Identification of Fungi in Oil Palm with Dry Frond Disease at PT Bumitama Gunajaya Agro

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Abstract. Oil palm is a plantation crop that produces palm oil and palm kernel oil which are Indonesia's main source of foreign exchange from the plantation sector. The practice of monoculture cultivation on oil palm plantations grown on various types of soil in Indonesia, has big challenges in the aspect of cultivation, especially pest and disease control. One of the diseases that cause losses in the cultivation of this commodity is dry frond. This disease has been found in oil palm trees in the plantation of PT Bumitama Gunajaya Agro grown on Quartzpsamments and Haplohemists soils. This pathogen causes the rachis and leaves to dry from the tip to the base of the frond. The spread of this disease is thought to be due to contact between infected to healthy fronds. In this study, 6 isolates were obtain from leaves and 7 isolates from infected rachis. Based on the macroscopic and microscopic characteristics of the isolates, one isolate from Sungai Langir Estate belonged to the genus Thielaviopsis sp. However, the postulate koch test showed that the thirteen isolates could not infect and cause symptoms of dry frond disease on the leaves and rachis of the tested oil palm plants.

Keywords: Oil palm disease, Haplohemists, Quartzpsamments, Thielaviopsis sp.

1. Introduction
Palm oil (Elaeis guineensis Jacq.) is an oil crop that produces crude palm oil and palm kernel oil which is Indonesia's main source of foreign exchange from the plantation sector. In Indonesia, oil palm plantations are spread across 26 provinces with a total land area of 15,081,021 Ha in 2021. Of the total area, 54.94% of oil palm plantations are cultivated by large private companies, 40.79% are cultivated by smallholders, and 4.27% are cultivated by large state companies [1].

The large area of oil palm plantations cultivated with monoculture planting systems on various types of soil in Indonesia has a big challenge in the cultivation process to obtain high productivity oil palm plants [2]. One of the obstacles in oil palm cultivation is the attack of dry frond disease. This disease has been reported to attack oil palm plantations in Sabah, East Malaysia [3], as well as East Kalimantan and North Sumatra, Indonesia. Symptoms of the attack are characterized by the occurrence of decay at the end of the frond then spread to the middle of the frond. When the rot has reached the middle of the frond, the frond will dry out and eventually fracture. When the frond is split crosswise, brown rotted areas are seen overgrown with a small amount of white mycelium [2].
Dry frond disease has been found to attack oil palm plantations in area 5, PT Bumitama Gunajaya Agro (BGA), Pangkalan Bun, Central Kalimantan, Indonesia in 2020. In the Kotawaringin Estate, this disease infects the oldest frond to frond no 17 on Quartzpsamments. Meanwhile in the Sungai Langir Estate plantation which was planted on Haplohemists this disease was found to attack oil palm suspected causing the death of the palm. Palms that are attacked by this disease experience dry rachis and leaves from the tip to the base of the frond. The attack starts from the oldest frond to the young frond and heavy attacks can cause the death of the oil palm plant. Until now, the main cause of dry frond disease in oil palm plants is not known, so that effective control techniques have not been obtained. The researchers suspected that this disease was caused by nutrient deficiency, water stress, nutrient uptake disorders, and the fungus *Thielaviopsis* sp. the disease-causing pathogen or the infecting fungi after the disease [2,3]. Thus, it is necessary to isolate and identify the fungi found in leaves and rachis that are attacked by this disease to determine whether the fungi that have been isolated are the main pathogenic fungi causing dry frond disease or secondary pathogenic fungi.

2. Materials and Methods

2.1. Sampling
Samples of infected rachis and leaves were taken from three infected palm in Sungai Langir Estate (SLNE) and Kotawaringin Estate (KTWE), Pangkalan Bun, Kotawaringin Barat, Central Kalimantan. Infected palms were found in Haplohemists and Quartzpsamments (Figure 1). Samples were taken by cutting the newly infected frond using oil palm sickle, then the leaves and rachis were separated.

![Figure 1. A. Palm infected with dry frond disease on Quartzsamments, B. Heavy attack of dry frond disease on Haplohemists, and C. Dead palm on Haplohemists suspected with dry frond disease](image)

2.2 Sample Isolation

Samples obtained from the block was sent to the Microbiology Laboratory of the Research Department PT Bumitama Gunajaya Agro and then washed with running water for 15 minutes. The washed samples were wiped with a tissue until clean and dry. Samples of leaves and rachis that have been cleaned are then cut 2 cm long on the newly infected parts. The sample was put in a laminar air flow cabinet and sterilized in a solution of NaClO for 10 seconds and 70% alcohol for 30 seconds. Then the sample was washed with sterile distilled water 3 times and placed in a sterile petri dish.

