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Full Length Research Paper

Occurrence and Pathogenicity of *Fusarium* spp. on the potato tubers in Malaysia

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Dry rot of potato caused by *Fusarium* species is a common and commercially important disease of potato tubers found in all production areas of the world, including Malaysia. The objective of this study was to identify *Fusarium* species associated with wet market potatoes and their pathogenicity on potato tubers in Malaysia. In this survey, 65 *Fusarium* strains were isolated and identified from diseases decayed potato's segments collected from different regions in Malaysia. All of these 65 isolates, which belong to two morphotypes of *Fusarium solani* species complex (FSSC) and *Fusarium oxysporum,* were evaluated to study their pathogenicity on healthy potato tubers. The tubers rot symptoms were observed on the 21st day after inoculation of *F. solani* and *F. oxysporum* strains on the tubers tested. In the tubers inoculation tests, lesion sizes were quite variable; therefore, the measurement was done to compare the depth and width of lesion expansion among the strains. The results of the pathogenicity test revealed that *F. solani* isolates FSO4, FSO12 (morphotype I), FSO18 (morphotype II) and *F. oxysporum* isolates FOX4 and FOX16 were strongly pathogenic to inoculated potato tubers. The inoculated fungi were re-isolated from the diseased potato tubers to prove the Koch's postulates. The present study showed that two morphotypes of *F. solani* were associated with tuber rot of potato in Malaysia. This is the first comprehensive report on identity and distribution of major pathogenic fungi causing potato dry rot in Malaysia.

Key words: Occurrence, pathogenicity, dry rot, potato, Malaysia.

INTRODUCTION

Annually it is estimated that over 22 million ha of cultivable lands of the world, are under potato (*Solanum tuberosum* L.) cultivation that producing more than 300 million tons of tuber and is considered to be a major diet ranking fourth after wheat, barley and rice (Anonymous, 2004; Khorasani et al., 2008). Potato is the most important daily food in Malaysia like many countries in the Southeast Asia (FAO, 2000).

The most important pathogens that cause dry rot in potatoes in the entire world are *Phytophthora infestans* (Fry, 2007), *Erwinia caratovora* ssp. *caratovora*, *E. caratovora* ssp. *artroseptica* (Zimnoch-Guzowska et al., 2000) and *Fusarium* spp. (Secor and Salas, 2001; Sadfi et al., 2002). *Fusarium* spp. is ubiquitous pathogens that can cause a variety of diseases in many agricultural, horticultural and forestry crops (Logrieco et al., 2002; Desjardins, 2006). *Fusarium* dry rot is an economically important disease worldwide, which is characterized by shrunken, collapsed diseased tissues that are usually dry. *Fusarium* spp. cause tuber rot, with severe reductions in crop yield, often estimated 25% annually (Lui and Kushalappa, 2002; Slininger et al., 2004). *Fusarium* dry rot of potato tubers is caused by several species of *Fusarium*, the most important of which are *F. solani*, *Fusarium sambucinum* and *F. avenaceum* (Nasr-Esfahani, 1998; Cullen et al., 2005). In North America and parts of Europe, *F. sambucinum* and *F. coeruleum* (Libert) Sacc. are considered to be the most significant causal agent of tuber dry rot (Secor and Salas, 2001). In Britain, *F. coeruleum* (Libert) Sacc. is more prevalent (Hide et al., 1992) and in Iran and South Africa, *F. solani* is the main causal species of potato dry rot (Theron and Holz, 1989; Nasr-Esfahani, 1998). *Fusarium* spp. can also produce mycotoxins which relevant to animal and human health and potato may become contaminated with different *Fusarium* toxins (Marasas et al., 1984). El-Banna et al. (1984) reported that trichothecenes such as deoxynivalenol (DON), acetyl-DON, and HT-2 toxin were

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Table 1. Place of sample collection, name of the pathogen identified and measurement of lesion expansion and rank of the tubers' condition 21 days after incubation from each sample.

