



# Mycotoxin Study from Contaminated Foods by Means of Fungus

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**Abstract:** *Natural toxins in food are an important part of our lives, as is the oxygen we breathe. It is very important to naturally neutralize these toxins from our diet in order to consume food safely. This can only happen if we are exposed to small, non-toxic amounts that our bodies can easily handle. Not only would it be impractical, but it would also be impossible to completely eliminate natural toxins from our diets. Fungi and bacteria are the main causes of food poisoning. Symptoms of diarrhea, stomach cramps, and fever will develop in about 3 days.*

**Keywords:** Aspergillus, Aflatoxins, Maize

## I. INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi. Due to their pharmacological activity, some mycotoxins or mycotoxin derivatives have been used as antibiotics, growth promoters, and others are considered chemical warfare agents. Some mycotoxins directly inhibit microbial growth. This review focuses on the most important diseases associated with human and veterinary diseases, including aflatoxin, citrinin, ergot alkaloids, fumonisins, ochratoxin A, patulin, trichothecenes and zearalenone, etc. The most important mycotoxins are aflatoxin (AFB1, B2, G1, G2), ochratoxin A (OTA), trichothecenes, zearalenone and fumonisins (B1, B2). Trichothecenes mainly include deoxynivalenol (DON or vomiting), nivalenol, and T2 toxin. Some other examples are HT2, 3-Ac-DON, DAS, Fusarenon-X toxins. MAS, Neosolaniol and 15-Ac-DON are also included in the scope of the analysis. Other mycotoxins mentioned are patulin, sterigmatocystin and cytochalasin E.

## II. AFLATOXINS

Aflatoxins are toxic metabolites produced by certain fungi in/on food and animal feed. They are probably the best known and most studied mycotoxins in the world. The presence of aflatoxin is influenced by a number of environmental factors; therefore, the degree of contamination will vary depending on the geographical location, agricultural and agronomic practices as well as the susceptibility of the product to fungal invasion during the pre-harvest, storage and handling stages and processing.

Aflatoxin is produced by many species of *Aspergillus niger*, especially *flavus* and *parasiticus*, as well as by members of the genera *Penicillium* and *Rhizopus*. Aflatoxin can contaminate corn, cereals, sorghum, peanuts and other oilseeds. *Aspergillus*, which produces aflatoxin, is one of the most common cereal molds.



However, aflatoxin's reputation as a potent poison may explain why it has been used in bioterrorism. Aflatoxin has been isolated and characterized following the death of more than 100,000 birds (turkey X disease) attributed to the consumption of peanut meal contaminated with mold (the four major aflatoxins are referred to as B1, B2, G1 and G2 as a function of their fluorescence under UV or green light) and relative chromatographic mobility during thin layer chromatography.

Aflatoxins are associated with a variety of diseases such as aflatoxinosis in livestock, companion animals and humans worldwide. Aflatoxins have received more attention than any other mycotoxin due to their demonstrated potent carcinogenicity in susceptible laboratory animals and acute toxicological effects in humans. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds. Aflatoxin, like other natural toxins found in food, cannot be completely eliminated from food. Aflatoxin poisoning has been reported in nearly all domestic and non-domestic animals and other non-human primates from all regions of the world. Additionally, aflatoxin inhalation is associated with disease and injury in both animals and humans, and genetic resistance to aflatoxin production has been identified in more than five major cultures. Recognized as a serious food safety hazard in the country, more than 50 countries have enacted or proposed regulations to control aflatoxin in food and feed. To the best of our knowledge, there is little evidence of the presence of aflatoxins in European grains or in a few other countries. Anything containing more than 20ppm should not be used in human diets or fed to dairy cows. Crop losses due to aflatoxin contamination are estimated to cost US growers an average of more than \$100 million annually. Recognizing that absolute safety can never be achieved, many countries have attempted to limit exposure to aflatoxin by imposing regulatory restrictions on commodities intended for food and feed use. It was made.

### III. MATERIALS AND METHODS

#### Part I: Preparation of Potato Dextrose Agar Medium

##### Requirements

|                 |       |
|-----------------|-------|
| Potato tubers   | 100g  |
| Dextrose        | 10g   |
| Agar            | 7.5g  |
| Peptone         | 1g    |
| Distilled water | 500ml |
| HCl             | 1N    |
| NaOH            | 1N    |
| Knife           |       |
| Muslin cloth    |       |
| Heater          |       |

Beaker (500ml capacity)

Borosil flask (500ml capacity)

**Procedure**

1. Take a potato, peel it and weigh 100 g. Use a knife to cut the tubers into small pieces.
2. Transfer the chopped potatoes to a cup containing about 200ml of distilled water. Etc Boil the contents using a heater for about 20 minutes.
3. Decant the supernatant, filter through four layers of cheesecloth, and collect the filtrate in a beaker. This filtrate is called potato extract. Because. Transfer the dextrose (10 g), agar (7.5 g) and peptone (1 g) to the extract, heat and gently swirl to dissolve the components. .
4. Finally, transfer the medium to a 500 ml graduated cylinder and make up to 500 ml by adding distilled water.
5. Measure the pH of the medium and adjust to 5.6 with 1N HCl or NaOH.
6. Pour the medium into the Borosil bottle, plug the cotton stopper and cover the stopper with aluminum foil/aluminum foil, autoclave at 121 ° C for 20 min.
7. Once the temperature has cooled, remove the vial and use as needed or store at room temperature.

