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by Asman Asman

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Antifungal activity of extracts of *Melia azedarach* and *Ageratum conyzoides* against *Lasiodiplodia pseudotheobromae* through in vitro test

Asman Asman[1,2] , A B Cahyani[1], A H Nufus[1], A Rosmana[1,2], A Fakhruddin[3] and N U Natsir[3]

[1] Department of Plant Pests and Diseases, Hasanuddin University, South Sulawesi, Indonesia
[2] Cocoa Research Group (CRG), Faculty of Agriculture, Hasanuddin University, South Sulawesi, Indonesia
[3] Agricultural Quarantine Major Service of Makassar, Indonesia

Email: asman@agri.unhas.ac.id

Abstract. *Lasiodiplodia pseudotheobromae* is one of the pathogens of the cocoa dieback disease. Currently, the disease is considered a significant disease in cocoa, which is a newly emerging disease in Sulawesi. The control tools and methods remain unexplored comprehensively. The main objective of this study was to evaluate *Melia azedarach* and *Ageratum conyzoides* leaf extract to inhibit the growth of the *L. pseudotheobromae*. Three different concentrations were applied for each weed extract, namely: 1%, 3%, and 5%. The experiment was conducted through the poison food technique method both in solid medium and liquid medium. *M. azedarach* and *A. conyzoides* were significantly inhibited the colony growth of *L. pseudotheobromae* in all concentrations in solid medium. However, *A. conyzoides* 5% performed well to suppress the colony growth of *L. pseudotheobromae* (42.7%), followed by *M. azedarach* 5% (16.0%). The mycelium biomass of *L. pseudotheobromae* was significantly inhibited by *M. azedarach* and *A. conyzoides* as well. *A. conyzoides* 5% showed a higher inhibition of the fungal biomass either wet biomass (90.3%) or dry biomass (95.5%), followed by *M. azedarach* 5% both wet biomass (85.6%) and dry biomass (78.1%). *M. azedarach* and *A. conyzoides* remain to inhibit the colony growth and fungal biomass regardless of the type of concentrations. *M. azedarach* and *A. conyzoides* can potentially be an option for controlling dieback disease caused by *L. pseudotheobromae*.

1. Introduction

Lasiodiplodia pseudotheobromae, a member of botryosphaeriaceae, causes destructive diseases such as leaf spot, dieback, and canker on many tropical plants [1–3]. One of the diseases is caused by *L. pseudotheobromae* is cocoa dieback. The disease is considered a newly emerging disease on a cocoa plantation in Sulawesi, Indonesia, and a new threat to cocoa production [4,5].

Finding effective and efficient control methods is necessary to prevent significant losses of the disease. Besides, microbes and fungicides have been studied to suppress pathogen growth and incidence [6,7] 1some of the plants are able to produce secondary metabolites that can act as antifungal compounds [8–11].

Much attention has focused on exploring in-situ/local weed around the cocoa farm, such as *Melia azedarach* and *Ageratum conyzoides*. The weeds are one of the main sources of potentially active secondary metabolites. The active compound has proved to inhibit fungal mycelia. *M. azedarach*, commonly known locally as mindi (Indonesia) or China tree (English), belongs to the Meliaceae family. The effectiveness of *M. azedarach* extract against a range of plant pathogenic microbes was studied in vitro against plant pathogenic fungi [8,12–14]. *A. conyzoides*, commonly called goat weed, is a member of the Asteraceae family. The weed has many active antimicrobial compounds which can inhibit the growth of microorganisms and serve as an antifungal as well [15,16]. However, very few studies have focused on their effect on *L. pseudotheobromae*.

The present study aimed to assess the antifungal activity of *M. azedarach* and *A. conyzoides* to *L. pseudotheobromae* through in vitro conditions. The research will present and improve the knowledge about the possibility of using local plant extracts to control *L. pseudotheobromae*.

