Assessment of aflatoxigeinic Aspergillus species in food commodities from local market of Addis Ababa

Article *in* Research · November 2014

DOI: 10.13070/rs.en.1.1195

CITATIONS
9

READS
446

6 authors, including:

Negero Gemeda Negeri
Ethiopian Public Health Institute
30 PUBLICATIONS **178** CITATIONS

SEE PROFILE

Yimtubezenash Woldeamanuel
Addis Ababa University
92 PUBLICATIONS **1,469** CITATIONS

SEE PROFILE

Daniel Asrat
Addis Ababa University
98 PUBLICATIONS **2,454** CITATIONS

SEE PROFILE

Research

Assessment of aflatoxigeinic Aspergillus species in food commodities from local market of Addis Ababa

Negero Gemeda[1] (4negero at gmail dot com) #, Yimtubezinash Woldeamanuel[2], Daniel Asrat[2], Asfaw Debela[1], Hirut Lemma[1], Yehualashet Belete[1]

[1] Traditiona and Modern Medicine Research Directorate, Ethiopian Public Health Institute, P. O. Box 1242, Addis Ababa, Ethiopia.

[2] Department of Microbiology, Immunology and Parasitology, Addis Ababa University, P. O. Box 9086, Addis Ababa, Ethiopia

: corresponding author

DOI http://dx.doi.org/10.13070/rs.en.1.1195
Date 2014-11-18
Cite as Research 2014;1:1195

Abstract

Background: Contamination of food commodities with spoilage fungi presents a problem of global concern, since the growth and metabolism of these organisms can cause serious food-borne illnesses and a rapid spoilage of food products. Aspergillus species, a type of opportunistic fungi linked to food spoilage is the leading cause of infection, mycotoxicosis and economic loss. Mycotoxins that produce are responsible for cancers and many diseases affecting the gastrointestinal, urogenital, vascular, kidney, and nervous systems. Within the African region, aflatoxin is a threat to public health. In parts of sub-Saharan Africa about 250,000-hepatocarcinoma-related deaths occur annually due to aflatoxin ingestion alone. Because the impact is so devastating, there should be continuous assessment mechanisms to monitor the occurrence of aflatoxigenic strains of Aspergillus species in food commodities. **Objective**: To assess the occurrence of aflatoxigenic Aspergillus species in food commodities collected from Addis Ababa. **Method**: The isolation of aflatoxin producing species of Aspergillus from food commodities sold in Addis Ababa market was investigated. A total of 108 food samples were bought from markets within Addis Ababa. All samples were separately analyzed for the presence of aflatoxigenic Aspergillus species. The isolation of these species was carried out using dilution plating and/or direct plating methods. Morphological and culture characters, along with secondary metabolite profiles derived from thin-layer chromatography, were used to sort and identify Aspergillus species cultured on potato dextrose and sabaroud dextrose agar along with their aflatoxin-producing potentials on SMKY medium. **Result**: A total of 90 Aspergillus species (A. flavus, A. parasiticus, A. niger and A. fumigatus) were isolated from 108 samples of food commodities. The least number (5 isolates of Aspergillus species isolates were counted from the sample cookies while the highest (19 isolates) were counted from both the peanut and emmer wheat flour samples. From 33 Aspergillus flavus isolates 9 isolates (27.3%) were isolated from the peanut sample, while the fewest were isolated from the pea flour and cookies sample (n=3 (9.1%)). Six out of eighteen samples of peanut, emmer wheat flour, maize, and roasted barley were contaminated with Aspergillus niger, accounting (22.2%). A total of 21 (23.3%) isolates of Aspergillus species produced aflatoxin in vitro in SMKY broth culture out of 90 different Aspergillus species isolated from food commodities. Among the aflatoxigenic species isolated, Aspergillus flavus were responsible for the majority of cases (66.67%) followed by Aspergillus parasiticus (33.33%). None of the Aspergillus fumigatus isolates were found to produce aflatoxin in vitro on SMKY broth medium. Within species, 36.36% of Aspergillus flavus isolates were found to produce aflatoxin in vitro, while 18% of Aspergillus parasiticus isolates produced aflatoxin in vitro on culture media. **Conclusion**: Our study conducted to evaluate the presence of toxigenic strains of Aspergillus species in food commodities in Addis Ababa, has indicated the presence of these strains. Further studies should be done to quantify the level of aflatoxin in food commodities and also to evaluate the presence of potential mycotoxigenic fungi other than Aspergillus species in commercial food commodities.

