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SHORT COMMUNICATION

Occurrence of microfungi on several dried fruits

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ABSTRACT

Aims: Dried fruits may harbour a variety of fungi especially those that can grow in low water activity conditions. Knowledge on the occurrence of fungi on dried fruits is an important step to predict the possibility of mycotoxin contamination, thus the present study was conducted to determine the occurrence of fungi on dates, figs, kiwi and raisins.
Methodology and results: Based on morphological characteristics, six genera of fungi comprising eight species, *Aspergillus niger*, *A. flavus*, *Penicillium corylophilum*, *P. glabrum*, *Chaetomium globosum*, *Fusarium sacchari*, *Acremonium strictum* and Mycelia sterilia were identified. Three most commonly species isolated were *A. niger* (19 isolates), *P. corylophilum* (seven isolates) and *A. flavus* (five isolates). The less common species were *C. globosum* (three isolates), Mycelia sterilia (three isolates), *A. strictum* (one isolate) and *P. glabrum* (one isolate).
Conclusion, significance and impact of study: Common mycotoxin producer are the fungi from the genera *Aspergillus*, *Penicillium* and *Fusarium*. The occurrence of toxigenic fungi on dates, figs, kiwi and raisins suggested that some of these fungi may be an important source of mycotoxin contamination.
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Keywords: diversity, fungi, dried fruits

INTRODUCTION

Dried fruit is known as low water activity or low moisture foods and soluble carbohydrate (Pitt and Hocking, 2009). It is preserved by removing the water content through sun drying or specialized dryers (Ozer *et al*., 2012). Although it is dried, the fruits may harbour a variety of fungi especially fungi that can grow in low water activity conditions. In addition to low water activity, these fungi can produced spores or remain latent until temperature and relative humidity are favourable for the fungal growth.

Many types of dried fruits in Malaysia are imported from other countries especially fruits that are not planted locally such as dates, figs, raisin, kiwi and many other dried fruits originated from temperate regions. Dried fruits are consumed directly, therefore it is important to determine that these imported commodities are not contaminated by toxigenic fungi as contamination can occur during storage, handling, drying and packaging processes (Pitt and Hocking, 2009).

Toxigenic fungi are widespread and have ecological link with food commodities (Pitt, 2000). Thus, the occurrence of toxigenic fungi on food commodities is influence by the genetics of the fungi, the host plant and environmental factors as well as processing practices (Simsek *et al.*, 2002). The most prevalent toxigenic fungal genera associated with food commodities are *Aspergillus*, *Penicillium* and *Fusarium*. Species of *Aspergillus* and *Penicillium* are commonly associated with food commodities during drying and storage while species of *Fusarium* are plant pathogens on many economically important crops (Pitt, 2000). Some species that belong to the three genera of the toxigenic fungi are well-known producer of mycotoxin such as *A. flavus* produced aflatoxin and *A. niger,* ochratoxin A which exhibit mutagenic and carcinogenic effects in humans and animals (Fisvad *et al.*, 2006). The most important mycotoxin produced by *Fusarium* spp. are trichothecenes, zearalenone and fumonisin B1 which are capable of inducing both acute and chronic effects (Antonissen *et al.*, 2014).

The presence of toxigenic fungi on food commodities might be used as an indicator of possible contamination of mycotoxins. Through understanding and determination of toxigenic fungal isolates presence on food commodities, preventive strategies can be developed. Thus, the objective of this study was to determine the occurrence of toxigenic fungi on several types of dried fruits.

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MATERIALS AND METHODS

Isolation and identification of fungi from dried fruits

Dried fruit samples were purchased from a hypermarket in Penang Island, Malaysia. A total of four types of dried fruits were chosen, namely dates, figs, kiwi and raisins. These products were chosen on the basis of their commercial availability and easily found.

Two methods, direct plating and surface sterilization, were used to compare the incidence of fungal contamination in dried fruits in different culturing methods. In direct plating method, pieces of the dried fruit samples were directly plated on potato dextrose agar (PDA) plates to allow fungal growth. For raisins, four whole fruits were plated onto PDA plates equidistant from the centre and from the edge of the plate. The other dried fruits which were bigger in size such as dates, figs and kiwi were cut into smaller pieces (approximately 1 cm) using a sterile scalpel and forceps before plated onto PDA plates in the same manner. Three replicates for each dried fruit were prepared. The plates with inoculated samples were incubated at 27±1 °C for 14 days and observed every day for signs of fungal growth.

