AFLATOXINS IN COCONUT PRODUCTS

UPALI SAMARAJEEWA

Department of Bacteriology, University of Ceylon
Peradeniya

Introduction

Fungi are present in large numbers in our environment. Some of these are beneficial providing antibiotics and modifying foods. Harmful species of moulds also occur among these. Some of them were found to attack animals, directly causing various diseases, while others produce toxic metabolites on various substrates consumed by animals and man. Many such pathogenic fungi were found to grow on rice (1) causing diseases. *Fusarium*, *Aspergillus*, and *Penicillium* are some of these toxin producing genera.

Earlier, these fungi were suspected to be the cause of sudden losses in poultry farms observed in different parts of the world. However no attention was paid to this problem until 1960. The sudden death of more than 100,000 turkey poult's in England during Christmas 1960 drew scientific attention to the then unknown “Turkey—X disease.” (2) The cause of this was traced back to a shipment of groundnut (peanut) meal exported to England from Brazil. Similar losses of ducklings were reported in Kenya and Uganda in 1961. (3)

This discovery lead to intensive scientific research on the toxic principle associated with these ground-nuts. (4) A toxic fungal metabolite was isolated and identified from this meal. Fungi responsible for the production of the toxin were found to be *Aspergillus flavus* or *Aspergillus parasiticus*. The toxic entity was hence named A-FLA-TOXIN.

This toxin is a group of chemical compounds having the dihydro-furo-benzo-furan ring system. In this group about 11 compounds were isolated and some have been named B₁ B₂ G₁ G₂ M₁ M₂ G₂a and B₂a.

The aflatoxin producing fungus *Aspergillus flavus* has a brownish yellow appearance on its third day of growth. In about seven days it takes a moss green colour associated with its sporulation. Fungi could be characterised under the microscope by their long, heavy walled, pitted, colourless conidiophores having globose, colourless vesicles which are fertile over the whole surface. The sterigmata are mostly biseriate and colourless and are closely packed. The rough walled conidia are yellowish green in colour and can be globose or subglobose.

*Aspergillus flavus* grows on a large variety of food products including peanut, rice, wheat, sorghum, beans, barley, sweet potato, red pepper (chillie), cocoa, coffee, coconut, maize and cottonseed.

This fungus has been found to grow at temperatures ranging from 12-40.5°C., and moisture contents above 12%. For peanut, the optimal conditions were established as 30-35°C and 15-20% moisture content(6). Highest yields were obtained in coconut after 8 days of incubation at 24°C (7). Once these temperature and moisture conditions are provided, aflatoxin production occurs readily on storage. In stored foods, the action of micro-organisms and insects leads to the formation of micro-atmospheres even in samples dried to moisture contents below 12%, and these micro-atmospheres can support fungal growth. This observation has been made in products like copra during storage. (8)
Fungal growth in coconut products

Coconut products can support the growth of these fungal types. Eyre on "Cultural studies of lipase production" has isolated several *Aspergillus* strains from copra samples. (9) Different strains of *Aspergillus* grow at different moisture levels of dried, semidried and wet copra. (7) Even at moisture contents of 20-50% *Aspergillus flavus* and *Aspergillus parasiticus* were found to grow on copra samples.

Copra, poonac and coconut oil samples collected from different parts of the Island on analysis showed aflatoxin contamination due to fungal growth.(10) On the basis of these findings the suitability of coconut as a medium for the experimental production of aflatoxin was investigated using high aflatoxin producing strains of *Aspergillus flavus*. The values obtained under different conditions for fresh coconut at 24°C are given in table 1. (7) Here the *Aspergillus flavus* strain ATCC 15546 was used for inoculation.

Table 1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Aflatoxin yield B&lt;sub&gt;1&lt;/sub&gt; + G&lt;sub&gt;1&lt;/sub&gt; ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static, 12 days</td>
<td>1960</td>
</tr>
<tr>
<td>Continuous Agitation, 13 days</td>
<td>8368</td>
</tr>
<tr>
<td>Continuous Agitation, 7 days</td>
<td>6434</td>
</tr>
<tr>
<td>Shaken once daily, 5 days</td>
<td>510</td>
</tr>
<tr>
<td>Static, 5 days</td>
<td>225</td>
</tr>
<tr>
<td>Shaken once daily, 12 days</td>
<td>2813</td>
</tr>
<tr>
<td>Continuous Agitation, moistened, 12 days</td>
<td>7500</td>
</tr>
<tr>
<td>Shaken once daily, 5 days</td>
<td>2813</td>
</tr>
<tr>
<td>Shaken once daily, 8 days</td>
<td>2110</td>
</tr>
</tbody>
</table>

These values on comparison with those for other products charted in table 2 appear to be much higher.