Topics

mycotoxins
food contamination

Introduction

Despite the advancement of medicine, food science, and the technology of food production, diseases caused by food-borne fungal pathogens have continued to present a major problem of public health [1] and agricultural production [2]. Among fungi, Aspergillus species have the potential to contaminate food items by producing hydrolytic enzymes. Various mycotoxins have been reported in Aspergillus-contaminated food commodities [3]. Consumption of such contaminated food leads to a serious cases of illness and mycotoxicoses [3]. Among these, aflatoxicoseis have received significant public attention because of their acute and chronic toxicological effect [1]. In acute doses aflatoxin causes hemorrhage, acute liver damage, edema, and death, while in chronic exposure it

causes induction of cancer, mutagenicity, immune suppression, birth defects, and estrogenic, gastrointestinal, urogenital, vascular, kidney, and nervous system disorders [2] [4]. Within African regions aflatoxins are at major public health threat [1]. In parts of sub-Saharan Africa about 250,000-hepatocarcinoma-related deaths occur annually due to aflatoxin ingestion alone [5].

The genus Aspergillus, which includes about 350 species, is important in public health as human pathogens and as toxin-producing food contaminants. They produce and release millions of spores small enough to be found in air, water, soil, plant debris, rotten vegetation, manure, sawdust waste, bagasse waste and animal feed, and on animals and in indoor air environments [6]. As Aspergillus spores swell with water and grow, they elongate, forming hyphae which secrete digestive enzyme and mycotoxins. Aspergillus spores can survive harsh environmental conditions, such as extreme dry conditions, that do not support normal mold growth [7] [8] [9] [10].

Among the genus of Aspergillus, only a few well-known species are recognized as important pathogens of humans or domestic animals. Of these, over 95% of all infections are caused by Aspergillus fumigatus, Aspergillus flavus, and Aspergillus niger [6]. Some other species of clinical importance include A. nidulans, A. terreus, A. oryzae, A. ustus and A. versicolor [11] [12].

Aspergillus species is one kind of fungi that is linked to infection, mycotoxicosis, and food spoilage. These fungi are a common contaminant of indoor and outdoor environments and they can be major threat to human worldwide. While we are frequently exposed to airborne spores of Aspergillus, in immunocompetent people they rarely lead to disease. However, in a study of immunocompromised individuals Aspergillus species were frequently recovered [13]. Aspergillus causes animal disease in three major ways: through localized or systemic infections; through induction of allergenic responses; and through the production of mycotoxins. Aspergillosis can cause a wide range of clinical manifestations, including allergic aspergillosis, colonization of cavities with or without the formation of a fungus ball (mainly in the lungs, paranasal sinuses, bronchiectasis), acute to chronic necrotizing invasive forms, ocular infections (keratitis), endocarditis, osteomyelitis, and skin infections [14]. Invasive aspergillosis in critically ill patients is associated with high in-hospital mortality rates ranges between 50% [15] and 75.7% [16].

From the time when primitive man began to cultivate crops and store food; spoilage fungi (Aspergillus and Penicillium) caused problems [17]. The genus Aspergillus is an important genus in foods, causing more spoilage and biodeterioration than other fungi [17]. Even today in the era of significant technological advancements in the food industries, Aspergillus food spoilage is a major problem of food in storage [18]. Almost all kinds of food including cereals, meat, milk, fruit, vegetables, nuts, fats, and products made with these foods are susceptible to contamination by Aspergillus species [19]. These food contaminations are more common in stored commodities in tropical and subtropical climates than in temperate climates [17]. Aspergillus contamination of food commodities has been identified as a major contributing factor for huge agro-economic losses in the world. It is estimated that about 25% of the world's food supply is lost through microbial activity alone [11] [20].

