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IN VITRO ANTAGONISM OF THE ISOLATE MUSHROOM *Trichoderma harzianum* TO *Fusarium oxyzporum f. sp. Cubense* CAUSES WILT DISEASE IN BANANA KEPOK *(Musa paradisiaca normalis)*

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ABSTRACT

Trichorderma harzianum is a soil native fungus that is beneficial because it has high antagonistic properties against plant pathogenic fungi. Control mechanisms can be: antibiosis, parasitism, competition and hyphae interference. T. harzianum as a biological control agent for the pathogenic fungus Fusarium oxyforum f. sp. Cubense is an alternative to control this pathogenic fungus, without causing a negative impact on the environment. The aim of the study was to determine whether Trichodernma harzianum isolates Piru, Kairatu and Uraur could suppress the growth of the fungus Fusarium oxyforum f. sp. cubense and Trichorderma harzianum which isolates (Piru isolate, Kairatu isolate and Uraur isolate) had faster inhibition against Fusarium oxyforum f. sp. cubense causes wilt disease on banana kepok (Musa paradisiaca normalis) in vitro with antagonism test. The research was conducted in 2 places, namely in the HPT BPTPH laboratory in Maluku province and continued in the microbiology laboratory at FMIPA Unpatti. The research method used was laboratory experimental in nature by using a completely randomized design with factorial pattern with three treatments and three replications. The results showed that the cause of wilt disease in banana plants was Fusarium oxyforum f. sp. Cubense. Antagonism test in vitro showed that T. harzianum Piru isolate showed the highest inhibition zone (70.87%), both Kairatu isolates (45.27%), and the lowest by Uraur isolate (32.61%) so that it can be said that T. harzianum has the potential to inhibit the growth of Fusarium fungus. oxyforum f. sp. Cubense.

Keywords: *fusarium, disease, trichorderma, antagonism.*

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INTRODUCTION

Fusarium wilt in bananas, often also called panama disease, is considered a very dangerous disease in banana plants worldwide. In Indonesia wilt disease has long been known, but in general people suspect that the cause of this disease is only one type, namely caused by bacteria (Semangun, 1996). This disease is capable of destroying banana plantations in a relatively short time. Banana wilt disease consists of two types, namely Fusarium wilt caused by the fungus Fusarium oxysforum f. sp. cubense and bacterial wilt caused by

the bacterium Pseudomonas solanacearum. Fusarium wilt is a soil-borne disease while bacterial wilt is vector-borne, but the spread of both diseases can be mechanically accelerated by humans (Dinas Pertanian, 2004). The use of pesticides on food crops and horticulture is currently considered to be excessive and uneconomical and can also cause harm to the environment. These losses are in the form of unexpected side effects such as pest resistance, pest resurgence, killing of natural enemies, leaving pesticide residues in production, environmental pollution, and others (Sudantha, 1993). In Semangun, the amount of losses caused by wilt disease is based on the calculation that the number of plants per clump that is cared for, for example, is 5 trees and each tree produces 6 combs while the average price is Rp. 5,000,- per comb, the loss borne by the community will increase if the selling price of bananas becomes more expensive and the area of disease attacks increases in the future.

Chemical control of Fusarium wilt has not yet been discovered because there are no pesticides that can kill this fungus. Alternative control that can be used is biological control, which utilizes microorganisms known as biological agents. One of the biological agents that can inhibit the growth of pathogens that cause disease in plants is an antagonist agent (Dinas pertanian, 2004). The antagonist agent used in the study was the fungus Trichoderma harzianum. According to Sudantha (Syahnen, 2006) the fungus Trichoderma harzianum in vitro was able to suppress the growth of the fungus Sclerotium oryzae on upland rice. Trichoderma viridae can control stem rot disease in pepper caused by Phytopthora capsici. Trichoderma koningii in addition to controlling white root fungus on rubber plants can also be used to control diseases that attack roots such as: Giberlla fujikori, Pythium ultimatum, Rhizoctonia solani, Sclerotium rolfsii and others (Dharmaputra, 1993).

The mechanism of pathogen control with Trichoderma spp is mostly microparasite events and aggressive competition. The growth of the Trichoderma spp mycelium will twist and fill the space around the host fungal hyphae, then the pathogenic hyphae will become empty. The fungi T. harzianum and T. hamantum act as microparasites against Rhizoctonia solani and Sclerotium rolfsii by producing β-(1-3)-glucanase and chitinase which cause exolysis in the host hyphae. The fungus T. hamantum also produces cellulose which is capable of parasitizing Pythium sp. Semangun stated that the fungus T. viridae produces gliotoxin and viridian antibiotics. This antibiotic is resistant to acidic soils, but quickly decomposes when the soil reacts alkaline. The use of antagonistic fungus Trichoderma spp is used to suppress the growth of pathogens that cause Fusarium wilt in banana plants.Based on the explanation above, the authors are interested in conducting research with the title "Testing in vitro antagonism of Trichoderma harzianum isolates against Fusarium oxysforum f. sp. cubense causes wilting disease in kapok banana plants (Musa paradisiaca normalis).

