http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5 (1): 69-74, 2002

Aflatoxins Production by *Aspergillus flavus*, Isolated from Different Foodstuffs Commonly Used in Jeddah Region, Saudi Arabia

F.M. Bokhari
Department of Biology, College of Science, King Abdul Aziz University,
P.O. Box-12161, Jeddah-21473, Saudi Arabia

Abstract: *Aspergillus flavus* isolated from 51 foodstuff samples was screened for aflatoxin production by TLC. It was found that 26.1% of the total isolates were toxic strains and the main toxin was found to be aflatoxin B_1. Of 46 foodstuffs commodities surveyed for natural contamination with aflatoxin by HPLC method, 26.1% of the samples were found to contain the aflatoxin and the most contaminated samples were poultry feed, cereal grains and oil seeds. The presence of four aflatoxins B_1, B_2, G_1 and G_2 was detected only in two of the tested samples. Bioassay of a toxigenic *A. flavus* strain (AFB_2) had been done on four different types of three seeds and grains, using four different soaking intervals 3, 6, 12 and 24 hours on culture filtrate of the fungus. Germinability of cow pea and chick pea had vigorously reduced when soaked on culture filtrate of the toxic isolate of *A. flavus*. Broad bean and maize were the most affected since the germination percentage were 0% and 15%, respectively. The toxigenic isolate (AFB_4) was used to detect their toxicity on 14 fungal and bacterial species. The toxigenic isolates inhibited the growth of two fungal species (*A. flavus* and *Penicillium digitatum*) and two bacterial species (*Klebsiella oxytoca* and *Staphylococcus aureus*).

Key words: *Aspergillus flavus*, aflatoxins, TLC, HPLC, bioassay, foodstuff .

Introduction

Aflatoxins have carcinogenic properties , so may be involved in the etiology of human cancer. On broad basis early studies on feeds contaminated with *Aspergillus flavus* established typical toxicity syndrome involving liver damage in many mammals, fish and birds (Wogan, 1965 and Lancaster, 1968). Aflatoxins initiation in tumors has been described in ducklings, rats, ferrets, trouts, guinea pigs, mice and sheep (De Scott, 1965 and Hsieh and Wong, 1994). Also, aflatoxin has been implicated in the induction of neoplasms in the glandular stomach, kidney, lung, salivary glands, lachrymal gland, colon and skin as reported by Butler and Brans (1966). In addition, aflatoxins proved to be immunosuppressive. Significant levels of aflatoxins and aflatoxicol in the blood and other body fluids of children were found in the tropics (Hendrickse, 1984; Coulter *et al.*, 1986). Very young children can be exposed to aflatoxins M1 through their mother milk consuming the toxin in their diets (Coulter *et al.*, 1984, Saad *et al.*, 1989 and Al-Julaifi and Al-Juwaied, 1999).

A. flavus is a common mold in tropical and subtropical countries and was found to cause aflatoxin contamination as a result of molding of badly stored commodities, such as groundnuts, cereal seeds, cotton and a wide range of nut trees such as cashew, almonds, pistachio, pecans, walnut, hazelnut, Brazil nuts and sunflower seed (Pohland and Wood, 1987; Bokhary, 1993; Lata, 1991; Hooda and Singh, 1992). Despite the extensive studies that have been done in many countries concerning natural contamination of different commodities such as cereal seeds, nut seeds, milk and dairy products by *A. flavus* and aflatoxins B_1, B_2, G_1 and G_2, little work has been done in Saudi Arabia (Bokhary, 1986; 1993; Gawad and Zohri, 1993; Ewaidah, 1998). So, this work was conducted for screening the association of *A. flavus* and its aflatoxins with some marketing cereals (maize, wheat and rice), nut seeds (almond, cashew, hazelnut, pistachio, walnut, groundnut and sesame), some herbal seeds and spices (cloves, cinnamon, thyme, red and black pepper, coriander, cardemon) and milk and dairy products.

Materials and Methods

Source of collected samples: All tested seeds , nut seeds , spices, milk and milk products were collected from different markets in Jeddah during 1997-1998. The samples were either immediately plated for fungus detection or stored at 3-5 °C until mycofloral determination.

