

Pervasiveness of Aflatoxin in Peanuts Growing in the Area of Pothohar, Pakistan

Mateen Abbas, Abdul Muqeet Khan, Muhammad Rafique Asi, Javed Akhtar

Abstract—Mycotoxin (aflatoxins) contamination of peanuts is a great concern for human health. A total of 72 samples of unripe, roasted, and salty peanuts were collected randomly from Pothohar plateau of Pakistan for the assessment of aflatoxin. Samples were dried, ground and extracted by acetonitrile (84%). The filtered extracts were cleaned up by MycoSep-226 and analyzed by high performance liquid chromatography with fluorescence detector. Quantification limit of Aflatoxin was 1 µg/kg and 70% Recovery was observed in spiked samples in the range 1–10 µg/kg. The screening of mycotoxins indicated that aflatoxins were present in most of the samples being detected in 82%, in concentrations from 14.25 µg/kg to 98.80 µg/kg. Optimal conditions for mycotoxin production and fungal growth are frequently found in the crop fields as well as in store houses. Human exposure of such toxin can be controlled by pointed out such awareness and implemented the regulations.

Keywords—Aflatoxin, HPLC, Pakistan, Peanuts, Punjab

I. INTRODUCTION

PEANUT (*Arachis hypogaea* L.) also called “The King of Oilseeds, is one of the leading leguminous oilseed crops grown in Pakistan belong to the family Fabaceae [1]. Pakistan is also considered the leading peanut producer in the world where Punjab contains the large groundnut cultivated area (84%). Pothohar plateau is the famous land for peanut cultivation in Punjab Province, Pakistan where the most prominent rain-fed (barani areas) areas for peanut production are Attock, Chakwal, Jhelum and Rawalpindi etc [2]. Peanut has diverse uses like confectionery, nimko, margarine; oil for cooking, making soaps, cosmetics and lubricants; seedcake and manure for livestock; Oil emulsion used for softening pharmaceutical products, thus plays a significant role for food economy, also called the “cash crop”.

The toxicity of aflatoxins, are becoming a great menace for both human and animals in all over the world due to the health and economic significance. Everybody is now more conscious for food safety and security to keep him healthy and energetic. Aflatoxin is a most frequently growing mycotoxin in a massive variety of food and feed [3]-[4]. It is the naturally occurring stable mycotoxins can grow in maize, peanut, cottonseed, spices and other feed grains [5]-[6], more commonly in peanuts [7]. Peanut, an important oilseed is the appropriate substrate for aflatoxin growth which unluckily suffers significant problems regarding the quality of peanuts worldwide.

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Approximately, 25% of the world’s food commodities are contaminated by aflatoxin each year [8]. The Codex Alimentarius commission (Joint FAO/WHO) approved 15 mg/kg as the toxic limit for total aflatoxin in peanut [9].

Storage conditions are also not appropriate in developing countries like Pakistan where temperature and humidity favor the aflatoxin growth. Therefore, the study was planned to explore the current scenario of aflatoxin contamination in peanut.

II. EXPERIMENTAL WORK

A. Collection of peanut samples

Three different types of Peanut samples (unripe, roasted & salty) were collected from different localities around four major cities of Attock, Chakwal, Jhelum and Rawalpindi. Six each type of peanuts were collected from all selected cities (6 x 3 x 4 = 72). Samples were collected in a clear air tight polythene bag and stored until analysis.

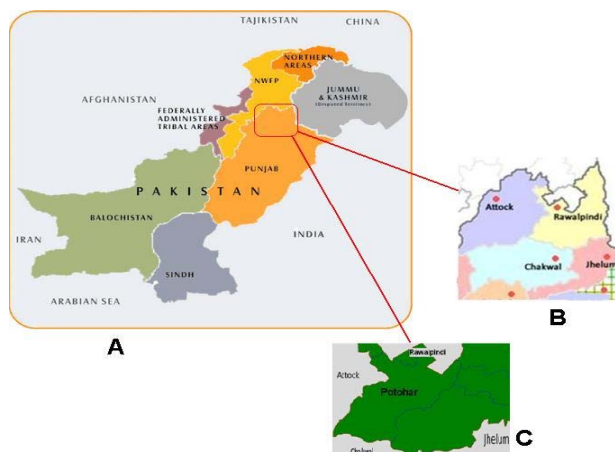


Fig. 1 (a) Pakistan map, (b) Showing sampling cities (Attock, Jhelum, Chakwal and Rawalpindi), (c) Showing Pothohar plateau surrounded by sampling cities

B. Standard, Chemicals and Columns

Aflatoxin mix standard (Aflatoxin Mix Kit-M) containing B₁, G₁, B₂ & G₂ was purchased from Supelco, USA. All other chemical (HPLC grade) were acquired from registered distributors of Merck, Germany. MycoSep (226 AflaZon+ Multifunctional) purification columns were purchased from Romer Labs., USA.

