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MYCOTOXINS IN FOOD[1]

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What we eat actually depends on what we get and not what we want. Approximately 20% food becomes unsafe before reaching the consumer. Moulds are known to produce a wide variety of biologically active compounds on agricultural and other consumable commodities. Some of these compounds which are mostly secondary metabolites (Mycotoxins) are higly toxic to the vertebrates. Mycotoxins are elaborated on cereals, pulses, oil seeds, dry fruits, spices, green fruits, and vegetables, dried fish and shrimps, milk and milk products, different types of meat as well as wide variety of other consumable articles. Most of these toxic substances find their way to human system through different routes. These harmful chemicals are in our food chain but unfortunately their long range effects have not been properly evaluated.

About a dozen mycotoxins are known to contaminate different types of food materials. Aflatoxins, ochratoxins, zeralenone, citrinin, sterigmatocystin, trichothecenes, patulin, penicillic acid are some of the more potent ones.

TABLE I

NATURAL OCCURRENCE OF MYCOTOXINS IN FOOD

Mycotoxin	Commodity	Reference
Aflatoxin	Maize	Krishnamachari *et al.*, 1975a. Misra, 1977. Sinha, K. K., 1980. Daradhiyar, 1980. Bilgrami *et al.*, 1981c. Mall *et al.*, 1983.
	Paddy	Sreenivasamurthy, 1975. Nusrath & Ravi, 1983.
	Wheat	Misra, 1977. Vora, 1978. Prasad *et al.*, 1982. Agarwal *et al.*, 1983.
	Sorghum	Tripathi, 1973. Bhadraiah & Rama Rao, 1983.
	Mung, Urad	Sinha, R. K., 1980.

[1]Lecture delivered on the occassion of award of Panchanan Maheshwari Medal for 1983 by the Indian Botanical Society at Bhubaneswar on December 29, 1983.

TABLE I (*Contd.*)

Mycotoxin	Commodity	Reference
	Groundnut	Sreenivasamurthy *et al.*, 1965. Rao *et al.*, 1965. Subrahmanyam & Rao, 1974. Mehan & McDonald, 1983.
	Groundnut oil	Dwarkanath *et al.*, 1969.
	Cotton seed	Raghavendra Rao *et al.*, 1970.
	Coconut	Narasimhan , 1968. Anonymous, 1980. Singh, Anjana, 1983.
	Dry fruits	Singh, Anjana 1983.
	Spices	Singh, Anjana, 1983.
	Fruits	Verma *et al.*, 1980. Sinha & Singh, 1982b.
	Vegetables	Anonymous, 1980. Sinha & Singh, 1982.
	Milk	Yadgiri & Tulpule, 1974.
Zearalenone	Maize	Bilgrami *et al.*, 1980.
	Paddy	Nusrath & Ravi, 1983.
	Dry fruits & spices	Singh, Anjana, 1983.
Sterigmatocystin	Paddy	Anonymous, 1980.
	Wheat	—do—
	Cotton seed	—do—
Ochratoxin	Paddy	Nusrath & Ravi, 1983.
	Dry fruits & spices	Singh, Anjana, 1983.
	Groundnut	Rao *et al.*, 1979.
Trichothecenes	Sorghum	Rukmini & Bhat, 1978.
Penicillic acid	Fruits	Anonymous, 1980.
Citrinin	Groundnut	Subrahmanyam & Rao, 1974.
	Dry fruits & spices	Singh, Anjana, 1983.

Ergotoxins from *Claviceps purpurea* (Fr.) Tul. is known since the time of recorded history. gangarene, nausea, giddiness, cramps, nervous break-down and convulsions are some of the common manifestations of Ergotism. Ergot is now no longer feared as a food poison rather it has proved to be a starting material for production of several important drugs. However, in India consumption of ergoty Bajra in parts of Maharashtra, Gujarat and Rajasthan results in vomiting, giddi-

ness and diarrhoea. In extreme cases it may be fatal (Bhat *et al.*, 1975). This ergot which is caused by *Claviceps fusiformis* Love is quite different in its chemical nature from the ergot of rye or wheat.

