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AFLATOXINS AND AFLATOXINS MANAGEMENT IN GROUNDNUTS, MACADAMIA NUTS, MAIZE, HERBS AND SPICES VALUE CHAINS

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AFLATOXINS AND AFLATOXINS MANAGEMENT IN GROUNDNUTS, MACADAMIA NUTS, MAIZE, HERBS AND SPICES VALUE CHAINS

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DISCLAIMERS

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FOREWORD

Aflatoxins are chemical poisons produced by moulds belonging to *Aspergillus* section *Flavi,* primarily *Aspergillus flavus* and *A. parasiticus*. Key segments of value chain actors that determine the fate of commodities in terms of safety remain minimally informed on the impact and practical approaches for mitigating aflatoxins. Past landscape analyses by UNIDO have identified gaps amongst producers, extension agents, regulators, traders and consumers in Kenya hence increasing the exposure of consumers to aflatoxins and stifling trade. Measures that improve food safety and quality both at household and market levels should be encouraged and implemented by actors in different parts of the value chains. Aflatoxin contamination begins in the field and may increase during post-harvest stages. Factors influencing aflatoxin contamination include on- and off-farm crop handling practices, weather conditions during crop growth, plant susceptibility, and storage conditions. Maize and groundnut, which are key staple crops in Kenya, are the most susceptible to aflatoxin contamination.

Besides the effects on health of consumers, aflatoxin contamination limits local, regional and international trade. In the national markets, aflatoxin contamination results in reduction in marketable volume, loss in value and revocation of business operating permits; while in regional or international markets there might be inadmissibility or rejection of consignments and finished products. The cost of surveillance and destruction of condemned produce and products is also a direct cost to the concerned agencies. Ingestion of contaminated feed by livestock leads to losses incurred from diseases, morbidity and mortality.

Effective control of aflatoxin requires a combined approach of various technologies that have been proven to be effective that straddle good agricultural practices (GAPs), good handling and storage practices, and good manufacturing practices (GMPs). There are also cross cutting measures such as enhancing capacity, strengthening policy and following through its implementation, surveillance, and strengthening the role of private sector remain key.

The EU in partnership with the EAC launched the Market Access Upgrade Programme (MARKUP) to support member countries improve market access of agro-food products to the EU and regional markets. UNIDO is the implementation partner for the Kenya-Partner States Window. Recent studies have analyzed the reasons for low productivity and competitiveness in these value chains such as the need for specialized extension services and a diffuse lack of knowledge on appropriate good agricultural practices. These value chains for exports are also lacking compliance with market requirements and standards. The MARKUP project aims to improve the institutional and regulatory framework for better conformity assessment services in Kenya's horticultural sector; increase revenue and MARKUP for Kenya's smallholder producers and enterprises in export-oriented horticulture sectors.

This training manual focuses on building capacity in the management of aflatoxins that has been a major hazard and impediment to safe trade both locally and internationally. In addition to nuts, herbs and spices, the manual also covers aflatoxin control in maize, due to its importance to food security in Kenya and its potential to hurt local and regional trade.

This training manual highlights the issue of aflatoxins from a local, regional and international perspective. It provides the needed support in equipping various stakeholders including but not limited to; regulators, extension officers, producers and traders. The manual offers an opportunity for trainees to interact with practical aspects of aflatoxins on aspects of appearance of moulds on the produce, management approaches and testing methods. This will enhance the learning curve enabling the trainees to identify aspects that are highly applicable in their area of jurisdiction. The training will combine instructor-led and practical/ hands-on training.

Implementation of the guidelines contained in this manual aims at minimizing aflatoxin contamination of groundnuts, macadamia nuts, maize, herbs and spices through adoption of integrated preventive or control measures in the production, handling, transportation, storage and processing of the crops. The guidelines also provide testing options – based on need - for determination of the levels and types of aflatoxins in foods and feeds with the aim of ensuring food safety and supporting local, regional and international trade.

Director General, AFA

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EXPECTED LEARNING OUTCOMES

- The Regulators/Inspectors are expected to:
  - Have a good understanding of aflatoxins and their effects on health, trade and food security.
  - Effectively inspect and test for aflatoxins in the groundnuts, macadamia nuts, herbs and spices and maize value chains.
  - Definitively explain the problem of aflatoxins and their management in groundnuts, macadamia nuts, herbs and spices and maize, including in their products.

- Extension agents are expected to:
  - Effectively disseminate information on aflatoxins, their impact, regulatory standards and their management in groundnuts, macadamia nuts, herbs and spices to farmers.
  - Identify specific needs in various value chains and how to implement aflatoxin mitigation measures.
  - Identify pre-harvest and post-harvest aflatoxin contamination risk factors in groundnuts, macadamia nuts, herbs and spices and maize value chains.
  - Effectively train farmers on mitigation approaches at pre-harvest and post-harvest stages in groundnuts, macadamia nuts, herbs and spices and maize.

- Traders and processors are expected to:
  - Understand the local and international regulatory requirements for the various value chains.
  - Have an in-depth understanding of the impacts of aflatoxins on health and trade.
  - Effectively identify contaminated produce as well as test for aflatoxins using rapid methods.
  - Understand practices that reduce the risk of aflatoxin contamination during post-harvest stages.
  - Clearly understand the management approaches for aflatoxins and how to apply them in their respective value chains.

- Producers are expected to:
  - Have an in-depth understanding of pre-harvest and post-harvest practices that reduce the risk of aflatoxin contamination in their respective value chains.
  - Understand the impacts of aflatoxins on health and trade.
  - Be aware of the local and international aflatoxins regulatory requirements for the various value chains.

- Consumers are expected to:
  - Have an in-depth understanding of post-harvest practices that reduce the risk of aflatoxin contamination.
  - Understand the impacts of aflatoxins on human and livestock health.

- Laboratory Technologists are expected to:
  - Effectively test for aflatoxins in the groundnuts, macadamia nuts, herbs and spices and maize value chains.
  - Understand the effects of aflatoxins on health, trade and food security.
  - Understand the local and international regulatory requirements for the various value chains.

- County Government officials and Policy Makers
  - Understand the effects of aflatoxins on health and trade.
  - Understand practices that reduce the risk of aflatoxin contamination during pre-harvest and post-harvest stages.
  - Understand the role of cross-border trade on exposure to aflatoxins.

ABBREVIATIONS AND ACRONYMS

ADON:	Acetyldeoxynivalenol
AFA:	Agriculture and Food Authority
ANIV:	Acetylnivalenol
AOAC:	Association of Official Analytical Chemists
Codex:	Codex Alimentarius Commission
COVID-19:	Coronavirus Disease
DNA:	Deoxyribonucleic Acid
DON:	Deoxynivalenol
DSP:	3,3'-Dithiodipropionic acid di (N-hydroxysuccinimide ester)
EAC:	East African Community
EAS:	East African Standard
EC:	European Commission
ECD:	Electron Capture Detector
ELISA:	Enzyme Linked Immunosorbent Assay
EQCM:	Electrochemical Quartz Crystal Microbalance
EU:	European Union
FAO:	Food and Agriculture Organization
FDA:	United States Food and Drug Administration
FDA:	Food and Drug Administration
FDSCA:	Food, Drugs and Chemical Substances Act
FID:	Flame Ionization Detector
FPIA:	Fluorescence Polarization Immunoassay
GAP:	Good Agricultural Practices
GC:	Gas Chromatography
HACCP:	Hazard Analysis Critical Control Point
HPLC:	High Performance Liquid Chromatography
HPTLC:	High-Performance Thin-Layer Chromatography
IR:	Infrared Spectroscopy
KDHS:	Kenya Demographic and Health Survey
KEBS:	Kenya Bureau of Standards
LCMS:	Liquid Chromatography Mass Spectrometry
LFD:	Lateral Flow Device
MARKUP:	Market Access Upgrade Programme
MLs:	Maximum Limits
MoA:	Ministry of Agriculture and Livestock Fisheries and Cooperatives
MS:	Mass Spectrometer
NIV:	Nivalenol
QCMs:	Quartz Crystal Microbalances
RIA:	Radioimmunoassay
SA:	Standards Act
SPR:	Surface Plasmon Resonance
TLC:	Thin-Layer Chromatography
UN:	United Nations
UNIDO:	United Nations Industrial Development Organization
WHO:	World Health Organization

CHAPTER 1: UNDERSTANDING MYCOTOXINS AND AFLATOXINS AS A SUB-TYPE

1.1. Mycotoxins: An overview

- Mycotoxins are a group of chemically diverse secondary metabolites produced by fungi, and exhibit a wide array of biological effects.
- Individual mycotoxins can be mutagenic, carcinogenic, embryo-toxic, teratogenic, oestrogenic or immunosuppressive.
- Mycotoxins that frequently occur in food and feeds include: aflatoxins (B1, B2, G1 and G2), deoxynivalenol, fumonisins (B1, B2 and B3), zearalenones and ochratoxins (A, B and C).
- Other common mycotoxins include citrinins, patulin and ergot alkaloids.
- The United Nations Food and Agriculture Organization (FAO) has estimated that up to 25% of the world's foods are significantly contaminated with mycotoxins.
- The detailed information on these mycotoxins is outlined in Table 1.

Table 1: Common mycotoxins contaminating foods and feeds, fungi producing them, most susceptible foodstuff and the toxins major effects on human and animal health

Mycotoxin	Producing Fungi	Affected Foodstuff	Health Effects
Aflatoxins (B_1, B_2, G_1, and G_2)	*Aspergillus flavus* *Aspergillus parasiticus* *Aspergillus nomius* *Aspergillus pseudotamarii* *Aspergillus bombycis*	Maize, groundnuts, wheat, rice, nuts, spices, oilseeds, and cottonseed	• Potent carcinogens • May cause stunted growth in children • Leads to immunosuppression • Acute poisoning causes death • Impaired productivity and reproductive efficiency in animals • Liver damage in humans and animals • Reduced weight gain in animals
Aflatoxin M_1	Metabolite of aflatoxin B_1	Milk and dairy products	
Ochratoxin A	*Aspergillus ochraceus* *Aspergillus carbonarius* *Aspergillus niger* *Penicillium verrucosum* *Penicillium nordicum* *Penicillium cyclopium*	Wheat, barley, oats, cocoa beans, coffee beans, fruits and fruit juice, dried fruits, and wine	• Liver damage due to accumulation of toxins • Immunosuppression • Inhibition of macromolecular synthesis • Increased lipid peroxidation (cellular damage) • Inhibits mitochondrial ATP production (effecting energy production)

Patulin	*Penicillium expansum* *Byssochlamys nivea* *Aspergillus clavatus*	Fruit and fruit juices (apple juice, grapes), cheese, and wheat	• Edema and hemorrhage in brain and lungs • Damage in the liver, spleen, and kidney • Paralysis of motor nerves • Convulsions
Zearalenones	*Fusarium graminearum* *Fusarium culmorum* *Fusarium cerealis* *Fusarium equiseti* *Fusarium verticillioides* *Fusarium incarnatum*	Maize, wheat, barley, rye, and animal feeds	• Hormonal imbalance of the body • Numerous diseases of reproductive system such as prostate, ovarian, cervical and breast cancers • Immunosuppression • Affects gut health in swine
Fumonisins (B1, B2, B3)	*Fusarium verticillioides* *Fusarium proliferatum*	Maize, rice, wheat, sorghum, barley, and oats	• They are cancer promoting metabolites • Chronic exposure of fumonisins in humans has been associated with throat cancer, esophageal cancer • Disruption of sphingolipid metabolism
Trichothecenes[a]	*Fusarium sporotrichiodes* *Fusarium langsethiae* *Fusarium graminearum* *Fusarium culmorum* *Fusarium cerealis*	Maize, wheat, barley, oats, grains, and animal feed	• Emesis • Food refusal and weight loss • Dermal effects • Immune suppression with secondary infection
Deoxynivalenol	*Fusarium graminearum* *Fusarium culmorum*	Maize, wheat, oats, barley, rice, sorghum, and beer	• Causes acute temporary nausea and vomiting • Diarrhea • Abdominal pain • Headache • Dizziness • Fever • Decreased body weight, weight gain, and feed consumption in animals • Increased serum immunoglobulin A (IgA) levels

T-2 toxin	*Fusarium sporotrichioides* *Fusarium poae* *Fusarium tricinctum* *Fusarium langsethiae* *Fusarium acuminatum*	Maize, wheat, oats, barley, and rye	• Inhibits protein synthesis • Disrupts DNA and RNA • Feed refusal mainly in swine • Lack of weight gain • Digestive disorders • Diarrhea • Acute exposure leads to death

[a] Trichothecenes belong to a large family of secondary metabolites, with over 150 members. They are primarily produced by species from the genus *Fusarium*, but also by isolates from the genera *Myrothecium*, *Stachybotrys* and *Trichoderma*. Trichothecenes are divided into two categories: Type A trichothecenes (e.g. T-2 toxin and HT-2 toxin), and Type B trichothecenes (e.g. Deoxynivalenol - DON, Acetyldeoxynivalenol-ADON, Nivalenol -NIV and Acetylnivaleno –ANIV)

1.2 Aflatoxins, aflatoxin producing fungi and aflatoxin types

- Aflatoxins are a group of mycotoxins that are highly toxic and are produced as secondary metabolites by various species of fungi found in the *Aspergillus* section *Flavi* group.
- The aflatoxin problem came to light in the 1960's when there was a severe outbreak of a disease, referred to as "Turkey 'X' Disease" in England, in which over 100,000 turkeys and other farm animals died.
- The cause of this disease was traced to a component in peanut meal contaminated with *Aspergillus flavus*.
- This singular event triggered intensive research on mycotoxins.
- Aflatoxins are mainly produced by two fungal species in the *Aspergillus* section *Flavi* group, *Aspergillus flavus* and *A. parasiticus*, shown in Figure 1.
- Other less common aflatoxin producing species in the group include *A. nomius*, *A. pseudotamarii* and *A. bombycis*.
- There are different aflatoxin types including B1, B2, G1, G2, M1 and M2 (Figure 2).
- Aflatoxins B1, B2, G1 and G2 have often been detected in groundnut, maize and other agricultural commodities and their products.
- Aflatoxin M1 (4-hydroxy derivative of aflatoxin B1) is of special importance because it is detected in milk from animals fed with aflatoxin B1 contaminated feed.
- Aflatoxin B1 is the most toxic type followed by aflatoxins G1, B2, and G2 in order of decreasing potency.

Aspergillus Flavus *Aspergillus Parasiticus*

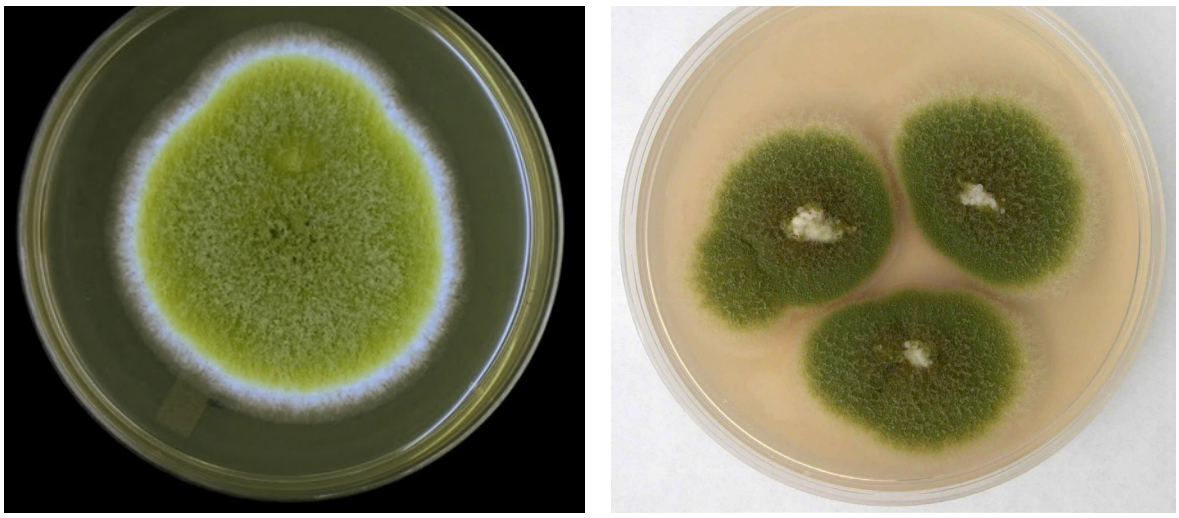

Figure 1: Cultures of the major aflatoxin producing Aspergillus species

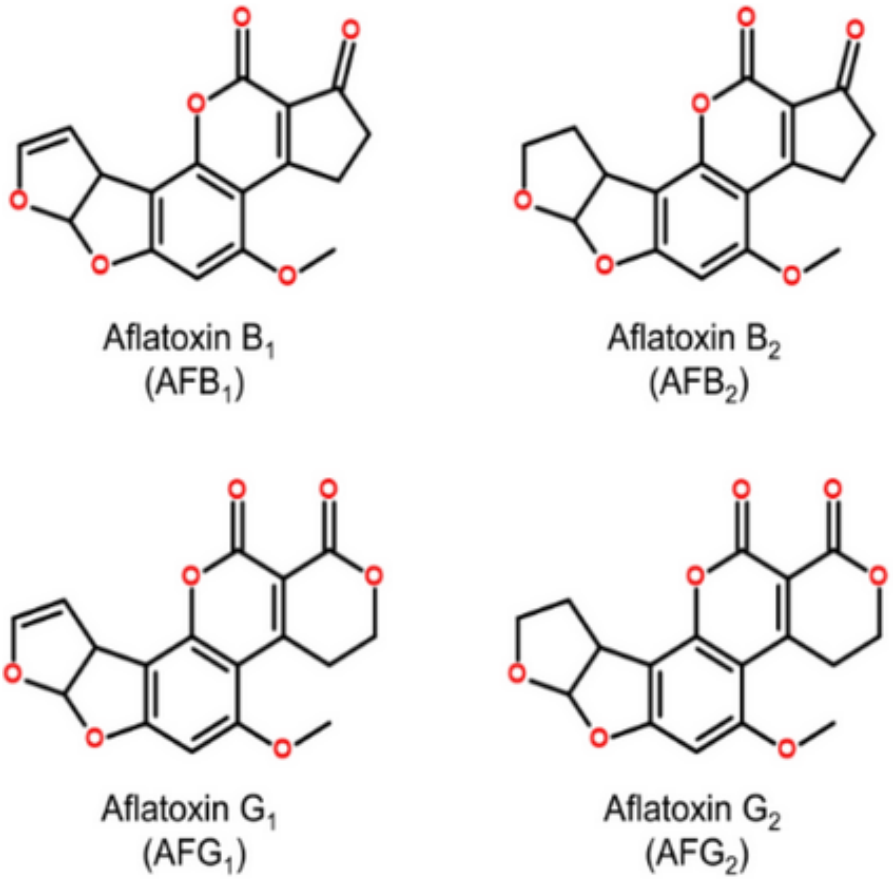

Figure 2: Molecular structures of four primary aflatoxin types

1.3 Factors affecting fungal growth and aflatoxin production

- The main factors that affect growth of aflatoxin producing fungi and aflatoxin production on a given food are:
    i. Moisture
    ii. Temperature
    iii. pH
    iv. The environment
- Production of aflatoxins is optimal at relatively high temperatures.
- Therefore, contamination is most prevalent and widespread in warm, humid climates highlighted in the Figure 3.
- Warm temperatures (32°C to 38°C) favour infection of grains and other susceptible crops compared to cool temperatures (21°C to 26°C).
- *A. flavus* will only grow when the moisture content exceeds 9%, at 80-85% relative humidity and above.
- *A. flavus* grows best between 10°C and 45°C at a relative humidity of 75% or more.
- However, the optimum conditions for aflatoxin production are between 25°C and 30°C, at 85% relative humidity.
- Aflatoxin producing moulds may invade agricultural products during plant growth (pre-harvest), harvest and post-harvest stages.

