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A Preliminary Mycological Evaluation of Organic and Conventional Foods

ABSTRACT

Selected conventional and organic foods were surveyed during the period 2009–2013. A total of 100 samples (50 conventional and 50 organic) were purchased from grocery stores and farmers' markets in southeastern Pennsylvania. The products investigated were popcorn, rice, corn, walnuts, almonds, peanuts, pumpkin seeds, green peas, flaxseeds, soybeans and cashew nuts. Direct plating was used in the study, with the following media: MEA, DRBC, DG18 and AFPA. After samples were incubated at 25°C for 3–5 days, the results were expressed as percentage of food particles infected by molds. Selected mold isolates were tested for the production of mycotoxins in laboratory media, and all food samples were screened for aflatoxin. The results of this preliminary study demonstrate no significant difference in the levels of mold contamination between organic and conventional samples. Twenty-two mold species representing eleven different genera have been identified. The most dominant mold genera were *Aspergillus, Penicillium, Alternaria* and *Cladosporium.* Some of the species identified have been found to produce their respective mycotoxins in laboratory media. A few samples in each food category were found to contain aflatoxins. There was no significant difference in mycotoxin production between mold species isolated from organic samples and those isolated from conventional samples.

INTRODUCTION

Filamentous fungi are a diverse group of organisms that are widely distributed in nature. They commonly contaminate agricultural commodities, foods and feeds, causing high economic losses worldwide. In addition, the growth of some filamentous fungal species on foods and crops presents a serious health hazard to humans and animals by producing mycotoxins. The major mycotoxigenic fungi are in five taxonomic genera: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps.* The *Aspergillus* group produces aflatoxins, ochratoxin A, sterigmatocystin, cyclopiazonic

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acid, and others. *Penicillium* species produce ochratoxin A, patulin, citrinin, citreoviridin, PR toxin and other less toxic substances. *Fusarium* species produce trichothecenes, deoxynivalenol (DON), T-2 toxin, zearalenone, fumonisins and others *(3, 10, 17)*. Aflatoxins have been shown to be genotoxic and hepatocarcinogenic in humans and animals *(9)*; they are produced mainly by *Aspergillus flavus* (aflatoxin B_1 and B_2) and *A. parasiticus* (aflatoxins B_1, B_2, G_1, and G_2). Aflatoxins are estimated to account for 28% of all cases of hepatocellular carcinoma, the most common form of liver cancer *(14)*. Ochratoxin A is linked to kidney damage (nephrotoxicity) in humans and animals and also has immunotoxic, neurotoxic and teratogenic effects. Fusarium toxins cause a wide range of toxic effects, including equine leucoencephalomalacia (ELEM) in horses, esophageal cancer in humans, and weakening of the immune system *(3, 4)*. Some of the most common foods and agricultural commodities that become contaminated by these fungi and potentially by their mycotoxins include cereals, nuts, beans, apples and apple products, aged meat products and spices.

The growth in sales of organic foods has increased over the past two to three decades, reaching over $28 billion dollars *(11, 24, 27)*. Organic foods are defined as foods that are grown without synthetic pesticides, antibiotics, growth hormones and genetic engineering. To enhance soil fertility and protect crops, organic farmers practice various methods such as crop rotation, tillage and cultivation practices, use of cover crops, use of animal and crop wastes, and any synthetic pest control chemicals that will be broken down by oxygen and sunlight *(15, 27)*. In the U.S., the Organic Foods Production Act (OFPA), introduced by the USDA in 1990 and implemented in 2002, outlines the methods, practices, and substances that could be used in the production, processing, handling, and labeling of organic foods.