2. Materials and methods

The pathogen was collected, and experiments were conducted in the laboratory at the Department of Plant Pests and Diseases, Hasanuddin University and Agricultural Quarantine Major Service of Makassar.

Preparation and extraction of M. azedarach and A. conyzoides

Leaves of *M. azedarach* and *A. conyzoides* were collected directly from the field. After that, the weeds were washing with tape water thoroughly, then leaves were sundried for three days, crushed and stored in a glass container. The crushed leaf materials of the two test plant species were soaked in methanol (1:2 w v-1) for seven days. After that, extracts were filtered through cloth. The organic solvent was evaporated using a vacuum rotary evaporator. During the process, A constant temperature and pressure were set at 60 ^{O}C and 85 rpm, respectively. The extract was transferred in amber bottles and stored in the refrigerator.

2.2. *In vitro inhibition on mycelial growth in PDA medium*

A 9 mm-diameter of mycelial-agar plug placed off the center of the petri dish contains potato dextrose agar (PDA) medium added with plant extracts on each concentration. There are three concentrations, namely: 1%, 3% and 5%. The desired concentration of each plant extracts was mixed thoroughly in 20 ml of PDA. A mycelial plug of *L. pseudotheobromae* was taken from the tips of 2 days old fungal culture. Controls were only PDA medium without extracts. Petri plates were wrapped and incubated at 25-27 °C. Each treatment was replicated four times. Observations were taken at 1-day intervals until the mycelia of *L. pseudotheobromae* was covered thoroughly on control.

Colony diameter was measured after 24-hour and 48-hour of inoculation by using measurement, and the percentage of inhibition of mycelial growth was calculated as the following formula [17].

$$I = \frac{C-T}{C} \times 100\% \quad (1)$$

Where C = diameter of the colony in check (average of both diagonals, T = diameter of the colony in treatment (average of both diagonals).

2.3. *In vitro inhibition on fungal biomass in PDB medium*

A mycelial plug of *L. pseudotheobromae* was taken from the tips of two days old fungal culture use a 9-mm cork borer and transferred to a 40-ml bottle. There concentration of each plant extract viz., 1%, 3%, and 5% were added to potato dextrose broth (PDB) medium. Controls were treated with no extracts. The bottles were wrapped and incubated at room temperature and prepared on the shaker for seven days [8]. Each treatment was replicated four times. After 7-day the fungal biomass in each bottle was filtered out through filter paper and weighed for wet weight on an electric scale. Then, the fungal biomass was dried at 60 °C in the electric oven for two days and weighed for dry weight on an electric scale. Per cent inhibition of fungal biomass was calculated as the following formula [17].

$$I = \frac{C-T}{C} \times 100\% \quad (2)$$

Where C = Biomass of the colony in check, T = Biomass of the colony in treatment

2.4. Statistical analysis

Data analysis regarding fungal growth both mycelial growth and fungal biomass inhibition were determined using factorial analysis of variance (ANOVA). 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

All concentrations *of M. azedarach* and *A. conyzoides* extracts tested on PDA medium against *L. pseudotheobromae* were found statistically effective reduced the growth of mycelia (Table 1). *A. conyzoides* was found most effective in inhibiting mycelial growth both 24-hour after inoculation (73.7, 45.7, 44.9) and 48-hour after inoculation (42.7, 10.5, 13.6) of *L. pseudotheobromae* at 5%, 3%, and 1%, respectively, followed by *M. Azedarach* extracts (53.4, 49.8, 13.4) at 5%, 3%, and 1%, respectively, on 24-hour after inoculation while on 48-hour after inoculation was 16.0, 9.1, 2.2 at 5%, 3%, and 1%, respectively.

The concentration of 5% of *A. conyzoides* was found significantly superior over other treatments. The concentration 1% of *M. azedarach* was the least effective in inhibiting mycelial growth of *L. pseudotheobromae* (2,2%).

Table 1. Effect of *M. azedarach* and *A. conyzoides* on mycelia growth of *Lasiodiplodia pseudotheobromae* by poisoned food technique on PDA medium.