The outer part of the sterilized rachis was then exfoliated and cut using a sterile scalpel to leave a newly attacked frond measuring 1x1 cm. The sterile leaf samples were cut to a size of 1x1 cm on newly
attacked leaves. Each sample of rachis and leaf was put in 5 pieces of Dichloran Rose Bengal Chloramphenicol (DRBC) Himmedia and repeated 3 times. The samples were then incubated in an incubator at 30°C for 14 days.

After the incubation period, isolates growing on each petri dish were re-isolated. Reisolation was carried out by drilling the isolates that grew from the sample in DRBC media with a cork drill, then transferred to Merck Potato Dextrose Agar (PDA) media with a sterile ose needle. The isolated isolates were incubated in an incubator at 30°C for 14 days.

2.2. Isolate Identification
The isolates that had filled the petri dish were then observed macroscopically by observing the color of the hyphae. Microscopic observations were carried out by taking a sample of the isolate with an ent needle and placing it on a glass preparation that had been dripped with lactophenol cotton blue, then the sample was covered with a cover glass. The preparations that have been made are then observed for the shape of the spores, the presence or absence of bulkheads on the hyphae, and the angle of branching of the hyphae on a compound microscope with a magnification of 100x. The characteristics obtained from each isolate were then matched with the appropriate fungal characteristics by referring to the book Soil and Seed Morphologies of Cultured Fungi and Key Species [4].

2.3. Koch Postulate Test
The isolates that had been obtained were then tested postulate koch to determine whether the isolates obtained were fungi that caused dry disease of oil palm fronds or not. Koch's postulate was carried out by drilling the isolates with a cork drill, then the isolates obtained from infected leaves were placed on the leaves with the hyphae in direct contact with the leaves. The isolates obtained from the palm rachis were placed on the rachis with the position of the isolates in direct contact with the previously injured oil palm rachis tissue. In this study, five months old seedling were used as test plants. The infected isolates were then incubated for 14 days to determine whether the infected isolates could infect oil palm leaves and fronds and gave the same symptoms as dry frond disease.

Drill isolate that infects then observed macroscopic characteristics of infection whether it is following with the symptoms of dry frond disease or not. Infected leaves and frond were isolated and purified to determine whether the infection was caused by previously infected isolates. If the results of isolation and purification obtained the same isolates, it can be concluded that the isolates were the cause of dry frond disease of oil palm.

2.4. Soil Analysis
For each subject, soil samples were taken at a depth of 0–15 cm at 3 points on the cycle as much as 500 g. The samples obtained were then composited and sent to the Analytical Laboratory of PT Bumitama Gunajaya Agro to analyze the soil nutrient content of infected palms.

2.5. Leaf nutrient Analysis
Leaf nutrient content tests were carried out on infected palms in Kotawaringin Estate. Leaf samples were taken on the 17th frond, then 20 leaves were taken on the right and left at the mid-point of the frond section. The leaf samples were then wiped clean and dried in an oven to dry and then sent to the Analytical Laboratory of PT Bumitama Gunajaya Agro to analyze the nutrient content of leaves on infected palms.

2.6. Observation of spore count of arbuscular mycorrhizal fungi (AMF)
Soil samples from each palm were taken as much as 50 g, then put in a 1000 ml measuring cup and added 800 ml of water. The sample was stirred for 1 minute, then poured on sieves of 850µm, 180µm, and 45µm, the same thing was repeated 3 times. After filtering, the soil retained on the 180 and 45 m sieves was placed in 3 petri dishes with a diameter of 6 cm and the number of AMF spores was observed with a 35x magnification stereo microscope.
3. Results and Discussion

Dry frond disease found in the Kotawaringin Estate was from 12 years old oil palm on Quartzpsamments. Based on vitual observation, infected palms had lower vegetative growth compared to normal palms. There is a positive correlation between oil palm frond drying scores and oil palm vegetative measurements of petiole size, leaf area, leaf length, and dry root mass collected from 50 and 150 cm from the palm base. This means, higher the severity of desiccation, lower the palm growth [3].

There was a decrease in sex ratio in infected oil palm, which was indicated by more male flowers compared to female flowers, so the number of fruit bunches decreased. This decrease in sex ratio resulted in a decrease in production of 12.5% in 2020. Production of oil palm fruit bunches on the sampling block had reached 19.28 tons/ha in 2019, but decreased to 16.87 tons/ha in 2020.

