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Diversity of fungi isolated from vegetable seeds

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ABSTRACT

Aim: Seed fungi can cause reduction in seed viability, decreased germination capability, physiological changes, and pre- and post-germination death. The present study was conducted to evaluate the diversity of seed fungi from different vegetable seeds and also to identify the fungal isolates based on their macromorphology and micromorphology characteristics.
Methodology and results: Seven vegetable seeds, namely bitter gourd (*Momordica charantia*), pumpkin (*Cucurbita pepo*), brinjal (*Solanum melongena*), tomato (*Lycopersicon esculentum*), okra (*Abelmoschus esculentus*), watermelon (*Citrullus lanatus*) and French bean (*Phaseolus vulgaris*) were assayed to evaluate the incidence of seed fungi using surface sterilization and blotter paper methods. Twenty-five fungal isolates were recovered from agar plate method and seven isolates from blotter paper method. Five genera and five mycelia sterilia were identified. *Aspergillus* comprised the highest percentage (50%) of the fungal isolates recovered, followed by *Penicillium* (25%), mycelia sterilia (15.6%), and 3.1% each for *Curvularia*, *Scopulariopsis* and *Trichoderma.*
Conclusion, significance and impact of study: In the present study, the seed samples were postharvest seed and thus, the seeds had a greater chance of being infected by the storage fungi once the storage conditions are not well regulated. The results showed that the fungal isolates recovered were internal seed fungi which might have infected the seeds during storage period.

Keywords: diversity, fungi, seed, vegetable

INTRODUCTION

Seed is important for crop production and harbour various types of fungi which can resulted in decrease germination, discolouration and decay seed which can lead to lower production of the crops as many seed fungi are parasites of seed primordia, maturing and stored seeds (Williams and McDonald, 1983; Quenton *et al*., 2003). Internal seed fungi are of concern because the establishment of fungi in the seed is difficult to detect and observe compare to external fungi which sometimes can form mycelia. Seed that harbor plant pathogens may cause crop losses and may spread the pathogen into new areas. It is also difficult to identify infected seed as the symptoms are difficult to observe on the seed surface (Quenton *et al*., 2003). The existence of various fungi from different types of seeds has been reported by several workers which suggested that seed borne diseases are able to spread easily. There are limited published studies on seed-borne fungi from seed samples in Malaysia. Thus, the objectives of the present study were to identify fungal isolates from vegetable seed samples and to compare the effectiveness of two different methods in recovering fungal isolates from the seed samples.

MATERIALS AND METHODS

Seed samples and fungal isolation

Seven types of imported seed samples, namely bitter gourd (*Momordica charantia*), pumpkin (*Cucurbita pepo*), brinjal (*Solanum melongena*), tomato (*Lycopersicon esculentum*), okra (*Abelmoschus esculentus*), watermelon (*Citrullus lanatus*) and French bean (*Phaseolus vulgaris*) were obtained from a shop in Georgetown, Penang, Malaysia. The seeds were chosen to be tested as the plants are mainly cultivated in Malaysia.

For the isolation of fungi from the seed samples, agar plate and blotter paper methods were applied. These two methods are among the methods used by International Seed Testing Association (ISTA, 1993) for isolation of seed fungi. The methods were chosen as comparison as agar plate method contains nutrient whereas blotter paper do not contain any nutrients.

For agar plate method, the seeds were surface sterilized with 1% sodium hypochlorite solution (NaOCl)

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for 2 min, rinsed three times with sterile distilled water, immersed in 70% ethanol for 1 min, rinsed again with sterile distilled water and were finally dried on sterile filter paper. The seeds were then plated onto Potato Dextrose Agar (PDA) and triplicate of each seed sample were done. The plates were incubated at 25±1 °C for 7 days with alternate cycle of 12 h day and night fluorescent light.

In blotter paper method, three layers of blotting paper (Whatman No. 1) were placed in Petri plates and moistened with sterile distilled water. The treated seeds were then placed in the Petri plates and triplicates of each seed sample were done. The seeds were then incubated at 25±1 °C for 7 days under alternating cycle of 12 h day and night fluorescence light.

