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Occurrence of *Fusarium* spp. on Vegetable Crops and Assessment of Their Pathogenicity

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ABSTRACT

Fusarium are among the fungal genera that can cause contamination or spoilage on vegetable crops. Therefore, it is important to identify the occurrence of *Fusarium* species on these commodities as some species are plant pathogen and some other are toxigenic. In the present study, 83 *Fusarium* isolates were recovered from rotting tissues of nine vegetable crops, namely, cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), okra (*Hibiscus esculentus)*, loofah (*Luffa acutangula*), bitter gourd (*Momordica charantia*), moringa *(Moringa olifel)*, brinjal (*Solanum melongena*), long bean (*Vigna sesquipedalis*) and red chilli (*Capsicum annuum*). The species identified were *F. oxysporum* (22 isolates), *F. semitectum* (19 isolates), *F. solani* (19 isolates), *F. proliferatum* (14 isolates), *F. pseudocircinatum* (four isolates), *F. sacchari* (two isolates), *F. equiseti* (two isolates) and *F. verticillioides* (one isolate). From pathogenicity test, only 21 isolates were found to be pathogenic, causing vegetable rot on their host. The present study showed that *Fusarium* species are prevalent on vegetable crops and the species might be pathogenic or epiphytic .

Keywords: Fusarium, vegetable crops, pathogenicity

INTRODUCTION

Many *Fusarium* species are plant pathogen and cause vascular wilts, root and fruit rots diseases on various types of vegetable crops. There are also opportunistic species or weak pathogen which colonize plant tissues after the plants have become stressed, especially the species associated with spoilage or postharvest disease on vegetables crops. After harvest, vegetables contain relatively high microorganism which includes spoilage and plant pathogenic fungi that can cause deterioration and reduction in quality, texture and loss of nutrients (Barth *et al*.,

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2009). It can also reduce shelf-life and the acceptability of the produce. Among the fungal pathogens, the *Fusarium* species is commonly found to be associated with losses caused due to rotting and spoilage of several types of vegetable crops. Theses include vegetables mainly belonging to the solanaceae and cucurbitaceae families (Snowdon, 1990; Tournas, 2005a, d, b; Naureen *et al*., 2009).

In Malaysia, occurrences of *Fusarium* species on vegetable crops have not been given much attention compared to other agricultural crops. Therefore, the present study was conducted to evaluate the occurrences of *Fusarium* species on several vegetable crops and determine if the isolates were pathogenic and caused vegetable rot.

MATERIALS AND METHODS

Isolation and Identification of the Fusarium species

Fusarium isolates were isolated from rotting tissues of nine vegetable crops, namely, cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), okra (*Hibiscus esculentus)*, loofah (*Luffa acutangula*), bitter gourd (*Momordica charantia*), moringa *(Moringa olifel)*, brinjal (*Solanum melongena*), long bean (*Vigna sesquipedalis*) and red chilli (*Capsicum annuum*) obtained from several markets and hypermarkets in Penang Island, Malaysia. The mycelium grow on the vegetable was transferred onto Peptone Pentachloronitrobenzene Agar, a semi-selective medium for isolation of *Fusarium*. The medium was incubated at 27±1°C for 4-5 days or until the mycelia growth were observed. The mycelia were then subcultured onto potato sucrose agar (PSA).

For identification, the procedures in The *Fusarium* Laboratory Manual (Leslie & Summerell, 2006) were adopted and single spore culture was used. Each isolate was cultured on Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA). The CLA was used to determine the shapes of microconidia and macroconidia, the number of septa and the shapes of the apical and basal cells of the macroconidia, formation of conidiogenous cell, the presence and the colour of sporodochia, and presence of chlamydospore. The cultures plated on CLA were incubated at 27±1°C for 4-5 days. On PDA, cultural characteristics and pigmentation were determined, in which the observations were made after 3 days of incubation at 27±1°C. Species descriptions in The *Fusarium* Laboratory Manual (Leslie & Summerell, 2006) were adapted for the identification of the *Fusarium* isolates to the species level.

