*Artykuł przeglądowy* *Review*

# Aflatoxins and possibilities for their biological detoxification*)

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Summary

**Mycotoxins are secondary metabolites of fungi, which may cause diseases in animals or humans. Aflatoxin $B_1$ is mycotoxin, which is known to frequently contaminate poorly stored food products destinated for human consuption. In nature, there exist microorganisms for which the aflatoxins are non-toxic. Aflatoxins are degraded through the microorganism's activity and the obtained products are probably utilized in their metabolism or the microorganisms have ability to bind aflatoxins to their surface. Components of herbs and spices have antiaflatoxigenic properties. They inhibit fungal development and subsequent aflatoxin production. The study reviews literature concerning the detoxification of mycotoxins by microorganisms and components of herbs and spices.**

**Keywords: Aspergillus flavus, detoxification, degradation**

In the 1960 more than 100,000 young turkeys on poultry farms in England died in the course of a few months from an apparently new disease that was termed „Turkey X disease". It was soon found that the condition was not limited to turkeys. Ducklings and young pheasants were also affected and heavy mortality was experienced.

A careful survey of the early outbreaks showed that they were all associated with a variety of feed, namely Brazilian peanut meal. An intensive investigation of the suspect peanut meal was undertaken and it was quickly found that this peanut meal was highly toxic to poultry and ducklings with symptoms typical of Turkey X disease. Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin. In fact, the toxin-producing fungus was identified as *Aspergillus flavus* (1961) and the toxin was given the name Aflatoxin by virtue of its origin (*A. flavis* → Afla).

Accumulation of aflatoxin B, has been reported from members of three diffrent groups of Aspergilli:

– Aspergillus section Flavi: *A. flavus*, *A. flavus var. parvisclerotigenus*, *A. parasiticus*, *A. toxicarius*, *A. nomius*, *A. pseudotamarii*, *A. zhaoqingensis*, *A. bombycis.*

– Aspergillus section Nidulantes: *Emericella astellata* and *Emericella venezuelensis.*

– Aspergillus section Ochraceorosei: *A. ochraceoroseus* and *A. rambellii.*

G type aflatoxins have only been found in some of the spices in Aspergillus section Flavi, while B type aflatoxins are common in all three groups (7). From the mycological point of view, there are great qualitative and quantitative differences in the toxigenic abilities displayed by different strains within each aflatoxigenic species. For example, Bennett and Klich (3) reported that only about half of *A. flavus* strains produce aflatoxins, while those that do may produce more than $10^6$ µg/kg. *A. flavus* is a common contaminant in agriculture, *A. parasiticus* occurs in warm areas, *A. nomius*, *A. pseudotamarii*, *A. bombycis* and *A. ochraceoroseus* are encountered less frequently (3, 11). Optimal conditions for aflatoxin production by *A. flavus* were observed at water activity 0.95-0.99 $a_w$ and temperature 25-30°C (11). Minimum water activity for aflatoxin production was 0,80 a (21) and temperature was > 7°C . Koehier (10) presented the conditions for growth and aflatoxin production by *A. flavus* and *A. parasiticus* (tab. 1).

Water stress, high-temperature stress and insect damage of the host plant are major determining factors in mold infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities and weed competition have been associated with increased mold growth and toxin production. Aflatoxin formation is also affected by associated

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growth of other molds or microbes. For example, pre-harvest aflatoxin contamination of peanuts and corn is favoured by high temperatures, prolonged drought conditions and high insect activity; while post-harvest production of aflatoxins on corn and peanuts is favoured by warm temperatures and high humidity.

