



Production of aflatoxins by *Aspergillus flavus* and *Aspergillus niger* strains isolated from seeds of pulses

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Abstract

Fourteen *Aspergillus flavus* and three *A. niger* strains were isolated from seeds of lupine (*Lupinus albus* L.), mung bean (*Vigna radiata* L.), faba bean and field bean (*Vicia faba* L.) and lentil (*Lens culinaris*). The aflatoxin production of isolates was tested by two different bioassay methods as measuring the inhibitory effect on okra seed germination and bacterial growth. Toxic effect has different degrees including delay of seed germination, yellowish plant and inhibition of *Bacillus subtilis* growth. Autoclaving of fungal filtrate, freezing or microwaves has no effect on bacterial growth inhibition. B1 and B2 aflatoxins were found in all fungal filtrates with concentrations ranging from 38 to 496 µg/litre (the permitted limit is 20 µg/litre), and G1 and G2 aflatoxins were also produced by some isolates. Results proved the stability of such toxins.

Key words: Aflatoxins B1, B2, G1, G2, fungi, mycotoxins, toxic effects, bioassay methods, bean, lupine, lentil seeds.

Introduction

Mycotoxins are harmful substances produced by fungi in various foods. As much as 25% of the world's crop each year is estimated to be affected by these toxins. Most of mycotoxins are produced by three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium*. Although over 300 mycotoxins are known, those of most concern based on their toxicity and occurrence are aflatoxin, vomitoxin, ochratoxin, zearaleone, fumonisin and T-2 toxin. These toxins may be carcinogenic, teratogenic, mutagenic, immunosuppressive, tremorgenic, hemorrhagic, hepatotoxic, nephrotoxic and neurotoxic².

Mycotoxins are chemically unrelated groups of fungal metabolites characterized by their ability to induce a toxic response in human and animals when contaminate food. Mycotoxins are produced by a number of genera of fungi, however, only the aflatoxins produced by the genus *Aspergillus* have drawn great attention almost universally. Mycotoxins are diverse range of molecules that are harmful to animals and humans. They are secondary metabolites secreted by moulds, mostly *Penicillium* and *Fusarium*. They are produced in cereal grains as well as forages before, during and after harvest, in various environmental conditions. Due to the diversity of their toxic effects and their synergetic properties, mycotoxins are considered as risky to the consumers of contaminated foods and feeds⁶⁸.

Mycotoxins are metabolized in the liver and the kidneys and also by microorganisms in the digestive tract. Therefore, often the chemical structure and associated toxicity of mycotoxin residues excreted by animals or found in their tissues are different from the parent molecule⁵⁰. A study in Swaziland about the possible relationship of aflatoxin contamination and the incidence of primary liver cancer is reported. Aflatoxin ingestion levels have been

determined in "food from the plate" samples collected over a one year period. A significant correlation between the calculated ingested daily dose and the adult male incidence of primary liver cancer in different parts of Swaziland has been established, and no region of the world escapes the problem of mycotoxins³⁴.

Aflatoxins are fluorescent compounds, they are chemically classified as difurocoumarolactones and their biosynthesis by the producing fungi is via the polyketide pathway⁶⁴. Four major aflatoxins produced in feedstuffs and foods are aflatoxins B1, B2, G1 and G2. The most potent and most frequently occurring of the four compounds is aflatoxin B1. Aflatoxin is a metabolite that occurs in various tissues and fluids from animals⁵³. Two major species of *Aspergillus*, *A. flavus* and *A. parasiticus*, are responsible for the production of aflatoxins in feedstuffs and food. Infection and production of aflatoxins in field crops by these species is often associated with drought stress and insect damage⁵³.

The physiological signs accompanied toxication by aflatoxin include liver damage characterized by enlargement, release of enzymes into the blood (for example, aspartase aminotransferase and alkaline phosphatase) and impaired protein synthesis⁹. When significant quantities of aflatoxin B1 are consumed in feed by lactating dairy cows, the metabolite M₁ appears in milk within 12 hours³². The No.1 concern among dairymen is the contamination of milk by aflatoxin M₁. To avoid contamination of milk, lactating dairy cows should not receive more than 20 ppb in the total ration. Aflatoxin affects also all poultry species. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually ingested. Young poultry, especially ducks and turkeys, are very susceptible. As a general rule, growing poultry should not receive more than

20 ppb aflatoxin in the diet. However, feeding levels lower than 20 ppb may still reduce their resistance to disease, decrease their ability to withstand stress and bruising and generally make them unthrifty³².