Pathogenicity Test

All the isolates of *Fusarium* successfully isolated from the nine vegetable crops were used in the pathogenicity test. Different types of healthy vegetables, namely, cucumber, tomato, okra loofah, bitter gourd, moringa, brinjal, long bean and red chilli were washed with running tap water, disinfected with sodium hypochloride (10%), rinsed with distilled water, and dried at 27 ± 1°C. Inoculations were performed on wounded and unwounded vegetables with three

replicates for each isolate. Mycelial plug (6 mm) was prepared from 5-day old culture and used as inoculum. Three replicates were made for each vegetable and the test was repeated twice. Uninoculated vegetable served as a control. Inoculated vegetables were incubated in a sterile plastic container (40 cm x 30 cm) at 27 ±1°C and disease symptoms were assessed daily through visual examination. Disease symptoms were recorded based on the following scale which was adapted (with some modifications) from Benyon *et al*. (1996): 1 = 20% diseased area, 2 = 50% diseased area, 3 = 80% diseased area, 4 = 100% diseased area. Based on the scale, the percentage of rotted areas was estimated. Reisolation of the fungi was made by direct isolation from the mycelia developed on the rotting tissues, and plated on PSA.

RESULTS AND DISCUSSION

Eighty three *Fusarium* isolates were isolated from rotting tissues of all the vegetable crops, in which 22 isolates were recovered from okra, 13 from tomato and bitter gourd, seven from brinjal, four from cucumber, three from moringa and loofa, and one from long bean. The *Fusarium* isolates were identified as *F. oxysporum* (22 isolates), *F. semitectum* (19 isolates), *F. solani* (19 isolates), *F. proliferatum* (14 isolates), *F. pseudocircinatum* (four isolates), *F. sacchari* (two isolates), *F. equiseti* (two isolates) and *F. verticillioides* (one isolate) (Table 1). The morphological characteristics of each species are presented in Table 2 and Fig.1.

TABLE 1
Fusarium species isolated from the rotting symptom of vegetable crops

Host	*Fusarium* species / number of isolates
Solanaceae	
Tomato	*F. oxysporum* (7)
	F. solani (5)
	F. proliferatum (1)
Chilli	*F. solani* (4)
	F. pseudocircinatum (2)
	F. proliferatum (2)
	F. sacchari (1)
	F. semitectum (1)
Brinjal	*F. proliferatum* (3)
	F. solani (2)
	F. equiseti (1)
	F. pseudocircinatum (1)
Cucurbitaceae	*F. semitectum* (2)
Cucumber	*F. solani* (1)
	F. oxysporum (1)
Loofa	*F. semitectum* (3)
Bitter gourd	*F. oxysporum* (6)
	F. solani (3)
	F. semitectum (2)
	F. proliferatum (2)
Malvaceae	*F. semitectum* (11)
Okra	*F. oxysporum* (7)
	F. proliferatum (5)
	F. solani (2)
	F. pseudocircinatum (2)
	F. verticillioides (1)
Moringaceae	*F. oxysporum* (1)
Moringa	*F. solani* (2)
Fabaceae	*F. semitectum* (1)
Long bean	

