

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



24 -27 February 2008



AFLATXINS: AWARENESS AND CONTROL

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AFLATOXIN

**Most references to
“mycotoxin”,
unspecified, refer to
Aflatoxin.**

Why to deal with aflatoxin ?

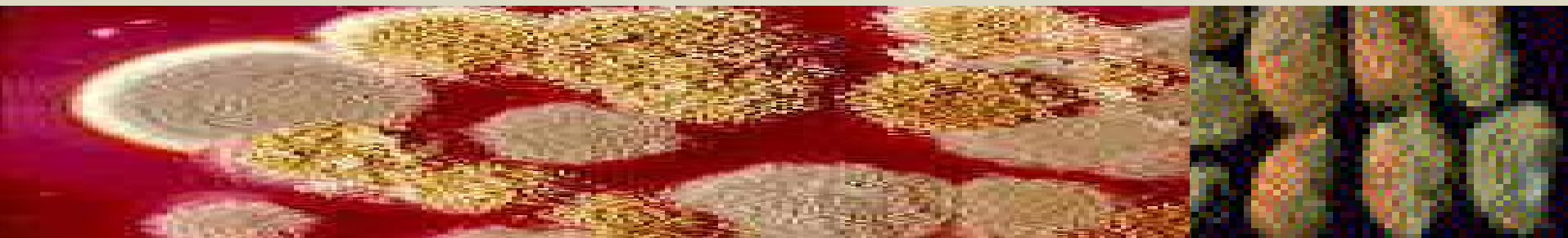
- **Mycotoxins are present in all stages of food production, processing and storage.**
- **These natural toxins occur primarily in those cereal crops (e.g. wheat, maize) which serve as main food items for the human population and as important raw materials of animal feeds.**
- **The risk posed mycotoxins consumed in low amount but continuously is not yet explored, however the danger is very high ('hidden killers').**

History of Aflatoxins

- 5,000 years ago in China.
- Early 1861, was reported in Russia.
- 1891, there was a report of mouldy rice in Japan.
- 1913, First describes but the toxin was not isolate.
- 1940, Aflatoxicosis in swine had been reported due to ingestion contaminated **Maize**.
- 1950, similar ingestion happened in Alabama, US.
- 1960, is the time of the discovery of aflatoxin in England (Turkey X Disease).

Sources of Aflatoxins

- Produced by 3 species of *Aspergillus*: *A. flavus*, *A. parasiticus*, *A. nomius*.
- Aflatoxin: A (*Aspergillus*) - fla (*flavus*) – toxin.
- Four toxins soon identified: Aflatoxin B₁, B₂, G₁, G₂ - blue or green fluorescence under UV-light.
- Aflatoxin B₁ most important - highly carcinogenic and widespread occurrence in foods
- (B₁ > M₁ > G₁ > B₂ > M₂ ~ G₂).
- Aflatoxin M₁ : hydroxylated product of B₁ appears in milk, urine, and feces as metabolic product.