Table 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conditions</th>
<th>Yield (total) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushed wheat</td>
<td>30°C, 7 days</td>
<td>750</td>
</tr>
<tr>
<td>Peanuts</td>
<td>30°C, 10-13 days shaken daily</td>
<td>265</td>
</tr>
<tr>
<td>Rice</td>
<td>28°C 5 days, continuous shaking</td>
<td>1510</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>25°C 8-10 days</td>
<td>1100</td>
</tr>
</tbody>
</table>

It is not known whether high aflatoxin production occurs in naturally contaminated products. However, the levels of toxicity of aflatoxin in coconut samples are very much lower than those observed during experimental aflatoxin production. The permissible levels of aflatoxin in food products suggested by the Tropical Products Institute, London (1) are as follows:

<table>
<thead>
<tr>
<th>Toxicity Level</th>
<th>Aflatoxin Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high toxicity</td>
<td>over 1 ppm of aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>High</td>
<td>0.25—1 ppm</td>
</tr>
<tr>
<td>Medium</td>
<td>0.05—0.25 ppm</td>
</tr>
<tr>
<td>Low or negative</td>
<td>below 0.05 ppm</td>
</tr>
</tbody>
</table>

Some of the copra, poonac and coconut oil samples collected from the field showed medium or high aflatoxin levels. (10) This indicates the need for controlling aflatoxin contamination of coconut products.

Similar medium and high toxicity levels of aflatoxin were reported by Baur and Armstrong in 1971 in their studies on methods for the examination of copra meal for aflatoxin. (11)

Studies on the distribution of aflatoxin during the mechanical extraction of coconut oil by using artificially inoculated coconuts showed that about 20% of aflatoxin passes into the oil phase. The same effect may be occurring in the extraction of coconut oil on a commercial scale. Low and medium toxic levels were recorded on analysis of coconut oil procured from the market. This may be the result of using contaminated copra for oil extraction. It is however probable that on refining coconut oil the aflatoxin present is destroyed.
The waste coconut meat ("pol kudu") collected by the Oils and Fats Corporation is an excellent medium for the growth of fungi. There is sufficient moisture in this medium and during sundrying airborne spores of *Aspergillus* could settle on it and grow during storage producing aflatoxin. The oil extracted by hexane from coconut waste is split under conditions of elevated temperature and pressure into glycerols and fatty acids. Aflatoxin is not likely to appear in these products on account of the extreme conditions required for this hydrolysis. The residue left after solvent extraction which is directly used as animal feed by mixing with other necessary ingredients, however, may contain aflatoxin. This may be one of the reasons for the reduction in the egg laying capacity of poultry and lowered weight gain in cattle and pigs observed in local farms.

Possible results of aflatoxin contamination of coconut

Coconut products (mainly desiccated coconut and coconut oil) make a substantial contribution to Ceylon’s exports. Any contamination of these products could affect our export market, with loss of valuable foreign exchange. In 1970 FDA detected the presence of aflatoxins in several consignments of coconut products imported to USA from the Philippines (12) leading to a rejection of those consignments. This underlines the importance of strict quality control measures for coconut products, especially for export.

Almost all our animal feeds are prepared from coconut poonac or solvent extracted coconut waste. The use of poonac from contaminated copra or coconut waste could lead to complications where animal feeds are concerned. The ultimate effect of feeding with toxic meals vary from sudden death of animals (1) to other problems such as carcinogenic effects (13) loss of weight of broilers (14) loss of weight gain and stunted nature of pigs (15) and lowering of egg laying in chicken. (16) The consumption of contaminated poonac by dairy cattle has been found to induce the production of a different aflatoxin metabolite in the milk produced by them. Such metabolites were found in the milk of goats (17) fed with aflatoxin containing diets. This product aflatoxin M₉ appearing in milk was shown to be as toxic as aflatoxin B₉ (18) As regards the susceptibility of poultry to aflatoxins their resistivity was observed to decrease in the order (19) chicks, young pheasants, goslings, turkey poult, and ducklings.

Effect on human population

Possible ingestion of aflatoxins by human beings could be either direct by consumption of contaminated foods or indirectly through animal products like milk from animals fed on contaminated foods. The effect of aflatoxin as a carcinogenic agent has not being proved directly with regard to humans, but indirect evidence and work with animals closely related to human beings has thrown some light on this problem. The fact that liver cancer is the greatest single cause of cancer in South East Asia (20) gives some clue to this. As against this, death due to liver cancer is only 4% of all cancer deaths in Europe and America, where the cold, less humid climate does not favour fungal growth. The climatic conditions in South East Asia encourages fungal growth in food commodities.

