Fungal Infection and Aflatoxin Contamination of Market-Sold Groundnut Seeds in Ibadan and Iwo, South West Nigeria.

Sangoyomi, T. E.

Department of Environmental Management and Crop Production, Faculty of Agriculture, Bowen University, Iwo, Osun State Nigeria.

Author’s email address: tsangoyomi@yahoo.com Phone number: +234 803 4510 695

ABSTRACT

Groundnut is an important food crop worldwide and constitutes an inexpensive source of protein, fat, minerals and vitamins in diets. However, production of the crop is low in Nigeria due to erratic rainfall, poor soil fertility, poor agronomic practices, and most importantly pests and diseases. Studies were carried out to assess the quality of groundnuts seeds sold in local markets in relation to fungal infection and aflatoxin contamination using the thin layer chromatography technique. Groundnut samples were obtained from three major markets in Ibadan Oyo state (Bodija, Ojoo and Oje markets) and three major markets in Iwo, Osun State Nigeria (Odo-Ori, Oluwo and Oja-Ale markets). Results showed that four fungi (Aspergillus flavus, Aspergillus niger, Fusarium spp. and Rhizoctonia solani) were frequently isolated from groundnut samples obtained from all surveyed markets. The presence of these fungi and the detection of Aflatoxins B1 and B2 with concentration levels ranging from 5.69 and 230.20 ppb in groundnut samples from some of the markets is an indication of microbial and mycotoxin contamination of market-sold groundnut. Farmers, traders and consumers should carry out adequate management practices on produce to reduce or prevent if possible contamination of groundnut in the field and during storage.

Key words: Groundnut, fungi, aflatoxin, contamination, market.

INTRODUCTION

Groundnut (Arachis hypogaeae L.) constitutes a major food legume cultivated in the world. It is also the most valuable source of vegetable oil used in the food, pharmaceutical and cosmetic industries (Wright and Bell, 1992). Groundnut is frequently predisposed to invasion by fungi and subsequent contamination by mycotoxin producing fungi especially in areas with warm or hot and humid climate that favours fungal growth (Bhatnagar and Ehrlich, 2002; Bankole et al., 2004). Drought at the end of the growing season and infection by root knot nematodes (Melodogyne spp) can also facilitate aflatoxin contamination of groundnut (Wilson and Stansell, 1983; Timper and Wilson, 2007). Fungi may invade the seeds at the pre-harvest stage, during the post-harvest curing and drying and most importantly during storage (Gonzalez et al., 2008). Moulds are probably responsible for food contamination and spoilage than any other group of microorganisms. Foods contaminated by fungi develop unpleasant taste and becomes unsafe for consumption as some produce mycotoxins although, presence of fungi on produce does not infer mycotoxin contamination (Offiah and Adesiyun, 2007).
Fungal infections and aflatoxins contamination of market-sold groundnut

Mycotoxins are low-molecular-weight secondary metabolites produced by certain fungi prior to harvest and/or during storage (Bhatnagar and Ehrlich, 2002). Contamination of food commodities by mycotoxins is of high economic importance due to their effects on health of man and livestock as well as the market value of agricultural products (Bhatnagar and Ehrlich, 2002; Bankole et al., 2004) thereby making them unsafe. Aflatoxins constitute a major group of naturally occurring toxins produced by common fungi such as Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius (Kim et al., 2000; Alborzi et al., 2006; Gurbay et al., 2006) and are very toxic to man and animal due to their mutagenic, teratogenic and carcinogenic potentials (Ditcher and Ehrlich, 1984; Sassahara et al., 2005). Aflatoxins have also been implicated as causative agents and risk factor of many human diseases (Ditcher and Ehrlich, 1984; Dominguez-Malagon and Gaytan-Graham, 2001; Sassahara et al., 2005) and pose serious threat to human and livestock health. Even at low levels, aflatoxins contaminations are of major concern and many countries have developed extensive monitoring programmes, legislative restrictions of contaminated products and have established maximum tolerance levels for total aflatoxin. The United States of America Food and Drug Administration has set 20 ppb as an aflatoxin limit for foods and most feeds and feed ingredients while the European Union has set a more stringent tolerance threshold of 2µg/kg for aflatoxin B1 and 4µg/kg total aflatoxin for nuts and cereals meant for human consumption (Van Egmond, 1989, Bankole and Adebanjo, 2003). Risks posed by aflatoxin contamination depend on the level and type of aflatoxin in the diet, strain and nutritional status of the animal (William et al., 2004). Aflatoxins B1, B2, G1, G2, M1 and M2 have been identified as the most common types of aflatoxins and of these, aflatoxins B1 and G1 as the most frequently occurring with B1 as the most dreaded (Mishra and Das, 2003). Groundnut is consumed in high quantities in Nigeria and in most cases, consumers buy raw groundnut seeds from market, roast, boil or extract the oil for cooking. This study was therefore conducted to assess the quality of groundnut seeds sold in local markets in relation to fungal infection and aflatoxin contamination.