Fungal isolates recovered from the seeds were identified based on the methods and descriptions of species in Food and Indoor Fungi (Samson *et al*., 2010) and Pictorial Atlas of Soil and Seed Fungi (Watanabe, 2010). For identification, all the fungal isolates were inoculated in three point position on culture plates and incubated at 25±1 °C for 7 days. For identification of *Aspergillus* species, differential media comprising Malt Extract Agar (MEA), Czapek Yeast autolysate Agar (CYA) and CYA incubated at 37±1 °C provide morphological data for identification of *Aspergillus* species (Klich, 2002; Samson *et al*., 2010). For *Penicillium* spp., three differential media were used, namely MEA, CYA and CREA (Creatine Sucrose Agar) in which CREA was used to distinguish closely related species. Ehrlich reaction was also carried out to differentiate *Penicillium* species by the detection of indole metabolites (Samson *et al*., 2010).

Potato Dextrose Agar was used for observation of morphological characteristics of *Scopulariopsis* and Mycelia sterilia. For identification of *Trichoderma* and *Curvularia*, morphological characteristics on MEA were observed.

RESULTS AND DISCUSSION

Agar plate method was found to be better in the recovery of seed fungi from the vegetable seeds compared to blotter paper method. Perveen and Ghaffar (1995), Niaz and Dawar (2009), Al-Kassim and Monawar (2000) and Summaiya *et al.* (2010) also found that agar plate method yielded more fungal isolates than blotter paper method. The seeds were surface sterilized before plating on both agar plate and blotter paper in order to remove surface mycoflora and also to increase the probability of isolating seed-borne fungi (Perveen and Ghaffar, 1995; Narayanasamy, 2010; Shakoor *et al.*, 2011).

Low recovery of fungi using blotter paper method could be due to lack of nutrient. Blotter paper moistened with distilled water did not contain any nutrients which can assist fungal growth. Slow growing fungi did not sporulate due to the unsuitable environment and fungi that reside in internal tissues of the seed would take time to sporulate and to be recovered due to the lack of nutrient.

Table 1: Fungal isolates recovered from different seed samples.

Seed sample	Fungal isolate	Total number of isolate
Bitter gourd	*Aspergillus flavus*	1
	Aspergillus fumigatus	1
	Mycelia sterilia	2
	Penicillium corylophilum	1
Pumpkin	-	-
Brinjal	-	-
Tomato	*Trichoderma longibrachiatum*	1
	Aspergillus flavus	1
Okra	*Aspergillus niger*	9
	Mycelia sterilia	2
	Penicillium aurantiogriseum	1
	Penicillium solitum	1
	Aspergillus niger	4
Watermelon	*Curvularia tuberculata*	1
	Penicillium aurantiogriseum	4
	Penicillium digitatum	1
	Scopulariopsis sp.	1
French bean	Mycelia sterilia	1
		32

Thirty-two fungal isolates were successfully isolated from five seed samples, namely bitter gourd, tomato, okra, watermelon, and French bean. Okra seeds yielded the most fungal isolates (14 isolates), followed by watermelon (11 isolates) and bitter gourd (five isolates) seeds. Only one isolate was recovered from both tomato and French bean's seeds. Fungal isolates were not recovered from pumpkin and brinjal seeds.

Based on morphological characteristics, five genera of microfungi and five Mycelia sterilia were identified. *Aspergillus* comprised the highest percentage (50%) of the fungal isolates recovered followed by *Penicillium* (25%), Mycelia sterilia (15.6%), and 3.1% each of *Curvularia*, *Scopulariopsis* and *Trichoderma* (Table 1). The colony morphology and microscopic characteristics of each species of microfungi and Mycelia sterilia recovered from the seed samples are shown in Tables 2-6.