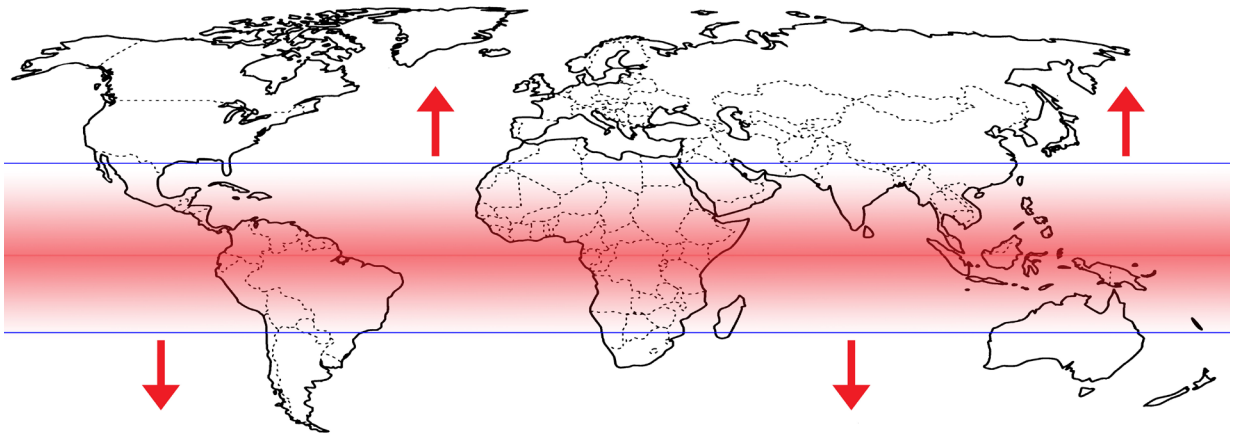

Figure 3: Regions of the world that lie between latitudes 40°N and 40°S (between the two blue lines) where the risk of aflatoxin contamination of foods is most prevalent and widespread

1.4 Aflatoxin contamination of groundnuts, macadamia nuts, maize, herbs and spices

- Biotic and abiotic factors, either nutritional or environmental, affect aflatoxin production in toxigenic *Aspergillus* species and host plants.
- Several factors predispose crops to accumulation of high levels of aflatoxins and particularly aflatoxin B1, which is highly potent.
- These factors include:
    i. Use of cultivars that are highly susceptible to infection by *Aspergillus*.
        o There are cultivars that have some resistance to infection by the pathogens.
    ii. Use of already infected planting material - where farmers do not use certified seeds.

iii. Environmental conditions during growth of the crop, especially high temperatures and moisture stress.
   - Such conditions favour pathogen growth and development leading to accumulation of aflatoxins.
   - Crops tend to be highly colonized by *A. flavus* and *A. parasiticus* species in warm and dry conditions with temperatures ranging from 25-35°C.

iv. Poor cultural practices such as lack of field sanitation contribute to crop infection contributing high fungal inoculum that result in aflatoxin accumulation in storage.
   - In addition, crop debris left in the field can act as a source of inoculum for the next season leading to infections in the field and higher levels of aflatoxin accumulation during storage.

v. High moisture content in the grains is also a predisposing factor.
   - Crops harvested for storage should be dried immediately before storage to a moisture content of <13.5%.

vi. Most of the *Aspergillus* species affect crops in the field and the effects are accelerated by damaged and shriveled kernels, drought related stress, delayed harvesting and insect wounds caused by field pests or birds.

vii. Poor storage conditions.
   - High temperatures and moisture during storage favour proliferation of *A. flavus* and *A. parasiticus* and consequently in afaloxin contamination.
   - There is therefore need to regulate temperature and moisture content during storage in order to curb growth of storage pathogens such as *Aspergillus.*

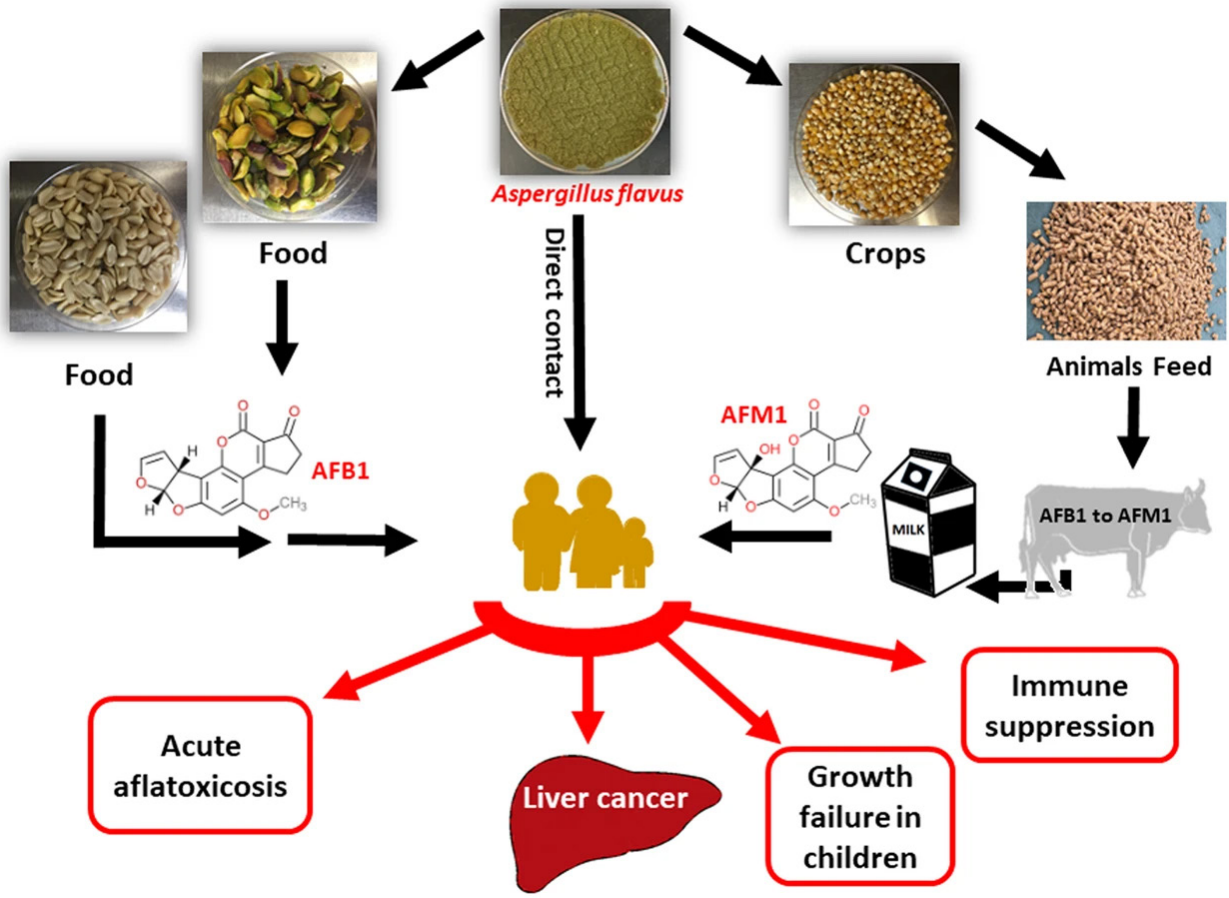

Figure 4: Schematic presentation of the major aflatoxin contamination/exposure routes and key adverse health effects to humans
Source: Alshannaq *et al*., 2018

1.4.1 Aflatoxin contamination of nuts

Groundnuts

- Groundnut flowers are formed and fertilized above ground, but downward growth of the pegs ensures that the fruit (pods and seeds) develop in the soil.
- Therefore, the pod is associated with soil microflora over an extended period of time which facilitates its invasion by various soil-inhabiting organisms.
- Aflatoxin contamination of groundnuts in Kenya has been associated with infection by *A. flavus* and *A. parasiticus* amongst other *Aspergillus* section *Flavi* fungi.
- Pre-harvest insect damage to the seeds/pods and drought predispose groundnuts to aflatoxin contamination.
- Drought causes plant stress by negatively impacting its physiology.
- This breaks down natural resistance mechanisms and increases susceptibility to infection by aflatoxin-producing fungi and aflatoxin contamination.
- During drought, groundnut kernels might strain and break providing entry for aflatoxin producing fungi.
- On the other hand, delayed harvesting reduces quality of the pods which makes them susceptible to fungal infection.
- Some pods get damaged during digging at harvest and this provides entry for aflatoxin producing fungi.

- Post-harvest, poor transportation and storage conditions of high moisture and temperature encourage growth of aflatoxigenic fungi in groundnuts.
- Moisture levels >10% produces a water activity of at least 0.85_{aw} which is conducive for the growth of *A. flavus* and *A. parasiticus* at 25 – 30°C.
- Contamination of groundnuts with aflatoxins has also been associated with the variety planted, with low aflatoxin levels generally being detected in oily hybrid varieties.
- Together with maize, groundnuts are a major ingredient of weaning foods in Kenya and are therefore a key route of aflatoxin exposure especially among infants.
- Among adults, the daily groundnut consumption is low compared to maize at an average of 1.1g/person/day.

Macadamia nuts and other tree nuts

- Kenya is the third largest producer of macadamia nuts globally (after South Africa and Australia), and the bulk of production is done by smallholder farmers.
- Tree nuts such as macadamia, walnuts and pistachio are also prone to aflatoxin contamination.
- When the nuts are still on the tree, the outer hull (dry outer covering of the nut) splits when the shell splits open (early-splits) and sometimes the hull is damaged by wind, insects or other pests.
- If insects or other pests damage the nutshell, then conditions exist for *Aspergillus* spores to invade and grow on the inner kernel and potentially produce aflatoxins.
- Macadamia nuts entering the European market must test below 2 µg/kg for aflatoxin B1 and 4 µg/kg for total aflatoxins.

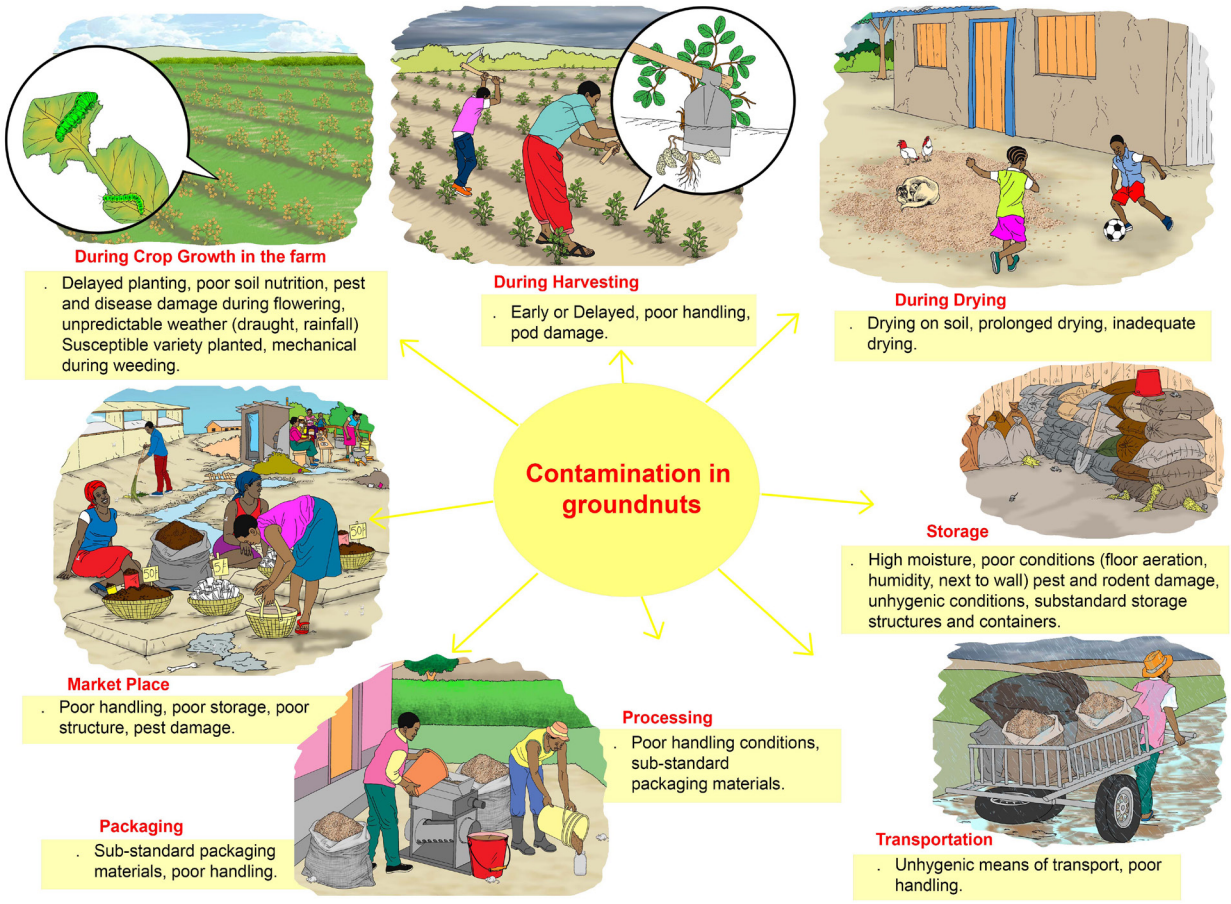

Figure 5: Diagrammatic representation of aflatoxin contamination of groundnuts and avenues of human exposure to the toxin

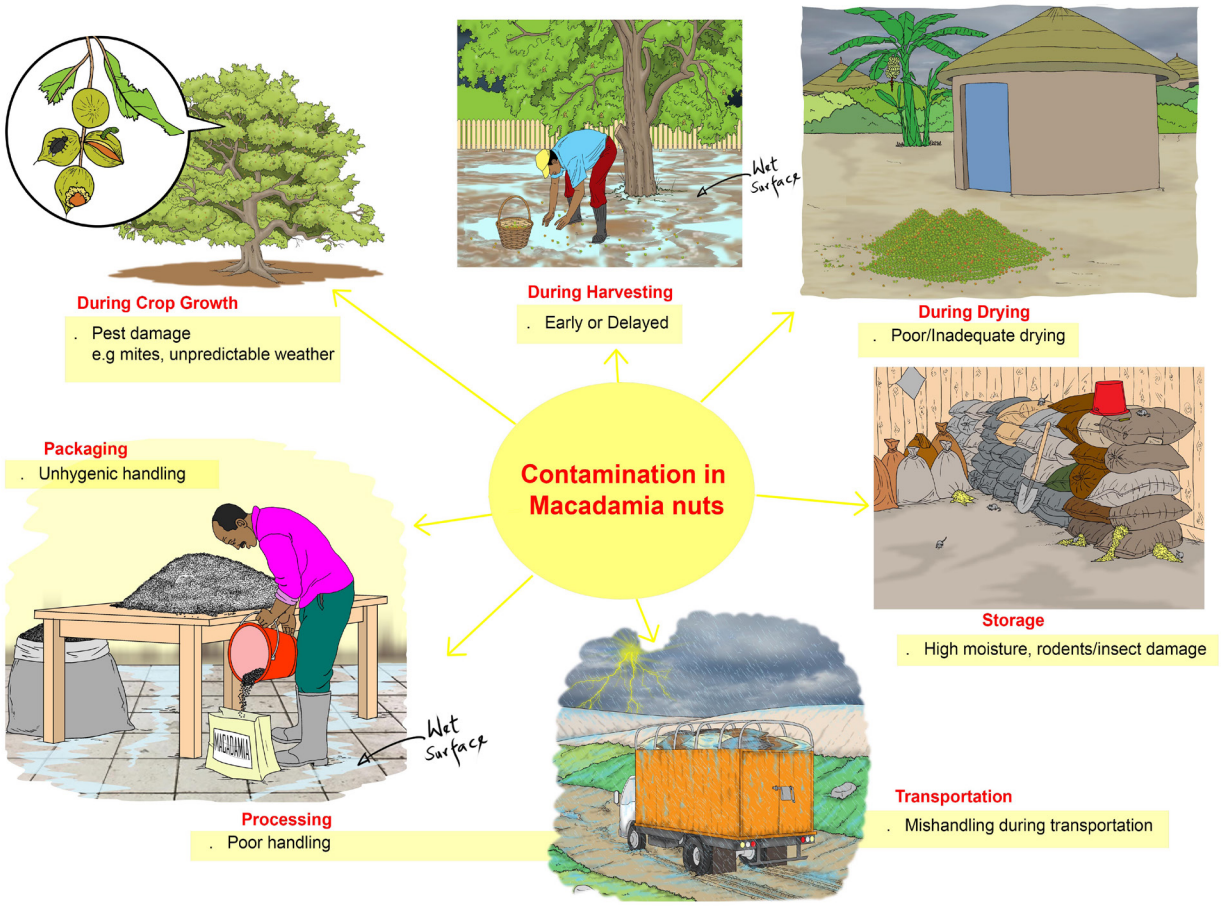

Figure 6: Diagrammatic representation of aflatoxin contamination of macadamia nuts and avenues of human exposure to the toxin

1.4.2 Aflatoxin contamination of maize

- Maize (*Zea mays* L.) is a staple food for majority of households in Kenya.
- It is also the major weaning food for infants in the form of porridge.
- However, quality and safety of maize and maize based products remains a concern due to fungal infection and mycotoxin contamination.
- Aflatoxins are the most important mycotoxins prevalent in Kenya that commonly contaminate maize.
- Kenya remains one of the world's hotspots for aflatoxins with numerous cases of aflatoxicoses outbreak for the last two decades, mainly attributed to maize.
- Acute aflatoxicosis outbreak in humans in Kenya was first reported in 1978 with subsequent cases in 1981, 1982 and 2001.
- During the devastating outbreak of 2004, 317 cases of aflatoxin poisoning were recorded, with 125 fatalities.
- The maize implicated during the 2004 aflatoxicosis outbreak was harvested during the off-season and early rains.
- The problem aflatoxicosis was exacerbated by poor storage of maize under damp conditions.
- The problem of aflatoxicosis is compounded by limited awareness on the health risks associated with consumption of aflatoxin contaminated maize and maize products.
- In January 2021, the Kenya Bureau of Standards (KEBS) banned 17 maize and porridge flour brands that had higher than the recommended 10µg/kg for total aflatoxin.
- Over-consumption of maize in Kenya also contributes to the country's growing burden of cancer.

- Maize meal consumption in Kenya is estimated at 400 g/person/day and has been incriminated for all aflatoxicoses cases recorded.
- Aflatoxin contamination of maize in Kenya has been associated with infection by *A. flavus* and *A. parasiticus* amongst other *Aspergillus* section *Flavi* fungi.
- These fungi are prevalent in warm and humid climates.
- Fungal and aflatoxin contamination of maize can occur in the field, at harvest, during processing, transportation and storage.
- The most important abiotic factors that predispose maize to infection by aflatoxigenic fungi include:
  i. Pre-harvest drought
  ii. High temperatures (heat stress)
  iii. Water stress
  iv. Delayed harvesting

- Contamination has also been due to the cultivation of maize in ecologically predisposed regions of the country as well as biophysical factors including:
  i. Type of soil
  ii. Plant genetic constitution and susceptibility
  iii. Composition of the fungal community

- Kenya experiences an erratic tropical climate characterized by periodic droughts, high humidity and high temperatures preceding harvests.
- The problem of aflatoxin exposure is expected to worsen with climate change, as the region becomes more prone to extreme weather - including drought and flooding.
- Drought conditions weaken the crop making it more vulnerable to fungal infection, while flooding compromises proper drying of the crop.

1.4.2.1 Key aflatoxin contamination risk factors for maize and other foods in Kenya

- Poor grain conditioning before storage
- Use of propylene storage bags
- Drying of grain on bare ground
- Insect infestation
- Inadequate drying before storage
  i. Fungal growth and aflatoxin contamination can occur within a few days if maize is not properly dried (to less than 13% moisture content) before storage
  ii. Adequately dry the maize immediately after harvest to <13% moisture content
  iii. Preventing contact of the maize with the soil
- Poor storage structures
- Poor transportation conditions
- Poor handling of produce
- Chronic poverty
- Poor storage conditions including:
  i. High humidity
  ii. High temperature
  iii. Insect damage
  iv. Poor aeration during drying and storage

  Storage in store that is not clean and dry
- Lack of sorting before storage
  i. Visibly mouldy, disfigured or damaged grains should be removed prior to storage

1.4.2.2 Challenge of aflatoxin testing of maize by traders

- There is an increasing number of food processing companies that test maize for aflatoxin levels before buying to avoid aflatoxin contamination in their products.
- But accurate testing remains a challenge because there is a lot of variation in aflatoxin across bags of maize, and even grains within a bag.
- In addition, testing for aflatoxin in maize at the factory gate poses unique challenges.
- When a consignment of maize is rejected by one company, it is sold to a company with less stringent requirements, or on the informal market.
- This implies that the lowest-cost food is often the most contaminated, and people with the least to spend are at the greatest risk of consuming unsafe food.
- At a larger scale, most of the maize consumed in Kenya is never tested for aflatoxin.
- This is because it is either purchased on the informal market, or consumed by households who have grown it.

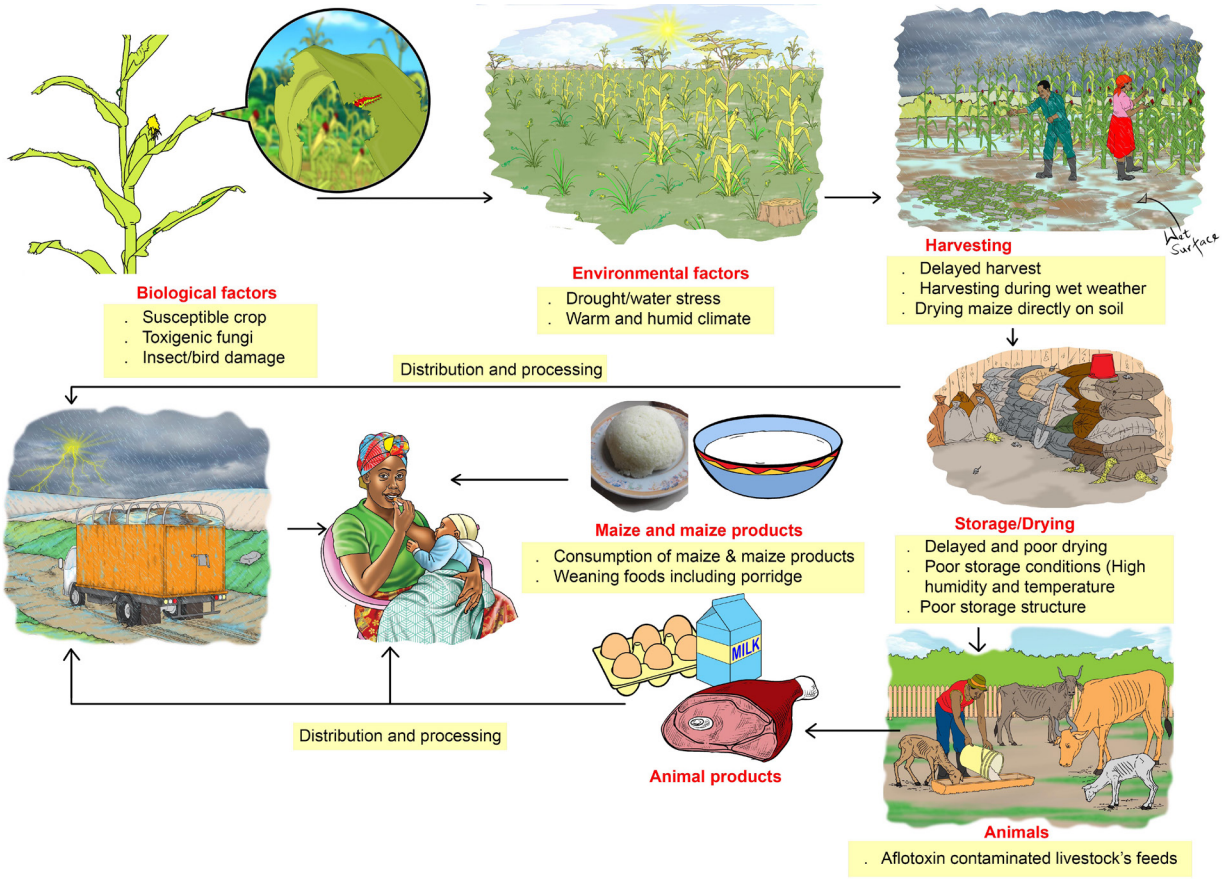

Figure 7: Diagrammatic representation of aflatoxin contamination of maize and avenues of human exposure to the toxin

1.4.3 Aflatoxin contamination of herbs and spices

- Because of their pre-harvest, postharvest, and storage conditions, herbs and spices are susceptible to contamination with mycotoxins.
- 5–10% of agricultural products in the world are contaminated by molds to the extent that

these products cannot be consumed by humans and animals.