MATERIALS AND METHODS
Sample source and preparation
Groundnut samples were obtained from six markets, Bodija, Oje and Ojoo markets in Ibadan, Oyo state as well as Odo-Ori, Oluwo and Oja-Ale markets in Iwo, Osun State, Nigeria. Five sub samples were purchased from randomly selected produce retailers from each of the six markets and mixed thoroughly in sample bags to obtain composite samples. Sampling was done twice and spread over a period of 28 days to avoid testing seeds from the same batch. Bags containing the composite samples were labeled and kept in the refrigerator for processing.
Sterilization of materials
All glass wares were sterilized in a dry oven at 120 °C for one hour. Forceps were dipped in 70% ethanol and flamed on a burner.

Surface sterilization of market-sold groundnut samples
Groundnut samples were surface sterilized to remove contaminants by dipping completely in 5% commercial bleach solution (0.05% sodium hypochlorite) for two minutes and rinsing twice (one minute each wash) in sterile distilled water (SDW) (Ruark and Shew, 2010). The samples were then placed on layers of sterile paper towels in a fume hood to dry for ten minutes.

Isolation and identification of fungi from market-sold groundnut seeds.
Four hundred (400) groundnut seeds were randomly selected from each composite sample; surface sterilized and plated on to 9cm Petri dish (10 seeds per plate) containing two layers of sterile filter paper moistened with sterile distilled water to maintain high relative humidity. The plates were covered, incubated at 27oC and monitored for 2-5 days for fungal growth. Mycelial growth from the groundnut seeds were described and sub-cultured into freshly prepared potato dextrose agar (PDA) to obtain pure culture for identification purpose. Fungi were identified based on their morphology, mycelia structure, nature of spores and other associated structures using the keys of Samson et al., 1988 and Barnett and Hunter, 1992. Data obtained were analyzed using frequency counts, simple averages and SPSS 16. Means were separated using standard deviation (SD) and Duncan Multiple Range Test (DMRT).

Determination of aflatoxin contamination in market-sold groundnut seeds
Twenty (20) grams each of ground-nutsamples was blended in 100 ml of 80% methanol for 3 minutes using a laboratory blender (Waring). The mixture was decanted into a 250 ml conical flask and sealed with parafilm. This was put on an orbital shaker for 30 minutes at 4x100 rpm and was then filtered. The filtrate was poured into a separating flask containing 25 ml of dichloromethane, shook gently and allowed to stand for the mixture to separate into top and bottom phases. The bottom phase was drained through a bed of 5gm anhydrous sodium sulphate into a 150 ml white plastic beaker and allowed to dry overnight in a fume hood. After drying, the extract was re-dissolved in 1 ml dichloromethane and 4µlof test sample. Aflatoxin standards were spotted on the thin layer chromatography (TLC) plate and developed in the TLC tank and thereafter ran through the TLC machine (CAMAG) for aflatoxin quantification.
RESULTS
Incidence of fungi on groundnut seeds obtained from six markets in South Western Nigeria.
The following fungi isolates; Aspergillus flavus, Aspergillus niger, Fusarium spp. and Rhizoctonia solani were found to grow from groundnut samples obtained from the sampled markets (Bodija, Oje and Ojoo markets in Ibadan, Oyo State and Odo-Ori, Oluwo and Oja Ale markets in Iwo, Osun States). Other fungi also observed on samples were Macrophomina phaseolina and Rhizopus stolonifer (Table 1). Groundnut seed samples obtained from Oluwo market showed incidence of 28% and 27% for A. flavus and R. solani respectively while A. niger and Fusarium occurred at 6% and 4% (Table 1). Rhizoctonia solani had the highest percentage incidence in samples obtained from Odo Ori, Oja Ale and Ojoo markets. Percentage incidences of Fusarium spp were also high in samples obtained from Bodija and Ojoo markets. Aspergillus flavus was observed in all samples while other fungi (M. phaseolina and R. stolonifer) were not observed on samples obtained from Oluwo and Ojoo markets (Table 1).

