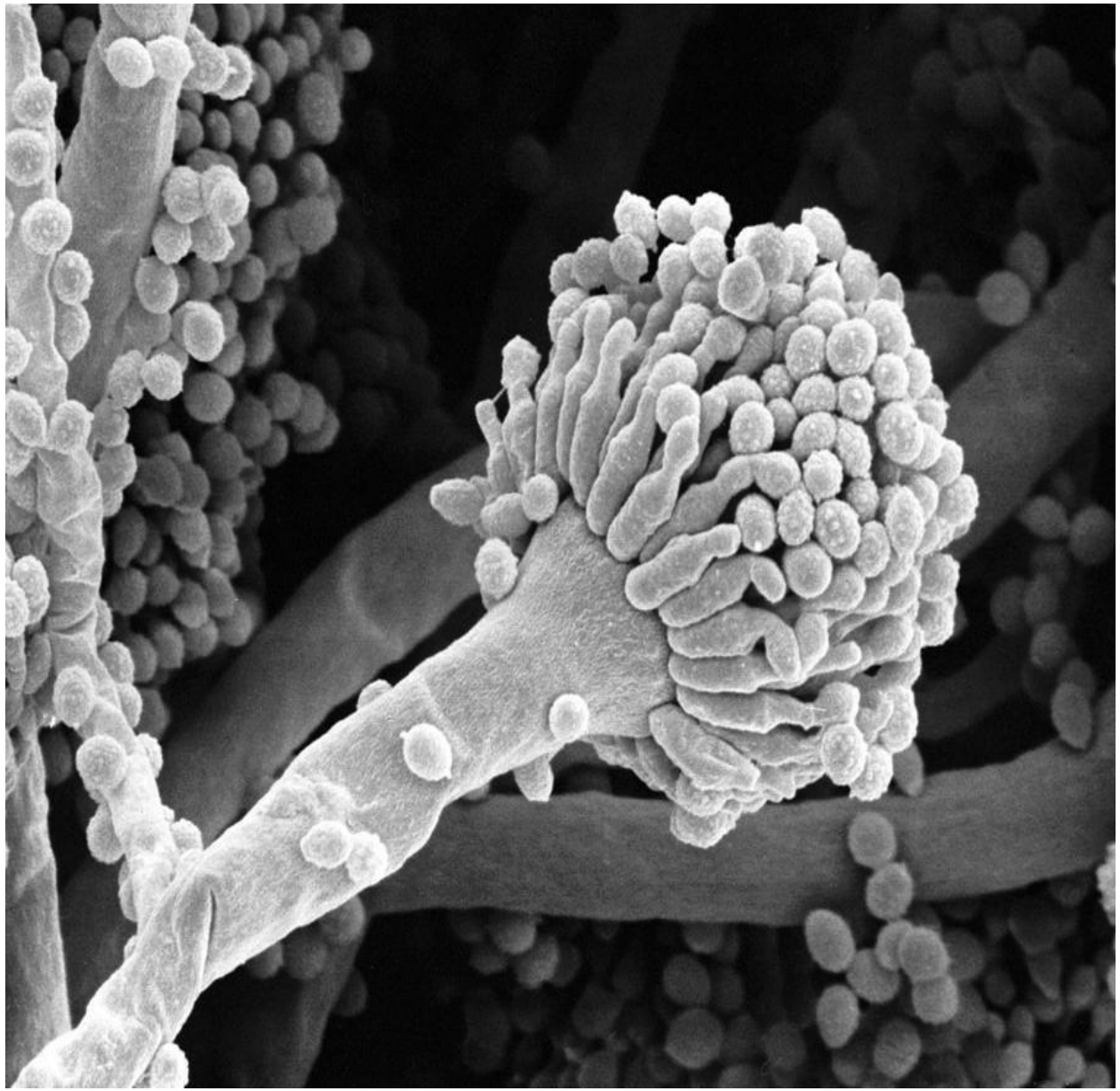


MYCOTOXIGENIC FUNGI & MYCOTOXINS

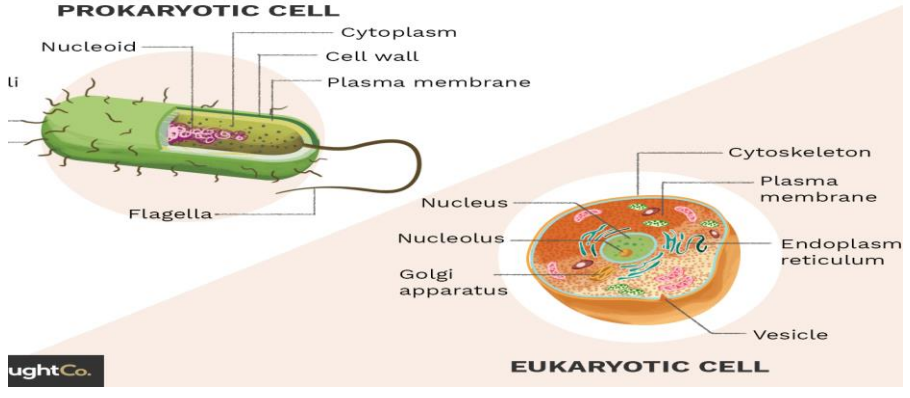
NM 26/04/2021

CONTENTS

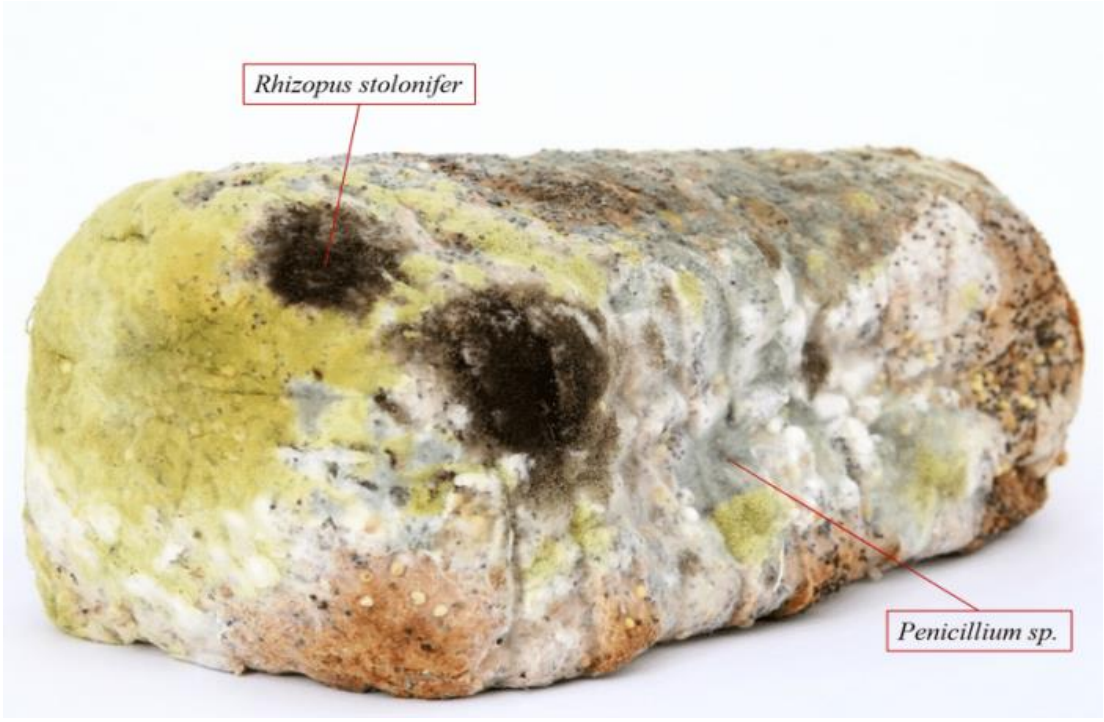
- Introduction to fungi and mycotoxins
- *Aspergillus* spp.
- *Fusarium* spp.
- *Penicillium* spp.
- Diseases
- Outbreaks
- Mechanism of action for aflatoxins
- Prevention method

WHAT IS FUNGI?

- Fungi is a kingdom that includes microorganism such as **yeasts, molds, and mushrooms**
- All fungi have a **filamentous structure** except yeast (a cell).
- Fungi consist of long thread-like structure known as **hyphae** and these hyphae together form a mesh-like structure known as **mycelium.**
- Fungi are **eukaryotic cells** & have a **cell wall**
- Fungi reproduce my means of spores (mold) and budding (yeast).
- Fungal classification is based on the **spore formation** (Zygomycetes, Ascomycetes, Basidiomycetes, Deuteromycetes)
- Spoilage and toxigenic fungi.



