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Full Length Research Paper

Mycotoxin production by fungi isolated from stored grains

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A study was conducted to determine the fungi associated with maize (*Zea mays*), rice (*Oryza sativa*) and millet (*Pennisetum typhoides*) in storage. Mycotoxin production by isolated fungi was subsequently evaluated using the thin layer chromatography technique. Eight different fungi were isolated altogether namely *Aspergillus terreus, Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Penicillium italicum, Penicillium spinulosum, Rhizopus stolonifer* and *Fusarium* sp. The results of this study show that all the fungi produced one toxin or the other as detected in the culture filtrates of isolated fungi. However, only three of these toxins were identified namely aflatoxin B1, fumonisin B1, and zearalenone. The retention factors (R_f) of all the toxins produced were determined.

Key words: Maize, rice, millet, storage fungi, mycotoxins.

INTRODUCTION

One of the most important effects of post harvest decays of fruits and vegetables and especially of seed and feed deterioration by fungi is the induction of mycotoxicoses. This is a disease of animals and humans following consumption of feeds and foods invaded by fungi that produce toxic substances called mycotoxins (Agrios, 1978; Moss, 1989). Some common mycotoxicoses caused by common and widespread fungi such as *Aspergillus, Penicillium, Fusarium* and *Stachybotrys* result in severe illness and death. *Aspergillus* and *Penicillium* produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field. Adams (1977) has reported that storage fungi especially *Aspergillus, Penicillium, Rhizopus* and *Mucor* species infect grains after harvest and can grow on them during storage.

The genera of mycotoxigenic fungi are mainly represented by *Aspergillus, Penicillium* and *Fusarium* but *Alternaria* among others are also important as food contaminants or pathogens of plants. *Alternaria* produces mycotoxins in grains such as rice and maize (Ramm et al., 1994). Many *Aspergillus, Penicillium* and *Cladosporium* species are known to produce mycotoxins. According to Dooley (2001), other toxigenic fungi frequently found on grains are *Alternaria, Trichoderma, Fusarium, Paecilomyces, Chaetomium* and *Acremonium*. Mycotoxins are secondary metabolites produced by filamentous fungi which may contaminate food, feeds or raw materials used in producing them.

Aflatoxin is about the most popular and widespread mycotoxin. Its name derives from the fact that it was originally found to be produced by *Aspergillus flavus* (Agrios, 1978), but is now known to be produced by other species of *Aspergillus*. Aflatoxin B1 is produced by *Aspergillus terreus,* though it may also be produced by *Aspergillus flavus* as well as *Aspergillus oryzae.* It is the most toxic, carcinogenic and most prevalent of the different aflatoxins. Generally, mycotoxins have been implicated as causative agents of different human and animal health disorders (Ciegler and Bennett, 1980). Both the toxigenic fungi and the mycotoxins they produce are potential problems from both health and economic perspectives. Fumes from burning molded hay may also affect animals and man and handling of such hay by farm workers causes in them a toxic dermatitis, conjunctivivtis, etc. Mycotoxin production by fungi in stored grains, therefore really posses a health problem in animals and man. The objective of this study is to isolate the fungi associated with rice, maize and millet in storage and

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Table 1. Organisms isolated from stored maize, rice and millet grains.

Grain	*A. terreus*	*R. stolonifer*	*A. oryzae*	*P. italicum*	*P. spinulosum*	*Fusarium* sp.	*A. niger*	*A. flavus*
Maize (White)	+	+	-	+	+	+	-	-
Maize (Red)	+	+	+	-	+	+	-	+
Millet	+	-	-	-	-	-	-	+
Rice	+	-	-	-	-	-	+	+

+ = Present; - = absent.

determine the ability of the isolates to produce mycotoxins. Findings will serve the purpose of alerting consumers on the dangers of consuming poorly stored grains.

MATERIALS AND METHODS

Isolation

Associated fungi were isolated from stored rice, maize and millet grains. The grains were surface-sterilized in NaOCl for 2 min and rinsed in two changes of sterile distilled water. The grains were blotted dry in between sterile Whatman No. 1 filter papers and plated on sterile potato dextrose agar (PDA) at the rate of 10 grains per plate. Thirty grains were plated per crop and incubated at room temperature of 25 ± 1°C. Sub-cultures were made from emerging colonies and pure cultures obtained for subsequent studies.

