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Research Article

Antifungal Effect of Leaf Extracts on the Groundnut Late Leaf Spot Pathogen *Cercosporidium personatum*[(Ж)]

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ABSTRACT

Late leaf spot of groundnuts is caused by the fungal pathogen *Cercosporidium personatum*. Efforts to prevent this disease generally use synthetic fungicides that can pollute the environment. This study aims to identify the fungal pathogen of groundnut late leaf spot and determine the antifungal effect of leaf extract from several plants on leaf spot disease suppression. Late leaf spot pathogen was identified based on hyphae and conidia morphological characteristics. Growth inhibition of leaf extracts from butterfly pea (*Clitoria ternatea*), Mexican sunflower (*Tithonia diversifolia*), basil (*Ocimum gratissimum*), and sugar apple (*Annona squamosa*) at a concentration of 25% were tested against *C. personatum in vitro*. The growth and intensity of groundnut disease attacks were assessed for seven weeks by inoculating *C. personatum* and applied with several leaf extracts. Results showed that the pathogen was identified as *C. personatum* with conidia characteristics of cylindrical to obclavate, short and slightly curved with four septa, and 35.75 × 7.11 µm in size. The inhibition percentage of *C. personatum in vitro* reached 94.03% by leaf extract of *C. ternatea*, 88.56% by *T. diversifolia*, 87.20% by *O. gratissimum*, and 84.10% by *A. squamosa*. Treatment of *C. ternatea* increased plant height by up to 12.85% and showed the highest reduction of infected leaves by up to 61.53%. *C. ternatea* leaf extract was found to be the most effective leaf extract to suppress *C. personatum* infection by 77.94%, while *T. diversifolia*, *O. gratissimum*, and *A. squamosa* reduced disease severity by 70.15%, 65.43%, and 57.76%, respectively.

Keywords: antifungal compound; fungicide; growth; inhibition; plant pathogen

INTRODUCTION

Late leaf spot due to the infection of *Cercosporidium personatum* (Berk. & M.A.Curtis) Deighton. is one of the main diseases of groundnut, *Arachis hypogaea* L. (Sumartini *et al.,* 2020). *Cercosporidium personatum* generally attacks groundnut leaves in the late growth phase which can decrease productivity (McDonald *et al.,* 1985; Sumartini *et al.,* 2020). In Indonesia, groundnut productivity is relatively low and has tended to decrease in the last five years. The Ministry of Agriculture, Republic of Indonesia (2018) reported that Indonesia's groundnut production in 2014 was 638,896

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tons, but production decreased to 512,198 tons in 2018. This decline is believed to be influenced by late leaf spot infestation.

Cercosporidium personatum can infect groundnut leaves resulting in dark brown to black spots accompanied by a circle of thin yellow surrounding them (Meswaet *et al.,* 2021). Therefore, fungicide application is important to suppress the emergence of late leaf spots in groundnuts, but excessive use can cause environmental pollution, health complications, pathogen resistance, and a decrease in agricultural product quality (Andreas *et al.,* 2018).

Plant-based bio fungicides can be used to reduce the negative impact of synthetic fungicides. Parts of plants, such as leaves, are commonly used as ingredients for making natural fungicides. The leaves of several types of plants, such as butterfly pea (*Clitoria ternatea* L.), Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray), basil (*Ocimum gratissimum* L.), and sugar apple (*Annona squamosa* L.). Apart from being easy to obtain and cheap, these plant species have been reported to have potential as bio fungicides against fungal pathogenic in plants. These four plant species have been reported to contain similar various antifungal compounds such as alkaloids, saponins, tannins, phenols, terpenoids, and flavonoids (Akinmoladun *et al.,* 2007; Awere *et al.,* 2021; Chakraborty *et al.,* 2017; Kalidindi *et al.,* 2015). Many studies testing these plant species' leaf extracts as bio fungicides against fungal pathogens have been done, such as *C. ternatea* against *Alternaria solani* on tomatoes (Suganda *et al.,* 2020), *T. diversifolia* against *Cercospora nicotianae* on tobacco plant (Apriyadi *et al.,* 2013), *O. gratissimum* against *Aspergillus niger*, *A. flavus*, and *Fusarium solani* on peanuts (Adjou *et al.,* 2013), and *A. squamosa* against *Fusarium oxysporum* on watermelons or bananas (Purwita *et al.,* 2013). However, the use of these four plant leaf species against *C. personatum* on groundnuts has not been reported before.

This study aims to determine *Clitoria ternatea*, *Tithonia diversifolia*, *Ocimum gratissimum*, and *Annona squamosa* leaf extracts' effectiveness as bio fungicides against *Cercosporidium personatum in vitro* and *in vivo* on *Arachis hypogaea*.