METHOD

This type of research was an experimental laboratory, which was carried out at the Plant Pests and Diseases Laboratory of the Food Crops and Horticulture Protection Agency (HPT BPTPH) in Passo–Ambon from June to July 2009 and the Microbiology Laboratory, FMIPA Pattimura University, Ambon from March to March 2010.

Tools and materials

The equipment used in this study consisted of: analytical balance, autoclave, hot air sterilizing oven, haemocytometer type “neubauer improve”, 1000 ml Erlemeyer, 500 ml Erlemeyer, 500 ml glass beaker, 500 ml measuring cup, petri dish, spatula, pipette volume, test tube, test tube rack, centrifuge, knife, plastic container, pan, hot plate, filter, funnel, in-flow, Bunsen lamp, light microscope, object glass, cover glass, loop needle, cork well, hand counter. The materials used were: infected banana stems, detergent, water, 70% alcohol, distilled water, potatoes, granulated sugar, agar bars, cotton, tissue, aluminum foil, paraffin, proclin (bleach solution).

Procedure

The fungal pathogen Fusarium oxyforum f. sp. cubense was isolated from infected plant parts by taking 4 pieces of tissue with a size of 2 x 2 mm. The piece is placed in a bleach solution (proclin) for 60 seconds to kill other unnecessary pathogens. Then using sterile forceps each piece is removed and dried on a piece of tissue paper. The pieces were then placed on the surface of the PDA media in the Petridish to observe their growth for 7 days.

Identification of Pathogenic Fungi

Pathogenic fungi were identified by observing the morphological characteristics of these fungi (mycelium and spores) using a light microscope and then matching with identification books "Illustrated Genera of Imperfect Fungi" (Barnett, 1955) and "Introductory Mycology" (Alexopoulus & Costantine John, 1907). Fungal colonies growing on the media were taken using an ose needle, then placed on a glass object at an angle of 45o to avoid the occurrence of air bubbles which would hinder observation. The visible mecelium and fungal spores were then observed for the type of mecelium, branching, spore size and spore location. The results of the observations were matched with the literature which has a mycelium that is insulated with regular branching, in each spore mycelium located at the end of the conidophore, relatively small in size and bluish green in color.

Purification of Pathogenic Fungi

Fungal colonies identified as Fusarium wilt disease pathogens were then transferred to PDA media to obtain pure cultures. The total density of growing mushroom colonies was counted using the “Neubauer Improve” Haemocytometer. The results of the purification were transferred to PDA media so that it was slanted which resulted in pure isolates. The isolates were then stored at room temperature for 1 week until the growth of the fungus sporulated completely.

Calculation of the Number and Density of Trichoderma harzianum Fungus Spores

The number of fungal spores per gram of media was calculated using the Gabriel and Riyatno (1989) formula:

$$S = \frac{.d}{n.0.25} x10_6$$

Information:

S = number of spores

t = the number of spores counted in the counting box (a, b, c, d, e)

d = dilution level (ml)

n = the number of small squares observed (= 80 small squares)

0.25 = standard size Haemocytometer (mm)

Calculation of spore density using the formula Gabriel and Riyatno (1989):

$$C = \frac{t.d}{n.0.25} x10_6$$

Data analysis

This study used a completely randomized design (CRD) in a factorial pattern, which consisted of two factors. Treatment of Trichoderma harzianum mushroom isolates is the first factor (A) which consists of 3 levels, namely:

A1 = Trichoderma harzianum isolate from Piru

A2 = Trichoderma harzianum isolate from Kairatu

A3 = Trichoderma harzianum isolate from Uraur

Time of inoculation of the fungus Trichoderma harzianum into the petridish as the second factor (B) consists of:

B1 = Trichoderma harzianum placed together with Fusarium oxyforum f. sp. cubense

B2 = Trichoderma harzianum placed 24 hours after Fusarium oxyforum f. sp. cubense

B3 = Trichoderma harzianum placed 48 hours after Fusarium oxyforum f. sp. cubense

Each treatment was repeated 2 (two) times so that the total experimental units were 18 experimental units. The combination of experimental units is arranged as follows:

