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Isolation, Identification, and Pathogenicity Test from *Fusarium oxysporum* f.sp. *cubense* Causing Banana Wilt

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This study aimed to isolate and identify *Fusarium oxysporum* f.sp. *cubense* from banana wilt and performed pathogenicity test on the banana leaves by detached leaf method. The fungus isolated from vascular strands from inner banana pseudostem of infected plant. The isolate showed colonies on PDA with a variable morphology, hairy to cottony and the colony color from whitish to yellow, pink or purple shades. Morphological characteristics of the isolate was observed under the light microscope and showed the fungus produces macroconidia, microconidia, and chlamydospores. The macroconidia are nearly straight, slender and thin-walled with 3 – 4 septa, a foot-shaped basal cell and a curved tapered apical cell. Microconidia are single celled, oval to kidney-shaped and are produced in false heads. Chlamydospores are formed as intercalary and terminal ones. Pathogenicity test proved this fungus causing wilt symptom thereafter it was inoculated on banana leaves.

Keywords: Banana wilt, *Fusarium oxysporum* f.sp. *cubense*

Introduction

Fusarium oxysporum is a pathogenic fungus common in soils around the world, cause of fusarium wilt of several agricultural and horticultural crops. This fungus invades the root system and the xylem vessel. Wilting is most likely caused by a combination of pathogen activities that comprise part of fungus, toxin production, and host defense responses, including production of gels, gums and tyloses, and vessel crushing by proliferation of adjacent parenchyma cells. Forms of *Fusarium oxysporum* are divided into forma speciales based on the specific hosts (O'Donnell *et al*. 1998).

Fusarium wilt of bananas, also known as Panama disease is caused by *F. oxysporum* f.sp. *cubense* (Foc). It only infects banana and relatives but it may survive as a parasite of non-host weed species. Three species of grass,

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Paspalum fasciculatum, *Panicum purpurascens* (*Brachiaria mutica*), and *Ixophorus unisetus* and *Commelina diffusa* have been implicated (Cheraghian A., 2016). There are 4 distinct races of this fungus, and one is divided into two strains. Tropical race 4 (TR4) is the most serious as it affects a large number of varieties, including the popular Cavendish. Infection due to *F. oxysporum* f.sp. *cubense* triggers the self-defense mechanisms of the host plant causing the secretion of a gel occurs followed by formation of tylose in the vascular vessels which blocks the flow of water to the upper part of the host plant (Stover, R.H., 1972). Two types of external symptoms are yellow leaf syndrome and green leaf syndrome (Stover, 1962; Pérez-Vicente, 2004). Yellow leaf syndrome is the classic symptom of Fusarium wilt on banana, the oldest leaves turned to a faint off-green to pale-yellow beginning with patches or streaks at the base of the petiole, close to the midrib and hang down, forming a skirt of death leaves around the pseudostem. Green leaf syndrome is contrast to the yellow leaf syndrome. The leaves remain green until the petioles bend and collapse. Other symptoms include irregular, pale margins on new leaves and the wrinkling and distortion of the lamina. Internodes may also shorten (Stover, 1962, 1972; Jones, 1994; Moore *et al.*, 1995). The characteristic internal symptom of Fusarium wilt is vascular discoloration. Discoloration varies from pale yellow in the early stages to dark red or almost black in later stages. The oldest and outermost pseudostem leaf sheaths in the early stages of disease to heavy discoloration throughout the pseudostem and fruit stalk in the later disease stages (Cheraghian A., 2016).

The leaf symptoms of Fusarium wilt can be confused with bacterial wilt. Fusarium affected disease symptoms progress from older to younger leaves. In plants affected by bacterial, the wilting can begin with any leaf and bacterial ooze can be observed on exposed cut surfaces (Ploetz, R. C. 2000).

As diseased plants die, the fungus grows out of the xylem into surrounding tissues. Many chlamydospores are formed which are returned to the soil as the plant decays. The fungus can survive for more than 30 years as chlamydospores in infested plant debris or in the roots of alternative hosts (Stover, 1962).

Materials and methods

Fungal isolate

To isolate *Fusarium oxysporum* f.sp. *cubense* from infected banana plant by the tissue transplanting technique. Cut the discoloured vascular strands from inner banana pseudostem into small pieces approximately 0.5 cm. and then rinsed 2 – 3 times with distilled water after soaked in 1% Sodium hypochlorite

for 2 – 3 min. Place the tissue sections in Water Agar (WA). When mycelium grown from the tissue sections were transferred to Potato Dextrose Agar (PDA) and incubated at 25 ºC for 7 – 10 days, until get pure culture.

Cultural and morphological identification

Isolates of *Fusarium oxysporum* f.sp. *cubense* were identified according to their cultural appearances and morphological characteristics such as microconidia, macroconidia, chlamydospores, etc.

Pathogenicity test

The banana leaves were detached from healthy plant, then surface sterilized with 70% ethyl alcohol. Each leaves were cut into 5 cm x 5 cm pieces and placed with the upper leaf surface in a sterile petri dish containing filter paper moist with distilled water to maintain high humidity. Wounding by sterile needle at the center of leaf pieces for easy access of the fungus.

Mycelial disc cut from 7 days old cultures of *Fusarium oxysporum* were inoculated on the wound of leaf pieces. Noninoculated controls were inoculated with an agar plug without the fungus. The petri dishes were then incubated at room temperature for 1 week. Six replications of each treatments.

Results and discussion

The cultural appearances were observed on potato dextrose agar (PDA), colonies have a variable morphology. It can be hairy to cottony and the color from whitish to yellow, pink or purple shades (Fig. 1A).

The fungus produces macroconidia, microconidia, and chlamydospores. The macroconidia are nearly straight, slender and thin-walled with 3 – 4 septa, a foot-shaped basal cell and a curved tapered apical cell (Fig. 1C). Microconidia are single celled, oval to kidney-shaped and are produced in false heads (Fig. 1D). Chlamydospores are formed in hyphae or conidia, usually globose single or in pairs with a coarse protective wall (Fig. 1B).

Infected leaves turned yellow. After that under and around the mycelial disc becomes brown (Fig. 2A). The noninoculated controls showed no symptoms, leaves remained green (Fig. 2B).

Pathogenicity tests showed that the fungus caused symptoms on banana leaves. As reported by several studies such as in Taiwan, Malaysia (Ong Kim Pin, 1996) and Indonesia (Hermanto, C. *et al*, 2011) which found and described this fungus is pathogenic to banana caused, by *Fusarium oxysporum* f.sp. *cubense*.

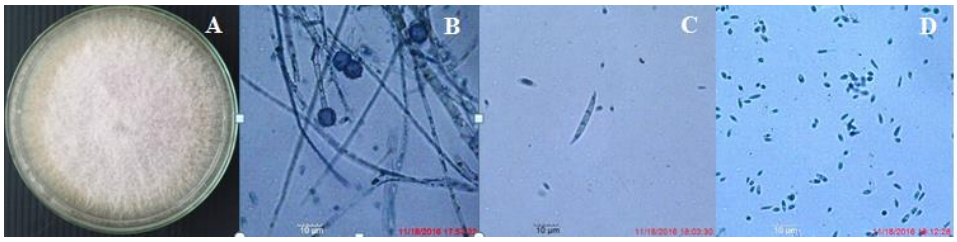

Fig. 1. Reproductive structures of *F. oxysporum* f.sp. *cubense*.A; *F. oxysporum* f.sp. *cubense* in PDA media. B; Chlamydospores C; Macroconidia D; Microconidia

Fig. 2. Pathogenicity test A; Control B; *F. oxysporum* f.sp. *cubense*

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