Mycotoxicosis is a disease caused by coming into contact with mycotoxins through eating contaminated food, dermal contact, or inhalation [21]. Currently more than 300 mycotoxins are known, and scientific attention has been focused mainly on those that have proven to be carcinogenic and/or toxic. The most significant genera of mycotoxigenic fungi are Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium and Stachybotrys [22]. During their lifecycle, over 40 species of Aspergillus have been listed as capable of producing a wide range of mycotoxins harmful to humans and animals that consume them [4], but the Aspergillus mycotoxins of greatest public health and agro-economic significance are aflatoxins (by Aspergillus flavus and Aspergillus parasiticus), ochratoxin A (by Aspergillus ochraceus, Aspergillus carbonarius and Aspergillus niger), sterigmatocystin (by Aspergillus versicolor), and cyclopiazonic acid (by Aspergillus flavus and Aspergillus tamarii) [4].

The mycotoxins produced constitute-a major risk to human and animal health [23]. Aflatoxin has carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive properties and can inhibit several metabolic systems. Aflatoxin is mainly found on certain fruits, peanuts, pistachio nuts, Brazil nuts and figs. The principal classes of mycotoxins include aflatoxin B1 (AFB1), the most potent hepatocarcinogenic substance known, and also show to be genotoxic [4]. Ochratoxin A (OTA), which is a nephrotoxin, teratogen, and carcinogen, has mainly been found in cereals as well as in other products like coffee, wine, dried fruits, beer, grape juice and meats [4]. Citrinins tend to affect kidney function. Cyclopiazonic acid has a wide range of effects, and tremorgenic toxins such as territrems affect the central nervous system [4].

Food commodities can be contaminated with mycotoxins [24] and their presence in food products is a chemical hazard of biological origin [25]. Mycotoxins are responsible for cancers as well as many different disorders affecting the gastrointestinal, urogenital, vascular, kidney, and nervous systems [4]. In addition, mycotoxins are responsible

for generating huge economic losses in the producing countries [26]. Some of the mycotoxins have also been implicated as chemical warfare agents.

In Africa, certain aflatoxin productions are associated with hot, dry agroecozones with latitudinal shifts in climate influencing the fungal community structure [27]. Aflatoxin producing fungi are native to warm arid, semi-arid, and tropical regions. About 5.2 million cancer deaths occur each year, 55% of which occur in developing countries. Most of the mycotoxin contamination problems occur in the sub-Saharan Africa in which about 250,000-hepatocellular carcinoma related deaths occur annually in parts of sub-Saharan Africa due to aflatoxin ingestion alone. In sub-Saharan Africa, the aflatoxins, fumonisin, and ochratoxin mycotoxins are the most common [28]. The impact of mycotoxin in Ethiopia dates back to 1978 when the gangrenous ergotism epidemic occurred from the consumption of grains contaminated with Claviceps purpureu resulting in 34% mortality [29]. Since then studies have reported aflatoxins [30] [31] [32], gangrenous ergotism [33] and ochratoxin [34] in the country.

Mycotoxins are unavoidably consumed or ingested by animals or humans [11]). Hence, regulations are important to minimize human exposure to mycotoxins that result in high economic loss to handlers, producers, processors, and marketers of food commodities. For this reason, assessing the presence of mycotoxin producing fungi in food commodities is vital. Therefore, this study was carried out to assess the occurrence of aflatoxigenic Aspergillus species in food commodities collected from Addis Ababa.

Materials and methods

Sample collection site

Commercial commodities of six types were collected from Addis Ababa including peanut, pea flour (shiro), barley & emmer flour (besso & mittin aja), cookies, maize and roasted barley (kolo and senef kolo). A total of 108 food products including six samples from each type of food commodity were collected from six open markets around Teklehaimanot, Merkato, Messalemia, Medhanialem, Addisu-Mikael, and Enkulal-Fabrika.

