Mycotoxins

In the broad sense, toxic substance of fungal origin.

Usually defined as fungal metabolites that are toxic to man and/or animals and are produced by molds growing on foodstuffs or in homes and work places.

Molds: Asexual fungi classified in "form phylum" Deuteromycota

A Few Words About Molds

Molds

- Fungi with septate mycelium where sexual reproduction is unknown or rare.
- Asexual reproductive spore = conidium (pl. conidia).
- Asexual spore not produced in a sporangium.

Definition:

- Sporangium: spore containing structure.
- Conidium: Asexual spore not produced in a sporangium.
- Conidia borne on specialized hyphae (conidiophore) in Penicillium example.

- "Form Phylum" Deuteromycota erected to classify these fungi.
- Sexual reproduction stage lost or rare.
- When sexual stage discovered, usually Ascomycota, less frequently Basidiomycota.
**Sexual spores of Basidiomycota and Ascomycota**

Basidium & basidiospores; ascus and ascospores

**A Few Words About Molds**

- After discovery species reclassified, i.e. moved from Deuteromycota to Ascomycota or Basidiomycota.
- This has never happened!

**Example of Deuteromycota**

- *Aspergillus variecolor* = Asexual stage classified in Deuteromycota

Chains of globose conidia borne on a swollen vesicle.

**Example of Deuteromycota**

- *Emericella variecolor* = Sexual stage classified in Ascomycota

Fruiting bodies of *Emericella* contains asci & ascospores

**Example of Deuteromycota**

- *Emericella variecolor* = Sexual stage classified in Ascomycota

Ascus with ascospores within Star-shaped ascospore

**Example of Deuteromycota**

- *Emericella variecolor* and *Aspergillus variecolor* are same species!
- *Aspergillus variecolor* name should have been eliminated and only *Emericella variecolor* should be correct name.
- Species never has been reclassified!
- Why?
Significance of Mycotoxins
- Mycotoxins can be cause of illnesses in workplace and home.
- Contraction of illnesses due to mycotoxin on spores.
- Has been major problem in urban areas, in high rises since 1970's.
- Complaints reached epidemic proportions during 1990's.
- Mycotoxins released into food products can cause illness or death when consumed.

Mycotoxins in Food
Thursday – Inhalation of Mycotoxins on Spores

Discovery of Mycotoxins
- Relatively recent discovery:
  - In early 1950's, hundreds of wild pigs died due to what is believed to be consumption of moldy corn.
  - Researchers isolated number of fungi from moldy corn.
    - Grew fungi on sterilized corn and fed them to pigs.
    - One species, Aspergillus flavus made pigs ill.

Discovery of Mycotoxins
- Relatively recent discovery:
  - Although published, (Burnside, et al, 1957), it seemed like old news. Why?
  - It would not be until 1960 when mycotoxin poisoning would be demonstrated.

Turkey-X Disease
- In 1960, approximately 100,000 turkeys and lesser number of domesticated birds mysteriously died in England.
- Initially thought to be a virus, it was named the Turkey-X disease.
- Cause of disease traced to Oil Cake Mills, Ltd.
  - Oil cake feed for turkey toxic.
  - Specifically, peanut was contaminated with mold.

Turkey-X Disease
- Fungus identified as source of toxicity was Aspergillus flavus.
- Chemist isolated mycotoxin and called it aflatoxin (from A. flavus + toxin).
- Damage caused.
  - Sensitivity to aflatoxin varied according to animals tested.
  - Small amounts of toxin consumed could caused damage to internal organs, especially liver. One effect was cancer.
Turkey-X Disease

- That peanut was implicated was of great concern to world.
  - Within United States, Food and Drug Administration (FDA) concerned.
  - Internationally, United Nations International Children's Emergency Fund (UNICEF) and other similar organization concerned because of:
    - Kwashiorkor severe malnutrition caused diet high in carbohydrate, low in protein.

- Within United States, Food and Drug Administration (FDA) concerned.

- Internationally, United Nations International Children's Emergency Fund (UNICEF) and other similar organization concerned (continued).
  - Peanut used to increase protein in diet.

- Quality control in United States ensured poisoning would be minimized.
  - Standards set for aflatoxin.

