

MYCOTOXINS IN FOOD¹

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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 highly 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 <i>et al.</i> , 1975a. Misra, 1977. Sinha, K. K., 1980. Daradhiyar, 1980. Bilgrami <i>et al.</i> , 1981c. Mall <i>et al.</i> , 1983.
	Paddy	Sreenivasamurthy, 1975. Nusrath & Ravi, 1983.
	Wheat	Misra, 1977. Vora, 1978. Prasad <i>et al.</i> , 1982. Agarwal <i>et al.</i> , 1983.
	Sorghum	Tripathi, 1973. Bhadraiah & Rama Rao, 1983.
	Mung, Urad	Sinha, R. K., 1980.

¹Lecture delivered on the occasion 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
Mycotoxin	Groundnut	Sreenivasamurthy <i>et al.</i> , 1965. Rao <i>et al.</i> , 1965. Subrahmanyam & Rao, 1974. Mehan & McDonald, 1983.
	Groundnut oil	Dwarkanath <i>et al.</i> , 1969.
	Cotton seed	Raghavendra Rao <i>et al.</i> , 1970.
	Coconut	Narasimhan, 1968. Anonymous, 1980. Singh, Anjana, 1983.
	Dry fruits	Singh, Anjana, 1983.
	Spices	Singh, Anjana, 1983.
	Fruits	Verma <i>et al.</i> , 1980. Sinha & Singh, 1982b.
	Vegetables	Anonymous, 1980. Sinha & Singh, 1982.
	Milk	Yadgiri & Tulpule, 1974.
	Maize	Bilgrami <i>et al.</i> , 1980.
	Paddy	Nusrath & Ravi, 1983.
Zearalenone	Dry fruits & spices	Singh, Anjana, 1983.
	Paddy	Anonymous, 1980.
	Wheat	—do—
Sterigmatocystin	Cotton seed	—do—
	Paddy	Nusrath & Ravi, 1983.
	Dry fruits & spices	Singh, Anjana, 1983.
Ochratoxin	Groundnut	Rao <i>et al.</i> , 1979.
	Sorghum	Rukmini & Bhat, 1978.
	Fruits	Anonymous, 1980.
Trichothecenes	Groundnut	Subrahmanyam & Rao, 1974.
Penicillic acid	Dry fruits & spices	Singh, Anjana, 1983.
Citrinin		

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*/*Gladosporium*) overwintered and snow covered grains, suffered with severe dermal necrosis, leucopenia, hemorrhagic rashes and destruction of bone marrow. 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 interesting 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 *Gladosporium* 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 designate it as "Turkey-X-Disease". It was, however, soon realized that the sudden doom of the Turkey poult 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 vigorous 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 fluorescence, their Rf values, chemical structure, toxic potency and

mode of action. Those recognized initially were named as B₁, B₂, G₁ and G₂. It was also established that aflatoxins were secondary aromatic and heterocyclic metabolites and toxicity was more pronounced in B₁ and G₁ 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 B₁ and B₂ were named as M₁ and M₂ or milk toxins. M₁ 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 M₁ 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. M₁ and M₂ were spotted in milk only when there was at least a threshold 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) deficiency.

At Bhagalpur, we started work on mycotoxins in 1975 when our attention was drawn towards high mortality of poultry birds 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

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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 changes 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 between 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°C. 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 surveillance 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 profound 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 xanthone nucleus. Ochratoxins (Ochratoxin-A is most toxic) are a group of isocoumarin amides. Citrinin is a-quinone methide while citreoviridin contains an α -pyrene chromophore. Patulin and penicillic acid are unsaturated lactone derivatives. Trichothecenes are complex group of sesquiterpenoids and zearalenone is β -resorcylic acid lactone having estrogenic 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 (Wyllie 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 B₁ have been examined and confirmed on *Ghlaymdomonas*, *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 assessing 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 B₁ which is the most potent carcinogen till date. Kojic acid, phenyl alanine, tyrosine, tryptophan, methionine, leucine, an acetate, mevalonic acid, C₁₈ polyketide single and double units as well as C₂₀ 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 B₁. The evidences suggest C₂₀ 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 B₁. We have examined the effects of various carbon and nitrogen compounds as well as vitamins and TCA cycle intermediates on biosynthesis of aflatoxin B₁. 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 B₁ even on comparatively resistant varieties of maize like Ganga-5 maize hybrid. Proper screening of this crop should, therefore, be undertaken 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 circumstantial. Sudden out-break of aflatoxicosis among the Bhil tribals of Western India in October, 1974 attracted the attention of Indian scientists because 106 of the affected victims died within few days. In most of the cases profuse gastrointestinal bleeding preceded 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. Possibility of adverse effects of aflatoxin M₁ 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 familiar 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, therefore, 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 cysteine 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, Premata, 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.