From both agar plate and blotter paper methods, *A. niger*, Mycelia sterilia and *P. aurantiogriseum* were commonly isolated from the seed samples. The number of *A. niger* isolated using agar plate method (13 isolates) was higher than blotter paper method (three isolates). Other fungal isolates such as *A. flavus*, *A. fumigatus*, *C. tuberculata*, *P. digitatum*, *P. solitum*, and *Scopulariopsis* sp. were recovered using agar plate method. Only *T. longibrachiatum* was obtained via blotter paper method (Table 7).

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Table 2: Colony morphology of *Aspergillus* spp.

Species	Colony on MEA			Colony on CYA			Colony on CYA37			Production of sclerotia	Growth on CREA	
	Diameter (mm)	Upper surface	Lower surface	Diameter (mm)	Upper surface	Lower surface	Diameter (mm)	Upper surface	Lower surface		Growth	Acid
A. niger	65	black	white, yellow or black	70	brown to black	white	65	black	white, yellow, brown to black	absent	poor	++
A. flavus	65	yellowish-green	cream	70	light green	light brown	70	light green	cream	absent	poor	+
A. fumigatus	67	grey-green	dark green	65	grey-green	cream to dark-green	65	grey	cream yellow	absent	poor	–

Acid production: – , no acid production; +, weak acid production; ++, strong acid production

Table 3: Microscopic characteristics of *Aspergillus* spp.

Species	Conidia		Conidial head	Shape / Diameter of vesicle (µm)
	Shape	Diameter (µm)		
A. niger	rough-walled, globose	3.70	globose to radiate, biseriate	globose (14 – 50)
A. flavus	rough-walled, globose to sub-globose	3.56	radiate to columnar, biseriate	globose (24.1)
A. fumigatus	rough-walled to echinulate, globose to sub-globose	2.30	radiate, biseriate	broadly clavate (17.44)

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Table 4: Colony morphology of *Penicillium* spp.

Species	Colony on MEA			Colony on CYA			Growth on CREA		Ehrlich test
	Diameter (mm)	Upper surface	Lower surface	Diameter (mm)	Upper surface	Lower surface	Growth	Acid	
P. corylophilum	28	grey green	cream	22	grey green	cream	good	-	negative
P. digitatum	25	grey green	pale	17	yellow green	pale to beige	poor	-	negative
P. aurantiogriseum	25	blue green	yellow	29	dark green	pale yellow	poor	++	negative

Acid production: –, no acid production; +, weak acid production; ++, good acid production

Table 5: Microscopic characteristics of *Penicillium* spp.

Species	Conidia			Conidiophore	
	Ornamentation	Shape	Diameter (µm)	Branching pattern	Stipe
P. corylophilum	smooth-walled	globose to elliptical	2.48	biverticillate	smooth-walled
P. digitatum	smooth-walled	ellipsoidal to cylindrical	2.50	terverticillate	short and hyline
P. aurantiogriseum	smooth	globose to subglobose	2.32	terverticillate	rough-walled

Table 6: Colony morphology and microscopic characteristics of *T. longibrachiatum, Scopulariopsis* sp., *C. tuberculata* and Mycelia sterilia.

Species	Colony PDA/MEA		Conidia		Conidiophore
	Upper surface	Lower surface	Diameter (µm)	Shape	Shape
T. longibrachiatum	green (MEA)	light yellow (MEA)	2.3	subglobose to ellipsoidal	Cylindrical
Scopulariopsis sp.	light brown (PDA)	light brown (PDA)	2.5	square-shaped	Penicillate-like
C. tuberculata	olive brown (MEA)	dark brown (MEA)	2.5	ellipsoid with four transverse septa	Erect, pigmented and paler at the tips
Mycelia sterilia	white to pinkish (PDA)	grayish (PDA)	2.8	–	–

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Three *Aspergillus* species were identified in which *A. niger* was the most prevalent species isolated from the seed samples (13 isolates) especially from okra and watermelon seeds. Both *A. flavus* and *A. fumigatus* were isolated from bitter gourd seeds. Rathod *et al.* (2012) also reported that *Aspergillus* species which include *A. niger, A. flavus* and *A. fumigatus* were among the most common species isolated from legume seeds.