MATERIALS AND METHODS

The study was carried out from September 2022 to March 2023 at the Plant Systematics Laboratory and the Sawitsari Study Station, Faculty of Biology, Universitas Gadjah Mada. This study used a completely randomized design (CRD) method consisting of five treatments with five repetitions to test growth inhibition *in vitro* and six treatments with four repetitions to test the effectiveness of plant leaf extracts *in vivo*. The treatment consisted of fungal species inoculation (*Cercosporidium personatum*) with 1.2 × 10^6 mL^{-1} conidia density (C), distilled water (P0) used for the control treatment, and the application of various plant leaf extracts. The leaf extracts used were *Clitoria ternatea* (P1), *Tithonia diversifolia* (P2), *Ocimum gratissimum* (P3), and *Annona squamosa* (P4), at a concentration of 25%. This concentration was selected because concentrations below 25% exhibited low inhibitory effects against several pathogenic fungi (Dongmo *et al*., 2021; Novianti, 2019; Reviani, 2021; Suganda *et al*., 2020). The growth inhibition test was carried out on PDA media *in vitro* with a combination of treatments CP0, CP1, CP2, CP3, and CP4. The effectiveness of plant leaf extracts *in vivo* used Gajah variety groundnut seeds (A) aged two weeks after planting (WAP) with treatment combination of AP0, ACP0, ACP1, ACP2, ACP3, and ACP4. The growth diameter of *C. personatum*, disease severity index, the incidence of leaf spots on groundnut leaves, and the plant height of groundnut were observed once a week for seven weeks.

Collection and Identification of Phytopathogenic Fungi

Cercosporidium personatum was isolated from groundnut leaves that showed symptoms of late spotting disease. Isolation was done by cutting 0.5 cm × 0.5 cm leaves, sterilized with 1% sodium hypochlorite (NaOCl) solution for one minute, rinsed with sterile distilled water, and air dried. Each petri dish with 10 ml of Potato Dextrose Agar (PDA) was used, then five pieces of leaves were arranged separately and incubated for seven days at 27 °C (Nayogyani & Kasiamdari, 2022). *Cercosporidium personatum* mycelia and conidia were identified based on macroscopic and microscopic characteristics. Fungal identification was done using keys by Barnett and Hunter's (1998) and other several credible references.

Preparation of Potentially Antifungal Plant Leaf Extracts

Plant leaves of butterfly pea (*Clitoria. ternatea*), Mexican sunflower (*Tithonia diversifolia*), basil (*Ocimum gratissimum*), and sugar apple (*Annona squamosa*) were washed, cut into small pieces, and weighed as much as 0.4 kg. Leaf pieces were dried in the oven for 20 min at 80 °C (Chege & Kimaru, 2021) and grinded to obtain leaf simplicia. A total of 25 g of leaf simplicia was added to 75 mL of sterile distilled water to obtain leaf extracts at a concentration of 25%. Extract solutions were homogenized, left for 24 h, and filtered to obtain filtrate (Reviani, 2021).

Growth Inhibition Test of Phytopathogenic Fungi with Plant Leaf Extracts

The *in vitro* inhibition test was carried out by mixing each plant leaf extract at a concentration of 25% with sterilized PDA media in a petri dish with a plant extract: PDA media ratio of 1: 10, then the petri dish was shaken until homogeneous while sterile distilled water mixed with PDA without leaf extract was used for the control (Andriyani & Purwanti, 2019). *Cercosporidium personatum* hyphae were cut into 5 mm diameter discs and placed in

the center of a petri dish containing treatment media. Incubation was carried out for seven days at a temperature of 27 °C and 80% humidity. The growth diameter of *C. personatum* colonies was measured four times at 1, 3, 5, and 7 Days After Inoculation (DAI). The percentage of growth inhibition of *C. personatum* colonies was calculated using the Achmad and Suryana (2009) formula as follows:

$$PI(\%) = \frac{D1 - D2}{D1} \times 100 \quad (1)$$

Note: *PI* = Inhibition percentage of pathogenic fungal growth; *D1* = Diameter of pathogenic fungal colonies without leaf extract treatment/control (mm); and *D2* = Diameter of pathogenic fungal colonies with leaf extract treatment (mm).

Effectiveness Test of Plant Leaf Extracts Against Fungal Infection on Groundnuts

One groundnut plant of Gajah variety was planted in 25 × 25 cm polybag with a planting medium consisting of a mixture of husk charcoal, manure, cocopeat, bamboo leaf humus, and fertile soil for each treatment. Plant arrangement used a completely randomized design (CRD) consisting of six treatments and four repetitions with distances of approximately 50 cm between each plant. Groundnut plants were watered and weeded while fertilizer was not used because nutrients were sufficiently provided by planting medium. Watering was done once a day in the morning until it reached 75% of planting media field capacity (Evita, 2012).

Cercosporidium personatum conidial suspension inoculation was carried out once on each 2 WAP groundnut seedling. The conidial suspension was prepared by suspending pure isolates of 7 days *C. personatum* from each petri dish in 100 mL sterile distilled water at a density of 1.2×10^6 mL^{-1}. As much as 5 mL of *C. personatum* conidial suspension was sprayed evenly on top and bottom of groundnut leaves, then

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covered for 24 h in transparent plastic to induce incubation. Groundnut seedlings that have been inoculated with pathogenic fungi were left for seven days until initial symptoms of *C. personatum* infection appeared. The first treatment of each leaf extract spray was carried out one week after the inoculation of *C. personatum*. As much as 5 mL of plant leaf extracts were sprayed once every five days for seven weeks of observation on the top and bottom of the groundnut leaves.

