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Jengkol Peel Extract (*Pithecellobium jiringa* (Jack) Prain) as a Biofungicide Against Fungus *Curvularia* sp., the Cause of Leaf Spot Disease on Oil Palm (*Elaeis guineensis* Jacq) Seedlings

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Abstract

Leaf spot disease of oil palm caused by fungus Curvularia sp. is a major disease in Oil Palm Seedlings (Elaeis guineensis Jacq). Implementation of best nursery practices is the key to prevent it. This study aims to determine the antifungal activity of Jengkol peel (Pithecellobium jiringa (Jack) Prain) extract against the fungus Curvularia sp. which is the cause of leaf spot disease in oil palm (Elaeis guineensis Jacq) seedlings. This study was carried out for five months at the Plantation Plant Protection Center (Balai Proteksi Tanaman Perkebunan, BPTP) in Pontianak. Jengkol peel extract was screened for its ability to inhibit Curvularia sp. growth by in vitro technique. The research method used a completely randomized design (CRD) with a single factor, namely the concentration of Jengkol peel extract, P0: as a control (without extract), P1: extract with a concentration of 10%, P2: extract with a concentration of 20%, P3: extract with a concentration of 30%, P4: extract with a concentration of 40%, and P5: extract with a concentration of 50%. Parameters observed were suppression of colony diameter and suppression of colony biomass. The results of the study showed that the administration of Jengkol peel extract in P5 treatment (50% extract concentration) had a major effect on suppressing the diameter of fungal colonies (36.44%) and showed a significant difference between each concentration ($p < 0.05$), while the P3 treatment (30% extract concentration) was able to suppress colony biomass up to 100%. The use of Jengkol peel (Pithecellobium jiringa (Jack) Prain) extract has potential as bio fungicide based on the effect found on the suppression of growth both from the diameter and biomass of the fungal colony of Curvularia sp.

Keywords

Antifungal; Curvularia sp.; Jengkol peel (Pithecellobium jiringa (Jack) Prain) extract; Oil palm seedlings; Elaeis guineensis (Jacq)

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1 Introduction

Oil palm (*Elaeis guineensis* Jacq) is one of the plantation crops that produces vegetable oil which has become the main and leading agricultural commodity in Indonesia. The development of oil palm in Indonesia has increased every single year. According to the Directorate General of Estate Crops[1], for West Kalimantan region itself, the area of oil palm in 2017 reached 1,187,354 ha with a total production of 2,784,180 tons, while in 2018 it was estimated to reach 1,245,070 ha with a total production of 2,929,360 tons and in 2019 it was 1,276,001 ha with a total production of 3,095,601 tons. Along with the wider growth of oil palm development, it will also be accompanied by an increasing risk of pests and diseases of the palm.

A good nursery is one of the means to achieve maximum production. By using good seeds, the hope to achieve maximum production will be easily obtained. One of the things that affect the quality of oil palm seedlings is disease attack during the seedling stage. The most common disease found in oil palm nurseries is leaf spot disease. This disease is detrimental because it can inhibit seedling growth, may result to stunted seedlings, prolong nursery age, increase mortality in planting, prolong immature plant period (TBM), and become a source of inoculum for other seeds[2]. Leaf spot disease in oil palm nurseries in Sanggau district which was observed in five sub-districts had reached between 4% to 38% which was caused by the fungus *Curvularia* sp. and *Glomerella* sp.[3].

The control of leaf spot disease that is currently being carried out is still dominated by the use of synthetic fungicides. One of the active fungicides that can suppress the development of leaf spot disease is difenoconazole[4]. The use of synthetic fungicides is favoured by farmers because of the results that can be seen immediately and its practical application. However, the use of synthetic fungicides can cause environmental pollution by chemical residues and pathogens will become resistant to these fungicides. Therefore, to reduce these adverse side effects, the use of synthetic fungicides can be reduced or replaced by using natural materials that are environmentally friendly, inexpensive, and widely available in nature. One of the plants that has potential as a vegetable fungicide is Jengkol (*Pithecellobium jiringa* (Jack) Prain) peel. Jengkol peel extract is known to contain active compounds, namely alkaloids, saponins, flavonoids, tannins, glycosides and steroids[5]. According to Nurussakinah[5] and Juariyah and Oktaviyani[6], Jengkol peel extract can be used as an antibacterial against *Streptococcus mutants*, *Staphylococcus aureus*, and *Escherichia coli* and can inhibit the growth of the fungus *Candida albicans*. Based on the content of active compounds contained in the Jengkol peel extract, it is expected to inhibit the growth of the fungus *Curvularia* sp. which can cause leaf spot disease in oil palm nurseries. The objective of this study is to determine the antifungal activity of Jengkol peel (*Pithecellobium jiringa* (Jack) Prain) extract against the fungus *Curvularia* sp. which is the cause of leaf spot disease in oil palm (*Elaeis guineensis* Jacq) seedlings.