Although no direct evidence exists on Aflatoxicosis in human beings, a survey on association between contamination in foods and frequency of hepatoma in Uganda (21) has shown some interesting results. 480 food samples collected from different districts were analysed for aflatoxin contamination. 29.6% of them showed detectable levels of aflatoxin. In Karamoja district where the incidence of hepatoma was highest the food were found to be highly contaminated. Calculating on the observed aflatoxin levels, consumption of 500 g of grain per person per day could lead to ingestion of 0.02 to 2.0 mg. of aflatoxin daily. This level is hepatotoxic to monkeys. It may be that the same effects occur with human beings. Foods stored and fermented, for beer production in these areas were also found to be highly contaminated with aflatoxin.

Experiments with human lung cultures has shown that 0.03 μg./ml. of aflatoxin B₁ could produce 51% reduction (22) in mitosis. Working with human embryonic liver cells using aflatoxin B₂ and G₂ which are less toxic than B₁, the LD₅₀ values were established as 35 and 10 μl./ml. respectively (23) on 48 hour exposure to toxins. However adult lung and liver cells were shown to be less sensitive. Adults are less susceptible than young ones in all species tested experimentally so far.
Observations made with other primates such as monkeys could be used to study possible effects of aflatoxin on human beings. Rhesus monkeys(24) fed with diets containing 1 mg. of aflatoxin per Kg. of body weight per day lived beyond 22 days without any external symptoms, but haemorrhagic necrosis was noted in the livers. However no tumours developed in any of the animals. Intragastric administration of aflatoxin B, (0.01 to 1.0 mg.) per day to African monkeys(25) till death produced histological patterns characteristic of hepatic toxicity. Working with Vervet monkeys(26) the LD_{50} given in 10 days was established to be 3.7 mg/Kg. of body weight.

It may be that aflatoxins undergo metabolic changes forming nontoxic or less toxic compounds in some animals. A comparative study of aflatoxin metabolism in rats and yellow baboons fed with diets containing aflatoxin and humans from rural areas in good health and liver conditions was done by Monjour, Giorgi and Toury.(27) Their work revealed that protein fractions from fungi consumed with food are not present in urine, faecal, blood or liver extracts in any of the types studied. Aflatoxin however was found to be present in urine, faeces and liver of rats but not in similar samples of yellow baboons. Analysis of urine from monkeys fed with aflatoxin B showed the presence of a new phenolic aflatoxin called aflatoxin p.(28)

Because of the differences in results obtained with different types of monkeys the exact effects of aflatoxin consumption by humans cannot be predicted. However, continuous consumption of aflatoxin may have some effect or other on humans, though they could be much more resistant than the animals studied.

Experiments in vitro have shown that aflatoxin binds rat DNA in chromosomes(29) and inhibits RNA polymerase activity. Similar effects may be occurring with human DNA probably at higher concentrations of aflatoxin than with animal RNA. However it is not known whether these reactions occur in vivo.

Analysis of breast milk from lactating rats fed with aflatoxin B showed the presence of aflatoxin M_{1}(30). Similar compounds were found in breast milk from mothers in India. The children fed on milk of these mothers showed cirrhosis of the liver leading to death.(31) Investigations revealed that the food consumed by some of the mothers was contaminated. All these experimental and clinical observations suggest the danger of fungal contaminations of foods.

Prevention of contamination

Only fungal growth can lead to aflatoxin contamination in food materials. Therefore prevention of Aspergillus growth should be done both at industrial level where severe economic losses could occur and at domestic level where health hazards could be the result. Most of the rural folk consider the "Yellow" fungal growth as a sign of good processing of copra. Others consider "Blue" fungal growth as an indication of copra being properly dried. It is essential that the people who handle copra especially labourers should understand this problem. Some form of propaganda is essential to make them understand the conditions under which fungal growth occurs. They should be made aware of the results of improper drying and storage of coconut products. Unless they have a proper understanding of the results of fungal growth, the way it affects the industry and thereby the economy of the country and the health of people the proper control of aflatoxin contamination will not be possible. Prevention of fungal growth on raw materials at the very beginning of handling and processing will be the most efficient and cheapest method of solving the problem.

