Overview and future prospects of aflatoxin research at ICRISAT

Farid Waliyar
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Outline of presentation

• Aflatoxin problem, effects & regulations
• Prevalence: A case study of Mali
• Aflatoxin research at ICRISAT
• Achieved results
• Diagnostics
• Future prospects
The Aflatoxin problem

• Most serious food safety issue

• Many countries lost the world groundnut market share due to aflatoxin contamination

• Order of toxicity: $\text{AFB}_1 > \text{AFG}_1 > \text{AFB}_2 > \text{AFG}_2$

• $\text{AFB}_1$ is most prevalent & categorized as a group 1 carcinogen
The Aflatoxin Effects

Aflatoxin contamination in food and feed

- Trade: Quality reduction, Trade Restrictions
- Vicious-Link: Malnutrition
- Health: Liver cancer, Reduced ability to cope with diseases, especially HIV/AIDS; Liver cirrhosis, immuno-suppression, blocks nutrient absorption, growth abnormalities, etc.; Synergistic interaction with Hepatitis-B & C

Malnutrition

Liver cancer
## Regulatory limits

<table>
<thead>
<tr>
<th>Country</th>
<th>Product</th>
<th>Aflatoxin</th>
<th>Maximum allowable limit (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China, Japan, Thailand, Egypt, Turkey</td>
<td>Groundnuts</td>
<td>Total</td>
<td>10</td>
</tr>
<tr>
<td>Indonesia, Malaysia, Taiwan province of China, Australia</td>
<td>Groundnuts</td>
<td>Total</td>
<td>15</td>
</tr>
<tr>
<td>EU</td>
<td>Groundnuts</td>
<td>Total</td>
<td>4 (direct consumption) 15 (further processing)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B1</td>
<td>2</td>
</tr>
<tr>
<td>Kenya</td>
<td>Groundnuts</td>
<td>Total</td>
<td>20</td>
</tr>
<tr>
<td>Russia</td>
<td>Groundnuts</td>
<td>B1</td>
<td>5</td>
</tr>
<tr>
<td>Canada</td>
<td>Nuts and Nut products</td>
<td>Total</td>
<td>15</td>
</tr>
<tr>
<td>India</td>
<td>All food products</td>
<td>Total</td>
<td>30</td>
</tr>
<tr>
<td>Philippines</td>
<td>Nuts and products</td>
<td>Total</td>
<td>20</td>
</tr>
<tr>
<td>Singapore</td>
<td>Nuts</td>
<td>Total</td>
<td>5</td>
</tr>
<tr>
<td>USA</td>
<td>All foods except milk</td>
<td>Total</td>
<td>20</td>
</tr>
<tr>
<td>Viet Nam</td>
<td>Food stuffs</td>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

(CODEX ALIMENTARIUS COMMISSION, 2013)
Complexity of the problem

- Cultivars and farming practices
- Weather conditions
  - Drought stress
- Time of harvest / pod removal
- Method of harvest / drying
- Mechanical / insect damage
- Storage conditions
- Conditions of packing and distribution

In many SAT countries, monitoring and enforcement of food safety standards are rare due to poor awareness and lack of human skills and infrastructure
Typical model for Groundnut value chains in Mali

1. Farmer Field
2. Farmer Granary
3. Traders
4. Processors
5. Whole salers
6. Markets
7. Consumers
Importance of aflatoxins
A case study: Pre-harvest groundnut aflatoxin contamination in different districts of Mali during 2009 & 2010

<table>
<thead>
<tr>
<th>Aflatoxin range (µg/kg)</th>
<th>% of groundnut samples in each category/district</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kayes</td>
</tr>
<tr>
<td>0-4</td>
<td>41.1</td>
</tr>
<tr>
<td>5-10</td>
<td>26.7</td>
</tr>
<tr>
<td>11-20</td>
<td>10.0</td>
</tr>
<tr>
<td>21-35</td>
<td>3.3</td>
</tr>
<tr>
<td>36-100</td>
<td>8.9</td>
</tr>
<tr>
<td>101-500</td>
<td>10.0</td>
</tr>
<tr>
<td>&gt;500</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Post-harvest aflatoxin contamination in groundnut (2009 rainy season crop) in farmers’ granaries in different districts of Mali, West Africa
Research on aflatoxins.....Priorities changed over time

Up to 1990
- Focus was mainly breeding
- Resistant levels were hovering between 20-50 ppb

1990-2000
- Breeding efforts continued (resistant levels around 20 ppb)
- Other management options explored including biocontrol and cultural practices

