

Research Article

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## Assessment of Leaf Spot and Anthracnose Diseases in Nurseries and It Relationship with Oil Palm Seedling Ages

Azizi Muhamad Nor Azlan<sup>1,2</sup>, Zainal Abidin Mior Ahmad<sup>1</sup>,  
Abu Seman Idris<sup>3\*</sup> and Ganesan Vadamalai<sup>1</sup>

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.

<sup>2</sup>Crop and Soil Science Research Centre, Malaysian Agriculture Research and Development Institute (MARDI), MARDI Kluang, P.O. Box 525, 86009, Kluang, Johor, Malaysia.

<sup>3</sup>Malaysian Palm Oil Board (MPOB), No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

\*Corresponding Author: [idris@mpob.gov.my](mailto:idris@mpob.gov.my)

### Abstract

Survey of leaf spot and anthracnose diseases were conducted in nine selected oil palm nurseries in Peninsular Malaysia. Studies covered four nurseries in Johor (Kluang, Bkt.Tongkat, Kahang and Ayer Hitam), three nurseries in Perak (Teluk Intan, Permatang Guntung and Kg. Bakar Arang) and two nurseries in Selangor (Sepang and Kuala Langat). The symptoms of leaf spot and anthracnose diseases were recorded and characterized. In this study, the leaf spot and anthracnose diseases were found to be associated with three pathogens; they are *Colletotrichum* spp., *Curvularia* sp., and *Pestalotiopsis* sp. The disease survey revealed that leaf spot disease was a major problem in oil palm seedlings with the highest incidence was 62.8% compared to anthracnose disease at 16.5%. For incidence of leaf spot disease, the highest were recorded in Kuala Langat, Selangor (90.3%) and Kluang, Johor (87.7%). The least incidences of leaf spot were recorded in three nurseries in Perak occurring between 50.0% to 55.0%. For anthracnose disease, the highest incidence was recorded in Kuala Langat, Selangor (45.0%) and the least were recorded in six nurseries which were located in Johor and Perak occurring between 8.0% to 14.0%. For assessment of disease severity (DS), the highest for leaf spot disease were recorded in Kluang (64.7%) and Kahang (59.2%), both in Johor. Besides, the least severity was recorded in Permatang Guntung, Perak with 19.2%. For DS of anthracnose disease, the highest were recorded in Kuala Langat, Selangor (9.1%) and Teluk Intan, Perak (6.5%) and the least DS were recorded in six nurseries which were located in Johor and Perak occurring between 1.0% to 2.5%. The study also found disease incidence of leaf spot and anthracnose diseases were both highly significant with different age of oil palm seedlings. As the seedlings became older, incidence of leaf spot and anthracnose diseases were reduced. For leaf spot disease, seedling <4 months old, the highest score percentage at 77.4% followed by 4-12 months old which was 72.8% and seedlings with age >12 month old were the lowest score of 49.0%. This was similar with disease incidence of anthracnose for <4 months old (21.5%), 4-12 months old (16.8%) and with the least incidence was observed in seedlings aged >12 months old at 14.8%. For interaction of seedling age with DS of leaf spot, study revealed that there were highly significant relationships with seedling ages. Seedling with age <4 months old were the most susceptible and severely affected (50.9%) followed by seedling

### Keywords

Leaf spot,  
Anthracnose,  
Oil palm,  
Disease Severity,  
Disease Incidence.

age 4-12 months old (39.8%) and the most resistance seedling age was the seedling >12 months old with only 18.4%. However, for anthracnose disease, there was no significant relationship between DS and seedlings ages.