[enlarge]

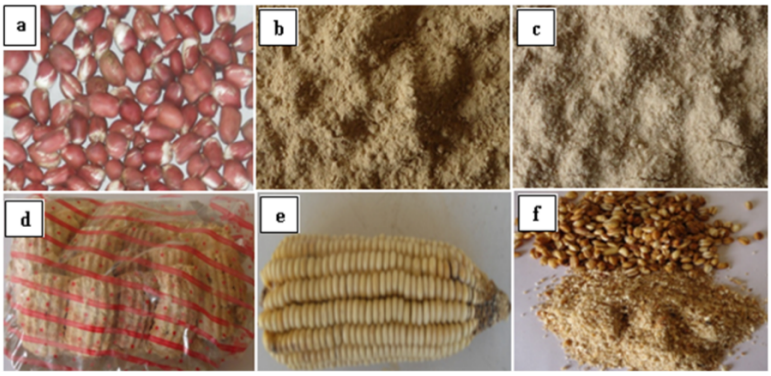

Figure 1. Food commodities used in study (a= Peanut, b= Pea flour, c= Barley, d=cookies, e=maize and f= roasted Barley).

Sample processing

A total of 108 samples were collected from Addis Ababa (Figure 1). About 1kg of each sample was bought from the market and aseptically taken with a sterile paper bag covered with a plastic bag. Codes were given to each sample and the samples were transported to the laboratory. Samples were kept in a refrigerator maintained between 4 to 8OC to minimize fungal growth or death until processing and mycological analysis of the food samples to be analyzed.

Culture and identification of Aspergillus species

Mycological analysis of the collected food samples was carried out according to Hocking macro-and micro-identification techniques [17]. Each sample was aseptically sub-sampled (25gm) for mycological analysis. Serial dilutions of food samples were prepared using 1:10 dilution (1gm of food in 9ml of sterile distilled water) and then homogenized by a vortex mixer. Two milliliters of the samples from each dilution were added into 18ml of PDA in a test tube and homogenized by a vortex mixer, then poured into 90mm Petri dish. After incubation the plates were observed for the presence of growth of visible fungal colonies. To better isolate the various fungal species from the samples, simple growth analyses made on mycological culture were carried out.

Variations in growth rate, color of the colony, and thermotolerance were used in identification of Aspergillus species. Aspergillus colonies are downy to powdery in texture. Aspergillus fumigatus is a thermotolerant fungus and grows well at temperatures over 40°C. This property is unique to Aspergillus fumigatus among the Aspergillus species. Aspergillus flavus can be readily distinguished from other Aspergillus species by lack of growth at 5 °C, by rapid growth at both 25 and 37°C, and by the production of a bright yellow-green conidial color. Macroscopically the surface and reverse color may vary depending on the species. A. flavus has a yellow-green surface and gold to reddish-brown reverse; A. fumigatus displays a blue-green to gray surface and white to tan reverse, and A. niger

colonies have a black surface and white to yellow reverse. Microscopic features were also used for identification. Microscopically, A. flavus have a colorless rough conidiophore, uniseriate or biseriate phialides, and a round radiate head vesicle; A. fumigatus have a short (<300 µm) smooth colorless or greenish conidiophore, uniseriate phialides and a round columnar head vesicle; and A. niger have long, smooth, colorless or brown conidiophores, biseriate phialides, and round radiate head vesicles.

Characterization of Aspergillus species for production of aflatoxin

Aspergillus species isolates from food commodities were screened for the production of aflatoxin following the protocol by Kumar and colleagues (35). The isolates were cultured separately in 25ml SMKY broth (sucrose 200g; $MgSO4 \cdot 7H2O$, 0.5g; $KNO3$, 0.3g and yeast extract, 7g; 1000ml distilled water) in a 100ml flask for 10 days. The contents of each flask were filtered and extracted with 20ml chloroform in a separating funnel. The extract was evaporated to dryness on a water bath and redissolved in 1ml chloroform. Aflatoxin production was detected by thin layer chromatography. Fifty microliters of chloroform extract were spotted on TLC plates and developed in the solvent system comprising chloroform: acetone (9:1 V/V). The plate was air dried and the intensity of aflatoxin was observed in UV-366.