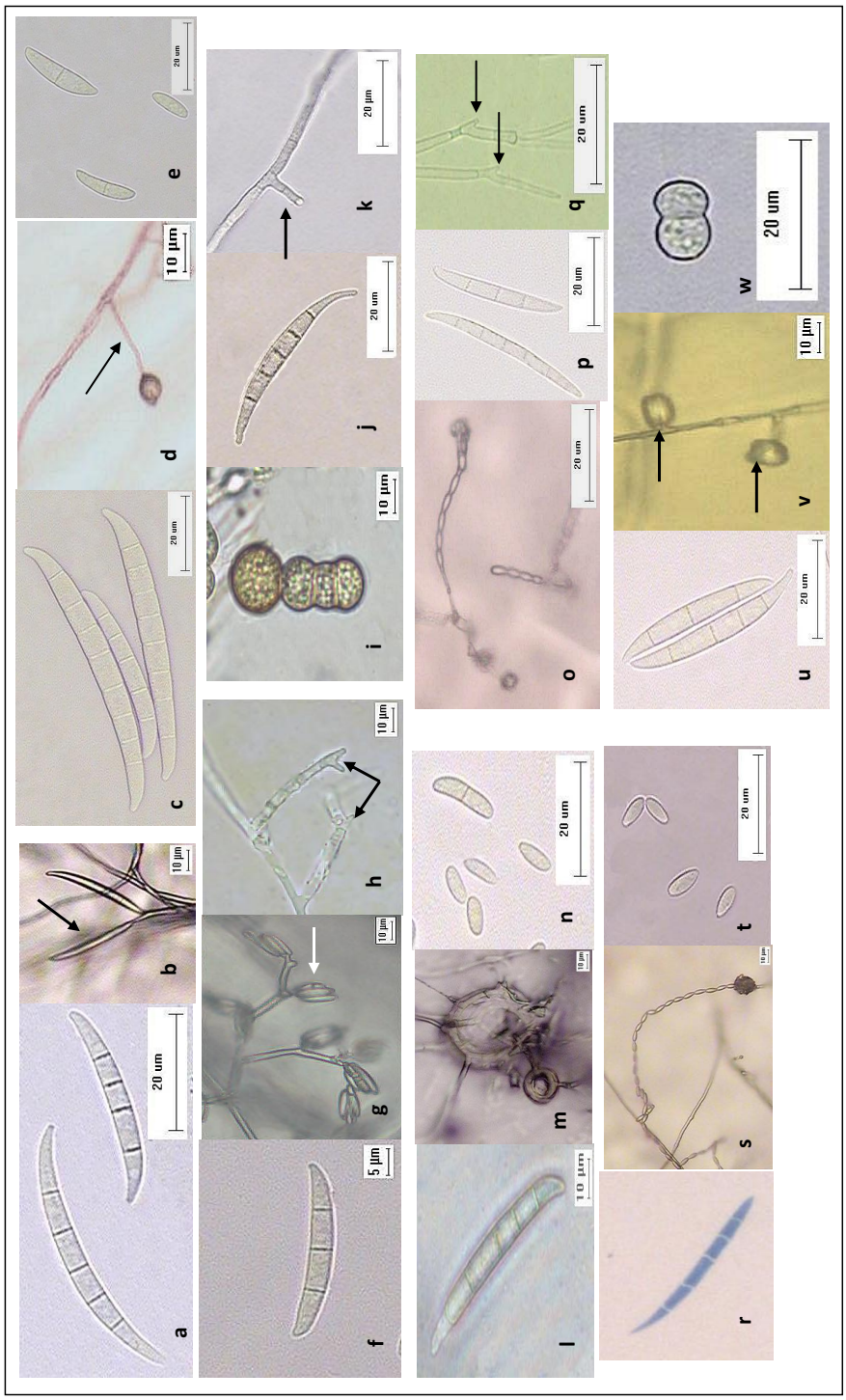

Fig.1: Morphological characteristics of *Fusarium* spp. isolated from rotting tissues of vegetable crops. **(a - b)** : *F. semitectum*, a: macroconidia, b: rabbit ear. **(c – e)** : *F. solani* , c: macroconidia, **d**: long moniphialide, e: microconidia. **(f – h)** : *F. sacchari*, f: macroconidia, g: mesoconidia, h:polyphiliades. **(i – k)** : *F. equiseti*, **i**: chlymadospores, j: macroconidia, **k**: monophialide. **(l – n)** : *F. pseudocircinatum*, **l**: macoconidia, **m**: coiled hyphae, **n**: microconidia. **(o – q)** *F. verticillioides*, **o**: conidia in chain, **p**: macroconidia, **q**: polyphialides. **(r – t)** : *F. verticillioides*, r: macroconidia, **s**: conidia in chain, **t**: microconidia. **(u – w)** : *F. oxysporum*, **u**: macroconidia, **v**: false heads, **w**: chlamydospores

TABLE 2
The morphological characteristics of *Fusarium* species isolated from nine vegetable crops

	Species			
Characteristics	*F. oxysporum*	*F. semitectum*	*F. solani*	*F. sacchari*
Microconidia	Abundant , formed in aerial mycelia, oval to kidney-shaped produced in false head	Scarce, presence of fusoid mesoconidia in aerial mycelia, rabbit-ear appearance.	Abundant, oval to kidney-shaped produced in the agar and carnation leaf.	Abundant, oval, produced only in false head. Presence of mesoconidia in false head.
Macroconidia	Abundant in sporodochia, slightly sickle-shaped, thin walled, tapered apical cell, foot-shaped basal cell	Relatively slender, curved apical cell and foot-shaped basal cell	Abundant, stout, cylindrical, blunt apical cell, distinct and rounded foot-shaped basal cell.	Abundant, slightly sickle-shaped to almost straight, curved apical cell and poorly developed basal cell.
Conidiogenous cell	monophailides	monophailides and polyphialides	long monophailides	polyphailides and monophailides.
Chlamydospore	Present, singly or in pairs.	Present	Present singly or in pairs.	Absent
Pigmentation	White to purple.	Beige to brown	Cream to white	White to purple.

