Prevalence of Aflatoxin Levels along the Groundnut Value Chain Actors in Different Agro-Ecology and Evaluation of Groundnut Varieties for Reaction to *Aspergillus flavus* and Aflatoxin Contamination in Eastern Ethiopia

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Contents of Presentation

1. Introduction
2. Justification
3. Research Questions
4. Objectives
5. Materials and Methods
6. Results and Discussion
7. Conclusion
8. Recommendations, and
9. Future Research Directions

1. Introduction

- **Groundnut** (*Arachis hypogaea* L.) is an **annual legume**.

Importance (USDA, 2010)

↓

Production (USDA-FAS, 2010)

↓

Worldwide (25.2 m ha & 35.9 m m tons)

↓

Asia (66%)

↓

Africa (25%)

↓

Ethiopia (67,062.40 ha & 103,939.53 tons, Avg. 1.550 tons/ha) (CSA, 2015).

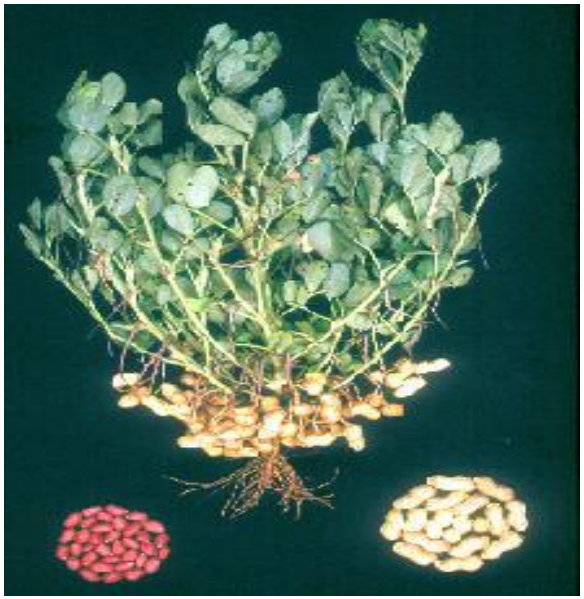

Figure 1. Groundnut plant, pods and seeds
(Source:http://agriorissa.blogspot.no/2011/09/groundnut-cultivation.html**)**

Cont'd…

❑ Constraints of Groundnut Production

➡ Biotic and Abiotic Factors

- Infection of groundnut seed by molds, *A. flavus* and *A. parasiticus*, results in Aflatoxin-contamination of seeds

- What is Aflatoxin? Afla-toxin ⟶ *A. flavus*-toxin

Importance of Aflatoxin

1. Health Implications Worldwide (Richard *et al*., 2003)

Humans and Animals

2. Economic Implications Worldwide (Coulibaly *et al*., 2008)

Affect Agricultural Industry Worldwide

In USA, income loss >US$100 million/year, where as in Africa >US$750 million/year (Cardwell *et al*., 2004; Coulibaly *et al*., 2008).

Cont'd…

- In **Ethiopia, a food processing company imported groundnuts** from **India** while **groundnut producers** in **Gursum, Fedis** and **Bablie** could **not find market to sell their crop** (Amare Ayalew, personal communication).

- **In Ethiopia**, an earlier report showed **mean levels of aflatoxin B1** of **34.7** and **105 µg/kg** in samples of **groundnut** and **peanut butter**, respectively (Bisrat and Gebre, 1981).

- Amare *et al.* (1995) reported **aflatoxin levels** of **5-250 µg/kg** in **groundnut seed** from **eastern Ethiopia.**

- Recently, Alemayehu *et al.* (2012) reported that **total aflatoxin levels** in ***Aspergillus flavus* positive** samples of **groundnut seed** varied between **15** and **11865 µg/kg**.

- These results indicated **heavy aflatoxin contamination of groundnut samples from Ethiopia**, at levels **much higher** than **any international acceptable standards, e.g. FAO** and **WHO acceptable limit being 15 µg/kg**.

Justification

- **Although groundnut** has **a huge potential**, its **market** is **declining** and **export** of the crop has come to **a standstill**.

- This is due to **aflatoxin** contamination of the crop and the **difficulty** of **meeting tolerance limits** by **importers** and **food processors,** leading to **rejection** of the crop and **reduction in market demand.**

- **Earlier reports from Ethiopia** have been based on **limited single-season survey of market samples** and there is **a need to extend the data base** both **spatially** and **temporarily to get conclusive evidence on the level of contamination.**

- Such studies would be **more meaningful** if they address the **entire groundnut value chain** covering major nodes **from production** through **storage** to **consumption (marketing)**, since they could **support decisions** on **targeting major points of aflatoxin contamination**.

Justification Cont'd...

- **Use of varities resistant to *Aspergillus flavus* and with diminished accumulation of aflatoxin** is the best option for the **resource constrained farmers** to **manage** the problems of aflatoxin contamination (Kwemoi, 2011).

- **Planting varieties** that are resistant to fungal infection or that does not support high aflatoxin synthesis offers **a sustainable, low cost approach for aflatoxin management that is suited for adoption by small scale groundnut producers.**

- **Amare *et al.* (1995)** reported **variations in terms of reaction to *A. flavus* infection among groundnut varieties widely cultivated in Ethiopia.**

- **But a number of groundnut varieties** have been **released since then** and **evaluation of their reaction to *A. flavus* infection and aflatoxin contamination could identify varieties that could be promoted for widespread use by groundnut producers.**

- Despite the **importance** of the **problem,** there are **no recommended research results** for **aflatoxigenic fungi** and **aflatoxin management** in **Ethiopia**.

- **Therefore, elucidating** the **reaction** of **existing groundnut varieties** to **infection** by **aflatoxigenic fungi** and to **aflatoxin accumulation** is **prudent** in order to **generate methods** of **aflatoxin management** suited for **adoption** by **groundnut growers** in **Ethiopia**.

Research Questions

- **What is the magnitude of aflatoxin levels along the groundnut value chain in different agro-ecological zones of Eastern Ethiopia?**

- **Is there variation among groundnut varieties in the level of resistance to *A. falvus* and aflatoxin contamination in Eastern Ethiopia?**

Objectives

- **To determine prevalence of aflatoxin levels in groundnut along the value chain actors in different agro-ecological zones of Eastern Ethiopia**
- **To evaluate groundnut varieties for reaction to field seed colonization by *Aspergillus flavus* and pre-harvest aflatoxin contamination in Eastern Ethiopia.**

2. Materials and Methods

2.1. Description of the Study Areas

- The study was involved **field work (Field survey, Planting & Groundnut sampling)** and **laboratory characterization**

- The **field work** were conducted in **Babile, Fedis and Gursum Districts**, **East Hararghe Zone, Oromia Regional State, Eastern Ethiopia (Figure 1).**

- **The areas** are **selected purposively** as they **represent the bulk** of **groundnut production** in **Ethiopia** (Alemaw and Alemayehu, 1991).