Surface sterilization was done by first soaking the dried fruit samples in 2% NaOCl (Chlorox) for 30 sec and then transferred into 70% alcohol for another 30 sec using a sterilized forceps. Finally, the samples were then rinsed twice using distilled water before plating them onto fresh PDA plates. The plates were then incubated at 27±1 °C for 14 days to be observed for signs of fungal growth. Three replicates were done for each dried fruit sample.

Fungal isolates successfully recovered from the four types of dried fruit were identified based on species description in the Food and Indoor Fungi lab manual (Samson *et al*., 2010) and the media used for identification were also adapted from the manual. For *Fusarium* species identification, the media recommended for species identification and descriptions of species in The *Fusarium* Laboratory Manual (Leslie and Summerell, 2006) were followed.

RESULTS AND DISCUSSION

A total of 39 fungal isolates were isolated from the four different types of dried fruits namely dates, figs, kiwi and raisins using direct plating and surface sterilization methods. A total of 26 isolates were obtained by the direct plating and 13 isolates by the surface sterilization (Table 1) which indicated that direct plating gave higher number of isolates. Unsterilized surface of the samples could have been contaminated with various microorganisms during exposure to the environment through handling, transport and storage. Surface sterilization method generally will reduce the number of fungi isolated as this method will removes most of the surface contaminants of the samples (Pitt and Hocking, 2009). The fungal isolates successfully isolated from surface sterilization method were considered as internal fungi which invade the dried fruits and grow inside the samples (Romero *et al*., 2005).

Based on morphological characteristics, six genera of fungi comprising eight species were identified namely *Aspergillus niger*, *A. flavus*, *Penicillium corylophilum*, *P. glabrum*, *Chaetomium globosum*, *Fusarium sacchari*, *Acremonium strictum* and Mycelia sterilia. The most common species recovered was *A. niger* (19 isolates) followed by *P. corylophilum* (7 isolates), *A. flavus* (5 isolates), three isolates each for *C. globosom* and Mycelia sterilia and one isolate each for *A. strictum* and *P. glabrum.*

The occurrence of the fungal isolates using direct isolation and surface sterilization methods on dried fruits samples of dates, figs, kiwi and raisin are shown in Table 1. From the present study, *Aspergillus* and *Penicillium* were the most common fungal genera isolated. Contamination of *Aspergillus* and *Penicillium* species in most of the dried fruits suggests that most of the fungal invasion happened during storage where water activity and moisture content in the substrate is lower than those in the field (Pitt and Hocking, 2009). Similar findings were also reported by Zohri and Abdel-Gawad (1993), Abdel-Sater and Saber (1999), Alghalibi and Shater (2004), Javanmard (2010) in which *Aspergillus* and *Penicillium* were isolated in higher frequency from different types of dried fruits.

In the present study, two *Aspergillus* species, *A. niger* and *A. flavus* were recovered from several types of the dried fruits (Table 1). *Aspergillus niger* was more prevalent compared to *A. flavus* which was not surprising as *A. niger* spores are resistance to sunlight and UV radi-

Table 1: Occurrence of fungi in dried fruit samples by direct plating and surface sterilization methods.

Fungal isolates	Number of isolates							
	Direct plating				Surface sterilization			
	Dates	Figs	Kiwi	Raisins	Dates	Figs	Kiwi	Raisins
Acremonium strictum	-	-	-	-	1	-	-	-
A. flavus	3	-	-	1	1	-	-	-
A. niger	11	-	3	1	4	-	-	-
C. globosum	1	-	-	-	2	-	-	-
F. sacchari	-	-	-	-	-	1	-	-
P. corylophilum	1	-	2	1	3	-	-	-
P. glabrum	-	-	-	1	-	-	-	-
Mycelia sterilia	-	-	1	1	1	-	-	-

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ation (Romero *et al*., 2005). Both species are common contaminant of agricultural products and contamination can occur during pre-harvest, processing and handling (Perrone *et al*., 2007). *Aspergillus niger* and *A. flavus* were also among the most common species isolated from muesli component (Dimic *et al*., 2005), some dried fruits in Yemen (Alghalibi and Shater, 2004), and dried figs in Iran (Javanmard, 2010).