Aflatoxin B1 (AFB1) induces mutation of the p53 gene at codon 249 (p53mt249) which is critical during the formation of hepatocellular carcinoma (HCC) following hepatitis B virus (HBV) infection³⁷. Exposure of rainbow trout, *Salmo gairdneri*, to the carcinogenic mycotoxin, aflatoxin B₁, resulted in a loss of B cell memory, but produced no change in the primary B cell antibody response. This effect was demonstrated both *in vivo*, in the generation of serum antibodies, and *in vitro*, in the generation of antibody-producing cells⁴.

The study aims at testing the ability of aflatoxin production of some fungi isolated from the seeds of pulses during the dry storage. Furthermore, two different bioassay methods to confirm the formation of aflatoxins were investigated.

Materials and Methods

Source of isolates: Some biological tests were conducted to study the ability of aflatoxin production of some fungal strains isolated from the seeds of some pulses during storage in Dammam Province in Eastern region of Kingdom of Saudi Arabia. These fungal isolates and corresponding pulse seeds are listed in Table 1.

Bioassay methods: The isolates were evaluated for their ability to produce toxigenic materials.

Okra seeds as a substrate for aflatoxin production: This bioassay was conducted according to the method of El-Akkad¹⁹. The tested isolates were individually grown on SMKY liquid medium for 10 days at 25°C. The culture was filtrated to eliminate the spores. Seeds were surface sterilized with 2% sodium hypochlorite for 2 min, rinsed thoroughly in sterile water and soaked in the desired culture filtrate of the tested isolates for 12 and 24 h. The seeds were then sown in sterilized plastic pots (7.5 cm x 6.5 cm), filled with autoclaved soil. Some other seeds were kept in SMKY medium to serve as a control. Ten seeds were grown

in each pot and three pots were employed for each treatment. All the pots were maintained under the greenhouse conditions at 25°C for 2 weeks, after which the percentage of germination was recorded. Also, yellowing percentage was calculated on some cotyledonary leaves of okra plants as follows: 0 = plants free of yellowing, 1 = 1-25% leaf area yellowish, 2 = 26-50% leaf area yellowish, 3 = 51-70% leaf area yellowish, 4 = 71-100% leaf area yellowish, 5 = Death of seedlings. Yellowing percentage = [(NPC x CR)/(NP x MSC)]x 100, where NPC = number of plants in each of class rate, CR = the respective class rate, NP = total number of plants, MSC = maximum class rate.

Sensitivity of *Bacillus subtilis* to the aflatoxin formation: The previous fourteen isolates of *Aspergillus flavus* fungus and three isolates of *A. niger* were individually grown on SMKY liquid medium for 10 days at 25°C as mentioned before to obtain the culture filtrate. Then, the filtrate of each isolate was extracted three times with equal volumes of ethyl acetate. The ethyl acetate was removed by evaporation and the residue was brought up in sterilized distilled water. The method described by Lenz *et al.*³⁸ was used as follows: A species of specific bacteria, i.e. *Bacillus subtilis*, was obtained from Bacterial Disease Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Equal disks (cm) of the tested bacterium were prepared from 10-days old bacterial cultures grown on TYG solid medium which consists of 50 g tryptone, 2.5 g yeast extract, one g glucose and 20 g agar dissolved in one litre of distilled water. A liquid medium of TYG was prepared, distributed into 500 ml Erlenmeyer flasks (200 ml/each) and autoclaved. After cooling, one disk (0.5 cm) of bacterial culture was added to each flask. All the flasks were incubated for 48 h at 30°C. Other flasks (250 ml) containing TYG solid medium (100 ml/each) were prepared and autoclaved. After cooling and before solidification, one ml of the previous bacterial suspension was added to each flask and shaken well. The inoculated medium was distributed into Petri dishes (20 cm) at the rate of 10 ml medium/plate. By using a cork borer (0.3 cm in diameter), a pore was made in the middle of each plate. One ml of the aforementioned filtrate of each tested fungal isolate was added

Table 1. Effect of culture filtrates of tested fungi on okra seedlings.