Each treatment was repeated 2 times so that the total experimental units were 18 experimental units. The combination of experimental units is arranged as follows:

A_1B_1	A_2B_2	A_3B_3
A_1B_2	A_2B_2	A_3B_2
A_1B_3	A_2B_3	A_3B_3

In this study, the variable measured was the percentage inhibition of the antagonistic fungus Trichoderma harzianum against the pathogenic fungus Fusarium oxyforum f. sp. cubense. Then the data will be analyzed and if it is significantly different a Least Significant Difference Test (LSD) will be carried out.Model matematika dari rancangan yang dipergunakan:

$$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij = \sum ijk ; \quad i = 1, 2, 3$$
$$j = 1, 2, 3$$
$$k = 1, 2, 3$$

DISCUSSION RESULT

The highest number and density of spores were found in the Piru isolate, second by the Kairatu isolate and the lowest was the isolate from Uraur which was calculated using the Gabriel and Riyatno formula (1989).

Table 1. Number and density of T. harzianum spores produced

Isolate	Spore (per gram media)	Spore Density (juta/ml)
Piru	875×10^6	8.75×10^6
Kairatu	685×10^6	6.85×10^6
Uraur	325×10^6	3.25×10^6

Antagonism test of the fungus Trichoderma harzianum against the fungus Fusarium oxysforum f. sp. cubense was carried out over an interval of 13 days because on that day the T. harzianum fungal colonies had filled the entire surface of the petri dish. It can be seen that between each isolate there is a difference in the percentage of inhibition where the highest inhibition occurs in T. harzianum isolates from Piru with an average value of 70.87%. then by T. harzianum isolate from Kairatu with an average value of 45.27% and the inhibition percentage occurred in T. harzianum isolate from Uraur with an average value of 32.61% while between the time of inoculation the lowest inhibition percentage was 24 hours after the fungus Fusarium oxysformis f. sp. cubense was inoculated into a petri dish. Each T. harzianum isolate did not show a significant difference between the three types of isolates, while the inoculation time of 0 hours was significantly different from the inoculation times of 24 and 48 hours and between the inoculation times of 24 hours and 48 hours did not show a significant difference (Table 2).

Table 2. Percentage of inhibition of Trichoderma harzianum against Fusarium oxyforum f. sp. cubense

Time Inokulasi	Inhibition Percentage (%)			mean
	A1	A2	A3	
A1	74.19	38.52	40.36	51.02 a
A2	63.76	48.88	26.83	46.49 b
A3	74.67	48.41	30.65	51.38 b
mean	70.87 a	45.27 a	32.61 a	49.63

Note: Numbers in the same column followed by the same letter are not significantly different from the test BNT at the rate of .05

The results of the analysis between the two factors (factor A = Trichoderma harzianum isolate, and factor B = incubation time) at the same time of inoculation showed a significant difference between 24 and 48 hours of inoculation with a P value <0.05 but between 24 and 48 hours of inoculation. did not show a significant difference with a P value > 0.05, while the type of isolate did not show a significant difference. Mushroom Fusarium oxysforum f. sp. cubense is one of the pathogens that cause disease in plants. Dharmaputra stated that the fungus Fusarium oxysforum can cause stem rot in potatoes, soybeans, leaf spot disease in bananas, vascular wilt in bananas, chilies and strawberries. This fungus is known as a soil borne pathogen. The fungus enters through wounds in the roots, then develops and damages the xylem vessels. Mycelial threads are mainly found in cells, especially in the vascular tissue of wood. As a result of the damage

and the presence of mycelium in the network, the transportation of water and food is disrupted, so that the plants wither and die (Tjahjadi, 1989). According to (Sudanta, 1992) Fusarium oxysforum f. sp. cubense is a soil fungus that can survive for a long time in the soil as chlamydospores which are abundant in the roots of diseased banana plants. After entering the root, the fungus develops along the root to the stem and here the fungus develops extensively in the vascular tissue before entering the pseudostem. At later stages of infection the mycelium may extend from the vascular tissue into the parenchyma. The fungus forms many spores in plant tissue and will spread throughout the plant.