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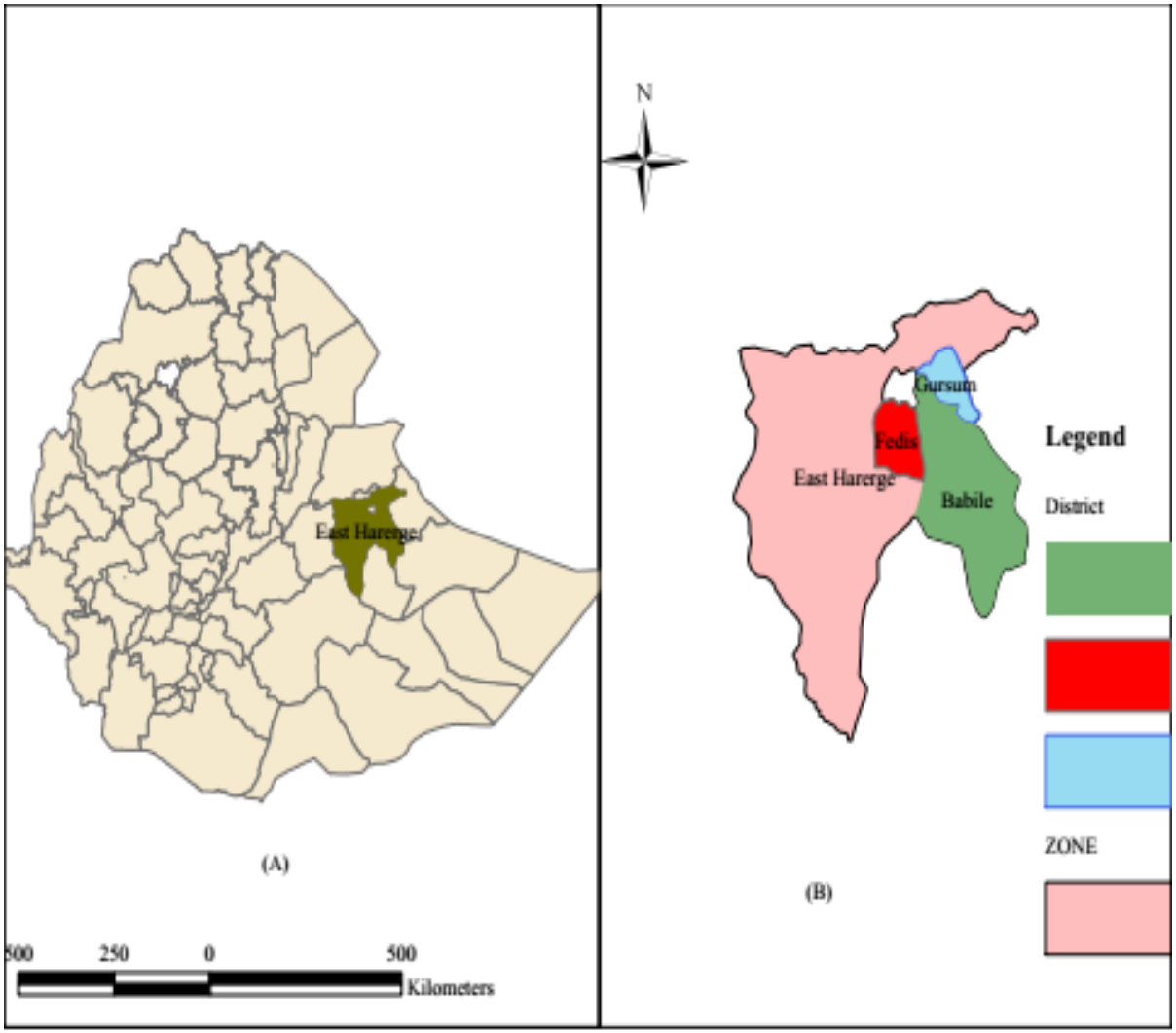

The Agro-ecological Zone:

- **lowland** (*kolla*, 30-40%)
- **midland** (*weyna dega*, 35-45%) and
- **highland areas** (*dega*, 15-20%).

Geographical Position:

- 09°02'52" N and 09°19' 11" N latitude and 42°06'03" E and 42°27'02" E longitude

Babile:

Altitude: 1401 to 1483 m.a.s.l
RF: 500-975 mm per year
T°: 28.27 and 14.18 °C

Fedis:

Altitude: 1501 to 1899 m.a.s.l
RF: 650-1000 mm per year
T°: 26 and 14 °C

Gursum:

Altitude: 1200 to 2950 m.a.s.l.
RF: 650-1050 mm per year
T°: 24 and 13 °C

Figure 1. Map of (A) Ethiopia with study zone and (B) location map of study zone and districts

2.2. Description of Groundnut Value Chain in Eastern Ethiopia

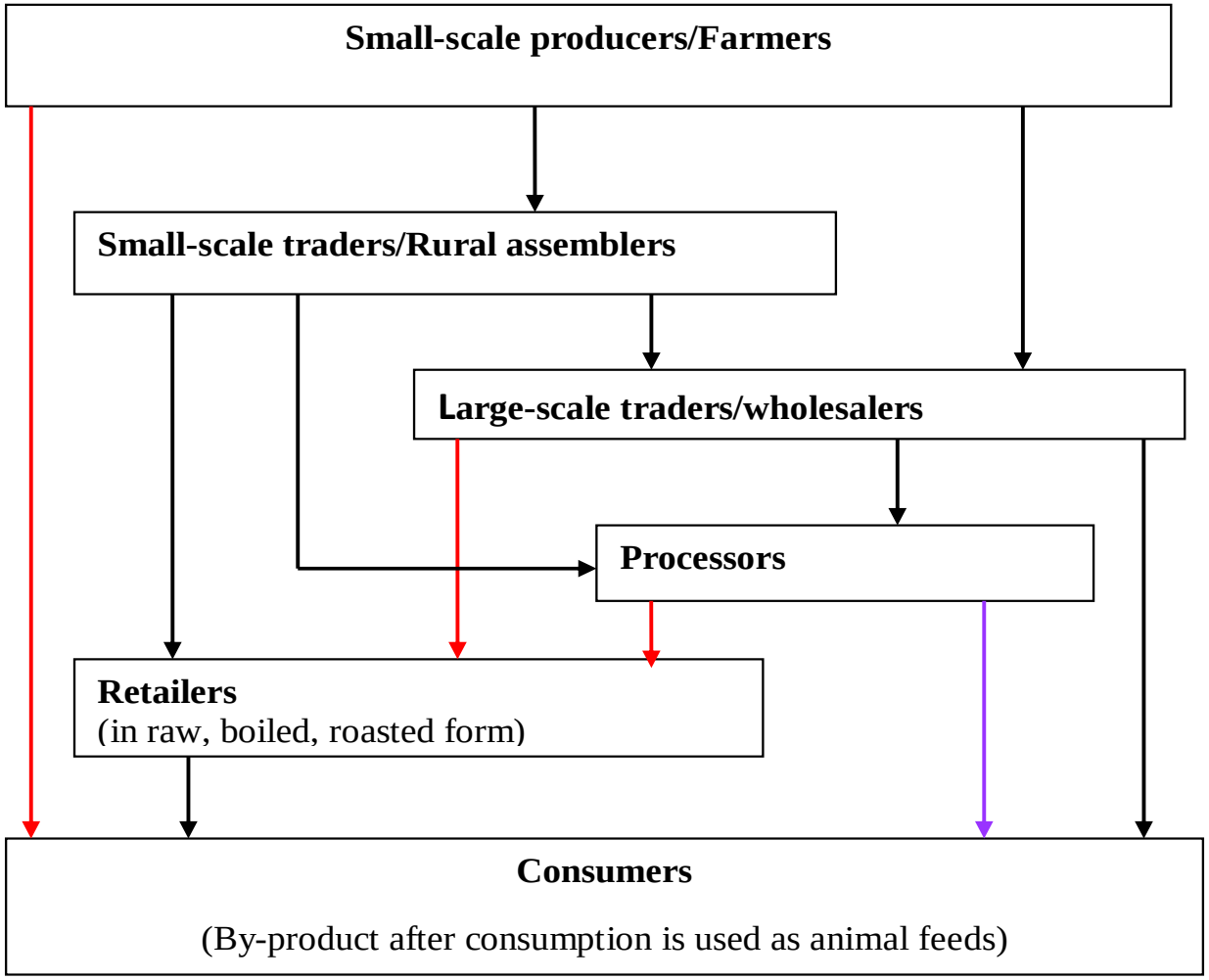

Figure 2. Flowchart of groundnut value chain and marketing channels process

2.3. Sampling

- **Samples** were collected **along the groundnut value chain in the three districts.**

- Accordingly samples were drawn from **farmers' fields at harvest,** from **farmers' storage,** from **traders (both wholesalers** and **retailers)** as well as from **rural** and **urban markets.**

- Samples were **1 kg each** comprising **five sub-samples** drawn from **different parts** of the groundnut lot **(composite samples).**

- The samples were placed in **cloth bags** to allow **air circulation** that **reduces condensation** and **limit fungal growth** after sampling.

