ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF *Phytophthora palmivora* FROM COCOA DISEASES

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ABSTRACT - *Phytophthora spp. are responsible for some serious diseases of cocoa including black pod, stem canker, seedling blight, leaf blight and chupon wilt. Eight species of Phytophthora have been isolated from diseased cocoa worldwide, even though only three species cause most losses in cocoa production which are P. palmivora, P. megakarya and P. citropthora. In this study, seven isolates of Phytophthora sp. were isolated from black pod, stem canker and seedling blight collected from Cocoa Research and Development Centre (CRDC) Jengka, Pahang, Malaysia. All isolates were incubated on potato dextrose agar (PDA), corn meal agar (CMA) and carrot agar (CA) to observed their macro-morphology on different media. They were then identified using their micro-morphological characteristics and it was concluded that all of the isolates are P. palmivora. The growth rate of all isolates were faster when incubated on CA, ranged from 1.17 – 1.42 mm/day, compared to CMA (1.07 – 1.20 mm/day) and PDA (0.43 – 0.75 mm/day). The colony morphology of P. palmivora in PDA is white fluffy with concentric rings and irregular margins, while on CMA is white and scanty fluffy colonies. Meanwhile, all isolates produced white dense rosettes of hyphae when grew on CA.*

Keywords*:* cocoa, Phytophthora palmivora, black pod, stem canker, seedling blight

INTRODUCTION

Phytophthora is a 'fungus-like' plant pathogen under the kingdom Chromista. It is oomycetes and synonymous as a 'plant destroyer'. One of the most devastating species of *Phytophthora* is *P. palmivora* which cause significant disease losses to global cocoa production. The infection of *P. palmivora* on the pods significantly reduces the number of cocoa beans and infected beans are not suitable for processing and must be discarded. This has led to cocoa yield reduction and the losses can reach up to 100% of annual production if no control measures are taken (Ndoumbe-Nkeng *et al*., 2004). In Malaysia, *P. palmivora* had cause three different cocoa diseases which are black pod, stem canker, and seedling blight.

The symptom of black pod is formation of pod lesions which begin as dark, hard and small spots on any part of pod and can spread the entire pod surface within a few days (Figure 1A). Meanwhile, stem canker cankers effect either on the main trunk, jorquettes or fan branches. Initial symptom of stem canker is the presence of a greyish brown water soaked lesion on the outer bark. Then, a reddish brown liquid exudes out from these lesions, which later dries up to form rusty deposits. Due to rotting, the tissues beneath the outer lesion show reddish brown discoloration (Guest, 2007) (Figure 1B).

Seedling blight symptoms is yellow spots that enlarge and coalesce with neighbouring spot to form wide necrotic lesions (Figure 1C) (Harni *et al*., 2020).

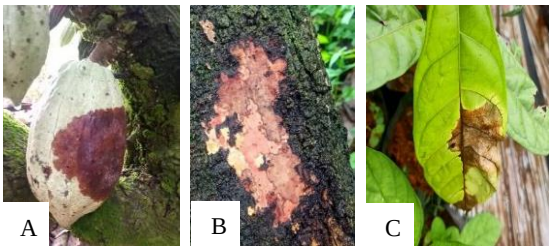

Figure 1: The symptoms of black pod (A), stem canker (B) and seedling blight (C).

This paper reports the isolation results and identification of *Phytophthora* spp. from cocoa diseases samples collected from Cocoa Research and Development Centre (CRDC) Jengka, Pahang, Malaysia. With identification of correct species, hopefully it will be able to recommended disease control strategy in cocoa plantation.

MATERIALS AND METHODS

Sample collection and Phytophthora isolation

The sample of black pod, stem canker and seedling blight were collected from cocoa field at CRDC Jengka, Pahang, Malaysia. The samples were kept in separate paper envelopes and brought to the

laboratory for further studies. From each sample, tissue pieces (5×5 mm) were excised from the margin of the lesions and surface sterilized using 0.5% sodium hypochlorite solution for 30 seconds and rinsed three times with sterile distilled water. The drained tissue pieces were dried on sterilized tissue paper and placed in potato dextrose agar (PDA). The PDA plates with diseased tissues were incubated at 27-30°C for 24-48 hours. Hyphal tips growing from the diseased tissues were cut and transferred to new PDA plates and incubated for 7 days to obtain a pure culture (Latifah *et al.,* 2018). Fungal isolates were identified by comparing colony growth patterns on PDA, cornmeal agar (CMA) and carrot agar (CA).