- Drought, improper storage conditions and high humidity during storage can favour the growth of *Aspergillus* species in herbs and spices.
- Spices are mostly produced in humid and tropical countries, conditions which are conducive for fungal growth.
- In the recent past, contamination of herbs and spices with moulds and mycotoxins has gained attention globally.
- Among the herbs and spices mostly tested for contamination with aflatoxins in documented studies include coriander, chillies, red pepper, black pepper, medicinal herbs, cumin, fennels, cinnamon, basils, celery, paprika, ginger and garlic among others.
- Studies conducted in Kenya report on fungal and mycotoxin contamination in medicinal herbs from street vendors, herbal clinics and supermarkets.
- Herb preparations, including liquid, powder, capsule, lotion, cream or syrup have been reported to be contaminated with toxigenic fungi and aflatoxins.
- Majority of the value chain actors in the spice commodities operate through informal set ups that are characterized by post-harvest storage and handling challenges.
- Data gathering on aflatoxins in herbs and spices in Kenya has been sparse, leaving a gap in data that should inform decision making.
- Nevertheless, the following diagrammatic representation shows where contamination can occur for herbs and spices.

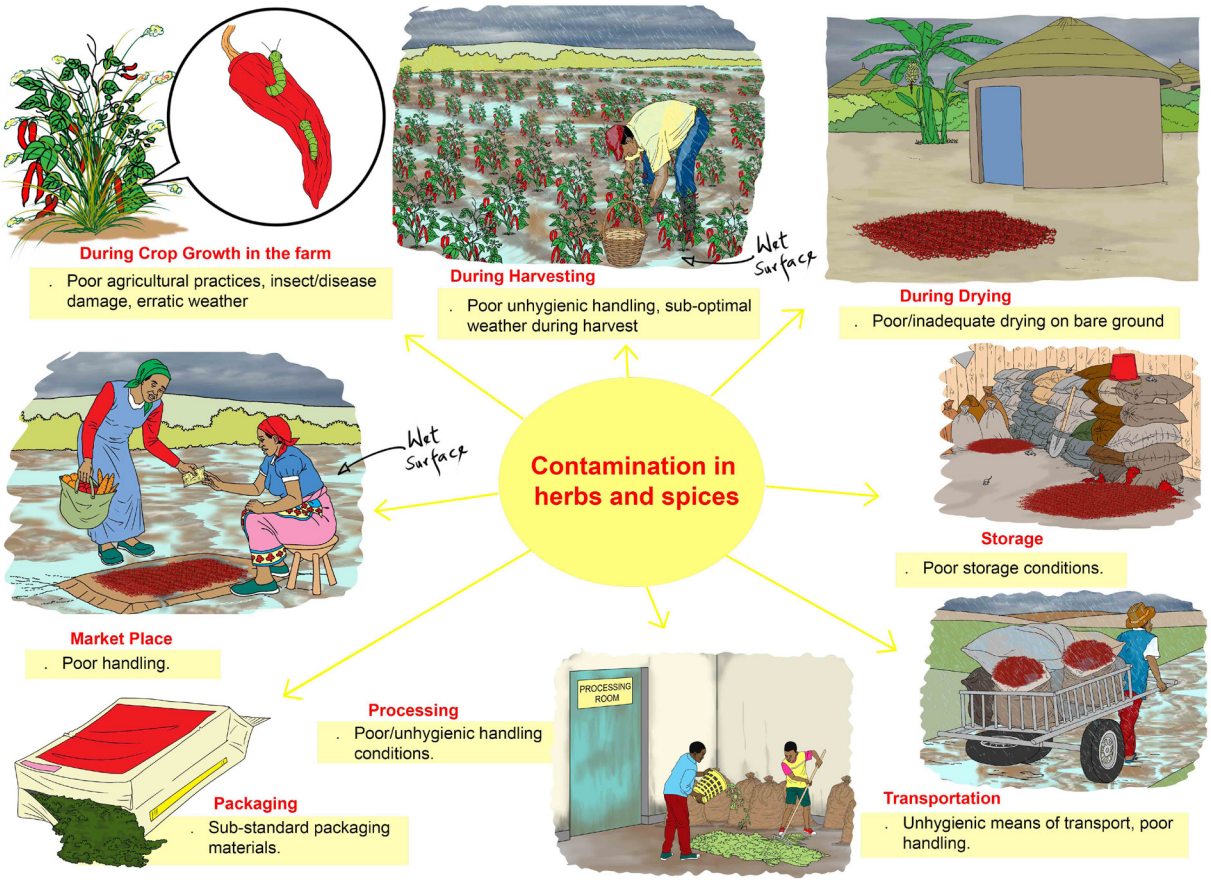

Figure 8: Critical factors and stages favouring fungal growth and aflatoxin contamination in herbs and spices value chains
Source: Costa *et al.*, 2019

1.4.4 Aflatoxin contamination of animal feeds

- Humans can be exposed to aflatoxins through consumption of animal products such as milk, eggs and meat.
- Exposure of animals to aflatoxins is usually through consumption of aflatoxin contaminated feeds.
- Maize is the major component of livestock and poultry feeds.
- This increases the risk of indirect human exposure to aflatoxins through regular consumption of animal products that contain aflatoxin residues.
- Elevated levels of aflatoxin B1 have been recorded in animal feeds in Kenya.
- The situation is exacerbated by animal feed processors and dairy farmers utilizing low quality grains (mould-damaged, rotten and insect infested) for the formulation of livestock rations.
- High levels of contamination of commercial animal feeds with aflatoxins (120.9 µg/kg and 49.7 µg/kg) have been reported in Kenya (Nyangaga, 2014).
- In addition, higher aflatoxin levels have been reported in processed than in non-processed feeds.
- This implies that some feed processing techniques (including handling and storage) predispose the feeds to aflatoxin contamination.

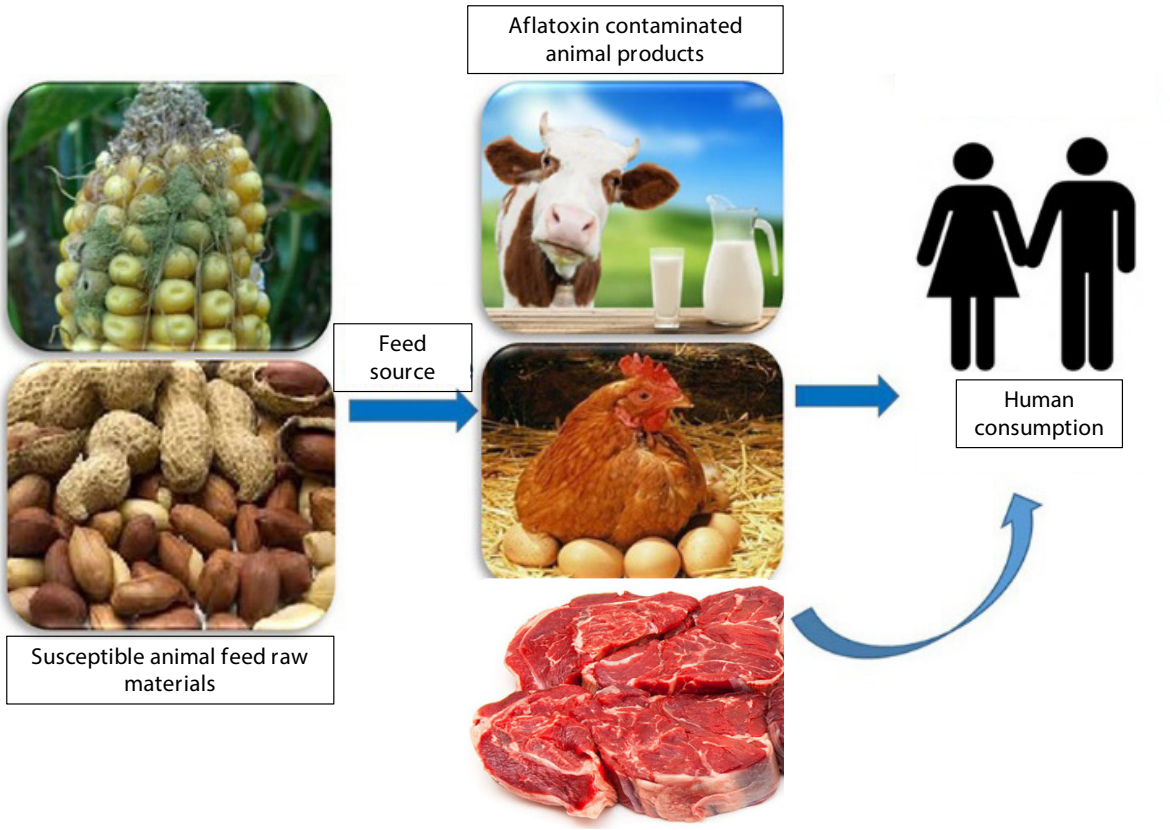

Figure 9: Diagrammatic representation of aflatoxin contaminated feeds as an avenue of exposure of humans to the toxins

1.5 Co-occurrence of aflatoxins with other mycotoxins

- Different types of mycotoxins can co-occur in foods, implying the possibility of compound health effects on the consumer due to synergistic action of the toxins.
- When aflatoxins co-occur with other mycotoxins in foods, there is an increased health risk of the consumer due to exposure to multiple toxins.
- Execution of control and management strategies of aflatoxins in foods and feeds should therefore target the other mycotoxins as well.
- In a study by Muriuki and Siboe (1995), two popular maize flour brands in Kenya were co-contaminated with aflatoxins B1 and B2, ochratoxin A and zearalenone.
- High (>20%) co-contamination of maize with aflatoxins and fumonisins has also been reported in: Siaya, Kakamega, Kisumu, Migori, Vihiga, Makueni; Meru, Machakos and Kitui counties (Kang'ethe *et al*., 2017).
- *Busaa* (a maize-based traditional beer), has been reported to be co-contaminated with aflatoxins, fumonisins and deoxynivalenol.
- Aflatoxins and fumonisins have also been reported to co-contaminate herbal preparations (liquid, oil, powder and capsule) sampled from Eldoret and Mombasa towns.
- In some instances, up to 100% of the samples (e.g. oily herbal samples from Mombasa) were contaminated with both mycotoxins.
- Co-occurrence of aflatoxins with A-trichothecenes, B-trichothecenes, fumonisins, zearalenone and ochratoxin A in animal feeds and feed ingredients have been reported in Kenya (Kemboi *et al*., 2020).
- In a study by Makau *et al*. (2016), both aflatoxin B1 and deoxynivalenol were detected in >50% of animal feed samples collected from actors in the informal sub-value chains of rural and peri-urban dairy systems in Nakuru County, Kenya.

CHAPTER 2: IMPACT OF AFLATOXINS ON HEALTH, TRADE AND FOOD SECURITY

2.1 Overview of impact of aflatoxins

- Presence of mycotoxins in grains, other staple foods and animal feeds has a great impact on human and animal health (Bennett and Klich, 2003).
- In response, over 100 nations have established maximum tolerable levels for aflatoxin in food.
- Some nations have set standards for 'total aflatoxins' (the sum of the concentrations of aflatoxin B1, B2, G1, and G2).
- Others regulate the most toxic and carcinogenic of the aflatoxins, aflatoxin B1 (AFB1).
- Others have standards for both AFB1 and total aflatoxins in foodstuffs.
- Additionally, several nations have set standards for aflatoxin M1 (AFM1): the metabolite of aflatoxin B1, which can be found in dairy products due to dairy animals' consumption of aflatoxin-contaminated feed.
- As a result, most countries have set the maximum acceptable limits for presence of certain mycotoxins in human food and animal feed.
- In addition, regulations on aflatoxin levels have an impact on regional and international trade as most countries, especially the developed ones, cannot allow an import of food or feeds that have mycotoxins beyond the specified levels.
- The regulations have been set based on the risk assessments that have been carried out on humans and animals for the specific mycotoxins.

2.2 Effect of aflatoxins on human health

- Once ingested through food and animal feeds, aflatoxins have major effects on humans and animals, respectively.
- While the bulk of human exposure to aflatoxins is through oral consumption of contaminated foods.
- Human beings can also be exposed to aflatoxins through skin penetration upon contact and inhalation.
- Aflatoxin M1 has been found to be secreted in milk, increasing the exposure to humans through milk consumption.
- Continued exposure of aflatoxins to humans and domestic animals in small amounts leads to chronic aflatoxicosis characterized by:
    i. Impaired food conversion
    ii. Stunted growth in children
    iii. Immune-system suppression
    iv. Cancer
- Acute exposure to aflatoxins may result in death.
- Uterine exposure of unborn children to aflatoxins has been associated with poor development resulting in birth defects such as low birth weight and small head circumference.

Table 2: Per capita food/aflatoxin consumption in Kenya compared to other East African countries

Foodstuff	Country	Per capita food consumption (g/ person/day)	Mean aflatoxin content (µg/kg)
Maize	Kenya	400	131.7
	Uganda	69	9.7
	Tanzania	405	49.7
Groundnuts	Kenya	1	-
	Burundi	65	12.5
	Uganda	65	15.0
	Tanzania	65	25.1
Milk	Kenya	750ml	0.8
	Tanzania	750ml	0.9

Adapted from the report by the East African Community's aflatoxin working group in April 2013 (Dar es Salaam-Tanzania, EAC/TF/405/2013)
* µg/kg is equivalent to parts per billion (ppb)

- Exposure to aflatoxins in Kenya starts at an early age with infants being breastfed on contaminated milk.
- The stunting rates in children younger than 5 years have been reported in Makueni (28.7%) and Nandi counties (18.5%); while the respective severe stunting rates in the two counties were 30.7%, 16.5%, respectively (Kang'ethe *et al*., 2017).
- These stunting rate in Makueni County was above the national average of 26%, while the severe stunting rates in the two counties were above the national average of 11% (KDHS, 2015).
- The underweight children (<5years) were 2.9% and 14.6% in Nandi and Makueni counties, respectively, with respective proportions of 3.9% each for severe underweight.
- The national average for underweight is 11% and 2% for severe underweight (KDHS, 2015).
- Exposure to high levels of aflatoxins have led to death due to aflatoxicosis.
- Cases of serious aflatoxicosis have been reported in Kenya in the last two decades, with the most severe outbreak being reported in 2004 where 125 people died in Eastern and Central regions of Kenya.
- About 317 people were affected by the outbreak which was as a result of aflatoxicosis from contaminated maize.
- The 2004 outbreak was followed by other smaller outbreaks in 2005 and 2006 leading to 53 deaths.
- Chronic exposure to aflatoxins has been associated with the country's growing burden of cancer.
- Aflatoxin B1 is a highly potent toxin with carcinogenic effects and has been associated with liver cancer in both humans and animals.
- Primary liver cancer is one of the most prevalent human cancers in developing countries.
- Epidemiological studies support the association between the incidence of hepatocellular carcinoma and consumption of foods contaminated with aflatoxin.
- It is currently known that there are synergistic effects between aflatoxin and hepatitis B virus (which causes jaundice) infection causing primary liver cancer.
- Long periods of exposure to aflatoxins in humans affects food digestion and absorption leading to stunted growth and in some cases, it affects the immune system.

Table 3: Aflatoxicosis outbreaks reported in Kenya over time

Affected group	Number affected	Region	Toxin source	Health/trade effects	Year	Reference
Humans, dogs	Not confirmed	Eastern Kenya (29 districts)	Suspected contaminated maize	Price fluctuation, grain trade breakdown, unconfirmed dog deaths in Nairobi	2010	Muthomi *et al*., 2010
Humans	5	Kibwezi, Kajiado, Mutomo	Maize	3 hospitalized, 2 deaths	2008	Muthomi *et al*., 2009
Humans	4	Kibwezi, Makueni	Maize	2 deaths in Makindu town, Mukueni County	2007	Wagacha and Muthomi, 2008
Humans	20	Makueni, Kitui, Machakos, Mutomo	Contaminated maize	Acute poisoning, 10 deaths in Mutomo and 9 in Makueni	2006	Daniel *et al*., 2011
Humans	75	Machakos, Makueni, Kitui	Maize	Acute poisoning, 75 cases, 32 deaths	2005	Azziz-Baumgartner *et al*., 2005
Humans	331	Eastern/ Central Machakos, Kitui, and Makueni areas	Contaminated maize	Acute poisoning, 125 deaths	2004	Daniel *et al*., 2011
Humans	6	Thika	Mouldy maize	6 deaths	2003	Lewis *et al*., 2005
Poultry, dogs	Large numbers	Coast	Contaminated feed	150 deaths	2002	Probst *et al*., 2007
Humans	3, 26	Meru North, Maua	Mouldy maize, contaminated maize	Severe liver damage, 16 deaths	2001	Probst *et al*., 2007
Humans	3	Meru North	Maize	Acute effects, 3 deaths	1998	Mutegi *et al*., 2018
Poultry	Large numbers	Kenya	Imported maize	Deaths	1984/ 1985	CIMMYT/ UNDP/ USAID, 1986; FAO,1988
Humans	12	Machakos	Poorly stored maize	Deaths	1981	Ngindu *et al*., 1982
Poultry, dogs	Large numbers	Nairobi, Mombasa, Eldoret	Poorly stored feed	Deaths	1977/ 1978	Muraguri *et al*., 1981
Ducklings	16,000	Rift Valley	Peanut ration	Deaths	1960	Peers and Linsell, 1973

Years are those in which the aflatoxicoses occurred rather than the years the data were published.

2.3 Effect of aflatoxins on livestock health

- Ingestion of contaminated feeds by livestock leads to losses incurred from diseases, morbidity and mortality.
- Chronic exposure to aflatoxins results in:
  i. Suppression of the immune system
  ii. Impaired growth
  iii. Reduced productivity
  iv. Reduced reproductive efficiency
  v. Reduced feed conversion efficiency
  vi. Anaemia
  vii. Reduced weight gain
  viii. Jaundice
  ix. Laying chicken may also develop enlarged fatty liver
- Animals fed on feed contaminated with aflatoxins also produce aflatoxin contaminated products such as eggs, milk and meat.
- Such animals have significantly reduced live weight, which is a direct and frequent loss to poultry producers.
- Acute exposure of animals to high levels of aflatoxin can lead to death.
- High doses fed to young ducklings also lead to death.
- Low doses fed to pigs, cows and sheep over a long period of time results in:
  i. Body weakness
  ii. Intestinal bleeding
  iii. Reduced feeding
  iv. Frequent abortions
  v. Reduced growth
  vi. Nausea

- Outbreak of aflatoxicosis was first reported in turkeys in the UK in 1960.
- Affected birds lost appetite, became lethargic and died within 7 days from the onset of symptoms.
- Aflatoxins were eventually recovered in East Africa (Kenya and Uganda) in peanut rations that caused substantial losses in ducklings.
- In 1994, over 200,000 chickens died around Hyderabad city in India due to inclusion of aflatoxin-contaminated maize and groundnut meal in their feed.
- There are also several reports on aflatoxicosis outbreaks in cattle.
- Initial symptoms appear as lesions, ultimately leading to diffuse cirrhosis of liver.
- Sheep do not appear to be susceptible to aflatoxins.
- Dogs and pigs are highly susceptible while mice are to a certain extent resistant to aflatoxins.
- It is important to note that acute toxicity due to aflatoxins for any given species of animal is influenced by factors such as:
  i. Age - Young animals tend to be more sensitive than mature animals
  ii. Size
  iii. Breed
  iv. Health condition of the animal
  v. Diet composition

2.4 Effect of aflatoxins contamination on local, regional and international trade

- Aflatoxin contamination of grain is an impediment to quality food production and trade across the globe.
- Aflatoxin contamination in food crops limits domestic, regional, and international trade in agricultural produce and results in economic losses.
- This is encountered through rejection of consignments and finished products as well as revocation of business operating permits.
- The cost of surveillance and destruction of condemned produce and products is also a direct cost to the concerned agencies.
- The direct economic impact of aflatoxin contamination in crops results mainly from:
    i. Reduction in marketable volume
    ii. Loss in value in the national market
    iii. Inadmissibility or rejection of products by the international market

- The cost of surveillance and destruction of condemned produce and products is also a direct cost to the concerned agencies.
- The regulations on acceptable aflatoxin levels can result in foregone trade revenues arising from the increased cost of meeting the set standards including:
    i. Cost of testing
    ii. Rejection of shipments
    iii. Eventual loss of admissibility into foreign markets

- Direct economic loss from aflatoxin contamination in crops results from reduced marketable volume as well as loss in value for both local and international markets.
- While it may seem that tighter phytosanitary measures imply more costs than benefits, once stakeholders understand the costs of non-compliance and bear them as a financial cost, greater benefits will arise including larger and more stable markets as well as reduced burden of disease.

2.4.1 Effect of aflatoxin contamination on local trade

- Aflatoxin contamination in food crops limits domestic trade in agricultural produce and results in economic losses.
- This is encountered through rejection of finished products as well as revocation of business operating permits.
- Estimates have been made in various studies on the cost of aflatoxin to farmers.
- Besides the direct costs, others are associated with livestock illnesses due to aflatoxin exposure.
- In the recent past, KEBS has banned various products from the market due to lack of compliance with the 10µg/kg acceptable threshold for total aflatoxin.
- For example, in August 2021, KEBS banned 27 maize and porridge flour products due to aflatoxin levels above the acceptable threshold.
- KEBS has previously ordered manufacturers to recall seven brands of peanut butter from the market citing high levels of aflatoxin.
- However, there are circumstances where enforcement of compliance with the set standards faces challenges; for example, at the peak of the COVID-19 pandemic in 2020, a period when the country was also experiencing maize shortage.
- Besides condemning the non-compliant products, KEBS may revoke the operating licenses of the responsible company.