2 Materials and Method

2.1 Research Location dan Materials

The research was carried out at the Disease Laboratory of the Plantation Plant Protection Center (BPTP) in Pontianak. The study was conducted from December 2018 to April 2019. The research materials consisted of Jengkol peel, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), sterile distilled water, 96% alcohol, isolate *Curvularia* sp., spiritus and filter paper. The tools used were oven, incubator, rotary evaporator, petri dish, beaker, 250 mL Erlenmeyer flask, autoclave, scales, knife, cork drill, hot plate, and measuring instruments.

2.2 Microorganisms Sources

Fungal isolate *Curvularia* sp. in this study was obtained from the Disease Laboratory of the Plantation Plant Protection Center (BPTP) in Pontianak. In this study, the finding showed that the macroscopic characteristic of the mycelium is white, with colonies of dark green to grey. Microscopic characteristics of *Curvularia*

sp. consist of three insulated conidia with a size of 23.02-24.66 μm × 12.23-12.30 μm.

2.3 Jengkol Peel Extraction

The Jengkol peel extract was produced by using a cold maceration method with 96% alcohol solvent. Jengkol peels that were washed, were then cut into smaller pieces and air-dried for three days. About 100 g of dried Jengkol peels were extracted using 96% alcohol (1 L) and allowed to stand for 72 hours. After that, it was filtered using filter paper and the filtrate was evaporated using a rotary evaporator at a temperature of 65°C until a concentrated extract was produced.

2.4 Antifungal Activity of Jengkol Peel Extract

Jengkol Peel extracts were screened for their ability to inhibit *Curvularia* sp. growth by *in vitro* technique. The research used a completely randomized design (CRD) with several treatments with concentrations of Jengkol peel extracts (10%, 20%, 30%, 40%, 50% and control) repeated four times. The parameters observed were suppression of colony diameter and biomass suppression by Jengkol peel extract against *Curvularia* sp. The treatments in this study were P0: no treatment (control), P1: extract with a concentration of 10%, P2: extract with a concentration of 20%, P3: extract with a concentration of 30%, P4: extract with a concentration of 40%, and P5: extract with a concentration of 50%.

Colony diameter suppression test was carried out by adding a test solution of Jengkol peel extract according to concentration (10%, 20%, 30%, 40%, 50% and control) into Potato Dextrose Agar (PDA) media solution. After that the growth media was allowed to harden, inoculated with the pathogen *Curvularia* sp. with a diameter of 6 mm[7]. Colony suppression test was measured by Equation 1[7].

$$x = \frac{b - a}{b} \times 100 \quad (1)$$

x = Percentage of inhibition of colony growth
a = Diameter of growth in treatment (mm)
b = Diameter of growth in control (mm)

The suppression of the colony biomass of *Curvularia* sp. was performed by using Potato Dextrose Broth (PDB) liquid media. Then, the Jengkol peel extract test solution was added according to the concentration. After the fungus *Curvularia* sp. was introduced into the medium, the culture was incubated in an incubator at 28°C for nine days. Furthermore, the grown fungal colonies that grew were taken and dried in an oven at 80°C for 48 hours. Finally, the percentage of inhibition of colony biomass was calculated with Equation 2[8].

$$P = \frac{K - T}{T} \times 100 \quad (2)$$

P = Percentage of inhibition of colony biomass
K = Colony biomass of control (gram)
T = Colony biomass of treatment (gram)

2.5 Statistical Analysis

The data obtained were analysed by using ANOVA test which was performed with the SPSS Version 17.0 software. Significant differences obtained by ANOVA analysis were further analysed based on the Least Significant Difference (LSD) test at $p < 0.05$.

3 Results and Discussion

Mycelium of *Curvularia* sp. in the control treatment was observed to grow into the full diameter length of the petri dish. Treatment with concentrations of 10% and 20% showed that the mycelium of *Curvularia* sp. had covered the petri dish surface similar to the control treatment. Meanwhile, at treatment concentrations of 30%, 40% and 50%, mycelium *Curvularia* sp. was unable to fill the medium in the petri dish.

Table 1. Average Growth and Growth Suppression of Colonies of *Curvularia* sp.