YEAST & MOLDS IN FOOD



Rhizopus oligosporus

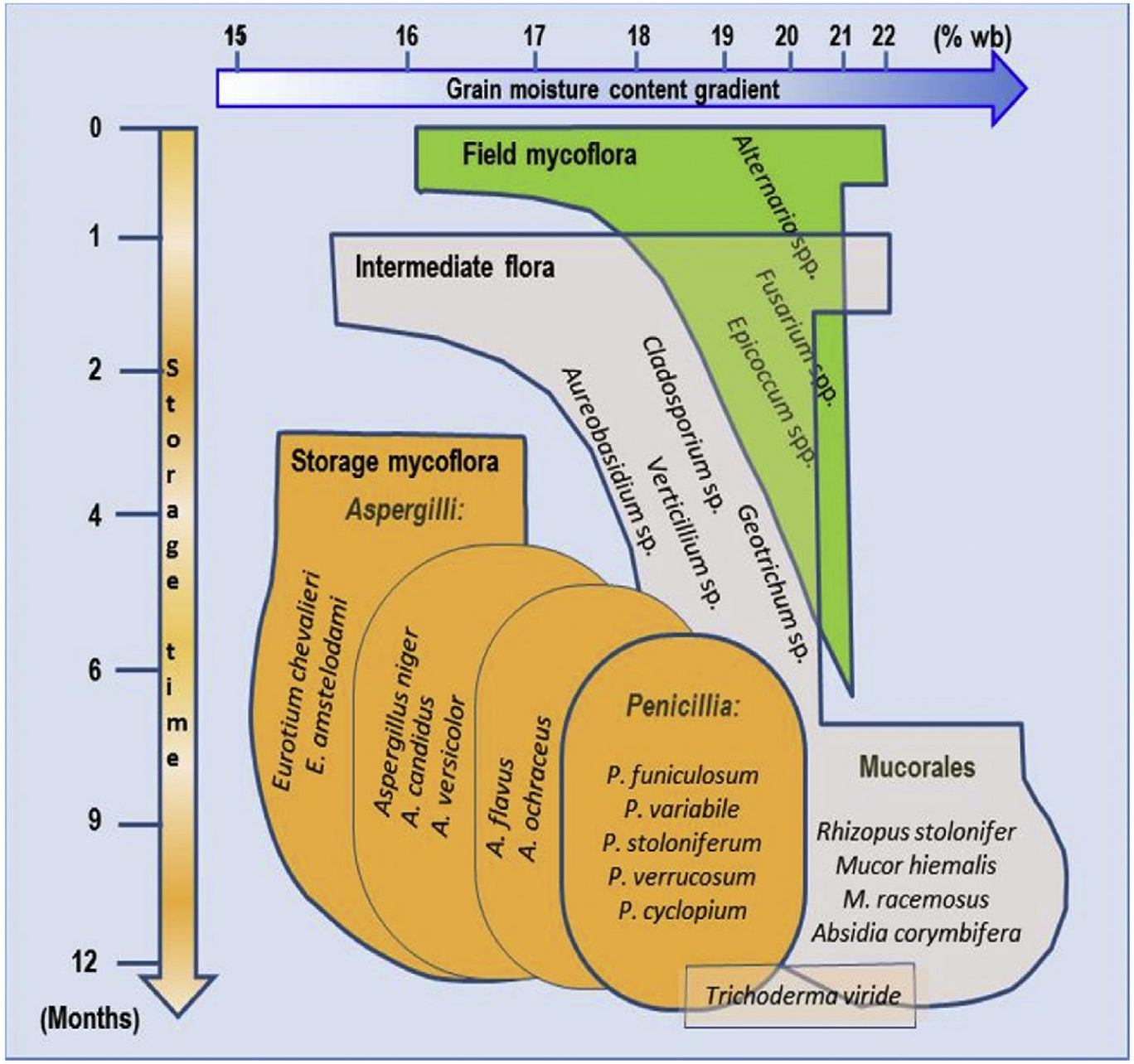
Penicillium roqueforti

Mycotoxigenic fungi

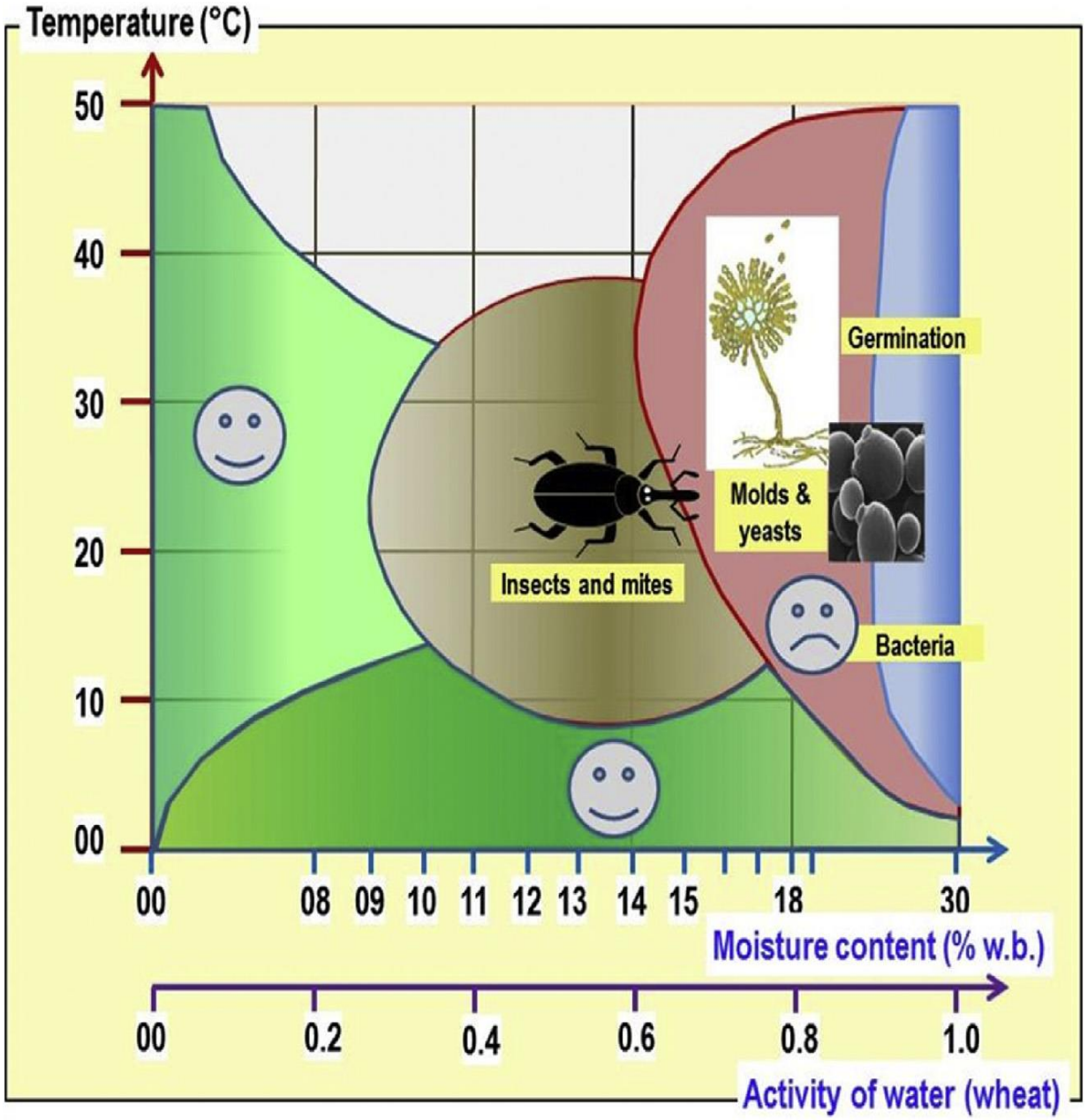
- Produce mycotoxins – toxigenic to humans
- Fungi can grow and produce mycotoxins on crops in the field, during storage, and transportation
- Mycotoxins are toxic secondary metabolites that are naturally produced by certain types of fungi.
  - NOT directly involved in the growth, development, and reproduction of the organism.

- Chemically stable and survive during food processing
- Adverse effect of mycotoxins:
  - acute poisoning
  - long-term effects such as development of cancer
- Main genus that produce mycotoxins: *Aspergillus, Fusarium, Penicillium, Alternaria*
- Mycotoxins: Aflatoxins, ochratoxins, patulin, fumonisins, zearalenone and deoxynivalenol.

Growth pattern of main fungi species contaminating stored grain during prolonged storage time



Pelhate, J., 1988. Ecology of the microflora of grains and seeds. In: Multon, J.L. (Ed.).Preservation and Storage of Grains Seeds and Their By-products. Lavoisier publishing Inc., New York, pp. 244-262.



- Growth condition
  - Optimum T for growth: 29 -35C
  - Relative humidity > 80%

- Mycotoxins are stable and resistant to heat processing such as pasteurization, UHT, roasting, and baking, also cold storage.