## Introduction

Oil palm (*Elaeis guineensis* Jacq.) or well known as 'kelapa sawit' in Malaysia is one of the most important species in the genus of *Elaeis* under the family of Palmae. The *Elaeis guineensis* Jacq. was derived from the Greek 'Elaion' which means oil and guineensis from its origin which is equatorial Guinea coast (Bazmi *et al.*, 2011). The palm oil industry has been identified to be a major contributor to the economics of the countries. Anyawu *et al.* (1982) reported the oil palm as one of the most important economic crops in the tropics and also a very important source of vegetable oil because of its capability as the highest production of oil compared to the other oil bearing plants (Soyebo *et al.*, 2005). Tan *et al.* (2009) reported that Malaysia and Indonesia contributed 83% of the world production and 89% of exports. In 2005, these two countries became the most important oil palm producer and contributing to 50% of total world production of palm oil. In Malaysia, oil palm planting area was recorded increased from 3.37 million hectares (ha) in 2000 to 4.05 million ha in 2005 then increased to 4.85 million ha in 2010 (Sulaiman *et al.*, 2012). Currently, there are 5.74 million ha total oil palm planted area in Malaysia was reported (MPOB, 2017).

Oil palm contributes many advantages and importance for the human population. It has been used for cooking oils, soap making and lamp oils. Palm kernel oil (PKO) is used for soap making, source of glycerine, manufacturing of margarine, cooking fats and for making pomade. The residue of oil called palm kernel cake (PKC) is very useful for livestock feeding (Soyebo *et al.*, 2005). Palm oil which is well known as world's leading vegetable oil in term of production and trade volume also nowadays recognized as good source of biofuel. The growing demand from the increasing of biofuel consumption is likely to increase and initiate the economies in China and India. Lower price compared to other alternative vegetable oils also contribute to the successful of oil palm (Kongsager and Reenberg, 2012).

Like any other crop, the oil palm is being threatened by various pest and disease problems. From seed germination until fruit harvesting, the crop is exposed to several pests and diseases including those caused by

fungal infections. Due to the advantages and contributions of the oil palm industries as mentioned previously, the sustainability of oil palm cultivation especially in the aspect of effective pests and diseases management to increase productivity is particularly important. To achieve this, research and development of the diseases of oil palm are being investigated. The main objective was to know the status of two leaf diseases, namely leaf spot and anthracnose, for their incidence and severity in relation to the oil palm ages.

## Materials and Methods

The surveyed study sites involved three states in Peninsular Malaysia: Johor, Perak and Selangor. Data were collected from four nurseries in Johor (Kluang, Bukit Tongkat, Kahang and Ayer Hitam), three nurseries in Perak (Kg. Bakar Arang, Permatang Guntung and Teluk Intan) and two nurseries in Selangor (Sepang and Kuala Langat).

### i. Assessments of disease incidence (DI) and disease severity (DS)

For survey of disease incidence (DI), numbers of infected plants in every selected nursery was counted and recorded. Percentage (%) of DI was compared between nurseries involved in this study. The frequency of DI of different diseases was also calculated and compared.

Disease Severity (DS) was also measured using the equation proposed by Kranz (1988) as follows:

$$DS = \frac{(a \times b)}{N \cdot Z} \times 100\%$$

(a × b) = Sum of the symptomatic plant and their corresponding score scale

N = Total number of sampled plant

Z = Highest score scale

### ii. Statistical data analysis

Percentage of DI and DS were calculated. The percentages were subjected to analysis of variance (ANOVA) test using SAS version 9.3. Where there

were significant differences, a Duncan Multiple Range Test (DMRT) was performed for means separations. For DS, data was incorporate in arcsine transformation prior to further analysis. For the statistical analysis, DI and DS in relation to age of seedlings, the data were compared with the aged recorded in each of the nurseries surveyed.

## Results and Discussion

The surveys of oil palm seedling diseases in nurseries indicated that the DI of leaf spot was generally common in comparison with anthracnose disease. The DI of anthracnose was lower but the epidemic proportion of the disease was severely affected on oil palm seedlings. Based on morphological identification, *Curvularia* sp., *Pestalotiopsis* sp. and *Colletotrichum* sp., mainly the pathogen to be associated with leaf spot and anthracnose disease in oil palm seedling. Kittimorakul *et al.*, (2013) also reported that *Curvularia* and *Colletotrichum* are the major foliar diseases recorded in oil palm seedling in Thailand. Studies on morphological identification of the fungi associated with the foliar disease were:

**i. Leaf spot disease –the following two fungi commonly identified to be associated with the disease, they were:**

### *Curvularia* sp.

Leaf spot caused by *Curvularia* sp. are the most frequent disease observed in oil palm nurseries.