Place of sample collection	Pathogen identified	Strain no	Measurement (cm)		Condition
			Width	Depth	
Pulau Penang	*F. solani*	USM7364	4.0	3.3	4
	F. solani	USM7391	0.5	0.7	2
	F. solani	FSO12	4.0	3.3	4
	F. solani	FSO22	1.8	1.5	3
	F. oxysporum	FOX16	4.0	3.3	4
	F. solani	FSO38	1.8	1.5	3
	F. oxysporum	FOX4	4.0	3.3	4
	F. solani	FSO19	0.7	0.5	2
	F. oxysporum	FOX1	0.0	0.0	1
	F. oxysporum	FOX11	0.7	0.5	2
Perak	*F. solani*	FSO44	0.7	0.5	2
	F. solani	FSO36	1.8	1.5	3
	F. solani	FSO66	0.0	0.0	1
	F. solani	FSO18	4.0	3.3	4
	F. oxysporum	FOX17	4.0	3.3	4
	F. oxysporum	FOX19	0.7	0.5	2
Sarawak	*F. oxysporum*	FOX23	0.0	0.0	1
	F. solani	FSO4	4.0	3.3	4
	F. oxysporum	FOX22	0.0	0.0	1
Terengganu	*F. solani*	FSO9	0.7	0.5	2
Negeri	*F. solani*	FSO28	1.8	1.5	3
Sembilan	*F. solani*	FSO53	0.0	0.0	1
	F. oxysporum	FOX21	0.7	0.5	2

1: No symptom, 2: minor dry rot symptom, 3: moderate dry rot symptom, 4: severe dry rot symptom.

produced in potato tubers inoculated with *F. solani* var. *coeruleum.* Therefore, the current study has been performed to pursue the following goals: (1) to determine the contaminating *Fusarium* spp. in potatoes from the wet market located in different regions in Malaysia, and (2) to check the pathogenicity of *Fusarium* species on tubers of potato in Malaysia.

MATERIALS AND METHODS

Sampling, isolation and identification of *Fusarium* spp

Infected potatoes were collected from the wet market in different regions of Malaysia (Table 1). In the lab, the potatoes were rinsed with several changes of sterile distilled water and after desiccation by filter paper. The potatoes were cut into small blocks (1.5 cm) prior to surface sterilization using 70% alcohol for 1 min. Then, the pieces tubers were sterilized with 1% sodium hypochlorite (NaClO) (3 min) and then being rinsed in 3 changes distilled water and ultimately air dried. All the sterilized pieces were placed onto general medium (water agar) (Burgess et al., 1994) and pentachloronitrobenzene agar (PPA) plates, a selective medium for *Fusarium* (Nash and Snyder, 1962). The plates were incubated at 25°C for 24 h. The resulting single-spore *Fusarium* colonies were transferred to fresh potato dextrose agar (PDA) plates and maintained at 4°C for further studies. To study the growth rates and pigment production of *Fusarium* spp. all strains were transferred onto PDA plates and incubated at 25°C. For microscopic observations, all strains of *Fusarium* were transferred to carnation leaf agar (CLA) (Fisher et al., 1982), spezieller nahrstoffarmer agar (SNA) (Nirenberg, 1976), and potassium chloride agar (KClA) (Fisher et al., 1983) medium. The species were identified on the basis of macroscopic characteristics. Identification of species was based on species description of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

Pathogenecity test of the selected strains

The healthy potato tubers (*Solanum tuberosum* L.) were used in this experiment. Initially, Tubers appearing healthy and uniform in size (100 - 120 g) were selected and washed to remove excess soil, surface sterilized in 0.5% sodium hypochlorite solution for 10 min and rinsed in 3 changes of sterile distilled water (Lui and Kushalappa, 2002; Lui et al., 2005) and then air dried. Then the tubers were wounded with a cork borer with a diameter of 4 mm to a depth of 4 mm (Choiseul et al., 2007; Peters et al., 2008a, 2008b) and inoculated with all of the fresh *Fusarium* mycelium by putting CLA blocks (7 mm). All the wounded potato tubers were wrapped in black polyethelene bags (Manici and Cerato, 1994; Lui and