- Reinstatement of the licenses is pegged on compliance with quality standards including levels of aflatoxins.
- Once products are found to be non-compliant, they have to be withdrawn from the market, a cost that is borne by the processor or the trader.
- Further costs accrue from disposal or destruction of the condemned products including the cost of incineration which is exorbitant and might not be affordable for most of the traders.
- Despite efforts by KEBS to enforce compliance, a further challenge is that a large portion of the population gets their food from the informal market including small-holder farmers and millers.
- Most of the actors in the informal trade do not have the capacity to carry out the required practices to prevent contamination of food with aflatoxins.
- Even where it might be determined that the product is contaminated with aflatoxin, small-scale millers may be unable to absorb the cost accruing from the disposal and destruction of the product.
- They may therefore release it into the market through the informal market.

2.4.2 Effect of aflatoxin contamination on regional trade

- There is vibrant cross-border trade between Kenya and her neighbours.
- Within the East Africa Community (EAC), there is uniform standard of 10µg/kg for total aflatoxin in food products.
- Failure to comply with the set standard results in refusal for entry of the non-complying product, resulting in producers and traders incurring losses.
- However, enforcement of compliance with the aflatoxin threshold within EAC has at times been challenging.
- For example, when KEBS banned seven brands of peanut butter in 2019, Uganda and Rwanda immediately banned the same products from their respective markets.
- Even after compliance, it becomes harder for such products to access the regional market as they would be subjected to stricter scrutiny, compared to compliance verification in the local market.
- Status of aflatoxin contamination has at times threatened regional trade with retaliation measures from partner trading countries.
- Allowing importation of agricultural produce (especially maize) from neighbouring countries makes maize produced in Kenya less competitive due to the comparatively higher cost of production.
- This negatively affects the livelihoods of Kenyan farmers and tilts the balance of agricultural trade in favour of the neighboring countries.
- Besides the impact of aflatoxin contamination on trade, it has other important consequences such as adverse health impacts in African populations as a result of inability to export aflatoxin contaminated foodstuffs.
- Such foodstuffs end up being consumed locally.

2.4.3 Effect of aflatoxin contamination on international trade

- Aflatoxin contamination of grain is an impediment to quality food production and trade across the globe.
- Over 100 nations have established maximum tolerable levels for aflatoxin in food, typically expressed in parts per billion (ppb).
- Some countries have different limits depending on the intended use, the tightest applying to human consumption and export market.
- The highest acceptable levels apply to industrial products.

- In addition, some countries have set standards for 'total aflatoxins' (the sum of the concentrations of aflatoxin B1, B2, G1, and G2).
- Others regulate the most toxic and carcinogenic of the aflatoxins, aflatoxin B1 (AFB1).
- Other countries have standards for both AFB1 and total aflatoxins in foodstuffs.
- Additionally, several nations have set standards for aflatoxin M1 (AFM1): the metabolite of aflatoxin B1, which can be found in dairy products due to dairy animals' consumption of aflatoxin-contaminated feed.
- These regulations can result in foregone trade revenues arising from:
    i. Increased cost of meeting the standards
    ii. Increased cost of testing
    iii. Rejection of shipments in case of failure to comply with the standards of the importing country
    iv. Loss of admissibility into foreign markets
    v. Extra cost of disposal of the contaminated products

- Foreign markets are attractive to local producers because they offer premium prices for macadamia nuts, herbs and spices produced in the country.
- However, there are instances where traders export products that comply with the set standards and distribute lower quality and con-compliant products to the local market.
- In some cases, developing countries have experienced market losses due to persistent mycotoxin problems or the imposition of new, stricter regulations by importing countries.
- It has been estimated that adoption of a uniform aflatoxin standard based on international Codex Alimentarius Commission (Codex) guidelines would increase trade of cereals (grains) and nuts by more than $6 billion, or more than 50 percent, compared with the divergent standards.
- In the international market, products that do not meet the aflatoxin standards are:
    i. Rejected at the border
    ii. Rejected in channels of distribution
    iii. Assigned a reduced price
    iv. Diverted to nonhuman or even non-fee uses.

2.5 Effect of aflatoxin contamination on food security

- Aflatoxin contamination of key staples such as maize and groundnuts can affect each of the four pillars of food security - availability, access, utilization, and stability.
- Contamination in staple foods such as maize, sorghum and groundnuts can directly reduce availability of food.
- Producers of the affected crops may also earn less because of:
    i. Product rejection
    ii. Reduced market value
    iii. Inability to gain access to the higher-value international trade and the formal market

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- Lower farmer income in turn limits ability to purchase food for the household.
- This results in reduced access to food.
- Contamination reduces use options for the affected produce through complete rejection or need to put it to other safe uses.

Table 4: Estimated losses arising from aflatoxin contamination of food and feed in Kenya

	Food/ Feed	%below standards	Kilo tonnes produced	Consumption kg/per capita/ year	Discarded [kg]	Would have fed
Human	Maize	22	3339	84	734,580,000	8,745,000
	Millet	6	21	2.5	1,260,000	504,000
	Sorghum	7	125	5	8,750,000	1,750,000
	Milk (cow)	10	3733	110	373,300,000	3,393,636
Animal	Feed farmer	73	806	–	588,380	–

Data shows production and consumption figures for the years 2015 and 2016.
Source: Sirma et al., 2018

CHAPTER 3: MANAGEMENT OF AFLATOXINS IN GROUNDNUTS, MACADAMIA NUTS, MAIZE, HERBS AND SPICES VALUE CHAINS

3.1 Management of aflatoxin contamination in the various value chains

Management of aflatoxins in the various value chains requires interventions at the pre-harvest, post-harvest stage and cross cutting approaches through policy and regulation. The interventions should therefore address all aspects of the food continuum from farm to plate. In principle, effective aflatoxin management should focus on the following:

i. Crop health

It is important to maintain a healthy crop during production, which requires the following practices:

a. Planting high quality/certified seed of improved varieties
b. Proper selection of the production sites (well drained fields that are not prone to flooding)
c. Good agricultural practices (GAP)
   - Early planting
   - Improving soil fertility
   - Timely weeding
   - Effective and timely pest control
   - Irrigation at critical growth stages where necessary
   - Timely harvesting at physiological maturity under dry weather conditions
   - Avoiding mechanical damage during cultivation and harvesting for nut crops

ii. Proper and timely drying after harvest

- High moisture in plant tissues favours proliferation of fungal pathogens posing a risk of aflatoxin contamination.
- It is important to adequately dry the produce immediately after harvest.
- Since aflatoxin producing fungi reside in the soil and in plant debris, the drying should not be done directly on the ground to avoid further exposure of the produce to fungal inoculum.
- For safe storage of most of the grains (such as groundnuts and maize), dry herbs and spices they should be dried to <13% moisture content.
- Storage of the produce with >13% moisture content would favour proliferation of fungal pathogens and pre-dispose the produce to aflatoxin contamination.

iii. Storage temperature and relative humidity

- Although the initial infection of crops with aflatoxin producing fungi occurs in the field, *Aspergillus* species proliferate during storage.
- Their growth during storage is favoured by high temperature and relative humidity (RH).
- There is therefore need to control temperatures and RH during storage to arrest fungal growth.
- This can be achieved by ensuring the following:
  a. Proper drying of the produce before storage
  b. Ensuring the store is dry without leaking roofs
  c. Proper aeration of the store – As agricultural produce respire, they release moisture which should not accumulate in the store
  d. Use of recommended storage materials – where the produce is stored in bags or containers, it is important to use the recommended material.

- The polypropylene (nylon) bags which are commonly used by farmers and traders should be avoided since they hold and promote accumulation of heat and moisture.
- Sisal and hermetic containers are recommended for storage of properly dried produce.

iv. Cross-cutting approaches

- Effective management of aflatoxin contamination requires policy and regulation intervention.

a. Policy intervention

- There is need for government policy to guide in implementation processes on food safety and ensure that food safety management is adequately addressed.
- The policy must also ensure that there are mechanisms to support implementation of the activities/interventions proposed in the management and control of aflatoxins in the groundnuts, macadamia nuts, maize, herbs and spices value chains.

b. Regulation

- There is need to support competent agencies to undertake surveillance through strengthening human capacity, processing and testing for aflatoxins.
- This can be achieved through
- Enhancement of the aflatoxin physical testing infrastructure
- Training to build the competence of the personnel to undertake the aflatoxin testing analysis
- Provision of funds to guarantee availability of rapid testing kits for routine surveillance

3.2 Management of aflatoxin contamination in groundnuts

Pre-harvest management practices

- Plant improved varieties
- Good agricultural practices such as;

i. Improving soil fertility

ii. Irrigation to reduce water/drought stress

iii. Regular and timely weeding

iv. Pest control

v. Timely harvesting (when crop is at physiological maturity, harvesting when it is dry)

vi. Ensure you have the right storage facilities before harvesting

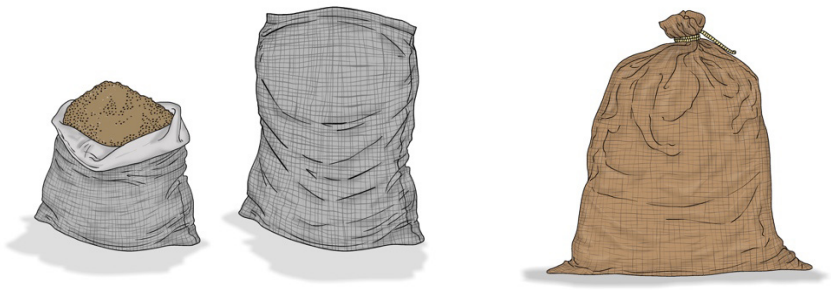
Post-harvest management practices

- Adequate and timely drying
- Transportation in dry, well aerated, cool conditions

Avoid drying groundnuts on the ground (dry them on tarpauline)

Avoid storing groundnuts on the ground

Storing groundnuts in the recommended storage bags



- Storing groundnuts in dry, well aerated, cool stores
- Controlling insects in the store
- At the processors' level;
    i. Sorting before shelling
    ii. Grading after shelling
    iii. Avoid using grade-outs
    iv. Practice good manufacturing practices
    v. Have a Hazard Analysis Critical Control Point (HACCP) system in place

3.3 Management of aflatoxin contamination in macadamia nuts

Pre-harvest management practices

- Plant improved varieties
- Good agricultural practices such as;
    i. Improved soil fertility
    ii. Pest control
    iii. Timely harvesting (when crop is at physiological maturity, harvesting when it is dry)

Post-harvest management practices

- Adequate and timely drying
- Transporting macadamia nuts in dry, well aerated, cool conditions
- Hulling of nuts should begin as soon as possible after harvest
- Avoid drying or storing macadamia nuts on the ground
- Storing macadamia nuts in dry, well aerated, cool stores
- Storing macadamia nuts without cracking
- Controlling insects in the store

- At the processing stage;
    i. Personnel involved in all stages of macadamia nuts processing should:
        a. Maintain a high degree of personal cleanliness
        b. Wear suitable protective clothing
        c. Be trained in food hygiene and general sanitation procedures to a level appropriate to the operations they are to perform in the processing facility
    ii. Areas where raw materials are received or stored should be separated from areas in which final product preparation or packaging is conducted as to preclude contamination of the finished product.
    iii. Processors should establish good quality control procedures at every step in the

processing sequence to avoid cross contamination of aflatoxins between various lots of nuts during processing.

i. Various visual (manual) and/or electronic sorting techniques should be used to remove foreign materials and nuts with various defects.
ii. The finished processed products (raw, shelled or in-shell, bulk or consumer ready) should be of the appropriate moisture.
iii. The finished processed products should also be packaged so as to maintain their quality under normal transportation and storage conditions without significant deterioration by decay, mould, or enzymatic changes.
iv. It is desirable that each plant has access to quality control facilities.
v. The amount and type of such control will vary depending on different nut products as well as the needs of management.

3.4 Management of aflatoxin contamination in herbs and spices

Pre-harvest management practices

- Plant improved varieties
- Good agricultural practices such as;
  i. Improved soil fertility
  ii. Irrigation to reduce water/drought stress
  iii. Regular weeding
  iv. Pest control

  Timely harvesting (when crop is at physiological maturity, harvesting when it is dry)

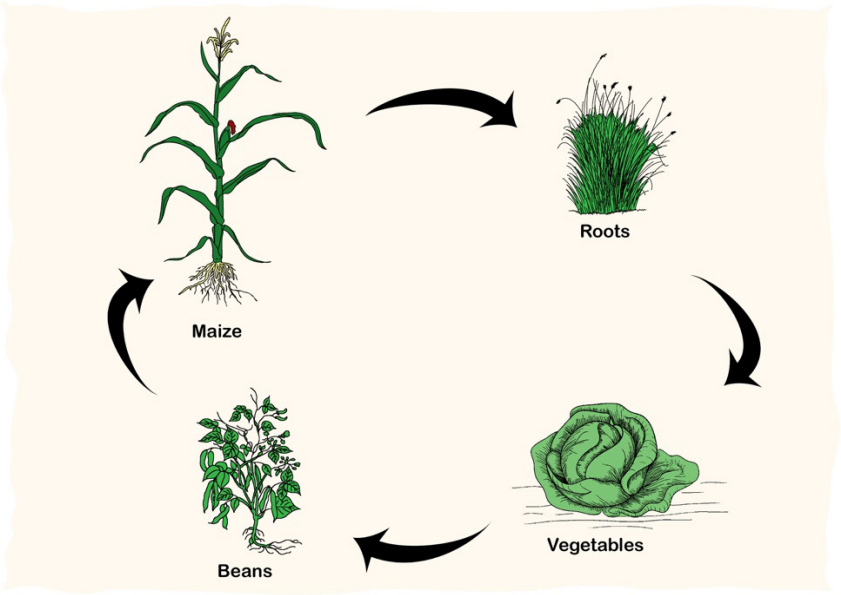
Post-harvest management practices

- Adequate and timely drying
- Avoid drying or storing herbs and spices on the ground
- Transporting herbs and spices in dry, well aerated, cool conditions adhering to cold storage where necessary
- Storing herbs and spices in dry, well aerated, cool stores

3.5 Management of aflatoxin contamination in maize

Pre-harvest management practices

- Plant improved varieties – fast maturing, drought tolerant
- Good agricultural practices such as;
  i. Practice crop rotation with non-cereal crops such as legumes, root and tuber crops, onions, solanaceous crops (tomatoes, potatoes, capsicums etc)



ii. Improving soil fertility
iii. Early planting
iv. Regular weeding
v. Control of aflatoxin producing fungi e.g. use of biological control

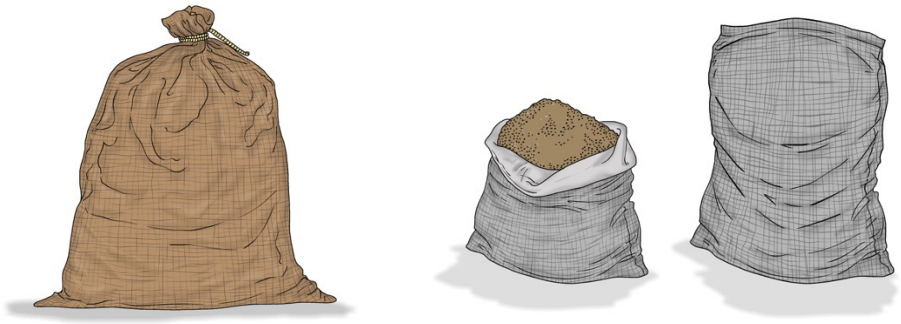
vi. Pest control
vii. Timely harvesting (when crop is at physiological maturity, harvesting when it is dry)
viii. Timely and proper drying of maize

Ensure you have the right storage facilities

Post-harvest management practices

- Adequate and timely drying
- Transporting in dry, well aerated, cool conditions

- Avoid drying or storing maize on the ground
- Storing maize in dry, well aerated, cool stores
- Storing maize in the recommended storage bags or containers



- Avoiding mixing freshly harvested maize with grains from previous seasons
- Controlling insects and rodents in the store
- Sorting maize grains before milling
- Storing maize flour in dry, well aerated, cool conditions

3.6 Emerging technologies in aflatoxin management

3.6.1 Nixtamalization as a method of reducing aflatoxin contamination in maize

- Nixtamalization is a traditional maize preparation process in which dried kernels are cooked and steeped in an alkaline solution, usually water and food-grade lime (calcium hydroxide).
- After that, the maize is drained and rinsed to remove the outer kernel cover (pericarp) and milled to produce dough that forms the base of numerous food products, including tortillas and tamales.
- The cooking (heat treatment) and steeping in the alkaline solution induce changes in the kernel structure, chemical composition, functional properties and nutritional value.
- For example, the removal of the pericarp leads to a reduction in soluble fiber, while the lime cooking process leads to an increase in calcium content.
- The process also leads to partial starch gelatinization, partial protein denaturation - in which proteins present in the kernel become insoluble - and a partial decrease in phytic acid.

Benefits of processing maize by nixtamalization:

In addition to altering the smell, flavor and color of maize products, nixtamalization provides several nutritional benefits including:

- Significantly reduced presence of mycotoxins such as aflatoxins and fumonisins
- Increased bioavailability of vitamin B3 niacin, which reduces the risk of pellagra disease
- Increased calcium intake, due to its absorption by the kernels during the steeping process
- Increased resistant starch content in food products, which serves as a source of dietary fiber
- Increased bioavailability of iron, which decreases the risk of anemia
    i. These nutritional and health benefits are especially important in areas where maize is the dietary staple and the risk of aflatoxins is high.
    ii. Removal of the pericarp helps reduce aflatoxin contamination levels in maize kernels by up to 60% when a load is not highly contaminated.
    iii. Additionally, nixtamalization helps to control microbiological activity and thus increases the shelf life of processed maize food products.

Figure 10: Key steps of the traditional nixtamalization process (Graphic: Nancy Valtierra/CIMMYT)

3.6.2 Biological control

- Biological control is a promising technology in the management of aflatoxins in key staple foods such as maize and groundnuts.
- An example of such a biocontrol product is Aflasafe KE01™ (Aflasafe[1]), which is registered in Kenya for the control of aflatoxins in maize.
- As of 2022, there are on-going field trials to have the product registered for aflatoxin control in groundnuts and sorghum.
- Aflasafe is a natural, fungal, anti-aflatoxin product that consistently reduces aflatoxin contamination by between 80 and 100%, when used alongside good agricultural practices, and when all the facilitative conditions are met.
- The active ingredient in Aflasafe are four non-aflatoxin producing strains of *A. flavus* that are native in Kenya.
- Sterilized sorghum grains are used as the carrier material.
- The variety of four atoxigenic *Aspergillus* strains makes Aflasafe resilient and effective across a wide range of conditions and environments.
- Aflasafe only needs to be applied once during a cropping season to be effective.
- Farmers apply about 10kg of Aflasafe on each hectare by hand broadcasting - throwing handfuls of Aflasafe onto the fields – 2 to 3 weeks before crop flowering/tussling.
- It is therefore important for farmers to know when the crop is due to flower for proper timing of the product's application.

1 https://aflasafe.com/2017/06/14/successfully-combatting-aflatoxin-in-kenyas-food-with-aflasafe-on-a-large-scale/

- This is necessary because flowering varies depending on the variety and geographical location.
- Application of Aflasafe should ideally be timed to coincide with rainfall and moist soils that help the fungus to sporulate.
- At the time of the crop growth when Aflasafe is applied, the population of aflatoxin producing *Aspergillus* is still low.
- After the product's application, the atoxigenic *Aspergillus* starts producing spores on the sorghum grains, which serve as a food source.
- These spores spread throughout the field.
- Because they arrive early, and with a food source, they are able to establish themselves well ahead of any other fungal strains – including aflatoxin producing fungi - residing in the field.
- As the maize (and other plants) begin to flower and later develop grains, the friendly fungi colonize these too, excluding other strains from colonizing the plant.
- This means that the vast majority of *Aspergillus* fungi living on the growing crop are safe Aflasafe strains that do not produce aflatoxin.
- In contrast, if Aflasafe is not used, as crops grow and mature they are colonized by the fungi present in the field, including aflatoxin producing *Aspergillus*.
- In addition, Aflasafe has been shown to provide protection to treated crops at post-harvest stages including during harvest, transportation and storage.
- However, it is important to note that Aflasafe works best in combination with other good practices such as proper drying and storage.
- With repeated Aflasafe applications, the composition of the fungal community in a field begins to shift, with the non-toxin-producers becoming more predominant.
- This positive effect spills over into neighbouring fields too.
- Although Aflasafe should be applied each cropping cycle to be effective, there is an ongoing benefit across seasons.