Morphological Identification

Phytophthora isolates were identified into species levels based on their macro and micro-morphological characteristics (Drenth and Sendall, 2001). The pure isolates that were grown on PDA, CMA and CA were recorded for their colony morphology and the growth rate was measured according to identification method by Diba *et al.,* (2007). Micro-morphological characteristics of *Phytophthora* isolates such as sporangia, oogonia, hyphae, and chlamydospores were observed from culture discs floated in sterile distilled water and examined by light microscopic (Rax Vision, model Y-100).

RESULT AND DISCUSSION

A total of seven isolates of *Phytophthora palmivora* have been successfully recovered from the sample of black pod, stem canker and seedling blight collected from CRDC Jengka. They were primarily selected based on the pigmentation and colony features on PDA after three days of incubation. The lumpy growth of colony with whitish colour were subcultured on PDA media to get a pure culture of *Phytophthora* sp. The pure cultures were then transferred on different media PDA, CMA and CA to observe their macro-morphology on different media (Table 1).

Table 1: Macro-morphological characteristics of *P. palmivora* isolates on potato dextrose agar (PDA), corn meal agar (CMA) and carrot agar (CA).

Isolate no.	Diseased sample	PDA		CMA		CA	
		Growth rate (mm/day)	Colony morphology	Growth rate (mm/day)	Colony morphology	Growth rate (mm/day)	Colony morphology
PC 62	Black pod	0.75	White fluffy colonies with concentric rings and irregular margins	1.16	White and scanty fluffy colonies	1.17	White dense rosettes of hyphae
PC 63	Black pod	0.74	White fluffy colonies with concentric rings and irregular margins	1.17	White and scanty fluffy colonies	1.21	White dense rosettes of hyphae
PC 64	Black pod	0.43	White fluffy colonies with concentric rings and irregular margins	1.11	White and scanty fluffy colonies	1.18	White dense rosettes of hyphae
PC 65	Black pod	0.71	White fluffy colonies with concentric rings and irregular margins	1.07	White and scanty fluffy colonies	1.19	White dense rosettes of hyphae
PC 66	Black pod	0.70	White fluffy colonies with concentric rings and irregular margins	1.20	White and scanty fluffy colonies	1.42	White dense rosettes of hyphae
PC 67	Seedling blight	0.57	White fluffy colonies with concentric rings and irregular margins	1.10	White and scanty fluffy colonies	1.21	White dense rosettes of hyphae
PC 68	Stem canker	0.67	White fluffy colonies with concentric rings and irregular margins	1.19	White and finely radiate colonies	1.21	White dense rosettes of hyphae

The colony morphologies of *P. palmivora* were similar to previous descriptions by Umayah and Purwantara (2006). On PDA, all isolates of *P. palmivora* examined produced fluffy colonies with concentric rings and irregular margins (Figure 2). The growth rate of *P. palmivora* also very slow (0.43 – 0.75 mm/day) on PDA. Slight variations in growth rates and patterns were noted among isolates when they grow on CMA and CA. All isolates grew most profusely on CA with growth rate range 1.17 – 1.42 mm/day and produced white dense rosettes of hyphae. In contrast, isolates of *P. palmivora* grew in CMA produced colonies with finely radiate growth patterns with growth rate ranged between 1.07 – 1.20 mm/day.

