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Identification of *Curvularia eragrostidis* (Henn.) J.A.Mey. The Leaf Spot Pathogen of Oil Palm (*Elaeis guineensis* Jacq.) and Its Control by False Elder (*Peronema canescens* Jack) Leaf Extract

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ABSTRACT

Oil palm is the main vegetable oil-producing crop in Indonesia. Leaf spot disease is one of the major diseases that attacks oil palm seedlings at all seedling stages. In Indonesia, leaf spot disease in oil palm nurseries is most commonly caused by the genus *Curvularia* with an infection intensity of up to 60–70%. The control of leaf spot disease usually uses chemical fungicides but its continuous use can cause the development of resistant pathogen fungi and have a long-term negative impact on the environment. False elder (*Peronema canescens* Jack*)* leaves have bioactivity as an antimicrobial control disease caused by fungal infections. In this study, *Curvularia* was isolated from oil palm seedlings infected with leaf spot disease from oil palm nurseries in South Sumatra. Effectiveness testing of *P. canescens* leaves conducted by a Completely Randomized Design method with five treatments and three replications. The fungi that caused leaf spot disease in oil palm were identified as *Curvularia eragrostidis*. *P. canescens* leaf water extract at 25% concentration was very ineffective to quite effective in controlling disease severity based on the average number of spots and diameter of spots in oil palm with the values 36.25–59.50% and 12.50–27.78%. *P. canescens* leaf water extract could reduce the average number of spots and diameter of spots started on day 30 after being sprayed three times with *P. canescens* leaf water extract so that it could be used as an alternative to control *C. eragrostidis* leaf spot disease in oil palm that is more friendly to the environment.

Keywords: *Curvularia eragrostidis,* disease, false elder, leaf spot, oil palm

INTRODUCTION

Oil palm is a leading crop producing the main vegetable oil in Indonesia and becoming the largest source of foreign exchange for the export of the agricultural sector. Total Indonesian crude palm oil exports in 2019 up to 36.17 million tons. Oil palm becomes a raw material for a variety of food products, oleochemicals, pharmaceuticals and health products, household products, and industrial products even about 16% of the world's oil palm is used as biodiesel (Goh *et al.* 2017). Therefore,

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keeping the quality and productivity of oil palm crops to grow to the maximum and profitable becomes important to pay attention. One problem that needs to be solved is overcoming infectious diseases that can decrease the quality of oil palm crops.

Leaf spot disease is one of the major diseases in oil palm cultivation. This disease infects oil palm seedlings at all seedling stages, both pre-nursery and main nursery (Priwiratama and Widiyatmoko 2022). The genus *Curvularia* is the genus most often found as the cause of leaf spot disease in oil palm nurseries in Indonesia (Priwiratama 2017). Leaf spots caused by *Curvularia* in oil palm nurseries have the highest severity of disease (Pornsuriya *et al.* 2013). If not properly controlled, the damage caused by *Curvularia* leaf spot on oil palm seedlings can reach 60–70%. Several pathogenic fungi that have been reported to cause oil palm leaf spots are *Curvularia lunata, C. eragrostidis, C. oryzae,* and *Cochliobolus carbonum* (Corley dan Tinker 2015; Susanto & Prasetyo 2013). Although *Curvularia* has been known to be a pathogen that causes leaf spot disease, but the species of *Curvularia* that infects oil palm leaves has not been clearly identified.

Today, chemical fungicides are still often used as the primary option in disease control, but continuous use of chemical fungicides can cause the development of resistant pathogen fungi and have a long-term negative impact on the environment such as contamination of cultivated land due to chemical residues (Zubrod *et al*. 2019). One way of controlling diseases that are friendly to the environment and can effectively control diseases is to use a natural fungicide derived from a plant, one of which is *P. canescens* leaf water extract. Secondary metabolite content in *P. canescens* leaf water extract could be one of the better alternatives because it is known to have minimal impacts on the environmental and humans compared to chemical fungicides. *P. canescens* leaves contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids (Emilia *et al.* 2023). In previous research conducted by Zarafi and Moumoudou (2010), the application of *Azadirachta indica* leaf extract which contained the same secondary metabolites as *P. canescens* leaves at 20% concentration could control *C. eragrostidis* infection by reducing the severity of the disease from initial 0.78% to 0.21%. The antifungal activity of *P. canescens* leaf water extract against *Curvularia* has never been studied and data related to *P. canescens* leaves an antifungal was still limited, so this research was important to carry out.