No.	Fungal isolate	Crop from which each fungus was isolated	Average percentage of okra seed germination after soaking periods (h)		Average percentage of cotyledonary yellowing after soaking periods (h)	
			12	24	12	24
1	<i>Aspergillus flavus</i>	Mung bean 1	95	90	28	62.67
2	<i>Aspergillus flavus</i>	Mung bean 2	100	100	45.33	54.67
3	<i>Aspergillus flavus</i>	Mung bean 3	95	90	43.33	68.67
4	<i>Aspergillus flavus</i>	Mung bean 4	85	80	61.33	62.67
5	<i>Aspergillus flavus</i>	Mung bean 5	100	95	42.67	54
6	<i>Aspergillus flavus</i>	Field bean 2	100	100	52.67	59.33
7	<i>Aspergillus flavus</i>	Field bean 3	100	95	58.67	58.33
8	<i>Aspergillus flavus</i>	Field bean 5	95	85	54.67	59.33
9	<i>Aspergillus flavus</i>	Faba bean 1	95	85	50	66
10	<i>Aspergillus flavus</i>	Faba bean 2	90	80	49.33	54
11	<i>Aspergillus flavus</i>	Faba bean 3	90	80	40.67	54.33
12	<i>Aspergillus flavus</i>	Lupine 3	100	100	49.33	57.33
13	<i>Aspergillus flavus</i>	Lupine 5	95	95	56	57.33
14	<i>Aspergillus flavus</i>	Green lentil	90	80	60.67	66.67
15	<i>Aspergillus niger</i>	Field bean 1	100	90	37.33	52.67
16	<i>Aspergillus niger</i>	Field bean 2	100	95	50.67	62.67
17	<i>Aspergillus niger</i>	Field bean 3	95	80	33.33	60
18	Control	-	100	100	0	0
	L.S.D. at 0.05%		1.63	2.05	2.72	2.69

to the pore. The same steps were repeated, but the fungal filtrate was autoclaved, freeze-dried for three days or treated with microwave for one min. Three plates were used for each treatment. Other Petri dishes containing only TYG medium without crude extract and individually inoculated with the tested bacterium were served as control, and all the dishes were incubated for 48 h at 30°C. The diameters of the inhibition zones were measured (in cm²) as an indicator of aflatoxin production.

Determination of qualitative and quantitative estimates of fungal toxins in the culture filtrate: Evident from the experiences of previous biological detection using okra seeds and bacteria *Bacillus subtilis* to detect the presence of toxins, the presence of biological materials vital in the culture filtrate were evaluated to inhibition of chlorophyll formation in okra seedlings and germination of seeds and also the growth of bacteria *B. subtilis*. From this standpoint an experiment was conducted to determine the quality of such biological material, if any, whether from fungal toxins (aflatoxins) or other. Extraction of aflatoxins from each fungi was carried out according to the method of Gertz²⁷, in collaboration with the Agricultural Research Centre of Arab Republic of Egypt.

Statistical analyses: Data obtained were statistically analyzed using SPSS Version 6. Treatment averages were compared at the 0.05 level of probability using LSD⁴¹.

Results and Discussion

Biological assay: The ability of fourteen isolates of *Aspergillus flavus* and three isolates of *Aspergillus niger* to produce biological toxigenic material in SMKY liquid medium was tested using two biological assay methods.

Effect on okra seedlings: Data represented in Table 2 proved that all fungal filtrates had adverse effect on okra seed germination. There was a proportional correlation between the degree of germination inhibition and the seed soaking period in fungal filtrates, starting from 12 h till 24 h which recorded the highest inhibition rate (80%). All the tested fungi can produce yellowish of okra leaves. The highest yellowish index (68.67%) was recorded

after the treatment of seeds by the filtrate of *Aspergillus flavus* (isolate No. 3). Our data assured the previous studies^{3, 23, 55}.

Ochratoxin A had an effect on some biochemical parameters, viz. chlorophyll, protein and nucleic acid concentrations during seed germination and seedling growth in mung beans (*Vigna radiata* (L.) Wilczek, var Pusa 119)⁶³. The inhibitory effect of aflatoxins on seed germination and growth of okra plants suggested that aflatoxins functioned as anti-auxins probably by inhibiting RNA synthesis⁵².