Thin layer chromatography for detection of aflatoxins:

Preparation of standard toxin solutions: Standard solutions of aflatoxins were prepared by dissolving pure crystalline aflatoxins B_1, B_2, G_1 and G_2 (Sigma) in ethanol (BDH, Spectrosol grade) at a rate of 1 mg ml^{-1}. Further dilutions were prepared using the same grade of ethanol and all stored at -20°C until use.

Preparation and development of TLC plates: For examination of extracts; aluminum-packed Kieselgel 60 (Merck, types 5554) was used as supplied for 20X20 cm^2 plates according to AOAC (1980). Plates were spotted along 1.5cm from the bottom with 10 μl aliquots of extract and standards. The plates were developed in one or more of solvent systems & (will mention latter) in equilibrated, unlined shandon TLC chromotanks at room temperature until the solvent front had reached a line marked 2 cm from the top of the plate. After development, plates were removed and air-dried in a fume-cabinet and then examined in a chlomatovue (Model cx-50) under visible light.

Solvent system for chromatogram development: The following solvent systems were used for development of TLC plates to achieve chromatographic separation of aflatoxins:
TEF- toluene: ethyl acetate: 90% formic acid (5: 4: 1, v/v/v).
TEC- toluene: ethyl acetate: chloroform (2: 1: 1).
TE- toluene: ethyl acetate (1: 3).
The distance traveled by the mycotoxin relative to that traveled by the solvent (RF value) was determined for particular pure mycotoxins and solvent system.

Detection and identification of aflatoxins on TLC plates: Developed chromatograms were examined under UV light, since aflatoxins B_1, B_2 visualized blue fluorescence and G_1, G_2 green fluorescence, without treatment.

Analysis of samples for aflatoxin contamination:

Extraction of aflatoxins: Aflatoxins B_1, B_2, G_1 and G_2 were extracted from each sample by blending 50 gm of substrate in 250 ml ethanol: water (55: 45 v/v) in a warming blender for 2 min and following the procedures described by best food (BF) and CB methods described in AOAC (1980).

Quantification of aflatoxins by HPLC: Aflatoxin content of extract

was determined by HPLC after the methods of Slubblefied and Shotwell (1977). Before quantification of aflatoxins B_1, B_2, G_1 and G_2 by HPLC, the extract was pipetted onto a sep-pak C_{18} cartridge for purification and the eluent was used for analysis. The chromatograph used was a Waters Delivery System (model 600) equipped with a Nova Park C_{18} column (3.4 x 150 mm^2), a scanning fluorescence detector (Ex. 360 nm, Em. 450 nm) linked to a millennium software programme for calculations. The solvent system was water: methanol: acetonitrite 3: 2: 1, which was degassed before use by a megason ultrasonic cleaner and the flow rate of the solvent was 0.75 ml min^{-1}. The samples (20 μl) were infected into the instrument using 25 μl glass syringe fitted with a waters infection needle. The presence of aflatoxins B_1, B_2, G_1 and G_2 was indicated by a peak with the same retention time as the standard.

Biological assay of aflatoxins:

Effect of toxigenic *A. flavus* on germination and growth of seedlings: Filtrates of *A. flavus* isolated from wheat seeds grown on PDA medium was used to investigate its effect on germinability of cowpea, corn, chickpea and broad bean. Seeds were treated with 1% mercuric chloride for 2 min, rinsed three times in sterile water then soaked in *A. flavus* filtrates for 3, 6, 12 and 24 hours. The liquid PD medium was also used for soaking the seeds for 24 hrs and represented the control treatment. Thereafter, all treated seeds were placed on water wetted cotton plates for germination. Twenty seeds were placed in each plate and three replicates were used for each treatment. Another treatment was used where the cotton was soaked with fungus filtrate in each plate used in germination. Percentage of germination was recorded after one week of planting.

Toxicity of *A. flavus* isolate on microorganisms: Partially purified filtrate of *A. flavus* toxigenic isolate which was found to contain B_1 and G_1 toxins was indexed for its toxicity to 14 pathogenic and non-pathogenic fungal and bacterial species; *Alternaria alternata*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium digitatum*, *Rhizoctonia solani*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter* sp., *Klebsiella oxyloca*, *Pseudomonas aerogenosa*, *Serratia morchii*, *Staphyllococcus aureus*, *Candida albicans* and *Saccharomyces cerevisiae*. Standard solution of B_1, G_1 toxins were also used to compare toxicity with the studied *A. flavus* isolate. The medium used for tested fungi was Czapek´s while it was nutrient agar medium for tested bacterial species. Sterilized discs of filter paper (Whatman No. 1) of 0.5 mm diameter were soaked in toxin solution and three discs were placed in each seeded Petri-dish. Thereafter, the fungal plates were incubated at 27°C while bacterial plates were incubated at 37°C. The plates were kept in incubation for 5 days, then inhibition zones were recorded.

