

Diversity of *Fusarium* Species Associated with Post-harvest Fruit Rot Disease of Tomato

(Kepelbagaian Spesies *Fusarium* yang Berasosiasi dengan Penyakit Lepas Tuai Reput Buah Tomato)

A.I. ABU BAKAR, M.Z. NUR AIN IZZATI* & Y. UMI KALSOM

ABSTRACT

Fusarium species is one of the common pathogens of post-harvest disease to cause rot on tomato and other perishable vegetable fruits. The objectives of this study were to determine the diversity of *Fusarium* isolated species from post-harvest diseases of tomato fruit, to identify the causal organisms by using phenotype characteristics and to verify the pathogens of *Fusarium* fruit of tomato based on pathogenicity test. Carnation leaf-piece agar (CLA) and potato dextrose agar (PDA) media were used for phenotype-based identification of the *Fusarium* isolates with emphasis for characterizations of the shapes and sizes of the macroconidia and microconidia, colony features, growth rates, conidiogenous cells and chlamydospores. A total of 180 *Fusarium* isolates were obtained from 13 locations throughout Selangor. *Fusarium solani* was most abundantly isolated (34%) followed by *F. semitectum* (31%) and *F. oxysporum* (31%), *F. subglutinans* (3%) while the least was *F. equiseti* (1%). Twenty seven isolates were tested for pathogenicity test by injecting 1 mL of the conidial suspension onto healthy tomatoes. All the tested *Fusarium* isolates were pathogenic on tomato with different severity levels. The non-inoculated controls showed no symptoms of fruit rot. The most virulent was *F. oxysporum* isolate B711T with DSI 93.75%, while the least were isolates of *F. solani* (B647T) and *F. oxysporum* (B727T) with DSI 37.5%. Majority of the isolated *Fusarium* species can potentially produce mycotoxins as their secondary metabolites. The potential production of mycotoxins by pathogenic isolates of *Fusarium* species in contaminated tomato fruits could pose health hazards when consumed.

Keywords: Disease severity index; fruit; *Fusarium*; pathogen; post-harvest; tomato

ABSTRAK

Spesies *Fusarium* merupakan salah satu patogen biasa lepas tuai yang menyebabkan penyakit reput buah tomato, sayuran dan buah lembut yang lain. Objektif kajian ini adalah untuk mengenal pasti kepelbagaian spesies *Fusarium* yang dipencilkan daripada penyakit reput lepas tuai tomato, mengenal pasti organisma penyebab berasaskan ciri fenotip dan mengesahkan patogen penyakit reput *Fusarium* tomato berasaskan ujian kepatogenesis. Agar keratan daun teluki (CLA) dan agar kentang dekstroza (PDA) digunakan untuk pengenalan pastian isolat *Fusarium* berasaskan ciri fenotip yang menumpukan bentuk dan saiz makrokoni diadankonidionidia, ciri koloni, kadar pertumbuhan, sel konidiogen dan klamidospora. Sejumlah 180 isolat *Fusarium* diperolehi dari 13 lokasi sekitar Selangor. *F. solani* adalah yang terbanyak dipencilkan (34%) diikuti oleh *F. semitectum* (31%), *F. oxysporum* (31%), *F. subglutinans* (3%), di mana yang paling sedikit adalah *F. equiseti* (1%). Dua puluh tujuh isolat *Fusarium* diuji dalam ujian kepatogenesis dengan menyuntik 1 mL ampaian konidia ke atas tomato sihat. Kesemua isolat *Fusarium* yang diuji adalah patogenik ke atas tomato pada tahap keseriusan yang berbeza. Tomato yang tidak diinokulat tidak menunjukkan sebarang simptom reput buah. Isolat B711T *F. oxysporum* merupakan isolat yang paling virulen dengan DSI 93.75%, sementara yang paling lemah adalah isolat *F. solani* (B647T) dan *F. oxysporum* (B727T) dengan DSI 37.5%. Majoritis spesies *Fusarium* yang dipencilkan berpotensi menghasilkan mikotoksin sebagai metabolit sekunder. Kebolehan menghasilkan mikotoksin oleh isolat yang patogenik pada buah tomato yang dijangkiti boleh menyebabkan keracunan dan masalah kesihatan jika dimakan.

Kata kunci: *Fusarium*; indeks keseriusan penyakit; lepas tuai; patogen; penyakit reput buah; tomato

INTRODUCTION

Vegetable fruits are highly perishable crops. Thus, post-harvest handling, storage, transportation and marketing are seriously affecting their quality in the market, where improperly handling, packaging, storage and transportation could cause decay and increase the production of micro-

organisms due to changing physiological state of the fruits and vegetables (Wilson et al. 1991). Fruits, due to their low pH, high moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxins (Moss 2002).

Tomato fruit rots are mainly caused by bacteria and fungi. However, some plant pathogens, viruses and nematodes could also be responsible for post-harvest losses but do not cause progressive deterioration of tomatoes in storage. Several fungal species can cause fruit decay in tomatoes such as the sour rot pathogens caused by *Geotrichum candidum*, Rhizopus rot caused by *Rhizopus stolonifer*, buckeye rot caused by *Phytophthora* species, black mold rot caused by *Alternaria arborescens* and Fusarium rots caused by *Fusarium* species (Mahovic et al. 2004).

Fusarium species can appear as saprophytes or pathogens on plants, animals as well as humans. They are well associated with a wide range of plants in their natural habitats such as tomato, banana, asparagus, barley, mango, pineapple, carnation, coffee, corn, grasses, legumes, oats, pine, rice, sorghum, sugarcane and wheat (Burgess et al. 1994; Nelson et al. 1990). Harvested tomatoes are susceptible towards infections caused by *Fusarium* species due to its succulent epicarp which enable the fungal hyphae to penetrate deeply into the fruit (Tournas & Katsoudas 2005). As a result, the yield of this economically important farm product is affected, hence lowering the production rate (Salleh & Mushitah 1991). *Fusarium* rot on tomato fruits are often caused by *Fusarium* species (Denis 1983; Mehrotra 1989; Sherf & Macnab 1986; Solunkhe & Desai 1984) and the disease symptoms include rots softer and extend into the center of the fruit. The rotted tissue is often water-soaked and becomes covered by white, yellow or pinkish mycelium externally while the infected tissue is discolored and appears pale brown (Denis 1983). The disease causes the vegetable fruits unmarketable as consumer will only choose those that are fresh and healthy (Nurulhuda et al. 2009). Hence, *Fusarium* species have been isolated from decaying tomato fruits (Oladiran & Iwu 1992). Tomato contaminated with *Fusarium* species are lethal for human and animals consumption since several species of them produce mycotoxins (Burgess 1985; Joffe 1986; Nelson et al. 1990).