Fleurat-Lessard, F. 2017. Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins- An update. Journal of Stored Products Research 71:22-40

Minimum water activity level that support fungal growth and mycotoxin production of storage mycotoxigenic fungi

Fungus species	Myco-toxin	Aw limit for growth	Related moisture content (%)	Aw limit for toxin production	Related moisture content (%)
Aspergillus flavus	AFB1	0.78-0.84	17.6-19.0	0.84	19.2
A. parasiticus	AFB1	0.84	19.0	0.87	20.2
A. ochraceus	OTA	0.77	16.5	0.85	19.6
Penicillium potulum	Patulin	0.81	18.0	0.95	27.0
P. expansum	Patulin	0.82-0.84	18.3-19	0.99	30.0
P. aurantiogriseum	OTA	0.82-0.85	18.5-19.6	0.87-0.9	20.2-22.0
P. verrucosum	OTA	0.80-0.81	18.0	0.83-0.86	18.8-19.8
Fusarium proliferatum	FB1	0.88	20.5	0.93	25.0
F. verticillioides	FB1	0.88	20.5	0.93	25.0

adapted from Beuchat, 1981; Cahagnier et al. 2005 and Magan et al. 2003

MYCOTOXINS

Field mycotoxin (*Fusarium*)

- Trichothecene
  - Deoxynivalenol
  - T2 Toxin
- Zeralenone
- Fumonisins
  - Fumonisin B1, B2

Storage mycotoxin (*Aspergillus, Penicillium*)

- Aflatoxin
  - Aflatoxins B1, B2, G1, G2
- Ochratoxins
  - Ochratoxin A

Scheme of the IARC Monographs evaluation of carcinogenic hazard (Mycotoxin)

EVIDENCE IN EXPERIMENTAL **ANIMALS**

EVIDENCE IN **HUMAN**

	Sufficient	Limited	Inadequate	Evidence suggesting lack of carcinogenicity
Sufficient	**Group 1 (carcinogenic to humans, AFB$_1$)**			
Limited	**Group 2A** (probably carcinogenic to human)	**Group 2B** (possibly carcinogenic, e.g: **Fumonisin B$_1$ and B$_2$, Ochratoxin A, AFM$_1$**)		
Inadequate	↑ 2A when strong evidence that mechanism also operates in humans **Group 2B** (possibly carcinogenic)	**Group 3** (not classifiable as to its carcinogenicity to human) **DON, patulin, Zearalenone, T-2 toxin, citrinin**)		
Evidence suggesting lack of carcinogenicity	**Group 3**			**Group 4** (probably not carcinogenic to human)

Maximum Permitted Level Of Mycotoxins In Malaysia

AFLATOXINS	Maximum level (µg/kg)		
	AFB1	Total aflatoxins	AFM1
Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled, for further processing)		15	
Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled, ready-to-eat)		10	
Cereal-based food for infants and children	0.1		
Milk			0.5
Infant formula and follow-up formula (ready-to-drink)			0.025

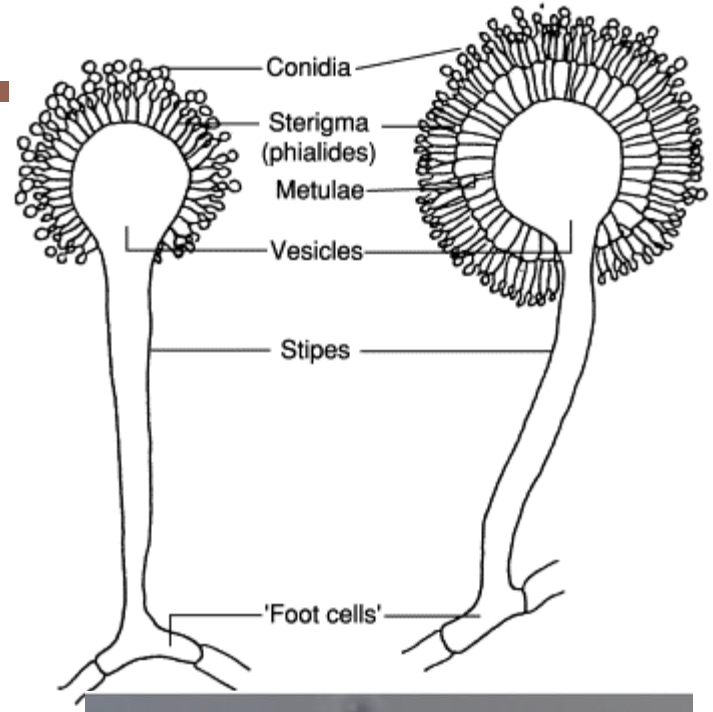
OCHRATOXINS	Maximum level (µg/kg)
Cereal-based food for infants and children	0.5
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape musts reconstituted, intended for direct human consumption	2
Coffee or ground coffee or coffee powder	5
Raw wheat	5
Instant coffee or soluble coffee, decaffeinated coffee	10
Currants, raisins and sultanas	10

PATULIN	Maximum level (µg/kg)
Apple juice (includes apple juices as ingredients in other beverages)	50

Food Act, 1983. Food (Amendment) (No.3) Regulations 2014.

Aspergillus spp.

- *Aspergillus* is a genus of filamentous fungi, prevalent in tropical countries
- The classification is based on the morphological characters, extrolites profiles, and genome sequencing.
- Currently, there are 4 subgenus and 19 sections in *Aspergillus*.
- Certain species is widely used in food industry; e.g. koji, soy source, sake (*A. oryzae)*
- Growth requirement: Temp. Range 12 – 48°C (Opt. 25 – 42°C), unable to grow below 10 =°C, moisture level (min 11%)

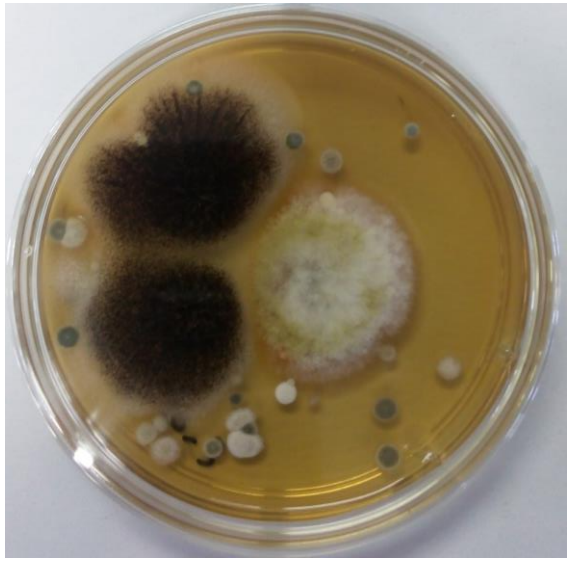


Aspergillus spp.

- Mycotoxin producer (subgenus *Circumdati*)
  - *Aspergillus* section *Flavi (e.g. A. flavus, A. parasiticus, A. nomius – produce aflatoxins)*
  - *Aspergillus* section *Circumdati (e.g. A. ochraceus – produce ochratoxins)*
  - Aspergillus section *Nigri* (e.g. *A. niger, A. carbonarius – produce ochratoxins)*
- Disease:
  - Aspergillosis – cause by *Aspergillus* infection esp. in the respiratory tract (symptom similar to asthma)
  - Mycotoxicosis – cause by ingestion of mycotoxin in contaminated food. (E.g. aflatoxin poisoning)

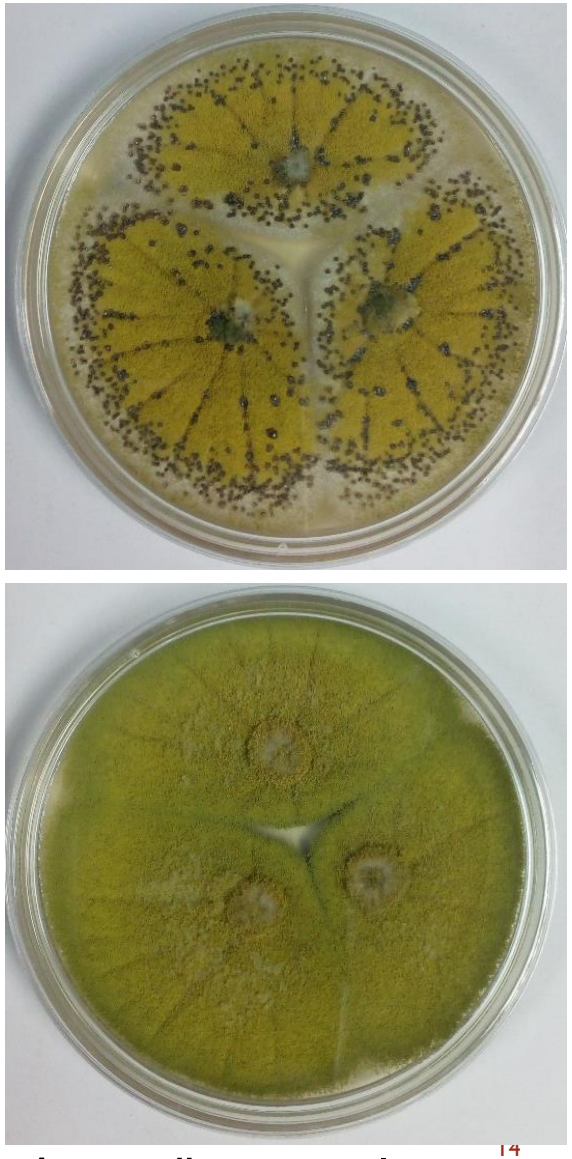
Subgenus	Section
Aspergillus	*Aspergillus* (Eurotium)
	Restricti (Eurotium)
Circumdati	*Candidi*
	Circumdati (Neopetromy
	Flavi (Petromyces)
	Flavipedes (Fennellia)
	Nigri
	Terrei
Fumigati	*Cervini*
	Clavati (Neocarpenteles,
	Fumigati (Neosartorya)
Nidulantes	*Aeni* (Emericella)
	Bispori
	Cremei[a] (Chaetosartorya)
	Nidulantes (Emericella)
	Ochraceorosei
	Silvati
	Sparsi
	Usti (Emericella)