Consumers perceive organic foods as more nutritious and safer than conventional foods. However, the literature includes only a few published studies that have compared differences between organic and conventional foods with respect to their microbiological and toxicological safety. These comparative studies fall mainly into two categories, based on the origin of the foods *(15)*; the food samples either have been purchased from local retail markets or have been directly obtained from organic or conventional farms. These studies have yielded inconclusive results because of lack of sufficient comparative data. Organic fruits and vegetables have been found to contain less synthetic agrochemicals than conventional ones; however, the levels of these chemicals in conventional foods are usually lower than the acceptable limits *(15)*. No differences have been found between organic and conventional foods with respect to their content of environmental contaminants such as heavy metals. In the few studies comparing mycotoxin levels in conventional and organic cereals and cereal products, the results are variable and not conclusive *(15)*. A study in Norway *(1)* found that organic cereals contain less contamination by *Fusarium* species than cereals from conventional farming systems. The authors attributed this difference to lack of crop rotation and use of mineral fertilizers in the conventional farming systems.

The main objective of this preliminary study was to compare the types and levels of fungal flora in selected samples of grains, nuts, and legumes that are labeled organic with those in samples of conventional foods. The mycotoxigenic properties of selected fungal isolates and the presence of aflatoxin in samples were also examined.

MATERIALS AND METHODS

Sampling

A total of 100 samples (50 conventional and 50 organic) were purchased from grocery stores and farmers markets in southeastern Pennsylvania during the period of 2009–2013. Foods tested were popcorn, rice, walnuts, almonds, peanuts, pumpkin seeds, green peas, flaxseeds, soybeans, cashew nuts, and corn *(Table 1)*. All the foods tested were dry. Products were identified as organic by the USDA organic logo on the label or by having been labeled organic by the stores.

Mycological examination

The direct plating technique *(20)* was used. The grains and nuts were aseptically plated on the four following solidified agar media: Malt Extract Agar (MEA), Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Dichloran 18% Glycerol Agar (DG18) and *Aspergillus Flavus Parasiticus* Agar (AFPA). For each sample, 50 particles were examined at the rate of 5 particles per plate. The plates were incubated upright at 25°C for 3–5 days. After incubation, the plates were examined and the number of contaminated particles were counted and reported as percentages. The major genera were isolated on appropriate agar media and identified by use of standard identification keys that are based on morphological and microscopic characteristics *(13, 19, 22)*. All analyses were done in duplicate.

Mycotoxin analysis

Selected isolates of *Aspergillus* and *Penicillium* species were tested for mycotoxin production by inoculating them in Yeast Extract Sucrose broth (YES), followed by direct detection on thin layer chromatography (TLC) plates *(5)*. Aflatoxins, ochratoxin A, citrinin and penicillic acid (Sigma Chemical Co., St. Louis, MO) standard solutions were prepared in chloroform at various concentrations (1, 5, 10, 20 and 50 ug/ml) and stored at 4°C. The extracts were spotted on TLC plates (20 × 20 cm, coated with a 0.25 nm thick layer of silica gel HPK; Watman, Clifton, NJ, U.S.). The plates were developed in toluene-EtOAc-90% formic acid (6 + 3 + 1). After development, the plates were exposed to ammonia fumes, dried, and exposed to long-wavelength ultraviolet light for detection. Sample spots were visually compared to mycotoxin standard spots. The detection limit for aflatoxin B_1

Table 1. Average mold incidence (%) in conventional and organic foods

Food Type	No. of Samples	Conventional Foods					Organic Foods				
		Media*				No. of different Species	Media*				No. of different Species
		MEA	DRBC	DG18	AFPA		MEA	DRBC	DG18	AFPA	
Popcorn	5	67.1	47.1	35.1	17.0	9	70.1	45.1	39.1	19.2	11
Walnuts	6	87.1	66.0	41.0	35.1	13	90.2	71.0	45.0	27.1	9
Rice	4	48.1	51.3	31.2	21.1	7	51.1	55.2	39.2	26.1	5
Almonds	4	35.4	33.1	31.1	29.3	4	41.4	37.1	38.1	28.3	3
Peanuts	7	80.0	76.1	72.0	27.3	15	83.1	79.1	76.2	19.1	16
Flaxseeds	5	34.3	32.5	36.3	23.1	4	40.3	39.5	37.3	18.2	6
Soybeans	4	87.3	79.3	76.1	14.3	17	89.3	80.1	78.1	11.3	15
Cashew Nuts	4	57.6	52.1	58.0	37.1	8	61.6	58.1	61.0	29.3	9
Corn	5	81.2	78.2	80.2	23.2	13	85.2	81.2	81.2	24.1	14
Green Peas	3	21.3	15.2	13.2	11.2	2	29.3	18.2	11.2	12.2	3
Pumpkin Seeds	3	34.2	35.2	27.6	18.3	4	39.2	40.2	25.6	20.3	5