Authentic reports about mould produced toxins in cattle (1924), Stachybotryotoxicoses in horses (1931), *Fusarium* toxicity in swine (1936), mouldy corn poisoning in horses (1940) started appearing in scientific literature since the early part of the present century. Maximum concern was, however, felt in the post second world war period when in Russia a disease broke in human beings which was named as Alimentary Toxic Aleukia (ATA). This disease was first recorded in eastern Siberia in 1913 but its deleterious effects were not fully realized at that time. Russians, eating mouldy (due to infestation of *Fusarium*/*Cladosporium*) overwintered and snow covered grains, suffered with severe dermal necorsis, leucopenia, hemorrhagic rashes and destruction of bone narrow. In acute cases nasal, gastric and intestinal hemorrhages may also occur and necrotic lesions are caused in the throat, on the lips and on skin of the nose, jaws and the eyes. Subsequently some intreasting observations were made and it was found that overwintered samples of cereals retained their toxicity even after seven years of mould infestation. The culture filtrates of *Fusarium* and *Cladosporium* grown at low temperature were more toxic than those maintained at room temperature. Highest toxicity was associated with materials collected at the time of maximum spore production (Joffe, 1972). In certain areas mortality was up to 60%.

Historically the year 1960 is very important because of the scientific concern generated about the mycotoxins since that time. It was in that year that about a lakh beautiful game birds-*Turkey* perished in England within a short span of time without leaving any trace about the cause of death. This mysterious disease greatly baffled the veterinary scientists who were unable to find out the cause or the cure of disease. Since it was difficult to give any scientific name to the disease, it was preferred to disignate it as "*Turkey-X-Disease*". It was, however, soon realized that the sudden doom of the Turkey poults was due to consumption of meal served to them. Systematic analysis of the meals was, therefore, initiated but it was found to be free of bacteria, viruses or any known toxic chemicals. The affected birds expressed loss of appetite, letharginess, weakening of the wings and drying of legs etc. It was found that the meal served to the Turkey birds was prepared of peanuts which was actually imported from Brazil by a ship named Rossette. The meal was thus nick-named as "Rossettee, meal".

A vigourus search by a team of British microbiologists lead by Sargeant resulted in identification of the causal organism and they came to the conclusion that the meal which had killed the birds was heavily infested by fungus *Aspergillus flavus* Link ex Fies (Sargeant *et al.*, 1961). This fungus is very well distributed in nature and its conidia are wide spread in air and soil. Unable to chemically characterize the nature of toxin, they named the toxic factor as AFLATOXIN (Sargeant *et al.*, 1963). These workers further reported that the toxic factors emitted blue and green fluorescence on TLC plates under UV-light. Further classification of aflatoxins was done on the basis of the colour of the fluoresence, their Rf values, chemical structure, toxic potency and

mode of action. Those recognized initially were named as B_1, B_2 G_1 and G_2. It was also established that aflatoxins were secondary aromatic and heterocyclic metabolites and toxicity was more pronounced in B_1 and G_1 series which was due to 2, 3-vinyl bond (Patterson, 1976). At present more than two dozen varieties of aflatoxins are known. All these findings have resulted in a rapid accumulation of literature on the subject.

Further investigations showed that the aflatoxins were sparingly soluble in water and these dissolved more readily in polar solvents like chloroform, methanol etc. Allcroft and Lewis in 1963 found their presence in milk of different types. These toxins, which are actually the hydroxylated derivatives of B_1 and B_2 were named as M_1 and M_2 or milk toxins. M_1 has also been reported from stored and freshly harvested corn (Shotwell *et al.*, 1976). A survey conducted by CFTRI, Mysore (Sreenivasamurthy, 1975) in the coastal districts of Karnataka has shown that heavy fungal infestations of foodgrains were associated with high incidence of liver enlargement in children. Spicules were observed in the RBC of such children. An important source of exposure to aflatoxins is through milk and milk products. Milk feeding starts from childhood and even human milk (Amla *et al.*, 1970, 1974) is not free of aflatoxins. It is suspected that Indian Childhood Cirrhosis (ICC) is due to breast feeding. Excretion of aflatoxin M_1 is reported in milk and urine of several farm and domestic animals (Bhat *et al.*, 1978). In milk these were developed due to consumption of mouldy fodder by the cattle. M_1 and M_2 were spotted in milk only when there was at least a threshhold level of 50 ppb of aflatoxin in the fodder. Foods fried in unrefined oil are a serious source of aflatoxin contamination (Dwarkanath *et al.*, 1969).