Mycotoxin production

The medium used for this study was potato dextrose broth (PDB). The medium was prepared routinely and sterilized. Twenty-five millilitres (25 ml) of the broth were dispensed into sterile conical flasks. The flasks were inoculated separately with 5 ml of any of the test fungi. The inoculated flasks were incubated on a shaker at a room temperature of 25 ± 1°C for 12 days. The filtrates of the test isolates were obtained and assayed for the presence of mycotoxins.

RESULT

The study shows that all the grains were infested to various degrees with storage fungi. A total of eight different fungi namely *A. terreus, A. flavus, A. niger, A. oryzae, P. italicum, P. spinulosum, R. stolonifer* and *Fusarium* species were isolated from the three grains studied. *A. terreus* was the most frequently isolated fungus followed by *A. flavus*. *R. stolonifer, P. spinulosum* and *Fusarium* sp. were equally frequent in the grains. Maize (*Zea mays*) was the most infested of the three grains assayed (Table 1) with the red maize being slightly more infested than the white while millet was the least infested.

Mycotoxin assay revealed that several toxins were produced in this study. However, only three of these toxins namely aflatoxin, fumonisin and zearalenone were identified in this study. All the fungi produced one toxin or the other. The retention factors (R_f) of all the toxins produced were determined. While *A. flavus*, produced both aflatoxin B1 and fumonisin B1, *A. terreus*, and *A. oryzae* produced only aflatoxin B1 (Table 2). *Fusarium* sp. and *P. italicum* produced zearalenone and fumonisin B1, respectively. Many of the isolates produced more than one toxin but some of them could not be identified within the scope of this study.

DISCUSSION

A total of eight fungi comprising five different genera were isolated from stored maize, rice and millet grains in this study. Four of these fungi were different species of the genus *Aspergillus*. According to Agrios (1978), the most common storage fungi are *Aspergillus* and *Penicilium* species. Seed infestation by microorganisms is a common and widespread phenomenon which has been variously reported. Amadi and Oso (1996) had reported *Aspergillus* spp., *Mucor hiemalis, Macrophomina phaseolina, Rhizopus oryzae, Alternaria longissima, Cochliobolus pallescens, Botryodiplodia theobromae* and *Colletotrichum* species in *Vigna unguiculata* seeds in Ibadan, Nigeria. Amadi (2002) had also reported 11 fungi including *Alternaria, Aspergillus, Fusarium, Rhizopus, Penicillium,* and *Mucor* species in *Saccharum officinarum* seeds. Storage fungi are usually not present in large quantities before harvest but are widely distributed and almost always present. Contamination occurs through small quantities of spores contaminating the grain as it is going into storage from the harvest in handling and storage equipment or from spores already present in storage structures (IRRI, 2006). Under high temperatures and moisture this small amount of inoculum can increase rapidly.

Several mycotoxins were observed in the present study including aflatoxin B1, fumonisin B1 and zearalenone. Some of the toxins, however, could not be identified. All the isolates produced one toxin or the other. Pitt (1995) has reported that *Fusarium* species produced mycotoxins in wheat, oats, barley, corn and rice and that it is a pre-harvest problem. Mycotoxins occur and exert their toxic effect in extremely small quantities in food stuffs. Mycotoxin literally means “fungus poison” and the fungi that produce mycotoxins do not have to be present to

Table 2. Mycotoxins produced by fungi isolated from stored grains.

Isolated Fungi	Retention factor (R_f)	Mycotoxin
A. flavus	0.44	Aflatoxin B1
	0.71	Fumonisin B1
A. terreus	0.44	Aflatoxin B1
	0.65	*
A. oryzae	0.44	Aflatoxin B1
	0.56	*
A. niger	0.60	*
	0.67	*
Fusarium sp.	0.40	Zearalenone
	0.62	*
P. italicum	0.71	Fumonisin B1
P. spinulosum	0.64	*
R. stolonifer	0.64	*

*Not identified.