*Malt Extract Agar (MEA), Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Dichloran 18% Glycerol Agar (DG18), *Aspergillus flavus Aspergillus parasiticus* Agar (AFPA)

on TLC was 3 ppb, while the detection limit for ochratoxin A, citrinin and penicillic acid was approximately 10 ppb.

All samples were screened for aflatoxin by use of the *Reveal* Test Kit (Neogen Corporation, Lansing, MI, www.neogen.com) and/or by TLC. The Reveal test, a single-step lateral flow immuno-chromatographic assay based on a competitive immunoassay format, is designed to screen samples for aflatoxin at the 20 ppb level. Samples that tested positive for aflatoxin using the Reveal Test Kit were mailed to a private analytical laboratory (Eurofins, Metairie, LA, USA) for confirmation by the Liquid Chromatography/Mass Spectrometry (LC/MS) method.

Statistical analysis

Each sample was analyzed three times and averages were obtained. The significance of the differences between means was determined by analysis of variance. The software Minitab 17 (Minitab Inc., USA) was used for statistical analysis.

RESULTS AND DISCUSSION

The average mold incidences in percentages for conventional and organic foods are shown in *Table 1*. In all four media used, no significant differences ($\alpha = 0.05$) were seen in the levels of contamination between organic and conventional foods *(Table 2)*. However, there were differences in the levels of contamination between the various foods within both organic and conventional samples. In both types of foods, the highest mold contamination levels were recorded in samples grown on MEA, followed by DRBC, DG18 and AFPA. MEA is considered a general-purpose medium that supports growth of a wide variety of fungi *(19)*, whereas DRBC contains both rose bengal and dichloran, which limits the spread of colonies and restricts rapid growth of *Mucor* and *Rhizopus* species *(19)*. The presence of glycerol in DG18 lowers its water activity and thus makes it particularly suitable for growth of xerophilic molds. AFPA is a selective medium used for detection and enumeration of potentially aflatoxigenic molds (*Aspergillus flavus, A. parasiticus and A. nomius*), which produce an orange-yellow color in the reverse of the colony; this color is due to the reaction of aspergillic acid with ferric ammonium citrate *(19)*. Other fungi, such as *A. niger* and *A. ochraceus*, can also grow on AFPA, but these exhibit different colors from that of *A. flavus* and *A. parasiticus* after 3 days of incubation. A number of mold isolates from AFPA were identified as *A. flavus* and *A. parasiticus.* Among the 11 foods examined, walnuts, peanuts, soybeans and corn showed the highest contamination levels in both conventional and organic foods. For conventional foods, the contamination frequency on MEA was 87.1% for walnuts, 87.3% for soybeans, 81.2% for

Table 2. *P*-values for average mold incidence in organic vs. conventional foods

Medium	*P*-value
MEA	0.3368
DRBC	0.3507
DG18	0.3892
AFPA	0.2651

Table 3. Major mold species isolated

Aspergillus flavus (43)*	*Penicillium aurantiogriseum* (40)
Aspergillus oryzae (10)	*Alternaria alternate* (8)
Aspergillus ochraceus (35)	*Cladosporium herbarum* (5)
Aspergillus niger (22)	*Paecilomyces varioti* (7)
Aspergillus parasiticus (17)	*Trichoderma* sp. (2)
Aspergillus restrictus (9)	*Rhizopus* sp. (21)
Aspergillus terreus (10)	*Fusarium graminarium* (7)
Penicillium glabrum (6)	*Fusarium solani* (5)
Penicillium islandicum (9)	*Botrytis cinerea* (8)
Penicillium cyclopium (10)	*Stachybotrys atra* (2)
Penicillium citrinum (13)	*Epicoccum* sp. (4)
Penicillium verrucosum (8)	