Usually cases of acute hepatitis are not etiologically confirmed even in well equipped hospitals and these are placed under a broad category of viral hepatitis. Cases of liver carcinoma in north Bihar are more frequent in post-monsoon period which is possibly because of consumption of mouldy maize.

Intensive histopathological studies of the affected birds and animals have shown that liver and kidney were the main targets of attack. Necrosis of hepatic cells, bile duct proliferation, dilation of veins were common in liver while kidney showed granular degeneration, dilation of tubules and proliferation of epithelial cells. Digestive and urinary tracts were also affected. Other parts to be affected were spleen, lymph nodes, bone marrow, nervous system, heart and lungs. Susceptibility of the animals to the aflatoxins is considerably enhanced by protein (Madhavan and Gopalan, 1965) and vitamin (Newberne *et al.*, 1968) difficiency.

At Bhagalpur, we started work on mycotoxins in 1975 when our attention was drawn towards high mortality of poultry brids in different parts of the state. We found heavy infestation of *A. flavus* in the feed that was served at Patna poultry farm and other places. The deaths were more severe in the post-monsoon period. Aflatoxins are a great threat to the poultry birds specially because maize and groundnut cake which are the two main constituents of the meal also happen to be very good substrates for proliferation of *A. flavus* and elaboration of aflatoxins. Association of toxigenic isolates of *A. flavus* with food commodities is quite common. Besides producing aflatoxins these fungi also

cause considerable damage to the nutritive quality of the infested substrates (Bilgrami *et al.*, 1979, 1981a, 1983 ; Singh and Sinha, 1982, 1983, Sinha and Singh, 1982a). Maize samples collected by us from different parts of Bihar yielded various forms of aflatoxins (Sinha, K. K., 1983). Reports from U. S. A. (Hasseltine, 1974; Shotwell *et al.*, 1976, 1977, 1980) as well as from various parts of India (Krishnamachari *et al.*, 1975a; Misra, 1977; Mall *et al.*, 1983) establish that maize is one of the very good substrates for aflatoxin production and hazard in this crop may be much more severe than that in the peanuts.

We conducted histopathological experiments with albino rats and noted the formation of liver carcinoma in the injected animals, necrosis in the liver cells, dialation of central veins and formation of hyper-chromatic nuclei. Such results have also been reported from other parts of the world. We suspect that the lungs of poultry birds are also possibly affected. Experimental confirmation is still awaited. Toxic effects of aflatoxin were also recorded on Swiss mice. A general decrease in body weight and loss in reflexes and sparking of coat hairs were common manifestations. A small tumour like protuberance was observed on right Pelvic gridle after 30 weeks of feeding. Histopathological studies of this tumour showed it to be a secondary carcinoma. Significant chan ges in the liver of affected animals were also recorded. Some of the central veins were dialated and surrounded by a large number of polymorphs and mononuclear cells. Mononuclear infiltration was also observed in the kidney.

Environment plays the most decisive role in natural contamination of mycotoxins because it affects the substrates as well as the activity of the moulds. Out of the various environmental factors, the role of temperature and humidity is most significant. A minimum of 85% RH is needed for aflatoxin elaboration while in case of grains and seeds the most suitable internal moisture level is between 15-20% (minimum 9%; maximum-30% in peanut). On basis of surveys conducted in different parts of the country it can be concluded that moist heat of monsoon is most conducive to mycotoxin elaboration on stored agricultural commodities. Incidence of aflatoxins and fusarial toxins was higher in paddy samples collected from coastal districts of Godavari and Andhra Pradesh (Nusrath and Ravi, 1983). Mall *et al.*, (1983) recorded a close correlation betwenn kernel moisture and *A. flavus* population in maize. A moisture level below 12% seems to be the safe limit for preventing aflatoxin formation. Aflatoxins are known to be elaborated on agricultural commodities from 12-40°G. but 28 to 30°C is the optimum range for paddy (Detroy *et al.*, 1971), peanuts (Diener and Davis, 1969) and maize (Om Prakash and Siradhana , 1978). This is the main reason for its being a major problem in a tropical country like India. Besides temperature and moisture, the pH alterations are also important so far as the toxin elaborating capacity of *A. flavus* is concerned.