Infected palms are found in the center of the block with symptoms of nitrogen, phosphorus and potassium deficiency. The infection of this disease starts from the oldest to the younger frond subsequently. The infection process starts from the tip of the frond, where the infected frond will rot and then dry up both the leaves and the frond. The infection process continues until the base of the frond and when the process of decay reaches the base of the frond, the dried leaf will break. The occurrence of frond fracture in this disease is very different from the fracture of the frond due to water stress. In addition, on the surface of the frond infected with this disease, white fungal mycelium can be found. When the newly infected petiole was cut crosswise, brown to black necrotic tissue was seen in the center of the petiole (Figure 2).

![Figure 2](image)

*Figure 2. A. Fronds infected with dry disease, B. Infected leaf, and C. Transverse view of infected petiole*

Severe attacks of this disease were found on eight years old plants planted in Haplohemist This disease has attacked the oil palm frond to the newly emerged frond and some palms that have died because all the fronds have dried up. Oil palm with infected fronds are found on palm near collection road. Oil palm with heavy attacks did not experience flowering or fruiting, this was presumably because oil palm focused on plant vegetative growth, especially in the formation of the frond.

After isolation and identification of isolates growing from leaves and fronds infected with dry frond disease, 6 isolates of fungus were obtained from infected leaves and 7 isolates of fungus from infected fronds. One isolate from the Sungai Langir Estate had characteristics that matched the genus *Thielaviopsis* sp. (Figure 3). Macroscopically, the isolate was dark brown in color and microscopically it had insulated hyphae, forming 45–90° branching, and had orange spores in the form of oval chains [2,3]. *Thielaviopsis* sp. is a pathogen that’s restricted to monocotyl plants that are stressed and plant organs that are senescing. The sexual stage of the fungus usually invades only parenchymal tissue rather than the denser meristem cells or bundle sheaths [5]. Dry basal rot at palm, caused by *Thielaviopsis* sp. can attack the stem, leaves, and fruits, also causing premature fruit drop [6].
Figure 3. Isolates of *Thielaviopsis* sp. from infected rachis

The thirteen isolates obtained from infected leaves and fronds were then tested for Koch's postulates to determine whether the isolates that were isolated from the stem dry disease were the pathogens causing the disease. After the Koch postulate test, the thirteenth isolate did not infect the leaves or frond of the tested oil palm. Thus, the thirteenth isolate obtained was not a fungus that causes dry palm fronds. Fungi obtained from infected leaves and fronds are thought to infect when leaves and fronds undergo decay and drying (secondary pathogen).

Based on the analysis of leaves nutrient on infected palm in Kotawaringin Estate, there was a nutrient deficiency in the parameters N, P, and K. On the parameters of Mg, Ca, Cu, and B obtained the optimum nutrient content [7] (Table 1). Deficiencies of these three nutrients were also seen in plant leaves during sampling. K deficient leaves are located on the old frond which is characterized by confluent orange spots that can transmit light. N deficiency is characterized by young leaves that are pale green and then gradually turn yellow as the deficiency becomes more pronounced. While P deficiency is characterized by reduced vegetative growth of plants, where leaves and fronds become shorter when compared to normal palms. In addition, P-deficient palms have stems that develop in a pyramidal shape [7].

**Table 1. Leaves Analysis**

| Nutrient Analysis of Leaf of frond 17th | Nutrient Content (Based on Dry Matter) | ppm on dry matter |
|--------------------------------------|--------------------------------------|-------------------|
| Type of palms | % on dry matter | Mg | Ca | Cu | B |
| Healthy | 2.66 | 0.165 | 1.2 | 0.38 | 0.65 | 8 | 19 |
| Infected | 2.33 | 0.148 | 0.78 | 0.37 | 0.94 | 6 | 18 |

The results of soil nutrient content on stems infected with dry frond disease in Kotawaringin Estate, showed low nutrient content in the parameters of N, available P, total P, K, and Mg. While in block Sungai Langir Estate has optimum soil nutrient content in parameters of N, available P, total P, K, and Mg, but low on soil pH parameters [7].

The low nutrient content of the Quartzpsamments in Kotawaringin Estate is thought to be because this soil has a coarse texture with a single grain structure and very few silt and clay fractions. These conditions will cause the low ability of the soil to retain water so that it is prone to drought and the low ability of the soil to retain nutrients so that nutrients will be easily washed out. The low ability to bind nutrients is indicated by the low CEC of the soil, so that it will have implications for the availability of
nutrients from fertilizers which will be washed away by percolation [8]. In addition, the low nutrient content of the soil is thought to occur due to the competition for nutrients between the oil palm tree and the weeds found in the plant row and wickets.