Morphology of *Aspergillus flavus*



Aspergillus sp. isolates on AFPA media

Positive isolate of *A. flavus*/ *A. parasiticus* (orange color on reverse plate)



Aspergillus sp. isolates on MEA media

Aflatoxins

- Aflatoxins are carcinogenic compound which mainly produced by *Aspergillus flavus* and *A. parasiticus*.
- AFB_1 is classified as a **Group I carcinogen by IARC** which is linked to the development of liver cancer (International Agency for Research on Cancer [IARC], 1993).
- Aflatoxicosis is a poisoning that results from the ingestion of aflatoxins in contaminated food.

Acute aflatoxicosis

- high dose exposure → rapid onset and obvious toxic response
- vomiting, abdominal pain, jaundice, liver damage, and finally death

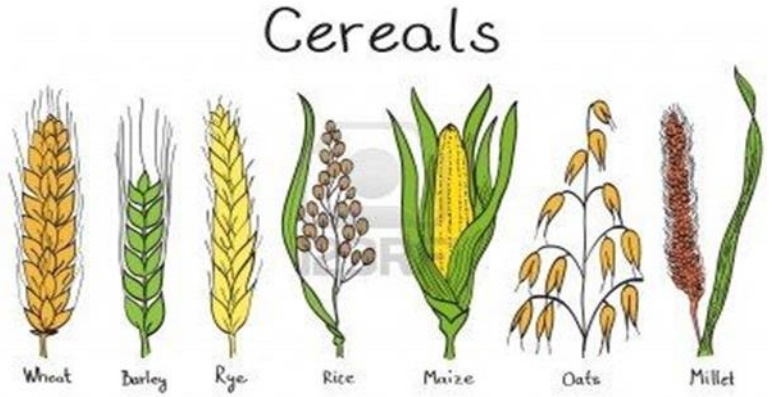
Chronic aflatoxicosis

- Low-dose exposure to aflatoxins over a long period of time
- Congenital malformation, mutation in the genetic code, and carcinogenic effect which can produce liver cancer (higher risk in patients with Hepatitis B virus), stunted growth in children.

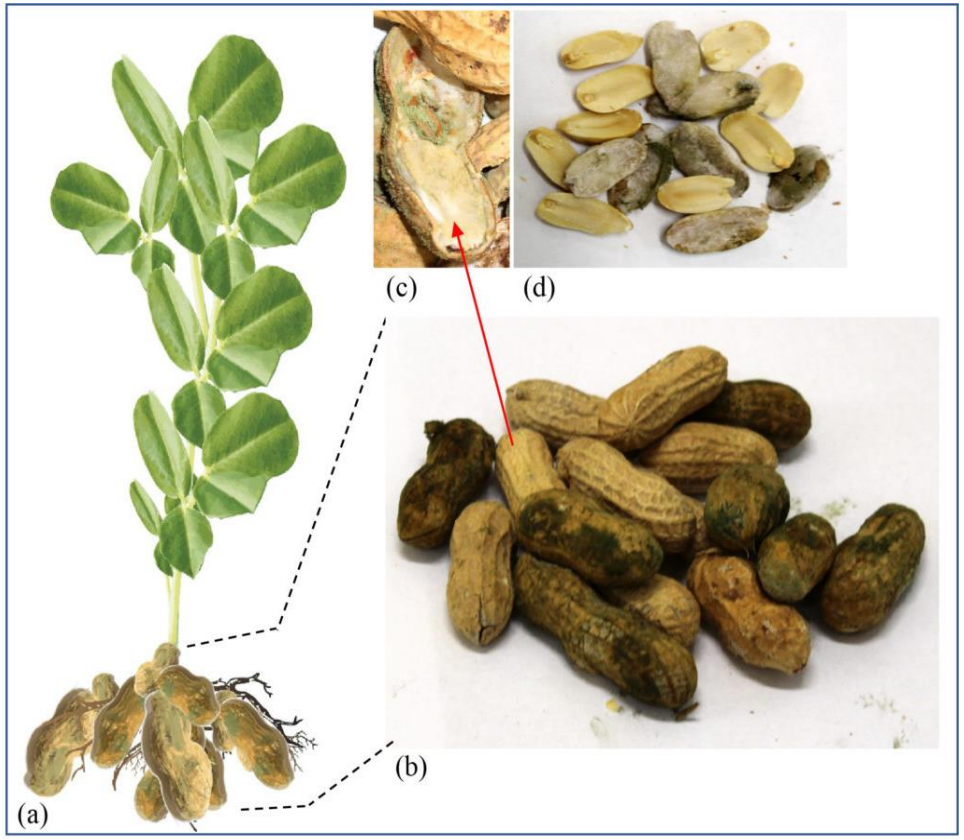
- However, the response is depending on the exposure level, health condition and age, duration of exposure, nutritional diet, and environmental factors.

Aflatoxins

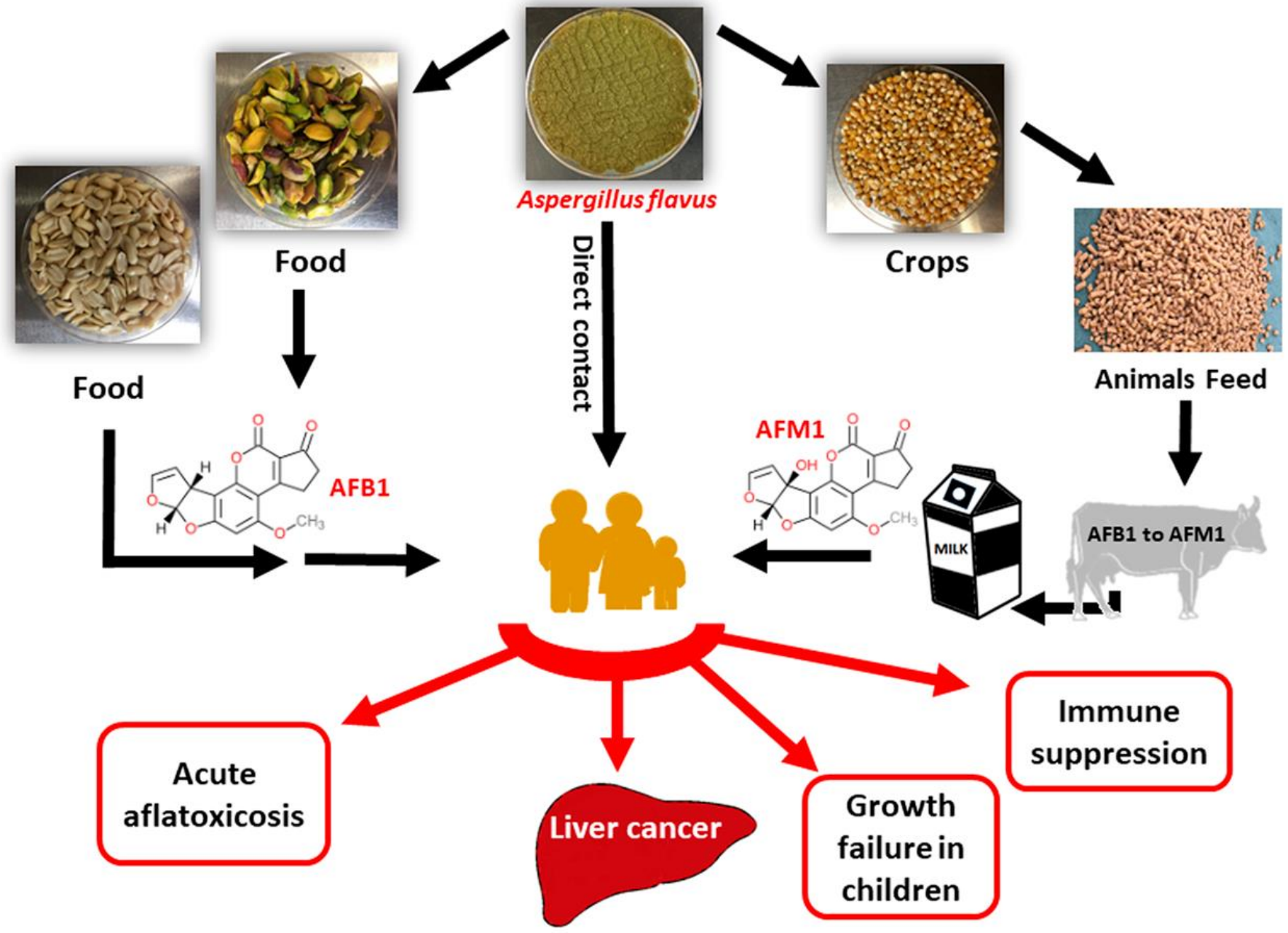
- Widespread aflatoxin contamination often occurs in hot and humid agro-ecological zones
- Occurrence of aflatoxins in foods:
  - AFB_1, AFB_2, AFG_1, AFG_2 – found in contaminated nuts, grains/cereals, oilseeds, spices, tree nut, groundnut, and dried fruits.
  - AFM_1 and AFM_2 – found in contaminated milk / milk products