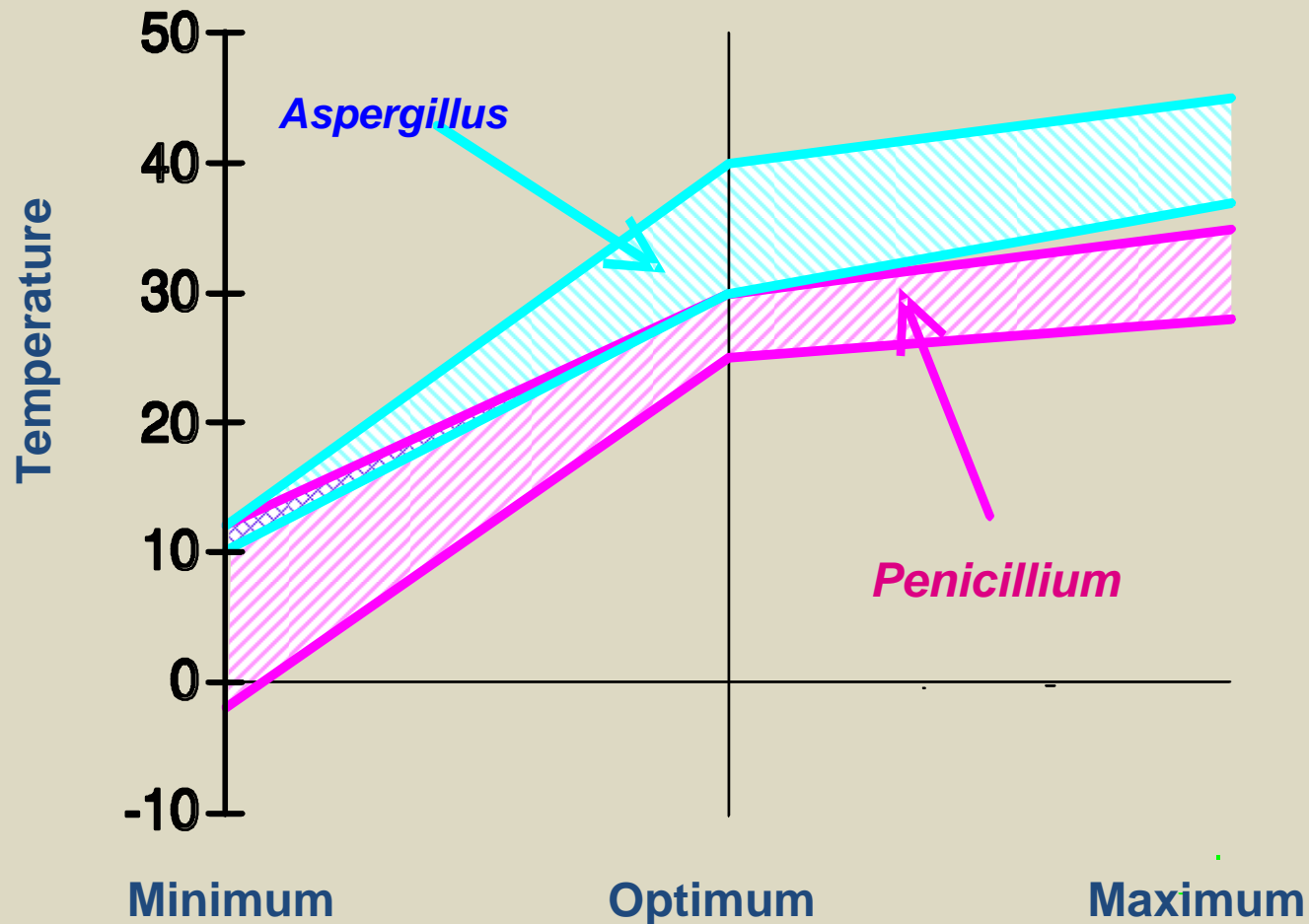


Physiological Characteristics of Fungi

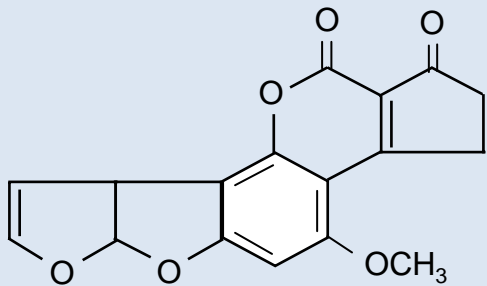
- **Growth temperature**
 - Opt. 25 ~ 30°C., Min. 4° C
- **Water activity (a_w)**
 - a_w – 0.62 ~ 1.0 (If substrate RH = 95%, a_w = 0.95).
- **pH**
 - pH 2 ~ 8.5 (pH 5.0 ~ 5.5).
- **Aerobic**
 - Oxygen (1-2%)



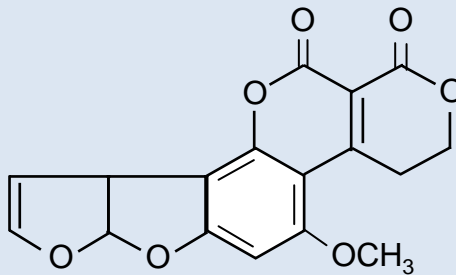
Temperature Range for Growth of Toxigenic Moulds



