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Aflatoxin contamination of dried red chilies: Contrasts between the United States and Nigeria, two markets differing in regulation enforcement

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ABSTRACT

Dried red chilies are among the world's most consumed spices. From farm to fork, chilies go through cropping, harvest, drying, processing and storage. Chilies are susceptible to infection by aflatoxin producing fungi and subsequent contamination by aflatoxins at every stage. Aflatoxins are highly regulated, hepatotoxic carcinogens produced by fungi in *Aspergillus* section *Flavi*. The current study examined prevalence of aflatoxin B_1 (AFB_1) in chilies from markets across the United States (US) and Nigeria, and determined predisposition of chilies to aflatoxins post-harvest. Aflatoxin B_1 was detected in 64% chilies from US markets (n = 169), and 93% of Nigerian chilies (n = 55) with a commercial lateral flow assay (Limit of Detection = 2 μg/kg). Two percent of US samples exceeded the aflatoxin regulatory limit of 20 μg/kg, while the highest concentration detected was 94.9 μg/kg. *Aspergillus* spp. could be recovered only from 40% of samples from the US, and aflatoxin levels did not correlate with quantities of *Aspergillus* section *Flavi* (Colony Forming Units g^{-1}), suggesting fungi associated with chilies in US markets were killed during processing. Both average AFB_1 concentrations and fungal quantities were significantly higher ($p < 0.01$) in Nigerian chilies. The most contaminated sample contained 156 μg/kg AFB_1. Aflatoxin concentrations in Nigerian chilies increased as an exponential function of the quantities of *Aspergillus* section *Flavi* ($r^2 = 0.76$). Results indicate that high rates of chili consumption may be associated with unacceptable aflatoxin exposure.

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1. Introduction

Dried red chili (*Capsicum* spp.), a member of the nightshade family of Solanacea, is used to enhance flavor, taste and aroma of foods. Chilies are native to the new world where they were domesticated about 6000 years ago (Perry et al., 2007). The economically notable species of *Capsicum* are *C. annuum, C. baccatum, C. chinense, C. frutescens,* and *C. pubescens* (Perry et al., 2007). Dried red chili is consumed across the globe in whole, crushed and ground forms. It stands second among spices in global consumption after black pepper (*Piper nigrum*) (Yogendrarajah, Jacxsens, De Saeger, & De Meulenaer, 2014). Over 80% of red chili is produced in tropical or sub-tropical regions (FAOSTAT, 2017). During the past decade, India, China and Thailand produced almost half of the world's dried chilies (FAOSTAT, 2017).

Fungal infection and subsequent mycotoxin contamination of chilies are affected by environmental conditions, with high temperatures and humidity favoring infection (El Mahgubi et al., 2013; Iamanaka, de Menezes, Vicente, Leite, & Taniwaki, 2007; Iqbal, Paterson, Bhatti, & Asi, 2010). Red chilies available in commercial markets are frequently contaminated with unacceptable concentrations of aflatoxins (Bircan, 2005; Paterson, 2007; Reddy, Kiran Mayi, Uma Reddy, Thirumala-Devi, & Reddy, 2001). This can be attributed to pre and/or post-harvest colonization of chilies by aflatoxin producing *Aspergillus* species. Inadequate conditions during drying, followed by poorly sheltered transport and storage can exacerbate contamination levels (Duman, 2010; Jalili & Jinap, 2012).

Aflatoxins in food and feed are of global concern because aflatoxins are highly toxic fungal metabolites that cause human cancer, immune suppression, and stunting (Khlangwiset, Shephard, & Wu,

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2011; Liu & Wu, 2010; Mehl et al., 2012; Schatzmayr & Streit, 2013; Williams et al., 2004; van Egmond & Jonker, 2004). Several *Aspergilli* belonging to section *Flavi* cause aflatoxin contamination of a wide range of crops including maize, groundnuts, tree nuts, cottonseed and spices (Doster & Michailides, 1994; Jaime-Garcia and Cotty, 2003; Probst, Bandyopadhyay, & Cotty, 2014; Tansakul, Limsuwan, Böhm, Hollmann, & Razzazi-Fazeli, 2013). Aflatoxin B_1 is the only mycotoxin classified as group 1 human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2002). Aflatoxins are stringently regulated in the developed world, resulting in huge economic losses. Aflatoxin contamination of food and feed can result in annual losses of more than $500 million in the US alone (Robens & Cardwell, 2003; Vardon, McLaughlin, & Nardinelli, 2003). In developing countries, aflatoxin is both an economic threat and a health concern. Many developing countries do not enforce aflatoxin regulations in crops, resulting in exposure of humans to chronic and acute health risks (Shephard, 2003; Williams et al., 2004). Food and feed contaminated with aflatoxin concentrations above legislated limits can face border rejections, and loss of both markets, and product value. The European Union (EU) regulates AFB_1 at 5 μg/kg and total aflatoxins at 10 μg/kg in spices (European Spice Association, 2004). Chili shipments to the EU from several nations are rejected every year due to aflatoxin contamination (RASFF, 2015).