[enlarge]

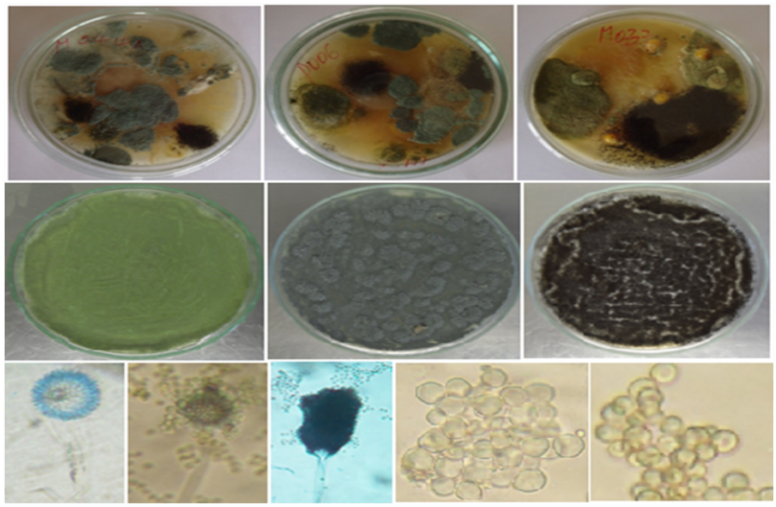

Figure 2. Culture and microscopy of Aspergillus species.

Statistical analysis

All measurements were replicated three times for each treatment. Data were entered into an Excel spreadsheet and presented as mean ± SE/SD. Significant differences between strains that were aflatoxin producers vs. non-producers were analyzed using statistical software (SPSS 20.0; Chicago, IL, USA) at 95% level of confidence by Chi-square analysis.

Results

Isolation of Aspergillus species from food commodities

Several fungal species became visible from the growth on PDA media after seven days of incubation. Only Aspergillus species (Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus) were sub-cultured for further study since these are the species are believed to be storage molds. Table 1 shows isolated Aspergillus species, percentage frequencies from total samples, and total number of Aspergillus species isolates from all samples using agar plate methods. Of the three Aspergillus species isolated from the food commodities, Aspergillus flavus were the most predominant with 36.6% (Table 1) isolated from 11 samples, followed by Aspergillus fumigatus collected from 10 samples (about 33.3%) while Aspergillus niger were recovered from 9 samples (about 30% of the samples collected for the study).

Food Sample	Number of Aspergillus isolates from food sample				
	A. flavus (n=33)	A. parasiticus (n=27)	A. fumigatus (n=10)	A. niger (n=20)	Total 90
Peanut	9(42.9)	6(28.6)	1(4.8)	5(23.8)	21(23.3)
Pea flour	3(27.3)	0(0.0)	3(27.3)	5(45.4)	11(12.2)
Barley flour (besso)	5(24.8)	6(28.6)	4(19.0)	6(28.6)	21(23.3)
Cookies	3(50.0)	3(50.0)	0(0.0)	0(0.0)	6(6.7)
Maize	8(42.1)	6(31.6)	1(5.3)	4(21.0)	19(21.1)
Roasted-Barley (Kolo)	5(41.7)	6(50.0)	1(8.3)	0(0.0)	12(13.3)
Total	33(36.7)	27(30.0)	10(11.1)	20(22.2)	90(100.0)

Table 1. Frequencies (%) of Aspergillus isolates from food samples in Addis Ababa.

As the microscopy and macroscopy (culture) characteristics of fungal isolates that are presented in Figure 2 and Table 1 show, a total of 90 Aspergillus species (A. flavus, A. niger and A. fumigatus) were isolated from 108 samples of food commodities. As seen in Table, 1 the least number (6 isolates) of Aspergillus species isolates were counted from the sample cookies while the highest (21 isolates) were counted from both peanut and barley flour

(besso) samples. Of a total of 33 Aspergillus flavus isolates, 9 (27.3%) were isolated from peanut sample, while the lowest numbers were isolated from the shiro and cookies sample (n=3, 9.1%). The food product with the highest number of Aspergillus fumigatus organisms was the barley flour (besso) samples (n=4, 40%), while Aspergillus niger was recovered most commonly from barley flour (besso) samples with the frequency of 6 (30%) (Table 1).