Results

Screening of *Aspergillus flavus* Toxicity: *Aspergillus flavus* was isolated from the majority of fifty-one samples (seeds, oil seeds, spices and milk and dairy products). The total isolates were amounted to 468 isolates as shown in Table 1. Results indicated that the lowest number of *A. flavus* isolates and also toxigenic isolates was recorded in spice samples. On the other hand, the highest number of *A. flavus* isolates was obtained from oil seeds amounting to 169 isolates while the toxigenic isolates were 29 only with 17.8% which was the lowest percentage.

Seventy-six *A. flavus* isolates were recovered from the tested seeds while toxigenic isolates were 47 with 61.8% being the highest percentage for toxigenic isolates. From milk and milk products *A. flavus* isolates were 155, while the number of toxigenic isolates was 29 matching 18.7% which was quite a low percentage. It was found (Table 2) that only B_1 and G_1 toxins were detected during screening by TLC test which represent 26.1 percent of 122 toxic isolates. No species of *A. parasiticus* strain was detected in all samples.

Naturally aflatoxins contamination in human and animal feeds: A total of 46 samples of various agricultural commodities were analyzed for four aflatoxins B_1, B_2, G_1 and G_2 detection. (Tables 2 and 3). The incidence and levels of aflatoxins given in Fig. 1, 2 and 3 show positive results for Sesame as well as in many other samples. Aflatoxin B_1 was detected most frequently in bean and nuts, followed by oil seed, poultry feed, and cereal grain (Table 3). The highest concentrations were found in poultry feed, sesame seed and cereal grain, followed by nuts and beans. Survey assayed only twelve samples (24%) were found contaminated with aflatoxins at a concentration range between 1.99-55.7 ppb. The highest total aflatoxin concentration was detected in bean sample. Aflatoxins B_1, B_2, G_1 and G_2 were found in most of the samples except the spices where no aflatoxins were detected (Table 2). In the survey of 46 samples analyzed, 34 gave aflatoxin negative results. The concentration of aflatoxin detected in the positive samples are presented in Tables 2 and 3 and Figs. 1, 2 and 3.

Bioassay studies of a toxigenic strain of *A. flavus:*

Effect on seeds germination: Broad bean, chick pea, cowpea seeds and maize grains were soaked for consecutive intervals ranging from 3 to 24 hours in *A. flavus* filtrates. Also, these seeds and grains were germinated on cotton pads, soaked with the seeds for 24 hrs in liquid medium. Data presented in Table 4 and Fig. 4,5 indicate that the culture filtrate of the toxic isolate of *A. flavus* had clearly affected the tested seeds and vigorously reduced their germinability as compared with the control which amounted to 100% in all tested seeds (Table 4). Seeds germination was decreased by soaking the seeds for consecutive intervals; 3, 6 and 12 hours while seed soaking for 24 hours, or using soaked cotton pad the germination percentage was 0% in all tested seed varieties. It was also observed that there was no germination after 3 hrs soaking in case of broad bean seeds and that is correct in all the treatments.

Maize grain germination was very low in 3 and 6 hrs treatment whereas they lost germinability in 12-24 hrs soaking and also on soaked cotton pads. Chick pea seeds germinated highly up to 12 hrs soaking as the percentage of germination was 50%. Cow pea seeds germinated only in 3 hrs and 6 hrs

Table 1: The number and percentage of toxigenic *A. flavus* isolates recovered from all tested foodstuffs.

Samples	Total no. of *A. flavus* isolates	No. of *A. flavus* toxic isolates	% of toxic isolates
Seeds	76	47	61.8
Nuts and oil seeds	169	29	17.8
Spices	68	17	27.4
Milk & milk products	155	29	18.7
Total	468	122	26.1

Table 2: Levels of natural occurrence of aflatoxins B_1, B_2, G_1 and G_2 in some food and feed stuffs in Saudi Arabia.