Basri *et al.*, (2003) also reported this disease is normally found during nursery stages and few cases observed in pre-nursery stage. Most of leaf spot diseases easily spread due to dissemination of their spores via wind or splashing water (Aji *et al.*, 2013).

The fungus was isolated from oil palm leaves with the symptoms of leaf spot. The fungus was isolated from small yellow-brown spot which may develop to oblong shaped lesion. This also supported by Howard and David (2005) stated that the symptom of *Curvularia* was observed as small yellow-brown spots on leaves that can expand to oblong lesions which commonly occur on leaf margins. The centre of the lesion turned to brown colour with the margin remaining green in colour (Figures 1a and 1b). Pornsuriya *et al.*, (2013) also describe the symptoms on leaf as the emergence of spots with yellowing that can rapidly turn into large, dark brown or black lesions. *Curvularia* sp. is a fast growing fungus on Potato Dextrose Agar (PDA). The colony has a wooly texture, grey to brown in colour on the surface and black colour on the reverse (Figure 1c). The conidia observed were curved in shape, with broad points at both ends. The cell in the middle of the curvature was often larger and darker than those at both ends (Figure 1d and 1e).

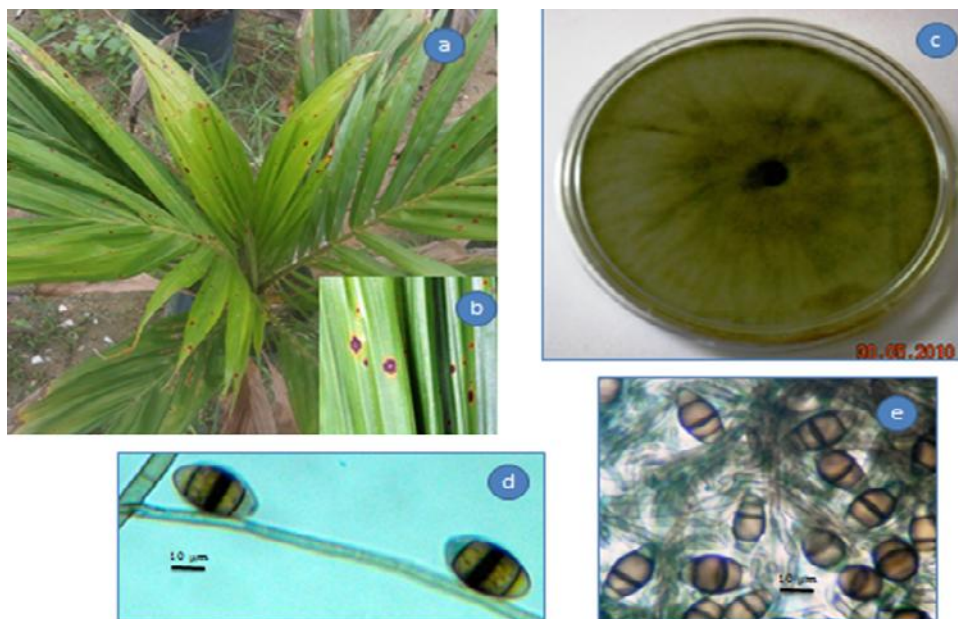


Figure 1: Leaf spot disease caused by *Curvularia* sp. Disease symptoms on leaf (a and b), colony morphology (c) and conidia of *Curvularia* sp. (d and e).