	Species			
Characteristics	*F. proliferatum*	*F. pseudocircinatum*	*F. verticillioides*	*F. equiseti*
Microconidia	Abundant, club shape with flattened base, in chain (10- 15 conidia) and false head.	Abundant, formed in aerial mycelia, oval, produced in false head and in short chain (5 – 10 conidia).	Abundant, formed in aerial mycelia, oval to club shaped, produced in long chain (more than15 conidia).	Absent
Macroconidia	Abundant, slender, almost straight, curved apical cell and poorly developed foot-shaped basal cell.	Scarce, slightly sickle-shaped to almost straight, curved apical cell and poorly developed basal cell.	Scarce, slightly sickle-shaped to almost straight, curved, tapered to a point apical cell and foot shaped basal cell.	Abundan, formed in aerial mycelia, long and quite slender, elongated apical cell and obvious foot shape basal cell.
Conidiogenous cell	polyphailides and monophailides	Usually monophailides. Presence of coiled hyphae	monophailides.	Monophailides
Chlamydospore	Absent	Absent	Absent	present, formed in clumps or chains.
Pigmentation	White to purple	White to light purple.	White to light purple.	Brown to dark brown

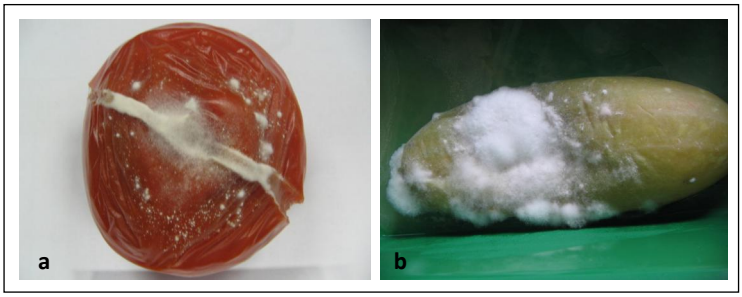

Fig.2: (a) Symptom on tomato inoculated with *F. proliferatum* (TMTC1) on non-wounded treatment (b) Symptom on cucumber inoculated with *F. solani* (TMN S1b) on wounded treatment

From the pathogenicity test, only 21 isolates were pathogenic to their host (Table 3) as these isolates were successfully reisolated from the rotting tissues, proving that the isolates were the causal pathogen of vegetable rot. Wounded treatment showed severed symptoms compared to unwounded treatment. Rotting symptoms shown on different types of vegetable were similar and characterized by the development of rotting areas with brown discolouration and water soaked appearance (Fig.2). Rotting symptoms were observed on the 7th day after inoculation and the size of the rotting areas gradually increased.

On Solanaceae crops, four species were pathogenic (namely, *F. solani, F. oxysporum*, *F. proliferatum* and *F. sacchari*). Four isolates of *F. solani*, three isolates of *F. oxysporum* and one isolate of *F. proliferatum* were pathogenic on tomato. On chilli, three isolates of *F. solani*, one isolate of *F. proliferatum* and one isolate of *F. sacchari* were pathogenic (Table 2). Disease severity ranging from 60% - 90% and only one isolate of *F. solani* (LMH T6) caused infection using both wounded and non-wounded treatment. On eggplant, one isolate of *F. solani* (TBP S3) was pathogenic (with 85% disease severity) using wounded treatment, while one isolate of *F. proliferatum* (TPJ T3) was pathogenic using both wounded and non-wounded treatment with 65% and 50% disease severity, respectively. Meanwhile, only one isolate of *F. solani* (TMT T3) was pathogenic on tomato using non-wounded treatment with 15% disease severity. Among the four species, *F. oxysporum* and *F. solani* are commonly reported to be associated with rotting of vegetable crops. *Fusarium oxysporum* has been recorded to cause fruit rot of tomatoes (Lockhart, 1970; Akinmusire, 2011) and peppers (Micosa & Ilag, 1977; Fletcher, 1994) and *F. solani* on eggplants, pepper (Ramdial & Rampersad, 2010) and brinjal (Pandey, 2010). Other *Fusarium* species have also been reported to be associated with the rot of Solanaceae crops such as *F. equiseti* on tomatoes and pepper (Adisa & Lekunze, 1986; Oladiran & Iwu, 1993), *F. chlamydosporum* (Oladiran & Iwu, 1993) and *F. avenaceaum* on tomatoes (Marras *et al*., 1979).