Plant extracts	Concentration	% of mycelial growth inhibition	
		24-hour after inoculation	48-hour after inoculation
M. azedarach	1%	13.4	2.2
	3%	49.8	9.1
	5%	53.4	16.0
A. conyzoides	1%	44.9	13.6
	3%	45.7	10.5
	5%	73.7	42.7
Averages for each Plant Extracts			
M. azedarach		38.8	9.1
A. conyzoide		54.7	22.3
Tukey's test		6.0	10.4
Averages for each concentration			
1%		29.1	7.9
3%		47.7	9.8
5%		63.5	29.3
Tukey's test		7.4	15.6
Analysis of Variance (*p*-value)			
Plant Extracts		**	*
Concentration		**	**
Plant Extracts x Concentration		**	ns

All the plant extracts tested on PDB medium at 1%, 3%, and 5% against *L. pseudotheobromae* reduced the fungal biomass significantly at all concentrations (Table 2). *A. conyzoides* was the most effective in inhibiting fungal biomass of *L. pseudotheobromae* in the study either on wet weight (90.3, 87.5, 77.5) or on dry weight (95.5, 91.3, 91.4) at 5%, 3%, and 1%, respectively, followed by *M. azedarach* extracts (85.6, 81.4, 65.8) at 5%, 3%, and 1%, respectively, on wet weight while on dry weight (72-hour after drying) was 78.1, 71.6, 69.6 at 5%, 3%, and 1%, respectively.

The concentration of 5% of *A. conyzoides* was found significantly superior over other concentrations. The concentration 1% of *M. azedarach* was the least effective in inhibiting fungal biomass of *L. pseudotheobromae*, both wet weight (65.8) and dry weight (69.6).

Table 2. Effect of *M. azedarach* and *A. conyzoides* on fungal biomass of *Lasiodiplodia pseudotheobromae* by poisoned food technique on PDB medium.

Plant extracts	Concentration	% of mycelial biomass inhibition	
		Wet weight	Dry weight (72-hour after drying)
M. azedarach	1%	65.8	69.6
	3%	81.4	71.6
	5%	85.6	78.1
A. conyzoides	1%	77.5	91.4
	3%	87.5	91.3
	5%	90.3	95.5
Averages for each Plant Extracts			
M. azedarach		77.6	73.1
A. conyzoide		28.4	92.7
Tukey's test		3.1	2.6
Averages for each concentration			
1%		71.6	80.5
3%		84.4	81.4
5%		87.9	86.8
Tukey's test		3.8	3.9
Analysis of Variance (*p*-value)			
Plant Extracts		**	**
Concentration		**	**
Plant Extracts x Concentration		*	ns

3.2. Discussion

L. pseudotheobromae is a significant pathogen that causes diseases in cocoa, including dieback. The disease is considered a newly emerging disease in cocoa in Sulawesi [5]. Several methods have been studied to minimize pathogen contamination, including using plant extracts which are eco-friendly management. To find out the possibilities of plant extracts as an effective control manner were tested in vitro against *L. pseudotheobromae*. In this study, two plant extracts were evaluated in vitro at 1, 3, and 5% concentrations.

A. conyzoides and *M. azedarach* in any concentrations were effective in inhibiting mycelial growth compared to the control. Both plants extracts can reduce fungal infection, with the result is vary depending on the concentration. Both plant extracts have been characterized by other studies for their antifungal properties against different fungi [12–16].

As far as we acknowledge, this study was the first attempt to test *A. conyzoides* and *M. azedarach* against *L. pseudotheobromae*. The plant extracts are able to suppress the mycelia growth of the

pathogen. However, further investigation should be undertaken, such as extraction of chemical compounds and testing on the plant.

4. Conclusion

*L. pseudotheobroma*e growth is effectively inhibited by *A. conyzoides* and *M. azedarach*. Application of the plant extracts should be the focus on plant extract doses.

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