Aflatoxin B₁ showed a profound effect on pollen germination and pollen tube morphology leading to only 40% germination and several morphological anomalies in *Catharanthus roseus* and *Haemanthus katherinae*. Embryogenic callus of *Santalum album* could give rise to only 60% of somatic embryos in the presence of 1 ml toxin with several abnormalities which is much less compared to 94% conversion to distinct bipolar embryos in case of control set without toxin. Only 49% of *Arachis hypogaea* seeds germinated in the presence of 1 ml aflatoxin in the germinating medium compared to 71% germination in control media¹³.

In a study on the relative susceptibility of 30 lettuce cultivars to inhibition by aflatoxin, seed germination was not inhibited by concentrations as high as 1,000 mg/ml in cultivar Imperial 44 or by 100 mg/ml in the remaining cultivars¹¹. Hypocotyl elongation was inhibited by 46 to 68% at a concentration of 100 mg of aflatoxin per ml. Seedlings exposed to aflatoxin did not become chlorotic. The similarity between the morphological reaction of plants to coumarin and aflatoxin suggests a common mode of action¹¹.

Macroscopic observations showed that aflatoxins affect certain plants by inhibition of seed germination⁶¹ and elongation of hypocotyls or roots of developing seedlings or both^{21, 51, 52} and by interference with chlorophyll synthesis^{21, 61, 65}. Similar inhibitory activities have been attributed to coumarin^{15, 45}, and since aflatoxins are derivatives of coumarin, analogous modes of action have been suggested for these substances¹⁶.

Aflatoxin has been associated with the development of albinism (virescence) in plants. Albinism has been induced in corn and citrus seedlings after infection with isolates of *Aspergillus flavus* of unknown toxigenicity^{18, 31, 33, 54}, however, albinism was not induced in citrus, tomato and several legumes with toxigenic and

Table 2. Effect of culture filtrates of tested fungi on growth of *Bacillus subtilis*.

No.	Fungal isolate	Crop from which each fungus was isolated	Inhibition zone (cm ²)			
			Not treated	Microwave	Autoclaving	Freezing
1	<i>Aspergillus flavus</i>	Mung bean 1	12.6	12.28	9.09	15.21
2	<i>Aspergillus flavus</i>	Mung bean 2	10.18	9.62	10.47	10.47
3	<i>Aspergillus flavus</i>	Mung bean 3	15.56	15.21	11.97	11.68
4	<i>Aspergillus flavus</i>	Mung bean 4	12.6	12.28	7.62	7.07
5	<i>Aspergillus flavus</i>	Mung bean 5	6.05	6.84	7.62	7.07
6	<i>Aspergillus flavus</i>	Field bean2	5.03	5.11	4.91	5.11
7	<i>Aspergillus flavus</i>	Field bean3	9.62	6.29	7.81	7.07
8	<i>Aspergillus flavus</i>	Field bean5	10.47	11.18	10.21	10.47
9	<i>Aspergillus flavus</i>	Faba bean1	9.09	7.06	8.08	9.09
10	<i>Aspergillus flavus</i>	Faba bean2	4.34	4.74	4.34	2.7
11	<i>Aspergillus flavus</i>	Faba bean3	10.19	10.13	9.35	9.9
12	<i>Aspergillus flavus</i>	Lupine3	13.53	10.89	7.07	9.35
13	<i>Aspergillus flavus</i>	Lupine5	12.6	10.89	6.29	9.35
14	<i>Aspergillus flavus</i>	Green lentil	14.19	11.74	7.07	9.35
15	<i>Aspergillus niger</i>	Field bean1	13.53	12.28	8.55	11.97
16	<i>Aspergillus niger</i>	Field bean2	9.62	9.08	7.62	9.62
17	<i>Aspergillus niger</i>	Field bean3	10.18	9.62	9.63	10.18
18	Control	-	0	0	0	0
L.S.D. at 0.05%			1.25	1.01	1.18	1.24

nontoxic strains of *A. flavus*. Schoental and White⁶¹ observed albinism in seedlings of *Lepidium* exposed to 10 µg of aflatoxin per ml.