- Samples were **properly labeled** and **relevant information on the locality, GPS coordinates, altitude, cultivar, date of sampling, date of harvest, type** and **duration of storage**, etc. were **recorded.**

❑Sampling from Farmers' fields and storage

- **Farmers'** groundnut samples were collected from **three sites** representative of **three agro-ecological zones (AEZs)** that have been selected from the **three districts (Table 1).**

- **A total of 150** groundnut seed samples were collected along the VC actors.

- **90 farmers'** groundnut samples were collected, i.e., **45** groundnut samples **(3 AEZs x 3 sites x 5 samples)** were collected from **farmers' fields at harvest** and **45 samples** were collected from **farmers' storage facilities.**

❑ Sampling from Traders

- **A total of 30 samples** were collected from **wholesalers** and **retailers** of each districts.

❑ Sampling from Venders

- **A total of 30 samples** were collected from **rural,** and **urban** and **semi-urban market places**.

- The samples were consisted of **five raw** and **five roasted seed** samples from markets in each of the three districts.

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Table 1. Description of Groundnut Seed Sampling in three Agro-ecologies of Eastern Ethiopia

District	Name of Site	Altitude (m)	Temperature(^{O}C)	Rainfall(mm)	Cropping sys	Agro-ecology
Babile	Shek Hussien	1401	20-28	<650	LCR	LLD
Babile	ShekAbdi	1419	20-28	<650	LLR	LLD
Babile	Kito	1420	20-28	<650	LLR	LLD
Fedis	Tuka Kanesa	1710	15-22	650-1000	IC	MLM
Fedis	Ido Basso-1	1841	15-22	650-1000	IC	MLM
Fedis	Ido Basso-2	1899	15-22	650-1000	IC	MLM
Gursum	Audal	2201	13-18	>1000	LLR	HLH
Gursum	Oda Oromia	2509	13-18	>1000	LLR	HLH
Gursum	Kassa Oromia	2525	13-18	>1000	LLR	HLH

*LCR=Legume-cereal rotation, LLR=Legume-Legume rotation, IC=Intercropping with cereals
*LLD=Lowland dry moist, MLM=Midland moist, HLH=Highland humid
(Source: Ngugi *et al*., 2002; Alemayehu and Reynold, 2006; Ayele, 2010)

2.4. Description of Field Experimental Procedures

- **A total** of **16 groundnut varieties** were grown in **a sick plot** field trial at **Babile experimental field of HU in 2015.**

- **The experiment** was laid out in **a RCBD with three replications.**

- **Each varieties** was grown in **four ridges** or **rows i.e.** plots were 3 m long by 2.4 m wide with **16 cm spacing between ridges or rows** and **two seeds** were sown at **10 cm spacing** along the ridges or rows.

- **Recommended agronomic practices (**seed bed preparation, planting, weeding, digging and inverting, threshing, pre-cleaning and drying or curing**) for groundnut production were applied**.

2.5. Inoculation of Groundnut Plots

- **Toxigenic *A. flavus* isolated from groundnut seed** was used as for **artificial inoculation of experimental plots.**

- **Inoculum** was prepared by the **organic-matrix (cracked corn) method** employed by (Will *et al*., 1994).

- **The inoculum** was **introduced into test plots** to **ensure the presence** of **sufficient aflatoxin-producing fungi** in the **groundnut pod zone.**

- **Artificial inoculation helps to ensure uniform testing conditions**, which **reduces the number of escapes** and **reduces variation in the data that could mask genetic differences.**

- **Each ridge/row within a plot at Babile** were treated with **200 g corn infested** with ***A. flavus*** to **soil around developing pods** at **mid-blooming** and about **4 weeks (20 to 30 days)** before **harvest.**

2.6. Testing for Field Seed Colonization by *A. flavus*

- **Levels of infection of groundnut seed by *A. flavus*** were determined for each varieties using plate count methods and **the percent infection of seed** was determined according to **Mehan and McDonald (1983).**

$$\text{PSI } (\%) = \frac{B}{A} \times 100$$

Where, A = total number of seeds and B = Number of seeds with sporulating growth of *A. flavus* on their surfaces.

- **Level of resistance** was tested according to **Mehan and McDonald (1983)**

2.7. Determination of Moisture Content

- **Moisture Content** of groundnut seeds was determined using **the oven drying method.**
- **The seeds were ground in a kitchen coffee grinder** (Coffee Grinding Mill, Armco USA Ltd, USA).
- **Two grams of the ground sample were placed on an aluminium dish**, which was placed in **a dry air oven** (Memmert ULM 500, Büchenbach, Germany).
- The samples were **dried at 105°C for 3 hours** and the **net weight of the dried sample** determined.
- **Each sample was replicated twice** and **the M.C. calculated** as follows:

$$\textbf{Moisture Content (\%)} = \frac{M0 - M1}{M0} \times 100$$

Where: M0 - initial weight; M1 - final weight, in grams of dried test portion

2.8. Aflatoxin (AFT) Analysis

- **Aflatoxin** concentrations in **groundnut seed samples** were determined using **ELISA kits**.

- The **ELISA tests** were done following **the procedure** described by **Waliyar *et al.* (2010)** and **Monyo *et al*. (2012),** at **ICRISAT laboratory** in **Malawi** **(Procedures: AFT Analysis using ELISA Test).**

2.9. Data Analysis

- **Data on total aflatoxin levels** in groundnut seeds were subjected to analysis by the **general linear model procedure** of **SAS Version 9.1 for Windows** and **Analyses of Variance (ANOVA)** using **Minitab version 17 for windows; means** were compared by **Fisher's protected least significant difference (LSD).**

- **Regression** and **correlation analysis** of **moisture contents and *A. flavus* infection with total aflatoxin levels** were done using **Minttab version 17 for windows.**

- **Percentage of seed infection by *A. flavus*** was determined using **Microsoft Excel.**

- **The results of AFT analysis were compared against with CODEX Alimentarious Commission Standard Limits (CODEX, 2004).**