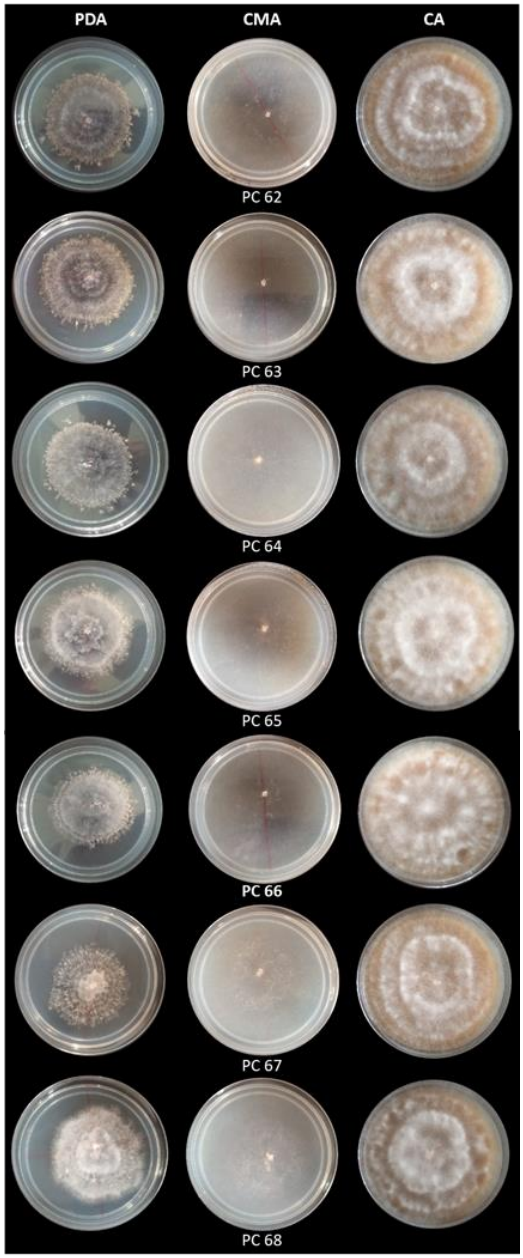

Figure 2: Colony morphology of 7 isolates of P. palmivora on PDA, CMA and CA media at 7th day incubation

Micro-morphological observation was carried out by doing slide cultures to examine the microscopic characteristics of *P. palmivora*. The observed characteristics are sporangia, oogonia, hyphae, and chlamydospores (Wongwan *et al.*, 2021). Sporangia of *P. palmivora* were produced in clusters sympodial, papillate and have ovoid form (Figure 3A-B). Morphological hyphae showed that various types of hyphal swellings (Figure 3C) were exist within isolates. According to Kuswinanti *et al.* (2020), hyphal swellings containing many nuclei (coenocytic) and no septate, generally were coralloid, coarse, torulose, smooth, regular, irregular and loops. They formed a branch point followed by a globular round swell with a thick cell wall called as chlamydospore (Figure 3D). All isolates of *P. palmivora* in this study were capable of producing abundant chlamydospores on PDA, CMA and CA. In contrast, other *Phytophthora* species recorded in Malaysia such as *P. meadii*, *P. hevea* and *P. botryosa* are not or rarely produce chlamydospores in media (Latifah *et al*., 2018). *P. palmivora* isolates also produced spherical oogonia with amphigynous antheridium (Figure 3E).

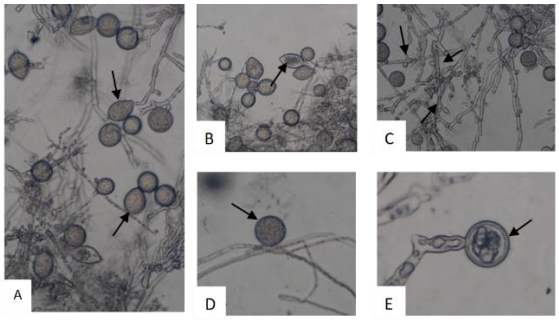

Figure 3: Micro-morphological structure of P. palmivora. A-B: Sporangia with spores; C: Hyphal swelling; D: Chlamydospore; E: Oogonium.

CONCLUSION

Seven isolates that obtained from CRDC Jengka showed a relatively same micro and macro-morphological characteristics. All of them are *P. palmivora*, the pathogen of black pod, stem canker and seedling blight disease in Malaysia.

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