Formerly, mycotoxins were considered to be essentially a storage problem but now it is well established that the problem in some of the crops like maize is quite acute under field conditions also. It is, therefore, essential that surveillence should be initiated from fields. We have noted that the areas which have high rainfall and are prone to floods show heavy infestation of *A. flavus* on maize under field conditions. More than 30% isolates are toxigenic out of

which 95% produced more than 30 ppb (safe limit fixed by WHO) of aflatoxin. Approximately 10% are high producers. Reports published from different parts of U. S. A. during the last ten years also confirm that aflatoxin elaboration on standing maize crops is quite frequent. Besides climate, the biotic factors like rodents, birds and-insects play a significant role in dispersal of the inoculum. Maize cobs damaged due to biotic factors were more severely affected and had invariably higher level of aflatoxins (Bilgrami *et al.*, 1978, 1980; Sinha, K. K., 1980). We have also recorded that the samples collected during the monsoon season have high incidence of aflatoxins and those collected in winters have greater frequency of fusarial toxin-zearalenone. Stressed growing conditions of crop, dense population and reduced level of fertilizers usually help in higher elaboration of toxins under field. Sudden fluctuation of winter temperature accompanied by light showers is very helpful for zearalenone production. Since aflatoxins can tolerate very high temperature and are degraded only after 268°C, many of the finished and market products like corn flakes have also substantially high concentration of this mycotoxin. *Aspergillus flavus* survives for years in naturally infested corn, even when it is kept dry (Hesseltine and Rogers, 1982).

In addition to maize, we have also undertaken surveys of dry fruits and spices in Bihar. Our findings show that coconut, *Euryale ferox* (Makhana), raisins and almond are some of the good substrates for aflatoxin elaboration and 15-25% samples were mycotoxin positive. Some samples had more than one mycotoxin. In addition to aflatoxin, ochartoxin and citrinin were elaborated on raisins and zearalenone on almond. Practically all the spices including coriander, black cumin, black pepper, cumin, fennel and chilli showed fairly high natural contamination of mycotoxins. The spices were in general better substrates for aflatoxin contamination than the dry fruits (Singh, Anjana, 1983). Danger from the dry fruits is, however, greater because most of these are consumed in uncooked stage. Fresh emblic fruits support good growth of *A. flavus* and other toxin producing moulds and natural contamination of three mycotoxins i.e., aflatoxins, ochratoxin and zearalenone was recorded on it. Careless handling, injury or unseasonal rains at the time of harvesting or drying greatly enhance the incidence of mould infestation and mycotoxin development. We have noted that in different agricultural belts of Bihar the storage practices vary and this has a profouund effect on the incidence of mycotoxins in agricultural produce. By and large the cultivators are not aware of mycotoxin hazards and if the moisture content is high in food and fodder during storage the mycotoxin level will also be high. Proper storage of food and fodder deserves highest priority specially in the areas which have high humidity and are prone to floods (Bilgrami, 1983).

Normally aflatoxin production is accomplished by *A. flavus* group of fungi including *A. parasiticus* but some other species of *Aspergillus, Penicillium, Rhizopus* and *Mucor* etc. are also known to elaborate this mycotoxin. Most of the potent mycotoxins are, however, elaborated by the species of *Aspergillus, Penicillium* and *Fusarium*. Why are the toxin producing potentials confined to few fungal genera only needs to be probed in depth. Besides aflatoxins, the other important ones are sterigmatocystin, oc-

hratoxin, citrinin, penicillic acid, patulin, rubratoxin, yellow rice toxins, zearalenone and trichothecenes. Sterigmatocystin, is structurally related to aflatoxin and consists of a zanthone nucleus. Ochratoxins (Ochratoxin-A is most toxic) are a group of isocoumarin amides. Citrinin is a-quinone methide while citreoviridin contains an α-pyreone chromophore. Patulin and penicillic acid are unsaturated lactone derivatives. Trichothecenes are complex group of sesquiterpenoids and zearalenone is β-resorcyclic acid lactone having esterogenic properties. Ergot alkaloids are derivatives of lysergic acid. Out of the various mycotoxins, aflatoxins and sterigmatocystin are potent carcinogens. Penicillic acid and patulin also have similar properties but of lesser dimension.