In Malaysia, many *Fusarium* species had been isolated from various vegetable fruits but are not well documented with detailed information on its diversity on tomato. Therefore, the objectives of this study were to determine the diversity of *Fusarium* species from *Fusarium* fruit rot (FFR) of tomato, to tentatively identify the causal organisms based on phenotype characteristics and to verify the pathogens of the disease by pathogenicity test.

MATERIALS AND METHODS

SAMPLING AND FUNGAL ISOLATION

Samples of tomato with post-harvest fusarium fruit rot symptoms were collected from 13 locations throughout Selangor. All samples were surface sterilized in 10% chlorox® and washed in sterile distilled water for 2 min each. The washed tomatoes were bolt dried using filter paper and transferred onto peptone pentachloronitrobenzene agar (PPA) media (Papavizas 1967). The samples were

subsequently incubated at room temperature for 7 days. Single spore isolation was carried out onto potato dextrose agar (PDA) by streaking technique. Single spored colony was transferred onto new PDA to obtain pure culture and incubated at room temperature for 7 days.

IDENTIFICATION OF *FUSARIUM* SPECIES

Pure cultures of the isolates were cultured onto carnation leaf-piece agar (CLA) for phenotype-based identification following *Fusarium* manual by Leslie and Summerell (2005). Cultures on CLA were incubated at standard condition which is room temperature (25±2°C) for 7 days. The isolates phenotype examined for identification were shapes and sizes of macroconidia and microconidia; number of septa and shapes of the apical and basal cells of the macroconidia, conidiogenous cells, growth rate, presence of chlamydo-spore, colony colour, growth and pigmentation on PDA.

DIVERSITY INDEX OF THE *FUSARIUM* SPECIES

The Shannon-Weiner index for diversity of the *Fusarium* species in each location was calculated using the formula by Venco and Meneses (1996):

$$H' = \sum_{i=1}^S p_i (\ln p_i),$$

where H' is the value of Shannon-Weiner Index, S is the number of species in the community, Pi is the relative abundance of the species in the community and ln is the natural log.

VIRULENCE TEST

Virulence test was conducted to verify the *Fusarium* isolates as pathogens or saprophytes on tomato fruits. Mature, fresh and healthy tomato fruits were surface sterilized and washed in the sterile distilled water both for 2 min each and subsequently placed on filter paper for drying. Four replicates for each of 27 selected isolates were used and were resub-cultured onto PDA and incubated at room temperature (25±2°C) for 7 days. The conidia were harvested using 20 mL sterile distilled water and only 1 mL conidial suspension was injected into the inner tissue of the tomato using the sterile syringe. The inoculated fruits were incubated for 7 days along with the uninoculated fruits as controls. The test was repeated 3 times. All inoculated and uninoculated tomatoes were kept in an individual sterilized container, 2 pieces/container with a cover and incubated at room temperature condition. The fungal colony from disease lesion was subcultured on PPA and PDA. The isolated fungal were identified based on their morphological characteristics including colony features, growth rates and pigmentation. Those isolated fungal that appeared the same as the inoculum were confirmed their pathogenicity on tomato fruits.

DISEASE ASSESSMENT

The disease symptoms were scored based on a disease scale from 0 to 4 devised by Amadi et al. (2009) with a slight modification for tomato. All scales were characterized with a particular symptom on tomato fruit rot labelled on it as shown in Table 1. The DSI data was then analysed using Friedman test of the non-parametric test of SPSS programme at $p < 0.05$ in order to compare the variation of the DSI distribution among the isolates.

RESULTS AND DISCUSSION

A total of 180 isolates of *Fusarium* were isolated from post-harvest disease of tomato fruit rot. Identification of all the isolates were based on phenotypic characterization. Five *Fusarium* species were identified namely, *F. solani*, *F. semitectum*, *F. oxysporum*, *F. subglutinans* and *F. equiseti*. Table 2 shows the phenotype of the five *Fusarium* species isolated from tomato throughout Selangor. Figure 1 shows the microscopic characteristics of the *F. solani*, *F. semitectum*, *F. oxysporum*, *F. subglutinans* and *F. equiseti*.

Table 3 shows the distribution of *Fusarium* isolates obtained from post-harvest *Fusarium* fruit rot of tomatoes in Selangor and their diversity based on Shannon-Weiner index. The highest distribution was in Sri Serdang with 77 *Fusarium* isolates followed by Kajang (39 isolates) while 8 *Fusarium* isolates each for Semenyih, Balakong, Shah Alam, Seri Kembangan and Ampang. The fewest was scored in Selayang, Puchong, Bandar Sunway, Subang Jaya, Rawang and Sungai Buloh with only 4 isolates. *F. solani* (34%) was dominantly isolated followed by *F. semitectum* (31%), *F. oxysporum* (31%), *F. subglutinans* (3%) and *F. equiseti* (1%). The community diversity of the *Fusarium* species in each location was however computed using Shannon-Weiner Index (H') with the highest value of the Shannon-Weiner index was recorded in Sri Serdang ($H' = 0.363$) followed by Kajang ($H' = 0.332$). The value of ($H' = 0.137$) each for Semenyih, Balakong, Shah Alam, Seri Kembangan and Ampang while the least value recorded in Selayang, Puchong, Bandar Sunway, Subang Jaya, Rawang and Sungai Buloh ($H' = 0.084$).