**Part II: Preparation of PDA Plates****Requirements**

PDA medium

Culture tubes (smooth mouth)

Test tube stand

Heater

Aluminum foil or paper

**Part III: Streak Plate Method to get Pure Culture**

The colonies on mixed plates are separated by spreading on a plate with good spacing each other using streak method.

**Requirements:**

Tripod and wire gauze Burner

Beaker of water Wire loop PDA pour plates

Mixed culture of fungi

**Procedure:**

1. Streak the plates following quadrant radiant or T-streak or continuous streak as shown in the Fig.
2. Keep the streaked plates in inverted position at 25°C for 24 – 48 hours.
3. Place the Petri dishes upside down to solve the problem of water condensation because if it drops down on the colonies, the organisms of one colony can spread on the other colony.

**Material:**

*Aspergillus niger* or *A. flavus* grown for 10 days on Czapek's broth supplemented with casein to give 0.5g nitrogen

/ L at pH 4.5 as stationary culture. Bucher funnel

Whatman No . 1 filter paper Chloroform

For Czapek (Dox) Broth (CDB) -

Sodium Nitrate : 1gm

Potassium dihydrogen phosphate : 0.5gm

Magnesium Sulphate : 0.25gm

Ferrous Sulphate : pinch

Sucrose : 15.0 gm

Distilled water : 500ml

**Method:**

To prepare Czapek broth, mix all ingredients required for preparation, adjust pH to 5.6 and sterilize for 15 minutes in an autoclave at 1.05 kg f/cm<sup>2</sup>.

Filter the medium through a Buchner funnel using Whatman filter paper No.1. Extract in triplicate 100 ml of the culture filtrate with equal volumes of chloroform. Cool the chloroform extract and evaporate to dryness on a rotary evaporator. Dissolve the residue in a minimum amount of distilled water. If direct biological assay or in chloroform for chromatographic separation is required, its toxicity to plant growth can be tested without further purification.

**Part V: Bioassay of Aflatoxins**

1. Extraction of Toxins
2. Preparation of TLC Plates
3. Qualitative Analysis of Aflatoxins
4. Seed Germination
5. Detection of Nucleic Acids (DNA and RNA)

**IV. RESULTS AND DISCUSSION****4.1 Sterilization of Maize Seeds**

Take the seeds in a conical flask. Wash properly with tap water and then with diluted Savlon or Dettol. Rinse twice with tap water. Wash with alcohol 70 % for half a minute. Transfer the seeds to a sterile conical flask containing 0.1% HgCL<sub>2</sub> solution. Cap the bottle and shake gently for 5-7 minutes. Remove the HgCL<sub>2</sub> solution from the vial. Wash seeds thoroughly 3-4 times with sterile DW. Transfer the seeds to a Petri dish. Seeds germinate in the dark, Calculate germination rate. Formed roots are kept in the light for further growth.

**Material**

*Aspergillus niger* or *A. flavus* grown for 10 days on Czapek's broth supplemented with casein to give 0.5g nitrogen/ L at pH 4.5 as stationary culture.



Bucher funnel Whatman No. 1 filter paper Chloroform

For Czapek (Dox) Broth (CDB) -

Sodium Nitrate: 1gm

Potassium dihydrogen phosphate: 0.5gm

Magnesium Sulphate: 0.25gm

Ferrous Sulphate: pinch

Sucrose: 15.0 gm

Distilled water: 500ml

### Method

To prepare the Czapek broth, mix all the ingredients required for its preparation, adjust the pH to 5.6, and sterilize for 15 minutes in an autoclave at 1.05 kg f/cm<sup>2</sup>. Filter the medium through a Buchner funnel using Whatman filter paper No.1. Extract three times 100 ml of the culture filtrate with equal volumes of chloroform. Cool the chloroform extract and evaporate to dryness on a rotary evaporator. Dissolve the residue in a minimum amount of distilled water. If direct biological assay or in chloroform for chromatographic separation is required, its toxicity to plant growth can be tested without further purification.

### 4.2 Bioassay of Aflatoxins

The toxicity of aflatoxin extracts obtained from culture filtrate to plant growth can be tested using seed germination and seedling growth tests.