3.6.3 Use of ozone in aflatoxin management

- Gaseous ozone is effective in reducing fungal and mycotoxin contamination in grains.
- Therefore, ozone can be used to treat grain contaminated with mycotoxins including aflatoxins.
- Ozone kills the aflatoxin producing moulds and also breaks down the aflatoxins.
- Such grains are safe for human or animal consumption.
- In addition, ozone can also be used as a fumigant to treat grain, to prolong shelf life, and to restore damaged grains.
- Ozone use in grain is a cost effective alternative and an organic chemical free method to treat grain.
- The penetration and adsorption of ozone depends on:
    i. Concentration
    ii. Exposure time
    iii. Flow rate
    iv. Temperature
    v. Grain characteristics
    vi. The presence of insects or mircobes on the seed surface
- The primary benefits of ozone include:
    i. Does not damage grain
    ii. Easy to implement
    iii. Destroys mycotoxins (e.g. aflatoxins and deoxynivalenol)
    iv. Stops mould growth
    v. Environmentally friendly
    vi. Works as a fumigant and insecticide

vii. leaves no residue on the food
viii. Does not cause nutritional changes in the food

3.7 Hazard Analysis Critical Control Points

Hazard Analysis Critical Control Points (HACCP) is a management system in which food safety is addressed through the analysis and control of biological, chemical and physical hazards in the following stages:

i. Raw material production
ii. Procurement and handling
iii. Manufacturing
iv. Distribution
v. Consumption of the finished product

- It is therefore a structured, systematic approach for the control of food safety throughout the commodity system from the plough to the plate.
- HACCP also identifies, evaluates and controls hazards which are significant for food safety.
- It requires a good understanding of the relationship between cause and effect in order to be more pro-active and it is a key element in Total Quality Management (TQM).
- HACCP builds on the foundations of well-established quality management systems such as:
    i. Good Agricultural Practice (GAP)
    ii. Good Manufacturing Practice (GMP)
    iii. Good Hygienic Practice (GHP)
    iv. Good Storage Practice (GSP)
- Specifically, HACCP is designed for use in all segments of the food industry from growing, harvesting, processing, manufacturing, distributing, and merchandising to preparing food for consumption.
- Food safety systems based on the HACCP principles have been successfully applied in food processing plants, retail food stores, and food service operations.
- Increasingly, regulatory bodies have recognized the usefulness of this tool and its 'principles' have been incorporated into legislative requirements by both the EU (in the General Hygiene regulations for managing food safety (93/43/EEC)), and the United States Federal Department of Agriculture (CPR - 123).
- The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) provided guidelines on HACCP including generic plans and decision trees in 1992.
- The Codex Alimentarius Commission adopted the HACCP system at its twentieth session in 1993.
- HACCP systems can be incorporated into other quality assurance systems such as the ISO 9000 series.
- Although conceived as a food safety system for both agricultural and processing systems, HACCP has found most application in the latter.
- This is primarily because it is much easier to apply a HACCP system in a factory where there is a single management or 'owner', and where it is possible to completely prevent a food safety hazard, or eliminate, or reduce it to an acceptable level.
- In the commodity system, there are often many disparate 'owners' of the commodity as it passes from the farm to the consumer, and complete control may be unobtainable.
- For successful implementation of a HACCP plan, top management must be strongly committed to the HACCP concept as it provides company employees with a sense of the importance of producing safe food.

HACCP pre-requisite programs

- Pre-requisite programs such as GAP, GMP and GHP must be working effectively within a commodity system before HACCP is applied.
- If these pre-requisite programs are not functioning effectively, then the introduction of HACCP will be complicated resulting in a cumbersome, over-documented system.

a. Good Agricultural Practices

Primary Production

- Primary food production should be managed to ensure that food is safe and wholesome for the consumer.
- It is essential that certain ground rules are followed.
- For example, land used for crop production should be fit for purpose and should not have previously been contaminated with heavy metals, industrial chemicals or environmental waste.
- Such hazards will be transferred into the food chain rendering the commodity unfit for human consumption.
- Farmers should control production so that contamination of the crop, proliferation of animal and plant pathogens and pests, do not compromise food safety.
- Good Agricultural Practices and Good Hygienic Practices, where appropriate, should be adopted to make sure that the harvested commodity will not present a food hazard to the consumer.

Good Storage Practices (GSP)

- GSP should be followed when the commodity is stored on the farm.
- Good Storage Practices should also be followed for storage throughout the commodity system.

b. Good Manufacturing Practices

Establishment Design and Facilities

The structure and location of a processing plant needs to be considered in relation to the nature of operations and risks associated with them.

- Food premises should be designed to minimize possibilities of contamination of commodity or product.
- Design and layout should permit maintenance, cleaning and disinfection of the site to minimize airborne contamination.
- All surfaces that come into contact with food should be non-toxic, as well as being easy to maintain and clean in order to prevent any additional contamination.
- Suitable facilities should exist for temperature and humidity control, when required.
- Effective measures should exist to prevent access by pests.

Control of Operation

Effective control measures should be in place to reduce the risk of contamination of the commodity or food supply such that it is safe and fit for purpose:

- Adequate time, temperature or humidity controls
- Food grade packaging
- Potable water supplies
- Maintenance of equipment

Maintenance and Sanitation

Procedures and work instructions should exist to demonstrate:

- An adequate level of maintenance of an establishment
- Efficient practices for cleaning
- Waste management
- Pest control

Overall, these operations will support the ongoing control of potential food hazards that may contaminate food.

Personnel Hygiene

- Measures need to be in place to ensure that food handlers do not contaminate food.
- This objective can be attained by maintaining an appropriate level of personal cleanliness and following guidelines for personal hygiene.

Transportation

- The method of transportation should be such that measures are taken to prevent any contamination or deterioration of the commodity.
- Commodities or products that need to be transported in certain environments should be appropriately controlled e.g. chilled, frozen, or stored under specific humidity levels.
- Containers and conveyors used for transporting food need to be maintained in good condition and be easy to clean.
- Containers used for bulk transfer should be designated and marked specifically for food use only.

Training

- All food handlers should be trained in personal hygiene, as well as in the specific operation with which they are working, to a level commensurate with their duties.
- Food handlers should also be supervised by trained supervisors.
- An ongoing training program for food handlers is paramount to the success of a Food Safety Management System.

Product Information and Consumer Awareness

- The end product should be accompanied by adequate information to ensure that personnel at the next stage in the food chain will handle, store, process, prepare and display the product safely.
- All batches of food should be easily identified, by a batch or lot number, to allow traceability of the commodity if required.

3.7.1 Basic principles of HACCP

There are seven discrete activities - referred to as the '**seven principles**' in the Codex Guideline (1997) - that are necessary to establish, implement and maintain a HACCP plan (FAO, 2001). The seven principles have been universally accepted by government agencies, trade associations and the food industry around the world.

Principle 1: Conduct a hazard analysis

- Identify hazards and assess the risks associated with them at each step in the commodity system.
- Describe possible control measures.

Principle 2: Determine the Critical Control Points (CCPs)

- A critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level.
- The determination of a CP can be facilitated by the application of a decision tree.

Principle 3: Establish critical limits

- Each control measure associated with a CCP must have an associated critical limit which separates the acceptable from the unacceptable control parameter.

Principle 4: Establish a monitoring system/procedures

- Monitoring is the scheduled measurement or observation at a CCP to assess whether the step is under control i.e. within the critical limit(s) specified in Principle 3.

Principle 5: Establish a procedure for corrective action

- This is necessary when monitoring at a CCP indicates a deviation from an established critical limit.

Principle 6: Establish procedures for verification to confirm the effectiveness of the HACCP plan

- Such procedures include auditing of the HACCP plan to review deviations and product dispositions, and random sampling and checking to validate the whole plan.

Principle 7: Establish record-keeping and documentation procedures

- This is important for documentation concerning all procedures and records appropriate to these principles and their application.

3.8 Policy and Regulation

- There is need for legal framework for food safety and particularly for residues and contaminants.
- The main laws surrounding food safety in Kenya are the Food, Drugs and Chemical Substances Act (FDSCA) and the Standards Act (SA).
- The provisions of the FDSCA relating to human food safety are worded in general language and therefore have wide effect.
- The FDSCA makes it an offence for a person to sell any food that, amongst other things, has “in or upon it any poisonous or harmful substance” or that “is unwholesome or unfit for human consumption”.
- Further it makes it an offence for a person to label, package, sell or advertise food that does not comply with a prescribed standard as food that complies with that standard.
- The accepted food standards are however developed under the Standards Act.
- The purpose of the Standards Act is to provide for the standardization of the specification of commodities, and also establish codes of practice.
- The Standards Act also sets up the Kenya Bureau of Standards (KEBS) whose mandate is to ensure that all commodities whether locally manufactured or imported comply with the provisions of the law dealing with standards of quality or description.
- KEBS has developed standards that provide for the maximum amount of aflatoxin that is permissible in foods in Kenya at 10µg/kg.

- Policy and regulation therefore play a key role in effective and consistent management of aflatoxins across all the value chains.
- Some of the policies and regulations that are important include;

i. Policy intervention

- Presently, the government of Kenya is in the processes of reviewing the Food Safety Policy 2013 and developing a food safety bill.
- The two instruments are expected to guide in implementation processes on food safety and ensure that food safety management is adequately addressed.
- The two instruments must ensure that there are mechanisms to support implementation of aflatoxin management and control in groundnut, macadamia nut, maize, herbs and spices value chains.

ii. Regulation

- For effective regulation of aflatoxins in the value chains, there is need to support competent agencies to undertake surveillance through strengthening human and infrastructure capacity, processing and testing for aflatoxins.
- Although there exists some capacity in the regulatory agencies, for consistency, it needs to be strengthened.
- This can be achieved through:
  a. Enhancement of the aflatoxin physical testing infrastructure – facilities such as laboratories and rapid aflatoxin testing kits
  b. Training to build the competence of the personnel to undertake the aflatoxin testing analysis. This capacity will be critical routine surveillance.

3.9 Additional interventions for sustainable aflatoxin management

Sustainable aflatoxin management and control also requires the following interventions;

- Promotion of dietary diversification to avoid overreliance on foods that are highly susceptible to aflatoxins
- Improvement of the healthcare system to manage exposure to aflatoxins
- Raising awareness on aflatoxin contamination for all the value chains
- Intensifying market surveillance for compliance across the value chains
- Enforcing compliance with aflatoxin standards as per the set regulations and market requirements
- Development of standard operating procedures in each of the value chains to address any existing gaps

3.10 Role of regulatory agencies in aflatoxin management

- To effectively address aflatoxin contamination in the country, there is need to address the existing institutional gaps.
- Most of the programs under the Ministry of Agriculture and Livestock Fisheries and Cooperatives (MoA) have been targeting increased productivity with inter-ministerial overlaps on regulation and policies.
- This has limited the effort to address issues concerning safety and quality emanating from increased productivity.
- A case in point is the Bura Irrigation Scheme where farmers experienced huge losses of maize worth Ksh. 12 million due to aflatoxin contamination.
- The World Food Programme declined to buy 48 metric tonnes of maize from farmers at the Bura Irrigation Scheme.
- This could have been averted if extension messages and farmer training on proper post-harvest maize handling had been implemented among other interventions.

- Food safety can be achieved through continuous monitoring and surveillance which requires investment in testing facilities and trained personnel.
- This capacity is lacking in majority of the regulatory agencies due to insufficient budgetary allocation.
- However, there is capacity within the private sector and research institutions which can be harnessed to ensure that monitoring is done.
- Although private capacity exists, sustained monitoring and surveillance for food safety is a public good and it is the responsibility of the Government implying the need for public/ private partnerships.
- Despite the formation of the National Food Safety Committee, lack of national Food Safety Authority implies that there is no overarching authority responsible for collecting and collating all information on aflatoxin prevalence, control and management.

3.11 Aflatoxin management regulatory infrastructure in Kenya and key challenges

- Assurance of food safety requires a fully functional and modernized food safety regulatory system supported by appropriate legislation.
- According to the FAO, an effective and efficient food regulatory system is comprised of four main components:
  i. A food control administration
  ii. Inspection services
  iii. Laboratory services
  iv. Information, education, communication and training
- Three options of organizational arrangements are recommended by the FAO for management of food-safety regulation in a country.
- These are a single agency system, an integrated system, or a multiple agency system.
- Kenya - like other EAC countries - operates a food-safety regulatory system based on the multiple-agencies model.
- Under the multiple-agencies system, food safety regulatory responsibilities are shared among government ministries including health, trade and industry, agriculture and livestock.
- Unfortunately, this structure has resulted in overlapping mandates, and has often produced conflicts among these agencies.
- This diffusion of food-safety responsibilities greatly hampers food safety at every level of the value chain, if not well coordinated and if functions are not clearly spelt out to remove duplication.
- Establishment of food standards in Kenya is vested with KEBS.
- Overlap and conflicts are most often encountered in areas of enforcement.
- This is because, although enforcement of food standards is mandated to various bodies under ministries, it is also either mandated or delegated to KEBS.
- KEBS is the major standards setting and enforcing agency, although other agencies under the Ministry of Public Health and the Ministry of Agriculture are also empowered to enforce the same standards.
- Enforcement of aflatoxin regulations in Kenya is hampered by:
  i. Inadequate public knowledge
  ii. Inadequate capacity within responsible institutions
  iii. Inadequate legislation
  iv. Political interference
  v. A weak inspectorate
  vi. Inadequate laboratory capacity
  vii. Inadequate human resources capacity
  viii. Low levels of awareness among stakeholders
  ix. Lack of adequate epidemiological evidence to support government food safety mandates

For effective regulation and enforcement of the standards, these gaps need to be adequately addressed.

3.12 Brief overview on national and international standards on aflatoxin levels in specific commodity and related products

- Regulation of aflatoxin contamination in food involves formulation and enforcement of maximum limits (MLs) tolerated in the food.
- Although most countries formulate their own MLs for aflatoxins in food, many of them rely on limits formulated by regional or international bodies.
- The European Union has set the limits at 5 ppb for aflatoxin B1 in human food.
- USA has set the limit for aflatoxin B1 in human food at 5 ppb except for milk where the limit is at 0.5 ppb.
- Fumonisins have a set legal limit of 1 ppm.
- The set regulations for the animals are set at higher levels with the values varying from one type of feed to another and from one animal to another.
- In East Africa, there are set regulations and standards that have been necessitated to harmonize the requirements that ensure food safety.
- The set standards are based on the East African Committee standards that have representatives from the national partner states, private sector and consumer organizations.
- Total aflatoxins are regulated at 10 ppb, aflatoxin B1 is regulated at 5 ppb whereas fumonisins are regulated at 1ppm.
- The set standards are regulated according to ISO 16050 East African Standard (EAS: 2011).
- The Codex specifies a maximum limit of 15 micrograms per kilogram, which is 15 parts per billion (15 µg/kg = 15 ppb) for total aflatoxins (sum of AFB1, AFB2, AFG1, and AFG2) in peanuts, Brazil nuts, hazelnuts, pistachios, and almonds for further processing.
- A maximum limit of 10 µg/kg is also set for ready-to-eat Brazil nuts, dried figs, hazelnuts, pistachios, and almonds.
- A level of 0.5 µg/kg is set for AFM1 in milk, signifying the importance of protecting children from aflatoxin exposure.
- However, for aflatoxins in staple foods, such as maize and rice, the Codex has not been able to formulate an internationally acceptable ML.
- Table 5 illustrates the national and international aflatoxin standards for specific commodities.

Table 5: Aflatoxin standards (µg/kg) for various commodities in different jurisdictions.

Crop	Kenya	EAC	EU	FDA	Codex
Groundnuts	10a, 5b	10a, 5b	4f, 15g	20	15
Spices	10a, 5b	10a, 5b	10h, 5b	20h	30i, 20j
Maize	10a, 5b	10a	4a	20a	
Millet	10a, 5b	10 a			
Milk (Aflatoxin M1)	0.05	0.05	0.05c, 0.4d, 0.02e	0.5	0.5
Infant foods	10a	10a	0.1b	20	15
Finished dairy animal feeds	20a	20a	10a, 5b	20a	20a

a- Total aflatoxins
b - Aflatoxin B1
c – Raw milk
d – Milk powder, condensed milk
e – Butter and cheese
f – Groundnuts intended for direct human consumption
g – Groundnuts to be subject to sorting or other physical treatment before human consumption or used as an ingredient
h – All spices
i - Nutmeg, chilli and paprika
j – Ginger, pepper, turmeric

CHAPTER 4: SAMPLING FOR AFLATOXIN ANALYSIS

4.1 Sample collection for aflatoxin analysis: Considerations and approach

- Sample variation is often the largest error in determining concentration of aflatoxins in food commodities.
- For small sample sizes, sampling is the largest source of error.
- Aflatoxins typically have a skewed or uneven distribution in foods and feeds, especially in whole kernels (or nuts).
- Only a small percentage of the kernels is contaminated.
- In a typical scenario, <0.5% of groundnut kernels contaminated in a lot with mean aflatoxin concentration of 5ppb.
- Yet the concentration in a single kernel could be >1,000ppb.
- It is therefore extremely difficult to collect a sample that accurately represents the mean batch concentration.
- Sampling plans have been developed for select mycotoxins such as aflatoxins, fumonisins and deoxynivalenol.
- The plans emphasize the importance of sample selection, sample size and the number of incremental samples.

Emphasis: Always aim at representative samples

Definitions

Lot means a food commodity delivered at the same time and has common characteristics, such as origin, variety, type of packing, packer or consignor.

Sublot means a designated part of a large lot in order to apply the sampling method on that designated part; each sublot must be physically separate and identifiable.

Incremental sample means a quantity of material taken from a single place in the lot.

Aggregate sample means the combined total of all the incremental samples taken from the lot

- Incremental samples should be taken at various places distributed throughout the lot or sublot.
- The aggregate sample is made up by combining the incremental samples.
- Replicate samples for enforcement and trade purposes are taken from the homogenized aggregate sample.

4.2 Sampling plan

- The sampling plan is defined by the number of samples tested from each food lot and the size of each sample.
- As the number and size of samples increases, the probability of getting a representative sample increases.
- However, the cost of sampling and analysis also increases.
- The first step associated with an aflatoxin sampling plan is the selection of a sample from a bulk shipment (lot).

Principle: Each kernel in the lot has an equal chance of being selected in the sample. There should be no biases in the sampling procedure.

4.2.1 Steps in sampling plan

- Typically the sampling plan consists of three independent steps:
  i. S**ampling step** – A sample (sometimes called a laboratory sample) is collected from the lot.
  ii. **Sample preparation step** - The entire sample is comminuted in a mill to reduce particle size and a subsample or test portion is removed from the comminuted sample.
  iii. **Analytical step** - Aflatoxin is extracted from the test portion and the aflatoxin in the extract is quantified.

4.2.2 Sampling error

- The total error associated with a sampling plan is the sum of sampling, sample preparation, and analytical errors.
- Increasing the size of the sample that is comminuted reduces the sampling error.
- Sampling variance can be halved each time the sample size gets doubled.
- In addition, a representative sample is extremely important.
- But there is need for a balance to ensure cost-effectiveness.

4.2.3 Sampling for surveillance

- Representative samples should be collected from carefully selected populations of food (e.g. batches or lots, marketplaces, farmers' stores).
- Ideally, a bulk sample should be composed of 100 primary samples.

Example: What should be the target of sampling groundnut kernels from a 20ton food lot? A 20kg sample produced by collecting 200g primary samples from 100 bags.

4.2.4 Sampling from different types of food lots

- Food commodities may be traded in bulk, containers, or individual packings such as sacks, bags, retail packings.
- The method of sampling may be applied to all the different forms in which the commodities are put on the market.
- The following formula may be used as a guide for the sampling of lots traded in individual packs such as sacks, bags, retail packings:

$$\text{Sampling frequency (SF) } n = \frac{\text{Weight of the lot} \times \text{Weight of the incremental sample}}{\text{Weight of the aggregate sample} \times \text{Weight of individual packing}}$$

— weight: in kg

— sampling frequency (SF): every n^{th} sack or bag from which an incremental sample must be taken (decimal figures should be rounded to the nearest whole number).

4.2.5 Sampling from cereals and cereal products

Determination of number of incremental samples

Subdivision of lots into sublots depending on product and lot weight

Commodity	Lot weight (tonne)	Weight or number of sublots	No of incremental samples	Aggregate sample weight (kg)
Cereals and cereal products	>300 and <1500	3 sublots	100	10
	≥50 and ≤300	100 tonnes	100	10
	<50	-	3 -100 (*[1])	1-10

Method of sampling for cereals and cereal products for lots <50 tonnes

Number of incremental samples to be taken depending on the weight of the lot of cereals and cereal products

Lot weight (tonne)	Number of incremental samples	Aggregate sample weight (kg)
≤ 0.05	3	1
> 0.05 - ≤ 0.5	5	1
> 0.5 - ≤ 1	10	1
> 1 - ≤ 3	20	2
> 3 - ≤ 10	40	4
> 10 - ≤ 20	60	6
> 20 - ≤ 50	100	10

4.2.6 Precautions during sampling

- In the course of sampling and preparation of the samples, precautions should be taken to avoid any changes, which would affect:
    i. The aflatoxin content
    ii. Make the aggregate samples unrepresentative
    iii. The food safety of the lots sampled

4.2.7 Packaging, labelling and transmission of samples

- Each sample should be placed in a clean, inert container offering adequate protection from contamination and against damage in transit.
- Precaution should be taken to avoid any changes in composition of the sample, which might arise during transportation or storage.
- Each sample taken should be sealed at the place of sampling.

CHAPTER 5: METHODS OF AFLATOXIN ANALYSIS

- Different methods have been used for detection and quantification of aflatoxins.
- The methods of analysis can be divided into:
    i. Qualitative
    ii. Semi qualitative
    iii. Semi quantitative
    iv. Quantitative
- Antibody-based assays and chromatography techniques have been used to detect the presence of mycotoxins.
- On the other hand, DNA-based assays have been used to detect the presence of the mycotoxins producing fungi whether or not they are toxigenic.
- The following factors should be considered in choosing the method of aflatoxin analysis:
    i. Need to determine total vis-a-vis specific aflatoxin types
    ii. Cost of running the analysis to ensure cost-effectiveness
    iii. Competence of the personnel to undertake the analysis. This should be linked to the preferred method of analysis
    iv. Sustainability of the testing method. To ensure a sustainable system of testing for aflatoxins, there is need to consider the target market and need for testing vis-à-vis the method of analysis. For example, for local traders, a rapid but reliable cost-effective testing method should be considered.
    v. Number of samples to be analyzed
- The following methods that are currently used widely for aflatoxin detection:

5.1 Qualitative methods

- These are the methods that determine presence or absence of aflatoxins without indicating the levels present.
- They can be best used by extension agents and producers/farmers to inform a decision.
- They can also be a pre-step for analysis as they ensure that proper use of resources is achieved.
- For example, samples with no aflatoxins do not have to be subjected to costly options for testing.
- They include:

5.1.1 Fluorescence Polarization Immunoassay (FPIA)

- Fluorescence polarization immunoassay (FPIA) is a homogeneous immunoassay useful for rapid and accurate detection of antibody or antigen.
- The principle of the assay is that a fluorescent dye (attached to an antigen or an antibody fragment) can be excited by plane-polarized light at the appropriate wavelength.
- The method was developed for the analysis of aflatoxins using an anti-aflatoxin B1 (AFB1) monoclonal antibody and a novel fluorescein-labeled aflatoxin B1 tracer.
- The tracer was an aflatoxin-fluorescein conjugate and the incubation time was 15 min.
- The group-specificity of anti-AFB1mAb indicated that the FPIA could potentially be used in a screening method for the detection of total aflatoxins, albeit not AFG2 and AFM2.
- The total time required for analyzing 96 samples in one microplate is less than 5 min.
- This makes FPIA a rapid and simple technique for monitoring aflatoxins.
- The procedure of the technique is illustrated in Figure 11 and Figure 12.