*Number of isolates in parentheses

corn, and 80% for peanuts. In organic samples, the highest contamination frequency levels were recorded for walnuts (90.2%), followed by soybeans (89.3%), corn (85.2%), and peanuts (83.1%).

In both conventional and organic foods, *Aspergillus* and *Penicillium* genera were the dominant mycoflora of the samples tested *(Table 3)*. Among the Aspergilli species, *A. flavus, A. oryzae, A. parasiticus, A. ochraceus, A. niger* and *A. terreus* were the most frequently encountered, followed by *A. restrictus, A. clavatus* and *A. ustus.* Among the *Penicillium* species, *P. aurantiogriseum, P. verrucosum, P. citrinum, P. cyclopium, P. islandicum* and *P. glabrum* were encountered the most frequently. Other non-dominant genera that were isolated from both categories of foods include *Alternaria, Cladosporium, Rhisopus, Fusarium, Botrytis* and *Epicoccum* *(Table 3)*.

Few published studies have compared conventionally grown foods with organic ones with regard to the mycoflora present. A mycological survey of Korean cereals grown conventionally reported that species of *Alternaria, Aspergillus, Chaetomium, Epicoccum* and *Fusarium* were the predominant fungal isolates *(26)*. A mycological investigation of organic and unlabeled (i.e., not labeled "organic") foods *(18)* showed no difference between the two food categories. The fungal flora was dominated by *Aspergillus glaucus, Aspergillus niger, Aspergillus flavus, Aspergillus candidus, Penicillium cyclopium* and *Pencillium viridicatum*, although it should be noted that in many earlier mycological studies, *P. viridicatum* was taxonomically confused with *P. verrucosum (19)*. *Alternaria* and *Cladosporium* were regularly isolated from organic and unlabeled foods, mainly wheat, brown rice and pinto beans. In both organic and conventional cornmeal, *Fusarium moniliforme* was the dominant fungal contaminant, while in both walnuts and pecans, *Rhizopus nigricans* and *R. oryzae* were regular contaminants in both foods *(18)*. A study of the mycology of rice (organic and non-organic) from the Swedish retail market

Table 4. Mycotoxin production by selected *Aspergillus* and *Penicillium* isolates

Species	Mycotoxin	Conventional		Organic	
		No. of Isolates	No. Positive	No. of Isolates	No. Positive
A. flavus/A. parasiticus	Aflatoxin B_1	27	3	28	4
A. ochraceus	Ochratoxin A	16	2	15	3
P. citrinum	Citrinin	7	1	5	1
P. aurantiogriseum	Penicillic acid	18	2	21	1

Table 5. Aflatoxin detection in conventional and organic foods

Food Type	Aflatoxin*			
	Conventional		Organic	
	No. of Samples tested	No. of Positive samples**	No. of Samples tested	No. of Positive samples**
Popcorn	5	0	5	0
Rice	4	0	4	0
Walnuts	6	1	6	0
Almonds	4	1	4	0
Peanuts	7	1	7	1
Flaxseeds	5	0	5	0
Soybean	4	0	4	0
Cashew Nuts	4	0	4	0
Corn	5	0	5	1
Green peas	3	0	3	0
Pumpkin seeds	3	0	3	0

*Total aflatoxin
**Samples containing at least 20 ppb

showed a predominance of the *Aspergillus* genus, followed by *Penicillium, Eurotium, Wallemia, Cladosporium, Epicoccum, Alternaria,* and *Trichotecium (8)*. One study showed that the presence of the pathogenic fungi Fusarium in organic crops was less than half of that in conventional crops *(2)*.