Characterization of Aspergillus species for the production of aflatoxin

A total of 21 (23.3%) isolates of Aspergillus species produced aflatoxin in vitro in SMKY broth culture out of 90 different Aspergillus species isolated from food commodities. Among the aflatoxigenic species isolated, Aspergillus flavus was responsible for the majority aflatoxin-positive samples (n=12, 66.67%) followed by Aspergillus parasiticus (n=6, 33.33%). None of the ten Aspergillus fumigatus or Aspergillus niger isolates were found to produce aflatoxin in vitro on SMKY broth medium (Table 2).

Peanut samples were the most frequently contaminated samples by aflatoxin-producing Aspergillus species (n=10) followed by maize samples (n=7) and barley flour (besso) (n=3). The least frequently contaminated samples were roasted barley (Kolo), pea flour, and cookies.

Within species, thirty six percent of Aspergillus flavus isolates were found to produce aflatoxin in vitro, while twenty two percent of Aspergillus parasiticus isolates produced aflatoxin in vitro on culture media. Sixty seven percent and fifty percent of Aspergillus flavus isolated from peanut and maize samples, respectively, demonstrated aflatoxigenic potentials. Aspergillus parasiticus isolated from peanut samples (50%) showed aflatoxigenic potentials followed by isolates from maize samples (33.3%).

Food Samples	Aspergillus species	No of strain isolated	No of aflatoxigenic strain
Peanut	A. flavus	9	6
	A. niger	5	0
	A. fumigatus	1	0
	A. parasiticus	6	3
Pea flour	A. flavus	3	0
	A. niger	5	0
	A. fumigatus	3	0
	A. parasiticus	0	0
Barley flour (besso)	A. flavus	5	1
	A. niger	6	0
	A. fumigatus	4	0
	A. parasiticus	6	1
Cookies	A. flavus	3	0
	A. niger	0	0
	A. fumigatus	0	0
	A. parasiticus	3	0
Maize	A. flavus	8	4
	A. niger	4	0
	A. fumigatus	1	0
	A. parasiticus	6	2
Roasted Barley (Kolo)	A. flavus	5	1
	A. niger	0	0
	A. fumigatus	1	0
	A. parasiticus	6	0
Total	A. flavus	33	12
	A. niger	20	0
	A. fumigatus	10	0
	A. parasiticus	27	6
	Total	90	18

Table 2. Toxigenic potentials of fungi isolated from foods in Addis Ababa.

Discussion

Aspergillus species are a major public health problem of modern mycology [19]. They are known to cause Aspergillus infection and mycotoxicosis especially upon the consumption of food contaminated with Aspergillus species.

Food commodities in Addis Ababa local markets were found to be frequently contaminated by Aspergillus species. Virtually all kinds of food commodities collected for this survey were colonized by Aspergillus species, though peanut samples represented a higher number of Aspergillus species. The major contributing factors include not being processed and stored in appropriate conditions [36]. On the other hand, cookies and shiro presented a lower number of Aspergillus species, likely due to the processing of the sample and low moisture content of the food.

According to a previous study, Aspergillus species were the major contaminant of human food in storage [36]. The relative frequencies of Aspergillus species may be higher because of their ability to grow at large range of temperatures, harvesting methods and storage conditions of food commodities, and also the ability of Aspergillus to colonize almost all food commodities [19]. As noted in a recent literature review, Aspergillus species colonization of food commodities may occur at any stage from flowering, harvest, storage, sorting or transport, and processing of

the foods [19]. This confirmed that Aspergillus species are truly storage fungi. Contamination of commercially purchased food commodities is clearly a cause for concern in our current turbulent time.