The aimed of this study was to identify the species of *Curvularia* that caused leaf spot disease in oil palms, and to analyze the effect of *P. canescens* leaf water extract on *Curvularia* infection in oil palms.

MATERIALS AND METHODS

Isolation of *Curvularia*

Samples of pathogenic fungi were taken from leaves of Costa Rica and D x P Bah Lias varieties oil palm seedlings infected with leaf spot disease in the oil palm nursery in Marga Baru Village, Muara Lakitan, Musi Rawas, South Sumatra, Indonesia. Isolation procedures adapted from Agustina *et al*. (2019). The infected leaves were cut in sizes of 0.5 x 0.5 cm^2. The cut leaf sections were disinfected with a chlorine solution (0.5%) for 2 minutes, then rinsed with sterile distilled water for 5 minutes 3 times, and then dried on sterile tissue paper. The leaf sections were then placed in PDA media in a petri dish and incubated for 7 days at room temperature. Colonies of *Curvularia* are characterized by brown, gray, or black of subcultures to identify the species (Sivanesan 1987).

Identification of *Curvularia*

Identification of morphological characteristics (Sivanesan 1987) was obtained by making semi-permanent slides. The slides were prepared with the surface of an object glass dripped glycerol, then placed a pure

culture of fungal mycelial using an ose needle, then applied trypan blue or taken fungal mycelial used tape and then placed on the surface of the object glass. The slides were covered with a cover glass and observed under a microscope with 400x magnification.

Preparation of *Peronema canescens* Leaf Water Extract

Preparation of *the* extract was made using the maceration method (Sari and Listiani 2022). *Peronema canescens* leaves were selected from mature leaves, and third to fifth leaves from shoots that showed no symptoms of disease. Two kg of *P. canescens* leaves were washed and then dried at 50 °C in the oven for 24 hours, then mashed with a blender. The leaf powder was then filtered using a 60-mesh filter. *P. canescens* leaf water extract with a concentration of 25% was made by weighing 25 g of leaf powder and then extracting it by macerating it with 100 mL of sterile distilled water. Maceration was conducted for 3 days. The macerated *P. canescens* leaves were then filtered to obtain the filtrate.

Effectiveness of *Peronema canescens* Leaf Water Extract against Leaf Spot Disease in Oil Palm

The effectiveness of *P. canescens* leaf water extract was adapted from Agustina *et al.* (2019) and Qaisar *et al*. (2023). The experiment was conducted by Completely Randomized Design method, with 5 treatments and 3 replications of each treatment. The treatments were negative control (Control), positive control 1 (infected with *C. eragrostidis* 1 with application of sterile distilled water) (C1S0), positive control 2 (infected with *C. eragrostidis* 2 with application of sterile distilled water) (C2S0), infection of *C. eragrostidis* 1 with application of *P. canescens* leaf water extract 25% concentration (C1S25), and infection of *C. eragrostidis* 2 with application of *P. canescens* leaf water extract 25% concentration (C2S25). The oil palm seedlings used five-month-old varieties of D x P Bah Lias.

Inoculation of *C. eragrostidis* was done by 1 x 1 cm^2 mycelial plug attached to the sterile abaxial side of oil palm leaves which had previously been wounded. The inoculation point was wrapped with sterile gauze then covered with plastic wrap and then covered with a transparent plastic bag for 48 hours. After 48 hours, the plastic bag and gauze were removed, and then the oil palm seedlings were kept in the greenhouse and observed every day for 7 days until symptoms of leaf spot appeared. Spraying was done after 14 days symptoms appeared and repeated every 7 days for 7 weeks in the afternoon. Observations were done every day by measuring the number of spots and the diameter of the spots on the leaves of oil palm seedlings in each treatment.