Food and feed materials	No. of estimated samples	No. of contaminated samples	Aflatoxin identification				Total concentration of AF μg
			B_1	B_2	G_1	G_2	
Oil seeds							
Sesames	5	1	+	–	+	+	1.49
Cereal grains							
Wheat (1)	5	1	+	+	+	+	2.98
Wheat (2)	6	1	+	+	+	–	0.39
Corn	8	1	+	+	+	–	0.64
Beans							
Broad bean	1	1	+	+	–	+	0.077
Lentil	1	1	+	+	+	–	0.52
Nuts							
Caju	4	1	–	–	+	–	0.29
Pistachio	5	3	+	+	–	–	0.28
Hazel nut	2	1	+	+	+	–	0.25
Spices							
Cinnamon	2	0	–	–	–	–	0.0
Black pepper	2	0	–	–	–	–	0.0
Poultry feed	5	1	+	+	+	+	11.14
Total No.	46	12					

– Not detected + = Detected

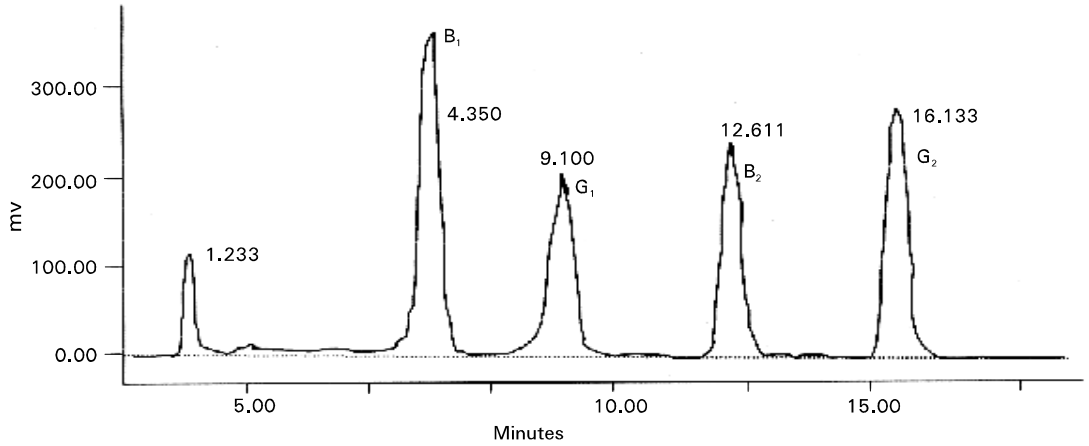

Fig. 1: High performance liquid chromatographic separation of aflatoxin standards B_1 (PK1), G_1 (PK2), B_2 (PK3), standard mixture of 5 μL injected for each aflatoxin.

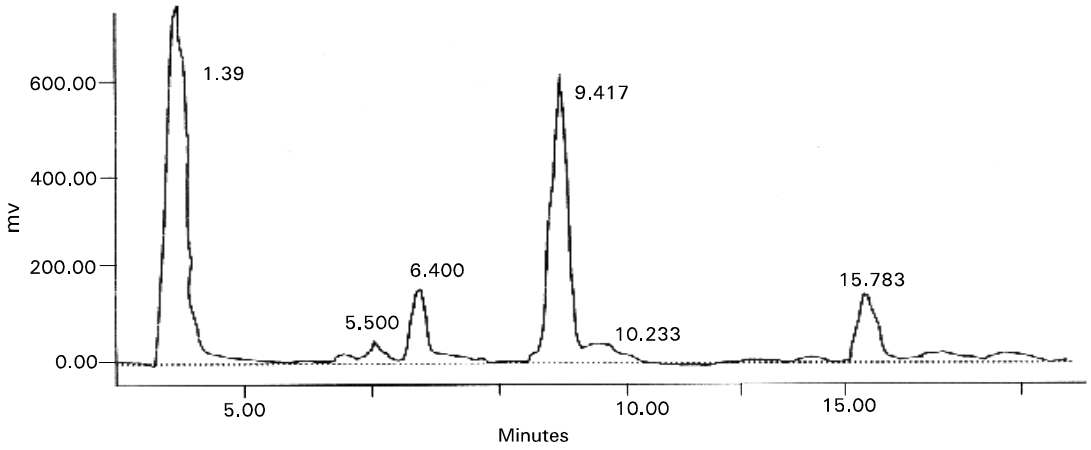

Fig. 2 High performance liquid chromatographic separation of the three naturally occurring aflatoxin in sesame sample.