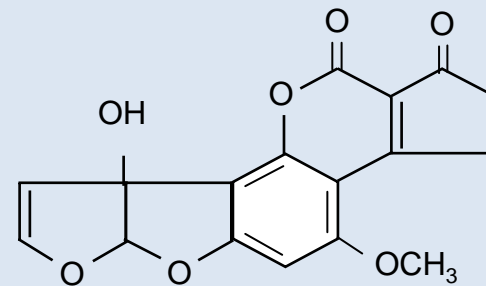
Aflatoxins



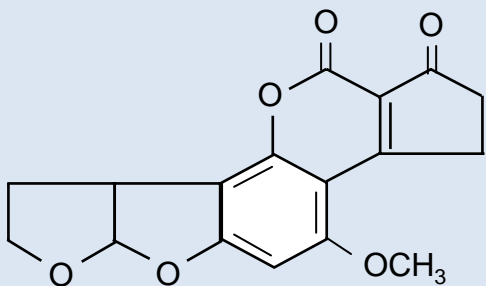
aflatoxin B₁



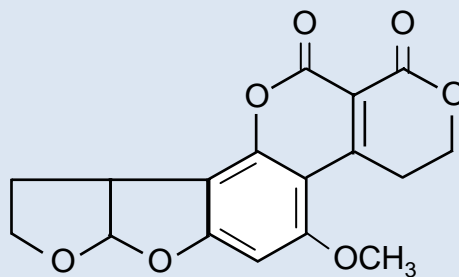
aflatoxin G₁



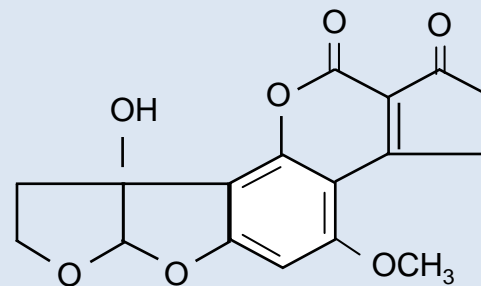
aflatoxin M₁



aflatoxin B₂



aflatoxin G₂



aflatoxin M₂

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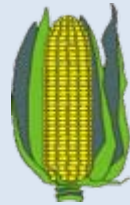
Chemical Properties

- AFs are **difuro-coumarin** (heterocyclic compounds), they are freely **soluble in chloroform and methanol**.
- Pure AFs are stable at High temp. and unstable when exposed to UV and air when dissolved in polar solvents.
- Chloroform and Benzene solution are stable for year if stored in dark cold place.

The important sites in AFB₁ molecule are:

- The 1st site: Double bond in position 8,9 of furofuran ring, (DNA and protein)
- The 2^{ed} site: Lacton ring in coumarin moiety
- Lacton ring is easily hydrolyzed (alkaline hydrolysis)

**Infection of plants
by moulds**



Mycotoxins entering the food chain

Contaminated feed



Toxin in the blood and organs

**Contaminated food of
plant origin**

**Contaminated food of
animal origin**



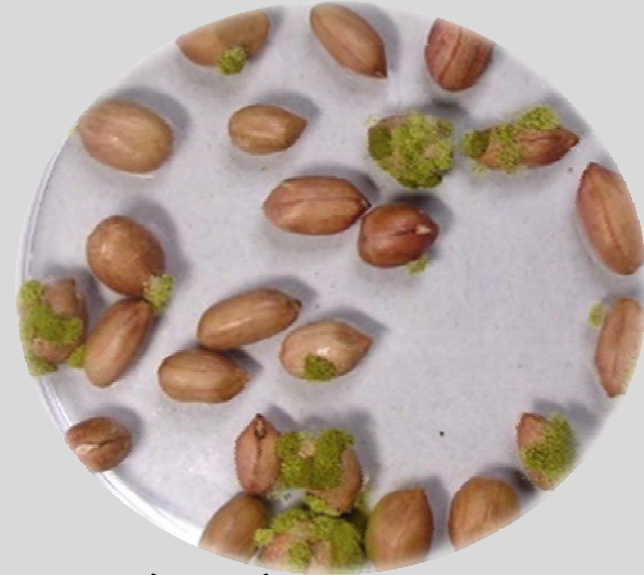
**aflatoxins
ochratoxin-A
zearalenone
fumonisins**