Two isolates of *F. solani* and one isolate of *F. oxysporum* were pathogenic on moringa, with disease severity ranging

TABLE 3. *Fusarium* isolates pathogenic to their host

Host	Isolate	*Fusarium* species	Pathogenicity			
			Wounded	Scale / Disease severity	Non-wounded	Scale / Disease severity
Tomato	TMT G7	F. solani	P	4 / 100%	NP	0
Tomato	TMT M1	*F. solani*	P	3 / 80%	NP	0
Tomato	TMT M5	*F. solani*	P	4 / 90%	NP	0
Tomato	TMT T2	*F. solani*	P	3 / 65%	NP	0
Tomato	TMT T3	*F. solani*	P	4 / 100%	P	1 / 15%
Tomato	TMT G3	*F. oxysporum*	P	3 / 80%	NP	0
Tomato	TMT M3	*F. oxysporum*	P	2 / 50%	NP	0
Tomato	TMT T1	*F. oxysporum*	P	4 / 90%	NP	0
Tomato	TMT C1	*F. proliferatum*	P	3 / 70%	NP	0
Chilli	LMH S1	*F. solani*	P	3 / 70%	NP	0
Chilli	LMH T3	*F. solani*	P	3 / 70%	NP	0
Chilli	LMH T6	*F. solani*	P	4 / 90%	P	2 / 50%
Chilli	LMH T4	*F. sacchari*	P	3 / 70%	NP	0
Chilli	LMH S4	*F. proliferatum*	P	3 / 60%	NP	0
Cucumber	TMN S1b	*F. solani*	P	4 / 85%	NP	0
Moringa	MNG R1	F. solani	P	1 / 7%	P	1 / 2%
Moringa	MNG R3	*F. solani*	P	3 / 65%	NP	0
Moringa	MNG R2	*F. oxysporum*	P	2 / 42%	P	1 / 5%
Eggplant	TBP S3	*F. solani*	P	4 / 85%	NP	0
Eggplant	TPJ T3	*F. proliferatum*	P	3 / 65%	P	2 / 50%
Long bean	KPJ N1	*F. semitectum*	P	4 / 100%	NP	0

* P – Pathogenic, NP – Non- pathogenic

from 2% - 65%. Although *F. solani* (MNG R1) showed infection using both wounded and non-wounded treatments, lower disease severity was observed with 7% and 2% severity, respectively. So far, there has been no report on the occurrence of *Fusarium* species that cause rotting of moringa pod.

Although *F. semitectum*, *F. solani* and *F. oxysporum* were recovered from cucumber and loofah, only *F. solani* (TMN S1B) was pathogenic on cucumber with 85% severity using wounded treatment. *Fusarium solani* has been found to be associated with rot of cucumber by Joffe and Plati (1972). In the present study, *F. oxysporum* was not found to be pathogenic on cucumber but it has been reported to be pathogenic on cucumber in the USA (Jenkins & Wehner, 1983; McMillan, 1986). In the present study, *F. semitectum* was not pathogenic to loofah. However, *F. semitectum* was found to cause decay on *Luffa cylindrica* (Tandon & Jamaluddin Bhargava, 1976) and was the most virulent species causing rotting on fruit tissues of *Luffa cylindrical* (Hilal *et al.,* 2003).

Pod rot of okra caused by *F. solani* has been reported by Esuruoso *et al*. (1975). However, in the present study, *F. solani* isolated from okra was not pathogenic, just like the six other species, namely, *F. oxysporum, F. proliferatum, F. solani, F. pseudocircinatum and F. verticilliodes*. Meanwhile, *Fusarium semitectum* was found to be the most common species isolated from okra but was non-pathogenic. This was not surprising as the species has not been known as an important plant pathogen although it has been reported to be pathogenic on several plants (Leslie & Summerell, 2006). In the present study, only *F. semitectum* recovered from long bean was pathogenic with 100% disease severity. On other types of legume, *F. semitectum* has been reported to have caused pod rot and seed rot of snap bean in India and field disease of common bean in Brazil (Dhingra & Muchovej, 1979; Dhingra *et al.,* 2002).