Data Analysis

Disease severity was calculated by assessing the percentage of the disease severity index. The disease severity index was measured using a modified score table from Kittimorakul *et al*. (2019), as seen in Table 1.

Table 1 Disease severity score

Score	Disease Severity
0	No disease symptoms
1	Some pinpoint brown spots on the leaf without any rotten tissues
2	Spot Diameter of 1 – <3 mm
3	Spot Diameter of 3 – <4 mm
4	Spot Diameter of 4 – <5 mm
5	Spot Diameter of ≥ 5 mm

After assessing the disease severity score, the disease severity index (DSI) was calculated (Izzati and Abdullah 2008):

$$\text{DSI (\%)} = \frac{\Sigma(Ax0)+(Bx1)+(Cx2)\ldots..(Fx5)}{\Sigma A+B+C\ldots..F \times 5} \times 100\%$$

where A, B, C and F are the number of plants to be multiplied by the 0–5 score.

The effectiveness of *P. canescens* was calculated using a formula modified from Stevic *et al*. (2017):

$$\text{Effectiveness (\%)} = \frac{X-Y}{X} \times 100\%$$

where X was the average number or diameter of leaf spots in controls and Y was the average number or diameter of leaf spots in the treatment. Category of effectiveness of *P. canescens*:

Ineffective	= 0
Very Ineffective	= 0 – 20%
Less Effective	= 20 – 40%
Quite Effective	= 40 – 60%
Effective	= 60 – 80%
Very Effective	= >80%

Statistical analysis was carried out using an analysis of variance (ANOVA) at the 5% level and Duncans Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

Isolation and Identification of Leaf Spot Disease in Oil Palm

Isolation of leaf spots on D x P Bah Lias variety of oil palm seedling resulted in isolate C1 (Figure 1) and leaf spots on the Costa Rica variety of oil palm seedling resulted in isolate C2 (Figure 2). Symptoms of leaf spot disease caused by C1 which come from oil palm seedling leaves of the D x P Bah Lias variety and C2 which come from Costa Rica variety were round spots that were initially light brown and then changed to dark brown with the edges surrounded by a yellowish halo (Figure 1a and Figure 2a). This was aligned with Priwiratama *et al*. (2017) who stated that the symptoms of oil palm seedling leaves infected with *Curvularia* leaf spot disease begun with the appearance of brownish spots which over time become dark brown necrotic spots with yellowish edges. Generally, the center of the spot was dark. The spots could combine to cause the leaves to dry out when the severity of the disease is very high. Pandey *et al*. (2014) also stated that the symptoms of oil palm seedling leaves infected with *C. eragrostidis* leaf spot disease begun with the appearance of small dark spots, then over time these spots enlarge and developed into irregular black necrotic spots that form extended along the edge of the leaf or several spots form on the leaf, and could unite to form a large spot surrounded by a yellow halo. The spots could merge to cause the leaves to become dry when the severity of the disease was very high.

The results of isolation from leaf spot resulted in colonies of grayish black or black and the texture of the colonies was hairy or cottony. According to Sivanesan (1987), the colony of *Curvularia* when grown on PDA media has colonies of brown, gray, or black, and texture of the colonies was hairy, cottony, or velvety (Figure 1b–c and Figure 2b–c). The two isolates had similar morphological characteristics except for the shape and size of the conidia and the rate of colony growth (Figure 1g–h and 2g–h). When compared with the literature, the two isolates have morphological characteristics that were similar to *C. eragrostidis* but were not similar to *C. lunata, C. oryzae,* and *C. carbonum* (Table 2). According to Sivanesan (1987), *C. eragrostidis* has hyphae septate, branched, subhyaline to brown. Conidiophores septate, branched, simple, straight or curved, brown to light brown. Conidia ellipsoidal or barrel-shaped, 3-distoseptate, end cells paler, central cells brown to dark brown. Turner (1971) reported that *C. eragrostidis* was the causal agent of leaf spot disease in oil palm nurseries in Sumatra.