To determine the *Cercosporidium personatum* severity level, late leaf symptoms on groundnuts were evaluated using the modified 9-point scoring method developed by Subrahmanyam *et al*. (1995) and modified by Inayati and Yusnawan (2016). Scoring system includes the description of disease symptoms, disease score, and the percentage of leaf area damaged by the disease on groundnuts. The scores used in this study to represent the percentage of damaged leaf area were as follows: 0% = 1; 1−5% = 2; 6−10% = 3; 11−20% = 4; 21−30% = 5; 31−40% = 6; 41−60% = 7; 61−80% = 8; and 81−100% = 9. The percentage of *C. personatum* disease severity index was calculated using the formula by Putri *et al.* (2016) as follows:

$$DSI\ (\%) = \frac{\sum (n \times v)}{(Z \times N)} \times 100 \qquad (2)$$

Note: *DSI* = Disease severity index of the leaf spot (%); *n* = Number of infected leaves having the same score; *v* = score for each category of leaf spot; *Z* = highest score assigned; and *N* = A total number of leaves observed.

The percentage of disease suppression was carried out to determine the ability of each plant leaf extract to suppress *Cercosporidium personatum* leaf spot disease which was calculated using the formula by Yulia *et al*., (2008) as follows:

$$P(\%) = \frac{I_c - I_T}{I_c} \times 100 \qquad (3)$$

Note: *P* = Suppression of disease attacks (%); I_C = Average disease severity on control (%); and I_T = Average disease severity in each plant leaf extract treatment (%).

To determine leaf spot disease incidence on groundnut leaves, the number of infected and total number of groundnut leaves was counted for each leaf extract treatment. The disease incidence value on groundnut leaves was calculated by dividing the number of infected leaves by the total number of leaves. Each leaf extract treatment's efficacy was measured by the percentage reduction of infected leaves.

Furthermore, the average plant height of groundnut was measured weekly over seven weeks to evaluate the potential impact of leaf extract on the plant's growth.

Data were analyzed using SPSS Statistics 25 with Analysis of Variance (One Way ANOVA) at $\alpha = 5\%$. If a significant difference occurred between treatments, the analysis was continued using the Duncan Multiple Range Test (DMRT) at $\alpha = 5\%$.

RESULTS AND DISCUSSION

Identification of Phytopathogenic Fungi

The results of isolating the leaves of groundnut plants infected with spots (Figures 1A and 1B) and the growth of the C2 isolate colony aged seven days (Figures 1C and 1D) were obtained. According to Bakhshi and Zare (2020) and Monguillot *et al.* (2023), the morphology colony of C2 isolate showed similar characteristics to *Cercosporidium personatum*, which had compact white to orange colonies, irregular to circular shapes, convex elevations with papillate colony surfaces, and smooth-textured mycelium (velvety).

There were similarities in the microscopic characteristics of the C2 isolate with *Cercosporidium personatum* according to McDonald *et al.* (1985) and Meswaet *et al.* (2021), specifically septate hyphae, branched and pale brown in color (Figure 2A). Conidia are cylindrical to obclavate shape, olivaceous in color, short, and slightly curved. The tip of the conidia was tapered (truncated), while the bases of the conidia were rounded. The conidia of the C2 isolate were 35.75 × 7.11 µm in size and had 4 septa (Figure 2B). Conidiophores were olivaceous in color, arranged in dense fascicles to form clumps, and unbranched (Figure 2C). These characters showed similarities to *C. personatum*, which had conidial size ranging from 20 – 70 × 5 – 9 µm and had 2 – 6 septa (McDonald *et al.,* 1985; Meswaet *et al.,* 2021). The morphological characteristics are important for distinguishing fungal species and species identification (Nayogyani & Kasiamdari, 2022).

Growth Inhibition Test of Phytopathogenic Fungi with Plant Leaf Extracts

Growth inhibition of *Cercosporidium personatum* with *Clitoria ternatea*, *Tithonia diversifolia*, *Ocimum gratissimum*, and *Annona squamosa* leaf extracts at a concentration of 25% on the seventh day are presented in Figure 3. It showed that each plant leaf

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extract treatment was able to inhibit the *C. personatum* growth, indicated by smaller diameter of colony growth compared to control treatment. It is widely accepted that the use of leaf extract as a growth medium *in vitro* can impede fungal colonies growth due to the depletion of media nutrients and the presence of foreign substances such as antifungal compounds within each leaf extract. These antifungal compounds were believed to disrupt the metabolic system of fungi and compromise the integrity of their cellular partitions, resulting in a reduced growth rate of fungal colonies.

Clitoria ternatea leaf extract had the most significant inhibitory effect on the growth of *Cercosporidium personatum* (Table 1). The highest to lowest *C. personatum* inhibition at 7 Days After Inoculation (DAI) were shown by *C. ternatea* (94.03%), *Tithonia diversifolia* (88.56%), *Ocimum gratissimum* (87.20%), and *Annona squamosa* (84.10%). *Clitoria ternatea* is believed to possess antifungal properties that inhibit *C. personatum* growth compared to the other three plant leaf extract treatments. *C. ternatea* contains various secondary metabolites, including alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins, terpenoids, and quinones, (Chakraborty *et al.,* 2017). Although the type and quantity of phytochemical content were not analyzed in this study, it is likely that the presence of antifungal compounds plays a significant role in the inhibitory power produced.