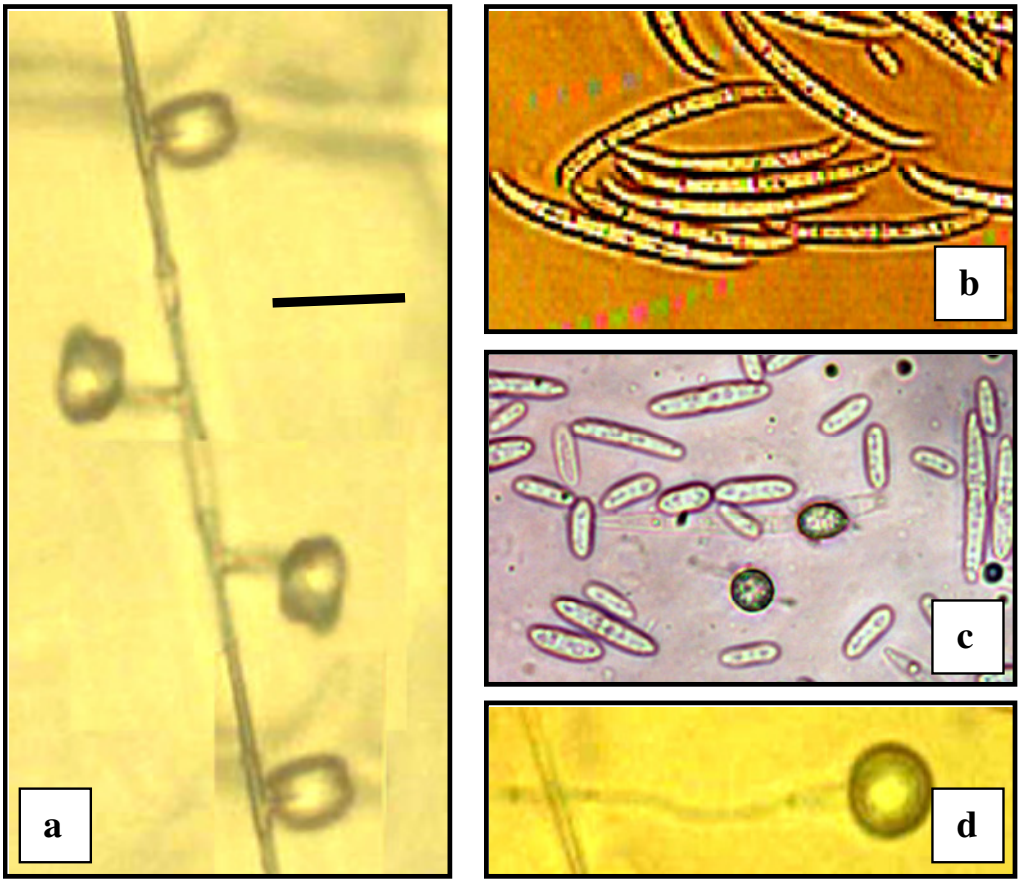

Figure 1. Morphological characters of *F. oxysporum*. a = Conidiophores, b = Macroconidia, c = Microconidia, d = Chlamydospore (scale bar = 20 µm).

Kushalappa, 2002) and incubated in the dark at 20°C for 3 weeks. For control we used *F. solani* isolates USM7364 and USM7391 maintained at the *Fusarium* Culture Collection Unit, Universiti Sains Malaysia (USM) were used as standard virulent and hypovirulent isolates, respectively, in this experiment.

RESULTS

A total of 65 *Fusarium* isolates were recovered from 50 diseased tubers potato samples. All the tubers potato samples were found positive for *Fusarium* species. Based on their morphological characteristics, the isolates were identified into two morphotypes of *Fusarium solani* species complex and *F. oxysporoum* (Table 1). *F. oxysporum* strains were identified based on their morphological characterization. This species showed floccose growth, abundant and pale violet aerial mycelia. Pigmentation of reverse colony is pale violet, short monophialides conidiogenous cells, macroconidia usually 3-septate and thin walled, the apical cell is short and basal cell is notch to foot shape, microconidia usually are no septate oval to elliptical, chlamydospores usually singly or in pair (Figure 1). *F. solani* species complex are also famed with its long phialides bearing false head (Figures 2 and 3). This character commonly observes *in situ* on CLA media. In this study two morphotyes of *F. solani* were isolated from potato tubers.