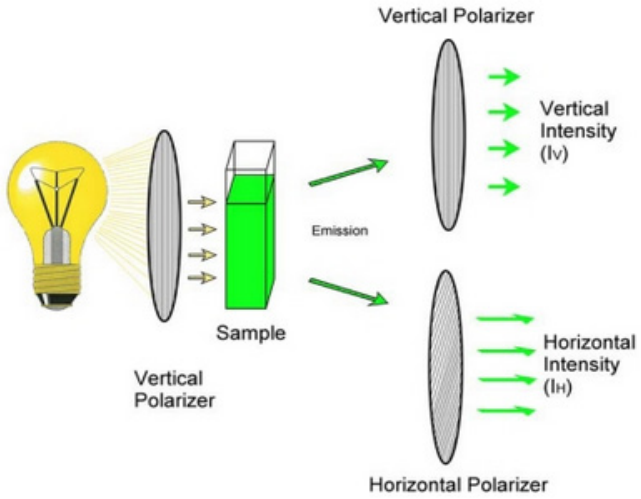


Figure 11: Schematic illustration of measurement of fluorescence polarization.
Source: Maragos, 2009

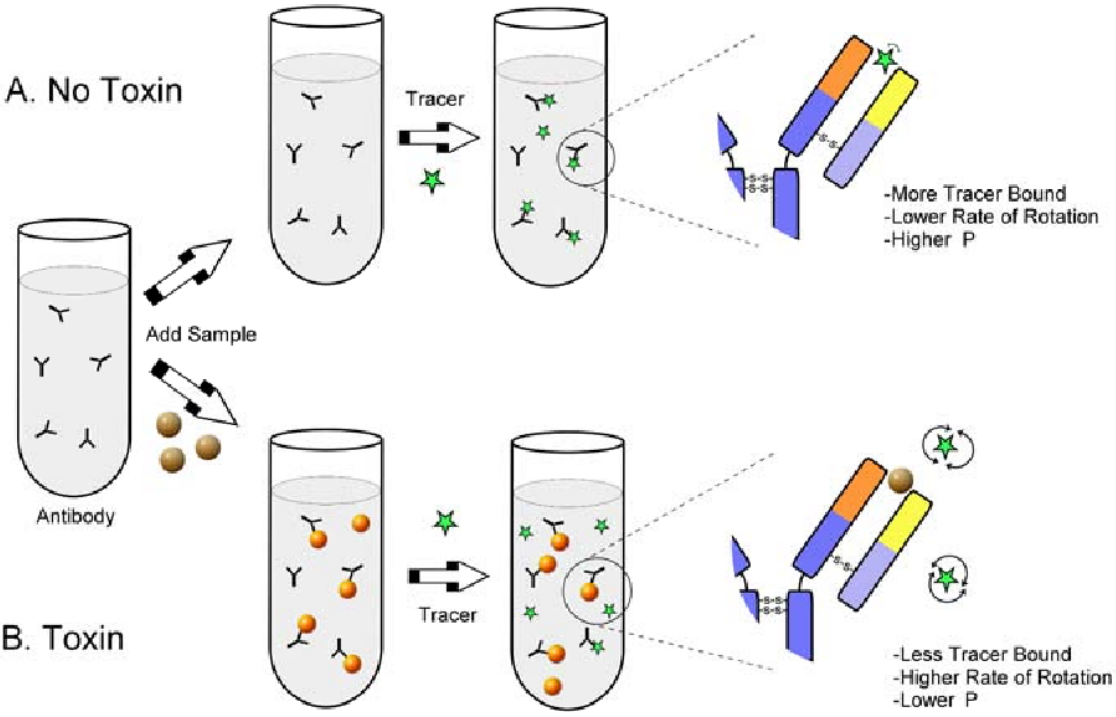


Figure 12: Schematic illustration of fluorescence polarization immunoassay.
Source: Maragos, 2009

The advantages and disadvantages of carrying out aflatoxin analysis using the FPIA method include;

Advantages

- It is a rapid method that can be used for analysis of many samples within a short time
- The method can simultaneously detect aflatoxins and zearalenone
- The method does not require separation or washing steps

Disadvantages

- The method cannot be used for quantification of aflatoxin levels in a sample

5.1.2 Fluorometry

- Fluorometry allows the identification of small substances by excitation with a beam of ultraviolet light.
- This is followed by detection and measurement of the characteristic wavelength of the fluorescent light emitted (Figure 13).
- This method is of particular interest when testing single samples within a short period of time, e.g. testing of incoming truck loads during the harvest season.

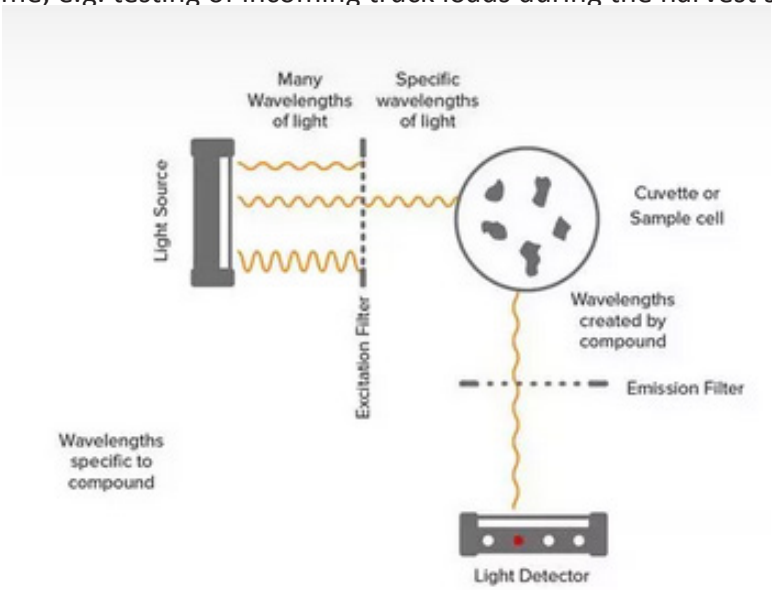


Figure 13: Schematic illustration of fluorometry where a sample is scattered with UV light and the emitted wavelength – specific to the sample - is measured
Source: Mycotoxin info, https://www.mycotoxins.info

Advantages of fluorometry method

- It is a rapid method results available in less than five minutes
- It can be used by untrained personnel
- No laboratory is required to carry out the analysis

Disadvantage of fluorometry method

- The method can only be used to determine total aflatoxin

5.2 Semi-qualitative methods

- These are methods that besides determining the presence or absence of aflatoxins in a sample, they can indicate the range of the of toxin levels.
- These methods are important for sample screening and determination of need for quantitative analysis based on suspected levels vis-à-vis need to comply with set standards.
- The can be best used by processors, traders, extension agents and producers/farmers to inform a decision.
- They can also be a pre-step for analysis as they ensure that proper use of resources is achieved.
- For example, samples with aflatoxin levels below the acceptable threshold do not have to be subjected to costly options for testing.
- They include:

5.2.1 Lateral Flow Devices (Immunodipsticks)

- Immunodipsticks are immunochromatographic assays, also known as lateral flow devices.
- The principle is based on the use of high sensitivity and specificity of antibody-antigen reactions for the rapid detection of analytes.
- Lateral flow devices contain (Figure 14):
  i. A porous membrane which ensures the flow
  ii. An absorbent pad that increases the volume of the flowing liquid
  iii. A sample pad that ensures contact between the liquid sample and the membrane
  iv. A rigid backing that gives support to the device
- Lateral flow devices use labels such as colloidal gold and gold coated with the antibody, which commonly provide red-colored binding zones.
- The liquid sample added to the sample pad moves towards the extreme end through the membrane by capillary flow to the absorbent pad.
- When the liquid component containing aflatoxins reaches the gold particles, the sample suspends the gold particles, and the aflatoxins bind to the particles, coloring the line red.
- Delmulle *et al*. (2005) developed a lateral flow device for detecting aflatoxin B1 in pig feed.
- The device would detect 5 µg/kg aflatoxin within 10 min, which is within the European Commission (EC) stringent limit fixed for feedstuffs.
- Another immunochromatographic method was developed by Ho and Wauchope (2002).
- The assay is based on competition between free AFB1 and AFB1-tagged dye-containing liposomes for the corresponding antibody.
- The device can detect 18 ng of the aflatoxin in less than 12 minutes.
- The device has also been adapted for use in the optical density scanning mode, which allows for quantitative determination of aflatoxins.
- The procedure is illustrated in Figure 15.

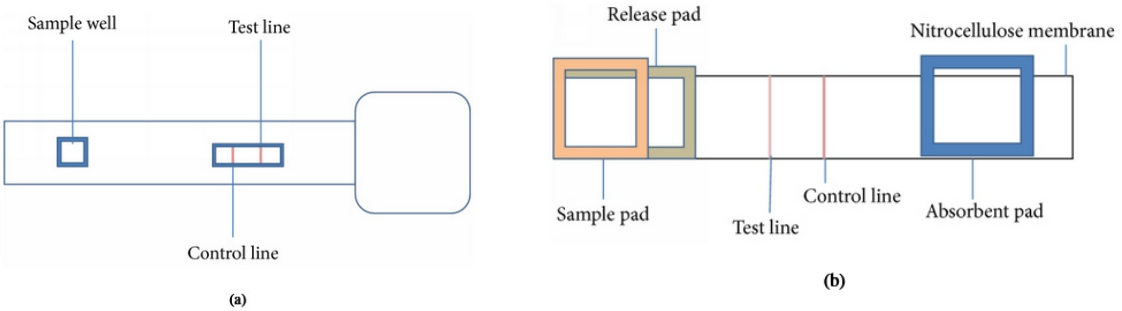


Figure 14: Schematic illustration of a lateral flow device in the dipstick format: (a) External details and (b) Internal details.

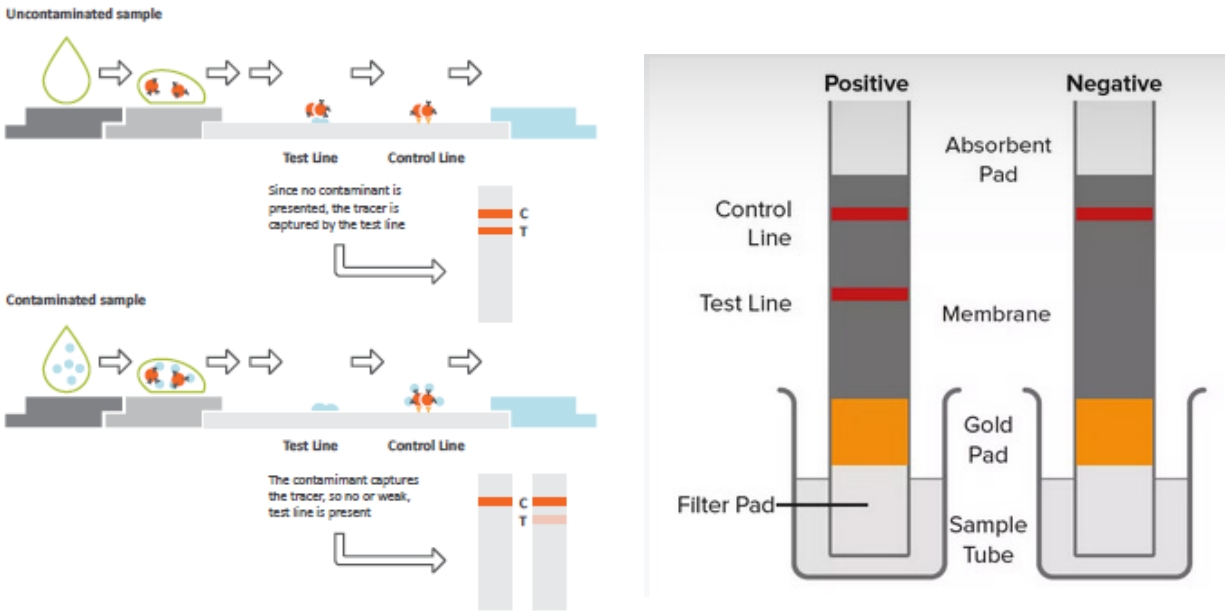


Figure 15: Illustration of a conventional lateral flow detection dipstick
Source: Tecna®, www.technalab.com

The advantages and disadvantages of carrying out aflatoxin analysis using the lateral flow technique include;

Advantages of using Lateral Flow Devices

- Rapid analysis provide quick on-site detection of aflatoxins (results available in 3 to 5 minutes)
- No special equipment necessary
- Lateral flow devices are easy to use
- They are cost-effective devices that can be adapted for day to day monitoring of aflatoxins
- Quantitative results can be obtained using a Lateral Flow Device (LFD) reader

Disadvantages of using Lateral Flow Devices

- There is a risk of matrix interferences (other substances in the solution that can alter results)

5.3 Semi-quantitative methods

These are methods that give an *estimation* of the approximate concentrations and levels of aflatoxin contamination. They can be used by traders, producers/ farmers and regulators to determine the need for further analysis. They include;

5.3.1 Frontier Infrared Spectroscopy (IR)

- Infrared spectroscopy relies on the alteration in molecular vibrations upon irradiation with infrared radiations (IR).
- The vibrations by the bonds within the molecule can be measured.
- Since the atomic size, bond length, and bond strength vary greatly from molecule to molecule, the rate at which a particular bond absorbs infrared radiation will differ from bond to bond and in the mode of vibration.
- For instance, the various bonds of organic molecules should vibrate at different frequencies, in tandem with the type of bond excited.
- So when an infrared spectrometer is used in the analysis of a compound, infrared radiations covering a range of different frequencies are passed through the sample and the radiant energy absorbed by each type of bonds in the molecules is measured.

- A spectrum is then produced normally consisting of plot of % transmittance against the wave number.
- No two organic compounds have the same infrared spectrum and thus individual pure compounds can be identified by examination of their infrared spectra.
- The use of Fourier transform infrared spectroscopy which employs attenuated total internal reflectance has been reported for analysis of aflatoxins in peanuts and peanut cake.
- It has also been used in transmittance and reflectance spectroscopy to detect aflatoxin in single maize kernels.
- More than 95% of the kernels analyzed were correctly categorized as having either high (>100 ppb) or low (<10 ppb) concentrations of aflatoxins.
- The IR procedure is illustrated in Figure 16.

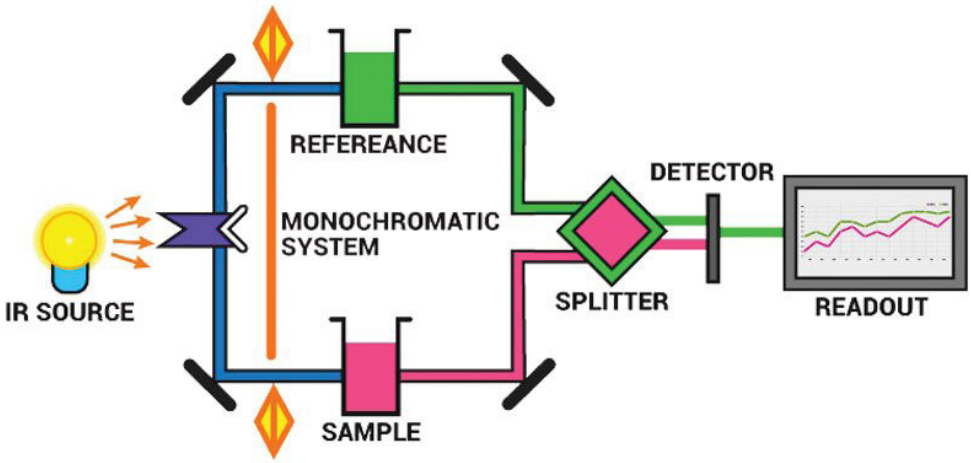


Figure 16: Illustration of the Frontier Infrared Spectroscopy technique
Source: BYJUS, https://byjus.com/chemistry/infrared-spectroscopy/

5.3.2 Radioimmunoassay (RIA)

- The radioimmunoassay technique relies on the principle of competitive binding between a radioactive-labeled antigen and a nonradioactive antigen.
- The radioactive-labeled antigen competes with unlabelled nonradioactive antigen for a fixed number of antibody or antigen binding sites on the same antibody.
- A known quantity of labeled antigen and unknown amount of unlabeled antigen from standards competitively react with a known and limiting amount of the antibody.
- The amounts of labeled antigen are inversely proportional to the amount of unlabeled antigen in the sample.
- Radioimmunoassay was the first immunoassay technique to be developed and was applied in the detection of insulin in human blood.
- Radioimmunoassay has also been used for analysis of aflatoxins in food samples.
- Radioimmunoassays have been used for the qualitative and quantitative determination of aflatoxin B1 levels and aflatoxin M1 levels.

The advantages and disadvantages of carrying out aflatoxin analysis using the RIA method include;

Advantages

- The method can be used to perform multiple analyses simultaneously with high levels of sensitivity and specificity

Disadvantages

- The method requires an antigen in a pure state
- A radioactive isotope is used as a label and is associated with potential health hazards
- The method is associated with the storage and disposing of the low-level radioactive waste

These disadvantages have limited the frequent use of RIA in the day to day analysis of aflatoxins.

5.3.3 Immunosensors

- An immunosensor is a biosensor that uses an antigen or antibody species as biological recognition components coupled to a signal transducer such as graphite, gold, and carbon that help to detect the binding of the complementary species.
- With respect to type of signal transduction in use, immunosensors may be grouped into piezoelectric, optical, and electrochemical sensors.

5.3.3.1 Piezoelectric Quartz Crystal Microbalances (QCMs)

- QCMs are label-free devices used for direct detection of antigens.
- The piezoelectric quartz crystal relies on changes in mass on the electrode surface when an antigen interacts with a cognate antibody immobilized on the quartz crystal surface.
- Since the change in mass is proportional to the concentration of the antigen-antibody complex, the method permits detection and quantification of the immune complex (Ab-Ag).
- Piezoelectric quartz crystal microbalance has been reported for aflatoxin B1 analysis.
- The procedure is illustrated in Figure 17.

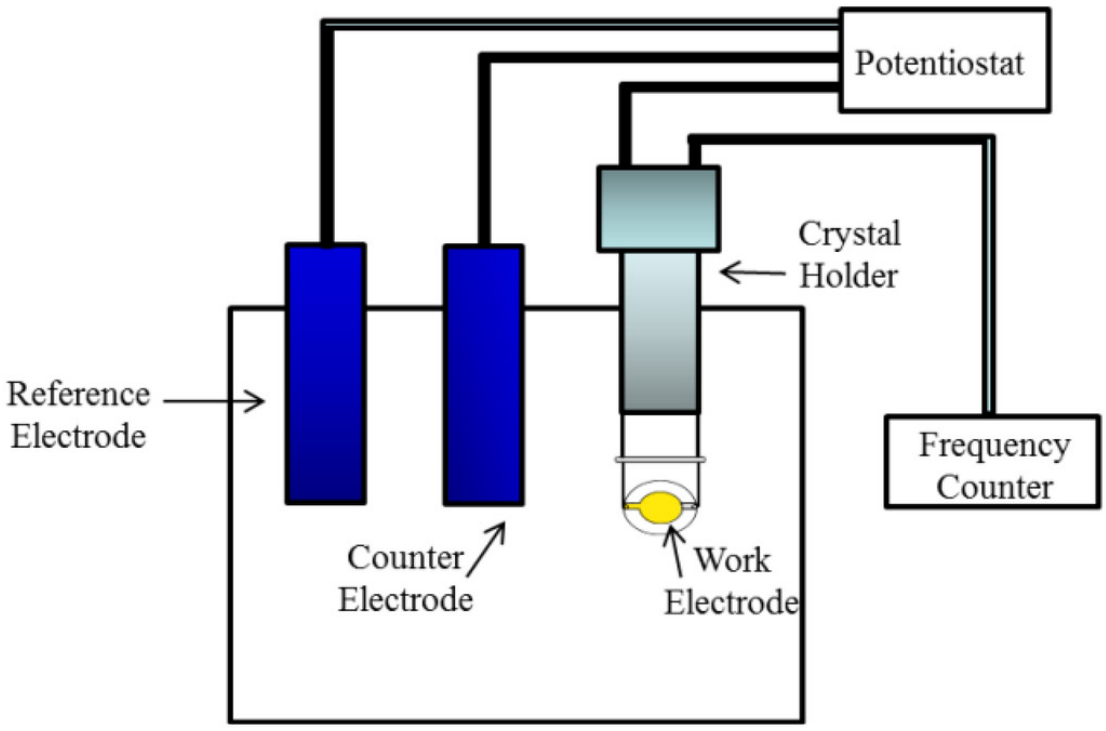


Figure 17: Schematic illustration of an electrochemical quartz crystal microbalance (EQCM) apparatus
Source: Marrazza, 2014

The advantages and disadvantages of carrying out aflatoxin analysis using the QCMs method include;

Advantages

- Quartz crystal microbalance is a good label-free technology

Disadvantages

- Use of Quartz crystal microbalance for direct detection of mycotoxins may be a challenge due to the small sizes of most mycotoxins

5.3.3.2 Optical Immunosensors

- A number of optical immunosensors have been developed for aflatoxins based on different transduction approaches.
- One of these optical immunosensors already developed for aflatoxin analysis is surface plasmon resonance (SPR) illustrated in Figure 18.
- Surface plasmon resonance platform relies on measurement of changes in refractive index produced by the binding of analyte to its biospecific partner immobilized on the sensor surface.
- When the analyte is flowed over the sensor surface, there is a shift in resonant SPR wavelength, which is proportional to the refractive change at the sensor surface and can be calibrated to the surface concentration of bound analyte.
- The SPR sensor surface contains a biorecognition layer that selectively binds either an antigen or antibody, which, in turn, causes parallel increase in the mass on the sensor surface that is proportional to an increase in refractive index.
- The increase in refractive index will be observed as a shift in the resonance angle.
- The measurable changes in concentration are those due to binding and dissociation of antibody to its target antigen.