Five *Penicillium aurantiogriseum* isolates and one *P. digitatum* isolate were isolated from watermelon seeds. The other *Penicillium* species, namely *P. aurantiogriseum* and *P. solitum* were recovered from okra seeds, and *P. corylophilum* from bitter gourd seeds. *Penicillium* species have been reported to be among the most frequent genera recovered from several legume seeds used as food in Saudi Arabia in which 14 species were identified as contaminant on bean, broad bean, lentil, lupine and pea seeds (Abdel-Hafez, 1984).

A total of five Mycelia sterilia were recovered from seeds of bitter gourd, okra and French bean. Abdel-Hafez (1984) and Bhattacharjee and Dkhar (2005) also recovered several Mycelia sterilia in their study on seed samples in Saudi Arabia and India, respectively. Other species of fungi isolated in lower frequency were *Curvularia tuberculata* and *Scopulariopsis* sp. from watermelon seeds, and *Trichoderma longibrachiatum* from tomato seeds (Table 1). These fungi have been isolated from seeds of several types of spices in India (Sumanth *et al*., 2010) and seed samples of lentil in Pakistan (Summiaya *et al*., 2010).

In the present study, the most common fungal genera recovered from the seed samples were *Aspergillus* and *Penicillium* which indicated that the seed become contaminated during storage. Both genera are common in indoor environment and regarded as storage fungi. The other fungal genera such as Mycelia sterilia, *Curvularia*, *Scopulariopsis* and *Trichoderma* are also common indoor fungi and can caused deterioration of many substrates. These fungi may invade seeds during storage due to improper storage conditions such as improper moisture content, temperature as well as the length of time the seeds are stored (Sweets, 1996). Fungal infection on the seeds commonly occurs through damage tissues in which warm and moist conditions favour the growth of these fungi. With the establishment of the fungi in the seeds, the metabolic activity of the seed may be disturbed and the nutritive tissues may be consumed by the fungi which could result in the deterioration of seed viability or atypical growth of the seedlings (Bhattacharjee and Dkhar, 2005). Contamination by seed fungi can cause deterioration, discolouration and affect the ability of the seeds to germinate (FAO, 2010).

When the seed-borne fungi prevail over the seed which is of high viability, a decrease in the ability to germinate is often seen in the seed. Pre- and post-emergence damping-off is the usual phenomenon observed and this result in a very weak plant stands in the field (Nene and Agarwal, 1978; Al-Kassim and Monawar, 2000). Discolouration of seed by fungi can happen either in the field before harvesting or after harvesting i.e. during storage period. Field fungi are responsible for contamination before harvesting whereby discolouration activity is confined to only the pericarps of seed. After harvest, contamination is caused by storage fungi which usually cause the discolouration of the whole seed (Nene and Agarwal, 1978). In the present study, the seed samples were postharvest seed and thus, the seeds had a greater chance of being infected by the storage fungi once the storage conditions are not well regulated. The results of the present study showed that the fungal isolates recovered were internal seed-borne fungi because the seed samples were all postharvest seed i.e. the seed samples might be infected during storage period.

The present study indicated that the seed samples were mainly contaminated with storage fungi as *Aspergillus* and *Penicillum* were recovered with higher percentage compared to the other fungal genera which were mostly field fungi. Bhattacharya and Raha (2002) reported that in storage, gradual decrease of field fungi were observed, and simultaneously population of storage fungi increased. The field fungi which comprised Mycelia sterilia, *Curvularia* and *Scopulariopsis* were isolated with lower percentage probably because these fungi were replaced by storage fungi after harvest and during storage (Bhattacharya and Raha, 2002). Lower recovery of field fungi from the seed samples may also be due to their interaction with storage fungi as *Aspergillus* and *Penicillium* are dominant during storage and may produce mycotoxin which can inhibit the growth of field fungi (Ehrlich *et al*., 1985).

The present study shows that seed samples were contaminated with storage fungi especially from the genera *Aspergillus* and *Penicillium*, two most common storage fungi. The occurrence of storage fungi in the seed may reflect poor harvesting method and improper storage conditions where the seeds are kept.

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