Mixing of mouldy copra of various standards collected from different sources at oil mills is one of the major causes of aflatoxin contamination of nontoxic products. In most of our mills poonac and coconut oil processed on different days from different batches of copra are pooled together. Among these there could be highly contaminated samples. The result of this is the distribution of aflatoxin into uncontaminated lots. Since the toxin is effective at very low concentrations (0.25-1 ppm) tons of uncontaminated material could be ruined by a few pounds of the contaminated product. At this stage any sampling for analysis becomes more difficult due to non-homogeneity of toxin distribution. Results obtained by analysis of samples from such heaps will show different aflatoxin levels in different areas of the same heap.
It is therefore essential to enforce strict quality control measures at the raw material stage, and frame appropriate laws whereby contaminated material could be rejected.

Coconuts are consumed in small quantities in our homes. The spores of *Aspergillus flavus* are present in the atmosphere and hence contamination could occur on exposure within a matter of hours, and significant amounts of aflatoxin produced within a day of fungal growth. Sometimes the yellow fungus appears within a day or two of exposure of the kernel and the usual habit is to scrape away these areas and use the meat for food preparations. Screening experiments have conclusively proved that diffusion of aflatoxin from the site of production occurs and that the toxin could be stable for several days. This implies that the scraping off of fungal areas from the endosperm would not remove residual toxicity. On the contrary, the scraper used could contaminate further fresh meat subsequently, as the fungal spores adhering to it could act as micro-media for the production of aflatoxin.

Another practice in rural areas is to keep waste coconut scrapings for days to be used as food for poultry and farmstock. These having high moisture contents could act as media for aflatoxin production. Any toxin present in such coconut waste could appear as aflatoxin M₁ in the milk of goats etc. Consumption of this milk by human beings (especially children) provides an indirect pathway for aflatoxin entry into the human body.

It is imperative therefore that coconut products constitute an excellent nidus for the growth of harmful fungi, and adequate precautionary measures should be taken to prevent fungal growth prior to consumption.

**Detoxification**

If contaminated meal is to be used in food-stuffs the only solution lies in detoxification. Laboratory experiments have shown that the detoxification could be brought about by reagents like hydrogen peroxide,(32) Ozone,(33) ammonia,(34) a mixture of acetone—Hexane —water,(35) isopropanol(36), aqueous ethanol(37), formaldehyde and sodium hydroxide(38) in contaminated peanuts and cottonseed products. Ultraviolet irradiation using high pressure mercury lamps (100 watts, 140,000 candles per sq. cm.) for 1-2 hours(39) was found to reduce toxicity in solution but was less effective for solids. It is suggested that this method could be applied for animal feeds in the form of a thin layer on a moving belt in industrial scale. Detoxification was also observed with peanuts containing 10% moisture on autoclaving at 15 lbs/sq. in. for half an hour.(40)

All these methods are expensive on the industrial scale (especially when working with small amounts) and are also not always effective. Further, such treatment could affect the nutritive quality of food materials, and important constituents like proteins destroyed and denatured.

A comparative study of the effects of ammoniation and methyl amine treatment on cottonseed meals was made by Mann *et al.*(41) On feeding rats with the treated meal ammoniation was found to lower protein efficiency whereas treatment with methyl amine gave even higher ratios of protein efficiency than the untreated products.

Similar treatment may be possible with coconut products, but on an industrial scale could involve economic losses and a reduction in food value. Since they constitute one of the major sources of protein supply to both human beings and animals their contamination with aflatoxin should be minimised by controlling the growth of toxic fungi.

**References**

COCONUT CULTIVATION BOARD

Among the major plantation crops in Sri Lanka coconut occupies the largest acreage. The Coconut Cultivation Board, functioning under the Ministry of Plantation Industries, has undertaken the task of improving the coconut plantations with a view to increasing the production level and also to establish new projects such as intercropping, livestock farming and pasture on coconut lands in order to get an additional income from coconut lands. As an initial step in achieving the aforesaid objectives, the following schemes are presently in operation:—

The Coconut Fertilizer Subsidy Scheme Free Advisory and Extension Service, Subsidy Scheme for the establishment of Pasture/Fodder under Coconuts, and the Subsidy Scheme for issuing Coconut Seedlings at subsidised rates operated by the Coconut Research Board.

For necessary information on the above Schemes and of the free Advisory Service regarding coconut cultivation, please contact the Coconut Development Officer in your area or any one of the Regional Offices of the Coconut Cultivation Board situated at Kurunegala, Kuliapitiya, Chilaw, Ambalangoda, Veyanagoda and Negombo, or please write directly to the Head Office of the Coconut Cultivation Board at the undermentioned address.

General Manager, Coconut Cultivation Board.
P.O. Box 1388, 1st Floor, Y.M.B.A. Building, Fort, Colombo-1.