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Diversity of *Fusarium* species Isolated from Soil Cultivated with Cucurbits within East Coast, Peninsular Malaysia

Siti Nordahliawate M. S.[1*], Nur Ain Izzati M. Z.[2], Nur Azlin A.[1] and Salleh B.[3]

[1]Department of Agrotechnology,
Faculty of Agrotechnology and Food Science,
Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu, Malaysia
[2]Biology Department, Faculty of Science,
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]School of Biological Science,
Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[*]E-mail: dahliasidique@umt.edu.my

ABSTRACT

Fungi in the genus *Fusarium* are well known as soil-borne pathogen with worldwide distribution. Therefore, this study focused on isolation of *Fusarium* species from soil cultivated with watermelons, muskmelon, pumpkins, and cucumber in the east coast of Peninsular Malaysia by using dilution plate technique, direct plating and debris plating. The highest number of *Fusarium* species isolated was *F. oxysporum* with 687 (26.2%) colonies counted based on colony formation unit (CFU); the colonies of *Fusarium*/g soil = mean of *Fusarium* colonies x dilution factor/weight of dried soil (g). Other *Fusarium* species isolated were *F. semitectum*, *F. solani*, *F. proliferatum*, *F. subglutinans* and *F. chlamydosporum*. Throughout the studies, peptone pentachloronitrobenzene (PPA) medium, potato dextrose agar (PDA) and carnation leaf-piece agar (CLA) were regularly used to identify each *Fusarium* species by morphological means. Based on the Shannon-Weiner Index, *Fusarium* species diversity is much higher in Besut, Terengganu (H'=1.59). *Fusarium* species can be considered as a functionally important biological component of Fusarium fruit rot disease study in cucurbits.

Keywords: ***Fusarium, rot disease, cucurbits, Soil Microbiology***

INTRODUCTION

Fusarium species cause a huge range of diseases on an extraordinary range of host plants. In the Family of Cucurbitaceae (e.g. watermelons, muskmelon, pumpkins, and cucumber), it can cause diseases known as vascular wilt, root rot and fruit rot (Vakalounakis & Chalkias, 2004). *Fusarium* is a genus of deuteromycetous fungi which are abundance in soils, whole parts of a plant, plant debris and other organic substrates (Summerell *et al*., 2003). It is found to be well and important genera living in the soils as free-living saprophytes, pathogens, and endophytes and known to produce a range of toxic compounds that can adversely affect livestock and humans (Summerell *et al*., 2001). Soil fertility is a main factor for the occurrence and virulence of *Fusarium* species. Meanwhile, Nik Mohd Izham *et al*. (2005) reported that *Fusarium* species are well distributed in soils planted with crops such as paddy, rubber, oil palm and vegetables compared to the unused

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[*]Corresponding Author

soils. A previous study by Lim (1971) was the first to report on the diversity of *Fusarium* species in Malaysian soils which had isolated 8 species with *F. solani* the widest spread species, and this was followed by *F. oxysporum*. It has been reported that various *F. oxysporum* pathotypes can survive successfully either in the soil or above ground by means of thick-walled chlamydospores that are either free or embedded in infected plant debris (Suarez-Estrella *et al*., 2004). Thus, this study was undertaken to determine: (i) *Fusarium* species isolated from the soils by using morphological identification, and (ii) its diversity in east coast, Peninsular Malaysia, especially the areas that are highly cultivated with cucurbits such as in Bachok, Pasir Mas, Tok Bali (Kelantan district), as well as Besut and Setiu (Terengganu district).

MATERIALS AND METHODS

Soil Samples

In this study, the soil samples were collected from areas cultivated with cucurbits (watermelon, muskmelon, pumpkin and cucumber) around the east coast of Peninsular Malaysia (Kelantan and Terengganu). Field sampling was done between January 2009 and April 2009. The soil samples were air-dried at room temperature (27±1°C) for 5 days and ground. The ground soil was then sieved with 0.5 mm sieve to separate larger particles such as debris. Both the soil and debris were kept in paper envelopes.

Isolation of Fusarium spp.

The isolation was based on three methods; namely soil dilution plate, soil direct plating and debris plating, as described by Leslie and Summerell (2006).