F. solani was the most common recovered *Fusarium* from tomato fruit rot. In Malaysia, *F. solani* has been

reported as a plant pathogen infecting tomato and other vegetable fruits (Nurulhuda et al. 2009) and was isolated from soil and also grasses where 39% isolated from cultivated and non-cultivated soil (Nik Mohd Izham 2008), 21.3% isolated from soil cultivated with cucurbits (Siti Nordahliawate et al. 2009) and 10.3% isolated from grasses (Nur Ain Izzati et al. 2009). In addition, *F. solani* was also reported to have been recovered from corn (Nur Ain Izzati et al. 2011) and root and stem rot of *Dendrobium* orchids (Latiffah et al. 2009). Besides damaging tomato fruits in storage (Amadioha & Uchendu 2003), *F. solani* was identified as a pathogen of a number of tropical agricultural crops such as potato, soybeans, peas and peppers (Grunwald et al. 2003). Leslie and Summerell (2005) reported that *F. solani* produces mycotoxins that are lethal to humans and animals health. *F. solani* has been known to synthesize compounds such as fusalanipyrene (Abraham et al. 1990), fusaric acid (Bacon et al. 1996), moniliformin (Chelkowski et al. 1990) and moreover, a number of chemically unidentified toxic compounds are produced by *F. solani* but its ability to produce trichothecene and zearalenone have not been confirmed (Marasas et al. 1984).

F. semitectum and *F. oxysporum* were the second most isolated *Fusarium* from decaying tomato fruits. *F. semitectum* was reported to have been isolated from decaying okra, bitter melon, loofah, red chilli and cucumber (Nurulhuda et al. 2009), corn (Nur Ain Izzati et al. 2011) and dragon fruit (*Hylocereus polyrhizus*) (Hawa et al. 2010). *F. semitectum* was however reported to have caused diseases on banana fruits (Wallbridge 1981), melons (McGovern 1994), beans (Dhingra & Muchovej 1979), sorghum (Gopinath et al. 1985), walnut (Seta et al. 2004) and storage rot of mushrooms (Seth & Shandilya 1978). *F. semitectum* has also been known to produce mycotoxins such as trichothecene and zearalenone (Marasas et al. 1984), beauvericin (Logrieco et al. 1998) and moniliformin (Rabie et al. 1982).

F. oxysporum was reported to have been isolated from tomato fruits (Fajola 2004), okra, bitter melon and tomato (Nurulhuda et al. 2009), corn (Nur Ain Izzati et al. 2011) and *Eurycoma longifolia* Jack (Nur Ain Izzati & Siti Nordahliawate 2010). Besides, it was also isolated from decay area of tomato fruit and the decay area appeared to

TABLE 1. Disease severity index (DSI) for disease assessment with a slight modification for tomato (Amadi et al. 2009)

Disease scale	Description	Inference
0	No visible symptoms on fruits	No infection
1	1 – 25% of inoculated area covered with slight necrotic	Mild infection
2	26 – 50% of inoculated area covered with necrotic and white mycelia of fungal	Moderate infection
3	51 – 75% of sample are necrotic with spore mass appeared	Severe infection
4	>76% necrotic tissue appears soft and decay with fungal mass	Very severe/Devastating

$$DSI = \frac{\sum(A \times n)}{\sum B} \times 100$$

A = disease scale (0, 1, 2, 3 or 4); N = number of tomato for each disease scale and B = total number of tomatoes

TABLE 2. Morphological characteristics of *Fusarium* species isolated from post-harvest tomato fruits

<i>Fusarium</i> species	Characteristics					Growth rate at 27°C
	Macroconidia	Microconidia	Conidiophores	Chlamydospore	Pigmentation on PDA	
<i>F. solani</i>	5-septate, wide, straight, robust and stout. Apical cell is blunt and rounded foot shaped basal cell	Oval and reniform shaped with 0 – 1 septa and abundant in the aerial mycelia	Long monophialide	Globose and exist singly with smooth and rough walled	White tinged with brown	1.7-5.4 cm
<i>F. semitectum</i>	5-septate and thin-walled with a curved dorsal surface and a slightly straighter ventral surface. Tapered apical cell and foot shaped basal cell	Oval and kidney shaped with 0-1 septa and scarce in the aerial mycelia	Monophialide and polyphialide	Globose, smooth and formed singly and in chains intercalary and terminal	Brown	2.1-5.8 cm
<i>F. oxysporum</i>	5 septate, wide and thin-walled with a curved dorsal surface and a straighter ventral surface. Apical cell is tapered and foot shaped basal cell	Oval and kidney shaped with 0-1 septa and usually abundance in false heads mycelia	Short monophialide	Oval and occurred singly and in chains with smooth and rough walled intercalary and terminal	White tinged with purple	3.1-5.6 cm
<i>F. subglutinans</i>	3 septate, wide and thin-walled with dorsoventral surfaces slightly parallel. Curved apical cell and foot shaped basal cell	Oval, single-celled, abundant and also occurred in false heads only	Monophialide and polyphialide	Absent	Dark purple	2.1-6.1 cm)
<i>F. equiseti</i>	5 septate, long, thick-walled and sickle-shaped. Tapered and whip-like apical cell and elongated foot shaped basal cell	Scanty and produce in false heads	Monophialide	Oval shaped and occurred singly, in chains and in clumps intercalary and terminally	Dark brown	2.7-5.5 cm

