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SHORT COMMUNICATION

Fusarium species Associated with Fruit Rot of Banana (*Musa* spp.), Papaya (*Carica papaya*) and Guava (*Psidium guajava*)

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ABSTRACT

A total of 60 isolates of *Fusarium* were isolated from fruit rot of banana (*Musa* spp.), papaya (*Carica papaya*) and guava (*Psidium guajava*). The most common species recovered from the fruit rot of the three fruit crops were *F. semitectum* (40 %), *F. solani* (38.3 %), F. *verticillioides* (11.7 %) and *F. oxysporum* (10 %). *Fusarium semitectum* was isolated from fruit rot of banana, papaya and guava; F. *oxysporum* from banana and papaya; *F. solani* from banana and guava and *F. verticillioides* from banana. From pathogenicity tests, *F. solani* and *F. semitectum* were pathogenic to both banana and papaya and *F. verticillioides* to banana. *F. oxysporum* was not pathogenic to banana and papaya and *F. semitectum* was not pathogenic to guava. The results of the present study showed the presence of several *Fusarium* spp. on fruit rot of banana, papaya and guava and several species are found to be pathogenic causing fruit rot on their hosts.

Keywords: Fusarium spp., banana, papaya, guava, pathogenicity

INTRODUCTION

Tropical fruits in Malaysia include a number of crops which are consumed fresh or turned into processed products such as juice, jam and chips. Among the well-known tropical fruits planted in Malaysia are banana (*Musa* spp.), papaya (*Carica papaya*) and guava (*Psidium guajava*) which are planted for local consumption as well as for export.

Quality of tropical fruits is commonly affected by post harvest disease such as fruit rot mostly caused by improper handling and storage, during transportation and marketing. One of the pathogenic fungi that are associated with fruit rot of tropical fruits is *Fusarium.* Fusarium rot on tropical fruits could posses a potential health risk as many *Fusarium* species are known to produce mycotoxins under suitable conditions. The greatest risk of mycotoxin contamination occurs when diseased produce is used in the production of processed food (Coates and Johnson, 1997). The presence of *Fusarium* species on tropical fruits received little attention and not well-documented in Malaysia although several species have been associated with rotting or decay on banana and papaya (Slabaugh, 1998; Nishijima, 1998). Therefore, the present study was conducted to determine the occurrence of *Fusarium* species on fruit rot of three tropical fruits, banana, papaya and guava and to test their pathogenicity.

MATERIALS AND METHODS

Fruit samples showing signs and symptoms of fruit rot were obtained from several markets and supermarkets in Penang Island, Malaysia. *Fusarium* isolates were isolated from the fruit rot using surface sterilization and direct isolation methods. For direct isolation, a scrape of the mycelia were taken out from the lesion or infected area by using sterile inoculation loop. The mycelia were then plated onto Peptone Chloro Nitro Benzene (PCNB) media. The plates were incubated at room temperature (27 ± 1 °C) for 4 to 5 days or until visible growth of mycelia were observed. For surface sterilization, pieces of tissues about 0.5 cm X 0.5 cm were cut at the boundary of infected and healthy tissue by using sterile scalpel. The tissues were then soaked into 10 % sodium hypochlorite for 3 min, dried on sterile filter paper and soaked again in 70% alcohol for 1 min. The tissues were rinsed three times with sterile distilled water, dried on sterile filter paper and plated onto PCNB. The plates were incubated at room temperature (27± 1 °C) for 4 to 5 days or until visible growth of mycelia were observed. Single spore isolation technique using conidial suspension was used to obtain pure culture.

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Identification of *Fusarium* isolates was based on the methods described in The *Fusarium* Laboratory Manual (Leslie and Summerell, 2006). Primary characters observed for identification were the shapes of the macroconidia and microconidia, formation of conidiophore cells i.e. monophialide or polyphialide, and the occurrence of chlamydospore on carnation leaf agar (CLA). The characteristics were observed using a light microscope (Olympus BX41). Secondary characteristics observed were pigmentation and, texture of the colony on potato dextrose agar (PDA).

Representative isolates of each *Fusarium* species were chosen for pathogenicity tests. The healthy fruits used for pathogenicity test were washed thoroughly, surface sterilized by soaking in 3 % sodium hypochloride for 2 min and washed with sterile distilled water. The fruits were then air dried in a laminar flow. After dried, the fruits were sprayed with 70 % alcohol, put in a tray and covered with a layer of plastic wrapper.