Effectiveness of *Peronema canescens* Leaf Water Extract in Reducing Leaf Spot Disease in Oil Palm

Based on Table 3, inoculation of *C. eragrostidis* 2 significantly showed higher disease severity than *C. eragrostidis* 1. After application of a 25% concentration of *P. canescens* leaf water extract, it could reduce the severity of disease caused by *C. eragrostidis* 1 and *C. eragrostidis* 2. On day 10, the severity of the disease was not reduced significantly and on days 30 and 50, the severity of the disease started to reduce but not significantly. The effectiveness of *P. canescens* leaf water extract against *C. eragrostidis* 1 and 2 on disease

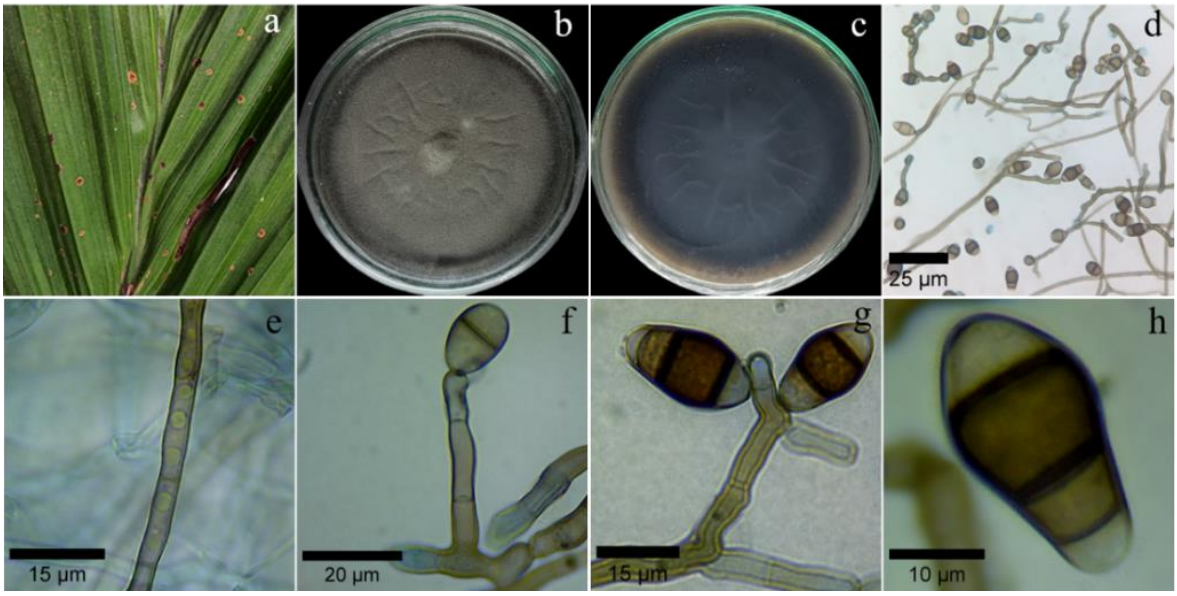

Figure 1 Symptoms and cultural characteristics of *Curvularia* isolate C1: a. Symptoms of leaf spot disease on D x P Bah Lias variety, b-c. Colonies isolate 1 on PDA after day 26, d. Hyphae and conidia e. Septate hyphae, f. Young conidia, g-h. Conidia.