This “bleaching” phenomenon was further studied by Slowatizki *et al.*⁶⁵ and proposed as a bioassay method for aflatoxin M. Mayer *et al.*⁴⁵ and Reiss⁵² observed some lightening of the coloration of *Lepidium* exposed to 100 µg of aflatoxin per ml but did not observe complete loss of chlorophyll. Lettuce seedlings did not exhibit albinism at concentrations as high as 1,000 µg/ml¹¹.

The aflatoxin molecule contains an unsaturated lactone ring structure postulated to be necessary for coumarin-like activity⁴³. The remaining portion of the aflatoxin molecule, however, is only distantly related to coumarin. This might explain aflatoxin’s lack of inhibitory activity towards lettuce seed germination but does not explain the observation⁶¹ that aflatoxin inhibited seed germination in *Lepidium*. Aflatoxin 100 µg per ml caused a 100% inhibition of germination. A light lag in the rate of lettuce seed germination occurred at a concentration of 1,000 µg of aflatoxin mixture per ml, an effective toxin concentration was 700 µg/ml. Reiss⁵², also using *Lepidium*, was unable to repeat the observations of Schoental and White⁶¹. Inhibition of seed germination and seedling elongation are the two physiological effects most often associated with coumarin activity. Aflatoxin does not affect seed germination but is inhibitory to hypocotyl elongation in lettuce.

In comparing the inhibitory effect of coumarin and various coumarin derivatives on seed germination in lettuce, Mayer and Evenari⁴³ found the derivatives to be less inhibitory than the parent compound. They postulated that the inhibitory effect of coumarin is a function of the unsaturated lactone ring of the coumarin molecule. Goodwin and Taves²⁸ studied the effects of coumarin and coumarin derivatives on seed germination and root growth in *Avena*. Some derivatives were active inhibitors of root growth but inactive as germination inhibitors, whereas other derivatives, which were very weak inhibitors of root growth, were as active as coumarin in inhibiting seed germination.

Effect of fungal filtrates on the bacterial growth: B. subtilis was used as an indicator of toxin effect. Table 2 proves that biological toxic material was produced in culture filtrates of all fungal isolates. The width (cm) of inhibition zones of *B. subtilis* growth varied from the tested fungus to another. Meantime, the effect of culture filtrate of *A. flavus* isolate No. 3 (isolated from Mung bean 3) was a large inhibition zone of bacterial growth being 15.56 cm² before boiling, 11.97 cm² after boiling, 11.68 cm² after freezing and 15.21 cm² after exposition to microwaves. Isolate No. 10, (isolated from Faba bean 2) showed the lowest inhibition effect, the zone being 4.34 cm² before and after autoclaving, 4.74 cm² after microwave treatment for one min and 2.7 cm² after freezing. After freezing at 0°C bacterial growth inhibition was increased by some *A. flavus* filtrates but other filtrates did not have any effect.

Ogunsanwo *et al.*⁴⁷ pointed to that there are three main possibilities for the aflatoxin loss as a result of heat application: heat liability of the aflatoxins, thermodynamically enhanced reactions between the aflatoxins and other constituents of the peanut seeds or thermal destruction of other constituents of the peanut seeds and less extractability of the aflatoxins in the presence of these products. The third explanation seems unlikely

since aflatoxins were detected even after extremely harsh conditions of roasting. Pyrolysis of aflatoxins, especially at elevated temperature, would lead to their decomposition. It will be difficult to predict the product of this pyrolysis since during this process, which is a very drastic condition of attack on any compound, bond breakage becomes indiscriminate. Results obtained in this work seem to support the above prediction with respect to thermolabilities of these compounds as there appears to be higher percentage reductions of AFG₁ than AFB₁ in the peanut seeds roasted under the same conditions. Similar observation has been reported by Hamada and Megalla²⁹.

In case of chemical reaction involving the aflatoxins, it is anticipated that the G series would be more reactive than their B counterparts. This could be predicted from the structural differences between these compounds. In the B series, there is just one ether linkage while in the G series there are two ether linkages. Ether linkages are susceptible to chemical attacks and therefore the presence of two of such sites in the G toxins makes them more susceptible to chemical attacks when compared to the B series. It is therefore expected that AFG₁ will be more thermo labile than AFB₁ and thus expected to suffer higher degradation⁴⁷. Peanut seeds were prepared with variations in roasting conditions. Positive correlations were obtained between loss of aflatoxins in the products and the roasting conditions. Seeds dry-roasted at 140°C for 40 min resulted in 58.8 and 64.5% reductions in AFB₁ and AFG₁, those roasted at 150°C for 25 minutes resulted in 68.5 and 73.3% reductions in AFB₁ and AFG₁, respectively. Roasting at 150°C for 30 min led to 70.0 and 79.8% reductions in AFB₁ and AFG₁, respectively⁴⁷. Reduction in the levels of aflatoxins in peanuts by roasting is in agreement with earlier reports^{22, 29, 35, 36, 67} that heating reduces aflatoxins in agricultural products.