REFERENCES

- AGARWAL, G. P., P. S. BISEN AND S. AWASTHY 1983. Aflatoxin producing potentials of *Aspergillus flavus* isolates associated with stored wheat grains. In: *Proc. Symp. Mycotoxin in Food and Feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 73-78.
- ALLOROFF, R. AND G. LEWIS 1963. Groundnut toxicity in cattle: Experimental poisoning of calves and a report on clinical effects in older cattle. *Vet. Record*, **75**: 487-493.
- AMLA, I., S. KUMARI, V. SREENIVARAMURTHY, P. JAYARAJ AND H. A. B. PARPIA 1970. Role of aflatoxin in Indian childhood cirrhosis. *Indian Pediatrics*, **7**: 262-268.
- AMLA, I., V. SREENIVARAMURTHY, A. P. JAYARAJ AND H. A. B. PARPIA 1974. Aflatoxin in Indian childhood cirrhosis: a review. *J. Trop. Pediatr. Environ. Child Health*, **20**: 28-33.

- ANONYMOUS 1983). *Survey for the presence of mycotoxins with special reference to patulin, sterigmatocystin, aflatoxin and penicillic acid in foods, food products animal feeds and concentrates available in the Tamil Nadu region of India. Final Technical Report (D.S.T.). Deptt. of Food Technology, Tamil Nadu Agric. Univ. Coimbatore.*
- BHADRAIAH, B. AND RANA RAO 1983. Aflatoxin production in preharvest, freshly harvested and stored sorghums. In : *Proc. Symp. Mycotoxins in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, P. 49-54.
- BHAT, R. V., D. N. ROY AND P. G. TULPUL 1975. Ergot contamination of bijra. *Proc. Nutr. Soc. India*, **19** : 7-11.
- BHAT, R. V., V. NAGARAJAN AND P. G. TULPUL 1978. *Health hazards of mycotoxin in India*. Indian Council of Medical Research, New Delhi, p. 58.
- BILGRAMI, K. S. 1983. Mycotoxin problems in food and feed some social obligations and strategy for future. In : *Proc. Symp. Mycotoxins in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 1-13.
- BILGRAMI, K. S. AND K. K. SINHA 1983. Aflatoxin contamination in agricultural commodities with special reference to maize and groundnut. In : *Recent Advance in Plant Pathology*. Eds. Akhtar Husain, B. P. Singh, Kishan Singh and V. P. Agnihotri. Print House, Lucknow, p. 253-263.
- BILGRAMI, K. S., R. S. MISRA, T. PRASAD AND K. K. SINHA 1978. Mycotoxin problem in standing maize crop in Bihar. *Nat. Acad. Sci. Letters*, **1** : 124.
- BILGRAMI, K. S., R. S., MISRA AND K. K. SINHA 1979. Aflatoxin production and loss in calorific value of maize seeds due to *Aspergillus parasiticus*. *Curr. Sci*, **48** : 642-643.
- BILGRAMI, K. S., T. PRASAD, R. S. MISRA AND K. K. SINHA 1980. *Survey and study of mycotoxin producing fungi associated with grains in the standing maize crop*. Final Technical Report. I. C. A. R. Project. Bhagalpur University.