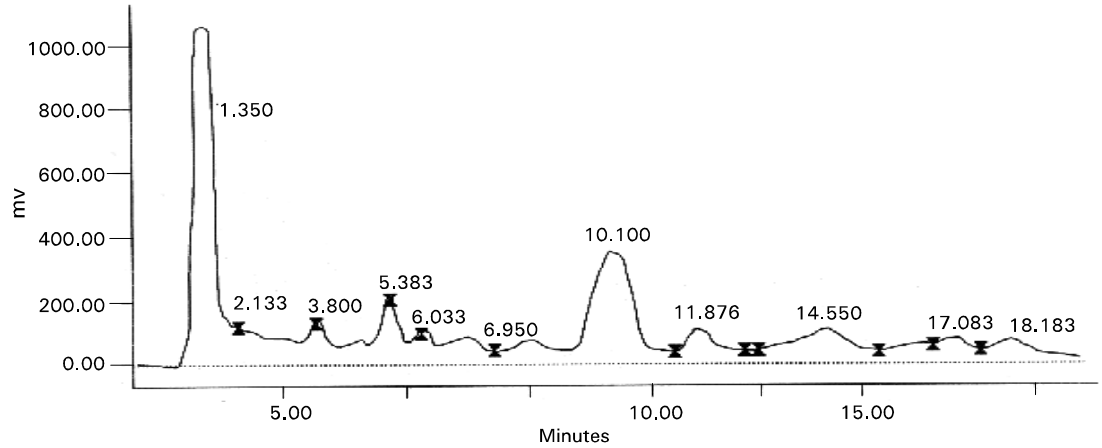

Fig. 3 High performance liquid chromatographic separation of the four naturally occurring aflatoxin in wheat sample.

Table 3: Level and frequency of detection of aflatoxin B_1 in common Saudi food and feed commodities.

Food and feed groups	No. of samples	Frequency of detection %	Measured range, ppb	Mean concentration ppb
Oil seeds	1	20	0.06-33.0	12.9
Cereal grains	3	15.8	0.08-49.0	8.02
Beans	2	50	0.08-4.0	1.99
Nuts	5	45.5	0.42-5.8	2.73
Poultry feed	1	20	2.9-90.0	55.7

Table 4: Effect of toxigenic *A. flavus* filtrates on germinability of four seeds after different periods (hours) of soaking.

Type of seeds	Control	3 hrs	6 hrs	12 hrs	24 hrs	Fungal filtrate on cotton
Broad bean	100%	0%	0%	0%	0%	0%
Chick pea	100%	75%	60%	50%	0%	0%
Cow pea	100%	55%	40%	0%	0%	0%
Maize	100%	15%	5%	0%	0%	0%

Fig.4: Effect of soaking broad bean seeds in filtrate of a toxigen *A. flavus* strain on germination after 12 hours intervals of soaking , comparing with control.

Fig. 5: The control treatment.

Table 5: Effect of the tested isolate toxins (B_1 + G_1) and standard B_1 and G_1 toxins on growth of some fungal and bacterial isolates.

Microorganisms	Toxin		
	B_1	G_1	B_1 + G_1 Tested isolate
Fungi			
Alternaria alternata	–	–	–
Aspergillus flavus	–	–	+
Fusarium oxysporum	–	–	–
Penicillium digitatum	–	–	+ +
Rhizoctonia solani	–	–	–
Saccharomyces cerevisiae	–	–	–
Candida albicans	–	–	–
Bacteria			
Bacillus subtilis	–	–	–
Escherichia coli	–	–	–
Enterobacter sp.	–	–	–
Klebsiella oxytoca	+	–	+
Pseudomonas sp.	–	–	–
Serratia nordii	–	–	–
Staphylococcus aureus	–	+ +	+ +

– No Inhibition + Inhibition

intervals where the germination percentage was 55 and 40%, respectively.

Toxicity of *A. flavus* on microorganisms: The toxigenic *A. flavus* isolate which produced B_1 and G_1 toxins was tested for its toxicity for 14 fungal and bacterial species. Data in Table 5 showed that the tested isolate has affected and inhibited two fungal species; *A. flavus* and *Penicillium digitatum* and two bacterial species; *Klebsiella oxytoca* and *Staphylococcus aureus*. It was also observed that B_1 and G_1 toxins separately did not affect the tested *A. flavus* and *P. digitatum* fungi while B_1 toxin had inhibited *Klebsiella oxytoca* and G_1 toxin inhibited the growth of *Staphylococcus aureus*.