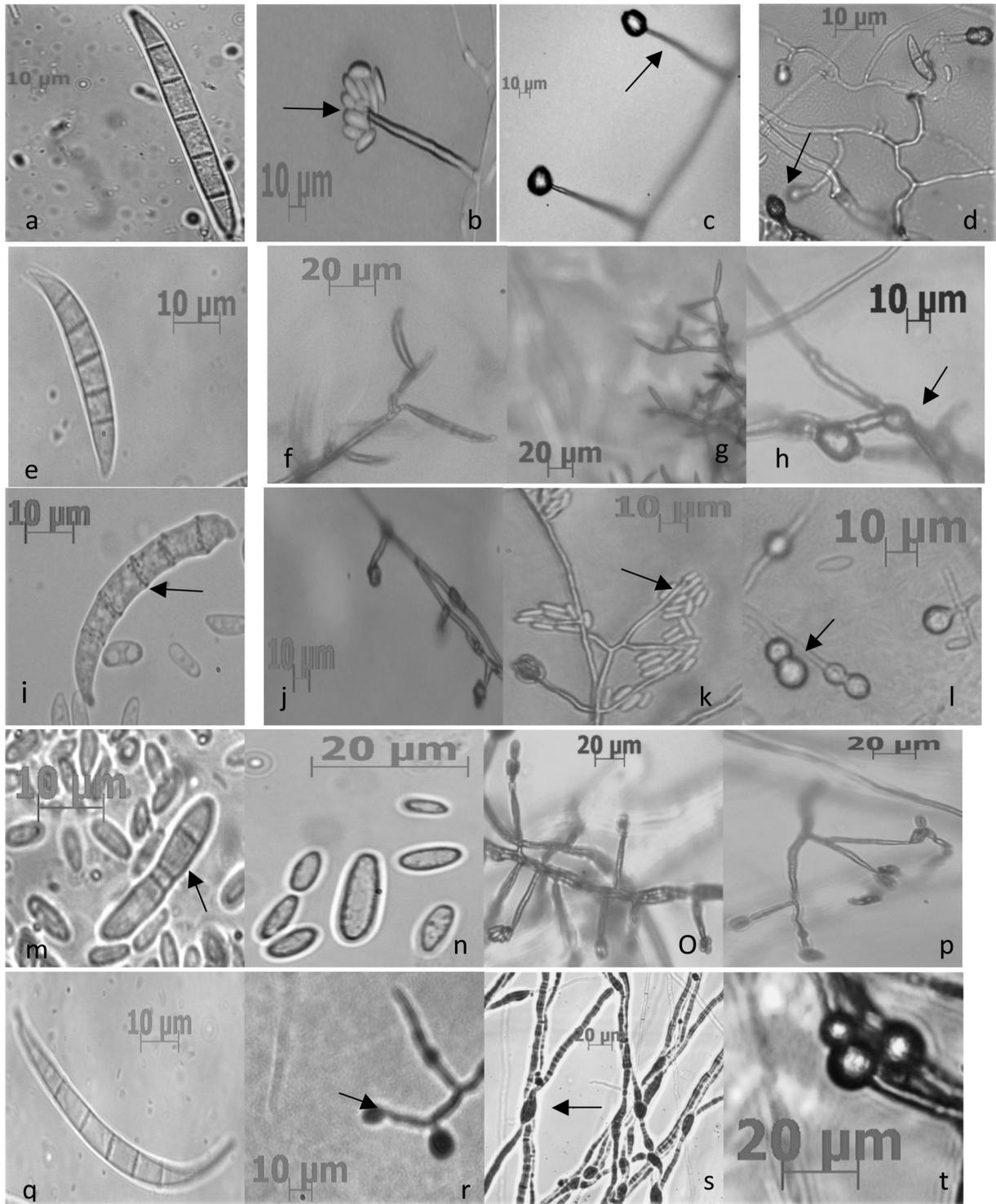


FIGURE 1. Microscopic characteristics of *Fusarium* species isolated from post-harvest disease of tomato *F. solani* a. (Macroconidia) b. (Microconidia in false heads) c. (Monophialide) & d. (Chlamydospore); *F. semitectum* e. (Macroconidia) f. (Monophialide) g. (Polyphialide) & h. (Chlamydospores in chain); *F. oxysporum* i. (Macroconidia) j. (Monophialide) k. (Microconidia in false heads) & l. (Chlamydospores in chains); *F. subglutinans* m. (Macroconidia) n. (Microconidia) o. (Monophialide) & p. (Polyphialide); *F. equiseti* q. (Macroconidia) r - s. (Microconidia in false heads) & t (Clustered chlamydospores)

TABLE 3. Distribution of *Fusarium* species isolated from post-harvest disease of tomato in Selangor and their diversity based on Shannon-Weiner index

Location	Number of isolate					Total	H'
	<i>F. solani</i>	<i>F. semitectum</i>	<i>F. oxysporum</i>	<i>F. subglutinans</i>	<i>F. equiseti</i>		
Sri Serdang	28	31	16	1	1	77	0.366
Selayang	0	0	0	4	0	4	0.084
Kajang	10	5	24	0	0	39	0.332
Semenyih	4	0	4	0	0	8	0.138
Puchong	0	0	4	0	0	4	0.084
Balakong	4	4	0	0	0	8	0.138
Shah Alam	0	8	0	0	0	8	0.138
Bandar Sunway	4	0	0	0	0	4	0.084
Subang Jaya	4	0	0	0	0	4	0.084
Seri Kembangan	0	0	8	0	0	8	0.138
Ampang	0	8	0	0	0	8	0.138
Rawang	4	0	0	0	0	4	0.084
Sungai Buloh	4	0	0	0	0	4	0.084
Total	62	56	56	5	1	180	1.889
Percentage (%)	34.44	31.11	31.11	2.78	0.56	100	

be water-soaked and slightly sunken (Snowdon 1991). *F. oxysporum* moreover causes decay on tomato fruits (Yeni et al. 2010), wilting, crown and root rot on tomato plants and symptoms of such infections are stunted growth, older leaves are often curved downward and subsequently the plants wilt and die (Denis et al. 1994; Momol et al. 2008). While crown and root rot stunted growth, yellowing and premature loss of cotyledons and lower true leaves, brown lesion, root rot, wilting and finally the death of the plants (Momol et al. 2008). *F. oxysporum* has also been reported to produce zearalenone and trichothecene (Marasas et al. 1984), beauvericin (Moretti et al. 2002) and moniliformin (Abbas et al. 1989).

F. subglutinans was the fourth *Fusarium* species isolated from tomato fruit rot in this study. It has been isolated from okra and red chilli (Nurulhuda et al. 2009), peppers (Mathur & Utkhede 2004), banana (Jimenez et al. 1993), rice (Nur Ain Izzati & Salleh 2009), sugar cane (Siti Nordahliawate et al. 2008), corn (Nur Ain Izzati et al. 2011), soybean (Schlub et al. 1981), millet (Onyike et al. 1991) and sorghum (Saubois et al. 1999). *F. subglutinans* produces beauvericin (Leslie et al. 2004), moniliformin (Lew et al. 1996) and little or no fumonisins (Reynoso et al. 2004).

F. equiseti was the least *Fusarium* isolated from post-harvest fruit rot of tomato and has been reported as a plant pathogen infecting tomato (Fajola 2004; Oladiran & Iwu 1992), pumpkin (Elmer 1996) and cucurbit (Adams et al. 1987) and moreover recovered from okra and cucumber (Nurulhuda et al. 2009) and corn (Nur Ain Izzati et al. 2011). *F. equiseti* was also reported to produce zearalenone (Hestbjerg et al. 2002), beauvericin (Logrieco et al. 1998) and trichothecene (Marasas et al. 1984).