In morphotype I of *Fusarium solani,* pigmentation of reverse colony is red, macroconidia mostly 5 septate, 37 - 58 × 3.5 - 4.8 µm and thick walls. The apical cell of macroconidia is tapered whereas the basal cell is pedicellate and foot shape. Microconidia are ellipsoid to truncate and clavate 0 - 2 septate. Chlamydospores are with smooth to rough outer walls (Figure 2). In morphotype II of *F. solani,* pigmentations of reverse colony are cram, macroconidia 3 - 4 septate but mostly 3 septate and 30 – 42 × 4.2 - 5.8 µm. Apical cell is round curved, short and basal cell is nearly notch shape. Microconidia were oval and kidney-shaped with 0 or 1 septa. Chlamydospores usually rough outer walls (Figure 3).

In the tuber potato inoculation tests, lesion sizes were quite variable and ranged from 0.5 to 4 cm^2. The standard virulent, *F. solani* isolate USM7364 and the standard hypovirulent, *F. solani* isolate USM7391 caused lesions of 4.0 and 0.5 cm^2, respectively. Therefore, based

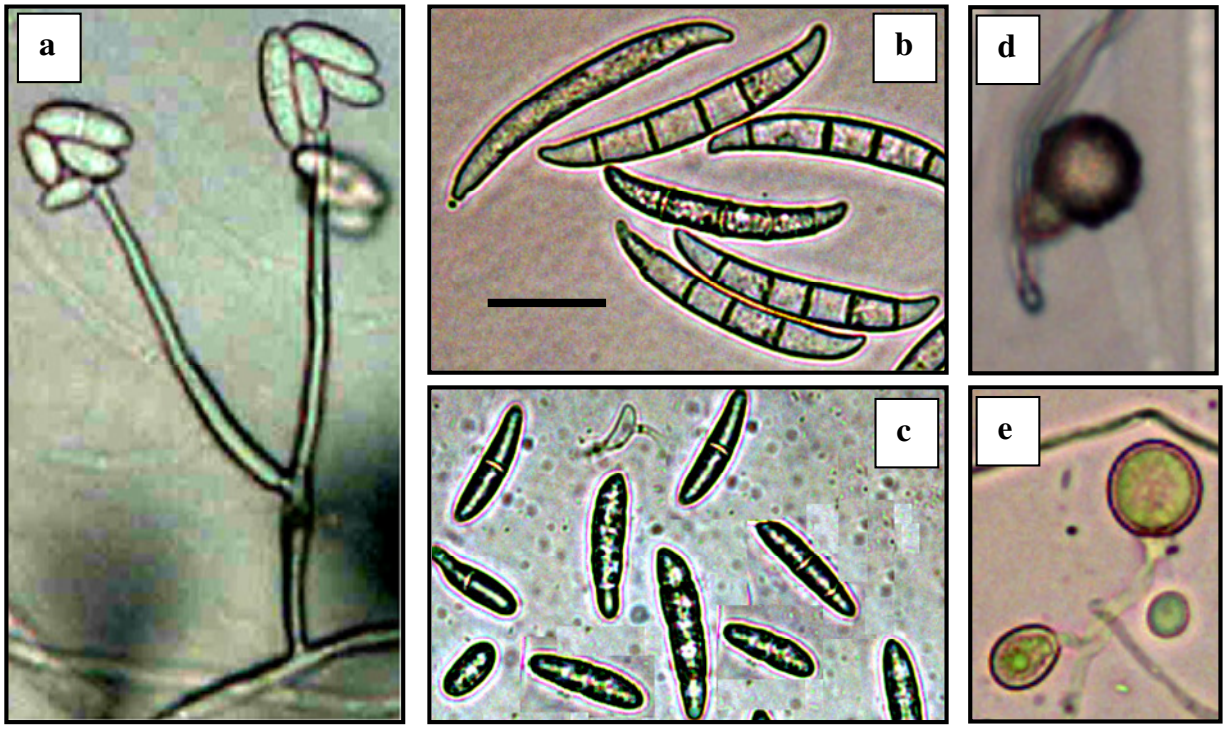

Figure 2. Morphological characters of *F. solani* (Morphotype I). a = Conidiophores, b= Macroconidia, c = Microconidia, d - e= Chlamydospore (scale bar = 25 µm).