Discussion

Four hundred and sixty eight isolates of *Aspergillus flavus* were tested for their ability to produce aflatoxins B_1, G_1, B_2 and G_2. The toxigenic isolates were 122 with percentage of 26.1%. The highest toxigenic isolates were found in seed samples amounting to 61.8%, followed by spices 27.4%, then milk and dairy products, 18.7% and the lowest for oil seeds 17.8%. Only B_1 and G_1 aflatoxins were detected from the toxigenic isolates while B_2 and G_2 were not detected. These findings are in agreement with those of Joe and Spanjer (1996), Adegoke *et al.* (1991), Kivanc (1990), Chourasia (1993), Park and Bullerman (1983) and Barrios *et al.* (1997), who studied aflatoxin production by *A. flavus* strains in many feed and foodstuffs.

HPLC chromatogram (quantitative assay for aflatoxins) of aflatoxins B_1 (PK_1), G_1 (PK_2), B_2 (PK_3) and G_2 (PK_4) were found to confirm the presence of four aflatoxins B_1, G_1, B_2 and G_2, respectively. Good resolution of the four compounds was obtained with no overlap of peaks. This is in contrast with other reports of HPLC of the aflatoxins by Seiber and Hsieh (1973). As evident from the results obtained natural substrates differ widely in their ability to produce mycotoxins and that result is similar to those reported by many authors (Diener and Davis, 1969; Hesseltine *et al.,* 1966 and Kriz, 1970). A wide variation in the measured range of aflatoxins from 4-90 was obtained from the twelve positive samples. Aflatoxin B_1 was the most dominant toxin on all samples. Several investigators (Borker *et al.*, 1966, Niles *et al.,* 1985), and Farag *et al.*, 1986) found that high carbohydrate substrates such as wheat and rice give larger yields of *A. flavus* aflatoxin than oil that are not immediately metabolized by toxigenic fungi. Differences in aflatoxin production can be attributed to factors such as the strain of the fungus, temperature, moisture, aeration and the method of analysis.

Biological assay was carried out for a toxigenic isolate of *A. flavus* (AF B_1) which has been isolated from wheat and proved to produce aflatoxins B_1 and G_1. This isolate used to study its effect on germination of four different seeds namely broad bean, chick pea, cow pea and maize seeds were soaked for different intervals between 3-24 hours in *A. flavus* culture filtrate. It was found that seed germination was significantly decreased by prolonged soaking intervals than control treatments. Germination failure of different seeds (groundnut, rice, cress, tinothy, wheat and maize) as a result of aflatoxin production has been also demonstrated by Schoeuaral and White (1965), Joffe (1969), Lalithakumari and Govindaswami (1970), Sulaiman and Hussain (1984), Lacey (1990) and Ewaidah (1992). The inhibitory effect of aflatoxins on seed germination suggested that aflatoxins had functioned as anti-auxins probably by inhibiting RNA synthesis. Similar conclusion was also suggested by Ciegler and Idllehoj (1968), Reiss (1971) and Rokesh *et al.*(1994).

The partially purified filtrates of the toxigenic *A. flavus* isolate was also used to study its effect on some selected fungal and bacterial species. This filtrate was found to contain both B_1 and G_1. Standards of pure B_1 and G_1 toxin solutions were also used. Seven fungal and seven bacterial species were used to study the effect of the toxigenic *A. flavus* isolate on their growth. The purified filtrate affected the tested fungal species; *A. flavus* and *Penicillium* whereas the rest of the tested fungi were not inhibited by the filtrate that contained B_1 and G_1. Also, all the tested fungal species were not affected by the standards B_1 and G_1 toxins. *Staphylococcus aureus* and *Klebsiella oxytoca* were also inhibited by the filtrate. *Staphylococcus aureus* was affected by the standard G_1 while

Klebsiella oxytoca was inhibited by B_1 toxin. Other tested bacterial species were not inhibited. In accordance with these results many workers have reported similar findings concerning inhibition or non-inhibition of aflatoxins to different fungal and bacterial species (Barmeister and Hesselltine, 1966; Arai *et al.,* 1967; Lillehoj *et al.*, 1967; Deshmukh and Agrawal, 1984 and Land *et al.*, 1987).