All leaf extract treatments showed significantly different, had stable values, and produced relatively high percentage of inhibition. Suganda *et al.* (2020) reported that *Clitoria ternatea* leaf extract at a concentration of 9% was able to inhibit *Alternaria solani* growth *in vitro* by 34.78%. Dongmo *et al.* (2021) revealed that *Tithonia diversifolia* leaf extract at a concentration of 10% inhibited *Bipolaris oryzae* and *Fusarium moniliforme* growth by 68.44% and 70.69%. Reviani (2021) revealed that *Ocimum gratissimum* leaf extract inhibited *Cercospora* sp. growth by 88.36% at concentration of 10%. *A. squamosa* leaf extract at 10% concentration inhibited *F. oxysporum* growth by 11.7% (Novianti, 2019). Several studies indicated that the four plant species used in this study could produce higher inhibition against *C. personatum*. Due to the lack of studies related to the inhibition of the four plant species used in this study against *C. personatum*, this study provides new information regarding their potential to inhibit the growth of *C. personatum in vitro*.

Cercosporidium personatum growth inhibitory in this study was possible due to the effect of antifungal compounds contained in each plant's leaves. The four types of plant leaves species werereported to contain the same phytochemical compounds, specifically alkaloids, saponins, tannins, phenols, terpenoids, and flavonoids (Akinmoladun *et al.,* 2007; Kalidindi *et al.,* 2015; Chakraborty *et al.,* 2017; Awere *et al.,* 2021). These phytochemical compounds were reported to disrupt peptidoglycan wall constituent components, inhibiting nucleic acid synthesis (Antonius *et al.,* 2017), causing leakage of intracellular material, interfering with nutrient absorption and metabolic processes in fungi (Septiadi *et al.,* 2013), and inhibit the synthesis of chitin during cell walls formation (Watson & Preedy, 2007).

Effectiveness Test of Plant Leaf Extracts Against Fungal Infection on *Arachis hypogaea*

The results showed that groundnut plants began to be infected with *Cercosporidium personatum* leaf spot disease at the age of 4 weeks after planting (WAP) or 2 weeks after inoculation (WAI). These results were similar to Saleh (2022) who revealed that *C. personatum* can attack groundnuts at the age of 3–5 WAP. The observed symptoms of *C. personatum* leaf spot disease was also reported by Sumartini (2008), Meswaet *et al.* (2021), and McDonald *et al.* (1985), who stated that there were spots with irregular shapes to circles, dark brown to black in color, and there was a thin yellow halo that encircling the spot. The spots that form on the adaxial side of the leaf were dark brown in color, while the spots on the abaxial side were black because it was thought to be the site of sporulation.

Disease severity in each treatment showed significantly different values every week until seven WAI (Figure 4). It was found that the four plant leaf species suppressed *Cercosporidium personatum* disease severity at 3 WAI and continued until the end of the observation. On the seventh week, the control treatment (C.p.) had the highest disease severity value of 26.25%. Disease severity of *C. personatum* treated by leaf extracts of *Clitoria ternatea*, *Tithonia diversifolia*, *Ocimum gratissimum*, and *Annona squamosa* had lower values of 5.79%, 7.82%, 9.07%, and 11.10% respectively at 7 WAI than the control treatment. Leaf extracts used in this study had a better inhibitory effects against *C. personatum* leaf spot disease on groundnuts because they showed lower disease severity value than the previous study. Apriyadi *et al.* (2013) reported that *T. diversifolia* leaf extract at a concentration of 75 g/L was able to control the development of *Cercospora nicotianae* in tobacco plants with a disease severity index of 9.62% at 65

Days After Planting (DAP). To this date, no data is available regarding the potency of *C. ternatea*, *O. gratissimum*, and *A. squamosa* leaf extracts against *C. personatum*.

Cercosporidium personatum leaf spot suppression by each treatment showed that leaf extract treatments resulted in significantly different values at 3 WAI until 7 WAI (Table 2). At the end of the observation, *Clitoria ternatea* leaf extract showed the highest disease severity suppression at 77.94%, followed by *Tithonia diversifolia* (70.15%), *Ocimum gratissimum* (65.43%), and *Annona squamosa* (57.76%). Based on previous research, the inhibition value of leaf extract against *C. personatum in vivo* was different from the results of inhibition *in vitro*. This difference can occur and is thought to be influenced by environmental factors such as temperature and humidity in the *in vivo* test. The value of disease inhibition will appear smaller when leaf spot disease can infect its host more quickly with the support of temperature and humidity. According to Sumartini *et al.* (2020), the emergence of late leaf spot disease in Indonesia is strongly supported by the tropical climate factor, which tends to be humid with a minimum, optimal, and maximum temperature range of 10 ^{o}C, 25 ^{o}C, and 31 ^{o}C. McDonald *et al.* (1985) also revealed that air temperatures ranging between 25 – 30 ^{o}C with high humidity support leaf spot disease emergence in groundnuts. Furthermore, groundnut leaf resistance against *C. personatum* infection plays a crucial role in determining the disease inhibition value. According to Sunkad & Kulkarni (2006), the resistance levels of groundnuts against leaf spot disease can be determined based on their morphological characteristics, including having a high number and large size of stomata, low number of epidermal cells, thick cuticle tissue and epidermis layers, and thicker wax layer on reproductive phase. This study focused on the Gajah variety of groundnuts, which is known to be vulnerable to *C. personatum* leaf spot. Consequently, the leaf extract inhibition value *in vivo* was relatively lower than the *in vitro* test.