### Reduction in Seedling Vigour

Sprouting seeds soaked in water in dishes. Select 12 germinated seeds with a uniform bud length of about 5 mm and place them in a dish lined with filter paper impregnated with 5 ml of culture filtrate or toxin extract. Maintain control with distilled water. After 6 days, measure the whole shoot growth of 10 seedlings avoiding the shortest and longest plants and measure the elongation over the 5-day incubation period. When aflatoxin extract was applied to corn kernels and growth of a specific fungus was observed. All 40% of the seeds germinated, of which only 15% showed seedling development. Corn plants grow closed and slow compared to standard plants.

### Result:

1. Percentage of germination of the standard seed of maize is = 0.70
2. Percentage of germination of the infected seed of maize by *Aspergillus niger* = 0.22
3. Percentage of germination of the infected seed of maize by *Aspergillus flavus* = 0.45

## IV. THIN LAYER CHROMATOGRAPHY

The separation and identification of organic compounds is routine work in many service laboratories. This layer chromatography (tlc) is an easy technique to apply for this purpose. It is useful in research laboratories to separate, identify and characterize unknown compounds. A wide range of small molecules such as amino acids, sugars, organic acids, organic acids, lipids, etc.



separated by TLC technique. The biggest advantage of TLC is the degree of separation achieved. When using volatile solvents, the time required to complete separation is only about 30 minutes, and with non-volatile solvents it is rarely longer than 90 minutes.

**Calculation:**

Rf = Distance (cm) moved by the solute from the origin

Distance (cm) moved by the solvent from the origin

For a fluoresce green (G) G1 & G2

Rf of *Aspergillus niger* =

14.6

$\frac{14.6}{20} = 0.73$

Rf of *Aspergillus flavus* =

15.3

$\frac{15.3}{20} = 0.77$

**Result:**

1. Rf of *Aspergillus niger* = 0.73
2. Rf of *Aspergillus flavus* = 0.77

**V. EXTRACTION AND DETECTION OF NULEIC ACIDS**

The nucleic acid consists of Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

**A) EXTRACTION AND DETECTION OF DNA FROM INFECTED SEEDS****Requirements:**

Radicals of germinating seeds

15 % TCA (Trichloro acetic acid)

10 % NaCl (Sodium chloride)

Chilled ethanol

EDTA (Ethylene diamine tetra acetic acid) powder

Grinder (motor and pestle)

Centrifuge

Test tubes.

**Chemical Preparation:**

1. 15%TCA:1.5gm of TCA in 30ml of distilled water.
2. 10%NaCl: 10gms of NaCl in 100ml of distilled water.

**Detection of nucleic acid**

The nucleic acids absorb strongly in the ultra violet region of the spectrum (due to conjugate double bond system).

They show characteristic maxima at 260 nm and minimum at 230nm.

**Result of OD:**

1. Standard material: 0.56
2. *Aspergillus flavus*: 0.66
3. *Aspergillus niger*: 0.45

**B) Extraction and Detection of RNA from plant material****Requirement:**

Maize seeds, saline EDTA (Ethylene diamine tetra acetic acid), Phenol 80%, chilled ethyl alcohol, centrifuge, pestle and mortar.

**Procedure:**

Weigh 1 g of corn and cut it into small pieces. Crush the ground corn kernels in 5 ml of EDTA saline or distilled water with a pestle and mortar. Add 10 ml of this 80% phenol. Shake the mixture and centrifuge it. Take the supernatant and 2 volumes of cold ethanol. Keep it in the refrigerator overnight. Centrifuge the next day and collect RNA ppt.

**Detection of RNA**

Dissolve the RNA crystals from the previous experiment in 1 ml of water. Also dissolve 0.5 g of RNA crystals. Use these two materials for the reduction chromatography on Whatman PAPER No. 1. After running the chromatography, allow to air dry for 4 h. And locate the bases under ultraviolet light. Both will be identical.

**Preparation:** 80% Phenol. Take 80ml of Phenol and 20ml of distilled water.

**Calculation:**

$R_f = \frac{\text{Distance (cm) moved by the solute from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$

$$R_f \text{ of Pure maize} = 8.1/8.9 = 0.91$$

$$R_f \text{ of } Aspergillus \text{ niger} = 7.2/8.4 = 0.84$$

$$R_f \text{ of } Aspergillus \text{ flavus} = 7.3/8.2 = 0.89$$

**Result:**

$$R_f \text{ of Pure maize} = 0.91$$

$$R_f \text{ of } Aspergillus \text{ niger} = 0.84$$

$$R_f \text{ of } Aspergillus \text{ flavus} = 0.89$$

**VI. CONCLUSION**

If seeds are infected with a fungal disease, the germination rate will decrease. Besides the germination rate, the nucleic acid composition in terms of DNA and RNA will be synthesized in less quantity, affecting the overall growth and development of the plant. Therefore, in order to sow



a seed, care must be taken to ensure that it is free of any kind of infection. As a precaution, seed sterilization can be used with mercuric chloride or a fungicide.

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