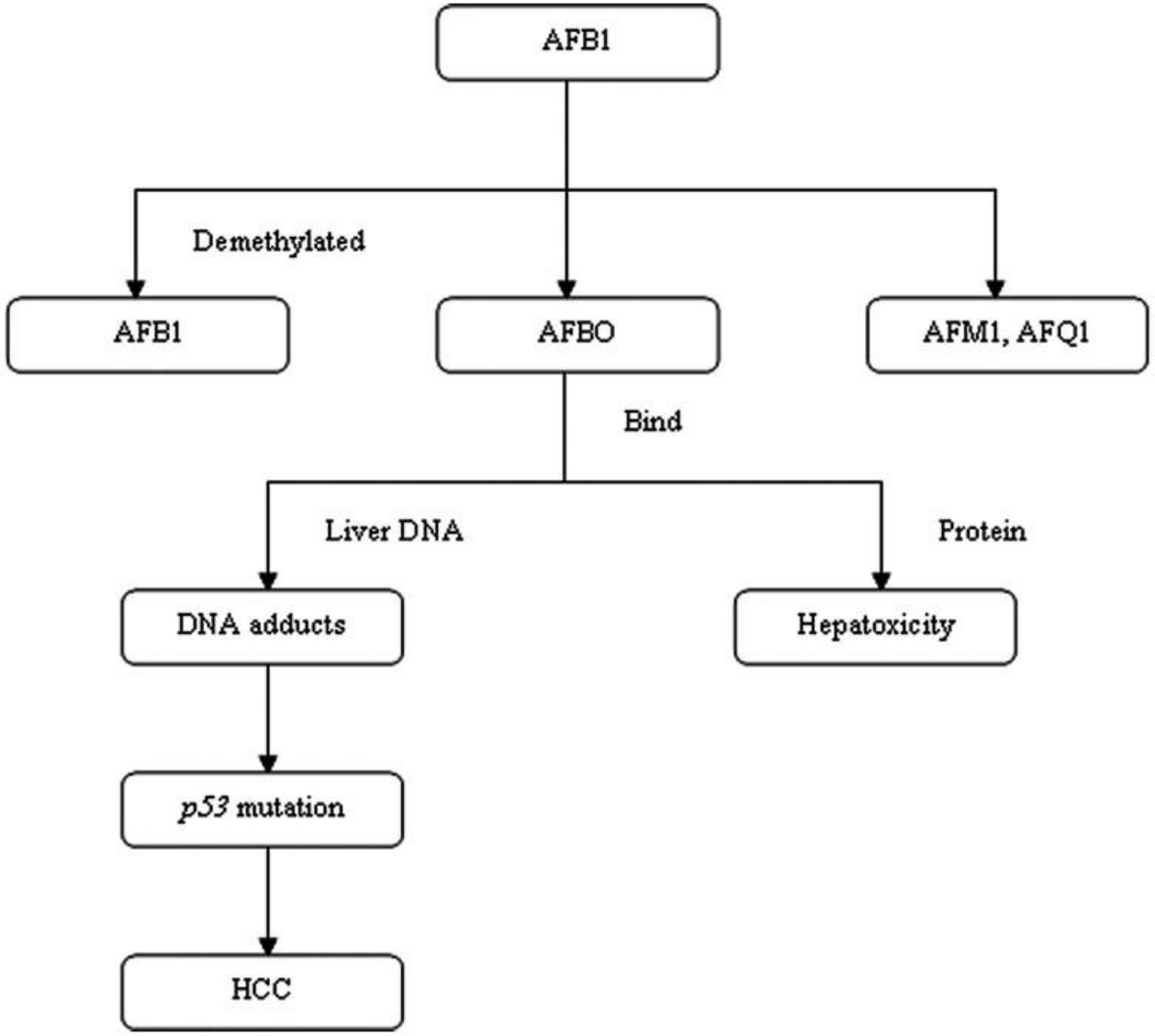
Aflatoxins in peanuts



Aflatoxins & fumonisins in maize

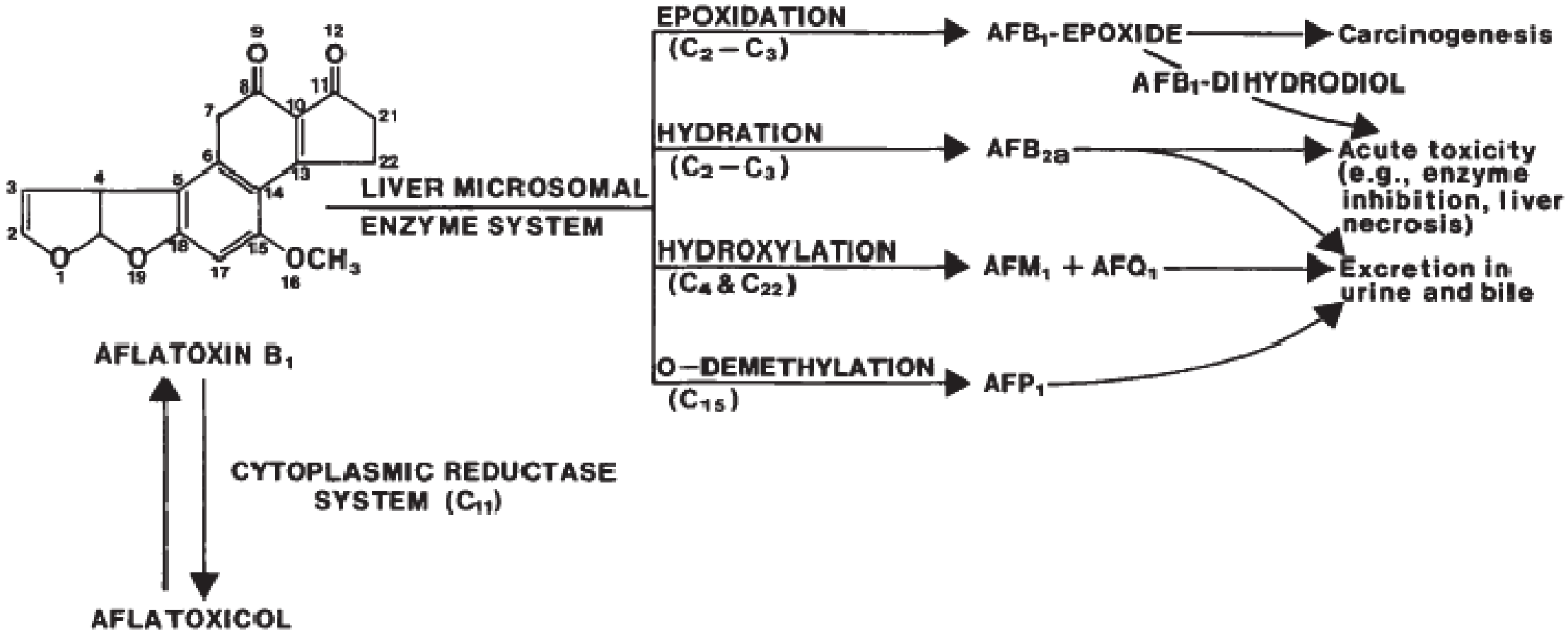

Aspergillus flavus
Food
Food
Direct contact
Crops
Animals Feed
AFB1
AFM1
MILK
AFB1 to AFM1
Acute aflatoxicosis
Liver cancer
Growth failure in children
Immune suppression