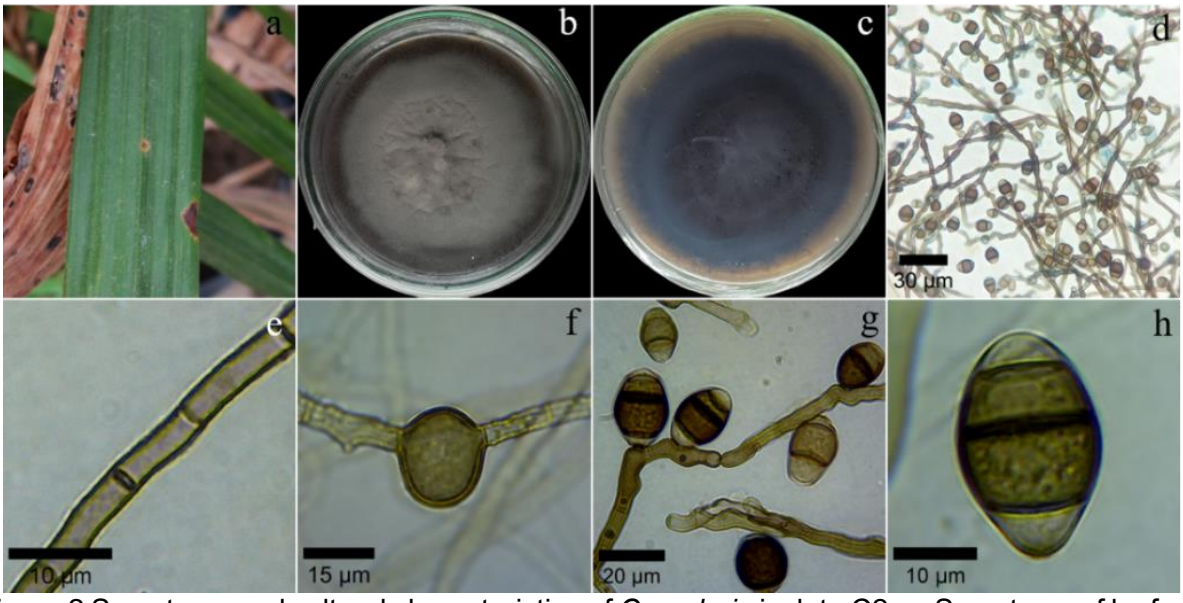

Figure 2 Symptoms and cultural characteristics of *Curvularia* isolate C2: a. Symptoms of leaf spot disease on Costa Rica variety, b-c. Colonies isolate 2 on PDA after day 26, d. Hyphae and conidia, e. Septate hyphae, f. Chlamydospores, g-h. Conidia.

Table 2 Morphological characteristics of *Curvularia* isolate C1 and C2 in comparison to *Curvularia* species references

No	Character	Isolate C1	Isolate C2	Reference *Curvularia lunata*: (Sivanesan 1987*; Salleh *et al.* 1996**)	Reference *Curvularia eragrostidis* (Sivanesan 1987*; Subramanian, 1953**; Salleh *et al.* 1996***)	Reference *Curvularia oryzae* (Sivanesan 1987*; Marin-Felix *et al*. 2020**)	Reference *Cochliobolus carbonum* (Sivanesan, 1987*, Elshafey *et al.* 2018**)
1	Septation of Hyphae	Septate	Septate	Septate*	Septate*	Septate*	Septate*
2	Branch of Hyphae	Branched	Branched	Branched*	Branched*	Branched*	Branched*
3	Pigmentation	Brownish	Brownish	Subhyaline-Brown*	Subhyaline-Brown*	Hyaline-Pale brown*	Hyaline-Brown*
4	Branch of Conidiophores	Branched	Branched	Simple/Branched*	Branched*	Simple/Branched*	Branched*
5	Shape of Conidiophores	Straight, Curved, Wavy	Straight, Curved, Wavy	Straight/Flexuous*	Simple, Erect, Straight**/Curved*	Straight/Flexuous*	Straight /Flexuous*
6	Septation of Conidiophores	Septate	Septate	Septate*	Septate*	Septate*	Septate*
7	Color of Conidiophore	Brown	Brown	Pale brown-Brown*	Brown-Light brown*	Brown/Dark brown*	Dark brown*
8	Shape of Conidia	Ellipsoidal/Barrel-shaped, Obclavate	Ellipsoidal/Barrel-shaped	Straight-Curved, Ellipsoidal, Obovoid/Clavate*	Ellipsoidal (Oval)/Barrel-shaped*	Straight, Ovoid, Obclavate/Elliptical*	Curved/Straigh, Cylindrical but broader in the middle & tapering toward the rounded end*
9	Size of Conidia	19–36 x 10–16 µm	18–25 x 8–18 µm	18–32 x 9,0–15 µm*	18–37 x 11–20 µm*, 17–26 x 11–16 µm**	24–40 x 12–22 µm*, 20–37,5 x 11–21,5 µm**	30–100 x 12–18 µm*
10	Septation of Conidia	Septate	Septate	Septate*	Septate*	Septate*	Septate*
11	Number of Septa Conidia	3 Septate	3 Septate	3/4-distoseptate*	3-distoseptate*	3-distoseptate*	7-8-distoseptate*
12	Color of Conidia	Brown (end cells paler, central cells brown)	Brown (end cells paler, central cells light-dark brown)	End cells pale brown, central cells brown/dark brown*	End cells paler, central cells brown-dark brown*	End cells paler, central cells brown/dark brown*	Dark brown*
13	Growth Rate	0.25–0.55 cm after day 4 or 0.1–0.45 cm after day 8	0.4–0.45 cm after day 4 or 0.4–0.5 cm after day 8	6.0–7.6 cm after day 4**	5.7–6.4 cm after day 4***	-	5.4 cm after day 8 **