FDA Levels for Aflatoxins (in part)

<table>
<thead>
<tr>
<th>Commodities</th>
<th>Levels (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut products intended for feed of beef cattle</td>
<td>300</td>
</tr>
<tr>
<td>Peanut products intended for feed of swine = more 100 lbs</td>
<td>200</td>
</tr>
<tr>
<td>Peanut products intended for breeding beef cattle, swine or mature poultry</td>
<td>100</td>
</tr>
<tr>
<td>Peanut products for usage unknown</td>
<td>20</td>
</tr>
</tbody>
</table>

Turkey-X Disease

- Quality control in United States ensured poisoning would be minimized.
  - Random samplings indicated that aflatoxin in peanuts and peanut products seldom occurred.
  - Aflatoxin poisoning did occur in third world countries without such controls.

About Aspergillus flavus

- Aspergillus flavus is actually a species complex of eleven species.
- Some of the species, e.g., A. oryzae, used in preparation of foods in orient.
  - Soy sauce and tofu are two examples commonly used in this country.
  - Were we slowly being poisoned by eating contaminated food products?

- Some of the species, e.g., A. oryzae, used in preparation of foods in orient (continued).
  - Commercial strains utilized in United States, of A. flavus, tested indicated aflatoxins not produced.
  - In strains, from households or village industry, tested from Philippines, all sample found to have aflatoxin.
About Aspergillus flavus
- Research indicated conditions that induce aflatoxin formation very narrow.
- Aflatoxin produced by A. flavus complex when growing alone on grain, i.e. pure culture.
- Normally, when occurring on grain, a number of species of fungi will be growing with A. flavus. Other species normally will invade first.

About Aspergillus flavus
- If grains are pre-invaded with other fungi, A. flavus contamination will not produce aflatoxin.
- Demonstrated at University of Minnesota.
  - Aspergillus flavus and other species grown on grains for purpose of feeding to experimental animals.
  - Ducklings, white rats and baby chicks fed contaminated grains.
  - No deaths occurred in animals! Animals gained weight.

About Aspergillus flavus
- When aflatoxin produced, amount is variable according to substrate:
  - More aflatoxin produced when A. flavus grows on peanut than in soybean, wheat corn barley, oat, etc.
  - Climatic conditions also a factor.
  - Different isolates of A. flavus will produce different amounts of aflatoxin. Some will not produce any.

About Aspergillus flavus
- Christensen (1972) sampled black pepper from all over the world.
  - In dilution plating, ground pepper was found to have mostly A. flavus, A. ochraceus and A. versicolor, all in species complex.
  - An average of 52,000 colonies/grams/black pepper.
  - If wheat had a few thousand, or barley 10,000 colonies per gram, they would be rejected for milling and beer making, respectively.

About Aspergillus flavus
- Christensen (1972) sampled black pepper from all over the world (continued).
  - If grains were as heavily contaminated as black pepper would have a musty odor and taste. Why not pepper?
  - Strong taste and odor of pepper conceals aflatoxin. True also of other spices.

About Aspergillus flavus
- Ochratoxin
  - Produced by Aspergillus ochraceus complex, composed of 9 species, and Penicillium viridicatum.
  - Like aflatoxin, low amounts can be lethal, e.g. will kill white rats.
  - Lower amounts that does not kill will cause severe liver damage.

Other Mycotoxins
**Other Mycotoxins**

- **Sterigmatocystin**
  - Produced by *Aspergillus versicolor*, which is part of *A. flavus* complex.
  - One of species found in black pepper.
  - Known to cause deaths in calves fed with grains contaminated by this species.
  - Causes tumors in lung, liver and kidney.
  - Contamination of barley, in malt house, in Scotland.

- **Fumagillin**
  - Produced by *Aspergillus fumigatus*.
  - Unusual in that it is a mycotoxin and antibiotic.
  - Used as an amoebicide, to rid body of amoebae that are human pathogens.
  - Too much and you get rid of the patient too!

**Other Mycotoxins**

- **The genus Fusarium**
  - Produces a number of important mycotoxins.
    - *Fusarium graminearum* produces *vomitoxin*, a *trichothecene*.
    - *Fusarium tricinctum* produces *Trichothecene* (T-2). Effects first discovered in Russia, in 1944(s(!)), associated with *alimentary toxic aleukia* (ATA).

- **Fusarium graminearum** produces *vomitoxin*, a *trichothecene*.