In the virulent test, a total of 108 tomato fruits were inoculated with 27 isolates of the *Fusarium* (Table 4) with the control fruits. All the inoculated and non-inoculated fruits were observed from day after inoculation (dai) 1 to 7 every 24 h. On dai 1, there was no growth either of necrosis or

mycelia on the epicarp of the majority of tomato fruits (0% DSI) except in 4 isolates with 6 - 7% DSI as shown in Table 5. On dai 2 and 3, there were growth and development of necrosis on most of the fruits (22 isolates with 7 - 25% DSI) with few exception associated with white mycelia of fungal (5 isolates with 37 - 50% DSI). The fungi were penetrated into the epicarp and lied within the fleshy parts of the mesocarp. The necrosis of the epicarp enlarges outwards and the fruit becomes water-soaked as the infestation of the fungi continued as revealed in Figure 2(a) and 2(b). Moderate infection dominated majority of the inoculated tomato fruits (18 isolates with 40 - 60% DSI) and others in few instances with spore mass appeared (5 isolates with 60 - 75% DSI) on dai 4 and 5. Isolates recorded with lower DSI were B645T (25%), B647T (37.5%), B721T (37.5%) and B727T (31.25%). Severe infection covered almost half of the fruits (12 isolates with 60 - 75% DSI) and few others very devastating in which their necrotic tissues were soft and decay with fungal mass appeared on dai 6 and 7 (11 isolates with >80% DSI). Isolates recorded with very lower DSI were B645T (43.75%), B647T (37.5%), B689T (56.25%) and B727T (37.5%). In severe devastating, the fungi extended deep into the seed cavity to destroying the seed coat and cotyledons, Figure 2(c). The non-inoculated controls showed no symptoms of fruit rot from dai 1 to 7.

The DSI data obtained from dai 1 - 7 was then analysed using Friedman test of the Non-parametric test of SPSS programme. The results revealed that there are no difference in the distributiouns of isolate, DSI and day. The DSI distributions are significantly different among the isolates ($p < 0.05$). The DSI data for dai 7 depicted that the *F. oxysporum* was the most devastating on tomato fruit rot (B711T - 93.75%) followed by *F. solani* (B684T - 87.50%), *F. subglutinans* (87.50%), *F. semitectum* (4 isolates with 75%) and *F. equiseti* (B607T - 68.75%) while the least severe however were recorded on *F. solani* (B647T - 37.50%) and *F. oxysporum* (B727T - 37.50%) (Table 5).

TABLE 4. Source of isolates used in the pathogenicity test

<i>Fusarium</i> species	Isolate identity	Locality
<i>F. semitectum</i> (5 isolates)	B602T	Sri Serdang
	B605T	Sri Serdang
	B609T	Sri Serdang
	B649T	Sri Serdang
	B675T	Sri Serdang
<i>F. solani</i> (8 isolates)	B616T	Sri Serdang
	B627T	Sri Serdang
	B647T	Sri Serdang
	B664T	Sri Serdang
	B684T	Kajang
	B698T	Kajang
	B721T	Semenyih
	B722T	Semenyih
<i>F. oxysporum</i> (10 isolates)	B633T	Sri Serdang
	B645T	Sri Serdang
	B688T	Kajang
	B689T	Kajang
	B711T	Kajang
	B713T	Kajang
	B717T	Semenyih
	B718T	Semenyih
	B725T	Puchong
	B727T	Puchong
<i>F. subglutinans</i> (3 isolates)	B658T	Sri Serdang
	B678T	Semenyih
	B679T	Semenyih
<i>F. equiseti</i> (1 isolate)	B607T	Sri Serdang

TABLE 5. Disease severity index (DSI) for day 1 - 7

<i>Fusarium</i> species	Isolate identity	Disease severity index (DSI %) for each day						
		1	2	3	4	5	6	7
<i>F. semitectum</i>	B602T	0	25.00	25.00	43.75	50.00	68.75	75.00
	B605T	0	25.00	25.00	43.75	50.00	68.75	68.75
	B609T	0	25.00	25.00	43.75	50.00	75.00	75.00
	B649T	0	25.00	37.50	50.00	50.00	75.00	75.00
	B675T	0	18.75	25.00	50.00	50.00	75.00	75.00
<i>F. solani</i>	B616T	0	25.00	25.00	50.00	50.00	75.00	81.25
	B627T	6.25	25.00	25.00	50.00	50.00	75.00	75.00
	B647T	0	12.50	18.75	18.75	37.50	37.50	37.50
	B664T	0	25.00	37.50	50.00	62.50	75.00	81.25
	B684T	0	12.50	25.00	50.00	75.00	87.50	87.50
	B698T	0	25.00	25.00	50.00	50.00	75.00	75.00
	B721T	0	18.75	25.00	25.00	37.50	50.00	62.50
	B722T	0	12.50	25.00	31.25	43.75	62.50	87.50
<i>F. oxysporum</i>	B633T	0	18.75	25.00	37.50	43.75	62.50	75.00
	B645T	0	0	18.75	25.00	25.00	43.75	43.75
	B688T	0	25.00	50.00	62.50	75.00	87.50	87.50
	B689T	0	25.00	25.00	37.50	43.75	50.00	56.25
	B711T	0	18.75	25.00	37.50	56.25	68.75	93.75
	B713T	0	25.00	25.00	37.50	50.00	62.50	87.50
	B717T	0	18.75	25.00	37.50	56.25	68.75	75.00
	B718T	0	25.00	25.00	50.00	50.00	68.75	81.25
	B725T	6.25	25.00	25.00	31.25	43.75	56.25	68.75
	B727T	0	0	25.00	25.00	31.25	31.25	37.50
<i>F. subglutinans</i>	B658T	0	25.00	25.00	43.75	50.00	68.75	87.50
	B678T	6.25	18.75	37.50	50.00	62.50	75.00	81.25
	B679T	6.25	25.00	50.00	50.00	75.00	75.00	81.25
<i>F. equiseti</i>	B607T	0	18.75	25.00	43.75	43.75	56.25	68.75

