The response of different fungicides against Lasiodiplodia pseudotheobromae causing dieback disease of cocoa through in vitro test

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Abstract. Cocoa dieback, caused by *Lasiodiplodia pseudotheobromae*, one of the significant cocoa diseases, which is a new disease in South Sulawesi and it is considered to be one of the important diseases in the field. Fungicides are one of the main methods to manage the disease on cocoa in South Sulawesi. However, the appropriate fungicides remain unexplored. Also, there are no data available and registered on the efficacy of fungicides on *Lasiodiplodia* cocoa dieback. Five different fungicides were used with three different concentrations namely fipronil + thiophanate-methyl + pyraclostrobin (0.5; 5.0; 50) ml/liter, sulphur (0.2; 2.0; 20) gr/liter, carbendazim + hexaconazole (0.04; 0.4; 4.0) gr/liter, carbendazim (0.1; 1.0; 10) ml/liter, and difenoconazole + azoxystrobin (0.1; 1.0; 10)) ml/liter. The results showed that the effectiveness of fungicides inhibit mycelial growth of *L. pseudotheobromae* was varied on each active ingredient. Fipronil + thiophanate-methyl + pyraclostrobin, carbendazim + hexaconazole and carbendazim showed excellent performances, followed by sulfur and difenoconazole + azoxystrobin. Higher concentration indicated excellent inhibition, followed by recommendation concentration and lower concentration. Fungicides can potentially be an option for dieback disease control in cocoa production areas that are at high risk of cocoa dieback disease.

1. Introduction

Cocoa is frequently influenced by a range of plant diseases wherever it is cultivated. One of them is dieback disease. Cocoa dieback disease is one of the significant diseases for cocoa production in the world [1, 2, 3, 4, 5], including in Indonesia [6]. The disease caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. and *Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous. *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous, a member of botryosphaeriaceae, one of the species of the genus *Lasiodiplodia* which can infect various host of plants and a cosmopolitan fungus that can cause diseases on plant such as leaf spot, dieback, and canker which can lead to plant death [7, 8, 9].

Chemical fungicides are the most prevalent control method of the fungal pathogen on cocoa farms in Indonesia. Due to cocoa dieback is an emerging disease in Indonesia, none of the fungicides has been registered officially and legally for the disease. Chemical fungicides are still considered an alternative

control to restrict fungal diseases on many important plants including cacao. Fungal pathogens can be controlled by fungicides through a number of different target sites and modes of action [10].

The present research aimed to assess response of *L. pseudotheobromae* towards different fungicides through *in vitro* conditions. The study will provide and improve the knowledge about the possibility of using chemical fungicides of controlling *L. pseudotheobromae*.

2. Materials and methods

2.1. In vitro inhibition

Five fungicides with different active ingredients belong to fipronil 250 g/L + thiophanate-methyl 225 g/L + pyraclostrobin 25 g/L, sulphur 80%, carbendazim 80%, carbendazim 50 g/L + hexaconazole 50 g/L, difenoconazole 125 g/L + azoxystrobin 200 g/L were tested individually on three different concentrations to evaluate their effect on inhibition of pathogenic growth. Fungicide concentrations were prepared according to the manufacturer's recommendations of commercial formulations for field application on their target plant; Subsequent concentrations were in the ratio 1:10 lower and higher from the recommended dose. Each fungicide was suspended in sterile distilled water and added to the sterilized potato dextrose agar (PDA) medium, the medium was cooled to 45-50 °C to obtain the appropriate volume of liquid fungicide at three different concentrations to be tested. In control, only PDA was used.

A disc (8-mm diameters) of 2-day old *L. pseudotheobroma* mycelium culture was moved to the center of the solidified PDA medium in the glass petri dish (90-mm diameter) with different concentrations of fungicides. Then the culture was kept in incubator at room temperature. There were four replication of each treatment. The observations on mycelial growth were recorded on 1, 2, 3, 4, 10, 20, and 30 days after inoculation. Fungal radial growth was measured as:

$$D = \frac{d1+d2}{2} \quad (1)$$

Information:
D = Diameter of fungi mycelia grown on PDA media
d1 = Vertical diameter of mycelia grown
d2 = Horizontal diameter of mycelial grown on PDA media

The results of the calculation of the diameter are used for the percentage of the inhibitory power calculated using the following equation [9]:

$$D = \frac{D1-D2}{D1} \times 100\% \quad (2)$$

Information:
D = Per cent inhibition (%)
D1 = the average diameter increase of fungal colony with control
D2 = the average diameter increase of a fungal colony in treatment

2.2 Statistical analysis

Data analysis regarding fungal growth and mycelium growth inhibition at 30 days after inoculation were determined using factorial analysis of variance (ANOVA) and standard error. If significant differences are detected, the data is further tested using Tukey's test at the 5% probability level.

3. Results and discussion

3.1. Results

Mycelium growth of *L. pseudotheobromae* was evaluated at 30 days after inoculation. The effects of different fungicides on fungal mycelium growth at 30 days after inoculation are reported in figure 1 and table 1. The results showed that among the five fungicides, fipronil 250 g / L + thiophanate-methyl 225 g/L + pyraclostrobin 25 g/L, carbendazim 80% and carbendazim 50 g/L + hexaconazole 50 g/L were significantly reduced the growth of pathogenic mycelium in the culture at all treatment concentrations with 0 mm mycelium growth each, followed by sulfur 80% and difenoconazole 125 g/L + azoxystrobin 200 g/L.