Some of the mycotoxins which contaminate our food have been found to be mutagenic (Wiyllie and Morehouse, 1978). There is every possibility that continued consumption of mycotoxin rich food may damage or might have already damaged the hereditary material of the consumers. If so, we may be unwittingly harming the human race. This possibility unfortunately has not been scientifically evaluated. Mutagenic effects of aflatoxin B_1 have been examined and confirmed on *Chlaymdomonas*, *Neurospora*, some prokaryotes and in mammalian tissue cultures. Teratogenicity and carcinogenicity of these toxins are further suggestive of their mutagenic property.

The mutagenic effects of mycotoxins in mammalian or human systems should be evaluated by adopting standard guidelines. Monitoring of mycotoxin- consuming human population for assesing the mutational effect would have been ideal but it is neither possible nor safe. Sinha, S. P. (1983) has suggested the use of suitable mammalian systems and he has given a detailed out-line for the experimental protocol.

The toxigenic properties of the mycotoxins are quite close despite variation in their chemical nature. Considerable attention has, therefore, been paid in the recent past on biosynthesis of mycotoxins especially of aflatoxin B_1 which is the most potent caroinogen till date. Kojic acid phenyl alanine, tyrosine, tryptophan, methionine, leucine, an acetate, mevalonic, acid, C_{18} polyketide single and double units as well as C_{20} polyketide (single unit) are some of the suggested precursors. Sincere efforts have been made by several biochemists including Pachler *et al.* (1976), Cox *et al.*, (1977) as well as Wan and Hsieh (1980) in the recent past to explore the biosynthetic steps of aflatoxin B_1. The evidences suggest C_{20} polyketide to be the possible precursor which undergoes folding and condensation to form a series of compounds including averantin, averufin, versiconal acetate, versicolorin "A", sterigmatocystin finally leading to formation of aflatoxin B_1. We have examined the effects of various carbon and nitrogen compounds as well as vitamins and TCA cycle intermediates on biosynthesis of aflatoxin B_1. Different compounds have varying effects and by and large the growth and aflatoxin producing potentials do not necessarily show a direct correlation.

Peptone, alanine, ammonium molybdate and ammonium carbonate are good supporters of aflatoxin production. Role of nitrogen sources is important because of easy conversion of amino acids to pyruvate and subsequently to acetyl coenzyme A which is a precursor

of aflatoxins (Sahay, 1983). Among the carbohydrates glucose and fructose are good for the growth of toxigenic strains as well as for aflatoxin production (Prasad, 1983). Nitrogen and carbon sources provided in different combinations support higher and faster production of aflatoxins than any individual source.

The basic constituents of agricultural commodities are important so far as production of aflatoxin is concerned but the environment and time of harvesting play a more decisive role. It is reflected from the fact that under natural conditions maize and rice are good substrates while wheat, barley and millets are comparatively poorer. Maize is one of the richest substrates for aflatoxin elaboration and cobs even in standing crops get high degree of infestation (Bilgrami and Sinha, 1983). Gaur and Siradhana (1983) reported elaboration of aflatoxin B_1 even on comparatively resistant varieties of maize like Ganga-5 maize hybrid. Proper screening of this crop should, therefore, be undretaken as it may prove to be risky for human beings as well as for the cattle. We have also examined the varietal effect and found that there was no such variety of maize which is totally resistant to mycotoxin elaboration. There are, however, a number of varieties like EH-2420, Ganga-5, Ganga-2 and Him-123 which are comparatively less susceptible (Bilgrami *et al.*, 1982a). Aflatoxin elaboration varies on different varieties on Sorghum also (Bhadraiah and Rama Rao, 1983).