Moreover, studies of authors (5, 11) also revealed that there are four major aflatoxins: $B_1$, $B_2$, $G_1$, $G_2$, plus two additional metabolic products, $M_1$ and $M_2$, that are of significance as direct contaminants of foods and feeds. $M_1$ and $M_2$ aflatoxins were first isolated from milk of lactating animals fed aflatoxin preparations, hence the M designation. B designation of aflatoxins $B_1$ and $B_2$, on the other hand, resulted from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light. These toxins have very similar structures and form a unique group of highly oxygenated, naturally occuring heterocyclic compounds. Their molecular formulas as established from elementary analyses and mass spectrometric determinations are: $B_1$ : $C_{17}H_{12}O_6$; $B_2$: $C_{17}H_{14}O_6$; $G_1$: $C_{17}H_{12}O_7$; $G_2$: $C_{17}H_{14}O_7$.

Aflatoxins $B_2$ and $G_2$ were established as the dihydroxy derivatives of $B_1$ and $G_1$, respectively, whereas aflatoxin $M_1$ is 4-hydroxy aflatoxin $B_1$ and aflatoxin $M_2$ is 4-dihydroxy aflatoxin $B_2$. Many substrates support growth and aflatoxin production by aflatoxigenic molds. Many authors (1, 3, 15, 30) have found aflatoxins occurring in the substrates: oilseeds (e.g. cottonseeds, soybeans, peanuts), nuts (e.g. walnut, almond), spices (e.g. peppers, mustard), cereals, maize, rice, dried fruits (e.g. figs), cocoa beans. Aflatoxins can also occur in liver, muscle, kidney, blood, milk, eggs and products containing these substances (11, 18). Aflatoxins have acute and chronic actions and the liver is the primary target organ of acute and chronic injury. Aflatoxins in the liver irreversibly bind to protein and DNA to form adducts such as $AFB_1$-lysine in albumin. Disruption of the proteins and DNA bases in hepatocytes causes liver toxicity. Acute aflatoxicosis is produced when approximately 10-20 mg of aflatoxins are consumed by adults. Symptoms include acute liver damage, acute necrosis, or in severe cases, acute liver failure and death.In humans, patients experience high fever, rapid progressive jaundice, edema of limbs, vomiting and alteration in digestion (3, 33).

According to $LD_{50}$ values the toxicity of aflatoxins is: $AFB_1 > AFM_1 > AFG_1 > AFB_2 > AFG_2$. The toxicity can by influenced by exposure level and duration of exposure, age, health, weight and the presence of other mycotoxins (11).

**Tab. 1. Limits for growth and aflatoxin production by *A. flavus* and *A. parasiticus***

| | Minimum | | Optimum | | Maximum | |
|---|---|---|---|---|---|---|
| | *A. flavus* | *A. parasitic.* | *A. flavus* | *A. parasitic.* | *A. flavus* | *A. parasitic.* |
| Growth | | | | | | |
| Temperature (°C) | 10-12 | 12 | 33 | 32 | 43 | 42 |
| Water activity | 0.8 | 0.80-0.83 | 0.98 | 0.99 | > 0.99 | > 0.99 |
| pH | 2 | 2 | 5-8 | 5-8 | > 11 | > 11 |
| Aflatoxin | | | | | | |
| Temperature (°C) | 13 | 12 | 16-31 | 25 | 31-37 | 40 |
| Water activity | 0.82 | 0.88-0.87 | 0.95-0.99 | 0.95 | > 0.99 | > 0.99 |
| pH | 2 | 2 | 6 | 6 | > 8 | > 8 |

## Detoxification

Because aflatoxin contamination is unavoidable, numerous strategies for their detoxification have been proposed. These include physical, chemical and biological methods. Microbial inactivation and fermentation methods have recently beene recognized in addition to separation, solvent extraction and adsorption from solutions.