3. Results and Discussion

3.1. Moisture Content of Samples

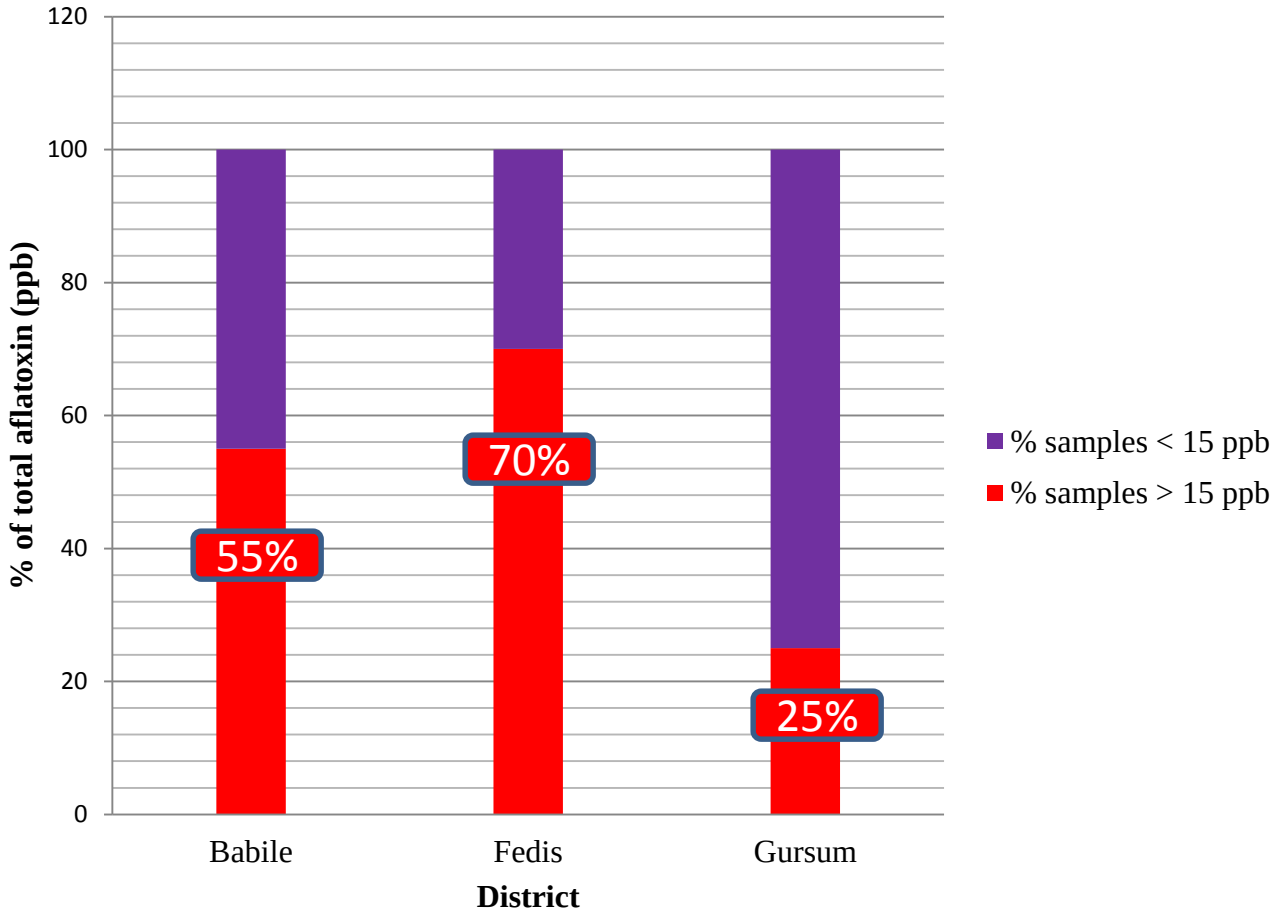
- **The seed moisture results showed that the moisture contents of seed samples ranged between 3 and 15% (Appendix _A).**

- **The highest was obtained from groundnut seed samples collected from farmers' fields at harvest in Fedis district & the lowest was from vendors in the three districts.**

- **All samples from vendors** were **below the maximum allowable Codex standard of moisture content (10%).**

- **This is because** samples from **vendors were roasted groundnut seeds and were used directly for consumption** & **during storage**, groundnut seeds **continued to dry as long as they were stored in well-aerated and protected structures.**

3.2.Prevalence of total aflatoxin at farmers' fields



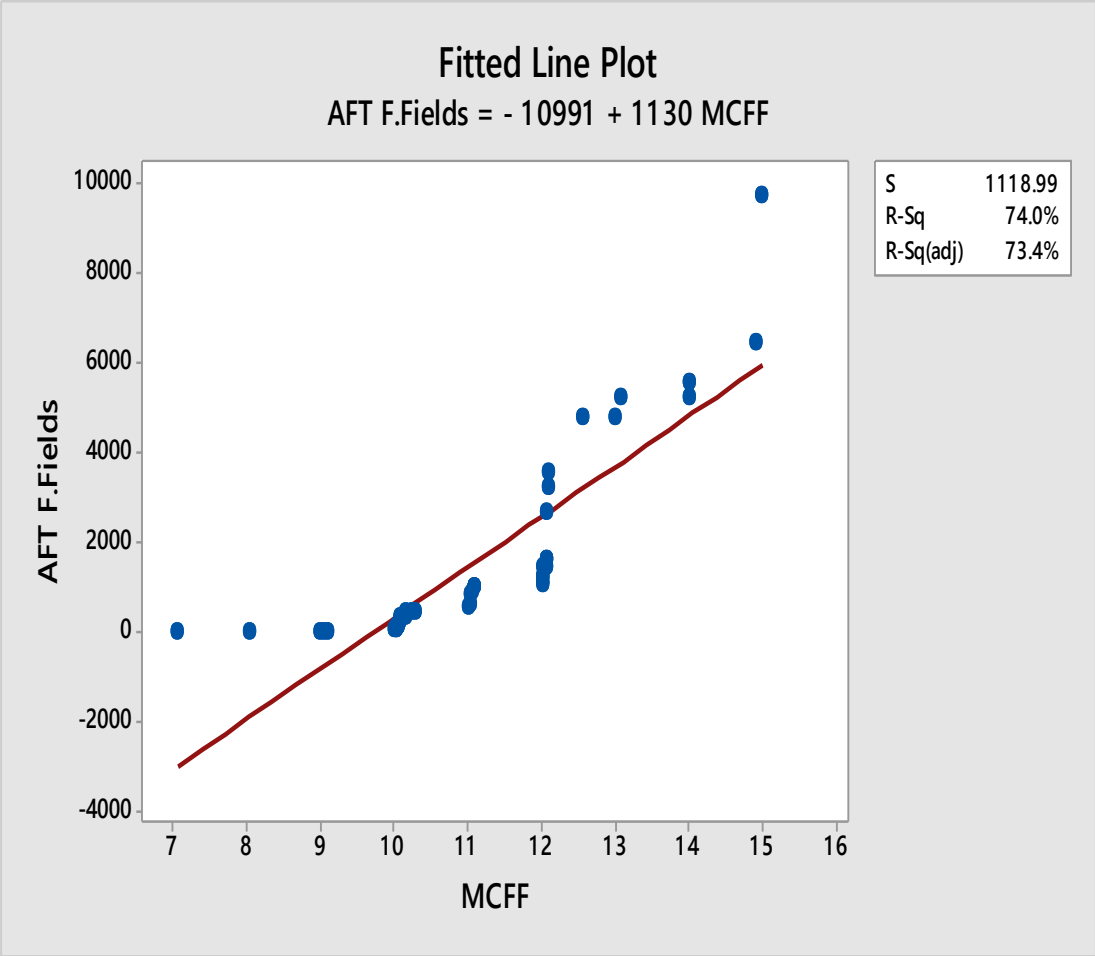
CODEX Limits:

AFT>15 ppb= Unfit for human consumption

AFT<15 ppb= Safe for human consumption

Figure 3. Percent of groundnut seed samples from farmers' fields with total aflatoxin levels above and below 15 ppb in the three districts

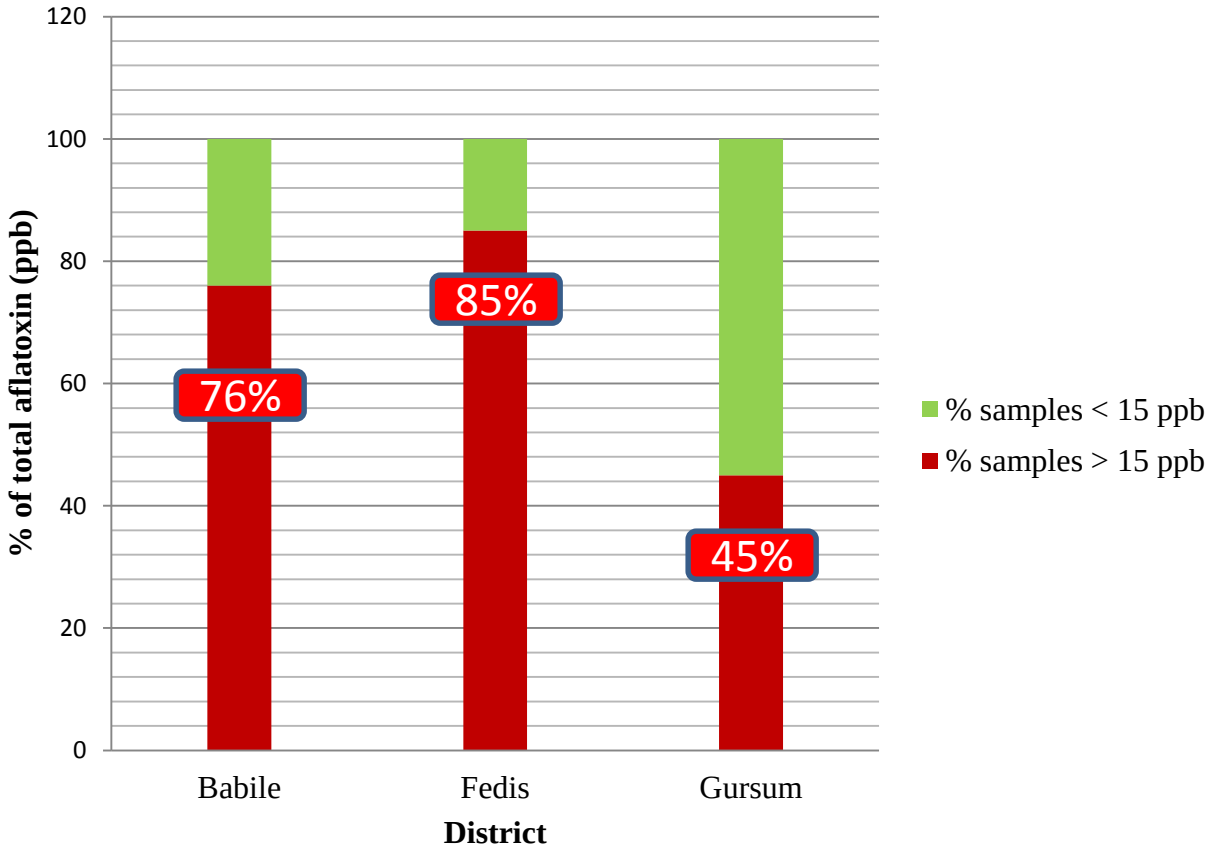
Regression analysis of MC with AFT at farmers' fields



MC with AFT levels were +vly correlated (r = 0.860) and significant ($p \leq 0.05$)

Figure 6. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at farmers' fields

3.3. Prevalence of total aflatoxin at farmers' stores



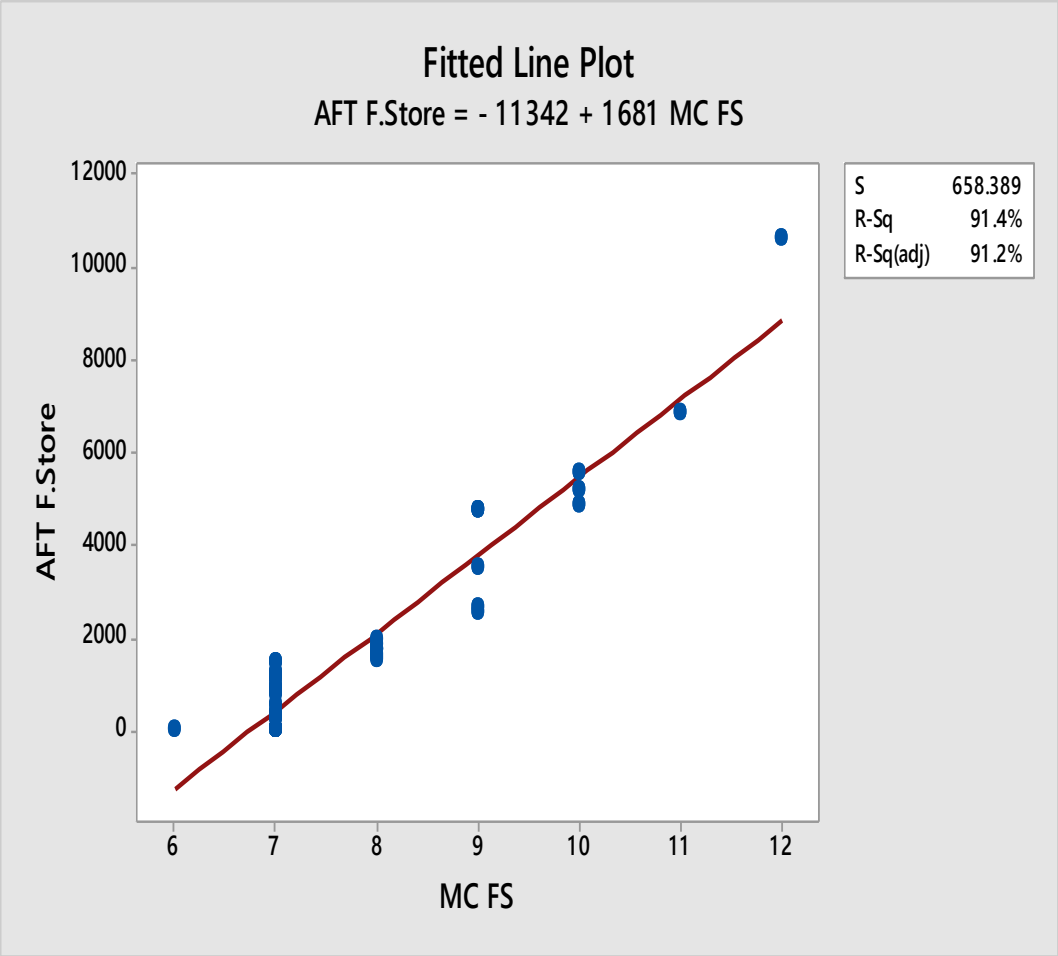
CODEX Limits:

AFT>15 ppb= Unfit for human consumption

AFT<15 ppb= Safe for human consumption

Figure 5. Percent of groundnut seed samples from farmers' stores with total aflatoxin levels above and below 15 ppb in the three districts