A very alarming facet of mycotoxin problem is their effect on human system and it is found that the human population has been affected from time to time due to aflatoxin infested food. Majority of these reports have come from less developed countries like India, Kenya, Swaziland and Mozambique.. Most of the evidences regarding liver cancer in human beings due to aflatoxin ingestion are circumstatntial. Sudden our-break of aflatoxicosis among the Bhil tribals of Western India in October, 1974 attracted the attention of Indian scientists bacause 106 of the affected victims died within few days. In most of the cases profuse gastrointestinal bleeding preceeded death. Enlargement of liver and spleen was noted and syndrome was similar to that of jaundice. Autopsy of liver showed proliferation of bile duct and multinucleate giant cells (Krishnamachari *et al.*, 1975 a, b, c). Aflatoxin source was maize which got drenched due to unseasonal rains creating congenial atmosphere for proliferation of moulds. The males are, by and large, more susceptible and incidence of liver cancer is higher in them as compared to the females. This variation in male and female sexes needs in depth studies because it is getting some support from the test animals also. Convincing genetical explanations are, however, wanting. Adverse responses in animals also vary with age, health, nutritional as well as hormonal status. Occasionally severe infant mortality has been reported from some parts of the country where symptoms similar to jaundice were recorded. Possiblility of adverse effects of aflatoxin M_1 can not be ruled out in such cases. Cases of liver carcinoma in North Bihar are more frequent in post-monsoon period (October to January) which is possibly due to consumption of mouldy maize. Unfortunately most of the physicians and veterinarians are not sufficiently familar with the disease syndrome and may falter to detect even the acute cases.

About 20% isolates of *A. flavus* and *A. parasiticus* are aflatoxin producers but it is not possible to distinguish the toxic and non-toxic strains on basis of their morphology. Some efforts were made in this direction by Rao *et al.* (1965), Tulpule (1969), Gupta *et al.* (1971), Mohan and Chohan (1973, 1974) but a definite solution is still wanting. Earlier some attempts have been made to segregate virulent and avirulent species/strains of some fungal genera on basis of their amino acid constituents. We have, there fore, tried to correlate the toxicity with the amino acid pattern of the mycelium. The fact that acetyl Co A or active acetate which is the chief precursor of aflatoxin and at the same time also an important intermediate for a number of primary metabolites like carbohydrates, proteins and fats was kept in view while undertaking these experiments. The results were quite encouraging (Sinha, R. K., 1983). We noted that serine, an aliphatic amino acid was present in the well known toxic strain of *A. parasiticus* i. e., NRRL-3240. Another aliphatic amino acid i. e., DL-alanine as well as sulphur containing amino acids like cystein and L-methionine were confined to most of the toxigenic isolates. Possibly these amino acids have a direct involvement in aflatoxin production because they require a comparatively shorter pathway for their conversion to acetyl Co A via pyruvate.

Three basic strategies i.e., prevention, inactivation and detoxification have been adopted to control the adverse effects of mycotoxins. While planning any strategy for control it is essential to ensure that control measures are cheap, convenient and nontoxic. Keeping these objectives in view we explored the possibility of exploiting the aqueous extracts of chlorophyll bearing plants, specially those which are shear weeds and with no apparent utility. Initially we screened 115 plants (Singh, 1981) out of which 14 were found to be quite effective. These were put to further test and finally 4 plants i.e., *Adiantum* sp., *Euphorbia hirta, Ricinus communis* and *Thuja orientalis* were found to be highly potent in minimizing aflatoxin elaboration of cereals. This inhibitory property can be attributed to the rich phenolic contents in these plants which mainly comprised tannic acid, pyrogallol, quinol, resorcinol, caffeic and salicylic acid (Singh, Premlata, 1983). Some mild phenolics like O-vanillin and ferulic acid also prevented aflatoxin elaboration by more than 70% on important cereals and oil-seeds (Bilgrami *et al.*, 1981b, 1982b). The phenolics are well known inhibitors of plant pathogens and their toxic metabolites. Therefore, further exploitation and efforts in this direction are expected to be quite rewarding in diluting and minimizing the adverse effects of mycotoxins on food products.

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