**Meat
Milk**

Egg

Occurrence of Mycotoxins in Foods

- Milk and milk products (*AFM₁*, *OTA*)
- Meat and meat products (*OTA*)
- Egg (*AFB₁*, *DON*, *CPA*)
- Cereals (*AFB₁*, *Fusarium toxins*, *OTA*)
- Oilseed (*AFB₁*)
- Wine, grape juice and grape products (*OTA*)
- Beer (*AFB₁*, *OTA*, *Fusarium toxins*)
- Fruits, juices, vegetables
(*AFB₁*, *OTA*, *citrinin*, *patulin*, *Fusarium toxins*)
- Coffee and cocoa (*AFB₁*, *OTA*)



HEALTH EFFECTS of AFLATOXINS

- The primary target of aflatoxins is hepatic system
- AFs induced Liver cancer and bile duct proliferation
- The chronic exposure to AFs lead to **(on both humans and livestock):**
 - 1- Impaired immunity
 - 2-Reduce food intake
 - 3- Reduce growth rate
 - 4- Reproductive disorders
 - 5- Increased susceptibility to infectious disease.
 - 6- Disorder of protein and lipid metabolism.
 - 7- Inhibits protein synthesis and stimulate lipid peroxidation
 - 8- Disfunction of enzyme function and their synthesis.
 - 9- Depressed complement and interferon production.

Factors Influencing The Harmful Effects of AFLATOXINS

- Chemical structure (accumulation)
- Concentration
- Time of exposure
- **Multitoxic effect** (interaction, synergism, additive effect)
- Individual sensitivity (Age, Sex, Species, Breed)
- Health status (Diseases)
- Nutritional status
- Vitamin supply
- Stressors

Target Organs of Some Mycotoxins

Mycotoxin

Target

Aflatoxin

Liver

Ochratoxin A

Kidney

Trichothecenes

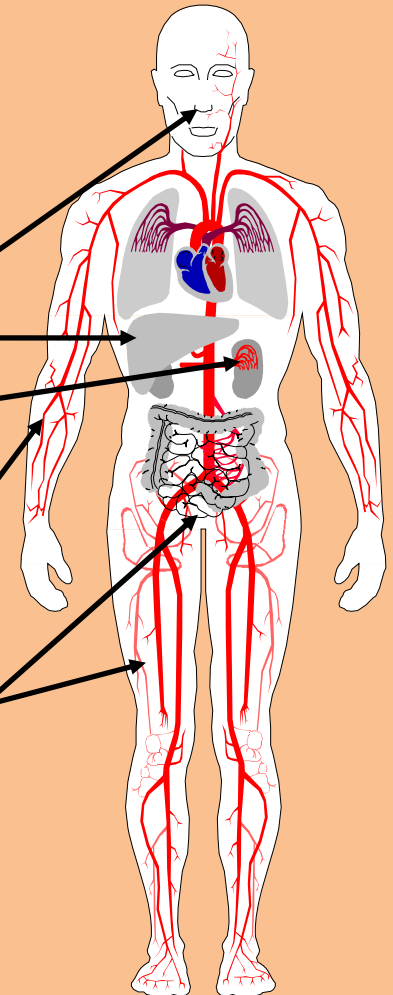
Mucosa

Ergot alkaloids

Peripheral vascular system

Zearalenone

Uro-genital tract



Aflatoxin Levels

- **Levels permissible in foods subject to legal limits in many countries**
- **Today, foods most frequently contaminated, routinely screened before processing or sale**
- **Permissible limits generally quite low (15-20 parts per billion)**
- **Some scientists feel that no detectable levels of aflatoxins should be permitted because of the carcinogenic effects**

Factors to Consider in Setting Regulatory Limits

- **Availability of data on incidence and occurrence**
- **Availability of toxicological data**
- **Availability of analytical methodology**
- **The need to maintain an adequate food supply at reasonable cost**
- **Knowledge of legislation in other countries involved in international trade**

Maximum Acceptable Levels for A Range of Aflatoxins In Foodstuffs for A Selection of Countries