- BILGRAMI, K. S., PRASAD, R. S. MISRA AND K. K. SINHA 1981 a. Changes in protein and amino acid contents of maize seeds infested with aflatoxin producing strain of *Aspergillus parasiticus*. *Seed Research*, **9** : 52-56.
- BILGRAMI, K. S., K. K. SINHA AND PREMALATA SINGH 1981 b. Inhibition of aflatoxin production by ferulic acid on some cereals and oil seeds. *Curr. Sci.*, **50** : 997-998.
- BILGRAMI, K. S., T. PRASAD, R. S. MISRA AND K. K. SINHA 1981 c. Aflatoxin contamination in maize under field conditions. *Indian Phytopath.*, **34** : 67-68.
- BILGRAMI, K. S., R. S. MISRA, T. PRASAD AND K. K. SINHA 1982 a). Screening of different varieties of maize for aflatoxin production by *Aspergillus parasiticus*. *Indian Phytopath.*, **35** : 376-378.
- BILGRAMI, K. S., K. K. SINHA AND PREMALATA SINGH 1982 b. Prevention of aflatoxin production on some cereals and oil seeds by O-vanillin. *Curr. Sci.*, **51** : 138.
- BILGRAMI, K. S., K. K. SINHA AND ANJANA SINGH 1983. Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link ex Fries. *Curr. Sci.*, **52** : 960-964.
- COX, R. H., F. CHURCHILL, R. J. COLE AND J. W. DORNER 1977. Carbon 13 n.m.r. studies of the structure and biosynthesis of versiconal acetate. *J. Amer. Chem. Soc.*, **99** : 3159-3166.
- DARADHIYAR, S. K. 1980. *Studies on aflatoxins associated with storage and standing crop of maize, poultry-feed and cattle cakes in the tribal belt of Santhal Pargana District*. Ph. D. Thesis, Bhagalpur University.
- DETROY, R. W., E. B. LILLEHOJ AND A. CIEGLER 1971. Aflatoxin and related compounds. In : *Microbial Toxins Vols. VI* Eds. A. Ciegler, S. Kadis and S. J. AJL. Academic Press, New York and London, pp. 3-178.
- DIENER, U. L. AND N. D. DAVIS 1959. Aflatoxin formation by *Aspergillus flavus*. In "Aflatoxin". Ed. L. A. Goldblatt. Academic Press, New York. pp. 13-54.
- DWARKANATH, C. T., V. SREENIVASAMURTHY AND H. A. B. PARIPIA 1969. Aflatoxin in Indian peanut oil. *J. Food Sci. Technol.*, **6** : 107-109.
- GAUR, V. K. AND B. S. SIRADHANA 1983. Aflatoxin B₁ formation by *Aspergillus flavus* Link ex Fries on leaves and stalks of Ganga-5 maize hybrid. In : *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 45-47.
- GUPTA, S. R., L. VISWANATHAN AND T. A. VENKATASUBRAMANIAN 1971. A comparative study of toxigenic and non-toxigenic strains of *Aspergillus flavus*. *J. Gen. Microbiol.*, **45** : 243-247.
- HASSETTINE, C. W. 1974. Natural occurrence of mycotoxins in cereals. *Mycopath. Mycol. Appl.*, **53** : 141-153.
- HESSETTINE, C. W. AND R. F. ROGERS (1982). Longevity of *Aspergillus flavus* in corn. *Mycologia*, **74** : 423-426.
- KRISHNAMACHARI, K. A. V. R., R. V. BHAT, V.