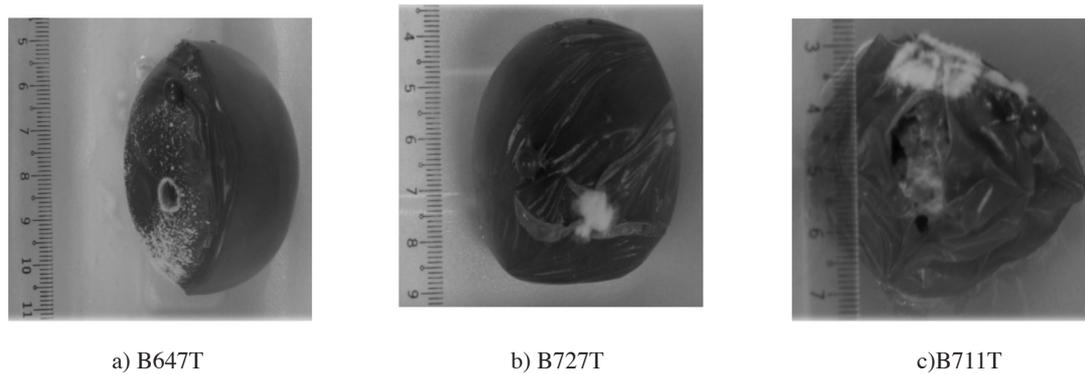


FIGURE 2. Mild to moderate infection in (a) and (b) (B647T and B727) while very severe/devastating in (c) (B711T)

All the isolates tested were pathogenic on tomato with different severity.

Tomato fruit rots caused by *Fusarium* was due to their utilization of ascorbic acid and soluble sugar contents in the fruit for their metabolic activities which showed a reduction trend with the days of incubation but there is a slight increase of soluble sugars on the 6th day after inoculation (Oladiran & Iwu 1992). Similarly Teisson et al. (1979) reported that fungal isolates utilized ascorbic acid in tomato fruits for their own metabolic activities. The reduction in ascorbic acid content also occurred in pineapples affected by black heart disorder (Abdullah et al. 1985, 1986). The carbohydrate content, especially the sugars, were reduced during the infection of sweet potatoes by *Botrydioplodia theobromae* (Arinze 1974). There is a decreased in sugar content of onion affected by *Pseudomonas fluorescens* (Oguntuyo 1981). The infection of pineapple fruits with black heart disorder was reported to have lower soluble solid than the uninfected fruits (Abdullah et al. 1986).

In conclusion, the present study therefore depicted that tomato fruits which form an important integral part of the human diet in providing basic food, essential vitamins, trace elements, food flavour, acceptability and could be used in many ways either in the raw or cooked state (Oladiran & Iwu 1992) are beset with decaying problems caused by micro-organisms. Fungi and bacteria have been isolated from decaying tomato fruits (Arinze 1986). The contamination of tomato fruits by fungi could pose threats on the biodiversity as the five *Fusarium* species isolated namely, *F. solani*, *F. semitectum*, *F. oxysporum*, *F. subglutinans* and *F. equiseti* were reported to produce mycotoxins. Mycotoxins are potentially toxigenic chemical compounds produced by some saprophytic *Fusarium* that affect humans and animals health when they entered food chains (Joffe 1986; Nelson et al. 1990). The findings of this study on the diversity of *Fusarium* species, morphological characteristics of the causal organisms and the virulence test is hope to provide an integrated information to formulating suitable control measure against the disease.

REFERENCES

- Abbas, H.K., Mirocha, C.J. & Gunther, R. 1989. Mycotoxins produced by toxic *Fusarium* isolates obtained from agricultural and non-agricultural Arctic areas of Norway. *Mycopathologia* 105: 143-152.
- Abdullah, H., Rohaya, M.A. & Zaipun, M.Z. 1985. Effects on modified atmosphere on black heart development and ascorbic acid contents in Mauritius pineapples during storage at low temperatures. *Association of Southeast Asian Nations Foundation Journal* 1: 15-18.
- Abdullah, H., Rohaya, M.A. & Zaipun, M.Z. 1986. Storage study of pineapple with special emphasis on black heart disorder. *MARDI Research Bulletin* 14: 132-138.
- Abraham, W.R., Knoch, I. & Witte, L. 1990. Biosynthesis of the terpenoid polyketide fusalaninpyrone. *Phytochemistry* 29: 2877-2878.
- Adams, G.C. Jr., Gubler, W.D. & Grogan, R.G. 1987. Seedling disease of muskmelon and mixed melons in California, USA caused by *Fusarium equiseti*. *Plant Disease* 71: 370-374.
- Amadi, J.E., Adebola, M.O. & Eze, C.S. 2009. Isolation and identification of a bacterial blotch organism from watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). *African Journal of Agricultural Research* 4(11): 1291-1294.
- Amadioha, A.C. & Uchendu, P.N. 2003. Post-harvest control of tomato fruit rot caused by *Fusarium solani* with extracts of *Azadirachta indica*. *Discovery and Innovation* 15: 83-86.
- Arinze, A.E. 1974. Studies on storage rot of sweet potato tubers in Nigeria. M.Sc. thesis, University of Lagos, Nigeria (unpublished).
- Arinze, A.E. 1986. Post-harvest diseases of tomato fruits in southern Nigeria. *Fitopatologia Brasileira* 11: 637-645.
- Bacon, C.W., Porter, J.K., Norred, W.P. & Leslie, J.F. 1996. Production of fusaric acid by *Fusarium* species. *Applied and Environmental Microbiology* 62: 4039-4043.
- Burgess, L.W., Nelson, P.E., Tousson, T.A. & Marasas, W.F.O. 1985. *Fusarium scirpi*: Amended description and notes on geographic distribution. *Mycologia* 77: 212-218.
- Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P. & Blackhouse, D. 1994. *Laboratory Manual for Fusarium Research*. University of Sydney. p.133.
- Chelkowski, J., Zawadzki, M., Zajkowski, P., Logrieco, A. & Bottalico, A. 1990. Moniliformin production by *Fusarium* species. *Mycotoxin Research* 6: 41-45.
- Denis, C. 1983. *Post-harvest Pathology of Fruits and Vegetables, Food Science and Technology, London, England: A Series of Monographs, Academic Press Incorporation Limited, 24-28 Oval Road, NW17DX.*