Aflatoxins	Country	Limit (µg per	Matrix
Aflatoxin B₁	EU	2	Nuts or dried fruit intended for direct human consumption or as an ingredient in foodstuffs
Aflatoxin B₁, B₂, G₁, G₂	EU	4	Nuts or dried fruit intended for direct human consumption or as an ingredient in foodstuffs
Aflatoxin B₁, B₂, G₁, G₂	Canada	20	Feeding stuffs for animals
Aflatoxin M₁	EU	0.025	Infant formulae including infant milk. The maximum level for infant formulae refers to the product ready to use
Aflatoxin M₁	US	0.5	Milk for human consumption

Action or recommended maximum concentrations of aflatoxins in animal feeds*

Commodity	Animal	Maximum conc. in diet
Corn, peanut products, other products or feed ingredients	Finishing (feedlot) beef cattle	300 ppb
	Breeding beef cattle, breeding swine, mature poultry	100 ppb
	Finishing swine > 100 lb	200 ppb
Corn, peanut products, other products or feed ingredients, excluding cottonseed meal	Immature animals	20 ppb
Cottonseed meal	Beef, cattle, swine, poultry (regardless of age)	300 ppb
All feeds or feed Ingredients	Dairy animals, animal species not listed above, uses not listed above, intended use unknown	20 ppb

* From: Hawk (2004)

MAXIMUM LIMITS OF MYCOTOXINS PERMITTED IN FOODS AND ANIMAL FEEDS – AFLATOXINS *In United Arab Emirates (GSO 841 / 1997)*



Mapping Specialists, Ltd.

Commodity	Maximum limits of Total aflatoxins
Foods	
Infants and children foods	0.05 µg/kg
Dried milk not used for infant foods.	0.5 µg/kg.
Liquid milk and its products. (except dried milk).	0.2 µg/kg.
Grains, nuts, oil seeds and their products (e.g peanuts, peanuts butter, pistachio, almond, sesame, cotton seeds, sunflower seeds, corn, wheat, rice, soya bean).	20 µg/kg
Other foods	20 µg/kg
Animal feeds	
Feeds for dairy cattles and small calves.	10 µg/kg.
Poultry feeds and other animal feeds.	20 µg/kg.

Strategies for Prevention & Control of Aflatoxins

- **Use “clean” procedures.**
- **Prevent contamination**
- **Inhibit mold growth**
 - **Drying**
 - **Refrigeration**
 - **Mold inhibitors**

Prevention of Aflatoxin Formation

- **Pre-harvest**

- Resistant varieties (if practicable).**

- Crop rotation , irrigation**

- Insect management**

- Minimize kernel damage during harvesting**

- **Post-harvest**

- Clean and dry corn to <9 ,for oily crops, and 13.5% , for corn, moisture**

- Facility with temperature-moisture control**

- Insect and pest management**

Decontamination Strategies

- **Physical separation**
 - **Sorting, washing, crushing, dehulling, Remove contaminated portions.**
 - **Diluting contaminated food with uncontaminated food!!!!**
- **Thermals Treatment**
- **Ammoniation**
- **Nixtamalization**
- **Radiation processing**
- **Binding Agents**
- **Sodium aluminosilicate, hydrated sodium calcium aluminosilicate, bentonites and activated carbons**
- **MUCH BETTER TO PREVENT FORMATION**

Heating and Cooking

- Food not largely affected by routine cooking temperature.
- Heating and Cooking under pressure can destroy 70% of AFBs in rice. Extrusion process in combination with 0.3% lime and H_2O_2 reduced AFBs level than lime and H_2O_2 alone.
- Roasting: dry and oil roasting can reduce 50 - 70% of AFB_1 .
- AFBs resists to higher temp. upto 260°C .
- Over heating affected essential nutrients

Nixtamalisation

- Maize cooked and steeped in alkaline solution of lime (0.3% CaOH).
- AFBs are reduced during Nixtamalization by 90%.

Ammoniation

- Treatment with aqueous NH_2OH (2%) at 121 °C. and 17 psi reduced AFB_1 by 99% in naturally contaminated yellow corn.
- Gaseous NH_3 (2%) showed lower efficiency at lower moisture content.