With various deficiencies possessed by Quartzpsamments, it causes water and nutrient retention which causes plants to macronutrient deficiency which causes stunted plant growth. With the occurrence of macronutrient deficiencies and lack of water in the Quartzpsamments make plant resistance to pests and diseases low, so that plants are susceptible to pests and plant diseases.

The results of soil nutrient analysis on Haplohemist showed a deficiency of K and low soil pH values. In addition, on the surface of the Haplohemist block A63 SLNE, a irreversible drying peat structure is found which is indicated by peat that looks like grains of sand. The decrease in the ability of peat to absorb water is caused by a decrease in the availability of carboxylate and OH-phenolic groups in the peat material. The two organic components are hydrophilic compounds, so that if the liquid phase has disappeared, the peat that was originally hydrophilic will turn into hydrophobic [9]. In this condition, peat is easily carried by water, does not have the ability to absorb water and nutrients, and is difficult to access by microbial decomposers [10].

| Parameter | Soil depth 0–15 cm Blok M59a | Soil depth 0–15 cm Blok A 63a |
|-----------|-----------------------------|-------------------------------|
|            | Healthy Plant | Infected Plant | Healthy Plant | Infected Plant |
| pH        | 4.36          | 5.49             | 4.13         | 3.94           |
| C-Organic | 1.42          | 3.02             | 31.53        | 33.41          |
| N Total % | 0.34          | 0.10             | 1.96         | 1.49           |
| C/N       | 4.18          | 30.20            | 16.09        | 22.42          |
| Available P (ppm) | 15.41       | 10.54            | 55.02        | 110.94         |
| P Total (ppm) | 97            | 181.18           | 279.75       | 570.84         |
| Ca-dd (cmol(+)/kg) | 0.43 | 0.56           | 0.82         | 1.02           |
| Mg-dd (cmol(+)/kg) | 0.14 | 0.14              | 0.21         | 0.40           |
| K-dd (cmol(+)/kg) | 0.14 | 0.11             | 0.09         | 0.31           |
| KTK (cmol(+)/kg) | 3.36 | 3.37              | 39.93        | 70.18          |

Oil palm naturally has a mutualism symbiosis with Arbuscular Mycorrhizal Fungi (AMF) [11]. AMF is a symbiotic mutualism between certain fungi and 90% of plants in the world [12]. AMF infection in oil palms begins with the formation of an appressorium on the root surface, then hyphae penetrate the epidermal wall of the plant roots [13]. Hyphae will grow in the root cortex to form vesicles and arbuscules. In addition, external hyphae located in the root rhizosphere help oil palm increase the absorption of nutrients N, P, K, Ca, as well as micro-nutrients needed by palms, prevent infection of root pathogens, help stimulate the increase in growth regulators, improve soil structure and aggregates, as well as increase the mineral cycle [14].

Based on the analysis of the arbuscular mycorrhizal fungi content in Kotawaringin Estate and Sungai Langir Estate samples taken in cycle with a depth of 0–15 cm, 84 spores/50 g of arbuscular mycorrhizal fungi were obtained in the KTWE sample and 31 spores/50 g in the sample of SLNE. In general, the type of FMA spores found in these two soil types was Glomus sp.. This is in line with the research of Rini et al. (2010), who obtained 33.8 FMA spores from oil palm plantations on privately owned peatlands with dominant spores of Glomus sp..

Viewed from the results of the nutrient content of soil and leaves on palms infected with dry frond disease in KTWE, there is continuity between the nutrient deficiency found in oil palm leaves taken
from the 17th frond and the nutrient content in the soil. Where N, P, K deficiency in the leaves also occurs in the soil. Thus, arbuscular mycorrhizal fungi present in the rhizosphere of oil palm roots infected with dry frond dry disease have not been able to assist oil palm roots in absorbing primary nutrients in Quartzpsamments soil.

4. Conclusion
From this study, 6 isolates were obtained from leaves and 7 isolates from the rachis that were infected with dry frond disease. Based on macroscopic and microscopic characteristics, one isolate from infected rachis in SLNE belonging to the genus Thielaviopsis sp. However, the postulate koch test showed that the thirteen isolates could not infect and cause symptoms of dry frond disease on the tested oil palm.

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