Although some destruction of aflatoxins B₁ and B₂ occurred during cooking and/or processing, the maximum amount of inactivation did not exceed 41% of the total and was generally in the range of 15-30%. Thus, results suggest that aflatoxins are quite stable during cooking and/or processing²⁵.

When peanuts or other objects are subjected to microwaves, the heating effect is from the center out. This is contrasted to conventional oven methods where hot gases are used to heat or roast the peanuts by convection. In turn, the interior of the kernel is heated by conduction. Experience has shown that microwave roasting produces a very uniform product in shorter time as aflatoxin content is reduced by conventional roasting. However, to reduce the aflatoxin content to an acceptable level often results in over roasting and decrease in commercial value because of darkening and/or loss of flavor and nutritional value. After microwave exposition of peanuts and yellow corn, deliberately infected by *Aspergillus flavus*, the contents of different aflatoxins in yellow corn were in the decreasing order B₁ = G₁ > B₂ > G₂. Infected peanuts were characterized by the highest B₁ level, and the rate of aflatoxin destruction increased with the increase of microwave oven power setting (low, moderate and high) and exposure time to microwaves²⁴.

Heat is relatively ineffective for destruction of aflatoxin, although normal roasting, as for the preparation of peanut butter, results in considerable reduction in aflatoxin content. The search is carried out intensively and the methods and technologies are created to stop the distribution of toxin-producing fungi and suppress their

negative effect on living organisms^{26, 39}.

Detoxification possibilities with the aid of microorganisms - various strains of bacteria and yeasts - are widely studied in various countries^{58, 59, 66}. Treatment with *Flavobacterium aurantiacum* removes aflatoxin and may be useful for beverages. Oxidizing agents readily destroy aflatoxin, and treatment with hydrogen peroxide may be useful. Treatment of defatted oilseed meals with ammonia can reduce aflatoxin content to very low or undetectable levels with only moderate damage to protein quality⁸. Great attention is paid to the regularity of toxin accumulation in feeding stock and fodder in Lithuania^{40, 46, 49, 62}.

Qualitative and quantitative estimates of fungal toxins in the culture filtrates:

It was found that all isolates of *A. flavus* and *A. niger* were able to produce aflatoxin B1 and B2, isolates No. 2, 3, 5, 6, 8 and 12 were capable of producing aflatoxin G1 and isolates No. 4, 5, 6, 7, 13, 16 and 17 produced G2 (Table 3). Generally, *A. flavus* isolate No. 10 was able to produce the largest concentration of aflatoxin B1 (496 ppm) and *A. flavus* isolate No.1 the least amount (38 ppm). *A. flavus* isolate No. 5 was able to produce the largest concentration of aflatoxin B2 (185 ppm), but *A. flavus* isolate No.10 was unable to produce aflatoxin B2. Concentrations of aflatoxin were higher than internationally recommended limits (20 µg/litre). These results are in agreement with those obtained by Diener and Davis¹⁷, who announced that 90% of *A. flavus* strains, previously isolated from different foodstuffs, produced high levels of B1, while 10% produced aflatoxin B1 and G1.

After four months commercial storage, 100 soybean samples from different places of Egyptian Governorates were assayed for filamentous fungi, and 73 species and 8 varieties belonging to 32 genera were isolated²⁰. The seeds were assayed for aflatoxin, ochratoxin A, sterigmatocystin, T-2 and zearalenone. Aflatoxin (5-35 µg/kg) was detected in 35% of soybean seed samples, but other mycotoxins were not detected.

Samples of dry-roasted groundnuts (DRG) in southwestern Nigeria were analysed for aflatoxin contamination⁷. Aflatoxin B1 was found in 64.2% of samples with a mean of 25.5 ppm. Aflatoxins B2, G1 and G2 were detected in 26.4, 11.3 and 2.8% of the samples

with mean levels of 10.7, 7.2 and 8 ppm, respectively, in contaminated samples.