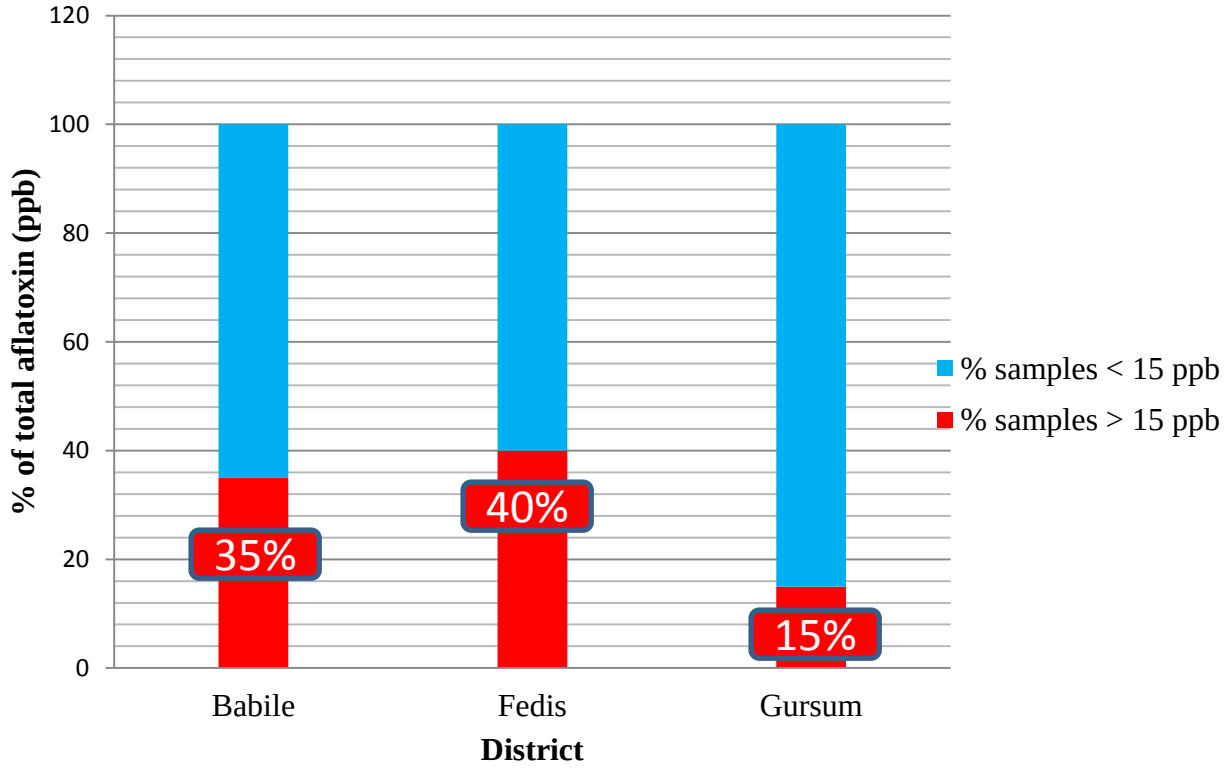
Regression analysis of MC with AFT at farmers' stores



MC with AFT levels were +vly correlated (r = 0.956) and significant ($p \leq 0.05$)

Figure 4. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at farmers' stores

3.4. Prevalence of total aflatoxin at market retailers



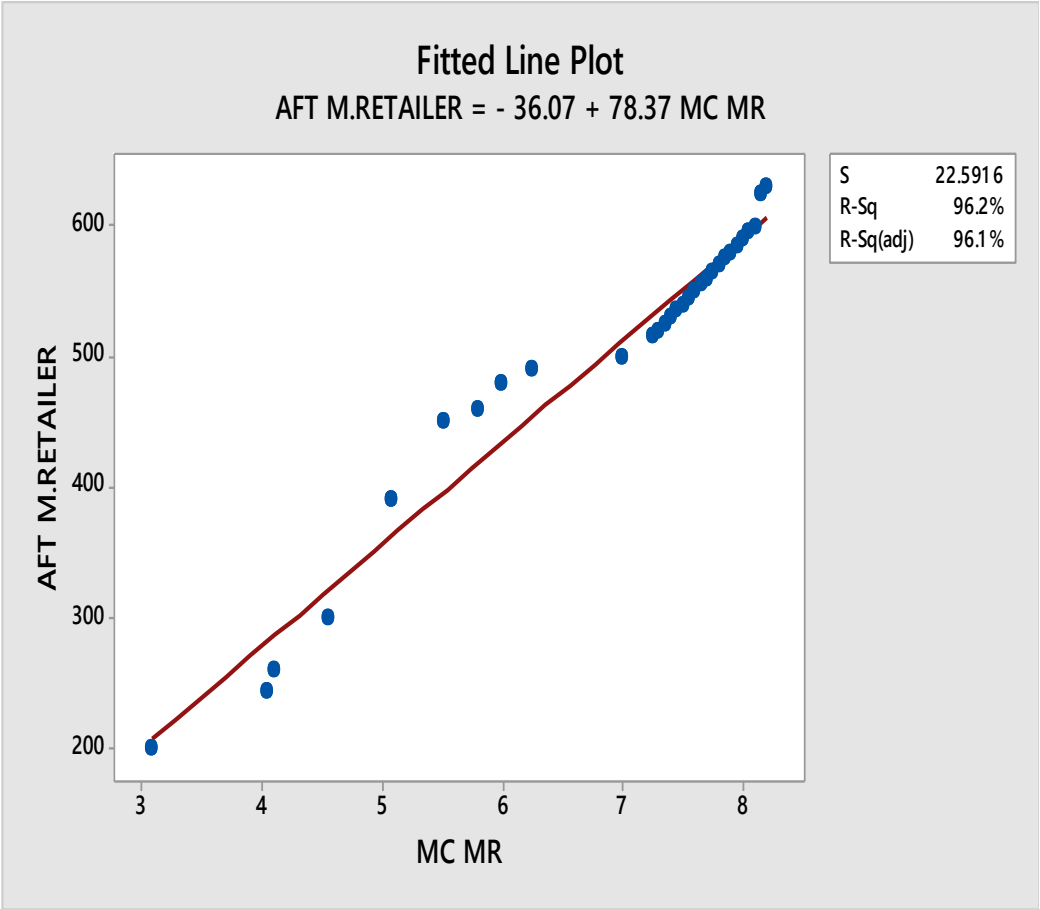
CODEX Limits:

AFT>15 ppb= Unfit for human consumption

AFT<15 ppb= Safe for human consumption

Figure 7. Percent of groundnut seed samples from market retailers with total aflatoxin levels above and below 15 ppb in the three districts