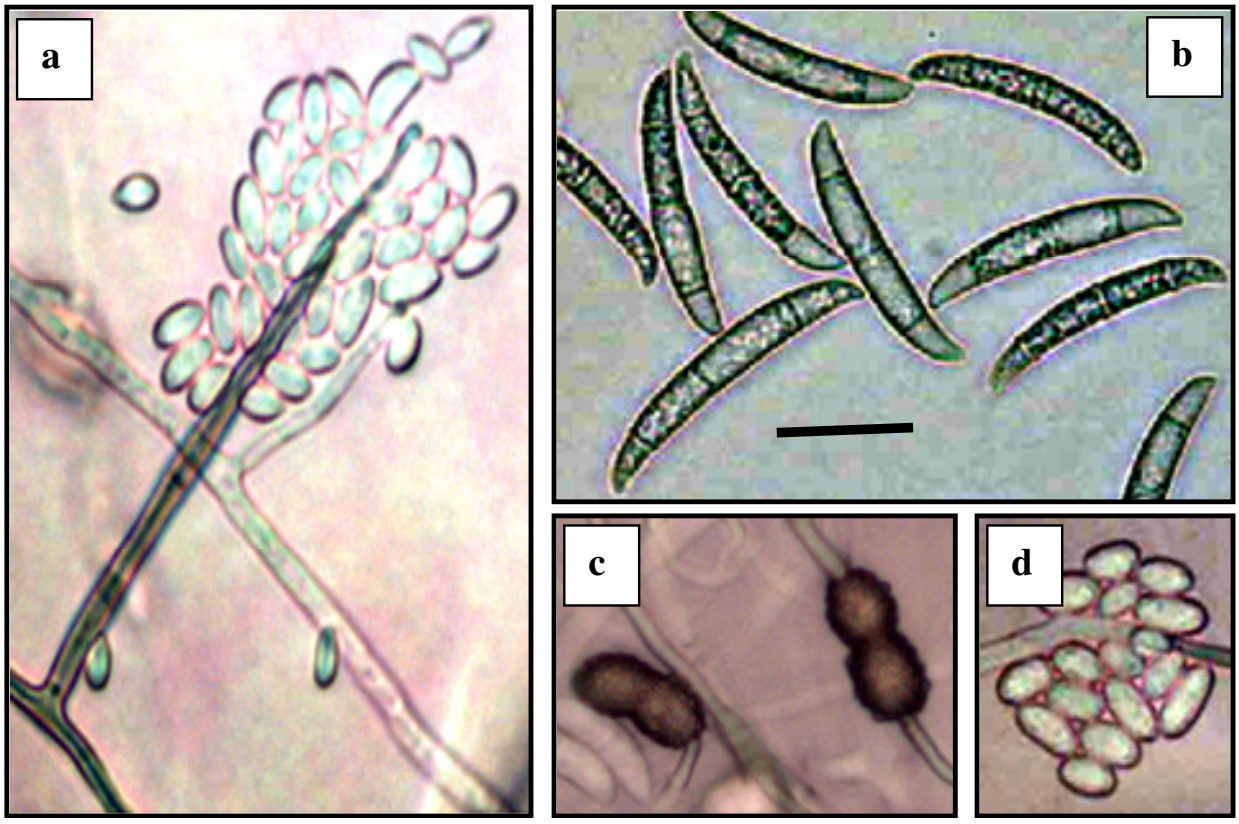

Figure 3. Morphological characters of *F. solani* (Morphotype II). a = Conidiophores, b = Macroconidia, c = Chlamydospores, d = Microconidia (scale bar = 25 µm).