References

Adegoke, G. O., A. K. Babalola and A. Kanni, 1991. Effect of sodium metabisulphite, hydrogen peroxide and heat on aflatoxin B_1 in lafun and gari-two cassava products. Die Nahrung., 35: 1044-1045.

Al-Julaifi, M. Z., and S. M. Al-Juwaied, 1999. Preliminary study on presence of aflatoxin in dairy milk. Saudi Biological Society (SBS), Nineteenth Annual Meeting on Biological Development of the Kingdom of Saudi Arabia.

AOAC., 1980. Association of Official Analytical Chemists. Official methods of analysis. 13th ed. Washington, D. C. p: 429.

Arai, T., T. Ito and Y. Kojama, 1967. Antimicrobial activity of aflatoxin. J. Bacteriol., 93: 59-64.

Barmeister, H. R. and C. W. Hesseltine, 1966. In "Microbial Toxins" Vol. VI. edited by Cciegler, A., Kadis, S. and Ajl, S. I., Academic press, New York, London.

Barrios, M. J., L. M. Medina, M. G. Goidoba and R. Jordano, 1997. Aflatoxin producing strains of *Aspergillus flavus* isolated from cheese. J. Food Prot., 60: 192-194.

Bokhary, F. M., 1986: Studies on thermophilic and thermotolerant fungi in the soil of the Kingdom of Saudi Arabia (Western Region). M. Sc. Thesis, Jeddah, The Girls University.

Bokhary, F. M., 1993. Studies on mycotoxins production by moulds in stored cereals and pulses. Ph. D. Thesis, Edinburgh Heriot-Watt University.

Borker, E., N. F. Insalata, L. P. Levi and J. S. Witzeman, 1966. Mycotoxins in feeds and foods. Advan. Appl. Microbiol., 8: 315-351.

Butler, W.H., and J.M. Brans, 1966. Carcinoma of the glandular stomach in rats given diets containing aflatoxin Nature, 209: 90.

Chourasia, H. K., 1993: Growth, sclerotia and aflatoxin production by *Aspergillus parasiticus* of food preservatives. Appl. Microbiol., 17: 204-207.

Ciegler, A. and E. B. Idllelhoj, 1968: Mycotoxins. Adv. Appl. Microbiol., 10: 155-219.

Coulter, J. B. S., S. M. Lamplugh, S.G.I. Uliman, B. I. Muktar and R. G. Hendrickse, 1986. Aflatoxin in liver biopsies from Sudanese children. Amer. J. Trop. Med. Hyg., 35: 360-365.

Coulter, J. B. S., S. M. Lamplugh, G. I. Suliman, M. I. A. Omer and R. G. Hendrickse, 1984. Aflatoxins in human breast milk. Ann. Trop. Paediatrics, 61-66.

De Scott, P. M., 1965. Studies on species producing ochratoxins. J. Food Prot., 44: 702-710.

Deshmukh, S.K. and S.C. Agrawal, 1984. Volatile metabolites of Aspergilli in relation to spore germination of some keratinophilic fungi. Experiertia, 40: 582-584.

Diener, U. L. and N. D. Davis, 1969. Production of aflatoxin on peanuts under controlled environments. J. Stored Products. Res., 5: 251-258.

Ewaidah, E. H., 1992. Aflatoxins survey of imported Maize in the Riyadh region. Arab Gulf J. Sci. Res., 10 : 77-86.

Ewaidah, E. H., 1998: Survey of poultry feeds for aflatoxins from the Riyadh Region. Arab Gulf J. Sci. Res., 6 : 1-7.

Farag, R. S., M. A. El-Leithy, A. E. Basyony and Z. Y. Daw, 1986. Effect of varied substrates on aflatoxin production by *Aspergillus parasiticus*. J. Am. Oil Chem. Soc., 63: 1024-1026.

Gawad, K.M.A. and A.A. Zohri, 1993. Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. Mycopathol., 124: 55-64.

Hendrickse, R. G., 1984. Trans Roy, coc. Trop. Med. Hyg., 78: 427-435.

Hesseltine, C. W., O. L. Shotwell, J. J. Ellis and R.D. Stubblefield, 1966. Aflatoxin formation by *Aspergillus flavus*. Bact. Rev., 30: 795-805.