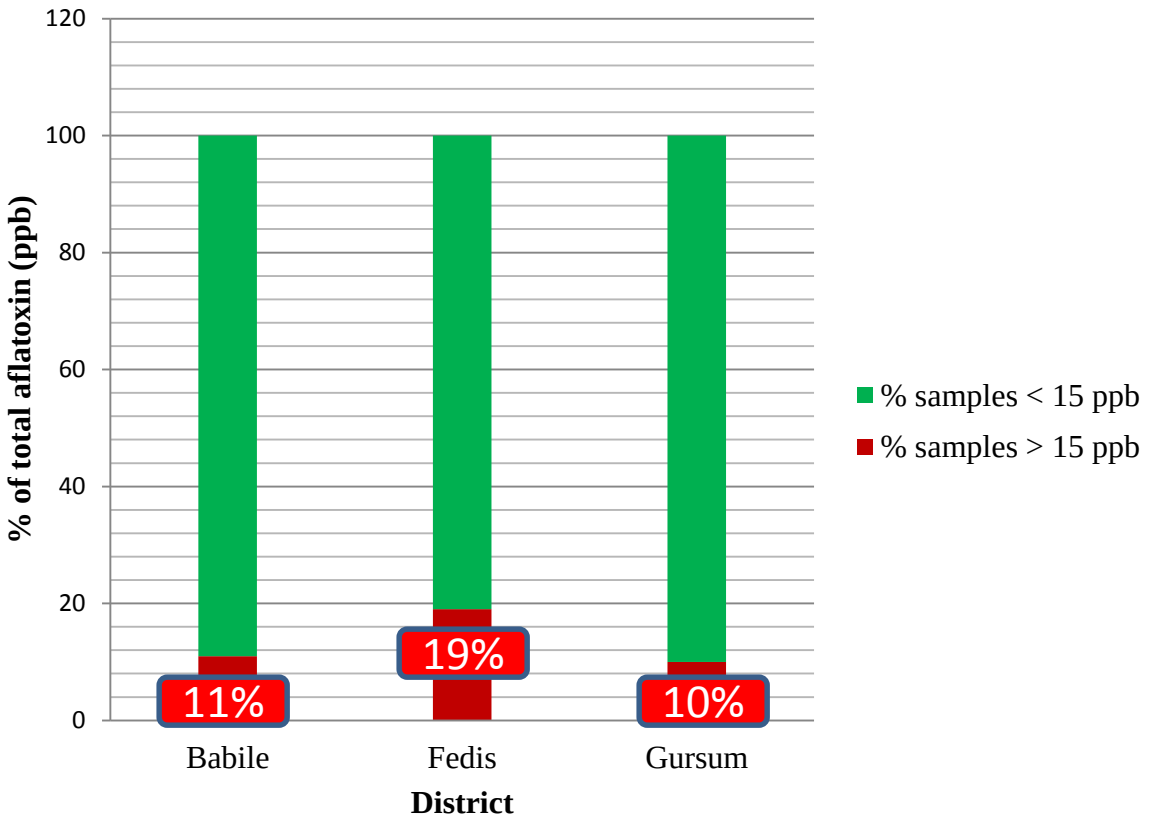
Regression analysis of MC with AFT at market retailers



MC with AFT levels were +vly correlated (r = 0.981) and significant ($p \leq 0.05$)

Figure 8. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at market retailers

3.5. Prevalence of total aflatoxin at venders



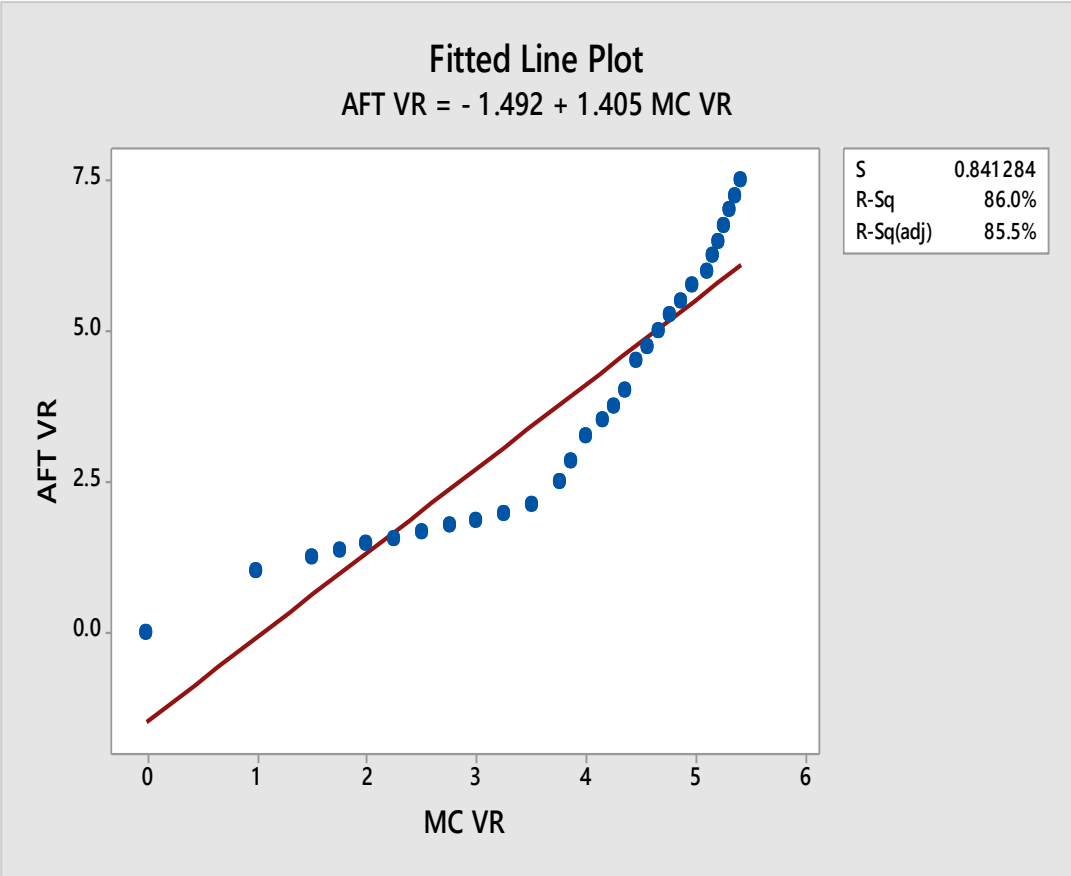
CODEX Limits:

AFT>10 ppb= Unfit for human consumption

AFT<10 ppb= Safe for human consumption

Figure 9. Percent of groundnut seed samples from venders with total aflatoxin levels above and below 10 ppb in the three districts

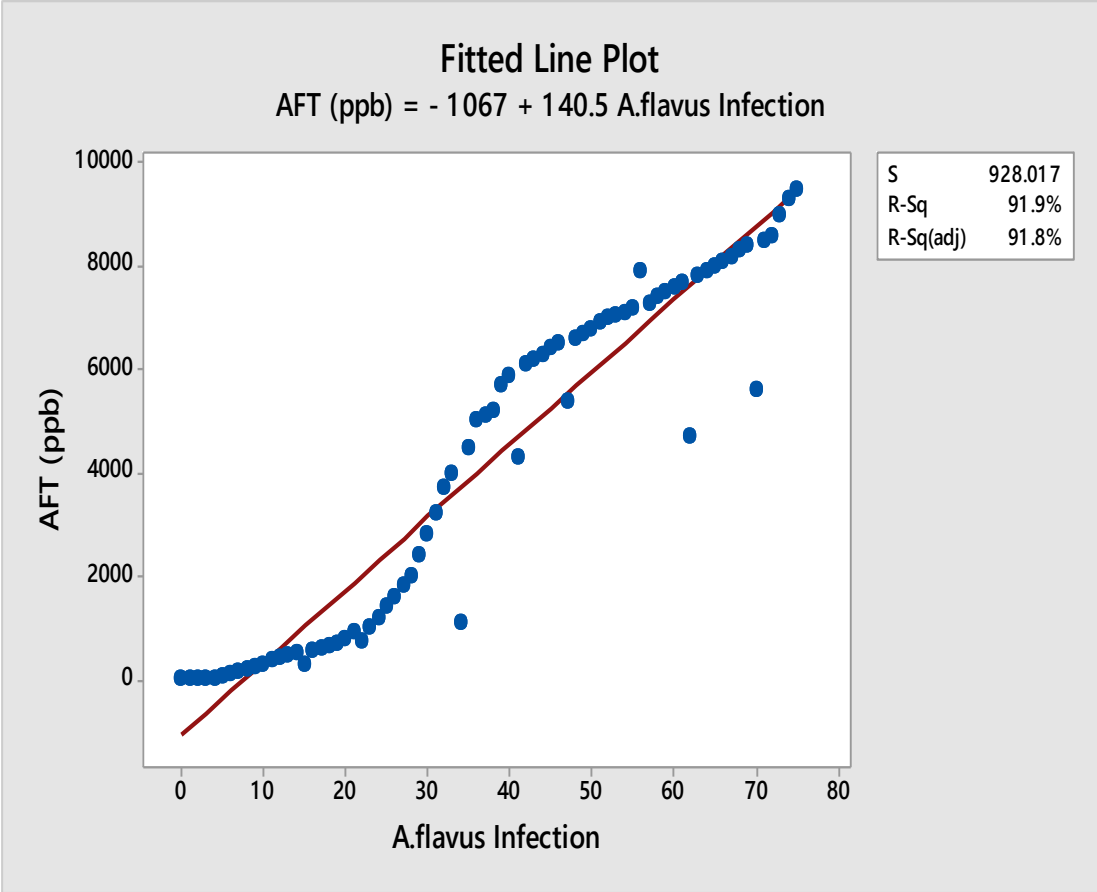
Regression analysis of MC with AFT at venders



