Study on *Aspergillus* Species and Aflatoxin Levels in Sorghum (sorghum bicolor L.) Stored For Different Period and Storage System in Kewet Districts, Northern Shewa, Ethiopia

Conference place Dessalgne Hotel
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Introduction

- Sorghum is grown for grain and fodder in the semi-arid tropics, mainly in Asian and African countries (Tekle and Zemach, 2014).

- It is a major food and nutritional security crop to more than 100 million people in the Horn of Africa (Katile et al., 2010).

- Ranks third among major cereal crops in terms of area and production next to teff and maize (Tigabu et al., 2012).
Lives of millions of Ethiopian depend on sorghum as a staple food crop (Tekle and Zemach, 2014).

Estimated to more than 1.6 million hectare of the land covered with sorghum production (CSA, 2010).

Subjected to a broad range of mould contamination

produce mycotoxin (Aspergillus flavus and Aspergillus parasiticus species) (Katire et al., 2010)
Objectives

General Objective

To evaluate the occurrence of Aspergillus species and aflatoxin levels in sorghum (sorghum bicolor L.) stored at different period and storage system in Kewet Woreda, Northern Showa, Ethiopia

Specific Objectives

To assess the occurrence and distribution of Aspergillus species in sorghum stored at different period and storage system.

To analyze the aflatoxins (B1, B2, G1, and G2) content of sorghum stored at different period and storage system.

To evaluate the association between aflatoxin levels with storage periods and storage system.
Methodology

Location of the study

- *Kewet* Woreda, North Showa Zone of Amhara Region
- 225 km to the north of Addis Ababa along the main road to Dessie
- Lowland and semi-arid area, Altitude range (1280 and 2700m)
- Lon. (10°00’N 39°54’E) and LAT. (10.000°N 39.900°E)
- Annual rainfall (600-700mm), Temperature of
- Total population 100,760 (CSA, 2007)
Apparatus and reagents

Apparatus

- SHIMADZU-HPLC-FID, 0.45µm filter paper…

Chemicals

- HPLC grade ACN and Methanol, n-hexane, Deionized water; MgSO4 anhydrous salt, NaCl, AFs(G1,G2,B1,B2) standard - sigma-Aldrich, Potato Dextrose Agar (PDA)
- 10% sodium hypochlorite solution, Ethanol absolute (99.7%)
Study Design

- Cross sectional study design was followed to assess the occurrence of *Aspergillus species* and Aflatoxin in Sorghum (*sorghum bicolor* L.) stored for different storage system and periods.
Nesting sampling method

North showa woredas (26)

Sorghum producing woredas

Ensaro
Ataye & Anstokiya
Kewet
Meda Oromo
Merahbete

Sorghum producing Kebles

Ashgne ena yegeda
Teri
Rassa
Yelen
Gerne fara
Sampling and sample preparation

- 30 samples of sorghum (15 samples from each storage type or 10 samples per each storage period) were collected purposively.

- storage period (<12 month, 1-2 year and ≥2 year)

- storage system (above ground and underground pit storage)

- questioners
samples were collected in plastic bag and were transported to the laboratory

Above ground storage  Underground pit storage

Figure 6. Diagram of sample collation from Kewet woreda
Method Validation

Selection of HPLC

- HPLC-FLD without post-column derivitization
- The optimized analytical conditions were evaluated in terms of :

\[
\% \text{ Recoveries} = \frac{\text{Total Analyte} - \text{Analyte Original Present}}{\text{Analyte Added}} \times 100
\]

LOD (signal / noise ratio = 3)

LOQ (signal / noise ratio = 10)
Preparation of Standard solution

- **Standard reagents** (AFG2, AFG1, AFB2 and AFB1), was dissolved in 
  \( ACN \) and \( MeOH \) (50:50) at 1mg/mL and stored at 4°C in the dark before use.

Chromatographic Condition

- **Mobile phase**: Milli Q water (100%) and acetonitrile (CAN) / methanol (MeOH) in the ratio of 71.5/28.5, v/v
- Flow rate of 1.0 ml min\(^{-1}\) and Injection volume (20μl)
- C-18 column (4.6 x250mm, 5μm particle size)
Determination of Moisture Content

Moisture content was determined according to AOAC (2000).

Determination of Aflatoxin in Sorghum sample

Method that was validated by GENT University Faculty of Pharmaceutical science Department (2014).

Isolation and Identification of Fungi

Isolates were identified to a species level based on morphological (phenotypic) features as described by Cotty (1994), Egel et al. (1994), Kurtzman et al. (1997), and Okuda et al. (2000).

For this purpose: Isolates representing each pure culture were grown on PDA agar at 25°C for 5-7 days.
**Statistical Analysis**

- Duplicate samples were analyzed by: "SPSS" 20.0 package
- One way-ANOVA for storage period
- Pared comparison for storage system
### Result and Discussion

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Frequency</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>mould contamination</td>
<td>14 16</td>
<td>46.3%</td>
<td>53.33%</td>
</tr>
<tr>
<td>color of mould</td>
<td>8 (Black) 6 (white)</td>
<td>26.6%</td>
<td>---</td>
</tr>
<tr>
<td>major critical problems</td>
<td>15 (Insects) 10 (Rodent) 5 (mould)</td>
<td>50% 33.33% 16.7%</td>
<td>----- ---- ----</td>
</tr>
</tbody>
</table>