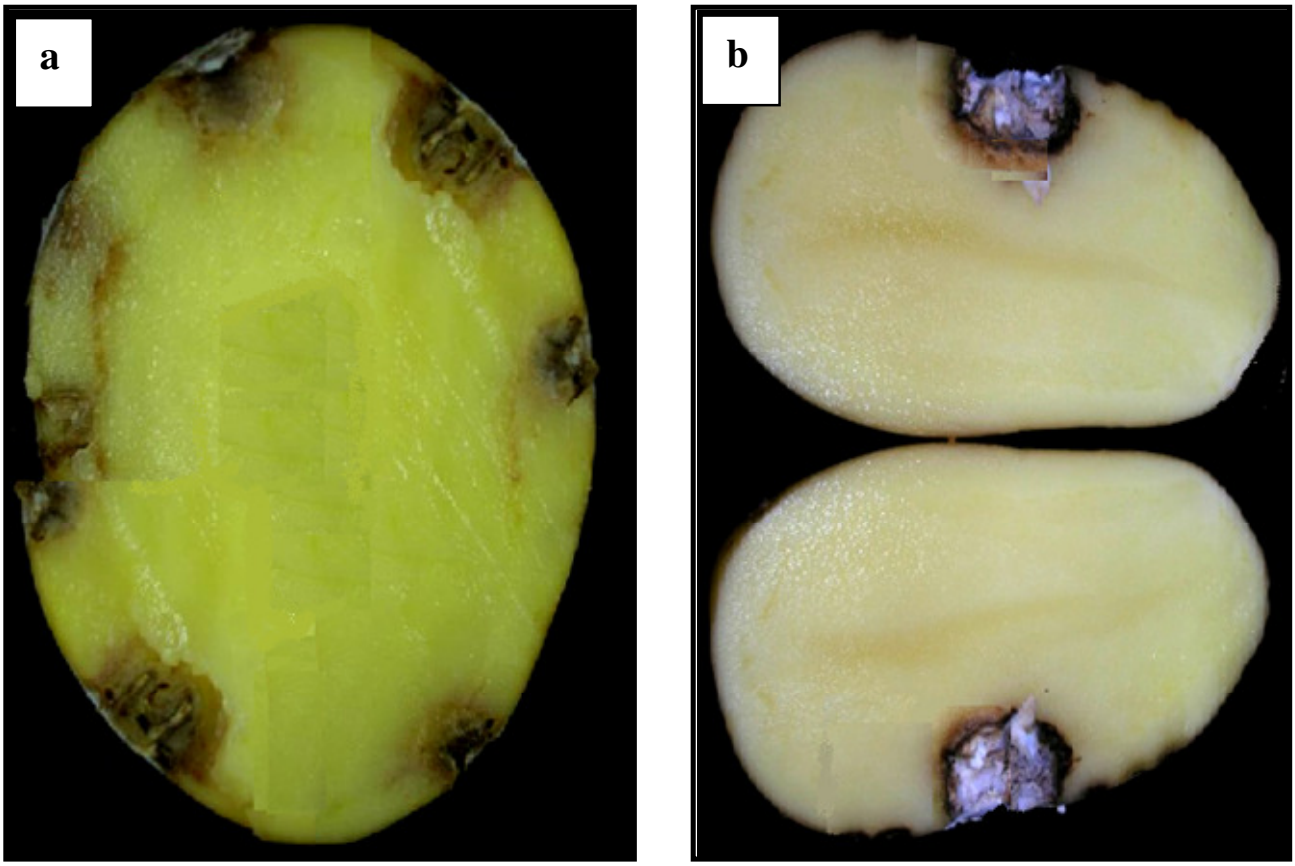

Figure 4. Diseases caused by pathogenic *Fusarium* spp. on potato. a = dry rot on potato by *F. oxysporum*, b = dry rot on potato by *F. solani* species complex.

on these two lesion sizes, the pathogenicity of *Fusarium* spp. isolates were divided into four groups: virulent (4.0 × 3.3 cm^2), moderately virulent (1.8 × 1.5 cm^2), hypovirulent (0.5 × 0.7 cm^2) and non virulent (0.0 - 0.0 cm^2). All the inoculated tubers were cut through the wound to observe the lesion expansion. Measurement consists of the lesion's depth and width was taken and recorded. Development of brownish lesions on the all of the tubers was observed after 3 weeks. *F. solani* isolates FSO4, FSO12 as morphotype I and FSO18 as morphotype II were caused lesions of 4.0 × 3.3 cm^2 and considered as virulent group. The results of the pathogenicity test revealed that *F. solani* isolates FSO22, FSO28, FSO36 and FSO38 as morphotype II were moderately pathogenic to inoculated potato tubers and considered as moderately virulent group and isolates FSO44, FSO9 and FSO19 as morphotype I, caused discoloration, necrosis and lesion of the tubers and therefore according to Table 1 was considered as hypovirulent group. *F. solani* isolate FSO53 and FSO66 as morphotype I three weeks after inoculation showed the tubers in the inoculation site generally showed no external symptoms and was considered as nonvirulent group (Table 1). The results of the pathogenicity test revealed that *F. oxysporum* isolates FOX4, FOX16 and FOX17 three weeks after inoculation showed inoculated potato tubers generally caused the most severe symptoms and *F. oxysporum* isolates FXO11, FX019 and FXO21 caused discoloration and necrosis of the potato tubers and therefore Table 1 was considered as virulent and moderate virulent groups, respectively whereas isolates FOX22 and FOX23 generally showed no external symptoms. The inoculated fungi were consistently isolated from the diseased plants again to prove Koch's postulates, but not from control plants and non virulent isolates (Figure 4).