- NAGARAJAN AND T. B. G. TILAK 1975a. Hepatitis due to aflatoxicoses-an outbreak in Western India. *Lancet* **1** : 1061-1063.
- KRISHNAMACHARI, K. A. V. R., R. V. BHAT, V. NAGARAJAN AND T. B. G. TILAK 1975b. Investigations into an out-break of hepatitis in parts of Western India. *Indian J. Med. Res.*, **63** : 1036-1048.
- KRISHNAMACHARI, K. A. V. R., R. V. BHAT, V. NAGARAJAN AND T. B. G. TILAK 1975c. Aflatoxicosis in Humans. *Proc. Nutr. Soc. India*, **19** : 18-22.
- MADHAVAN, T. V. AND C. GOPALAN 1965. Effect of dietary protein on aflatoxin liver injury in weanling rats. *Arch. Pathol.*, **80** : 123-126.
- MAJJI, O. P., K. K. RANAWAT AND S. K. CHAUHAN 1983. Mould flora and aflatoxin contamination in maize kernels. In *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 37-44.
- MEHAN, V. K. AND J. S. CHOIHAN 1973. Aflatoxin B₁ producing potential of isolates of *Aspergillus flavus* Link ex Fries from cotton, maize and wheat. *Mycopath. Mycol. Appl.*, **49** : 263-274.
- MEHAN, V. K. AND J. S. CHOIHAN 1974. Virulence of aflatoxigenic and non-to-xigenic isolates of *Aspergillus flavus* Link ex Fr. on groundnut seedlings. *Phytopath. Medit.*, **13** : 108-109.
- MEHAN, V. K. AND D. McDONALD 1983. Mycotoxin contamination in groundnut-prevention and control. In *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 237-250.
- MISRA, R. S. 1977. *Aflatoxins contamination of some agricultural commodities in Tarai and biochemical effects of aflatoxin B₁ on maize seeds*. Ph. D. Thesis, G. B. Pant Univ. of Agric. & Technol., Pantnagar.
- NARASIMHAN, M. J. 1968. On the presence of aflatoxins in Copra. *Hindustan Antibiot. Bull.*, **2** : 104-105.
- NEWBERNE, P. M., A. E. ROGERS AND G. N. WOGAN 1968. Hepatorenal lesions in rats fed a low lipotrope diet and exposed to aflatoxin. *J. Nutrition*, **104** : 331-342.
- NUSRATH, M. AND V. RAVI 1983. Incidence of mycotoxins in paddy from Andhra Pradesh. In *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 15-22.
- OMPRAKASHI AND B. S. SIRADIHANA 1978. Factors affecting formation of aflatoxin B₁ by *Aspergillus flavus* on Ganga-5 maize hybrid. *Curr. Sci.*, **47** : 695-696.
- PACHLER, K. G. R., P. S. STEYN, R. VLEGGAAR, P. L. WESSELS AND D. B. SCOTT 1976. Carbon 13₀ nuclear magnetic resonance assignments and biosynthesis of aflatoxin B₁ and sterigmatocystin. *J. Chem. Soc., Perkin Trans.*, **1** : 1182-1189.
- PATTERSON, D. S. P. 1976. Structure, metabolism and toxicity of the aflatoxins : a review. *Cah. Nutr. Dietet.*, **2** (Suppl.) : 71-76.
- PRASAD, R. B. 1983. Influence of carbon sources on growth and aflatoxin production by *Aspergillus parasiticus* (NRRL-3240). In : *Proc. Symp. Mycotoxin in food and feed*, Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 207-215.
- PRASAD, T., M. K. TILAKUR, R. B. PRASAD AND M. SAHAY 1982. Aflatoxin contamination in wheat. *Biol. Bull. India*, **4** : 107-108.
- RAGHAVENDRA RAO, S., A. S. INDULKAR AND H. S. VEDANAYAGAM 1970. *Mycotoxins of Aspergillus flavus and production of aflatoxin in cotton seed*. Final Tech. Report (Pl-480 project). Regional Research Laboratory, Hyderabad.
- RAO, E. R., S. C. BASAPPA AND V. SREENIVASAMURTHY 1979. Studies on the occurrence of ochratoxins in food grains. *J. Food Sci. Technol.*, **16** : 113-114.
- RAO, K. S., T. V. MADHAVAN AND P. G. TULPULE 1965. Incidence of toxigenic strains of *Aspergillus flavus* affecting groundnut crop in certain coastal districts of India. *Indian J. Med. Res.*, **53** : 1196-1202.
- RUKMINI, C. AND R. V. BHAT 1978. Occurrence of T-2 toxin in *Fusarium* infested sorghum from India. *J. Agric. Food Chem.*, **26** : 647-649.
- SAHAY, MANJU 1983. Effect of nitrogen sources on growth and aflatoxin production by *Aspergillus parasiticus* (NRRL-3240). In : *Proc. Symp. Mycotoxin in food and feed* Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 109-206.
- SARGEANT, K., A. SHERIDAN, J. O'KELLY AND R. B. A. CARNAGHAN 1961. Toxicity associated with certain samples of groundnuts. *Nature* **192** : 1096-1097.
- SARGEANT, K., R. B. A. CARNAGHAN AND R. ALLCROFT 1963. Toxic properties in groundnuts: Chemistry and origin. *Chem. Ind.*, **11** : 53-55.
- SHOTWELL, O. L., M. L. GOULDEN AND C. W. HESSELTINE 1976. Aflatoxin M₁ occurrence in stored and freshly harvested corn. *Agric. Food Chem.*, **24** : 683.
- SHOTWELL, O. L., M. L. GOULDEN, E. B. LILLEHOJ, W. F. KWOLEK AND C. W. HESSELTINE 1977. Aflatoxin occurrence in 1973 corn at harvest.