Most dry chilies in retail markets across the US are imported (FAOSTAT, 2017). Over 100,000 tonnes of dried red chili are imported annually into the US. Aflatoxins are regulated at 20 μg/kg in the US in foods for human consumption. This regulation can have a severe impact on the value of crops intended for US markets. Nigeria accounts for about 50% of chili production on the continent of Africa (FAOSTAT, 2017; Mohammed, Abdulsalam, & Ahmed, 2015). Chilies are an integral part of Nigerian cuisine. Although, Nigeria regulates AFB_1 at 20 μg/kg (FAO, 2004), these regulations are less effectively enforced, leading to chronic exposure (Omojokun, 2013; Williams et al., 2004). The current study sought to: (i) contrast prevalence of aflatoxin contamination in dried red chili from markets in US with those from markets in Nigeria, (ii) determine the relationship between quantities of *Aspergillus* section *Flavi* and aflatoxin concentrations, and (iii) test potential for post-harvest contamination of market-purchased chilies.

2. Materials and methods

2.1. Dried red chili samples

Dried red chili was purchased from retail markets in the United States (n = 169) and Nigeria (n = 55). Samples from US were collected during 2014–15 from retail markets in California (n = 68), Minnesota (n = 3), New York (n = 34), and Arizona (n = 64), and consisted of whole (n = 60), ground (n = 78) and crushed (n = 12) chili, and paprika (n = 19). Fifty-eight percent of chili samples from US were labelled as imported from various countries (Table 1). All samples from Nigeria consisted of whole red chilies (n = 55), which were purchased from rural, small-scale markets in Kaduna (n = 50) and Lagos (n = 5) states during 2015–16. Chili samples averaged 200 g and ranged from 70 to 300 g. Nigerian samples were transferred to zippered plastic bags immediately after purchase, kept under ambient conditions, and shipped within a week of purchase. These samples were imported to the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) laboratory, at the University of Arizona, Tucson, under permits issued by the USDA Animal and Plant Health Inspection Service (APHIS). Samples were dried in a forced air oven (40 °C) to below 8% water content and sealed in plastic bags to prevent fungal growth after receipt. Whole and crushed chili samples were finely ground in a laboratory mill (Retsch Grindomix GM200, Newtown, PA) for 30 s at 10,000 rpm for fungal isolation and aflatoxin B_1 quantification. Chili and paprika, that were purchased ground, were analyzed for both fungi and aflatoxin B_1 with no further processing.

2.2. Aflatoxin extraction and quantification

Aflatoxin B_1 in chilies was detected and quantified using an immunochromatographic assay (Reveal Q+ for Aflatoxin testing, Neogen Corporation, Lansing, MI) approved by the USDA, Grain Inspection, Packers and Stockyards Administration (GIPSA) (Anonymous, 2015). Each ground chili sample was thoroughly mixed, and a 25–50 g sub-sample, depending on the quantity available, was analyzed for AFB_1 content following manufacturer's instructions. Briefly, AFB_1 was extracted with 65% ethanol by blending ground chili with either 125 ml or 250 ml of the solvent (for 25 g and 50 g of ground chili, respectively). The slurry was shaken on a rotary shaker (HS501digital, IKA LABORTECHNIK, Germany) at full speed for 3 min and allowed to settle for an additional 3 min. The supernatant was filtered through Whatman No. 1 filter paper and AFB_1 was immediately quantified using the immunoassay and the AccuScan Pro reader.

2.3. Analytical performance- spike and recovery

Aflatoxin quantification was validated by spike and recovery experiments. Ground red chili (5 g) with no detectable aflatoxin was spiked to either 50 or 100 μg/kg of aflatoxin B_1 (AFB_1 in methanol, Supelco, Bellefonte, PA). Aflatoxin was extracted and quantified as described above. Spike and recovery was performed in four replicates. Recovery rates were estimated using the following equation:

$$\%\text{Recovery} = (\text{Aflatoxin B1 concentration measured in spiked sample}/\text{Spiked concentration}) \times 100$$

Precision of the analytical method was expressed as relative standard deviation (RSD) of replicated results.

2.4. Quantification of Aspergillus section Flavi

Members of *Aspergillus* section *Flavi* were recovered and quantified through dilution plate technique on modified Rose Bengal agar (Cotty, 1994). Briefly, up to 10 g of ground chili was suspended in 50 ml sterile de-ionized water containing 0.01% Tween-80 by stirring for 20 min. A 200 μl aliquot of the resulting suspension from each sample was plated in triplicate on modified Rose Bengal agar and incubated for 3 days at 31 °C in the dark. Adjustments were made to aliquot volume and/or crop quantity to allow no more than 10 *Aspergillus* section *Flavi* colonies per plate for accurate enumeration. After incubation, *Aspergillus* section *Flavi* colonies were microscopically identified and enumerated (Colony forming units (CFU) g^{-1}). Fungal isolations were performed at least twice for each chili sample. Up to five discrete colonies of section *Flavi* per isolation were sub-cultured onto 5–2 agar (5% V-8 juice; 2% agar; pH 6.0) and incubated at 31 °C for 5–7 days in dark. Isolates were saved and stored as 3 mm agar plugs of sporulating culture in a vial containing sterile distilled water (2 ml). Limit of detection of fungi belonging to *Aspergillus* section *Flavi* was 1.1 CFU g^{-1} of chili.

2.5. Evaluation of contamination of chilies post-harvest

Red chilies may go through several steps of post-harvest processing before reaching various markets. These include drying,