DISCUSSION

Fusarium species are ubiquitous in soil and may exist as saprophytes or pathogens in plant tissues and residue or as opportunistic pathogens awaiting stress in their host (Palmer and Kommedahl, 1969). Since, young developping potato crops are more susceptible to injury by *Fusarium*. Accurate identification of the species is necessary in order to develop proper management practices to control the pathogenic *Fusarium* species. In our study identification of *Fusarium* species and their

pathogenicity on potato was investigated from the wet market in different regions of Malaysia. The fungal isolation assays made on potato tubers samples collected throughout the Pulau Penang, Selangor, Johor, Perak, Perlis, Sarawak, Terengganu, Negeri Sembilan and Kedah provinces clearly indicate that the members of *F. solani* species complex and *F. oxysporoum* could be pathogenic to the potato and suggested that *F. solani* could be the main causal agent dry rot in potatoes. Several studies have shown that *F. solani* can be readily isolated from potato tubers and according this subject, results obtained in this study are in agreement with the previous findings (Hide et al*.,* 1992; Hansen et al., 1996; Lynch et al., 2003). *F. solani* is one of the most frequently found *Fusarium* species on European, North America and Asia (Manici and Cerato, 1994; Ershad, 1995; Secor and Salas, 2001). In this study we also identified *F. solani* isolates in all of the regions of Malaysia. In this survey the member of *F. oxysporoum* also comprised the most frequencies after *F. solani* species complex that were very important in pathogenicity assay. This result is in agreement with other international studies in the USA, Canada, Argentina (Manici and Cerato, 1994; Song et al., 2008). Environmental conditions and saprobic ability of *Fusarium* species are important attributes in determining their successful distribution and colonization in the soil niches (Sangalang et al., 1995). Also one possible explanation to these facts is that type of soil and rainfall determines the availability of water for fungi, to allow the spores to germinate and other activities. On the other hand the weather in Malaysia is warm with high humidity. This condition was suspected to be one of the factorsdetermining type of species confine to dry rot in this region. Of course, *F. oxysporum* is one of the most frequently found *Fusarium* species on potato where it is more common in wet and warm climate of Italy (Manici and Cerato, 1994). So, our study indicates an agreement with the previous literatures (Manici and Cerato, 1994). Besides, a study done by Song et al. (2008) also cited that *F. oxysporum* is the predominant species causing dry rot. These findings are parallel with this study since *F. oxysporum* was observed to be pathogenic besides of *F. solani*.

This study concludes that *Fusarium* spp. especially *F. solani* species complex and *F. oxysporoum* can cause dry rot on potato crop and may produce mycotoxins which have impact on human and animal health. The occurrence of mycotoxins in potato by *Fusarium* species is of great concern worldwide and their presence in processed feeds and foods seems unavoidable (El-Banna et al., 1984; Kim and Lee, 1994).

To our knowledge, this is the first report of potato tuber dry rot caused by two morphotypes of *F. solani* species complex in Malaysia and this is the first comprehensive report on identity, pathogenicity and distribution of *Fusarium* spp. from potato in Malaysia. Also, the strains of *F. solani* and *F. oxysporum* tested in this study were found to be weakly pathogenic to tubers. From several studies, it has been confirmed that *F. oxysporum* have antagonistic and biologically control potential against a diversity of soil borne pathogens (Marley et al., 1999; Mandeel and Baker, 1991; Nagao et al., 1990; Blok et al., 1997). We believe that this study will serve as a basis for the further identification of *Fusarium* species using molecular techniques and developing proper management strategies to control *Fusarium* diseases by non-pathogenic strains and reduce the risks of mycotoxin contamination. Mycotoxin profiles produced by these species are under progress.

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