2000 onwards
- Board approved strategy (Integrated Aflatoxin Management)
- Breeding efforts continued and found genotypes <10 ppb consistently
- Occurrence/distribution and value chains improvement
- Transgenics research
- Transcriptomics initiated very recently
ICRISAT’s Integrated Approach to Mitigate Aflatoxin Contamination

**Global Approach**
- Host resistance
  - Conventional breeding,
  - Transgenic approach with anti-fungal and anti-mycotoxin genes, Genomics-assisted breeding
- Bio-control Agents
  - Trichoderma, Pseudomonads, Atoxigenic strains
- Cultural Practices
  - Soil amendments (gypsum, compost)
- Harvesting and Post-harvesting Technologies
  - Drying and Storage

**Technology Transfer / Socio-economic issues**
- Regional studies & monitoring
- Public awareness
- Trade implications
- Advisory panels
- Consultation to Industries
- Training

**Assessment / Implementation at Regional level**
**Devising Appropriate Regional Package and Promotion**
- Pre- and Post-harvest Aflatoxin Management
  - No-cost
  - Low-cost
  - High-cost
Host Plant Resistance

Conventional Breeding

• Screening of various germplasm sets (core/mini core collections)
• Testing advanced breeding lines in Asia and Africa
• Incorporation of “R”
Groundnut breeding lines resistant to aflatoxin contamination at Mali and Niger (2008-2011)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Aflatoxin (µg/kg)</th>
</tr>
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<tbody>
<tr>
<td>ICGV 91324</td>
<td>1.8</td>
</tr>
<tr>
<td>ICGV 94379</td>
<td>2.3</td>
</tr>
<tr>
<td>ICGV 93305</td>
<td>2.6</td>
</tr>
<tr>
<td>ICGV 91284</td>
<td>2.8</td>
</tr>
<tr>
<td>ICGV 91278</td>
<td>2.8</td>
</tr>
<tr>
<td>ICGV 91315</td>
<td>3.1</td>
</tr>
<tr>
<td>ICGV 91317</td>
<td>3.1</td>
</tr>
<tr>
<td>ICGV 91279</td>
<td>3.3</td>
</tr>
<tr>
<td>ICGV 91283</td>
<td>3.5</td>
</tr>
<tr>
<td>ICGV 92302</td>
<td>4.0</td>
</tr>
<tr>
<td>JL 24</td>
<td>696.5</td>
</tr>
</tbody>
</table>
Performance of selected mini core accessions against pre-harvest aflatoxin contamination from 2008-2013 at Sadore, Niger

Mean of six years, and JL 24 had 584 ppb
Host Resistance: Transgenic Approach

Incorporation of rice chitinase gene in elite varieties

Evaluation of transgenic groundnut with rice chitinase gene for resistance to *A. flavus* by IVSC
Host Resistance: Transgenic Approach

- Transformation of groundnut with anti-aflatoxin genes [lypoxygenase (13-LOX)] for inhibiting the production of aflatoxins (Sowmini Sunkara et al.)
  (Research— in collaboration with the University of Wisconsin)

- The other work underway
  - “defensins” (in collaboration with Donald Danforth Plant Science Center)
  - “forisomes” (in collaboration with International Center for Genetic Engineering and Biotechnology)
Cultural Methods

- Adjusting planting dates
- Box ridges for moisture retention
- Soil amendments
  - FYM
  - cereal crop residues
  - Gypsum/Lime
- Conjunctive use of FYM & cereal crop residues

Soil Suppressiveness
- Pod wall resistance
Influence of agronomic/cultural practices in aflatoxin reduction

<table>
<thead>
<tr>
<th>Agronomic practice</th>
<th>Aflatoxin reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal crop residues (CCR) (2.5 t/ha)</td>
<td>28</td>
</tr>
<tr>
<td>Farmyard Manure (FYM) (2.5 t/ha)</td>
<td>42</td>
</tr>
<tr>
<td>Lime (400 kg/ha)</td>
<td>72</td>
</tr>
<tr>
<td>FYM + CCR</td>
<td>53</td>
</tr>
<tr>
<td>Lime + CCR</td>
<td>82</td>
</tr>
<tr>
<td>FYM + Lime + CCR</td>
<td>83</td>
</tr>
<tr>
<td>FYM + Lime</td>
<td>84</td>
</tr>
</tbody>
</table>

Waliyar et al., 2006 & 2007
Use of Biocontrol Agents (BCAs)

- Fluorescent Pseudomonads
- Actinomycetes
- Trichoderma

Identified potential biocontrol agents

- Good candidates for identification of anti-fungal metabolites
BCAs for reducing Aflatoxin Contamination