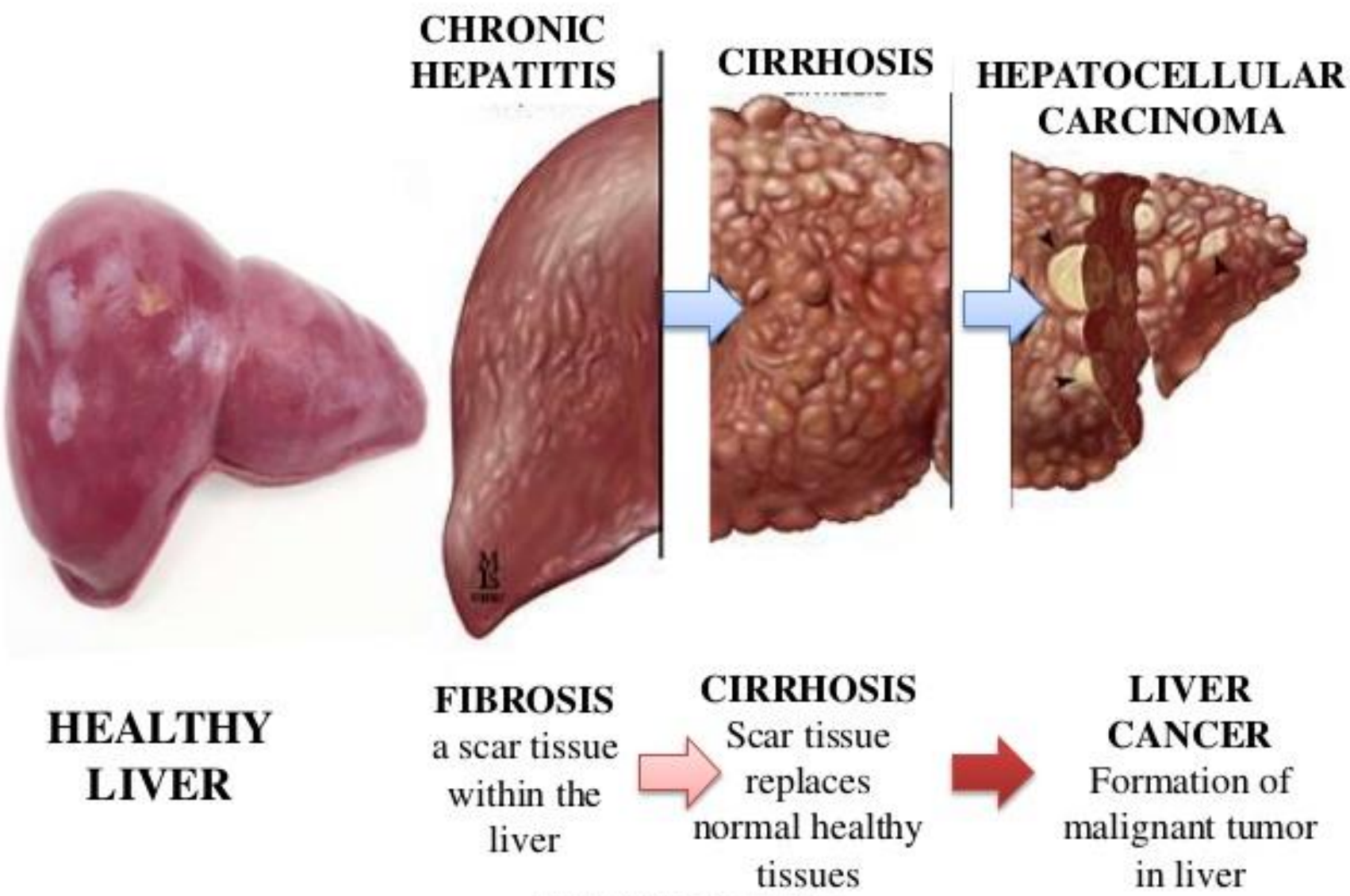
Metabolism Of Aflatoxin In The Liver



- Aflatoxins are absorbed across the cell membrane in the GI tract →enter the blood stream → spread to the **liver** (main target for AFB1 metabolism)
- AFB_1 is metabolized by the cytochrome-P450 enzymes to form reactive epoxide intermediate (AFBO), or hydroxylated to less harmful compound such as AFM_1 and AFQ_1.
- AFBO is a highly reactive genotoxic compound which binds to liver cell DNA and form DNA adduct → leads to DNA damage
- DNA adduct interact with guanine base of the DNA and cause mutation in the *p53* tumor suppressor gene. → results in formation of hepatocellular carcinoma (HCC)/ tumor.

Metabolism Of Aflatoxin In The Liver

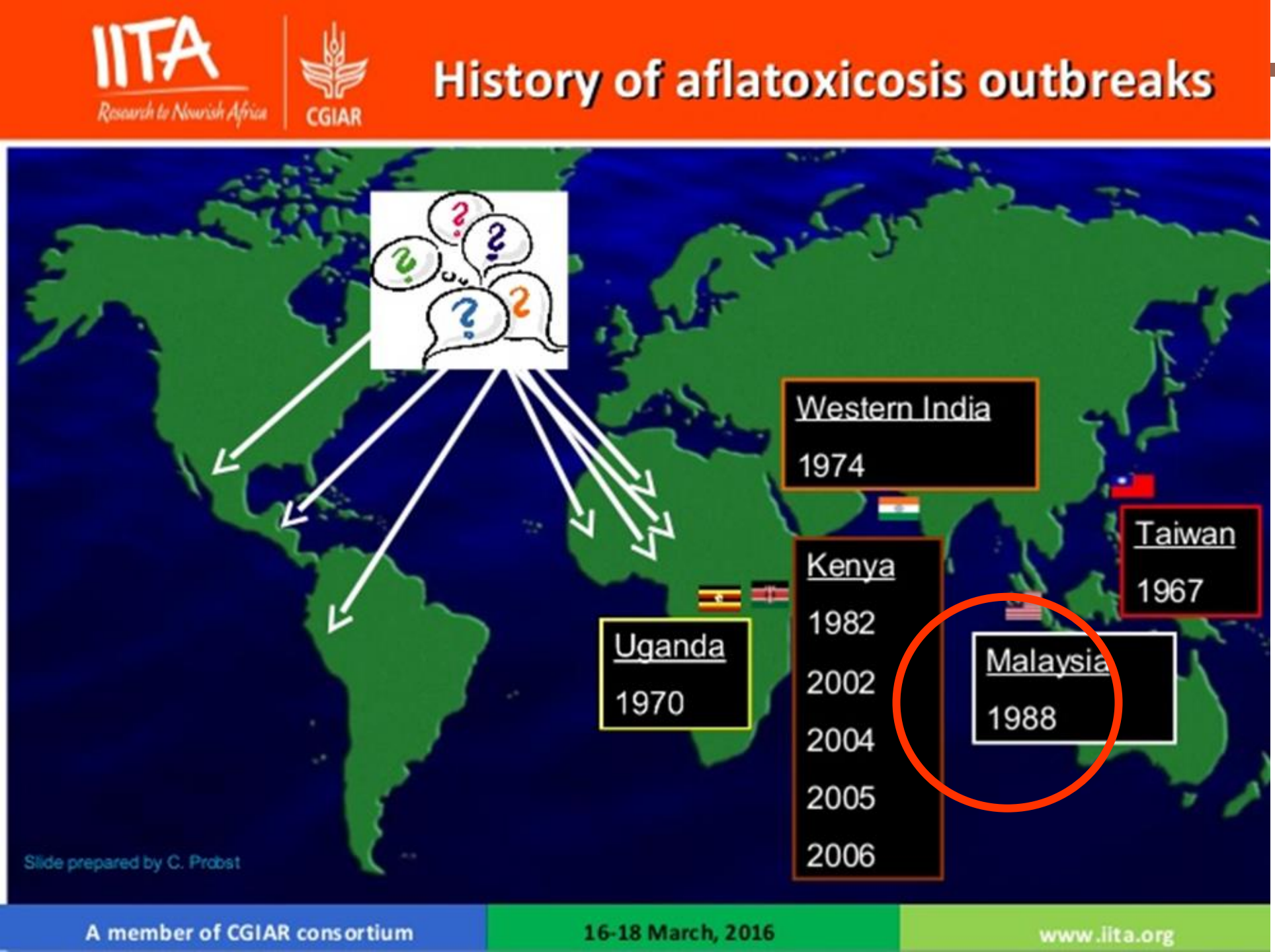




Cirrhosis is a late stage of scarring (fibrosis) of the liver caused by many forms of liver diseases and conditions, such as hepatitis and chronic alcoholism.

There is a synergistic carcinogenic interaction between hepatitis B virus (HBV) infection and dietary exposure to aflatoxin B1 (AFB1)

- Chronic HBV infection may induce the cytochrome P450s to metabolize inactive AFB_1 to the mutagenic AFB_1 epoxide.