severity based on the average number of spots has a value of 36.25–59.50%, whereas if based on the average diameter of the spots was 12.50–27.78% (Tables 4 and 5). According to Sugama and Rochjadi (1989) in Elfina *et al.* 2015), the percentage of effectiveness included the category of very ineffective to quite effective. The ineffective application of *P. canescens* leaf water extract in reducing leaf spot disease in oil palm seedlings because the application time was not appropriate namely after the spots appeared due to infection by *C. eragrostidis* 1 and *C. eragrostidis* 2. This was supported by research conducted by Zarafi and Moumoudou (2010) who reported that the application of cold water *Azadirachta indica* leaf extract 2 days before inoculation and 2 days after inoculation resulted in a significant reduction in the incidence and severity of spot disease caused by *C. eragrostidis* compared to the application at the time of appearance symptom.

Table 3 Average disease severity score and average disease severity index from *in vivo* test

Treatment	Average Disease Severity Score (Day)				Average Disease Severity Index (Day)			
	0	**10**	**30**	**50**	**0**	**10**	**30**	**50**
Control	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
C1S0	0^{a}	2^{b}	2.3^{b}	2.3^{b}	0^{a}	40^{b}	46.7^{b}	46.7^{b}
C1S25	0^{a}	2^{b}	2^{b}	2^{b}	0^{a}	40^{b}	40^{b}	40^{b}
C2S0	0^{a}	2.3^{b}	3^{bc}	3.7^{c}	0^{a}	46.7^{b}	60^{bc}	73.3^{c}
C2S25	0^{a}	2^{b}	2.7^{bc}	2.7^{bc}	0^{a}	40^{b}	53.3^{bc}	53.3^{bc}

Value in the average disease severity score and average disease severity index that are not significantly different ($p < 0.05$) have the same superscript letters ($N = 3$).
0 = No disease symptoms; 1 = Some pin-point brown spots on the leaf without any rotten tissues; 2 = Spot Diameter of 1 – <3 mm; 3 = Spot Diameter of 3 – <4 mm; 4 = Spot Diameter of 4 – <5 mm; 5 = Spot Diameter of ≥ 5 mm (Modified from Kittimorakul *et al.* 2019).