The first investigations studying the degradation activity of microorganisms ascertained that among the 100 various species of microorganisms there exists only one, namely the strain *Flavobacterium sp.* NRRL B 184, that is capable of detoxifiing aflatoxin $B_1$ after 94 hours of incubation. Subsequent studies confirmed that *F. aurantiacu* detoxified aflatoxin in contaminated milk, oil, peanut, butter and corn. The peroxidase enzyme seems to participate in this mode of aflatoxin degradation. Additionly aflatoxin was degraded by the intestinal microflora of rats and by *Corynebacterium rubrum* ATCC 14898 and by *Streptococcus lactis.* Among yeasts, attention should be drawn to the *Candida lipolitica* IMM No 151 strain, which degraded 79% of the original amount of aflatoxin during a 20--day incubation (26).

Peltonen et al. (16) reported that specific bacterial strains removed aflatoxins from media by physical binding. *Lactobacillus strain* bound 17.3 to 59.7% $AFB_1$, *Bifidobacterium strain* bound 18 to 48.7% and *Lactococcus strain* bound 5.6 to 41.1% $AFB_1$. *Lactobacillus amylovorus strain* CSCC 5160 and strain CSCC 5197 and *Lactobacillus rhamnosus* bound more than 50% $AFB_1$. The stabilities of the $AFB_1$ bacteria complexes were evaluated by determining the amount of $AFB_1$ remaining which bound five subsequent washes. *Lactobacillus amylovorus* and *Lactobacillus rhamnosus* retained 17.4% and 32.2% $AFB_1$, respectively (12).

Similarly Haskard et al. (9) reported that *Lactobacillus* strains removed aflatoxins from media by binding to the surface components of bacteria. *Lactobacillus rhamnosus* strain GG and strain LC-705 removed $AFB_1$ from media most efficiently. *Lactobacillus rhamnosus* strain GG bound 78.9% $AFB_1$ and

LC-705 strain bound 76.5% $AFB_1$. Heat-treated or acid-treated bacteria bound higher amounts of $AFB_1$. After five washes up to 72% of the total $AFB_1$ remained bound by acid-treated bacteria.

*Kluyveromyces* isolates Y(14) and Y(16) reduced the percentage of germination of *Aspergillus* strains, produced an increase of germination lag phase and lag phase of growth as well as a decreased growth rate of *Aspergillus* strains. No aflatoxins were produced (17) at water activities 0.937 and 0.994.

The results of the research carried out by Shanta (23) demonstrated that *Phoma* sp., *Mucor* sp., *Trichoderma harzianum*, *Trichoderma* sp. 639, *Rhisopus* sp. 663, *Rhisopus* sp. 710 and *Alternaria* sp. could inhibit aflatoxin production ≥ 90%. *Phoma* sp. was the most efficient destroying about 99% $AFB_1$.

The bacteria that degrade $AFB_1$ effectively are *Nocardia corynebacterioides* DSM 12.676 and DSM 20.151, *Rhodococcus erythropolis* and *Mycobacterium fluoranthenivorans* sp. nov. DSM 44.556 (T). Cell free extract of *N. corynebacterioides* DSM 12.676 degraded 60% $AFB_1$ after 24 hours, while 90% degradation was observed with *N. corynebacterioides* DSM 20.151 over the same time. Cell free extracts of *R. erythropolis* and *M. fluoranthenivorans* have shown more than 90% degradation of $AFB_1$ within 4 hours, while after 8 hours $AFB_1$ was practically not detectable (29). Also Alberts et al. (2) investigated the degradation of $AFB_1$ by *R. erythropolis*. A significant reduction of $AFB_1$ was observed after 72 hours in the presence of *R. erythropolis* extract (33% residual $AFB_1$). Extracellular enzymes from edible mushroom *Pleurotus ostreatus* also degraded aflatoxin (13).

Taylor and Draughon (28) showed the ability of *Nannocystis exedens* (myxobacterium commonly found in soil) to antagonize *A. flavus* and *A. parasiticus*. According to the author, zones of inhibition developed between *N. exedens* and mold colony, when bacteria was grown in close proximity with aflatoxigenic mold. *N. exedens* caused lysis of a mold colony, when bacteria was added to the centre of the colony. The maize endophyt *Acremonium zeae* is also antagonistic to *A. flavus*. Chemical studies of an organic extract from maize kernel fermentations of *A. zeae*, revealed that the metabolites accounting for this were antibiotics pyrrocidines A and B (31).