Two techniques of inoculation were used namely, mycelial plug and conidial suspension. For the mycelial plug method, the inoculum was prepared by using 0.6 mm cork borer. The mycelial plugs were inoculated on the surface of the healthy fruits. While for spore suspension method, conidial suspension with concentration of 5 X 10^6 conidia per ml were inoculated on the fruits. Pathogenicity test were conducted with three treatments, wounded, unwounded and control. For wounded treatment, the fruit was cut about 2.5 cm on the surface using sterile scalpel. Three replicates were made for each treatment. The fruits were put into trays, incubated at 27±1^{0}C and observation of disease symptoms were made every day. Disease severity was based on 0 – 4 scales, with the following index: 0 = no disease symptom or rotting on the fruit, 1 = 1-10 % rotting, 2 = 11-20 % rotting, 3 = 21-30 % rotting and 4 = 30 % rotting and more (Illeperuma & Jayasuriya, 2002). Isolation was made from the lesion or rotting to confirm the causal agent.

RESULTS AND DISCUSSION

A total of 60 *Fusarium* isolates were isolated from rotting symptom of banana, papaya and guava in which four *Fusarium* species were identified (Table 1). The most common Fusarium species isolated was *F. semitectum* (40 %), followed by *F. solani* (38.3 %), F. *verticillioides* (11.7 %) and *F. oxysporum* (10 %). *Fusarium semitectum* was isolated from fruit rot of banana, papaya and guava; *F. oxysporum* from banana and papaya; *F. solani* from banana and guava and *F. verticillioides* from banana.

Fusarium semitectum isolates were identified based on spindle shaped macroconidia and the appearance of 'rabbit ears' on the polyphialide. *Fusarium solani* isolates were differentiated from *F. oxysporum* isolates by the false head borne on the monophialide and the shape of the macroconidia. *Fusarium solani* isolates produced false head on long monophialide and the macroconidia were stout and robust whereas *F. oxysporum* isolates produced false head on short monophialide and the macroconidia were sickle-shaped. *Fusarium verticillioides* isolates produced long chain of microconidia and the macroconidia were long and slender, and occasionally 'rabbit ear' appearance was observed on the monophialide.

Table 1: *Fusarium* species successfully isolated from banana, papaya and guava

Fruits	*Fusarium* species
Banana	*F. oxysporum* (3 isolates)
	F. solani (10 isolates)
	F. semitectum (15 isolates)
	F. verticillioides (7 isolates)
Papaya	*F. solani* (13 isolates)
	F. semitectum (8 isolates)
Guava	*F. oxysporum* (3 isolates)
	F. semitectum (1 isolates)

Table 2 shows the results of the pathogenicity test of Fusarium species on banana, papaya and guava. Symptoms of fruit rot caused by *F. solani* and *F. semitectum* on banana and papaya, and *F. verticillioides* on banana were similar. Dark brown lesion appeared on the second day of the inoculation and the lesions grow larger with water soaked appearance. At this stage, mycelia covered the dark brown lesion.

From the pathogenicity test, F. *oxysporum* (USM BGi1) from banana and *F. oxysporum* (USM GPS1) from guava were not pathogenic to their host. *Fusarium solani* isolates (USMBTS1 and USMBD230) from banana and from papaya (USM PT1, USM PDA9 and USM PDA8) were pathogenic causing rot to both fruits. *Fusarium semitectum* isolates from banana (USM BGi5 and USM B2) and from papaya (USM PDA1) were pathogenic causing rot on both fruits but isolate from guava (USM GPS5) was not pathogenic. *Fusarium verticillioides* (USM BA230) was pathogenic to banana.

Generally, wounded treatments for both mycelia plug and spore suspension techniques produced more severe infection compared to unwounded treatment (Table 2). Wounded treatment for both mycelia plug and spore suspension techniques showed index of severity ranging from 3 to 4. For unwounded treatment using spore suspension technique, only *F. solani* isolates (USM BTS1 and USM BD230) from banana produced rotting symptom (index of 2). The other *Fusarium* species did not produce any disease symptoms by using unwounded treatment.