Based on Table 4, the application of *P. canescens* leaf water extract could reduce the average number of spots but not significantly. On day 10, the number of spots still increased and began to reduce but not significantly on day 30 after sprayed 3 times with *P. canescens* leaf water extract. On day 50, the inoculation treatment with *C. eragrostidis* 1 had the same average number of spots as on day 30 whereas in the inoculation treatment with *C. eragrostidis* 2, the average number of spots reduced but not significantly. Inoculation with *C. eragrostidis* 1 had a higher average number of spots than *C. eragrostidis* 2. Spot infection on positive control oil palm seedlings and those applied with *P. canescens* leaf water extract was spread in the area around the inoculation *C. eragrostidis*. This type of spot infection was a local type of infection. The local type of infection was an infection that caused physiological or structural changed within a limited period of time in the host tissue around the site of infection (Sharma 2023).

Based on Table 5, application of *P. canescens* leaf water extract could reduce the average diameter of spots but not significantly. On day 10, the diameter of the spots still increased in size and began to reduce but not significantly on day 30 after sprayed 3 times with *P. canescens* leaf water extract and the value remained the same on day 50. Inoculation with *C. eragrostidis* 1 had a smaller average diameter of spots than *C. eragrostidis* 2. There was an average reduction in the number and diameter of spots oil palm seedling leaves given the *P. canescens* leaf water extract indicating the presence of antifungal activity in *P. canescens* leaf

water extract. That activity was caused by antifungal compounds contained in *P. canescens* leaf water extract. *P. canescens* leaves contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids (Emilia *et al.* 2023). According to Utami *et al*. (2022), plants that contain secondary metabolite compounds such as steroids, alkaloids, saponins, tannins, phenolics, flavonoids, and triterpenoids could function as antifungals, namely substances that could inhibit and kill fungal growth so they could be used to control leaf spot disease caused by *C. eragrostidis.*

Table 4 Average number of leaf spots on oil palm seedlings after application of *P. canescens* leaf water extract concentration of 25%

Treatment	Average Number of Leaf Spots			Effective-ness on Day 50 (%)	Category
	10	30	50		
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-
C1S0	17.33 ± 2.30^{def}	24.00 ± 8.67^{ef}	26.67 ± 10.79^{f}	-	-
C1S25	15.33 ± 8.14^{cde}	17.00 ± 7.81^{cdef}	17.00 ± 7.81^{cdef}	36.25	Less Effective
C2S0	12.00 ± 5.29^{bcd}	13.00 ± 7.00^{bcde}	14.00 ± 7.54^{bcde}	-	-
C2S25	3.00 ± 2.00^{ab}	6.00 ± 2.64^{abcd}	5.67 ± 2.30^{abc}	59.50	Quite Effective

Value in each column and row that are not significantly different (p<0.05) have the same superscript letters (N = 3).

Table 5 Average diameter of leaf spots on oil palm seedlings after application of *P. canescens* leaf water extract concentration of 25%

Treatment	Average Diameter of Leaf Spots			Effectiveness on Day 50 (%)	Category
	10	30	50		
Control	0.00 ± 000^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	-	-
C1S0	1.33 ± 0.58^{ab}	2.40 ± 1.01^{bc}	2.40 ± 1.01^{bc}	-	-
C1S25	1.00 ± 0.00^{ab}	2.07 ± 0.92^{bc}	2.07 ± 0.92^{bc}	12.50	Very Ineffective
C2S0	1.83 ± 1.04^{abc}	3.30 ± 1.63^{c}	3.57 ± 1.70^{c}	-	-
C2S25	1.00 ± 0.00^{ab}	2.63 ± 1.50^{bc}	2.63 ± 1.50^{bc}	27.78	Less Effective

Value in each column and row that are not significantly different (p<0.05) have the same superscript letters (N = 3).

CONCLUSION

The fungi that caused leaf spot disease in oil palm were identified as *Curvularia eragrostidis*. False elder (*Peronema canescens* Jack) leaf water extract at 25% concentration was very ineffective to quite effective in controlling disease severity based on the average number of spots and diameter of spots in oil palm with the value of 36.25–59.50% and 12.50–27.78%. *P. canescens* leaf water extract could reduce the average number of spots and diameter of spots started on day 30 after sprayed 3 times with *P. canescens* leaf water extract.

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