Soil Dilution Plate Technique

One ml of the soil suspension that had been diluted from 10^{-2} to 10^{-4} was spread on the surface of peptone pentachloronitrobenzene (PPA) medium with 7 replicates for each dilution factor. The dilution plates were observed daily for 7 days for colony counting. *Fusarium* spp. colonies were counted based on the formula below for colony formation unit (CFU) (Nash & Snyder, 1962):

$$\text{Colonies of } \textit{Fusarium}\text{/g soil} = \frac{\text{Means of } \textit{Fusarium} \text{ colonies x dilution factor}}{\text{Weight of dried soil (g)}}$$

Soil Direct Plating

10 mg of the sieved soil were spread evenly on the surface of PPA medium and incubated. This was followed by observation within 7 days for colonies formation.

Debris Isolation Technique

After soil sieving, the collected debris was suspended in the running water for 24 hours with sieve as a separator to remove the soil particles that might be attached to debris. Then, the debris was air-dried on sterile paper and placed on the surface of PPA medium. Both direct plating and debris plating techniques were used for the qualitative data.

All *Fusarium* spp. that were isolated from those three techniques were then identified on PDA and CLA. The identification was based on the morphological characteristics from Leslie and Summerell (2006).

Measurement of Species Diversity

Fusarium species diversity within the east coast of Peninsular Malaysia was calculated by using the Shannon-Weiner Index (Spellerberg, 2008):

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where: $\sum$ refers to "the sum of"
there are *s* species in the community
p_i = is the relative abundance (proportion) of the i species in the community
ln = natural log

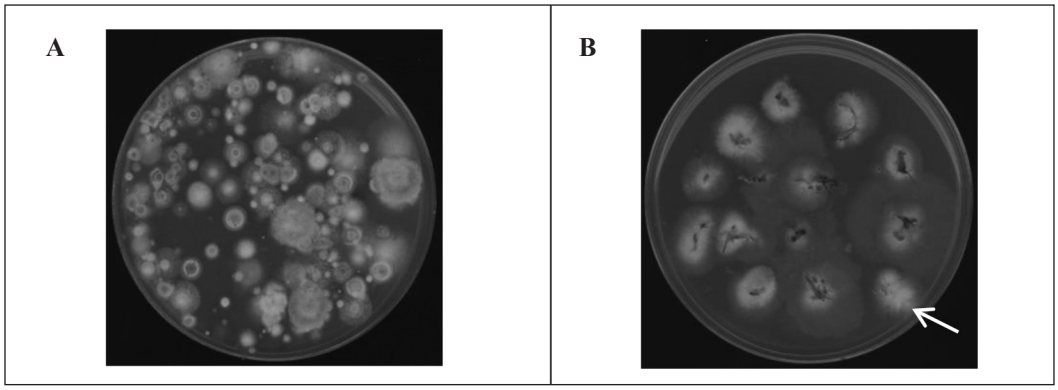

Fig. 1: (A) Colonies of Fusarium *species from soil dilution plate technique on PPA medium; (B)* Fusarium *species from debris on PPA medium (arrow)*

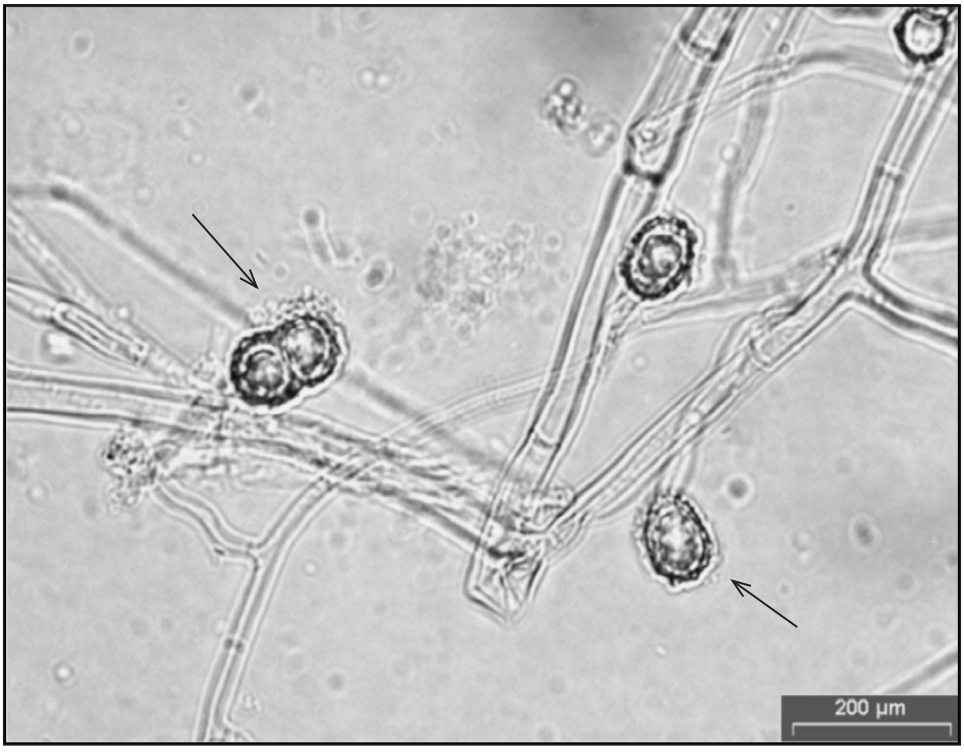

Fig. 2: The thick-walled chlamydospores of F. oxysporum *(arrow) that is either free or embedded in infected plant debris*