**Other Mycotoxins**

- Estimated that 10% of population contracted "disease", which was fatal.
- **Symptoms:**
  - Nausea, vomiting and diarrhea.
  - Hemorrhaging in many organs.
  - Low white blood cell count.
  - Bleeding from nose and throat.

**Other Mycotoxins**

- Symptoms were observed prior to WWII, but did not reach epidemic proportions until end of WWII.
- Believed to be due to inadequate number of people harvesting fall grain.
- Because grain was not harvested until following spring it was heavily contaminated with fungal growth.

- It would not be until 1965 that symposium on mycotoxin would this story be told.
- Discovery of aflatoxin in 1960 generated interest in learning about mycotoxin throughout world.
Yellow Rain

- **Trichothecene Mycotoxin**

- **Yellow Rain**
- Exposure reportedly caused heavy bleeding from the nose and gums, blindness, tremors, seizures, other neurological symptoms, and death.
- Similar incidents later reported in Cambodia in 1978.

- **Yellow Rain**
- Incidents investigated by U.S. medical teams 4-6 weeks later.
- Unable to determine cause of illnesses and deaths.

Yellow Rain

- Responsible for large scale deaths greater than ATA of 1944.
- Following WWII, when Russian soldiers returned home, there was no food other than contaminated grain.
- Consumption of contaminated grain led to mycotoxicosis of as many as a million soldiers.

- **Yellow Rain**
- Reports of terrorist attacks with variety of toxic agents dropped from low flying aircrafts.
- About 70% of eyewitnesses reported seeing oily yellow liquid making sound like rain when it fell = Yellow Rain.
## Yellow Rain

- In 1981, U.S. Army toxicologist Dr. Sharon Watson recognized symptoms as being same as trichothecene mycotoxin poisoning.
  - Found evidence in analysis of dead and living victims and plant samples.
  - Haig accused Soviet Union of supplying trichothecene to Vietnam and Laos.

## Yellow Rain

- News media became suspicious of accusations and investigated evidence.
  - Subsequent samples later submitted also tested positive, but with different concentrations.
  - U.S. Army report in 100s of samples detected no trichothecenes.
  - Government reported that there were 150-200 reports of such incidents, but few witnesses.

## Yellow Rain

- News media became suspicious of accusations and investigated evidence.
  - Investigative teams carried out "improper forensic methods".
    - Researchers made intentions of research known to villagers.
    - Asked leading questions.
    - Non-random selections of witnesses.
    - Samples to be analyzed collected by tribes people.

## Yellow Rain

- Independent investigation from other countries occurred:
  - Most did not reveal results.
  - Britain, France and Canada results endorsed U.S. findings.
  - Australia was very skeptical about accusations after investigation.

## Yellow Rain

- Another theory:
  - Yale entomologist and expert on Southeastern Asian bees concluded yellow rain was bee feces.
  - U.S. scientists said that toxin was incorporated into feces to aide in dispersal.
  - Yale botanist claimed pollen concentration too high to keep them airborne.
Another theory:

- Analysis of feces did not come up with trichothecene.
- Many accounts from entomologists and others on local bees defecating *en masse* as a “cleansing flight” usually lasting for several minutes. Harmless!
- "Defecation" occurred at same time as attack and became associated with yellow rain?

Conclusion:

- Reagan administration was accused of fabricating “yellow rain” controversy.
- Used controversy to increase defense spending budget.

Aftermath:

- Former Soviet scientist has testified that USSR was experimenting with trichothecene as biological weapon as early as 1958.

More on Trichothecenes:

- Trichothecene and Population of Europe:
  - Population stagnation in Europe
    - Before 1750, in England, life expectancy of member of British Peerage was about 36.7 years.
    - Conditions worst among common folks
    - A hundred years later, expectancy had risen to 58.4 years and population of Europe almost doubled.

- Trichothecene and Population of Europe:
  - Population stagnation in Europe
    - Before 1750, population was in constant fluctuation.
    - Many mortality crisis occurred: disease, famine, natural disaster.
    - After each crises, individuals married younger and more children were borne and population stayed even.

- Trichothecene and Population of Europe:
  - Believed changes in people's diet led to increase.
  - During 18th Century, various grain breads were main source of food.
  - Matossian is suggested people's fertility affected due to consumption of contaminated food.
More on Trichothecenes

- Trichothecene and Population of Europe:
  - Population stagnation in Europe
  - It would not be until change of diet that this trend would end.
  - Recall between 1750-1850 was also time Irish population doubled.