Similar with *Aspergillus*, two species of *Penicillium* were also isolated from dates, kiwi and raisins. *Penicillium corylophilum* is found on food and indoor environment (Samson *et al.*, 2010) and has been found to cause spoilage on jams and frequently isolated from barley, paddy rice, wheat and flour (Pitt and Hocking, 2009). Marin *et al*. (2002) reported that *P. corylophilum* was one of fungal spoilage of bakery products. *Penicillium glabrum* is also often encountered in food and indoor environment, and has been recovered from fruits, nuts, frozen cakes (Samson *et al*., 2010) as well as from muesli components (Dimic *et al*., 2005).

Members of the genus *Chaetomium* are fungi often found in the environment and *C. globosum* is the most common species occurring in indoor environment. Although *C. globosum* is a common fungi in the environment, the fungus has been isolated from nuts (Pitt and Hocking 1997), spices (Mandeel, 2005) as well as various other agricultural commodities such as wheat, barley, beans and soybean (Pitt and Hocking, 1997) and in the present study, *C. globosum* was isolated from dates. These findings suggested that *C. globosum* could also contaminate various types of food stuff. *Chaetomium globosum* has also been reported to produce toxic compounds such as chaetoglobosins A and C (Pitt and Hocking, 2009; Samson *et al*., 2010).

Mycelia sterilia isolates were recovered from dried kiwi, raisins and dates. From other food product, a few mycelia sterilia have been isolated from various imported fresh fruits and dried fruits as well as imported vegetables (Raudoniene and Lugauskas, 2005; Lugauskas *et al*., 2006). Therefore, like any other common spoilage fungi, mycelia sterilia may also contaminate food product for human consumption.

From fig only one isolate of *F. sacchari* was recovered. The dried fig sample could be contaminated by chance in the fields. So far, there is no report on the occurrence of *F. sacchari* on fig. However, in a study by Moretti *et al*. (2005) and Moretti *et al*. (2011), five *Fusarium* species, namely *F. lactis, F. subglutinans, F. solani*, *F. ramigenum* and *F. proliferatum* were found to be associated with contamination of dried figs.

Acremonium strictum is a field fungus and are commonly found on food and indoor environment (Samson *et al*., 2010). Only one isolate of this fungus was recovered which was from dates. *Acremonium acremonium* has been isolated from wheat, barley, rice, banana, peanuts, hazel nuts and walnuts (Pitt and Hocking, 1997)

Both *Aspergillus* and *Penicillium* are commonly known as storage fungi and are ubiquitous, causing contamination when processing conditions are inadequate and moisture content is too high (Pitt and Hocking, 2009). Other fungal genera such as *Fusarium, Acremonium, Chaetomium* and mycelia sterilia isolated in the present study can be considered as opportunistic fungi as they are capable of growth when the conditions are suitable such as availability of moisture on the dried fruits. These opportunistic fungi are commonly found in the indoor environment.

Although *Aspergillus* and *Penicillium* are well-known mycotoxin producer compared to other fungal genera isolated in the present study, only *A. niger* and *A. flavus* are well-known mycotoxins producer related to food and feed. Some strains of *A. niger* produce both fumonisin B2 and ochratoxin which has an implication on food safety (Frisvad *et al*., 2007). *Aspergillus flavus* is a well-known producer of aflatoxins which have been reported in many types of food in tropical countries (Frisvad *et al*., 2006) and on dried fruits (Pitt and Hocking, 1997). *Penicillium glabrum* and *P. corylophilum* are not known to produce mycotoxin (Samson *et al*., 2010). Major *Penicillium* mycotoxins related to food are chaetoglobosins which produced mainly by *P. expansum* and *P. discolor* (Frisvad *et al*., 2006).

CONCLUSION

Common toxigenic fungi such as fungi from the genera *Aspergillus*, *Penicillium* and *Fusarium* can easily grow on dried fruits when suitable conditions favour their growth. Therefore, knowledge on the occurrence of toxigenic fungi on dried fruits is an important step to predict the possibility of mycotoxin contamination. The production of mycotoxin is often species specific, thus identification up to species level is very important as the information will assist in determining the mycotoxins produced (Frisvad *et al.*, 2006). The information on the occurrence of toxigenic fungi on dried fruits is important to formulate methods such as proper storage conditions to prevent mycotoxin contamination as mycotoxin on dried fruits may have the potential of health hazards and economics losses.

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