Antifungal activity of crude extracts of *Ageratum conyzoides* and *Chromolaena odorata* for management of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* through in vitro evaluation

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**Abstract.** *Lasiodiplodia* is an important genus of fungi causing destructive diseases on perennial crops, including cocoa. Two crucial species of *Lasiodiplodia* that cause diseases in cocoa are *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae*. A variety of weeds is the potential to be applied as botanical fungicides to control the pathogens. The main objective of this study was to evaluate *Ageratum conyzoides* and *Chromolaena odorata* leaf extract to inhibit the growth of *L. theobromae* and *L. pseudotheobromae* on a synthetic medium. Solvent organic was methanol for weed extraction with a ratio of 1:5. The experiment was conducted through the poison food technique method, both in the solid and liquid medium in three different concentrations, 1, 3, and 5%. The result showed that *A. conyzoides* and *C. odorata* were significantly inhibited the colony growth of both *Lasiodiplodia* in all concentrations in a solid medium. *A. conyzoides* performed better than *C. odorata* in all concentrations of both *Lasiodiplodia* in inhibition. *A. conyzoides* 5% performed well to suppress the colony growth of *L. pseudotheobromae* (100%), followed by *A. conyzoides* 3% and 1%. *A. conyzoides* 5% able to inhibit the colony growth of *L. theobromae* until 100%, followed by *A. conyzoides* 3% and 1%. Meanwhile, *A. conyzoides* and *C. odorata* extract tested on PDB medium at 1, 3, and 5% reduced the fungal biomass significantly at all concentrations. *C. odorata* was found most effective in inhibiting fungal biomass of both pathogens either on wet weight or on dry weight at 1, 3, and 5% %. *A. conyzoides* and *C. odorata* can manage the growth of *L. theobromae* and *L. pseudotheobromae* through in vitro conditions.
1. Introduction

Cocoa (*Theobroma cacao* L), a source of chocolate, is frequently attacked by a range of plant fungal pathogens wherever it is planted [1]. Together with major quantities of milk, sugar, almonds and peanuts, it supports a multi-billion dollar chocolate industry [2,3]. Among the wide range of important fungal pathogens that affect cocoa production are members of the Botryosphaeriaceae [4–9]. *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* are a member of botryosphaeriaceae, causes diseases such as leaf necrosis, dieback, and canker on many tropical plants [4–9]. In cocoa, one of the diseases is caused by *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* is cocoa dieback. The disease is considered a threat to cocoa production in Sulawesi, Indonesia [8,9].

As a newly emerging disease, options of the control method remain limited against the pathogen. Providing a range of effective and efficient control methods is urgent to inhibit the pathogens. Recently, microbial antagonists and fungicides have been tested in suppressing the pathogen growth and incidence [10,11]. Meanwhile, Some of the plants are able to produce secondary metabolites that can act as antifungal compounds [11–14].

Utilization of local resources for controlling the pathogen is considered more effective and efficient, one of them is weeds around the cocoa farm such as *Ageratum conyzoides* and *Chromolaena odorata*. *A. conyzoides* or better known as goat weed has antifungal properties which can inhibit the growth of microorganisms [15,16]. *C. odorata*, commonly called kirinyuh or siam weed, is a member of the Asteraceae family that is considered a competitor of the cultivation plants. Antifungal activity of *C. odorata* extract against a range of plant pathogenic microbes has been performed by several previous studies [17–20].

This study aimed to evaluate the antifungal activity of *A. conyzoides* and *C. odorata* against *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* through *in vitro* conditions. The research will provide knowledge about the possibility of using weed extracts for controlling *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae*.

2. Materials and methods

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

2.1. Preparation and extraction of *A. conyzoides* and *C. odorata*

*A. conyzoides* and *C. odorata* leaves 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 (1:5 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 50 °C and 90 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 mycelial-agar plug of 9 mm-diameter of the pathogen was taken from two-day-old culture and 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. 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* and *L. theobromae* were 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 [21].

\[
I = \frac{C - T}{C} \times 100\% \tag{1}
\]

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 disc of \(L.\) pseudotheobromae and \(L.\) theobromae were taken from the tips of two days old fungal culture use a 9-mm cork borer and transferred to a 40-ml bottle. There is three 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 [22]. 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 [21].

\[
I = \frac{C - T}{C} \times 100\% \tag{2}
\]

where \(C\) = Biomass of the colony in check, \(T\) = Biomass of the colony in treatment

### 2.4. Statistical Analysis

The data were analyze by factorial analysis of variance (ANOVA). The mean comparison was made using Tukey’s test at 0.05 level of significance.