Results indicate leaf extracts had the potential as an antifungal to suppress *Cercosporidium personatum* leaf spot disease on groundnuts. The high disease suppression by *Clitoria ternatea* leaf extract was possible due to the effect of antifungal compounds contained in the leaves, such as alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins, terpenoids, and quinones (Chakraborty *et al.,* 2017). Different disease suppression values in each treatment can also be caused by the presence of specific compounds such as essential oils. It was reported that *C. ternatea* leaves contain several kaempferol compounds, such as kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-neohesperidoside, kaempferol-3-O- rhamnosyl-glucoside, and kaempferol-3-O-rhamnosyl-galactoside (Mukherjee *et al.,* 2008). The role of kaempferol had been tested and previously reported to have a significant antifungal activity to inhibit some fungal growth (Galeotti *et al.,* 2008). It is imperative to conduct further research studies to establish whether the kaempferol compound exhibits a similar inhibitory effect on the growth of *C. personatum* in *C. ternatea* leaves. This critical inquiry will aid in establishing the role of kaempferol in the plant's antifungal properties, providing crucial insights into the development of more effective fungicides. However, it is strongly suspected that the presence of antifungal phytochemicals in *C. personatum* influences the disease-suppressing ability. Based on several references, it was previously found that the leaf extracts contained some phytochemical compounds (Akinmoladun *et al.,* 2007; Awere *et al.,* 2021; Chakraborty *et al.,* 2017; Kalidindi *et al.,* 2015), especially terpenoids. Terpenoids were one of the secondary metabolites that were reported to inhibit the growth of fungi by interfering with the growth, development, and formation of fungal spores (Lutfiyanti *et al.,* 2012).

The application of plant leaf extracts at a concentration of 25% can reduce the infected leaves (Table 3). The leaves of groundnut plants began to be infected with *Cercosporidium personatum* spot disease at 2 WAI until 7 WAI. Plant leaf extract treatments reduced the percentage of infected leaves from 3 WAI. At the end of the observation, percentage of infected leaves with the highest value was shown in the control treatment (*C. personatum*) of 18.30%. Percentage of infected leaves with the lowest to the highest value at the end of the observation sequentially was *Clitoria ternatea* leaf extract (7.04%), *Tithonia diversifolia* (7.71%), *Ocimum gratissimum* (8.54%), and *Annona squamosa* (10.46%) at 7 WAI. From these results, *C. ternatea* leaf extract produced the lowest percentage of infected leaves compared to other treatments possibly because of antifungal compounds (Chakraborty *et al.,* 2017), which was more effective in inhibiting fungal infection.

The four plant leaf extracts with a concentration of 25% were effective in reducing the number of leaves infected with *Cercosporidium personatum* leaf spot based on the decrease in the percentage of infected leaves (DP). At the 7 WAI, *Clitoria ternatea* leaf extract could reduce the highest percentage of infected leaves by 61.53%, followed by *Tithonia diversifolia* (57.84%), *Ocimum gratissimum* (53.34%), and *Annona squamosa* (42.82%). To this date, there are no studies before that reveal the percentage value of leaves infected with disease produced by four plant leaf extracts against *C. personatum*, this study can provide information about the potential of four plant leaf extracts to reduce percentage of infected leaves in groundnut plants.

Based on Figure 5, the plant height from each treatment showed significantly different values every week, except at 3 and 4 WAI. It appeared that each plant leaf extract treatment resulted in a higher average plant height compared to the negative control. That was appropriate with Korwa *et al.* (2009), where untreated groundnut plants affected by *Cercospora* sp. showed lower plant height compared to the results obtained in this study. Groundnut plant height produced by leaf extract treatments at 7 WAI ranged between 56.25–62.33 cm, whereas Korwa *et al.* (2009) only resulted in heights ranging between 38–48.8 cm at the age of 10 WAP. The application of leaf extract in this study produced higher height growth compared to control plants. The height of the groundnut plant can also be affected by several other factors, such as plant variety, type of planting media, availability of nutrients/nutrients, growth regulators, light intensity, competition with weeds, and spacing (Kasno *et al.,* 2015).

The leaf extracts treatment resulted in plant heights that were not significantly different from each other at the end of this study. The average plant height produced by each extract treatment in the seventh week with the largest to smallest values sequentially was *Clitoria ternatea* (62.33 cm), *Tithonia diversifolia* (61.98 cm), *Ocimum gratissimum* (61.00 cm), and *Annona squamosa* (56.25 cm). *Clitoria ternatea* leaf extract was found to increase the plant height by 12.85%, *T. diversifolia* by 12.22%, *O. gratissimum* by 10.45%, and *A. squamosa* by 1.85%. In addition to their potential to inhibit the attack of the fungal disease of *Cercosporidium personatum*, leaf extracts besides being able to be used as natural fungicides on groundnut plants were also safe on cultivated crops because they do not have negative impact on plant growth. This result is in accordance with Sudarmo (2005) where plant-based fungicides can inhibit disease development and was also safe for humans, the environment, and agricultural plants because they can easily decompose and leave no residue on agricultural products.