Percentage incidence of fungi in groundnut seed samples across six markets in South Western Nigeria.
When percentage incidence of fungi on groundnut seeds were compared across all markets, results showed that incidence of R. solani (36%) was significantly higher than all other fungi while those of A. niger, A. flavus and Fusarium spp. between 20% and 25% but were not significantly different from each other (figure 1). Macrophomina phaseolina and R. stolonifer had the least percentage incidence across all markets (5%).

Table 1 Incidence of fungi in groundnut seed samples obtained from six markets in Ibadan and Iwo, South Western Nigeria

<table>
<thead>
<tr>
<th>Fungi</th>
<th>OLUWO Mean SD</th>
<th>ODO ORI Mean SD</th>
<th>OJA ALE Mean SD</th>
<th>OJE Mean SD</th>
<th>BODIJA Mean SD</th>
<th>OJOO Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. flavus</td>
<td>0.28 ± 0.254</td>
<td>0.27 ± 0.17</td>
<td>0.22 ± 0.12</td>
<td>0.34 ± 0.26</td>
<td>0.12 ± 0.86</td>
<td>0.22 ± 0.13</td>
</tr>
<tr>
<td>A. niger</td>
<td>0.06 ± 0.63</td>
<td>0.24 ± 0.20</td>
<td>0.31 ± 0.21</td>
<td>0.13 ± 0.26</td>
<td>0.28 ± 0.17</td>
<td>0.41 ± 0.20</td>
</tr>
<tr>
<td>Fusarium</td>
<td>0.04 ± 0.73</td>
<td>0.37 ± 0.23</td>
<td>0.30 ± 0.19</td>
<td>0.03 ± 0.16</td>
<td>0.48 ± 0.16</td>
<td>0.42 ± 0.19</td>
</tr>
<tr>
<td>R. solani</td>
<td>0.20 ± 0.27</td>
<td>0.65 ± 0.21</td>
<td>0.42 ± 0.25</td>
<td>0.05 ± 0.19</td>
<td>0.46 ± 0.13</td>
<td>0.56 ± 0.18</td>
</tr>
<tr>
<td>Others</td>
<td>0.00 ± 0.00</td>
<td>0.02 ± 0.04</td>
<td>0.03 ± 0.07</td>
<td>0.18 ± 0.56</td>
<td>0.01 ± 0.03</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
Determination of aflatoxin contamination in market-sold groundnut seeds
During the first sampling period, Aflatoxins $B_1$ and $B_2$ were detected in groundnut samples obtained from Bodija market at 230.20 ppb and 44.52 ppb respectively while only aflatoxin $B_1$ was detected in groundnut sample obtained from Odo-Ori market (5.69 ppb). Quantities of aflatoxins $B_1$ and $B_2$ were below detection limit in samples from Oje, Ojo, Oluwo and Oja axe markets during the sampling period (Table 2a). Only the groundnut sample obtained from Ojo market had aflatoxins $B_1$ and $B_2$ contaminations of 74.13 ppb and 10.04 ppb respectively during the second sampling period, all others were below detection levels (Table 2b). Aflatoxins $G_1$ and $G_2$ contaminations were below detection limits in samples obtained from all the markets at the time of this study (Tables 2a and 2b).