Aspergillus species that infect food commodities in Addis Ababa significantly produce aflatoxin. The aflatoxin-producing Aspergillus species were identified on the basis of all lines of evidence examined. However, the color of conidial heads may suffice for their differentiation. Our study confirmed that Aspergillus flavus were the most frequent producers of aflatoxin at a detectable concentration in vitro on culture medium as also reported by Legesse [32]. Only some Aspergillus flavus strains are genetically capable of producing aflatoxin, and external factors play a major role in the expression of the gene for aflatoxin production. In this study, of all Aspergillus species isolated from food commodities, Aspergillus flavus predominated, confirming the reputation of this mould as a ubiquitous spoilage mycotoxin-producing organism, even though Aspergillus parasiticus, Aspergillus niger, and Aspergillus fumigatus were also isolated. Even though aflatoxins are the among the first mycotoxins known to mankind, our finding reminded us that there is still much work to be done in controlling its appearance in food commodities [4]. Aspergillus species that produce aflatoxins are increasing because of favorable environmental conditions such as drought-stress, humidity, and insect infestation of the crops at various stages [12] [27]. Moreover, poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of mycotoxin production [1].

Conclusions

Our study of the presence of toxigenic strains of Aspergillus species in food commodities in Addis Ababa has indicated the presence of these strains. Aspergillus was more frequently isolated from peanut food samples. Peanut and maize were frequently contaminated by toxigenic Aspergillus species. Aspergillus flavus was the most frequent aflatoxin-producing species isolated from food commodities from Addis Ababa. Aspergillus and their mycotoxins are a growing threat to human and animal health around the world, not just in Ethiopia. Tests to detect contamination need to be adopted on wide scale in the country. Moreover, there should be control and regulation mechanism to limit the consumption of food contaminated by aflatoxigenic Aspergillus species and aflatoxin.

Acknowledgements

Financial support for this study was provided by Addis Ababa University and by the Ministry of Finance and Economic Development through EHNRI (through project number 342/02/04/01/012). We are thankful the staff of the Traditional and Modern Medicine Directorate, EHNRI for their help and support during our study. We would like to thank Mr. Awoke Kebede, for his generous permission to use his aflatoxin standard (Aflatoxin Mix Kit-M). The authors are grateful to Department of Microbiology, Immunology, and Parasitology (DMIP), College of Health Science, AAU, and EHNRI for their facility and financial support. We would like to thank Dr. Edward A. Rose for his edition for English on our manuscript.

References

1. DeWaal CS, Robert N. Global and Local: Food Safety Around the World. Washington: Center for Science in the Public Interest. 2005;1-68.
2. Leslie JF, Bandyopadhyay R, Visconti A. Mycotoxins-Detection Methods, Management, Public Health and Agricultural Trade UK: CAB International; 2008.
3. Wild C, Gong Y. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010;31:71-82 pubmed
4. Zain M. Impact of mycotoxins on humans and animals. JSCS. 2011;15:129-44.
5. Wagacha J, Muthomi J. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. Int J Food Microbiol. 2008;124:1-12 pubmed publisher
6. Bennett JW. An Overview of the Genus Aspergillus. In: Machida M, Gomi K, editors. Aspergillus Molecular Biology and Genomics. UK: Caister Academic Press 2010;1-17.
7. Castón-Osorio J, Rivero A, Torre-Cisneros J. Epidemiology of invasive fungal infection. Int J Antimicrob Agents. 2008;32:S103-9 pubmed publisher
8. Dainty R. Chemical/biochemical detection of spoilage. Int J Food Microbiol. 1996;33:19-33 pubmed
9. Pagano L, Caira M, Valentini C, Posteraro B, Fianchi L. Current therapeutic approaches to fungal infections in immunocompromised hematological patients. Blood Rev. 2010;24:51-61 pubmed publisher
10. Lestari T, Ryll S, Kramer A. Microbial contamination of manually reprocessed, ready to use ECG lead wire in intensive care units. GMS Hyg Infect Control. 2013;8:Doc07 pubmed
11. Murphy PA, Hendrich S, Landgren C, Bryant CM. Food mycotoxins: an update. J Food Sci. 2006;71:R51-R65.
12. Pattron DD. Aspergillus, Health Implication & Recommendations for Public Health Food Safety. IJFS. 2006;8:19-23.
13. Pfaller MA, Pappas PG, Wingar JR. Invasive fungal pathogens: current epidemiological trends. Clin Infect Dis. 2006;43(Suppl. 1):S3-14.
14. Muñoz P, Guinea J, Bouza E. Update on invasive aspergillosis: clinical and diagnostic aspects. Clin Microbiol Infect. 2006;12(7):24-39.