Hooda, K. S. and M. Singh, 1992. Quality of seeds from farmers own home saved seed in relation to seed-borne diseases of wheat. Crop Res., 5: 401-405.

Hsieh, D. P. H. and J. J. Wong, 1994. Pharmacokinetics and excretion of Aflatoxins. In D. L. Eaton and J. D. Groopman (eds.), The Toxicology of aflatoxins: Human health, veterinary and agricultural significance. Academic Press, New York.

Joe, M. S. and M. C. Spanjer, 1996: Determination of aflatoxin B in pistachio kernels and shells. J. A. O. A. C. Sntr., 79: 1360-1364.

Joffe, A. Z., 1969. Effects of *Aspergillus flavus* on groundnuts and other plants. Phytopathology, 64: 321-326.

Kivanc, M., 1990. Mold growth and presence of aflatoxin in some Turkish cheese. J. Food Safety, 10: 287-294.

Krishnamachari, K. A., R. V. Bhat , V. Nagarajan and T. B. J. Tilak, 1975. Lancet, 1061-1062.

Kriz, H., 1970. Effect of aflatoxins on the vitamin A stored in the livers of chickens. Acta Veterinaria (Brno). 39: 131-134.

Lacey, J., 1990. Mycotoxins in UK cereals and their control. Asp. Appl. Biol., 2: 395-405.

Lalithakumari, D. and C. V. Govindaswami, 1970. Role of aflatoxins in ground nut seed spoilage. Curr. Sci., 39: 308-309.

Lancaster, M. C., 1968. Effect of mycotoxins on experimental animals. Cancer Res., 28: 2288.

Land, C. J.J., R. Fuchs, S. Hagelberg and H. Land Storm, 1987. Thermogenic mycotoxins from *Aspergillus fumigatus* as a possible occupational health problem in Sawmills. Appl. Enviromicrobiol. Washington, D. C., Amer. Soc. Microbiol., 53: 787-790.

Lata.,1991. Seed mycoflora of some cereal grains from Garalc hpur. Nat. Acad. Sci. India, 61: 4.

Lillehoj, E. B., A. Ciegler and H.H. Hall, 1967. In "Microbial toxins" Vol., VI, edited by Ciegler, A. Kadis, S. and Aji, S. J., (1971), Academic press, New York, London.

Niles, E. V., A. Joanna, Norman and D. Pimbley, 1985: Growth and aflatoxin production of *Aspergillus flavus* in wheat and barley. Trans. Br. Mycol. Soc., 84: 259-266.

Park, K. Y. and L. B. Bullerman, 1983. Effect of cycling temperatures on aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus* in rice and cheddar cheese. J. Food Prot., 48: 889-896.

Pohland, A. E. and G. E. Wood, 1987: Occurrence of mycotoxin in foods. Academic press, New York.

Reiss, J., 1971: Increase of β-Indolacetic acid activity by aflatoxin B_1. Z. Pflanzenphysiol. 64: 260-263. (c.f. Microbial Abst., 6: A12798).

Rokesh-Kumar, P.P. Gupta and R. Kumar, 1994. Effect of seed mycoflora on seed germination and seedling mortality in crucifers. Int. J. Trop. Agric., 12: 136-139.

Saad, A. M., A.M. Abdelgadir and M.O. moss, 1989: Aflatoxin in human and milk in Abu Dabi, U. A. E. mycotoxin Res., 12: 252-262.

Schoeuaral, R. and A.F. White, 1965. Aflatoxins and 'Albinism' in plants. Nature, 205: 57-58.

Seiber, J.N. and D.P.H. Hsieh, 1973. Application of high-speed liquid chromatography to the analysis of aflatoxins. J. Assoc. Off. Anal. Chem., 56: 827-830.

Slubblefied, R. D. and O. L. Shotwell, 1977. Reverse phase. Analytical and preparative high pressure liquid chromatography of aflatoxins. J. Assoc. Off. Chem., 60: 784-790.

Sulaiman, E. D. and S.S. Husain, 1984. Pathogenicity and effect on germination caused by *Aspergillus* and *Penicillium* species on wheat, rice, barley and corn. Pakistan J. Sci. Ind. Res., 27: 359-362

Wogan , G.N, ed., 1965. Mycoxins in foodstaff. M.I.T. press, Cambridge Massachusetts.