Most of the *Fusarium* isolates infected the vegetable crops on wounded treatment, indicating that the *Fusarium* species associated with vegetable rot are weak pathogen that causes infection when the crops are weakened or stressed through mechanical injuries and impact damage (Coates & Johnson, 1997). Moreover, some vegetable crops such as tomato, chilli, brinjal and cucumber have thin skin which causes them to become more prone to injuries. Injuries on the surface of vegetables are caused by cuts or abrasion during harvesting, handling operations, storage pressure and impact damage as well as poor sanitary practice and contamination during transportation and marketing (Coates & Johnson, 1997; Eckert, 1978; Barth *et al*., 2009).

The injuries and presence of pathogenic microbes on vegetable crops, combined with suitable environmental factors, provide the conditions for disease expression and development by spoilage fungi including *Fusarium* species. From non-wounded treatment, three *F. solani* isolates, one *F. oxysporum* isolate and one *F. proliferatum* isolate were found to be pathogenic to their host. These *Fusarium* isolates might produce pectin-degrading enzymes to degrade pectin component of the cell wall which assist the pathogen to penetrate the host. In the *Fusarium* species, endopolygalacturonases are among the enzyme produced during infection especially for tissue penetration and colonization (Mariotti *et al*., 2009).

Although only 21 isolates were found to be pathogenic, the isolates showed variation in term of their degree of pathogenicity. Most *F. solani* and *F. oxysporum* isolates were pathogenic on different types of vegetable crops and showed variation in their pathogenicity. The range of variation in pathogenicity could be associated with genetic diversity as both *F. solani* and *F. oxysporum* are regarded as species complex (Baayen *et al*., 2000; O'Donnell, 2000). Species in a species complex exhibit high level of genetic diversity. Moreover, both species occur on a wide host range and have several forma specialis and races which infect specific plant species and cultivars. The same condition can be applied to *F. proliferatum* isolates which were pathogenic

on tomato, chilli and brinjal. *Fusarium proliferatum* is grouped in *Gibberella fujikuroi* species complex and can be found on a wide host range as well as pathogenic on various agricultural crops.

Other *Fusarium* species isolated with low frequency from vegetable rot were *F. sacchari, F. pseudocircinatum, F. verticillioides* and *F. equiseti*. Among the three species, only *F. equiseti* and *F. verticillioides* have been reported to be associated with vegetable crops. *Fusarium equiseti* has been isolated from rotten tomato fruits (Oladiran & Iwu, 1993) and the host range includes several numbers of Leguminosae (Goswani *et al*., 2008). *Fusarium verticillioides* has been recovered from internal fruit rot of pepper (Howard, 2005) and from apical segment of asparagus (Elmer, 2000).

The non-pathogenic *Fusarium* isolates recovered from the rotting tissues of the vegetable crops could be part of epiphytic mycoflora which occur naturally on the surfaces of the vegetables. Epiphytic mycoflora occurs on the plant surfaces of vegetables as vegetables have high water activity (more than 0.99) and the pH ranges from 4.9 – 6.5 which allow the growth of many fungi (Lund, 1992). Most epiphytic fungi including *Fusarium* are benign to the crops and in many ways can provide a barrier to infestation by plant pathogenic microbes (Janisiewicz & Korsten, 2002).

The present study showed that the *Fusarium* species are prevalent on vegetable crops. Many isolates are not pathogenic or not capable of causing diseases, while some species are opportunists. Opportunistic species can colonize plant tissues and this leads to infection by *Fusarium* when the crops are predispose to abiotic and biotic factors.

Fusarium spp. are among toxigenic fungi causing contamination on vegetables and fruits. Although detected at low level, *Fusarium* mycotoxins have been reported in asparagus, herbs, fig, potato, celery, beans, chilli, ginger, coriander and medicinal plant. The occurrence of *Fusarium* species on these crops may contribute to an intake of *Fusarium* mycotoxins (Logrieco *et al*., 2003). The ability of toxigenic species to produce mycotoxins depends on the substrates. Mycotoxin-producing *Fusarium* species are known as field fungi which require very high moisture content for growth on the substrate and for production of mycotoxin (Logrieco *et al*., 2003). These conditions make vegetables a suitable substrate for toxigenic *Fusarium* growth as the crops have ideal water activity and low pH which are conducive for fungal growth.

Thus, the knowledge on the presence of *Fusarium* on vegetable crops can provide a basis for proper harvesting and storage practices as unsuitable harvesting practices and poor storage conditions may cause growth and proliferation of the mycotoxin-producing *Fusarium* species.

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