CONCLUSIONS

Results showed that treatments of four leaf extracts had the potential as natural fungicides against leaf spot disease caused by *Cercosporidium. personatum* on groundnuts. Leaf extract of *Clitoria ternatea* showed the highest growth inhibition of *C.*

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personatum in vitro by 94.03%. Leaf extract of *C. ternatea* was found to be the most effective leaf extract to suppress leaf spot disease on groundnut plants by 77.94%, followed by *Tithonia diversifolia*, *Ocimum gratissimum*, and *Annona squamosa* by 70.15%, 65.43%, and 57.76%, respectively. Leaf extract of *C. ternatea* treatment produced the lowest disease severity value of 5.79% while also increased plant height up to 12.85%, and reduced the most optimal percentage of infected leaves up to 61.53%.

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APPENDIX

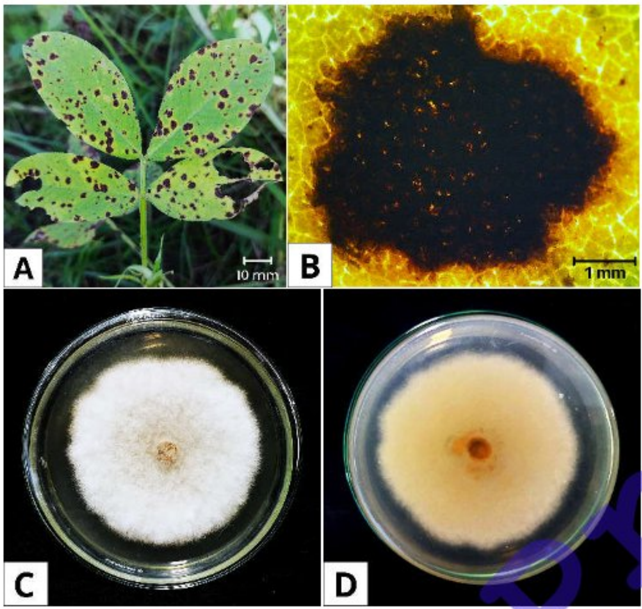

Figure 1. Symptom of leaf spot and pure culture of *Cercosporidium personatum:* leaf spots on groundnut leaves (A); leaf spot under a stereo microscope at 4× magnification (B); the colony of C2 isolates aged seven days on PDA media, front view (C) and back view (D)

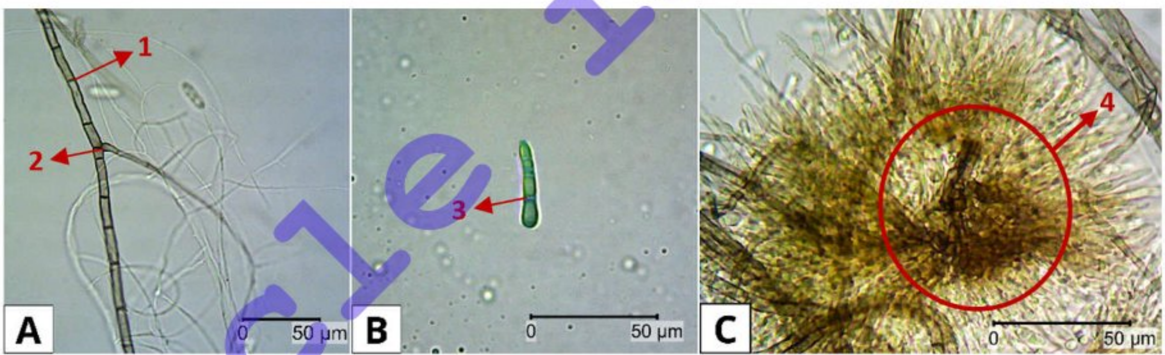

Figure 2. Microscopic morphology structure of *Cercosporidium personatum:* hyphae (A); conidia (B); and conidiophores (C); 1= hyphae septum, 2= hyphae branching, 3= conidia septum, and 4=conidiophores

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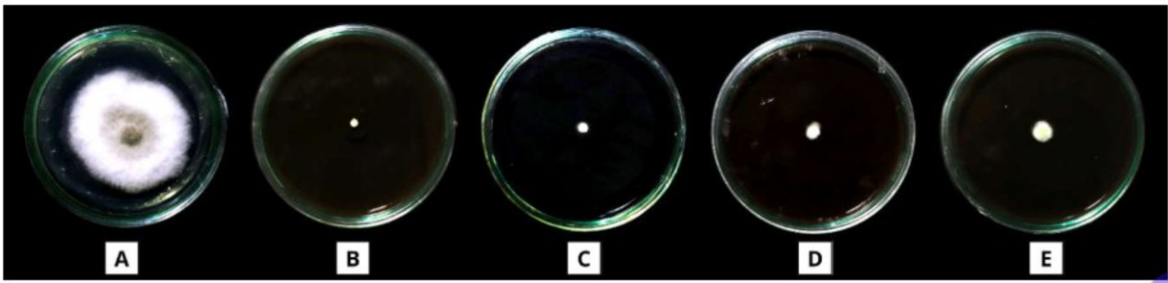