Radiation Processing



Purposes of Food Irradiation

- Low doses (< 1 kGy):

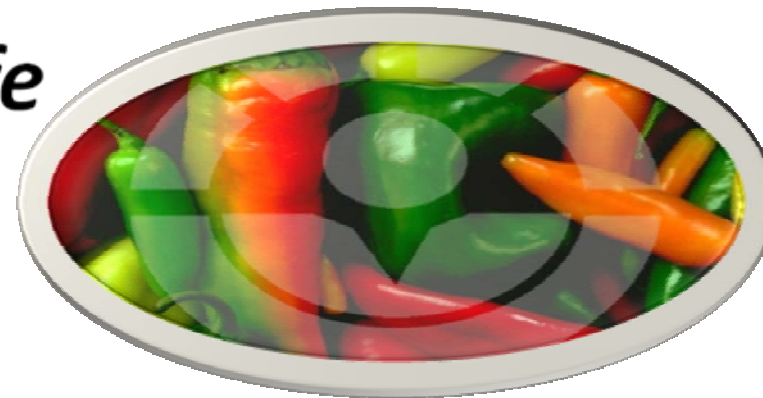
*control of insects and
delay of ripening*

- Medium doses (1 - 10 kGy):

*reduction of foodborne pathogens
extension of shelf life*

- High dose (> 25 kGy):

sterilization



Purposes of Food Irradiation



- Wheat flour – control of mold

- White potatoes – inhibit sprouting



- Pork – kill Trichinina parasites

- Fruit and Vegetables – insect control; increase shelf life



- Herbs and Spices - sterilization

- Poultry – bacterial pathogen reduction



- Meat – bacterial pathogen reduction



Radiation Processing

- Effective process, safe, and improve the safety of our food supply
- Inexpensive , approved by international organizations (WHO, FAO, IAEA, FDA and Others).
- Suppression and detoxified foodstuffs AFs in contaminated food.
- 5 kGy significantly detoxified 44 - 48% of AFB₁.
- 10 kGy detoxified AFB₁ by 62 – 88%.
- 20 kGy was quite enough for complete detoxification.

150 + irradiation facilities

40+ countries



Dietary Modification

- The carcinogenic effect of AFB₁ is affected by dietary factor such as:
- Vit. Am C and riboflavin reduce the biding of AFB₁ to DNA.
- Sulfur containing AA^S inhibit AFB₁ mutagenicity in microbial systems
- Essential oil extracted, from **Nutmeg, celery, black pepper and cardumon** , Inhibit AFB₁-DNA formation.
- **Micronutrient**, such as copper, manganese, zinc and selenium, inhibit mutugenicity.

Dietary Modification

- **Plant flavonoids, such as flavone, flavonol, isoflavon inhibit AFB₁-DNA formation.**
- **Butylated hydroxyanisole contained diet resulted in lower AFB₁-DNA formation.**
- **Butylated hydroxytoluene lower cancer incidence**
- **Broccoli sprouts, green tea, medicinal herbs lower cancer incidence.**

Control of Aflatoxins

Cooperation between:

Ministry of health

Ministry of Agriculture

Drug Administration

National Environment Committee board

Consumer Protection Committee Boared

Legislative body

International organizations

Awareness Campaigns

Information Dissemination to:

Farmer

Food and feed factories

Exporter

Middlemen

Non Governmental Organization

Public service association

Health care providers

School

Early Warning System

**EWS is intended to be safety net as supplemented to quality management system like ISO 9002, HACCP, GMP
EWS helps to prevent future outbreaks.**

EWS is able to detect potential food contamination

Any EWS should involve :

- **Monitoring AFs in food sources and individuals**
- **Response protocol to prevent further AF exposure**
- **inclusion of key members from various government agencies, health care sector and non governmental organization**

CONCLUSION

MUCH BETTER TO PREVENT FORMATION

- Selection of fungal resistant hybrids of crops
- Dry of commodities after post harvest is most effective means
- Gamma irradiation of commodities
- Raising awareness of AFs and disseminating information to formers, traders and consumers
- Collaboration between agricultural and public health communities and also between local, regional, national and international governing bodies.

**Don't forget who you heard this
from!!!**



Prof. Dr. M. Diaa El-Din H. Farag , Cairo, Egypt

NCRRT



Thank You