***Pestalotiopsis* sp.**

The fungus was isolated from oil palm leaves with leaf spot symptoms. Generally the size of the spot appeared to be larger than leaf spot caused by other fungal pathogens. Under severe conditions these spots will coalesce to form larger lesions. Pornsuriya *et al.*, (2013) also reported the symptom of leaf spot caused by *Pestalotiopsis* sp. showed the large lesion, irregularly shaped and orange-red in color. However, brownish with dark borders was observed in infected seedling as reported by Elliot *et al.*, (2004). The presences of black specks on lesions are associated

with the acervuli of the fungus. Symptoms of attack were more frequently observed on leaf margins, petioles and rachis (Figure 2a). On PDA agar, the colony of *Pestalotiopsis* sp. is white in colour colony on the surface of agar and yellowish on the reverse side. It is fast growing fungus which produces abundant spores in culture conditions (Figure 2b). The spores (conidia) produced by *Pestalotiopsis* sp. spores are oblong in shape with a thick posterior end. It has a pair of flagella on both end making it easily dispersed by water and also wind. Spores were found to be septate or having compartments as shown in Figure 2c.



Figure 2: Leaf spot disease caused by *Pestalotiopsis* sp. Disease symptom on leaf (a), colony morphology (b) and conidia of *Pestalotiopsis* sp. (c).

**ii. Anthracnose disease – Only fungus *Colletotrichum* sp. were found to be associated with the disease.**

During the first four months of nursery development, seedlings may be affected by species of *Botryodiplodia*, *Glomerella* (sexual stage of *Colletotrichum* sp.) and *Melanconium* causing early leaf disease commonly known as anthracnose (Turner, 1981). Anthracnose is capable of destroying the whole nursery if left uncontrolled and the incidence of this disease was commonly between 5 to 20% due to transplanting shock (Basri *et al.*, 2003). Over watering also resulted in up to 80% of plants becoming affected (Turner and Gillbanks, 1974).

The fungus was consistently isolated and identified showing symptoms of severe lesions in the margin of leaves (Figure 3a). Such symptoms may also appear as spots initially, but these can coalesce to form larger lesions under severe conditions. Previously studies also reported the first symptom of anthracnose appears as a small circular brown spots then elongated with yellow halo or dark brown (Kuinkel *et al.*, 2016; Corley and Tinker, 2003 and Pornsuriya *et al.*, 2013). *Colletotrichum* sp. colony on PDA is fast growing fungus, white colour becoming orange and this may later turn whitish grey in colour (Figure 3b). Brownish to black colour was observed on reverse side of colony. Orange spore masses were produced outwards from the center of the colony and can be easily observed. The spores were conidia which were rod shape (Figure 3c).

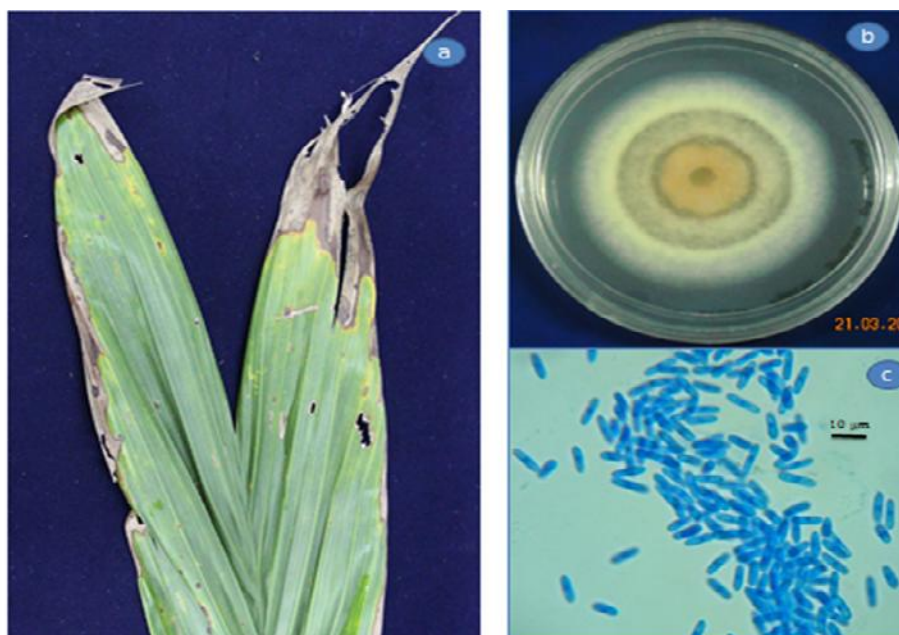


Figure 3: Anthracnose disease caused by *Colletotrichum* sp. Colony morphology (a) and conidia of *Colletotrichum* sp.(b).