In addition to the economic losses caused by fungal food spoilage, the presence of filamentous fungi in foods and crops raises a serious food safety concern because of the possible presence of mycotoxins. Many of the fungal species isolated in this and other studies are known to produce mycotoxins, including aflatoxins, ochratoxin A, fumonisins, sterigmatocystin, DON, T-2 toxin, patulin, penicillic acid, and others. Of these mycotoxins, aflatoxins, especially aflatoxin B1, produced mainly by *A. flavus* and *A. parasiticus*, are the most toxic. Aflatoxin B1 is known to be highly mutagenic, carcinogenic and teratogenic.

In this study, selected *Penicillium* and *Aspergillus* species isolated from both organic and conventional foods were found to produce some of their respective mycotoxins in laboratory medium *(Table 4)*. A total of 86 *Aspergillus* species and 51 *Penicillium* species were screened for the production in laboratory media of their respective mycotoxins, namely aflatoxin B_1, ochratoxin A, citrinin and penicillic acid. Of the *A. flavus* and *A. parasiticus* isolates tested for aflatoxin B_1 production, only three from conventional foods and only four from organic foods were found positive. Few isolates of *A. ochraceus, P. citrinum* and *P. aurantiogriseum* isolated from both food categories were found to produce ochratoxin A, citrinin and penicillic acid, respectively *(Table 4)*. No differences were observed in mycotoxin production between *Aspergillus* and *Penicillium* species isolated from the organic and the conventional foods. It should be noted that production of mycotoxins by these isolates in laboratory media does not necessarily imply that these species produce these mycotoxins in foods.

In addition to screening of the fungal isolates for mycotoxin production in a laboratory medium (YES), all food samples were screened for total aflatoxins. Aflatoxins were detected in three conventional foods (walnuts, almonds and peanuts) and in two organic foods (peanuts and corn) *(Table 5)*. The presence of aflatoxins in these samples was confirmed and quantified by the LC/MS method. The total amounts of aflatoxins (B_1, B_2, G_1, G_2) in the three conventional samples of walnuts, almonds and peanuts were 564, 306 and 538 ug/Kg, respectively, while the total of aflatoxins was 524 ug/Kg in the organic peanut sample and 465 ug/Kg in the organic corn sample. Currently, the health significance of fungi and mycotoxins in conventional and organic foods is still not clear and on a theoretical basis is controversial. Relatively few published studies have dealt with comparison of conventional and organic foods with regard to their mycological and mycotoxicological quality. It is assumed that because synthetic fungicides are not used in organic farming, and because organic crops contain higher concentrations of carbohydrates because of low nitrogen application, they will be more prone than conventional crops to fungal infections *(21)*. Tubajika et al. *(25)* reported that corn contains more aflatoxin B_1 when less nitrogen fertilization is used. However, in another study, barley was found to contain more ochratoxin A when higher-nitrogen fertilizer levels were used *(12)*. To add to the controversy, a study by Elmholt *(7)* reported that organically farmed soils contain five times more mycotoxigenic fungi than conventionally farmed soils, while other studies like this one, found no significant differences in the levels of viable fungi between organic and conventional foods *(16, 18)*. A study in Germany by Schollenberger et al. *(23)* found that conventionally produced bread contained more DON than bread made with wheat produced organically. However, a study in the UK *(6)* found no significant differences in the levels of DON and zearalenone between organic and conventional wheat samples.

In conclusion, this preliminary study showed no significant differences between the conventional and organic food samples tested with respect to the types and levels of fungi or the mycotoxigenic properties of the fungal isolates. However, it is important to note that this study was limited in scope and that the samples analyzed, of both conventional and organic foods, were purchased from retail markets, so that factors such as farming conditions, environmental factors such as weather conditions, and storage and processing conditions were not taken into consideration. For full assessments of mycological and myco-toxicological quality of conventional and organic foods, further studies are needed, using larger numbers of samples over an extended period of time and taking into consideration the growing, environmental, storage, and processing conditions.

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