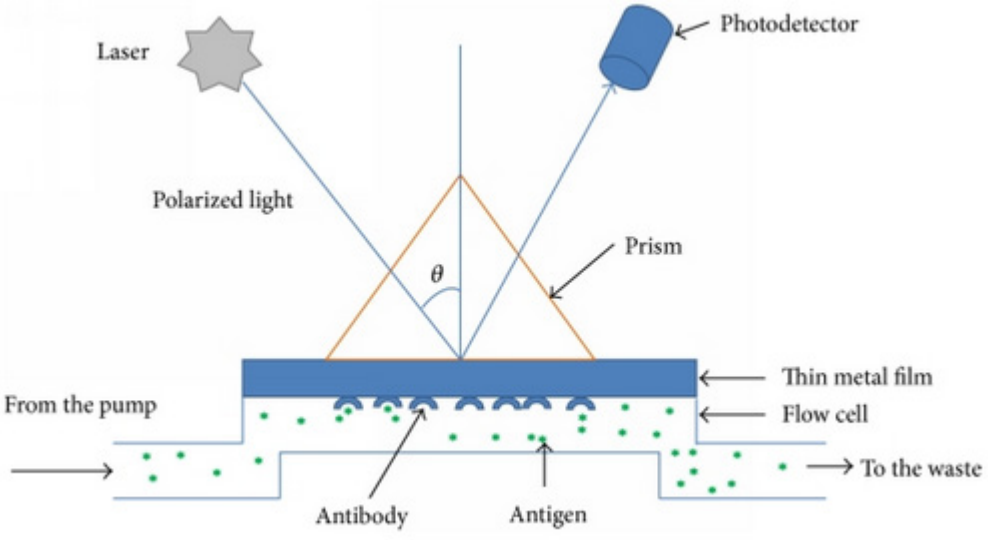


Figure 18: Surface plasmon resonance spectroscopy commonly used for the detection of antigen-antibody interactions in a buffered sample

The advantages and disadvantages of carrying out aflatoxin analysis using the SPR method include;

Advantages

- SPR immunosensor can be used for detection of multiple mycotoxins
- SPR immunosensors should offer label-free detection of aflatoxins

Disadvantages

- The SPR immunosensor immobilized with monoclonal antibodies encounters regeneration problems at the sensor surface due to the high-affinity binding of the monoclonal antibodies. However, this is not a challenge with polyclonal anti-aflatoxin B1 antibodies

5.3.3.3 Electrochemical Immunosensors

- An electrochemical immunosensor is a device that uses antibodies incorporated into a biorecognition layer to produce electroactive signals detectable by transducers (amplifiers), which generate measurable signals.
- The signal is generated in the form of a membrane potential when ions bind to a sensing membrane.
- The potential difference is then measured.
- A logarithmic relationship exists between the potential difference (pd) and concentration.
- The signal measurement can be in the form of:
  i. Differential pulse voltammetry
  ii. Cyclic voltammetry
  iii. Chronoamperometry
  iv. Electrochemical impedance spectroscopy

  Linear sweep voltammetry
- A number of electrochemical immunosensors have been reported to be used in aflatoxins analysis.
- Most of them involve immobilization of antibodies onto the surface of an electrode.
- Although majority of the electrochemical immunosensors developed for aflatoxins analysis use enzymes as active biological component to generate signals developed a nonenzymatic sandwich form of an electrochemical immunosensor.
- The sensor in the nonenzymatic sandwich type was developed through modification of glassy carbon electrodes using chitosan, gold nanoparticle, anti-aflatoxin B1, and iron III oxide (Fe_3O_4) magnetic core with a gold shell functionalized with 3-((2-mercaptoethylimino) methyl) benzene-1,2-diol and labeled with AFB1.
- This immunosensor achieved aflatoxin B1 detection range of 0.6–110 ng/mL and a detection limit of 0.2 ng/mL.
- Another form of nonenzymatic electrochemical immunosensor was developed by Linting *et al*. (2012).
- This type of immunosensor was developed by electrodepositing of graphene oxide and gold nanoparticles, respectively, on the surface of gold electrode.
- Aflatoxin B1 antibody immobilized on the conducting polymer film and ionic liquid and chitosan solution dropped onto this electrode.
- This immunosensor attained a dynamic range of 3.2–0.32 picomoles and detection limit of one femtomole with excellent long-term stability.
- The procedure is illustrated in Figure 19.

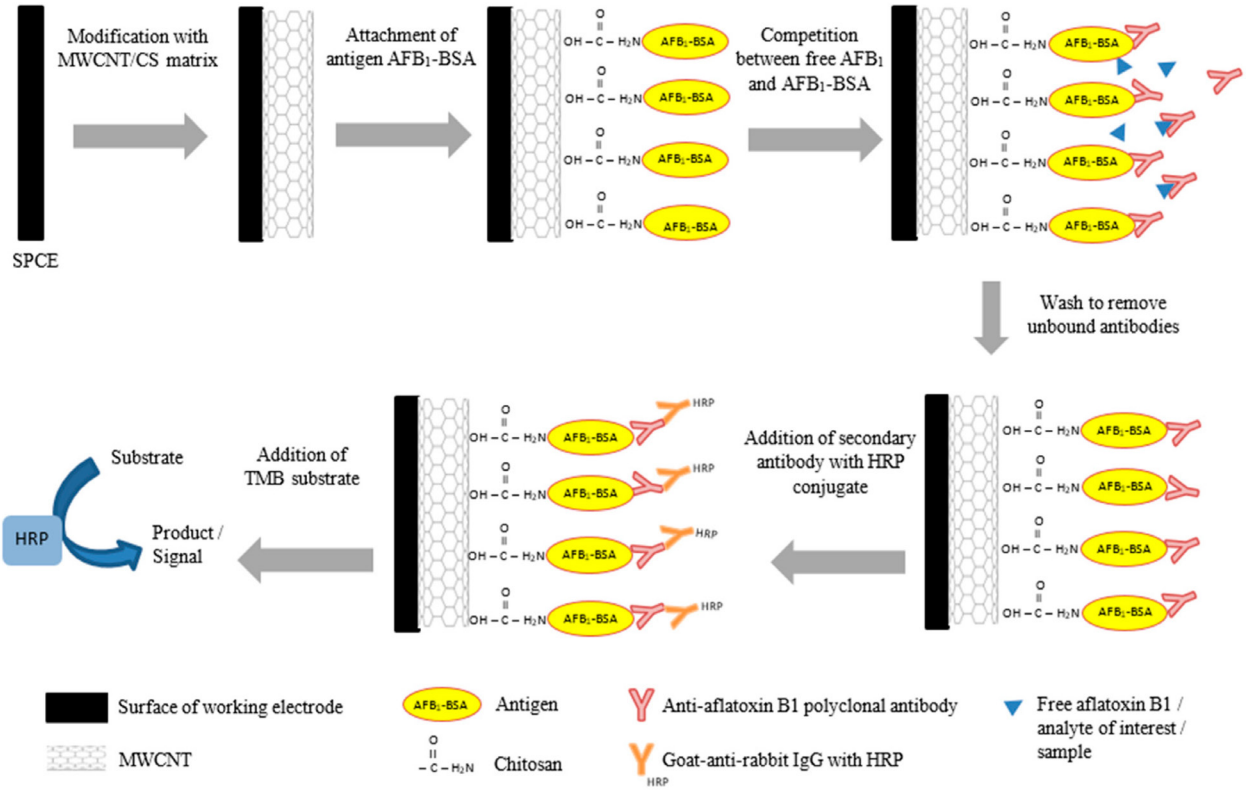


Figure 19: Schematic illustration of the electrochemical immunosensor technique in detection of aflatoxin B1
Source: Azri et al., 2017

5.3.4 Quantitative strip assay

- Most of the strip tests are qualitative although a new trend can be seen towards semi-quantitative strip tests led by a strong urge from industry.
- To meet the requirement, a few methods have integrated chromatographic separation as well as electrochemical, fluorescence, or optical detectors for rapid quantitative detection of aflatoxins.
- These approaches offer a greater sensitivity, better quantitative capability, and dynamic ranges compared to conventional strips.
- However, environmental pollution can happen due to heavy metals from these approaches.
- As detector-free approaches, a semi-quantitative ICA (Islet cell antibodies) has been developed.
- The dose ranges can be simply encoded to different numbers of a colored ladder on the assay strip, and a pH sensitive dye is used as the end-of-assay indicator.
- A potential problem of this technique is the time of the end-of-assay with a pH sensitive indicator that may vary from person to person and can cause a disparity in result determination.
- To overcome this, a detector-free (semi-) quantitative strip (DFQ-strip) was constructed considering AFB1 as the target analyte.
- The visual detection limit of this assay was 0.06 ng/mL.
- Rapid AgraStrip kits were also validated to test total aflatoxins in maize by using different cutoff value.
- These types of strip membrane usually contain a test zone and a control zone.
- A positive sample with total aflatoxins greater than the cutoff will result in no visible line in the test zone.

- In contrast, a negative sample will form a visible line in the test zone with total aflatoxins less than the cutoff value.
- Results can be obtained in only 5 minutes from this rapid test.
- Another kit for detecting and quantifying aflatoxin is aflakit.
- This kit is based on an adsorbent-coated dip-strips (polyester film) technique.

The advantages and disadvantages of carrying out aflatoxin analysis using the quantitative strip assay technique include;

Advantages of using quantitative strip assay

- Results can be obtained in only 5 minutes from this rapid test

Disadvantages of using quantitative strip assay

- There is a risk of environmental pollution due to heavy metals from these approaches
- The time of the end-of-assay with a pH sensitive indicator may vary from person to person and can cause a disparity in result determination

5.4 Quantitative methods

- These emphasize objective measurements and the statistical, mathematical, or numerical analysis of aflatoxins.
- They provide accurate determination of the levels of contamination.
- They can be used by regulators and traders to make critical decisions based on the aflatoxins present.
- Quantitative methods of aflatoxin analysis include;

5.4.1 Enzyme Linked Immuno-Sorbent Assay (ELISA)

- Enzyme Linked Immuno-Sorbent Assay (ELISA) is an antibody-based assay that is used to quantify mycotoxins.
- It is usually a competitive assay in which the mycotoxin of interest from a sample competes with a labeled mycotoxin for a limited number of specific antibody-binding sites.
- Since the assay is competitive, presence of the toxin is usually measured by the absence of color.
- ELISA is one of the more affordable methods for detecting mycotoxins.
- There are different types of ELISA including Direct, Indirect, Competitive and Sandwich ELISA.
- The ELISA procedure is illustrated in Figure 20.

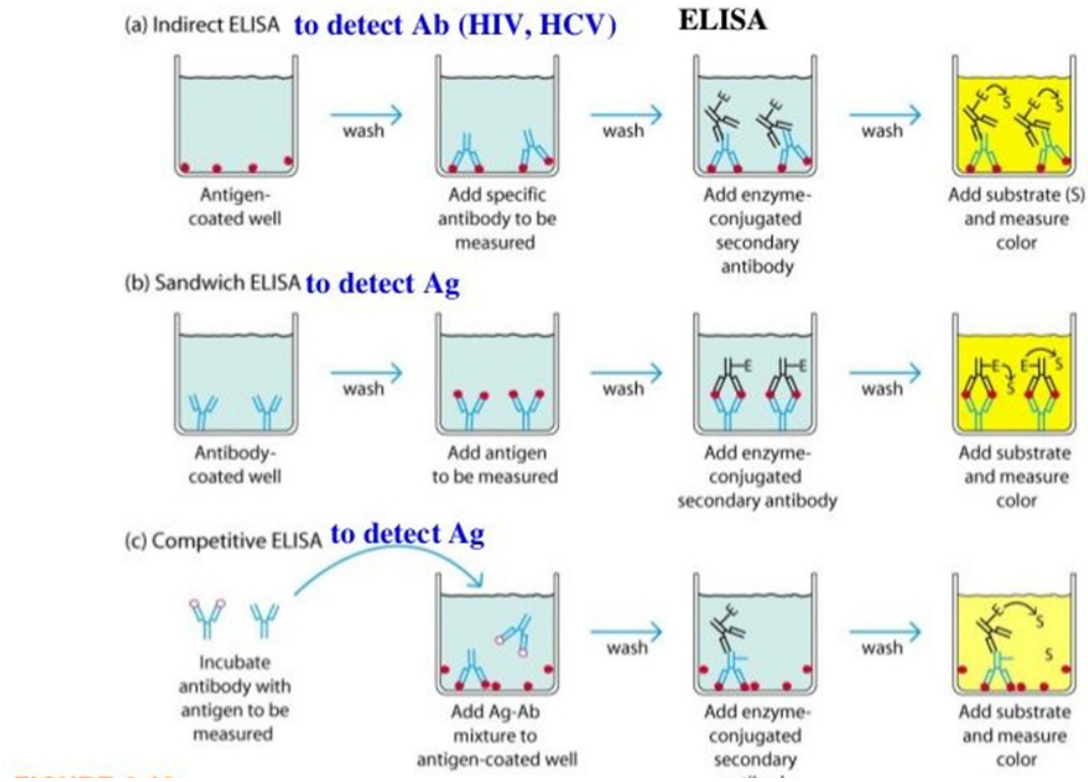


Figure 20: Illustration of the ELISA technique

There are several advantages and disadvantages of carrying out aflatoxin analysis using the ELISA technique;

Advantages

- ELISA is easy to perform with simple procedure
- It has high specificity and sensitivity
- It has high efficiency
- Simultaneous analysis can be performed without complicated sample pre-treatment
- It is generally safe and eco-friendly
- Radioactive substances and large amounts of organic solvent are not required
- It is a cost-effective assay as the reagents are relatively low cost

Disadvantages

- It is labor-intensive and expensive to prepare antibody
- It has a high possibility of false positive/negative due to insufficient blocking of immobilized antigen
- Antibody instability where storage conditions are not keenly observed
- Refrigerated transport and storage are required as an antibody is a protein

5.4.2 High Performance Liquid Chromatography (HPLC)

- HPLC is one of the most widely used methods for mycotoxin detection and quantification in food safety laboratories.
- The method separates a mixture of compounds on a stationary column using a carrier solvent such as methanol or acetonitrile.
- The mycotoxins are detected and quantified in the sample as they pass through a specific detector.
- The HPLC analysis procedure is illustrated in Figure 21.

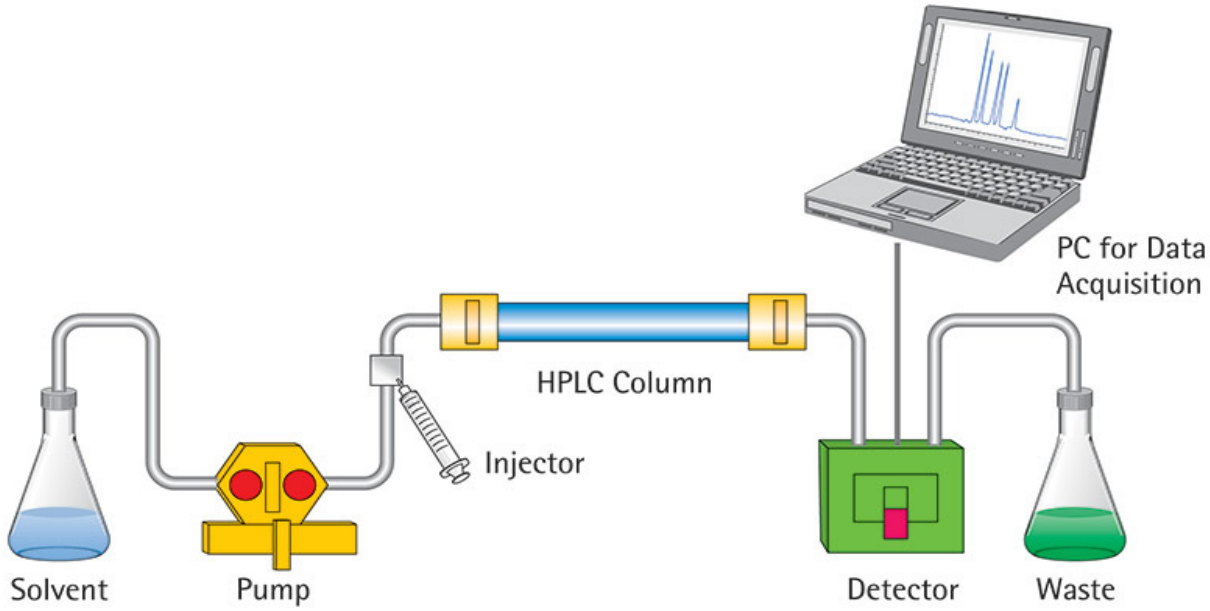


Figure 21: Illustration of the HPLC technique

Advantages of HPLC

- HPLC is extremely fast and efficient as compared to the other chromatographic techniques.
- It takes 10 to 30 minutes on average and delivers a high resolution.
- It is a highly accurate and reproducible separation technique for organic molecules.
- HPLC is a versatile and extremely precise analytical method to identify and quantify varied chemical and organic components.
- It requires a small sample size.
- Offers high reliability with a detection limit of less than 0.05 ppm for many mycotoxins.

Disadvantages of HPLC

- Despite its advantages, it could be costly, requiring large quantities of expensive organics.
- Requires highly skilled training to operate the equipment.
- Chances of coelution may occur interfering with the results.

5.4.3 Liquid Chromatography Mass Spectrometry (LCMS)

- Multiple mycotoxins can also be detected using Liquid Chromatography Mass Spectrometry (LCMS).
- Liquid chromatography coupled with mass spectrometry has been greatly used to detect multiple mycotoxins.
- Several conditions are used to achieve the right LCMS results and there seems not to be universally accepted conditions.
- The choice of ion source and mobile phase is dependent on the compounds used in the method.
- Electron Spray Ionization (ESI) is the most commonly used ion source.
- The procedure for LCMS analysis is illustrated in Figure 22.

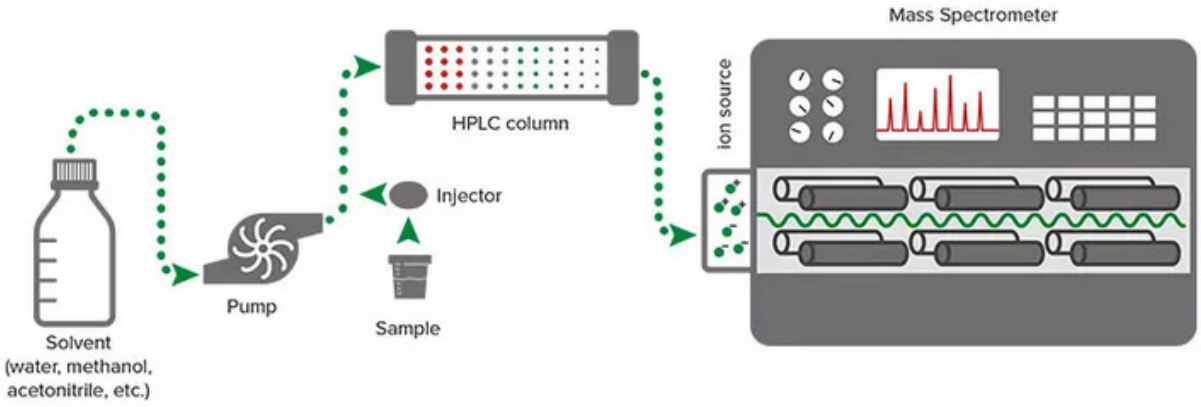


Figure 22: Illustration of the Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique
Source: Mycotoxin info, https://www.mycotoxins.info

Advantages of aflatoxin analysis using the LC-MS/MS technique
Liquid chromatography has many advantages in that;

- It has high selectivity
- It is highly sensitive
- It has low detection limits
- It gives qualitative and quantitative results
- It can detect a wide range of mycotoxins
- It generates structural information
- Minimal sample treatment required
- Applicable to complex matrices

Disadvantages of aflatoxin analysis using the LC-MS/MS technique

- It is difficult to develop conditions that are suitable for all mycotoxins
- It is not easy to reduce the matrix effects thus the method development and validation must be well established
- The difference in operation systems makes it difficult to transfer the eluate from the liquid chromatography column to the mass spectrometry source
- It is expensive
- Requires highly trained personnel to carry out the analysis
- It is time consuming compared to rapid tests

5.4.4 Thin-Layer Chromatography (TLC)

- Thin-layer chromatography was first used by de Iongh *et al*. in 1964.
- It has been regarded by the Association of Official Analytical Chemist (AOAC) as the method of choice since 1990.
- The method is one of the most widely used separation techniques in aflatoxins analysis.
- It consists of a stationary phase made of either silica or alumina or cellulose immobilized on an inert material such as glass or plastic, called the matrix.
- The mobile phase is comprised of methanol: acetonitrile: water mixture, which carries the sample along as it moves through the solid stationary phase.
- In TLC, the distribution of aflatoxins between the mobile and stationary phases is based primarily on differences in solubility of the analytes in the two phases.
- Different analytes, depending on their molecular structures and interaction with the stationary and mobile phases, either adhere to the stationary phase more or remain in the mobile phase, thereby allowing for quick and effective separation.