Figure 3. Growth of *Cercosporidium personatum* colonies *in vitro* on the 7th day after incubation on PDA media treated by leaf extract at a concentration of 25%, control (no leaf extract) (A); *Clitoria ternatea* (B); *Tithonia diversifolia* (C); *Ocimum gratissimum* (D); and *Annona squamosa* (E)

Table 1. Effect of leaf extracts of several plants at a concentration of 25% in PDA media on growth inhibition of *Cercosporidium personatum in vitro* at 7 days after inoculation

Leaf extract (25%) of	**Growth Inhibition (%)**						
	1 DAI	**2 DAI**	**3 DAI**	**4 DAI**	**5 DAI**	**6 DAI**	**7 DAI**
C. ternatea	94.17 ± 0.97[d]	92.56 ± 3.03[c]	94.70 ± 1.69[d]	94.55 ± 1.62[c]	93.03 ± 1.89[c]	93.32 ± 1.24[c]	94.03 ± 0.96[c]
T. diversifolia	90.83 ± 1.35[c]	87.50 ± 1.49[b]	88.13 ± 1.94[c]	88.12 ± 1.78[b]	87.71 ± 1.63[b]	87.99 ± 1.08[b]	88.56 ± 1.00[b]
O. gratissimum	85.11 ± 1.78[b]	85.48 ± 1.75[b]	84.79 ± 1.38[b]	86.20 ± 1.49[b]	87.04 ± 0.99[b]	86.91 ± 1.17[b]	87.20 ± 1.16[b]
A. squamosa	81.20 ± 1.63[a]	78.73 ± 1.26[a]	79.48 ± 2.50[a]	81.81 ± 1.92[a]	83.54 ± 1.60[a]	84.26 ± 1.73[a]	84.10 ± 1.31[a]

Note: DAI = Days After Inoculation. Numbers followed by the same letter in each column are not significantly different based on Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

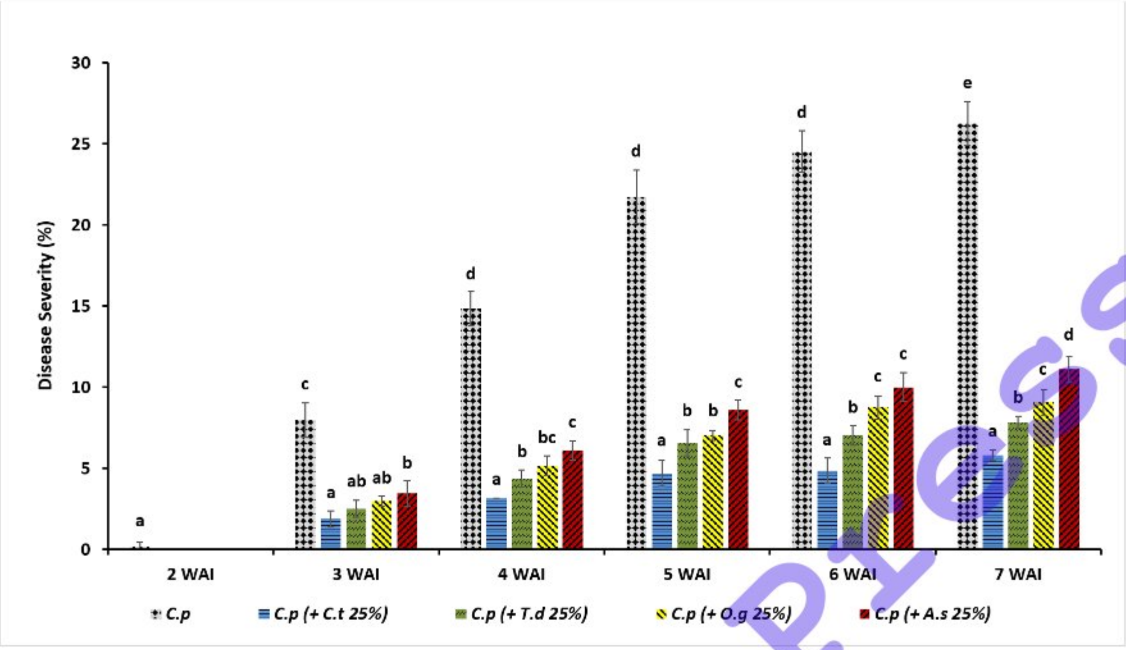

Figure 4. Disease severity index of cescospora leaf spot on groundnuts at seven weeks after inoculation (WAI) of *Cercosporidium personatum* (*C.p*) and treated by leaf extract (25%) of *Clitoria ternatea* (*C.t.*), *Tithonia diversifolia* (*T.d.*), *Ocimum gratissimum* (*O.g*), and *Annona squamosa* (*A.s.*). Note: Bar charts followed by the same letters indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

Table 2. Percentage of *Cercosporidium personatum* leaf spot disease suppression on groundnuts after plant leaf extract treatments at a concentration of 25% during seven weeks after inoculation (WAI)