*Trichoderma viridae*
Compost + *Pseudomonas aeruginosa* (CDB 35)(5t/ha)
Gypsum (500 kg/ha)
Cereal crop residues (5 t/ha)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2002*</th>
<th>2003*</th>
<th>2004*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. viridae</em> (T)</td>
<td>947 (98)</td>
<td>92 (93)</td>
<td>351 (91)</td>
</tr>
<tr>
<td>Gypsum (G)</td>
<td>320 (79)</td>
<td>329 (68)</td>
<td>497 (65)</td>
</tr>
<tr>
<td>G + Bacteria (B)</td>
<td>264 (99)</td>
<td>137 (94)</td>
<td>416 (97)</td>
</tr>
<tr>
<td>B + T</td>
<td>174 (89)</td>
<td>204 (63)</td>
<td>271 (69)</td>
</tr>
<tr>
<td>G + B + Crop residues</td>
<td>626 (81)</td>
<td>190 (90)</td>
<td>428 (79)</td>
</tr>
<tr>
<td>G + T + B</td>
<td>601 (55)</td>
<td>188 (71)</td>
<td>483 (59)</td>
</tr>
</tbody>
</table>

*Decrease in aflatoxin levels (µg kg-1) from control to application
* Percent reduction values in parenthesis
A novel biocontrol agent, *Streptomyces cavourensis* (CDA 19)

A. *flavus* mycelial shrinkage with culture filtrate of CDA 19

Control

*Harini et al., 2011*
Post-harvest management in reducing aflatoxin contamination

• Does it really matter?

• How important for a smallholder farmer?

• Can simple techniques minimize aflatoxin accumulation during storage?

• Feasibility and viability of approaches

• Awareness is the key
Post-harvest management: Existing drying practices

India

Traditional Field Drying

West Africa

Postharvest heaping
Improved Batch Drying

- Demonstrations in Kolokani and Kayes, Mali
- Drying pods: facing the Sun

- Improved Batch Drying
- (Pods facing the sun)
Effect of Drying Method and Varieties on Aflatoxin Content

<table>
<thead>
<tr>
<th>Farmer</th>
<th>55-437 (Resistant)</th>
<th>47-10 (susceptible)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traditional</td>
<td>Improved (% reduction)</td>
</tr>
<tr>
<td>Savado</td>
<td>8.08</td>
<td>3.03 (63)</td>
</tr>
<tr>
<td>Coumb</td>
<td>9.90</td>
<td>2.32 (77)</td>
</tr>
<tr>
<td>Kande</td>
<td>8.01</td>
<td>1.67 (79)</td>
</tr>
<tr>
<td>Seydou</td>
<td>5.78</td>
<td>0.31 (95)</td>
</tr>
<tr>
<td>Yaya</td>
<td>5.70</td>
<td>2.17 (62)</td>
</tr>
</tbody>
</table>
Aflatoxin Management Strategies: Challenges

**Host resistance**
- Need to identify resistant markers
- Deployment of genomic tools
- Strengthening Seed systems

**Transgenics**
- Regulatory issues
- Lack of appropriate capacity
- Lack of private sector investments in Africa

**Integrated Management**
- Transfer of Technologies (Funding required)
- Awareness
- Policy support
Aflatoxin Diagnostics

Why Immunological methods are advantageous over chromatographic and other methods???

• Simple
• Cost-effective
• Sensitive
• Rapid
• Versatile

- Hence we at ICRISAT developed competitive ELISAs with the help of in house raised antibodies
  - Indirect competitive ELISA
  - Direct competitive ELISA
A step further.....
Assessment of aflatoxin exposure in humans

- A fruitful collaboration between NIZAM’S Institute of Medical Sciences (NIIMS), Hyderabad, India and ICRISAT
- Developed in-house antibodies for aflatoxin-albumin adduct and standardised ELISA to assess aflatoxin exposure in humans

The association between exposure to aflatoxin, mutation in TP53, infection with hepatitis B virus, and occurrence of liver disease in a selected population in Hyderabad, India

S. Anitha, D. Raghunadhara, F. Waliyar, H. Sudini, M. Parveen, Ratna Rao, P. Lava Kumar
Future Prospects

• A comprehensive concept note on “Holistic Aflatoxin Solutions for Impacts on Health And Markets (HASHAM)” has been prepared and discussed at length
• My opinion is that ICRISAT should focus on

- Upscaling the best bet technologies to reach massive number of farmers in SSA and Asia
- Develop new technologies using modern tools
Future Prospects..... Data Base

- Aflatoxin database (24 yrs. of research at WCA): Good meat for Modelling and to draw valid conclusions

- Long term data on resistant trails, IDM packages, occurrence and distribution

- Already 6 peer reviewed journal articles are in different stage of publication
Thank you!

• To all ICRISAT staff, friends and colleagues, with whom I worked
• To all my students
• My parents and my wife and children