### 3. Results and discussion

#### 3.1. Results

There is an effect of the plant extracts on mycelia growth of \(L.\) pseudotheobromae and \(L.\) theobromae both on PDA and PDB medium where the influence was a range from significant to highly significant on 24-hour after inoculation and 48-hour after inoculation whereas concentration effect was also significant to highly significant at 24-hour after inoculation and 48-hour after inoculation. However, the interaction (plant extracts × concentration) effect was mostly not significant in any evaluation event.

All the plant extracts tested in vitro at 1, 3, and 5% were significantly inhibited the fungal growth of \(L.\) pseudotheobromae and \(L.\) theobromae at all concentrations (Table 1,2,3,4). Between two plant extracts, A. conyzoides was the most inhibitor mycelial growth of two Lasiodiplodia species on solid medium both 24-hour after inoculation (83.3, 96.4, 100) and 48-hour after inoculation (78.7, 93.9, 100) at 1, 3, and 5%, respectively. Meanwhile, \(C.\) odorata was also found the second most effective in reducing mycelial growth both 24-hour after inoculation (78.8, 93.9, 100) and 48-hour after inoculation (45.1, 72.3, 87.5) at 1, 3, and 5%, respectively.

Among three concentrations, the concentration of 5% was found significantly superior over other concentrations. The concentration 1% of \(C.\) odorata was the least effective in inhibiting the mycelial growth of \(L.\) pseudotheobromae and \(L.\) theobromae.
Table 1. Effect of *A. conyzoides* and *C. odorata* on mycelia growth of *Lasiodiplodia pseudotheobromae* by poisoned food technique on PDA medium.

| Plant extracts | Concentration | 24-hour after inoculation | 48-hour after inoculation |
|---------------|--------------|---------------------------|---------------------------|
| *A. conyzoides* | 1%           | 83.3                      | 78.7                      |
|               | 3%           | 96.4                      | 93.9                      |
|               | 5%           | 100.0                     | 100.0                     |
| *C. odorata*  | 1%           | 61.1                      | 45.1                      |
|               | 3%           | 79.0                      | 72.3                      |
|               | 5%           | 100.0                     | 87.5                      |

Averages for each Plant Extracts

| Plant Extracts | % of mycelial growth inhibition |
|----------------|--------------------------------|
| *A. conyzoides* | 93.3 90.9                      |
| *C. odorata*    | 80.0 68.3                      |
| Tukey’s test    | 10.7 6.3                       |

Averages for each concentration

| Concentration | % of mycelial growth inhibition |
|---------------|--------------------------------|
| 1%            | 72.2 61.9                      |
| 3%            | 87.7 83.1                      |
| 5%            | 100.0 93.8                     |
| Tukey’s test  | 16.0 7.7                       |

Analysis of Variance (*p*-value)

|                      | Plant Extracts | Concentration | Plant Extracts x Concentration |
|----------------------|----------------|---------------|-------------------------------|
|                      | *              | **            | **                            |
|                      | **             | **            |                               |

Table 2. Effect of *A. conyzoides* and *C. odorata* on mycelia growth of *Lasiodiplodia theobromae* by poisoned food technique on PDA medium.

| Plant extracts | Concentration | 4-hour after inoculation | 8-hour after inoculation |
|---------------|--------------|--------------------------|--------------------------|
| *A. conyzoides* | 1%           | 78.5                     | 75.8                     |
|               | 3%           | 98.5                     | 95.5                     |
|               | 5%           | 100.0                    | 100.0                    |
| *C. odorata*  | 1%           | 72.1                     | 43.2                     |
|               | 3%           | 84.2                     | 74.6                     |
|               | 5%           | 87.6                     | 86.2                     |

Averages for each plant extract

| Plant extract | % of mycelial growth inhibition |
|---------------|--------------------------------|
| *A. conyzoides* | 92.4 90.4                      |
| *C. odorata*    | 81.3 68.0                      |
| Tukey’s test    | 10.6 7.6                       |

Averages for each concentration

| Concentration | % of mycelial growth inhibition |
|---------------|--------------------------------|
| 1%            | 75.3 59.5                      |
| 3%            | 91.4 85.1                      |
| 5%            | 93.8 93.1                      |
| Tukey’s test  | 15.8 11.4                      |
A. conyzoides and C. odorata extracts tested on PDB medium at 1, 3, and 5% against L. pseudotheobromae and L. theobromae reduced the fungal biomass significantly at all concentrations (Table 3,4). C. odorata was found most effective in inhibiting fungal biomass of L. pseudotheobromae and L. theobromae either on wet weight (88.7, 94.0, 98.3) and (93.4, 93.5, 98.4) or on dry weight (62.9, 82.7, 92.2) and (86.7,88.3,95.7) at 1, 3, and 5% %, respectively.