- III. Aflatoxin distribution in contaminated, insect damaged corn. *Cereal Chem.*, **54** : 620-626.
- SNOTWELL, O. L., M. L. GOULDEN, C. W. HESSELTINE, J. W. DICKENS AND W. F. KWOLEK 1980. Aflatoxin : Distribution in contaminated corn plants. *Cereal Chem.*, **57** : 206-208.
- SINGH, ANJANA 1983. Mycotoxin contamination in dry fruits and spices. In : *c. Proc. Symp. Mycotoxin in food and feed* Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur. p. 55-68.
- SINGH, ANJANA AND K. K. SINHA 1982. Biochemical changes in musambi fruits inoculated with species of aflatoxin producing *Aspergilli*. *Curr. Sci.*, **51** : 841-842.
- SINGH, ANJANA AND K. K. SINHA 1983. Biochemical changes and aflatoxin production in guava fruits by *Aspergillus flavus* and *A. parasiticus*. *Indian Phytopath.*, **36** : 365-366.
- SINGH, PREMLATA 1981. *Studies on the effect of some plant extracts on aflatoxin production*. Ph. D. Thesis, Bhagalpur University.
- SINGH, PREMLATA 1983. Control of aflatoxin through natural plant products. In : *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 307-315.
- SINHA, K. K. 1980. *Survey and study of aflatoxin producing isolates of Aspergillus flavus associated with maize grains in storage and standing crop*. Ph. D. Thesis, Bhagalpur University.
- SINHA, K. K. 1983. Aflatoxin problem in storage and standing maize crops. In : *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur, p. 23-36.
- SINHA, K. K. AND ANJANA SINGH 1982a. Biochemical changes in orange fruits during infestation with *Aspergillus flavus* and *Aspergillus parasiticus*. *Nat. Acad. Sci. Letters*, **5** : 143-144.
- SINHA, K. K. AND ANJANA SINGH 1982b. Aflatoxin producing potentials of *Aspergillus flavus* and natural occurrence of aflatoxins in some fruits and vegetables. *Nat. Acad. Sci. Letters*, **5** : 213-214.
- SINHA, R. K. 1983. Differentiation of aflatoxin producing isolates of *Aspergillus parasiticus* on basis of amino acid pattern of mycelium. In : *Proc. Symp. Mycotoxin in food and feed*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur. p. 217-225.
- SINHA, S. P. 1983. Mycotoxins as mutagen—A need for confirmation using mammalian test-system. In *Proc. Symp. Mycotoxin in food and feeds*. Eds. K. S. Bilgrami, T. Prasad and K. K. Sinha. Allied Press, Bhagalpur. p. 123-128.
- SREENIVASAMURTHY, V. 1975. Mycotoxins in foods. *Proc. Nutr. Soc. India*. No. 19 : 1-6.
- SREENIVASAMURTHY, V., A. JAYARAMAN AND H. A. B. PARPIA 1965. Aflatoxin in Indian peanuts: analysis and extraction. In : *Mycotoxins in Feed-stuffs*. Ed. G. N. Wogan. M. I. T. Press, Cambridge. p. 251-260.
- SUBRAHMANYAM, P. AND A. S. RAO 1974. Occurrence of aflatoxin and citrinin in groundnut (*Arachis hypogaea* L.) at harvest in relation to pod condition and kernel moisture content. *Curr. Sci.*, **43** : 707-710.
- TRIPATHI, R. K. 1973. Aflatoxins in sorghum grains infected with head moulds. *Indian J. Exp. Biol.*, **11** : 361-362.
- TULPULE, P. G. 1969. "Aflatoxicosis". *Indian J. Med. Res.*, **57** : 102-114.
- VERMA, R. A. B., J. S. PRASAD AND S. K. VERMA 1980. Aflatoxin production in fruits under market conditions. *J. Indian bot. Soc.*, **59** (Suppl.) : 28.
- VORA, V. C. 1978. *A survey of toxin producing fungi and mycotoxins associated with post harvest deterioration of field crops grown for human or animal consumption*. Final Report (PI-480 scheme). G. D. R. I., Lucknow.
- WAN, N. C. AND D. P. H. HSIEH 1980. Enzymatic formation of the bisfuran structure in aflatoxin biosynthesis. *Appl. Environ. Microbiol.*, **39** : 109-112.
- WYLLIE, T. D. AND L. G. MOREHOUSE (Eds.) 1978. *Mycotoxic fungi, mycotoxins, mycotoxicoses*. Vol. 3. Marcel Dekker, Inc. New York.
- YADGIRI, B. AND P. G. TULPULE 1974. Aflatoxin in buffalo milk. *Indian J. Dairy Sci.* **27** : 293-297.