Acute toxicity

High fever, high coloured urine, vomiting, jaundice

Chronic toxicity

Human hepatic cell carcinoma (HCC)
Genetic mutation

Table 1 Summary table of fungi, associated mycotoxins, and clinical disease

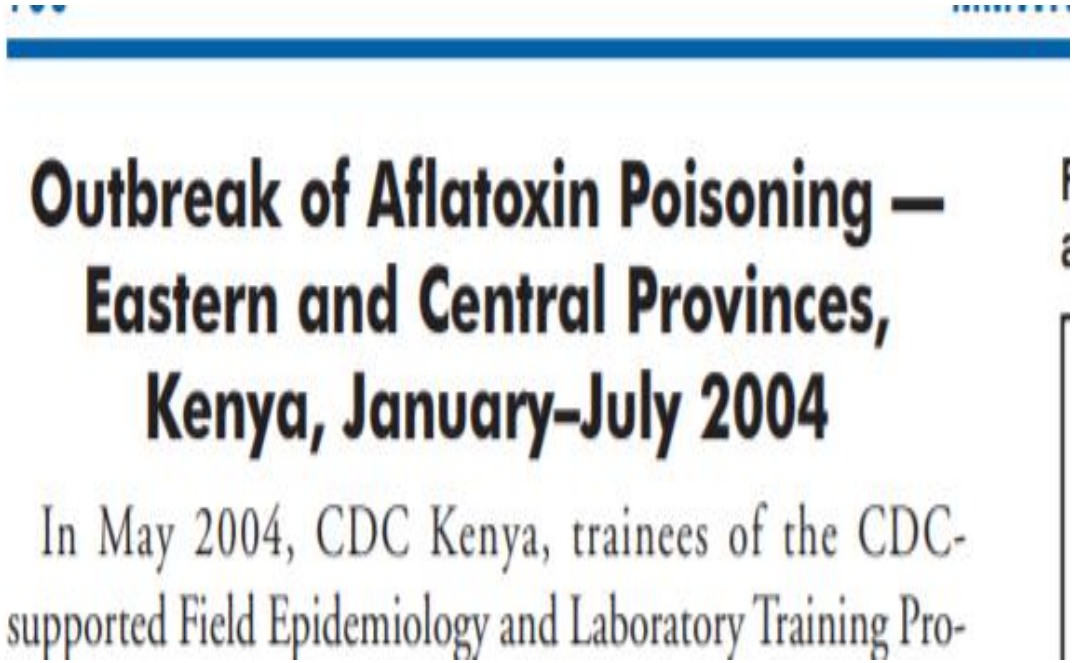
Fungal species	*Mycotoxin classification*	*Mycotoxins identification*	*Commodity*	*Clinical signs/symptoms*
Aspergillus flavus, *Aspergillus parasiticus*	Aflatoxins	B1, B2, G1, G2 M1, M2	Corn/maize, peanuts, tree nuts, cottonseed, rice, copra, heat, spices, cocoa (chocolate products) Milk products and tissues	Liver toxicity, poor weight gains, immunosuppression, hemorrhages, cancer, death
Fusarium sp. *(F. graminearum, F. Poae, F. acuminatum, F. sporotrichiodes, F. equiseti)*	Trichothecenes	DON (deoxynivalenol) or vomitoxin, nivalenol, 3- and 15-acetylDON, T-2 toxin, HT-2 toxin, T-2 tetral, DAS (diacetoxyscirpenol), neosolaniol	Wheat, maize, hay, straw Overwintered grains	Gastroenteritis, vomiting, diarrhea, immunosuppression, poor growth
Fusarium graminearum, *F. culmorum*, *F. equiseti*	Zearalenone	Zearalenone	Wheat, maize/corn, hay	Hyperestrogenism, infertility
Fusarium verticillioides (syn. *F. moniliforme*), *F. proliferatum*	Fumonisins	FB1, FB2, FB3	Corn Corn-based foods	Mild liver toxicity, mild kidney lesions, leukoencephalomalacia (horses), pulmonary edema (swine), possible carcinogen in humans
Claviceps sp. *C. purpurea [C. fusiformis, C. paspali] [Neotyphodium coenophialum in tall fescue grasses]*	Ergot alkaloids	Ergopeptine alkaloids: ergotamine, ergocristine, ergosine, ergocryptine, ergovaline, ergoline alkaloids: lysergic acid, lyergol, lysergic acid amide, ergonovine, clavines	Rye, wheat, cereals, grasses	Vasoconstriction with loss of extremities (ears, feet, tails in livestock), agalactia (reduced milk production in animals), abortions (humans), gastroenteritis, hallucinations (humans)
Aspergillus alutaceus var. alutaceus (formerly *A.ochraceus*), *A. westerdijkiae* and *A. Steynii* and *Penicillium verrucosum* (formerly *P. viridicatum)*	Ochratoxins	Ochratoxin A	Cereal grains (wheat, barley, oats, corn), dry beans, peanuts, grapes, raisins, cheese, apple juice Swine tissue	Liver and kidney toxic, immunosuppressive
Penicillium expansum, *Aspergillus* sp., *Byssochlamys fulva*, *B. nivea*	Patulin	Patulin	Rotten fruits, apple juice, applesauce	Immunotoxicity, liver, spleen, and kidney damage

Summary table of fungi, associated mycotoxins, and clinical disease

https://doi.org/10.1016/B978-0-12-384947-2.00480-3

Outbreak

Outbreak of Aflatoxin Poisoning in Kenya



Outbreak of Aflatoxin Poisoning — Eastern and Central Provinces, Kenya, January–July 2004

In May 2004, CDC Kenya, trainees of the CDC-supported Field Epidemiology and Laboratory Training Pro-

- 317 cases had been reported with 125 death
- 22.1% were aged <5 years
- significant association between the development of jaundice and consumption of homegrown maize
- high levels of aflatoxin (20 - 8,000 ppb) in maize samples collected from patient households
- Aflatoxin contamination occurred during storage of the maize under damp conditions

Aflatoxins outbreak in Malaysia

Pig farm

1960s

Outbreak of disease in two pig farms in Malacca which cause severe liver damage to the animals (Hamid, 1997).

Lim and Yeap (1966) reported the presence of **aflatoxins** in the feed ingredients.

Loh shi fun

1988

13 children died after consuming contaminated noodles called called *loh shi fun.*

The death was due to **acute hepatic encephalopathy** (*a decline in brain function as a result of severe liver damage due to the accumulation of toxins in the bloodstream*)

3 mg of aflatoxin was detected in a single serving of *loh shi fun.* (Lye et al. 1995)

The main ingredient, wheat :

→poor storage and processing,

→ promote the growth of ***Aspergillus* sp.** and subsequently the production of **aflatoxins**.

Ochratoxins

- Ochratoxins – a group of mycotoxins (Ochratoxin A, B, and C) produced by certain fungi such as *Aspergillus carbonarius, A. ochraceus* and *Penicillium verrucosum*.
- Ochratoxin A (OTA) has been classified as a possible group 2b human carcinogen (IARC 1993).
- It is known to contaminate cereals, coffee, dried fruits, grapes and **wine.**
- Source of OTA in wine – contaminated grapes in the vineyard.
  - Ochratoxigenic fungi invade the grapes though skin damage caused by insects and pathogenic fungi (e.g *Rhizopus stolonifera, Botrytis cinerea*)

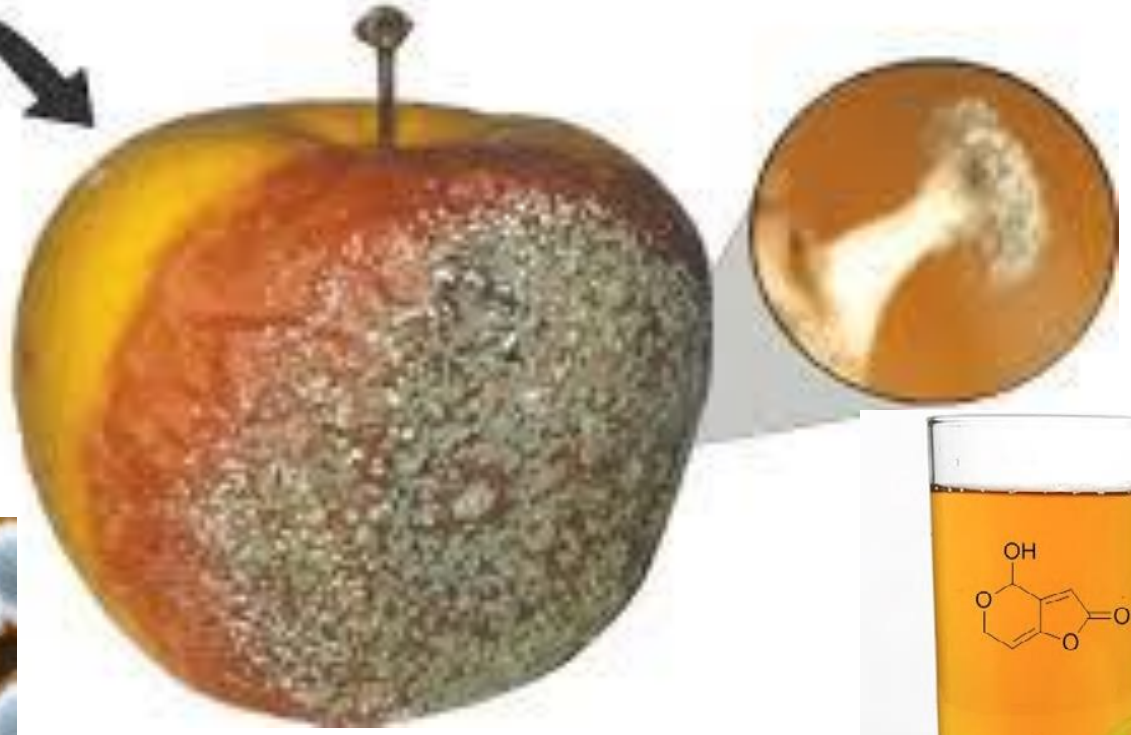
Penicillium spp.