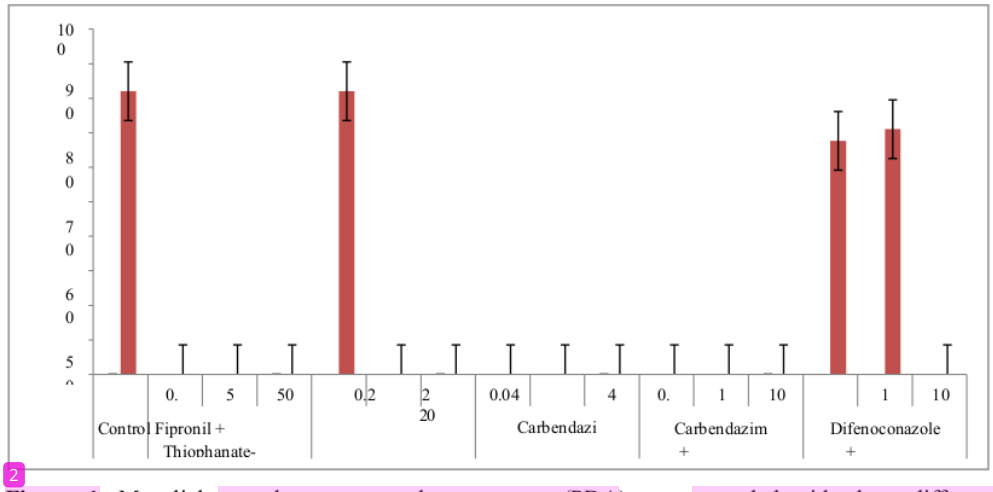

Figure 1. Mycelial growth on potato dextrose agar (PDA) was amended with three different concentrations of different fungicides (expressed in ml/g L^{-1} and reported on the x-axis), after a 30-day of incubation.

There is an effect of the fungicides (factor 1), concentrations (factor 2), and the combination on *L. pseudotheobromae* mycelial growth where the response was highly significant. Fipronil 250 g / L + thiophanate-methyl 225 g/L + pyraclostrobin 25 g/L, carbendazim 80% and carbendazim 50 g/L + hexaconazole 50 g/L were the most effective fungicides to inhibit *L. pseudotheobromae* mycelial growth (100%). Meanwhile, Higher concentration showed excellent inhibition, followed by recommendation concentration and lower concentration (table 1).

Table 1. Mycelial growth inhibition of *Lasiodiplodia pseudotheobromae* on potato dextrose agar (PDA), mixed with three different concentrations of different fungicides after 30-day of incubation.

Fungicides	Concentration (mg L^{-1})	% of Mycelial growth inhibition
Fipronil + Thiophanate-methyl + Pyraclostrobin	0.5	100
	5	100
	50	100
Sulfur	0.2	0
	2	100
	20	100

Carbendazim	0.04	100
	0.4	100
	4	100
Carbendazim + Hexaconazole	0.1	100
	1	100
	10	100
Difenoconazole + Azoxystrobin	0.1	18
	1	13
	10	100
Averages for each fungicides		
Fipronil + Thiophanate-methyl + Pyraclostrobin		100.0a
Sulphur		66.7b
Carbendazim		100.0a
Carbendazim + Hexaconazole		100.0a
Difenoconazole + Azoxystrobin		43.6c
Tukey's test		3.83
Averages for each concentration		
Lower		63.5c
Recommendation		82.7b
Higher		100.0a
Tukey's test		3.83
Analysis of Variance (*p*-value)		
Fungicides		**
Concentration		**
Fungicides x Concentration		**

Numbers in the same column followed by the same letter are not significantly different by Tukey's test analysis ($p<0.05$); ** indicate statistical significance at $p< 0.01, 0.05$.

3.2. Discussion

This study provides new information about the sensitivity of *L. pseudotheobromae* pathogens that cause dieback in cocoa. Pathogens were obtained from isolation from cocoa grown in Sulawesi, Indonesia. Since the disease was identified the first time several management methods have been studied, including chemical management to minimize pathogen contamination. The use of fungicides has become the most common cocoa disease control applied by almost all cocoa farmers. However, there are no registered fungicides to control the dieback of *Lasiodiplodia* in cocoa. In this study we tested the efficacy of five fungicides in vitro, the results showed that among the five fungicides, fipronil 250 g/L + thiophanate-methyl 225 g/L + pyraclostrobin 25 g/L, carbendazim 80% and carbendazim 50 g/L + Hexaconazole 50 g/L were fungicides that effectively suppresses the growth of pathogens followed by sulfur 80% and difenoconazole + azoxystrobin.

Among the fungicides assessed for their efficacy against the pathogen, fungicides with active ingredients of fipronil + thiophanate-methyl + pyraclostrobin and carbendazim in any concentrations were highly effective. The fungicides were previously studied as being effective to *Lasiodiplodia* species [11, 12]. Thiophanate-methyl is a type of pesticide from the thiofanate group with the work system to interfere with mitosis and cell division in the mitotic phase (β-tubulin) and systemic fungicides that belongs to the MBC-Fungicides (Methyl Benzimidazole Carbamates) group of fungicides [10], another member of the group is carbendazim, while pyraclostrobin is a fungicide from the type of strobilurin group which can inhibit mitochondrial [13, 14]. The second most effective tested fungicides were sulfur, followed by difenoconazole + azoxystrobin. These two fungicides were registered to cocoa.

4. Conclusion

*L. pseudotheobroma*e growth is effectively inhibited by several fungicides. Application of fungicides should be a focus on fungicide doses.

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