 30 and 40% compared to the negative control treatment indicating the presence of compounds in metabolites secondary which is able to inhibit the growth of the pathogenic *Foc* The chitinase enzyme produced by the*Trichoderma* sp. shows higher effectiveness compared to the chitinase enzyme produced by other organisms inhibits Fusarium proliferatum [20]. *Trichoderma* sp. has the potential to produce toxic compounds that are effective for controlling pathogen growth and use of *Trichoderma* sp filtrate. containing enzymes *chitinase* and ÿ-1,3- *glucanase* which are able to inhibit the growth of pathogens [21]. Enzymes and the toxin produced by *Trichoderma* sp. able to inhibit pathogens by damaging cell walls [22], [23].

Table 2. Inhibition percentage (%)

Treatment	Inhibition percentage (%) (day after inoculation)							
	1	2	3	4	5	6	7	8
Control	28,33 a	67,67	79,26 a	84,75 a	86,78 a	88,89 a	89,96 a	90,73 a
10%	16,67 a	60,90	75,12 a	81,69 a	81,32 a	86,23 a	87,55 a	88,51 a
20%	15,00 a	54,14	71,89 a	80,34 a	82,47 a	85,27 a	86,68 a	87,70 a
30%	0,00 b	61,65	76,50 a	82,71 a	85,34 a	87,68 a	88,86 a	87,70 a
40%	0,00 b	41,35	45,62 b	34,58 b	42,53 b	51,69 b	56,33 b	59,68 b

Note: Numbers followed by the same letter in the same column showed no significant difference at 1% DMRT.

The treatment of secondary metabolite concentration of *Trichoderma* gave the same value compared to fungicide treatment. The four treatments given showed that the administration of metabolites was able to inhibit the growth of *Foc* 87.70 - 90.73%. While synthetic fungicide treatment can only inhibit pathogens by 59.68%. Several studies on secondary metabolites of *Trichoderma,* mycotoxin biosynthesis can also be inhibited by *Trichoderma* fungi which have antagonistic properties against pathogenic fungi [24]. It can inhibit *Phytophora capsici* fungi by 72.53% with a concentration of 40% [25]. Secondary

metabolites are natural compounds produced by microorganisms and plants that are synthesized from primary metabolites. The formation of secondary metabolites is a defense against environmental disturbances and other organisms. The percentage of fusarium wilt attacks on tomato plants can be suppressed by the use of *Trichoderma* Sp. up to 100% [26]. The metabolites from *Trichoderma* sp. contain substances such as lysis enzymes which are known to be able to degrade the cell walls of host fungi. Administration of secondary metabolites of *T. harzianum* with a concentration of 10% can suppress Phomopsisvexans fruit rot disease in eggplant by 51.65%, increasing the concentration to 30% can suppress by 69.63% % [27].

Secondary metabolites from *Trichoderma* have many useful enzymes. Enzymes contained include protease, cellulase, cellobiase, chitinase, and 1,3-ß-glucanase [28]–[30], which play an important role in controlling plant diseases *Trichoderma* sp. and secondary metabolites released in the rhizosphere have an impact on plant growth and nutrition, stimulate systemic resistance and function as biocontrol agents against pathogenic microorganisms. Although the effect of *Trichoderma* sp. on the root system is not visible in the soil, the direct effects of this fungus can be observed in in vitro studies..

4. Conclusions

The secondary metabolites of *Trichoderma* sp. have been proven to effectively inhibit the growth of *Foc*. The most effective treatment was observed at a 10% concentration, which resulted in the lowest mycelial growth diameter of 0.58 cm and achieved an in vitro inhibition rate of 90.73% against Fusarium wilt in banana plants. This study supports new findings for implementing sustainable agriculture to minimize the use of chemical pesticides. Future research will focus on testing the effectiveness of the treatment through in vivo assay in banana plantations.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, G.F. and A.W.; methodology, G.F.; software, A.S.; validation, E.S., G.F. and A.W.; formal analysis, E.S.; investigation, T.R.; resources, R.R.; data curation, E.W.; writing—original draft preparation, G.F.; writing—review and editing, G.F.; visualization, G.F.; supervision, E.S.; project administration, T.R; funding acquisition, R.R. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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