MC with AFT levels were +vly correlated (r = 0.927) and significant ($p \leq 0.05$)

Figure 10. Regression analysis of moisture contents of groundnut seeds with total aflatoxin levels at venders

3.6. Regression Analysis of *A. flavus* Infection with AFT Levels



A.flavus **with AFT were +vly correlated (r = 0.959) and significant ($p \leq 0.05$)**

Figure 11. Regression analysis of *A. flavus* infection of groundnut seeds with total aflatoxin levels

3.7. Reaction to Field seed colonization by *A. flavus*

Table 1. Field seed colonization by *A. flavus* of the 16 groundnut seed varieties evaluated

No.	Varieties	Mean%of *A.flavus* Infection	Level of Resistance
1	NC-343	48.21[a]	Susceptible
2	Tole-2	45.01[b]	Susceptible
3	Baha Jido	41.10[c]	Susceptible
4	Werer 961	39.09[d]	Susceptible
5	Werer 963	35.16[e]	Susceptible
6	Lote	33.00[f]	Susceptible
7	Roba	32.50[g]	Susceptible
8	Shulamiz	32.20[g]	Susceptible
9	Werer 962	30.17[h]	Susceptible
10	Oldhele	27.63[i]	Susceptible
11	Bulki	27.00[j]	Moderately Resistant
12	Fayo	20.18[k]	Moderately Resistant
13	Fetene	19.00[l]	Moderately Resistant
14	Sedi	14.23[m]	Resistant
15	Sartu	14.00[m]	Resistant
16	Baha Gudo	13.70[m]	Resistant
Mean		**29.86**	

S = 3.70
LSD at p = 5%, 0.00
CV(%) = 12.39
***Significant at $p \leq 0.05$**

Means that do not share a letter are significantly different

According to Mehan and McDonald (1983), the level of resistance to invasion and colonization by *A. flavus* as follows:

Resistant = Sporulating growth on less than 15% of the seed number.
Moderately resistant = Sporulating growth on 16-30% of the seed no.
Susceptible = Sporulating growth on 31-50% of the seed number.
Highly susceptible = Sporulating growth on over 50% of the seed number

3.8. Reaction to Aflatoxin levels (ppb)

Table 2. Aflatoxin levels (ppb) of the 16 groundnut seed varieties evaluated

No.	Varieties	Mean of AFT in ppb	Level of Resistance
1	NC-343	841.90[a]	Susceptible
2	Tole-2	839.10[b]	Susceptible
3	Baha Jido	838.10[c]	Susceptible
4	Werer 961	808.10[d]	Susceptible
5	Werer 963	792.10[e]	Susceptible
6	Lote	724.10[f]	Susceptible
7	Roba	721.10[g]	Susceptible
8	Shulamiz	630.30[h]	Susceptible
9	Werer 962	584.20[i]	Susceptible
10	Oldhele	300.70[j]	Susceptible
11	Bulki	273.20[k]	Susceptible
12	Fayo	273.90[l]	Susceptible
13	Fetene	93.20[m]	Susceptible
14	Sedi	6.40[n]	Resistant
15	Sartu	3.70[o]	Resistant
16	Baha Gudo	1.93[p]	Resistant
Mean		**481.20**	

S = 55.58
LSD at p = 5%, 0.00
CV(%) = 11.55
***Significant at $p \leq 0.05$**

Means that do not share a letter are significantly different

According to CODEX STANDARD LIMIT (CODEX, 2014):

AFT < 15ppb = Resistant to AFT

AFT > 15ppb = Susceptible to AFT

4. Conclusion

- **Percent prevalence of AFT concentration were higher at farmers' stores at Fedis district, indicating heavy contamination of groundnut by aflatoxin beyond the maximum tolerable level by the CODEX (15 ppb).**

- **MC with AFT levels were positively correlated (r = 0.956) and significant ($p \leq 0.05$).**

- **Moreover, *A. flavus* infection with AFT levels were positively correlated (r = 0.959) and significant ($p \leq 0.05$).**

- **This study revealed that there was higher risk of exposure to aflatoxin through raw than roasted groundnut seeds.**

- The **16 tasted varieties** were **significantly different ($p \leq 0.05$)** in response to ***A. flavus* infection** and **aflatoxin contamination.**

- **Baha Gudo (13.70%), Sartu (14%)** and **Sedi (14.23%) were resistant to *A. flavus* infection**, sporulating growth <15% of the seed number.

- **Moreover**, **Baha Gudo (1.93 ppb), Sartu (3.70 ppb)** and **Sedi (6.40 ppb) were resistant to pre-harvest aflatoxin contamination, AFT** <15 ppb.

5. Recommendations

- **Application of proper pre-harvest and post-harvest management practices of aflatoxin contamination at farmers' fields at harvest and farmers' stores for high quality maintenance in the groundnut value chain and to avert human and animal health risks is necessary.**

- **The varieties that showed resistance could form part of an integrated management of aflatoxin contamination in Ethiopia.**

- **Further verification of groundnut varities (Baha Gudo, Sedi and Sartu) that exhibited resistance to field seed colonization and infection by *A. flavus* and pre-harvest aflatoxin contamination across years and locations would generate conclusive results.**

6. Future Research Directions

- **Further research on aflatoxin levels in groundnut and other cereals along the value chains and temporal and spatial distribution of it are needed to expand the data base at the country level.**

- **Further evaluation of resistance of different groundnut varities to *A. flavus* infection and pre-harvest aflatoxin contamination should be done in other areas of the country to come up with full picture at the country level. Also there is a need to elucidate the mechanisms of resistance to *A. flavus* infection and pre-harvest aflatoxin contamination in order to promote dissemination of the varieties.**

PARTIAL VIEW OF SOME OF THE ACTIVITIES

Field Layout & Sowing

Partial View Cont'd…

Emerged Seed

Two to Three Leaf Stage

Partial View Cont'd…

Flowering and Pegging

Field Visit

Partial View Cont'd…

Harvesting and Treshing

I THANK YOU!