TABLE 1
Number of *Fusarium* species colonies isolated per gram soils (CFU/g soil) by using soil dilution plate (10^{-2}) technique and species diversity (Shannon-Weiner Index)

Soil Locality	*Fusarium* species colonies						$p_i \ln p_i$
	[1]*F. chl*	[2]*F. oxy*	[3]*F. pro*	[4]*F. sem*	[5]*F. sol*	[6]*F. sub*	
Bachok, Kelantan	0	55	75	142	71	48	1.52
Pasir Mas, Kelantan	0	146	185	122	74	115	1.57
Tok Bali, Kelantan	24	101	34	114	65	5	1.50
Besut, Terengganu	18	190	74	132	191	53	1.59
Setiu, Terengganu	1	195	79	121	159	35	1.49
Total	43	687	447	631	560	256	

[1]*F. chlamydosporum*, [2]*F. oxysporum*, [3]*F. proliferatum*, [4]*F. semitectum*, [5]*F. solani*, [6]*F. subglutinans*

RESULTS AND DISCUSSION

Only the number of colonies from the dilution factor 10^{-2} was chosen as an optimum concentration of soil dilution for CFU counting on PPA medium. After five days, more *Fusarium* spp. colonies appeared in the PPA medium from soil dilution plate technique (*Fig. 1a*) and also debris (*Fig. 1b*). A total of 2,624 *Fusarium* colonies with 26.2% *F. oxsporum* was isolated, and this was followed by *F. semitectum* (24.0%), *F. solani* (21.3%), *F. proliferatum* (17.0%), *F. subglutinans* (9.8%) and *F. chlamydosporum* (1.7%).

The highest number of *Fusarium* species colonies isolated from the soil cultivated with cucurbits was *F. oxysporum,* with a total of 687 colonies (Table 1). Based on the Shannon-Weiner Index, even though all five locations in the east coast of Peninsular Malaysia had the same species richness, the *Fusarium* species diversity was found to be much higher in Besut, Terengganu (H'=1.59), followed by Pasir Mas, Kelantan (H'= 1.57), Bachok, Kelantan (H'= 1.52), Tok Bali, Kelantan (H'= 1.50) and Setiu, Terengganu (H'= 1.49) (Table 1).

The survival of *F. oxysporum* in soil and debris has been proven by Vakalounakis and Chalkias (2004), whereby it was found that *F. oxysporum* f. sp. *radicis-cucumerinum* could survive as a successful soil-inhabiting fungus for more than 13 months and cause root and stem rot of cucumber. *F. oxysporum, F. semitectum, F. solani and F. chlamydosporum* produced chlamydospores (*Fig. 2*) that may survive successfully in plant debris and could act as inoculums source when the environment is suitable for dispersion from one growing season to the next. Cucurbits fruits attached to the soil were easily infected with Fusarium fruit rot (FFR) disease; a recent study showed that 54% of *F. solani* and 46% of *F. oxysporum* were isolated from the east coast of Peninsular Malaysia, i.e. from the samples with FFR disease (Siti Nordahliawate *et al*., 2009).

Meanwhile, some research has focused on the survival of various *F. oxysporum* pathotypes in soils. Among other Roncero *et al.* (2003) used *Fusarium* as a model system to understand the process of root infection and disease development in soil-borne plant pathogens. Their ubiquitous distribution in soil may contribute as saprophytic decomposition in the process of nutrients cycling, while some species such as *F. oxysporum* have been found to beneficial in soil denitrification (Takaya *et al.,* 2002; Steven *et al*., 2008). These studies would become a platform for researchers and students to understand the importance of *Fusarium* species as a soil-borne microorganism that might contribute to the economically important cucurbit disease in the east coast of Peninsular Malaysia. In addition, soil treatments, crop rotation and other measure controls can be considered to control the diseases caused by *Fusarium* species, specifically at the highest inoculums area.

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