- The method has been used in determination of aflatoxin levels as low as 1-20 ppb.
- The advantages and disadvantages of carrying out aflatoxin analysis using the TLC method include;

Advantages

- It is simple, cheap and fast
- Multiple samples can be run simultaneously
- Several types of mycotoxins can be analyzed in a single test sample
- It has excellent sensitivities in mycotoxin detection

Disadvantages

- It requires skilled personnel
- The method requires pre-treatment of the sample
- TLC equipment is expensive
- The method lacks precision due to accumulated errors during sample application, plate development, and plate interpretation

5.4.5 High-Performance Thin-Layer Chromatography (HPTLC)

- Attempts to improve TLC have led to the development of automated form of TLC, called the high-performance thin-layer chromatography (HPTLC).
- The HPTLC has since overcome the problems associated with the conventional TLC techniques through automation of sample application, development, and plate interpretation.
- This makes HPTLC one of the most efficient and precise methods in aflatoxin analysis.
- Nevertheless, HPTLC is still faced with the following challenges that limit it to the laboratory making it inapplicable in field situations:
    i. The requirement for skilled operators
    ii. The costs of the equipment
    iii. Bulkiness
    iv. Extensive sample pretreatment

5.4.6 Gas Chromatography (GC)

- In gas chromatography, the mobile phase is a carrier gas and the stationary phase is a liquid coated onto inert solid particles.
- As with other chromatographic methods, sample analysis by GC is based primarily on differential partitioning of analytes between the two phases.
- The stationary phase consists of inert particles coated with a layer of liquid and is normally confined to a long stainless steel or glass tube called the column, which is maintained at appropriate temperature.
- The sample to be analyzed is vaporized into gaseous phase (mobile phase) and carried through the stationary phase by a carrier gas.
- The different chemical constituents in the sample will distribute themselves between the mobile phase and the stationary phase.
- Substances will separate according to their ability to cross the stationary phase (a process known as elution).
- The components of the samples mixture with higher affinity for the stationary phase are retarded in their movement through the column.
- Components of low affinity pass through the column less impeded.
- For that matter, each component of the analyte should have a specific partition coefficient, which, in turn, will govern its rate of passage through the column.
- Once separation has been achieved, the detection of the volatile products is carried out using either a flame ionization detector (FID) or an electron capture detector (ECD) and mass spectrometer (MS).
- The procedure is illustrated in Figure 23.

- A sample is carried by a gas into a heated glass column coated with a non-volatile liquid.
- Different substances will cross the column at different rates.
- The different substances will generate peaks that are read by the detector and shown on the computer.

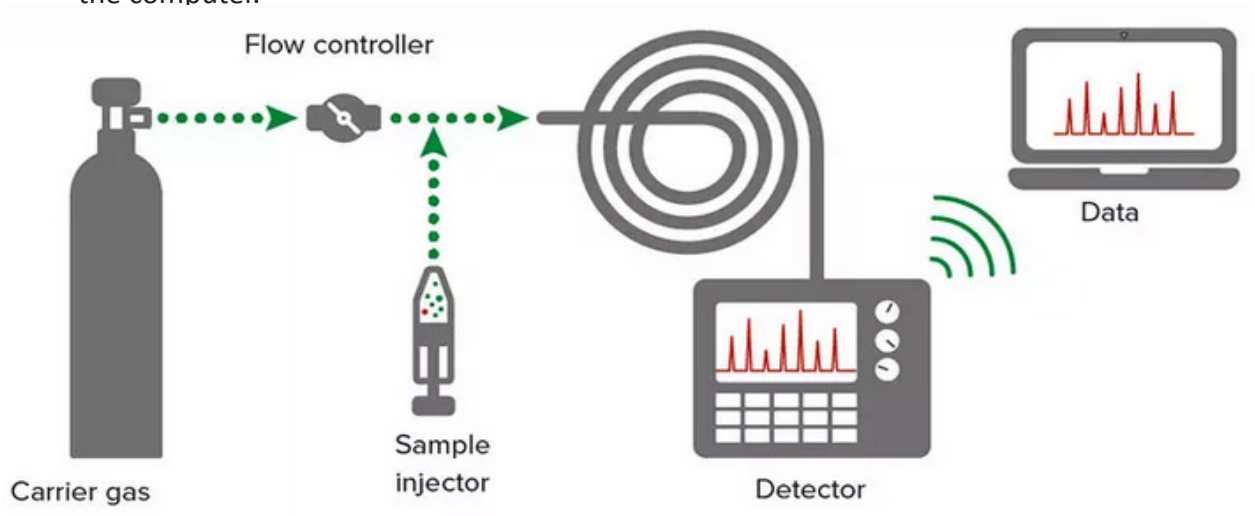


Figure 23: Illustration of the gas chromatography (GC) technique
Source: Mycotoxin info, https://www.mycotoxins.info

The advantages and disadvantages of carrying out aflatoxin analysis using the GC technique include;

Advantages

- High sensitivity
- High specificity (low interference)

Disadvantages

- It requires skilled personnel for mycotoxin analysis
- Owing to their nonvolatility in nature, aflatoxins may need derivatization in order to be detected
- The method is expensive and therefore less common in commercial analysis of aflatoxins due to the existence of other cheaper chromatographic methods
- Gas chromatography also requires a preliminary cleanup step before analysis
- It is therefore limited to analysis of a few mycotoxins, such as A-trichothecenes and B-trichothecenes
- Even in such analyses, the GC has such disadvantages as nonlinearity of calibration curves, drifting responses, memory effects from previous samples, and high variation in reproducibility and repeatability
- Data analysis is time-consuming and prone to errors

CHAPTER 6: TRAINING METHODOLOGIES/APPROACHES AND THEIR JUSTIFICATION

6.1 Training methodologies

There are various methods and approaches that instructors can use to deliver content contained in this manual:

6.1.1 Instructor-led training

In this method;

- Instructor-led training is used especially for complex topics outlines in Chapter One of this training manual.
- The instructor-led training will take place in a classroom setting as the instructor and trainees share information using various dissemination channels in interactive sessions.
- This approach will be used to train the regulators, extension agents, policy makers and laboratory technologists, traders and producers.
- Instructors will have an opportunity to answer specific questions or direct the trainees to further resources
- This approach is encouraged amongst traders and farmers/producers.
- The instructors will also engage the trainees in group activities to test their understanding on the focus topics as they progress with the training.
- Group activities approach is particularly effective for traders and farmers/producers.

6.1.2 Hands-on training

Hands-on training will include experiential training focusing on the individual needs of the target groups. This method will be used to cover Chapter Three of the module touching on methods of aflatoxin testing. In this method;

- The instructors will demonstrate testing methods using materials and equipment such as the rapid test kits.
- Demonstration of testing methods will be a key activity for the regulators/inspectors, laboratory technologists as well as traders.
- The trainees will have an opportunity to do a mock testing using the materials provided. This will help in enhancing understanding amongst the regulators/ inspectors, laboratory technologists, extension agents as well as the traders.
- Using samples contaminated with aflatoxins, the trainees will have an opportunity to collect data and interpret the results.
- This will be applicable for the traders, farmers, regulators, laboratory technologists and the extension agents.
- The samples provided will give the trainees an opportunity to interact with materials and try to visually distinguish fungal infected and non-infected materials, an aspect that will be vital for all the stakeholders.
- The approach will enable the inspectors and regulators to carry out mock inspections with the help of the trainers. This will be specific for the regulators.

6.2 Instructional materials and equipment

The following materials and equipment will be required for effective training:

- Laptop(s)
- LCD projector
- Printer and printing paper
- Flip charts
- Manila papers

- Mark pens
- Stationery – Note books, pens
- Pictorials
- Posters
- Hand-outs
- Contaminated and visually clean samples of groundnuts, macadamia nuts, maize, herbs and spices
- ELISA reader
- ELISA testing kits

For effective training and the expected outcomes to be achieved, the following instructional materials, samples and equipment will be used:

- Traditional resources will largely be used and will involve lectures with enough room for the trainer-trainee engagement.
- The trainers will use charts, pictorials and other reference materials.
- Print documents and posters that will be used during classroom training and after the training.
- Electronic/digital equipment where the instructors will use computers, projectors and PowerPoint presentations especially in the pedagogical training.
- Contaminated samples of groundnuts, macadamia nuts and herbs and spices will be used for illustrations
- Testing kits will be required for illustrations on testing methods.

6.3 Training duration

Training of trainers (ToTs) will take four days with 3 days being pedagogical while the fourth day will involve practical aspects and mock inspections.

6.4 Dissemination channels

During the training, various dissemination channels will be used. These include;

- Lectures where the instructors will use PowerPoint presentations and print references.
- Audio visual content where videos and digital illustrations will be given in a classroom setting.
- Print content will be used by the instructors to relay some information in print documents, carefully selected peer reviewed articles and simplified posters that can be used after the training.
- Digital content will also be shared with the participants after the training for reference.

6.5 Mode of assessment of the training

- The trainers will use testing resources such as classroom assignments to be handled both individually and in groups.
- The instructors will also test the understanding and competency of the trainees by allowing them to handle the mock inspections and testing, and then compare results.
- At the end of the training, a standardized evaluation test will be administered.

6.6 Training approaches for different categories

Category of Trainee	Training Methodology	Materials and Methods	Duration (days)	Assessment/ Monitoring
Inspectors/ Regulators	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples	• 3 days instructions • 2 days demonstrations, mock inspection and interpretation of aflatoxin analysis results	Questionnaire
Extension officers	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples	• 3 days instructions • 1 day demonstrations, mock inspection and interpretation of rapid aflatoxin analysis results	Questionnaire
Traders	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples • Field demo for product handling & storage	• 2 days instructions • 1 day demonstrations, mock inspection and interpretation of rapid aflatoxin analysis results • 1 day field demo for product handling, sampling & storage	Questionnaire
Processors/ Laboratory Techno-logists	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples • Field demo for HACCP	• 2 days instructions • 2 days demonstrations, mock inspection and interpretation of aflatoxin analysis results • 1 day field demo for HACCP	Questionnaire

Producers/ Farmers	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples • Posters	• 1 day instructions • 1 day demonstrations on sampling, rapid aflatoxin analysis and interpretation of analysis results	Questionnaire-led by instructor
Consumers	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples • Posters	• 1/2 day instructions (awareness raising) • 1/2 day demonstrations on aflatoxin analysis and interpretation of analysis results	Questionnaire-led by instructor
County Government Officials/ Policy Makers	• Instructor led • Practicals	• Power point presentation • Laboratory demonstrations • Samples	• 1 day instructions • 1 day demonstrations and interpretation of aflatoxin analysis results	Questionnaire

6.7 Training time table

A sample ToT Training Schedule on Aflatoxins and Aflatoxins Management in Groundnuts, Macadamia Nuts, Maize, Herbs and Spices Value Chains

DAY ONE			
Time	**Sunday**	**Duration**	**Remarks/Facilitator**
Afternoon/ Evening	Arrival of participants		Boarding of participants UNIDO
DAY TWO			
Time	**Monday**	**Duration**	**Remarks/Facilitator**
8.30-9.00 am	Arrival of participants and registration	30 Minutes	Training venue & materials ready for use Facilitators/UNIDO
9.00-9.30 am	Welcome participants Self-introduction and levelling expectations	5 Minutes 25 Minutes	Lead Facilitator
9.30-9.45 am	Official opening	15 Minutes	UNIDO Representative
9.45-10.00 am	Brief on the training program Formation of working groups	10 Minutes 5 Minutes	Lead Facilitator
10.00-10.30 am	**Health Break & Group Photograph**		
	Module 1: Mycotoxins - Types, Impact and Management Strategies		
10.30-11.30 am	Overview on mycotoxins	1 Hour	Facilitator
11.30–12.30 pm	Aflatoxins: Aflatoxin producing fungi, aflatoxin types and co-occurrence with other mycotoxins	1 Hour	Facilitator
12.30-1.00 pm	Factors affecting aflatoxin contamination of groundnuts and macadamia nuts	30 Minutes	Facilitator
1.00-2.00 pm	**Lunch Break**		
2.00-3.00 pm	Factors affecting aflatoxin contamination of maize, herbs, spices and animal feeds	1 Hour	Facilitator

3.00-3.45 pm	Group Work: Participants to identify and enumerate characteristics of provided samples contaminated with aflatoxin/ mycotoxin producing fungi *Each group to present results (5 minutes per group)*	45 Minutes	Facilitator
3.45-4.30 pm	Management of aflatoxin contamination in groundnuts, macadamia nuts, maize, herbs and spices value chains	45 Minutes	Facilitator
4.30-5.00 pm	**Health Break**		
DAY THREE			
Time	**Tuesday**	**Duration**	**Remarks/Facilitator**
8.30-9.00 am	Recap	30 Minutes	Facilitator
9.00-9.30 am	Impact of aflatoxins on health, trade and food security	30 Minutes	Facilitator
9.30-10.00 am	Hazard Analysis Critical Control Points (HACCP): Concept, pre-requisite programs and the seven principles	30 Minutes	Facilitator
10.00-10.30 am	**Health Break**		
10.30-11.00 am	Aflatoxin management policies and regulatory infrastructure in Kenya and key challenges	30 Minutes	Facilitator
11.00-11.15 am	Module review	15 Minutes	Facilitator
	Module 2: Sample Collection for Aflatoxin Analysis		Facilitator
11.15-12.15 pm	Considerations and approach to sample collection for aflatoxin analysis	1 Hour	Facilitator
	Module 3: Methods of Aflatoxin Analysis		Facilitator
12.15-1.00 pm	Overview of aflatoxin analysis: methods and justification	45 Minutes	Facilitator
1.00-2.00 pm	**Lunch Break**		

2.00-3.00 pm	Rapid quantitative methods of aflatoxin analysis: *Example of ELISA*	1 Hour	Facilitator
3.00-4.00 pm	Analytical quantitative methods of aflatoxin analysis: *HPLC, LCMS, TLC, GS*	1 Hour	Facilitator
4.00-4.45 pm	Group Work: Outline the steps and approaches in collecting representative maize or groundnut samples from a market for aflatoxin analysis *Each group to present results (5 minutes per group)*	45 Minutes	Facilitator
4.45-5.15 pm	**Health Break**		
DAY FOUR			
Time	**Wednesday**	**Duration**	**Remarks/Facilitator**
8.30-9.00 am	Recap	30 Minutes	Facilitator
9.00-10.00 am	Semi-quantitative methods of aflatoxin analysis: *Frontier Infrared Spectroscopy, Radioimmunoassay, Immunosensors*	1 Hour	Facilitator
10.00-10.30 am	**Health Break**		
10.30-11.30 am	Qualitative methods of aflatoxin analysis: *Fluorescence Polarization Immunoassay, Fluorometry*	1 Hour	Facilitator
11.30-12.15 pm	Semi-qualitative methods of aflatoxin analysis: *Immunodipsticks*	45 Minutes	Facilitator
12.15-1.00 pm	Group Work: Outline the key considerations in choice of aflatoxin analysis method(s) *Each group to present results (5 minutes per group)*	45 Minutes	Facilitator

1.00-2.00 pm	**Lunch Break**		
2.00-2.45 pm	Overview on national and international standards on aflatoxin levels in specific commodity and related products	45 Minutes	Facilitator
2.45-3.00 pm	Module review	15 Minutes	Facilitator
3.00-4.30 pm	Practical one: Isolation and identification of aflatoxigenic *Aspergillus* species	1.5 Hours	Accredited Laboratory
4.30-5.00 pm	**Health Break**		
DAY FIVE			
Time	**Thursday**	**Duration**	**Remarks/Facilitator**
8.30-9.00 am	Recap	30 Minutes	
9.00-11.00am	Practical two: Demonstration of aflatoxin analysis using ELISA method; interpretation of results; and dilution of sample extracts	2 Hours	Accredited Laboratory
11.00-11.30 am	**Health Break**		
11.30-1.30pm	Practical session: Visit the Aflasafe KE01™ Modular Manufacturing Plant	2 Hours	Aflasafe KE01™ Modular Manufacturing Plant, KALRO Katumani
1.30-2.30 pm	**Lunch Break**		
2.30-3.00 pm	Training Evaluation/Feedback	30 Minutes	Lead Facilitator
3.00-4.00 pm	Review of the Training Manual	1 Hour	Lead Facilitator
4.00-4.30 pm	Issuance of certificates and workshop closure	30 Minutes	UNIDO Representative
4.30-5.00 pm	**Health Break**		
PARTICIPANTS LEAVE AT THEIR PLEASURE			

REFERENCES

Alshannaq AF, Gibbons JG, Lee MK, Kap-Hoon Han K-H, Hong S-B and Yu J-H. 2018. Controlling aflatoxin contamination and propagation of *Aspergillus flavus* by a soy-fermenting *Aspergillus oryzae* strain. Scientific Reports 8: 16871. https://doi.org/10.1038/s41598-018-35246-1.

Azri FA, Selamat J and Sukor R. 2017. Electrochemical immunosensor for the detection of aflatoxin B1 in palm kernel cake and feed samples. Sensors 2017, 17(12), 2776; https://doi.org/10.3390/s17122776.

Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, Rubin C, Slutsker L and Aflatoxin Investigative Group. 2005. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environmental Health Perspectives 113(12): 1779-1783.

Bennett JW and Klich M. 2003 – For uniformity, delete the first colon (:) after the Journal name; it should read as follows: Toxins 11(1): 27

Daniel JH, Lewis LW, Redwood YA, Kieszak S, Breiman RF, Flanders WD, Bell C, Mwihia J, Ogana G, Likimani S, Straetemans M and McGeehin MA. 2011. Comprehensive assessment of maize aflatoxin levels in Eastern Kenya, 2005-2007. Environmental Health Perspectives 119(12): 1794-1799.

De Iongh H, Vles RO and Van Pelt JG. 1964. Milk of mammals fed an aflatoxin-containing diet. Nature 202(4931): 466-467.

Delmulle BS, De Saeger SMDG, Sibanda L, Barna-Vetro I and Van Peteghem CH, 2005. Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed. Journal of Agricultural and Food Chemistry 53(9): 3364-3368.

FAO, Food and Agriculture Organization of the United Nations. 2001. Manual on the Application of the HACCP System in Mycotoxin Prevention and Control. FAO, Rome, Italy. https://www.fao.org/3/y1390e/y1390e00.htm.

Ho J-AA and Wauchope RD. 2002. A strip liposome immunoassay for aflatoxin B1. Analytical Chemistry 74(7): 1493–1496.

International Maize and Wheat Improvement Center (CIMMYT)/United Nations Development Programme (UNDP)/ United States Agency for International Development (USAID). 1986. In: Proceedings of the Workshop: Aflatoxins in Maize, CIMMYT, El Batan, Mexico.

Kang'ethe E, Korhonen H, Marimba K, Nduhiu G, Mungatu J, Okoth S, Joutsjoki V, Wamae LW and Shalo P. 2017. Management and mitigation of health risks associated with the occurrence of mycotoxins along the maize value chain in two counties in Kenya. Food Quality and Safety 1(4): 268-274.

KDHS, Kenya Demographic and Health Survey. 2015. Kenya National Bureau of Statistics. https://dhsprogram.com/pubs/pdf/fr308/fr308.pdf

Kemboi DC, Ochieng PE, Antonissen G, Croubels S, Scippo ML, Okoth S, Kangethe EK, Faas J, Doupovec B, Lindahl JF and Gathumbi JK. 2020. Multi-mycotoxin occurrence in dairy cattle and poultry feeds and feed ingredients from Machakos Town, Kenya. Toxins 12(12): 762. doi: 10.3390/toxins12120762.

Linting Z, Ruiyic L, Zaijun L, Qianfang X, Yinjund F and Junkang L. 2012. An immunosensor for ultrasensitive detection of aflatoxin B1 with an enhanced electrochemical performance based on graphene/conducting polymer/gold nanoparticles/the ionic liquid composite film on modified gold electrode with electrodeposition. Sensors and Actuators B: Chemical 174: 359-365.

Maragos C. 2009. Fluorescence polarization immunoassay of mycotoxins: A review. Toxins 1(2): 196-207.

Marrazza G. 2014. Piezoelectric Biosensors for Organophosphate and Carbamate Pesticides: A Review. Biosensors 4(3): 301-317.

Muraguri N, Omukoolo LC, Kenji GM and Condier GA. 1981. A survey of mycotoxins in human and animal foods. East African Medical Journal 58(7): 484-488.

Muriuki GK and Siboe GM. 1995. Maize flour contaminated with toxigenic fungi and mycotoxins in Kenya. African Journal of Health Sciences 2(1): 236-241.

Mutegi CK, Cotty PJ and Bandyopadhyay R. 2018. Prevalence and mitigation of aflatoxins in Kenya (1960-to date). World Mycotoxin Journal 11(3): 341–357.

Muthomi JW, Mureithi BK, Chemining'wa GN, Gathumbi JK and Mutitu EW. 2010. *Aspergillus* and aflatoxin B1 contamination of maize and maize products from Eastern and North Rift regions of Kenya. In: Proceedings of the 12th KARI Biennial Conference, Nairobi, Kenya, pp. 344-352.

Muthomi JW, Njenga LN, Gathumbi JK and Chemining'wa GN. 2009. The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in Eastern Kenya. Plant Pathology Journal 8(3): 113–119.

Ngindu A, Johnson BK, Kenya PR, Ngira JA, Ocheng DM, Nandwa H, Omondi TN, Jansen AJ, Ngare W, Kaviti JN, Gatei D and Siongok TA. 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet. 12:1(8285): 1346-1348.

Nyangaga DK. 2014. Traders' awareness and level of aflatoxins in human foods and cattle feeds in selected markets and stores in Nairobi County, Kenya. MSc Thesis, Kenyatta University, Kenya.

Lewis L, Onsongo M and Njapau H. 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspectives 113(12): 1763-1767.

Peers FG and Linsell CA. 1973. Dietary aflatoxins and liver cancer-a population based study in Kenya. British Journal of Cancer 27(6): 473-484.

Probst C, Njapau H and Cotty PJ. 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. Applied Environmental Microbiology 73: 2762-2764.

Wagacha JM and Muthomi JW. 2008. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology 124(1): 1–12.

MARKUP

EU-EAC MARKET ACCESS UPGRADE PROGRAMME

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