Thin Layer Chromatography (TLC) technique was used for detection of mycotoxins. Both the filtrates and the standards were spotted on the prepared TLC plates at a distance of 1.5 cm from the lower edge and left for 1 min to dry. The solvent used was chloroform/methanol (97:3 and 50:50, v:v) to detect the mycotoxins produced by the test organisms. The TLC plates were removed when the solvent reached a mark 1cm from the upper edge of the plates. The plates were left to dry up and the solvent front marked across the plate. The color spots were marked with pencil and visualized under UV lamp. The retention factors (R_f) of the compounds/toxins detected in the filtrates were calculated. R_f = distance traveled by the compound/distance traveled by the solvent.

cause harm (Anon, 2003). The mycotoxin produced by each of these fungi may differ from each other in chemical formula, products in which they occur, conditions under which they are produced, their effects on various animals and humans and in degree of toxicity (Agrios, 1978). Several different fungi, however, produce some of the same or closely related toxins. *Aspergillus* and *Penicillium* species particularly produce similar toxins.

Aflatoxin, a metabolite of *A. flavus* and a precursor of liver cancer was first isolated from groundnut by Blount in 1961 and named by Splensley (1963). According to Alabi (1989), of the four aflatoxins (B1, B2, G1, and G2), B1 is the most common and most toxic with lethal dose (LD50) of less than 0.5 mg/kg body weight in ducklings which are particularly susceptible. Aflatoxin B1 is an important contaminant of food and feed crops before, during and after harvest (Shanahan et al*.,* 2003) and is very hazardous to human health. Fumonisins, a family of food-borne carcinogen are another important mycotoxins produced by *A. flavus* and *P. italicum.* Fumonisin B1 is the most abundant and may be related to esophageal cancer in humans. They are contaminants of natural and or processed maize used as human or animal feed and have a negative impact on the immune system (Dombrink-Kurtzman et al*.,* 1999; Paul and Gary, 2002).

Zearalenone is a field mycotoxin produced by *Fusaruim* spp. These species are common on cereals and tend to develop particularly during cool, wet growing and harvest seasons (AOAC, 2002). This estrogenic mycotoxin affects reproductive efficiency but generally not feed intake and seems to be most toxic to swine in which it causes abnormalities and degeneration of the genital system.

REFERENCES

Adams JM (1977). A review of the literature concerning losses in stored Cereals and pulses. Trop. Sci. 19(1): 1-27.

Agrios NG (1978). Plant Pathology. Academic Press, New York, 703p.

Alabi RO (1989). Mycology and National Development: Mobilization of Fungal Products for Life More Abundant. Inaugural Lecture, University of Ilorin, Ilorin, Nigeria, May 25, 1989.

Amadi JE (2002). Studies on the mycoflora of sugarcane (*Saccharum officinarum*) seeds and their importance in the nursery. NISEB J. 2(1): 89-95.

Amadi JE, Oso BA (1996). Mycoflora of Cowpea Seeds (*Vigna unguiculata* L.) and their effects on seed nutrient content and germination. Niger. J. Sci 30: 63-69.

Anon (2003). Botany 135 Syllabus: www.botany.hawaii.edu/faculty

AOAC (2002). Official Method 2001.04, Determination of Fumonisins B1 & B2 in corn and corn flakes, AOAC International 2002.

Ciegler A, Bennett JW (1980). Mycotoxins and Mycotoxicoses. Bioscicence 30(8): 512-515.

IRRI (2006). International Rice Research Institute: www.knowledgebank.irri.org/ppfm/storage/6.B.- fungi.htm

Moss MO (1989). Mycotoxins of *Aspergillus* and other filamentous fungi. J. Appl. Bacteriol. 67(Symposium Suppl): 695-815.

Paul V, Gary P (2002). What are mycotoxins. In: Mycotoxin in Grain. University of Kentucky Cooperative Extension Service, p. 7.

Pitt JI (1995). Under what Conditions are mycotoxins produced? 1. Field Fungi Austr. Mycotoxin Newsl. 6(2): 1, 2.

Shanahan JF, Brown WW, Blunt TD (2003). Aflatoxins. Colorado State University Cooperative extension. Crop series production. No 0.306.

Splensley PC (1963). Aflatoxin, the active principle in Turkey X diseases. Endeavour *22*: 75-79.