DISCUSSION
Groundnut is an important food component in Nigeria. The dried roasted groundnut can be consumed alone or combined with popcorn, gari, coconut, bread, banana or roasted plantain. Groundnut paste is also a cherished accompaniment with garden egg fruit. The seeds are found abundantly in local markets across Nigeria all through the year. Farmers and marketers preserve the seeds to ensure availability hence may be predisposed to fungal contamination.
Fungal infections and aflatoxins contamination of market-sold groundnut

Table 2a: Aflatoxin quantification from market-sold groundnut samples (First Sampling Period)

<table>
<thead>
<tr>
<th>Market</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodija</td>
<td>230.20</td>
<td>44.52</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oje</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ojoo</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Odoori</td>
<td>5.69</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oja Oba</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oja Ale</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note:
   i. Recovery of toxin = >85%
   ii. 0.00 means the aflatoxin level is below detection limit of the analytical method (1ppb)

Table 2b: Aflatoxin quantification from market-sold groundnut samples (Second Sampling Period)

<table>
<thead>
<tr>
<th>Market</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodija</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oje</td>
<td>74.13</td>
<td>10.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ojoo</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Odoori</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oja Oba</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Oja Ale</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note:
   i. Recovery of toxin = >85%
   ii. 0.00 means the aflatoxin level is below detection limit of the analytical method (1ppb)

The isolation of different fungi species in high incidences from groundnut samples obtained from markets in Ibadan and Iwo is an indication of fungal contamination and it is consistent with previous reports of McDonald, (1970); Samson et al., 1988 and Fingani et al. (2004). The pathogens observed on seeds could be seed borne or soil borne (Ruark and Shew, 2010) and fungi could also invaded groundnut during post-harvest curing, drying and in storage (Goncalez et al., 2008). Groundnut seeds are usually sold in their fresh form but greater quantities are dried and stored for some time before sales and/ or consumption. Stored commodities can get contaminated.
with fungal spores due to the abundance of spores in the environment. Previous report also indicated that groundnut is susceptible to a variety of fungi such as A. niger, Fusarium spp., Pythium spp., and R. solani causing pre and post emergence damping off (Kokalis-Burelle et al., 1997). High incidence of R. solani in groundnut seeds shows the need for adequate fungicide treatment to prevent seedling disease and crop failure. Losses of up to 50% have been reported when untreated seeds were planted (Shokes 1995; Spear et al., 2002; Melouk, and Shokes, 1995). The isolation of A. niger, A. flavus and Fusarium should be of concern to consumers because of their ability to produce mycotoxins (Fingani et al., 2004). Rhizopus stolonifer was observed and classified among other fungi encountered in the study. Although Rhizopus spp. do not produce mycotoxins, R. stolonifer is one of the most common Zygomycetes isolated from dried stored products, including peanuts (Pitt and Hocking, 1997; Fingani et al., 2004). Aflatoxins are toxic secondary metabolites produced by some strains of Aspergillus flavus Link ex. Fries and A. parasiticus Speare (Woloshuk and Prieto, 1998) and their contamination in staple foods compromises food safety (Williams et al., 2004). The detection of aflatoxins B1 and B2 in quantities above the limits of 20 ppb set by the US Food and Drug Administration for foods, feeds and feed ingredients in groundnut samples obtained from some of the markets is of great concern. Thus serious attention from stakeholders (government and non-governmental organizations, researchers, extension agents and produce merchants) to expand on awareness of the potential dangers of aflatoxin contamination, is a welcome idea. Therefore, enhancement of produce monitoring of food and feed ingredients that are sold in markets and pre and post-harvest practices that will reduce mycofloral infection and subsequently mycotoxin contamination should be given high consideration.

CONCLUSION
Fungi growth observed on groundnut seed samples is an indication that seeds were contaminated and the isolation of Aspergillus and Fusarium species necessitates screening for presence or absence of aflatoxin although, presence of fungi on produce does not infer aflatoxin contamination. Farmers are also discouraged from planting untreated seeds as it may enhance field to storage inoculum build up and crop failure. Consumption of contaminated groundnut should be avoided because of health risks of mycotoxins. More effort should be made in carrying out efficient awareness campaigns to enlighten producers as well as consumers on the dangers of fungal and mycotoxin contaminations.

REFERENCES
Fungal infections and aflatoxins contamination of market-sold groundnut