Yan et al. (32) found that cyclo (L-leucyl-L-propyl) produced by *Achromobacter xylosoxidans* inhibited production of norsolorinic acid, precursor of aflatoxin.

*Trametes versicolor* used as a „healing mushroom" is able to inhibit the toxin production by *A. flavus* from 40% to over 90%. This basidiomycete containes beta-glucans which are responsible for the stimulation of the host immune response and could be involved in aflatoxin inhibition (34). Similar findings have been reported by Reverberi et al. (20) who investigated mushroom *Lentinula edodes.*

Considerable interest has developed during recent years on the preservation of food by the using of herbs and spices to effectively retard growth and mycotoxin production. Effects of herbs and spices were diffrent in some studies and depended on test conditions.

Nielsen and Rios (14) reported that mustard essential oil and clove oleoresin reduced growth of *A. flavus* with 100% and 40%, respectively. Garlic essential oil had less than 10% inhibitory effect.

Soliman and Badeaa (24) investigated the antifungal effects of essential oils extracted from some medicinal plants and found that oils of thyme and anise (≤ 500 ppm), cinnamon (≤ 1000 ppm), caraway (≤ 2000 ppm), spearmint, basil and marigold (≤ 3000 ppm) induced the total inhibition of growth of *A. flavus* and *A. parasiticus*. One percent oils of thyme, anise and two percents oil of cinnamon completely inhibited aflatoxin production in wheat grains. Rasooli and Owlia (19) found that the static effect of essential oils from *Thymus eriocalyx* against *A. parasiticus* was at 250 ppm, and the lethal effect was at 500 ppm and aflatoxin production was inhibited at 250 ppm.

According to Tantaoui-Elaraki and Beraoud (27), thyme, cinnamon, oregano and cumin essential oils were able to stop mycelial growth at 0.1% in the medium; coriander, black pepper, mugworth, bay and rosemary essential oils caused the growth to stop at concentrations between 0.1-1%. Aflatoxin production was inhibited by all the essential oils.

Soni et al. (25) proved that extracts of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) inhibited aflatoxin production considerably (more than 90%) at concentrations of 5-10 mg/ml. According to Bilgrami et al. (4) maximum inhibition in the mycelial growth occured with garlic extract (62%), whereas the inhibition of aflatoxin production was highest (60%) with onion extract in liquid medium. Eugenol was most suitable for inhibiting aflatoxin production (60%) on maize grains. The mycelial growth of *A. flavus* and *A. parasiticus* and aflatoxin production were completely inhibited by a Welsh onion extract at a concentration of 10 mg/ml (6).

Hasan (8) reported that the aflatoxins produced by *A. parasiticus* in de-tannin-coffeinated coffee and black tea were five times more concentrated than in regular coffee and tea. Tannin and coffein induced 95% inhibition in aflatoxins at 0.3% and 0.6% concentrations respectively.

Sanchez et al. (22) investigated the effects of extracts of *Agave asperrima* and *Agave striata* on the growth and aflatoxin production of *A. flavus* and *A. parasiticus*. Extracts from flowers exhibited minimal inhibition concentrations of 0.5 to 2 mg/ml. Half of the minimal inhibition concentration inhibited 99% of the production of aflatoxins.

The development of microorganisms expressing $AFB_1$ – degrading enzymes or binding $AFB_1$ may be used in the feed, food and fermentation industry. With

the application of molecular biology techniques, microbial strains with multi-functional properties can be engineered to significantly improve the quality, safety and acceptability of fermented foods and beverages.

The components of herbs and spices should find a practical application in the inhibition of fungal growth and its production in some kinds of food. These components could be used as a substitute for chemical fungicides since they are natural and non-toxic to humans.

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