The spread of wilt disease is mainly due to the roots of healthy plants related to spores released by diseased plants, the use of diseased plant material can transmit fungal diseases so that fungal diseases can be carried by soil attached to agricultural tools or spread by wind, or irrigation water (Anggriani, 1993). The results of this study showed that the three T. harzianum isolates were able to suppress the mycelium growth of the pathogenic fungus Fusarium oxysforum f. sp. cubense. As reported by (Nasution, 2017), that the fungus, T. harzianum was effective in suppressing the fungus Fusarium oxysforum f. sp. lycopersici on tomato plants in the field. This is because the Trichoderma fungus can quickly colonize in the soil. It was also reported by (Syahnen, 2006), that the fungus T. harzianum in vitro was able to suppress the fungus Sclerotium oryzae. The fungus T. harzianum lives and carries out activities in soil conditions that have a pH of 3.7-4.7 and among the genus Trichoderma, the fungus T. harzianum is generally found in areas with hot climates and has faster growing characteristics. The highest inhibition percentage was shown by the isolate from Piru (70.87%), the second isolate from Kairatu (45.27%) and the lowest by isolate from Uraur (32.61%). This is due to the different storage time of each isolate, where the ages of the isolates used after isolation include Piru = 3 months, Kairatu and Uraur = 12 months. Syanen said that to prevent a decrease in the quality of the culture (amount, density and spore viability) of the fungus, it is necessary to avoid storage for a long time (more than 6 months) so it is better to use a mushroom culture that is approximately 3 months old after isolation. The three isolates had different densities, namely Piru isolate = 8.75x106 million/ml, Kairatu isolate = 6.85x106 million/ml, and Uraur isolate = 3.25x106 million/ml. The success of using fungi in biological control is determined, among other things, by the density and viability of the spores that will come in contact with the body/tissue (Ferron, 1981) and the type of mushroom isolate.

According to (Nasution, 2017) that the Trichoderma fungus is good as an antagonist in soybean plants attacked by Sclerotium rolfisii. This happens because Trichoderma will concurrently and will entangle the entire hyphae of the pathogenic fungal host. In accordance with the graph of the average percentage inhibition of the fungus T. harzianum against the fungus Fusarium oxysforum f. sp. cubense, it was clear that the three different T. harzianum isolates could inhibit the growth of the mycelium of the fungus Fusarium oxysforum f. sp. cubense, generally the fungus T. harzianum is a fast-growing colony capable of destroying pathogenic fungal hyphae in a short time. The results of data analysis using a completely randomized design in a factorial pattern showed that the three isolates, namely Piru, Kairatu and Uraur, were not significantly different in inhibition of the pathogenic fungus Fusarium oxysforum f. sp. cubense. The fungus T. harzianum on artificial media will form colonies quickly and will form a soft, wet white mycelium, then whitish green to bright green colonies. At first the Trichoderma mushroom was white and then dark green. This fungus has the ability to reproduce rapidly so that it has excellent spatial competition and is effective in suppressing the growth of other fungi (Directorate General of Food Crops, 2008). The fungus T. harzianum is actually already in the soil, living saprophytes on the remains of organic matter, it's just that the population may still be low. For this reason, efforts are needed to increase the effectiveness of these mushrooms by conducting mass breeding and inoculating them back into the soil (Syahnen, 2006). In addition, the microenvironment also affects sporulation. Walstad, Anderson and Stambaugh (1970) reported that the optimum growth and sporulation of T. harzianum occurs at 25o-30oC and 100% humidity. Also added by Junianto and Sri Sukamto (1955) that sporulation requires high humidity. At 100% humidity sporulation develops within 4 days, while at 92.5-98% humidity sporulation occurs 5 days or longer. Another factor that helps the mushroom mycelium grow properly is the provision of a good and sterile medium, which provides nutrients for the metabolism of the fungus as well as the potential for the fungus itself to grow (Sudantha, 1992).

The percentage of inhibition obtained showed that the three isolates of T. harzianum were effective in suppressing the growth of wilt disease pathogens. The mechanism of antagonism that occurs between the fungus T. harzianum and the fungus Fusarium oxysforum f. sp. cubense is competition for space and nutrients, where on the 12th day after the antagonistic fungus T. harzianum is inoculated, the pathogenic fungus undergoes lysis and eventually dies. This is due to the production of the antibiotic compounds trichodermin and peptin by the antagonistic fungus T. harzianum which inhibits the growth of the mycelium and destroys the hyphal cell walls of pathogenic fungi.

CONCLUTION

1. The three isolates of the antagonistic fungus Trichoderma harzianum can suppress the growth of the pathogen Fusarium oxysforum f. sp. cubense on banana plants in vitro.
2. The highest inhibition percentage was owned by the fungus Trichoderma harzianum Piru isolate, namely 70.87%, followed by the inhibition percentage by Kairatu isolate 45.27% and the lowest inhibition percentage by Uraur isolate 32.61%.
3. Analytically, analysis of variance percentage inhibition of the fungus Fusarium oxysforum f. sp. cubense by Trichoderma harzianum for the three isolates (Piru, Kairatu and Uraur) did not show a significant difference at the 5% level.

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