- Denis, M.P., Cooke, T. & House S. 1994. Disease of Vegetable Crops in Australia. Australia: CSIRO Publishing.
- Dhingra, O.D. & Muchovej, J.J. 1979. Pod rot, seed rot and root rot of snap bean and dry bean caused by *Fusarium semitectum*. *Plant Disease Reporter* 63: 84-87.
- Elmer, W.H. 1996. *Fusarium* fruit rot of pumpkin in Connecticut. *Plant Disease* 80: 131-135.
- Fajola, A.O. 2004. Post-harvest fruit rots of tomato (*Lycopersicon esculentum*) in Nigeria. *Wiley On-line Library, Food/Nahrung* 23: 105-109.
- Gopinath, A., Prakash, H.S. & Shetty, H.S. 1985. Grain mold of sorghum: Role of associated fungi on the health and viability of seeds. *International Journal of Tropical Plant Diseases* 3: 177-182.
- Grunwald, N.J., Coffman, V.A. & Kraft, J.M. 2003. Sources of partial resistance to *Fusarium* root rot in the *Pisum* core collection. *Plant Disease* 87: 1197-1200.
- Hawa, M.M., Salleh, B. & Latiffah, Z. 2010. Characterization and intraspecific variation of *Fusarium semitectum* (Barkeley and Ravenel) associated with red-fleshed dragon fruit (*Hylocereus polyrhizus* (Weber) Britton and Rose) in Malaysia. *African Journal of Biotechnology* 9(3): 273-284.
- Hestbjerg, H., Nielsen, K.F., Thrane, U. & Elmholt, S. 2002. Production of trichothecene and other secondary metabolites by *Fusarium culmorum* and *Fusarium equiseti* on common laboratory media and a soil organic matter agar: An ecological interpretation. *Journal of Agricultural and Food Chemistry* 50: 7593-7599.
- Jimenez, M., Logrieco, A. & Bottalico, A. 1993. Occurrence and pathogenicity of *Fusarium* species in banana fruits. *Journal of Phytopathology* 137: 214-220.
- Joffe, A.Z. 1986. *Fusarium Species: Their Biology and Toxicology*. New York: John Wiley and Sons.
- Latiffah, Z., Nur Hayati, M.Z., Salleh, B. & Maziah, Z. 2009. Identification and pathogenicity of *Fusarium* species associated with root rot and stem rot of *Dendrobium orchids*. *Asian Journal of Plant Pathology* 3(1): 14-21.
- Leslie, J.F. & Summerell, B.A. 2005. *The Fusarium Laboratory Manual*. Tygerberg, South Africa: W.F.O. Marasas, Pomec Unit, Medical Research Council.
- Leslie, J.F., Zeller, K.A., Logrieco, A., Mule, G., Moretti, A. & Ritieni, A. 2004. Species diversity and toxin production by strains in the *Gibberella fujikuroi* species complex isolated from native Prairie grasses in Kansas. *Applied and Environmental Microbiology* 70: 2254-2262.
- Lew, H., Chelkowski, J., Pronczuk, P. & Edinger, W. 1996. Occurrence of the mycotoxin moniliformin in maize ears infected by *Fusarium subglutinans*. *Food Additives and Contaminants* 13: 321-324.
- Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A. & Chelkowski J. 1998. Beauvericin production by *Fusarium* species. *Applied and Environmental Microbiology* 64: 3084-3088.
- Mahovic, M., Sargent, S.A. & Bartz, J.A. 2004. Identifying and controlling post-harvest tomato diseases in Florida. HS866 document, one of a series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Revised edition: 2004. Available from EDIS web site at <http://edis.ifas.ufl.edu>. (accessed on 14 Oct. 2009).
- Marasas, W.F.O., Nelson, P.E. & Toussoun, T.A. 1984. *Toxigenic Fusarium species: Identity and Mycotoxicology*. U.S.A: The Pennsylvania State University Press, University Park, Pennsylvania. pp. 8-9.
- Mathur, S. & Utkhede, R. 2004. Molecular detection of *Fusarium subglutinans*, the causal organism of internal fruit rot in greenhouse peppers. *Canadian Journal of Plant Pathology* 26: 514-521.
- McGovern, R.J. 1994. First report of corky dry rot of cantaloupe caused by *Fusarium semitectum* in Florida. *Plant Disease* 78: 926.
- Mehrotra, R.S. 1989. *Plant Pathology, Six Reprint*. New Delhi, India: Tata McGraw-Hill Publishing Company Limited.
- Momol, T., Ji, P., Pernezny, K., McGovern, R. & Olson, S. 2008. Three soil borne tomato diseases caused by *Ralstonia* and *Fusarium* species and their field diagnostics. U.S.A: p. 205. Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 2008. Available on-line at EDIS Web site <http://edis.ifas.ufl.edu>. (accessed on 20 Oct. 2009).
- Moretti, A., Belisario, A., Tafuri, A., Ritieni, A., Corazza, L. & Logrieco, A. 2002. Production of beauvericin by different races of *Fusarium oxysporum* sp. melonis, the *Fusarium wilt* agent of muskmelon. *European Journal of Plant Pathology* 108: 661-666.
- Moss, M.O. 2002. Mycotoxin review 1 *Aspergillus* and *Penicillium*. *Mycologist* 16: 116-119.
- Nelson, P.E., Burgess, L.W. & Summerell, B.A. 1990. Some morphological and physiological characters of *Fusarium* species in sections liseola and elegans and similar new species. *Mycologia* 82: 99-106.
- Nik Mohd Izham, M.N. 2008. Distribution, morphological characteristics and mycotoxin profiles of *Fusarium* species from soils in Peninsular Malaysia. M.Sc. thesis. University Sains Malaysia (unpublished).
- Nur Ain Izzati, M.Z., Siti Nordahliwate, M.S., Nur Azlina, J., Darnetty, A.A. & Salleh, B. 2011. Isolation and identification of *Fusarium* species associated with *Fusarium* ear rot disease of corn. *Pertanika Journal of Tropical Agricultural Science* 34(2): 325-330.
- Nur Ain Izzati, M.Z. & Siti Nordahliwate, M.S. 2010. *Fusarium* species isolated from *Eurycoma longifolia* Jack. In proceedings of the 11th symposium for the *Malaysian Society of Applied Biology* 2010 - Biological balance towards life sustainability. Kota Bahru, Kelantan. pp. 563-566.
- Nur Ain Izzati, M.Z., Siti Nordahliwate, M.S., Nur Azlin, A. & Salleh, B. 2009. Distribution and diversity of *Fusarium* species associated with grasses in Peninsular Malaysia. *Biotropia* 16(2): 55-64.
- Nur Ain Izzati, M.Z. & Salleh, B. 2009. Genetic variability amongst *Fusarium* species in the section liseola from Bakanae-infected rice in Malaysia and Indonesia by RAPD analysis. *Journal Malaysian Applied Biology* 38(1): 71-79.
- Nurulhuda, M.S., Latiffah, Z., Baharuddin, S. & Maziah, Z. 2009. Diversity of *Fusarium* species from vegetables fruits. *Journal Malaysian Applied Biology* 38(1): 43-47.
- Oguntayo, O.O. 1981. Studies on microbial spoilage of onions. PhD thesis, University of Lagos, Nigeria (unpublished).
- Oladiran, A.O. & Iwu, L.N. 1992. Changes in Ascorbic acid and carbohydrate contents in tomato fruits infected with pathogens. *Plant Foods for Human Nutrition* 42: 373-382.
- Onyike, N.B.N., Nelson, P.E. & Marasas, W.F.O. 1991. *Fusarium* species associated with millet grain from Nigeria, Lesotho and Zimbabwe. *Mycologia* 83: 708-712.
- Papavizas, G.C. 1967. Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. *Phytopathology* 57: 848-852.