Treatment	Average Colony Growth (mm)	Average Colony Growth Suppression (%)
Control Extract	88.5	0.0[a]
10% Extract	85.0	3.95[b]
20% Extract	80.0	9.60[c]
30% Extract	71.5	19.20[d]
40% Extract	65.25	26.27[e]
50% Extract	56.25	63.44[f]

Note: Values followed by the same letters in the same column are not significantly different at $p = 0.05$ based on ANOVA and LSD

Based on the results of the ANOVA test in Table 1, there was a significant difference as indicated by ANOVA ($p < 0.05$) and LSD tests. Hence, this indicates that the Jengkol peel extract can suppress the growth of the fungus *Curvularia* sp. which showed a significant difference between each concentration.

It can be seen that the 50% extract treatment had the largest suppression percentage, namely 63.44% and the smallest was 10% extract with a suppression percentage of 3.95%. Each increase in the concentration of the extract was accompanied by an increase in the suppression of the growth of *Curvularia* sp. This is due to an increase in the content of active substances in the extract with a higher concentration, where more extract diffuses into the fungal cell so that it has a greater ability to suppress growth[9,10].

Table 2 shows the average suppression of the colony biomass of *Curvularia* sp. The 10% extract treatment had the lowest biomass suppression ability with a percentage of 25.10% which was indicated by the LSD test where there were significant differences between the different concentrations. Meanwhile, the 30% extract treatment was able to suppress colony biomass up to 100%.

Table 2. Average Biomass and Biomass Inhibition Colonies of *Curvularia* sp.

Treatment	Average Colony Biomass (mg)	Average Suppression of Colony Biomass (%)
Control	119.4	0[a]
10% Extract	89.4	25.10[b]
20% Extract	21.1	82.32[c]
30% Extract	0	100[d]
40% Extract	0	100[d]
50% Extract	0	100[d]

Note: Values followed by the same letters in the same column are not significantly different at $p = 0.05$ based on ANOVA and LSD

The outcome of the effect of peel extract on the growth of test fungus *Curvularia* sp. showed similarity as reported by other researchers who have applied different plant extracts to study the effect on growth and reproduction of different pathogenic fungi. Only few reports were found on the effect of Jengkol peel extract (*Pithecellobium jiringa* (Jack) Prain) on growth of other fungi of other crops. Siswandi et al.[11] reported that administration of Jengkol peel extract is effective for controlling fungal pathogens (*Colletotrichum capsici*, *Fusarium oxysporum* and *Cercospora capsici*) by *in vitro* that caused disease in red chili plants. Refilda et al.[12] reported that fermented plant extract from Jengkol peel has potential as organic liquid fertilizer and biopesticide in tomato plant.

According to Nurussakinnah[5] and Refilda et al.[12], Jengkol peel extract is

known to contain active compounds, namely alkaloids, saponins, flavonoids, tannins, glycosides, terpenoids and steroids. Alkaloids, flavonoids, saponins and terpenoids are known to have antifungal activity so that they can suppress the growth of colonies of *Curvularia* sp.[13]. Alkaloids, saponins, and flavonoids can damage and inhibit the process of forming membranes and fungal cell walls so that they will interfere with the growth process of fungi[14,15]. According to Juariah and Oktaviyani[6] and Idris and Nurmansyah[7], tannins have the ability to inhibit the synthesis of chitin which is used for the formation of fungal cell walls which are needed to elongate hyphal tips, branching and spore formation. The inhibited development of conidia causes the development of the next generation to be disrupted, because conidia is an asexual development in the Deuteromycetes class[7]. Terpenoids can diffuse into pathogens and damage cell membrane structures resulting in its death[16]. Terpenes are the active antimicrobial compounds of plants. The action mechanism of this class of compounds is speculated to involve membrane disruption and also destroy the fungal mitochondria. Antifungal action from phenolic compounds happens through cell membrane disruption which depolarized mitochondrial membrane potential in a concentration-dependent manner[17].

4 Conclusion

Based on the results of the study, it showed that the use of Jengkol peel extract had an effect on the suppression of growth both from the diameter and biomass of the fungal colony of *Curvularia* sp. The largest diameter suppressive strength was found at 50% extract concentration with a suppressive strength of 36.44%, while the highest biomass suppressive strength was 100% with a minimum extract concentration of 30%. Further studies are warranted to evaluate the effects of Jengkol peel extract as a biocontrol agent in the biological control of other fungal diseases.

Conflict of Interest

The authors reported that there is no conflict of interest in this work.

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