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Table 2: Pathogenicity test and disease severity of *Fusarium* species on banana, papaya and guava

Fruits	*Fusarium* species	Mycelial plug		Spore suspension	
		Wounded	Unwounded	Wounded	Unwounded
Banana	*F. oxysporum* (USM BGi1)	0	0	0	0
	F. solani (USM BTS1)	3	2	4	2
	F. solani (USM BD230)	3	2	4	2
	F. semitectum (USM BGi5)	4	2	4	0
	F. semitectum (USM B2)	4	2	4	0
	F. verticillioides (USM BA230)	4	2	4	0
Papaya	*F. solani* (USM PT1)	4	3	4	0
	F. solani (USM PDA9)	4	3	4	0
	F. solani (USM PDA8)	4	3	4	0
	F. semitectum (USM PDA1)	4	2	4	0
Guava	*F. oxysporum* (USM GPS1)	0	0	0	0
	F. semitectum (USM GPS5)	0	0	0	0

Index: 0 = no symptoms; 2 = 11 – 20 % rotting; 3 = 21 – 30 % rotting; 4 = 31 % > rotting

Only *F. semitectum* was isolated from the three fruit crops, however, F. *semitectum* from guava was non-pathogenic. *Fusarium semitectum* was one of the most common species of *Fusarium* recovered from banana. The species have been isolated from lesions of banana fruits collected from markets in the USA and Italy (Vesonder *et al*., 1995) and were the most predominant species recovered from banana samples commercially available in Italy and Spain (Jimenez *et al*., 1993). In addition to banana fruit rot, *F. semitectum* also caused crown rot and fungal scald of banana (Jones and Slabaugh, 1998; Jones and Stover, 2000). In the present study, *F. semitectum* was also pathogenic, causing fruit rot of papaya. Other than the present study, there is no report of *F. semitectum* associated with fruit rot of papaya.

F. solani isolates were pathogenic to banana and papaya. Similar with *F. semitectum*, *F. solani* was also one of the most common *Fusarium* species associated with fruit rot of banana. The *Fusarium* species has been isolated from fruit rot of banana by Jimenez *et al*. (1997), from banana samples commercially available in Italy and Spain (Jimenez *et al*., 1993) and associated with rotting of banana fruits in the market (Datar and Ghule, 1988). *Fusarium solani* was also found to be associated with fruit rot of papaya. The species has been reported to cause severe rotting of papaya fruit in Nigeria (Echerenwa and Umechuruba, 2004) and causing dry lesion on papaya fruit after harvest (Alvarez and Nishijima, 1987). Fruit rot of papaya caused by *F. solani* is most common and widespread in Hawaii, India and the Philippines (Nishijima, 1998).

Fusarium verticillioides was only isolated from fruit rot of banana and was pathogenic to banana fruits. *Fusarium verticillioides* has been recovered from rotten fruit of banana imported to Japan (Hirata *et al*., 2001) and was identified as one of the most frequently isolated species from banana fruits marketed in Italy (Jimenez *et al*., 1993).

Fusarium oxysporum isolated from banana and guava was not pathogenic on both fruits. Although, F. *oxysporum* was not pathogenic on banana, the species was among the *Fusarium* species found on banana samples marketed in Italy and Spain (Jimenez *et al*., 1993). In the present study, *F. oxysporum* was found to be non-pathogenic to guava fruits however, *F. oxysporum* var. *psidi* was reported to cause post harvest rot (Sumia *et al*., 2006) and causing Fusarium wilt disease of guava plant.

Fusarium fruit rot of banana, papaya and guava can be a problem as the disease reduces the quality and lower the marketability of the fruits. *Fusarium* species possess saprophytic ability and can survive on the host for a long period as many species produced chlamydospores. *Fusarium* species causing fruit rot is a weak pathogen and often needed predisposing factor such as injuries before it become established (Nishijima, 1998). Therefore, *Fusarium* species associated with fruit rot is often regarded as secondary invader especially on lesions caused by other fungi, insects and any other mechanical means such as cuts, abrasion occur during harvesting and handling. For this reason, fruits showing sign of injuring or rotting should be discarded. The results of the present study showed that four *Fusarium* species, namely *F. semitectum*, *F. solani*, F. *verticillioides* and F. *oxysporum*

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were recovered from fruit rot of banana, papaya and guava.

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