15. Garnacho-Montero J, Amaya-Villar R, Ortiz-Leyba C, León C, Alvarez-Lerma F, Nolla-Salas J, *et al*. Isolation of Aspergillus spp. from the respiratory tract in critically ill patients: risk factors, clinical presentation and outcome. Crit Care. 2005;9:R191-9 pubmed
16. Khasawneh F, Mohamad T, Moughrabieh M, Lai Z, Ager J, Soubani A. Isolation of Aspergillus in critically ill patients: a potential marker of poor outcome. J Crit Care. 2006;21:322-7 pubmed
17. Hocking AD. Aspergillus and related teleomorphs. In: Blackburn CdW, editor. Food Spoilage Microorganism. England: Woodhead Publishing. 2006;451-487.
18. Dao T, Dantigny P. Control of food spoilage fungi by ethanol. Food Control. 2011;22:360-8.
19. Pitt JI, Hocking AD. Fungi and Food Spoilage. New York: Springer Science, 3rd edition 2009.
20. Karabulut OA, Gabler FM, Mansour M, Smilanick JL. Postharvest ethanol and hot water treatments of table grapes to control gray mold. Postharvest Biol Tec. 2004;34:169-77.
21. Pittet A. Natural occurrence of mycotoxins in food and feeds - an updated review. Rev Med Vet. 1998;149:479-92.
22. Steyn P. Mycotoxins, general view, chemistry and structure. Toxicol Lett. 1995;82:843-51 pubmed
23. Dantigny P, Marin S, Beyer M, Magan N. Mould germination: data treatment and modelling. Int J Food Microbiol. 2007;114:17-24 pubmed
24. Miller JD. Fungi and Mycotoxins in Grain: Implications for Stored Product Research J stored Prod Res. 1995;31(1):1-16.
25. Garcia D, Ramos A, Sanchis V, Marin S. Predicting mycotoxins in foods: a review. Food Microbiol. 2009;26:757-69 pubmed
26. Bhat RV, Vasanthi S. Mycotoxin food safety risks in developing countries. Food Safety in Food Security and Food Trade Vision 2020 for Food, Agriculture and Environment, Focus 10, brief 3 of 17, pp 1-2. 2003.
27. Cotty P, Jaime-Garcia R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int J Food Microbiol. 2007;119:109-15 pubmed
28. Wild C. Aflatoxin exposure in developing countries: the critical interface of agriculture and health. Food Nutr Bull. 2007;28:S372-80 pubmed
29. King B. Outbreak of ergotism in Wollo, Ethiopia. Lancet. 1979;1:1411 pubmed
30. Besrat A, Gebre P. A preliminary study on the aflatoxin content of selected Ethiopian foods. Ethiop Med J. 1981;19:47-52 pubmed
31. Fufa H, Urga K. Screening of aflatoxins in Shiro and ground red pepper in Addis Ababa. Ethiop Med J. 1996;34:243-9 pubmed
32. Legesse E. Aflatoxin Content of Peanut (Arachis hypogaea) in Relation to Shelling and Storage Practices of Ethiopian Farmers. Addis Ababa: Addis Ababa University; 2010.
33. Urga K, Debella A, W/Medihn Y, Agata N, Bayu A, Zewdie W. Laboratory studies on the outbreak of Gangrenous Ergotism associated with consumption of contaminated barley in Arsi, Ethiopia. Ethiop J Health Dev. 2002;16:317-23.
34. Ayalew A, Fehrmann H, Lepschy J, Beck R, Abate D. Natural occurrence of mycotoxins in staple cereals from Ethiopia. Mycopathologia. 2006;162:57-63 pubmed
35. Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK. Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innovat Food Sci Emerg Tech. 2008;9:575-80.
36. Njobeh P, Dutton M, Koch S, Chuturgoon A, Stoev S, Seifert K. Contamination with storage fungi of human food from Cameroon. Int J Food Microbiol. 2009;135:193-8 pubmed

ISSN : 2334-1009