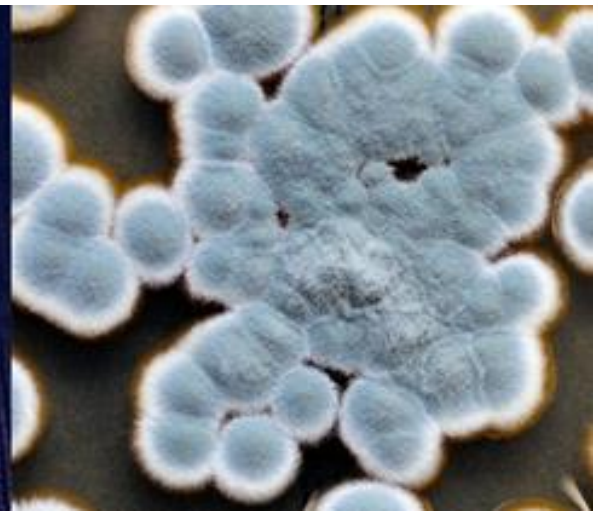
- *Penicillium* is a diverse fungal genus and contain more than 350 species.
- Certain species is widely used in food (Camembert/Roquefort cheese) and drug industry (penicillin)
- Growth requirement: Temp. 5 – 37°C (Opt. 20 – 30°C); pH 3 – 4.5.; aw 0.78 – 0.88
- Mycotoxin producer:
  - *P. verrucosum* – ochratoxin A & citrinin in stored cereals
  - *P. expansum* – patulin in fruits (grapes, oranges, apples, pear) , wine, juices & citrinin in cereals
  - *P. citrinum* – citrinin in cereals

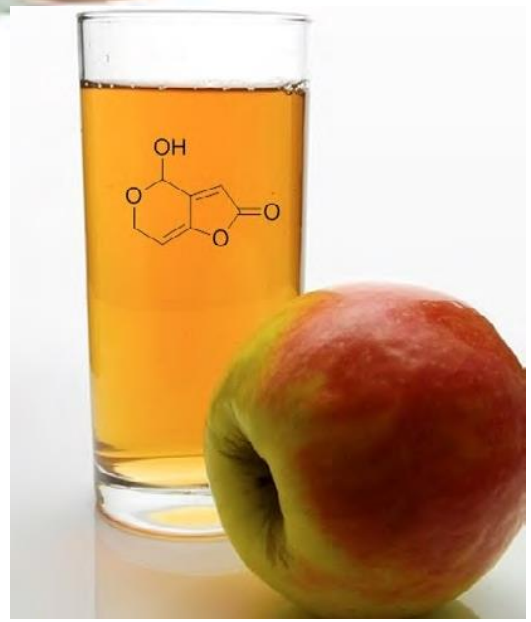
Camembert cheese - Penicillium camemberti

Roquefort cheese -Penicillium roqueforti

Patulin





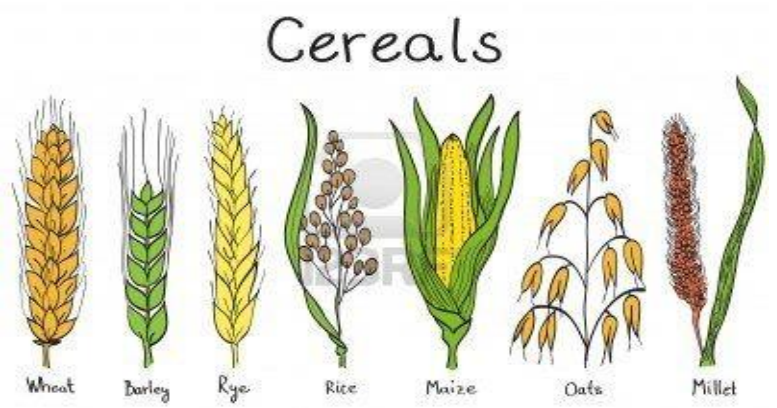


Fusarium spp.

Fusarium head blight

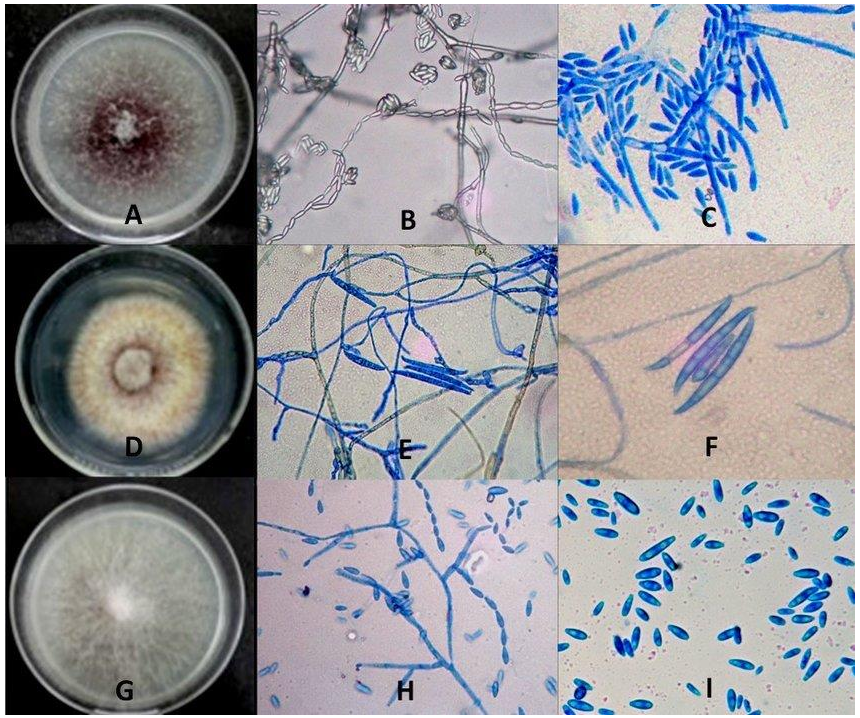
Fusarium ear rot

- *Fusarium* is a filamentous fungi that is widely distributed in the soils and plants (field fungi)
- The genus contain more than 20 species (14 species are significant to crops)
- *Fusarium* is a major agricultural plant pathogen of temperate growing regions
  - Cause *Fusarium* head blight disease and *Fusarium* ear rot
  - Infected plant is often associated with mycotoxin contamination
- Growth requirement:
- *Fusarium* toxins:
  - Fumonisins – *F. verticillioides, F. proliferatum*
  - Zearalenone – *F. graminearum, F. culmorum*
  - Trichothecenes group (Deoxynivalenol and nivalenol) – *Fusarium* spp.
- *Fusarium* toxins are commonly found in grains/cereals such as wheat, barley, maize, oats, sorghum



Fusarial-toxicosis outbreak

- India (1995) – affecting 1424 people due to consumption of **sorgum** and **maize** contaminated with **fumonisins.**



F. verticillioides

Pascual, C. B. , Barcos, A. K. S. , Mandap, J. A. L. , Ocampo, E. T. M. 2016. Fumonisin-producing *Fusarium* species causing ear rot of corn in the Philippines. Philipines Journal of Crop Science., 41:12-2.

Mycotoxin mitigation strategies

Pre-harvest

- Use of resistant varieties (e.g. Florunner, Tifguard peanut)
- Timely irrigation to decrease soil temp. (high temp + drought are conducive to Aspergillus growth)
- Insect management to avoid damage to the crops / transfer of spores from infected plant to healthy plant
- Early harvesting to avoid raining season
- Crop rotation to break the infection life cycle and reduce fungal inoculum in soil
  - the crop should not be a host to fungal pathogen that infect both crops
- Weed management – some weed can be the alternate host to *Aspergillus* spp.

Post-harvest

- Storage condition
  - Dry crop to 7% moisture content to control fungal growth
  - stored at 70% relative humidity, temp 25 -27C
  - Control pest
- Equipment sanitization – fungi can survive in soil/mud attached to harvesting equipment
- Packaging – use hermetic zero oxygen packaging
- Separation of infected and healthy pods

Prevention method for mycotoxin contamination in food

- Food manufacturer should do aflatoxin screening for raw materials that are prone to aflatoxin contamination. E.g. peanuts
- Manual / electric color sorting - Inspect the whole grains, nuts, dried fruits for the evidence of fungi & discard the moldy one.
- Avoid hot and humid storage area for grain/cereals
- Buy only reputable brands of nuts and nut butters
- Avoid making fruit juice from moldy fruits

THANK YOU