- Rabie, C.J., Marasas, W.F.O., Thiel, P.G., Lubben, A. & Vlegaar, R. 1982. Moniliformin production and toxicity of different *Fusarium* species from southern Africa. *Applied and Environmental Microbiology* 43: 517-521.
- Reynoso, M.M., Torres, A.M. & Chulze, S.N. 2004. Fusaproliferin, beauvericin and fumonisin production by different mating populations among the *Gibberella fujikuroi* species complex isolated from maize. *Mycological Research* 108: 154-160.
- Salleh, B. & Mushitah, A. 1991. *Fusarium* species in section *Liseola* in Malaysia. *Journal of Bioscience* 2: 1-8.
- Saubois, A., Laforet, E.P., Nepote, M.C. & Wagner, M.L. 1999. Mycological evaluation of a sorghum grain of Argentina with emphasis on the characterization of *Fusarium* species. *Food Microbiology* 16: 435-445.
- Schlub, R.L., Lockwood, L. & Komada, H. 1981. Colonization of soybean seeds and plant tissue by *Fusarium* species in soil. *Phytopathology* 71: 693-697.
- Seta, S., Gonzalez, M. & Lori, G. 2004. First report of walnut canker caused by *Fusarium incarnatum* in Argentina. *Plant Pathology* 53: 248.
- Seth, P.K. & Shandilya, T.R. 1978. Mycoflora associated with the deterioration of the cultivated mushrooms in storage. *Indian Journal of Mycology and Plant Pathology* 8: 209-210.
- Sherf, A.F. & Macnab, A.A. 1986. *Vegetable Diseases and Their Control*. 2nd ed. New York, U.S.A: A Wiley Interscience Publication, John Wiley and Sons Incorporation Limited.
- Siti Nordahliawate, M.S., Nur Ain Izzati, M.Z., Azmi, A.R. & Salleh, B. 2008. Distribution, morphological characterization and pathogenicity of *Fusarium sacchari* associated with Pokkah Boeng disease of sugar cane in Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science* 31(2): 279-286.
- Siti Nordahliawate, M.S., Nur Ain Izzati, M.Z., Nur Azlin, A. & Salleh, B. 2009. Diversity of *Fusarium* species in section *elegans* causing *Fusarium* Fruit Rot in the family of Cucurbitaceae. In *Proceeding of International Congress of Malaysian Society for Microbiology*, Grand Plaza Parkroyal, Penang, Malaysia. p. 379.
- Snowdon, A.L. 1991. *A Colour Atlas of Post-harvest Disease and Disorders of Fruits and Vegetables* Vol. 2: Vegetables. Wolfe Scientific.
- Solunkhe, D.K. & Desai, B.B. 1984. *Post-harvest Biotechnology of Vegetables, Volume 1*. Florida, U.S.A: CRC Press Incorporation Boca Raton.
- Teisson, C., Combress, J.C., Martin-Prevell, P. & Marchal, J. 1979. Internal browning of pineapples, III Symptoms IV Biochemical approach. *Fruits* 34: 315-338.
- Tournas, V.H. & Katsoudas, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology* 105: 11-17.
- Venco, G. & Meneses, E. 1996. Diversity pattern of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil* 183: 315-322.
- Wallbridge, A. 1981. Fungi associated with crown rot disease of boxed bananas from the Windward Islands during a two year survey. *Transactions of the British Mycological Society* 77: 567-577.
- Wilson, C.L., Wisniewski, M.E., Biles, C.L., McLaughlin, R. Chalutz, E. & Dorby, S. 1991. Biological control of post-harvest diseases of fruits and vegetables: Alternative to synthetic fungicides. *Crop Protection* 10: 172.
- Yeni, I.J., Dele, O.S., Ademola, I.J. & Adeniran, A.J. 2010. Allelopathic effect of leaf extract of *Azadirachta indica* and *Chromolaena odorata* against post-harvest and transit rot of tomato (*Lycopersicon esculentum* L). *Journal of American Science* 6: 1595-1599.

A.I. Abu Bakar, M.Z. Nur Ain Izzati* & Y. Umi KAlsom
 Department of Biology, Faculty of Science
 Universiti Putra Malaysia
 43400 Serdang, Selangor D.E.
 Malaysia

A.I. Abu Bakar
 Department of Integrated Science
 School of Science Education
 Federal College of Education (Technical) Bichi
 P.M.B 3473, Kano State
 Nigeria

*Corresponding author; email: izzati@science.upm.edu.my

Received: 16 April 2012

Accepted: 15 November 2012