**Assessments of incidence and severity of leaf spot disease**

Table 1 summarizes the incidence of leaf spot disease in oil palm seedlings. It showed a highly significant difference between locations and incidence of leaf spot disease. The highest incidences were recorded in Kuala Langat, Selangor (90.3%) and Kluang, Johor (87.7%). The least incidence of leaf spot was recorded

in three nurseries in Perak occurring between 50.0% to 55.0%. Table 1 also summarizes the severity of leaf spot disease in oil palm seedlings. It showed a highly significant difference between locations and severity of leaf spot disease. The highest disease severities were recorded in oil palm seedling at Kluang (64.7%) and Kahang, Johor (59.2%). The least disease severity of leaf spot was recorded in Permatang Guntung, Perak with 19.2%.

**Table 1: Disease incidence (DI) and severity (DS) of leaf spot in oil palm seedlings at different locations in Peninsular Malaysia.**

Location:	Incidence Disease (%)	Severity Disease (%)
Kluang, Johor	87.7a	64.7 ± 38.6a
Bkt. Tongkat, Johor	66.0cd	29.5 ± 38.9cd
Kahang, Johor	77.0b	59.2 ± 41.3a
Ayer Hitam, Johor	61.0de	34.9± 38.9cd
Teluk Intan, Perak	55.0ef	36.5 ± 41.2c
Permatang Guntung, Perak	53.0f	19.2 ± 32.0f
Kg. Bakar Arang, Perak	50.7f	21.7 ± 34.5ef
Sepang, Selangor	70.7bc	30.3 ± 31.0de
Kuala Langat, Selangor	90.3a	52.0 ± 38.9b
F-test	***	***

Notes: Non-significant (NS), significant (\*), high significant (\*\*) or highly significant (\*\*\*) at P 0.05, respectively. Means separation within columns and factors by Duncan Multiple Range Test (DMRT) at P 0.05. Mean values in each column with the same letter are not significantly different at P<0.05 according to DMRT.

### Assessments of incidence and severity of Anthracnose disease

Table 2 summarizes the incidence of anthracnose disease in oil palm seedlings. It showed a highly significant difference between locations and incidence of anthracnose disease. The highest incidence was recorded in Kuala Langat, Selangor (45.0%). The least incidence of anthracnose was recorded in six nurseries which is located in Johor and Perak occurring between

8.0% to 14.0%. Table 2 also summarizes the severity of anthracnose disease in oil palm seedlings. It showed a highly significant difference between locations and severity of anthracnose disease. The highest disease severities were recorded in oil palm seedlings at Kuala Langat, Selangor (9.1%) and Teluk Intan, Perak (6.6%). The least disease severity of anthracnose was recorded in 6 nurseries which is located in Johor and Perak occurring between 1.0% to 2.5%.

**Table 2: Disease incidence (DI) and severity (DS) of anthracnose disease in oil palm seedlings at different locations in Peninsular Malaysia.**

Location:	Incidence Disease (%)	Severity Disease (%)
Kluang, Johor	12.7cd	1.7 ± 6.6c
Bkt.Tongkat, Johor	9.3cd	1.0 ± 6.0c
Kahang, Johor	8.5d	1.4 ± 5.8c
Ayer Hitam, Johor	15.0c	2.1 ± 7.7c
Teluk Intan, Perak	14.0cd	6.6 ± 19.2a
Permatang Guntung, Perak	14.0cd	1.7 ± 6.4c
Kg. Bakar Arang, Perak	12.0cd	2.5 ± 8.8c
Sepang, Selangor	26.0b	4.8 ± 13.0b
Kuala Langat, Selangor	45.0a	9.1 ± 13.9a
F-test	***	***

Notes: Non-significant (NS), significant (\*), high significant (\*\*) or highly significant (\*\*\*) at P 0.05, respectively. Means separation within columns and factors by Duncan Multiple Range Test (DMRT) at P 0.05. Mean values in each column with the same letter are not significantly different at P<0.05 according to DMRT.