Leaf extract (25%) of	**Disease Suppression (%)**				
	3 WAI	**4 WAI**	**5 WAI**	**6 WAI**	**7 WAI**
C. ternatea	75.75 ± 8.41^{b}	78.87 ± 1.49^{c}	78.36 ± 3.98^{c}	80.29 ± 2.86^{c}	77.94 ± 1.58^{d}
T. diversifolia	68.57 ± 5.57^{ab}	70.29 ± 4.98^{b}	69.73 ± 3.70^{b}	71.24 ± 3.36^{b}	70.15 ± 2.50^{c}
O. gratissimum	61.94 ± 8.11^{ab}	65.13 ± 4.80^{ab}	67.54 ± 1.99^{b}	64.25 ± 3.56^{a}	65.43 ± 3.31^{b}
A. squamosa	56.01 ± 12.05^{a}	58.60 ± 6.82^{a}	60.32 ± 3.15^{a}	59.14 ± 4.27^{a}	57.76 ± 1.65^{a}

Note: WAI = Weeks After Inoculation. Numbers followed by the sameletter in each column are not significantly different based on Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

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Table 3. The incidence of leaf spot disease on groundnut leaves artificially inoculated with *Cercosporidium personatum* and treated with leaf extracts at a concentration of 25% at seven weeks after inoculation (WAI)

Leaf extract (25%) of	**Disease Incidence (%)**											
	2 WAI	**DP (%)**	**3 WAI**	**DP (%)**	**4 WAI**	**DP (%)**	**5 WAI**	**DP (%)**	**6 WAI**	**DP (%)**	**7 WAI**	**DP (%)**
No treatment	0.84	0.00	20.83	0.00	18.22	0.00	18.31	0.00	18.80	0.00	18.30	0.00
C. ternatea	0.00	100.00	6.70	67.82	6.86	62.34	7.76	57.64	6.89	63.37	7.04	61.53
T. diversifolia	0.00	100.00	10.19	51.08	9.87	45.84	8.54	53.35	8.16	56.62	7.71	57.84
O. gratissimum	0.00	100.00	10.61	49.05	9.81	46.14	9.32	49.13	8.97	52.29	8.54	53.34
A. squamosa	0.00	100.00	12.72	38.96	10.41	42.86	10.27	43.91	10.10	46.27	10.46	42.82

Note: WAI = Weeks After Inoculation, DP = Decreasing Percentage of Infected Leaves.

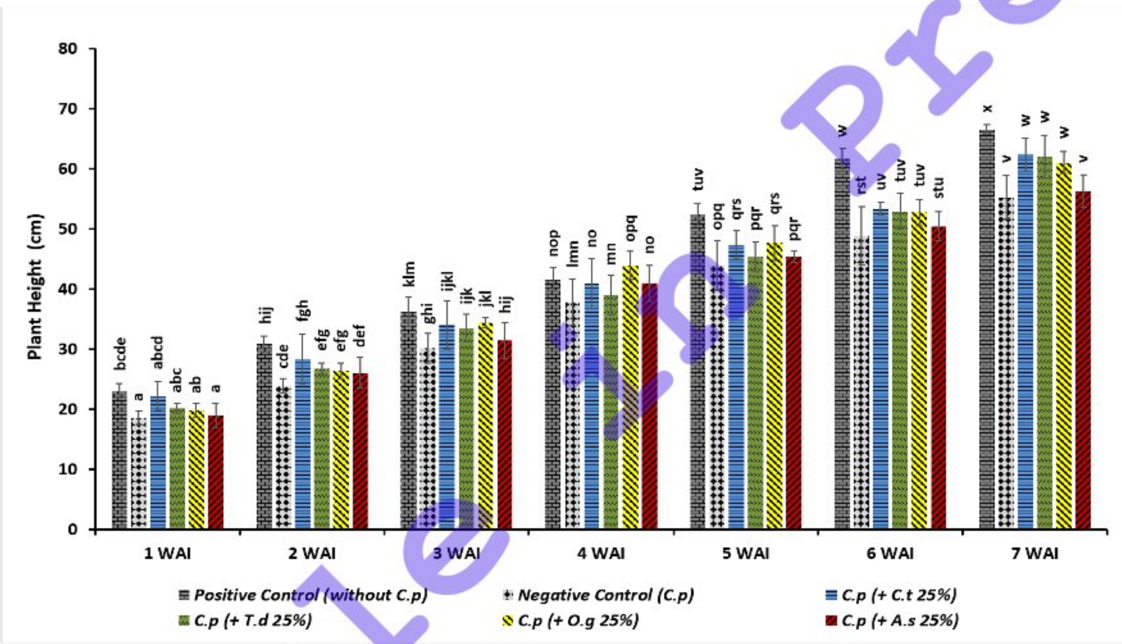

Figure 5. Height of groundnut at seven weeks after inoculation (WAI) of *Cercosporidium personatum* (*C.p*) and treated by leaf extract (25%) of *Clitoria ternatea* (*C.t.*), *Tithonia diversifolia* (*T.d.*), *Ocimum gratissimum* (*O.g*), and *Annona squamosa* (*A.s.*). Note: Bar charts followed by the same letters indicate no significant difference based on Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

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