Moreover, Bailey *et al.*⁶ found that AFB₁ is three times more carcinogenic than aflatoxicol (AFL); whereas relative tumorigenic potencies of aflatoxins were AFB₁ 1.0, AFL 0.936, aflatoxin M₁ 0.086 and AFL M₁ 0.041⁵. AFB₁ is a polycyclic aromatic hydrocarbon that is associated with hepatic carcinogenesis and immunomodulation in a broad spectrum of vertebrates⁴⁸; so, exposure to AFB₁ resulted in the reduction of cytokine, macrophage function and lymphocyte activity, i.e. trout exposed to very low concentrations of AFB₁ in feed or exposed as embryos had a very high incidence of carcinogenesis.

Anyhow, Sarcione and Black⁶⁰ suggested that serum alpha-fetoprotein measurements may be useful to confirm the appearance of hepatocellular carcinoma in experimental fish carcinogen-assay system and to detect hepatocellular neoplasia in high-risk wild fish populations exposed to carcinogenic pollutants. Abd-Allah *et al.*¹ also suggested that the Comet assay is a useful tool for monitoring the genotoxicity of mycotoxins such as AFB₁ and for evaluating organ specific effects of these agents in different species.

Chavez-Sanchez *et al.*¹⁰ reported that Nile tilapia reflected decreases in growth and food intake in direct relation to AFB₁ intake (0-3 ppm). The liver was severely affected and showed fatty liver, nuclear and cellular hypertrophy, nuclear atrophy, increase in number of nucleoli, cellular infiltration, hyperemia and necrosis. In kidneys congestion, shrinking of glomeruli and melanosis were observed. Additionally, Nile tilapia fed a diet contaminated with crude aflatoxins for 22 successive weeks showed a significant decrease in growth rate, PCV, Hb conc., erythrocyte count, total leukocyte count and lymphocytes. The mortality rate was 60% and aflatoxin residues were detected in fish at the end of week 16⁴². Also, Indian major carps (*Labeo rohita*) reflected immunosuppressive effect at a very low dose of AFB₁ (1.25 µg/kg body weight), since they revealed a reduction of total protein, globulin levels, bacterial agglutination titre, nitroblue tetrazolium assay and serum bactericidal activities, as well as an enhanced albumin-globulin ratio without change in albumin concentration⁵⁶.

Table 3. Aflatoxin concentration in the filtrate of tested fungi.

No.	Fungal isolate	Crop from which each fungus was isolated	Aflatoxin conc. p.p.m.			
			B1	B2	G1	G2
1	<i>Aspergillus flavus</i>	Mung bean 1	38	5	0	0
2	<i>Aspergillus flavus</i>	Mung bean 2	251	71.5	5	0
3	<i>Aspergillus flavus</i>	Mung bean 3	109	60	8	0
4	<i>Aspergillus flavus</i>	Mung bean 4	169.5	39.5	0	7
5	<i>Aspergillus flavus</i>	Mung bean 5	94	185	8	14
6	<i>Aspergillus flavus</i>	Field bean2	86	164	14	16
7	<i>Aspergillus flavus</i>	Field bean3	238.5	156	0	5.5
8	<i>Aspergillus flavus</i>	Field bean5	316	71	83	0
9	<i>Aspergillus flavus</i>	Faba bean1	225	37	0	0
10	<i>Aspergillus flavus</i>	Faba bean2	496	0	0	0
11	<i>Aspergillus flavus</i>	Faba bean3	337.5	20.5	0	0
12	<i>Aspergillus flavus</i>	Lupine3	259.5	56	21	0
13	<i>Aspergillus flavus</i>	Lupine5	249	53.5	0	3.5
14	<i>Aspergillus flavus</i>	Green lentil	146.5	26	0	0
15	<i>Aspergillus niger</i>	Field bean1	109.5	21	0	0
16	<i>Aspergillus niger</i>	Field bean2	139.5	31	0	0.5
17	<i>Aspergillus niger</i>	Field bean3	302	18.5	0	7.5
L.S.D. at 0.05%			5.76	37.94	72.54	5.568

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