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25/06/2015
Geremew Tassew                                                        Msc defense
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Frequency</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure taken to control</td>
<td>25 (Insect sides)</td>
<td>75%</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>5 (Others)</td>
<td>25%</td>
<td>----</td>
</tr>
<tr>
<td>Location</td>
<td>15 (Field)</td>
<td>50%</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>5 (inside house)</td>
<td>16.7%</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>10 (Courtyard)</td>
<td>33.3%</td>
<td>----</td>
</tr>
<tr>
<td>Storage system</td>
<td>9 (above ground)</td>
<td>30%</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>10 (Pit storage)</td>
<td>33.3%</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>11 (both system)</td>
<td>36.7%</td>
<td>-----</td>
</tr>
</tbody>
</table>
2. Average total aflatoxin concentration sorghum

Concentration of total aflatoxins by storage period

- **<12 month**: 102.23 µg/kg
- **1-2 year**: 139.52 µg/kg
- **≥ 2 year**: 123.29 µg/kg
Cont’d

2.1. Average level and % of aflatoxins contamination in sorghum sample

<table>
<thead>
<tr>
<th>AFs</th>
<th>Average level of AFs (µg/kg)</th>
<th>% of contamination level</th>
<th>Average M.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFG2</td>
<td>3.22-52.84</td>
<td>90</td>
<td>8.8-12.86 %</td>
</tr>
<tr>
<td>AFG1</td>
<td>9.87-139.64</td>
<td>96.7</td>
<td></td>
</tr>
<tr>
<td>AFB2</td>
<td>1.17-91.82</td>
<td>93.33</td>
<td></td>
</tr>
<tr>
<td>AFB1</td>
<td>3.95-153.72</td>
<td>96.6</td>
<td></td>
</tr>
</tbody>
</table>
Cont’d

- The incidence of aflatoxins contamination reported for sorghum and maize (Ayalew et al., 2006) and Quitet et al. (1993) in which the aflatoxin contamination reached up to 1000 µg/kg for sorghum and 1388 µg/kg for wheat, respectively.

- The maximum aflatoxin B1 content found in this research (153.72 µg/kg) was below the highest amount reported by Habtam and Kelbesa (692 µg/kg) (Habtamu & Kelbesa, 2001).

- However, the amount of aflatoxin B1 (153.72 µg/kg) in the sorghum samples in this research was much relatively lower than the finding reported by Alpert et al., (1971) which was as high as 1000µg/kg.
2.2. Effects of Storage Periods on the Level of Aflatoxin Contamination in Sorghum

<table>
<thead>
<tr>
<th>Duration of Storage period</th>
<th>Aflatoxin content (µg/kg)</th>
<th></th>
<th></th>
<th></th>
<th>Average total aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFG2</td>
<td>AFG1</td>
<td>AFB2</td>
<td>AFB1</td>
<td></td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>20.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>64.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-2 year</td>
<td>24.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥ 2 year</td>
<td>22.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

➢ Means in the same column with different letter superscripts indicate significant difference (P<0.05)
2.4. Effects of Storage System on the Level of Aflatoxin Contamination in Sorghum

<table>
<thead>
<tr>
<th>Storage type</th>
<th>Average aflatoxin content(µg/kg)</th>
<th></th>
<th></th>
<th></th>
<th>Total aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFG2</td>
<td>AFG1</td>
<td>AFB2</td>
<td>AFB1</td>
<td></td>
</tr>
<tr>
<td>Above ground</td>
<td>49.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pit storage</td>
<td>30.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Means in the same column with different letter superscripts indicate significant difference (P<0.05)
**Storage type** also did not significantly affect the level total aflatoxin \((p>0.05)\)

However, in this study, storage system was not resulted in significant variation between the mean aflatoxin contents of stored sorghum grains at different storage system and periods.
3. Isolation and Identification of *Aspergillus Species*

*Aspergillus spp.* isolation representing each pure culture were grown on PDA Agar at 25°C for 5-7 days.

- **A. niger**
- **A. flavus**
- **A. parasiticus**
3.1. The occurrence of *Aspergillus spp.* in different storage periods and storage system

<table>
<thead>
<tr>
<th><em>Aspergillus spp.</em></th>
<th>Number of sample</th>
<th>Total % of <em>Aspergillus spp.</em> found</th>
<th>Duration of storage period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Aspergillus species (%)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 12 months</td>
</tr>
<tr>
<td><strong>A. flavus</strong></td>
<td>17</td>
<td>56.66</td>
<td>13.33</td>
</tr>
<tr>
<td><strong>A. parasiticus</strong></td>
<td>5</td>
<td>16.66</td>
<td>6.67</td>
</tr>
<tr>
<td><strong>A. niger</strong></td>
<td>7</td>
<td>23.33</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>29</td>
<td>96.65</td>
<td>30.0</td>
</tr>
</tbody>
</table>
4. Evaluation of Aflatoxins Results against Different International Standards

- **E.U** legislation are specified for aflatoxin B1 (5µg/kg) and maximum total aflatoxin (10µg/kg) (EFSA, 2013; Herzallah, 2009).
- **FDA** have established maximum acceptable level of 20 µg/kg for aflatoxin in maize, sorghum and other cereals for **human consumption** (Grybauskas et al., 2000).
- East African standard specification (CD-ARS 462:2012(E) for sorghum….total aflatoxin (10µg/kg) and with AFB1 not exceeding 5µg/kg
- **Codex Alimentarius Committee** … total aflatoxin in food maximum limit (20µg/kg)
Conclusion & Recommendation

- The level of total Aflatoxins in sorghum samples was above tolerable limits set by different organizations.

- This can be more hazardous to individuals who are more sensitive and prone to toxic effects of such highly carcinogenic food contaminants.
Therefore, this situation clearly demands wider national or international programs for the control of Aflatoxin contamination in sorghum.

In conclusion, Aflatoxin control programs should focus on addressing all the factors that contribute to fungal growth across the value chain (i.e. pre-harvest to household practices).
Acknowledgement

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Thank you