### Interactions between leaf spot incidences and severities with different oil palm seedling ages

Table 3 summarizes the incidence and severity of leaf spot disease in relation to seedling ages. It showed a highly significant relationship between disease incidences of leaf spot with different stages of seedling ages. Seedling <4 months old score the highest percentage (77.4%) followed by 4-12 months old (72.9%) and seedling >12 months old are most resistance with the lowest score 49.0%.

There is also significant interaction between disease severities of leaf spot with different stages of seedling ages. Seedling with age <4 months old are most susceptible and severely affected (51.0%) followed by seedling age 4-12 months old (40.4%) and seedling >12 months old are most resistance with only 18.40%. This finding is strongly related with the previously study by Turner (1981) which was reported that the oil palm foliar diseases are primarily affected young seedlings up to 3 months old and newly transplanted seedling.

**Table 3: Disease incidence and severity of leaf spot disease in relation to oil palm seedling ages.**

Age of oil palm seedling	Disease incidence (%)	Disease severity (%)
< 4 months old	77.4a	51.0 ± 41.2a
4-12 months old	72.9b	40.4 ± 40.0b
> 12 months old	49.0c	18.4 ± 31.1c
Interaction	***	***

Notes: Non-significant (NS), significant (\*), high significant (\*\*) or highly significant (\*\*\*) at P 0.05, respectively. Means separation within columns and factors by Least Significant Difference (LSD) at P 0.05. Mean values in each column with the same letter are not significantly different at P<0.05 according to LSD.

Table 4 summarizes the incidence and severity of anthracnose disease in relation to seedling ages. It showed a highly significant relationship between disease incidences of anthracnose with different stages of seedling ages. Seedling <4 months old score the highest percentage (21.6%) followed by 4-12 months

old (16.9%) and seedlings >12 months old are most resistance with the lowest score 14.9%. Therefore, there is no significant relationship between age and disease severity of anthracnose disease were recorded, <4 months old (3.5%), 4-12 months old (3.8%) and >12 months old (3.3%).

**Table 4: Disease incidence and severity of anthracnose disease in oil palm seedlings in different range of age.**

Age of oil palm seedling	Disease incidence (%)	Disease severity (%)
< 4 months old	21.6a	3.5 ± 10.6a
4-12 months old	16.9b	3.8 ± 13.0a
> 12 months old	14.89b	3.3 ± 9.3a
Interaction	***	ns

Notes: Non-significant (NS), significant (\*), high significant (\*\*) or highly significant (\*\*\*) at P 0.05, respectively. Means separation within columns and factors by Least Significant Difference (LSD) at P 0.05. Mean values in each column with the same letter are not significantly different at P<0.05 according to LSD.

## Conclusion

The surveys of foliar diseases in nurseries reported that leaf spot and anthracnose diseases were the most abundance in oil palm seedlings. The higher incidence of leaf spot diseases were recorded as compared to anthracnose. It was indicated that leaf spot disease can cause more serious damage to the oil palm seedlings compared to anthracnose disease. However, leaf spot disease appeared to spread more slowly and the damage caused by this disease was less in the oil palm seedlings. Anthracnose disease can cause severe symptom of chlorosis, wilting and stunted in growth of oil palm seedling. As for the interactions of leaf spot and anthracnose disease with age of oil palm seedlings, the study found highly significant correlation by both. Therefore, as the seedlings become older, the resistance to these diseases was found to increase.

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