The concentration of 5% of C. odorata was found significantly inhibitive over two other concentrations. The concentration 1% of A. conyzoides was the least effective in inhibiting fungal biomass of two species of Lasiodiplodia, both wet weight and dry weight.

Table 3. Effect of A. conyzoides and C. odorata on fungal biomass of Lasiodiplodia pseudotheobromae on PDB medium.

| Plant extracts | Concentration | % of fungal biomass inhibition |
|----------------|---------------|-------------------------------|
|                |               | Wet weight                    | Dry weight (72-hour after drying) |
|----------------|---------------|-------------------------------|----------------------------------|
| A. conyzoides  | 1%            | 75.1                          | 43.4                             |
|                | 3%            | 85.4                          | 64.1                             |
|                | 5%            | 93.6                          | 66.6                             |
| C. odorata     | 1%            | 88.7                          | 62.9                             |
|                | 3%            | 94.0                          | 82.7                             |
|                | 5%            | 98.3                          | 92.2                             |

Averages for each plant extracts

A. conyzoides 84.7 58.0
C. odorata 93.7 79.3
Tukey’s test 2.9 7.2

Averages for each concentration

1% 81.9 53.2
3% 89.7 73.4
5% 96.0 79.4
Tukey’s test 4.4 10.9

Analysis of Variance (p-value)

|                        | p-value |               |               |
|------------------------|---------|---------------|---------------|
| Plant Extracts         | **      |               |               |
| Concentration          | **      |               |               |
| Plant Extracts x Concentration | ns | ns           |
Table 4. Effect of *A. conyzoides* and *C. odorata* on fungal biomass of *Lasiodiplodia theobromae* on PDB medium.

| Plant extracts | Concentration | % of fungal biomass inhibition |
|----------------|---------------|--------------------------------|
|                | Wet weight    | weight (72-hour after drying)  |
| *A. conyzoides*| 1%            | 84.3                          | 66.0                          |
|                | 3%            | 88.9                          | 68.2                          |
|                | 5%            | 92.9                          | 79.2                          |
| *C. odorata*   | 1%            | 93.4                          | 86.7                          |
|                | 3%            | 93.5                          | 88.3                          |
|                | 5%            | 98.4                          | 95.7                          |

Averages for each Plant Extracts

| Plant Extracts | % of fungal biomass inhibition |
|----------------|-------------------------------|
| *A. conyzoides*| 88.7                          |
| *C. odorata*   | 95.1                          |
| Tukey’s test   | 1.7                           | 6.5                           |

Averages for each concentration

| Concentration | % of fungal biomass inhibition |
|---------------|-------------------------------|
| 1%            | 88.8                          |
| 3%            | 91.2                          |
| 5%            | 95.7                          |
| Tukey’s test  | 2.6                           | 9.8                           |

Analysis of Variance (*p*-value)

|                      | **    | **    |
|----------------------|-------|-------|
| Plant Extracts       |       |       |
| Concentration        |       | *     |
| Plant Extracts x Concentration | ns | ns |

3.2. Discussion

*L. pseudotheobromae* and *L. theobromae* are significant pathogens that causes diseases in many trees, including cocoa. A variety of the symptoms caused by the pathogens, such as dieback and [4–9]. The dieback disease on cocoa is considered a future threat in cocoa in Sulawesi. As an emerging disease, control method needs to be explored, including using local plant extracts which are easy to access by cocoa farmers. To evaluate the possibilities of plant extracts as an effective control were tested in vitro against *L. pseudotheobromae* and *L. theobromae*.

All plant extracts at 1, 3, and 5% concentrations were found statistically effective to inhibit mycelial growth and fungal biomass over control. Both *A. conyzoides* and *C. odorata* can suppress fungal growth in any concentration with different inhibition. Either *A. conyzoides* or *C. odorata* have been studied for their antifungal properties against different fungi [15–19].

This research is the first effort to evaluate *A. conyzoides* and *C. odorata* against *L. pseudotheobromae* and *L. theobromae* isolated from cocoa. The plant extracts can inhibit the mycelia growth and fungal biomass of the pathogens. However, extraction of chemical compounds of the extracts and testing on the plant would be better for supporting the in vitro result.

4. Conclusion

Inhibition of *L. pseudotheobromae* and *L. theobromae* through *in vitro* is influenced by plant extracts and concentrations. *A. conyzoides* and *C. odorata* are proved effective in reducing mycelial growth and fungal biomass.
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