C. Instrument

Agilent (1100 series, USA) High Performance Liquid Chromatography (HPLC) was used for quantitative estimation of aflatoxin in peanuts. Agilent 1100 series equipped with degasser (Model: G1379A; Serial # JT40724009), Quaternary pump (Model: G1311A; Serial # DE43632988), auto-sampler

and auto-injector (Model: G1313A; Serial # DE33229943), column compartment (Model: G1316A; Serial # DE43647656) and fluorescent detector (Model: G1321A; Serial # DE43607390).

D. Extraction Procedure of Aflatoxin from peanuts

Peanuts were ground completely by using sieve size 50M. Aflatoxin was extracted from peanuts (25 g) by acetonitrile (100 ml; 84%), containing sodium chloride (5 g). Then the sample was placed in orbital shaker for 1 hr for complete missing and extraction. Filtered the sample and passed through MycoSep® cleanup column for purification. Sample was then derivatized with Tri Fluore Aceticacid (TFA) and analyzed by HPLC-FLD.

E. Chromatographic Conditions

Four different types of Aflatoxin B₁, G₁, B₂ & G₂ were separated through LiChrospher® Merck C18 column having internal diameter 4.6 mm, column length 250 mm and particle size 5µ. Double distilled water, methanol and acetonitrile (60:20:20 v/v) was utilized as a mobile phase and eluted with a flow rate of 1 ml/min. Column temperature was set at 30°C during run. Aflatoxin was quantitatively determined by fluorescent detector using excitation wavelength 360 nm and emission wavelength 440 nm.

F. Validation of HPLC Method

Working standard of aflatoxin was prepared in benzene:acetonitrile (98:2 v/v) and stored at -20°C after rapping with aluminum foil till analysis. Analytical method was validated by spiking the toxin (1, 2, 4, 6, 8 and 10 µg/kg,) in peanuts. The extraction and purification of the spiked peanuts (n = 5) were performed as described above.

G. Statistics

Data was analyzed using the Microsoft Excel program 7.0 version, calculated the mean, Standard Deviation (SD), maximum and minimum values. Comparative charts/graphs were also prepared to show the differences [10].

III. RESULTS

Peanuts are consumed in routine life by everyone as raw, cooked (roasted) or mixed with other foods like biscuits or confectionery. Contamination of aflatoxin has been a great problem particularly in developing countries due to unappropriate storage [11]. As peanut is one of the most vulnerable host of food materials for aflatoxin growth, a number of scientists have explored the presence of aflatoxins, particularly Aflatoxin B₁ [5], [7], [11]-[14].

Method was validated by spiking the aflatoxin standard in peanuts (1.0–10 µg/kg) in this study. A linear response was observed for all aflatoxin ($r^2 = 0.997$). Limit of detection was 0.8 µg/kg and limit of quantification was 1.0 µg/kg in peanuts.

Recovery of aflatoxin in spiked samples (n = 5) was 70% (ranged 68.20 to 72.10 µg/kg), shown in Table I.

TABLE I
RECOVERY OF AFLATOXIN B₁ SPIKED IN PEANUTS

Conc. of Aflatoxin added	Conc. of Aflatoxin found (µg/kg)		% Recovery	
	Mean	± SD	Mean	± SD

(µg/kg)	1	0.72	0.04	71.60	3.65
	2	1.36	0.05	68.20	2.33
	4	2.81	0.05	70.30	1.14
	6	4.33	0.11	72.10	1.79
	8	5.68	0.09	70.98	1.09
	10	7.14	0.06	71.36	0.64

Table II summarized the results of aflatoxin B₁ in peanut samples (roasted, salty and unripe) collected from different linked areas of Attock, Chakwal, Jhelum and Rawalpindi. Average concentration (µg/kg) of aflatoxin B₁ in peanuts collected from Attock was 25.93 ± 13.03 µg/kg (roasted), 18.22 ± 5.31 µg/kg (salty) and 42.46 ± 29.21 µg/kg (unripe).

TABLE II
OCCURRENCE OF AFLATOXIN B₁ IN ROASTED, SALTY AND UNRIPE PEANUTS COLLECTED FROM DIFFERENT CITIES

CITY	Mean (µg/kg)	Roasted	
		Range (µg/kg)	Positive Samples
Attock	25.93 ± 13.03	15-47	5 (83%)
Chakwal	45.85 ± 32.13	16-98	6 (100%)
Jhelum	37.06 ± 21.04	14-56	4 (67%)
Rawalpindi	71.28 ± 14.86	53-89	4 (67%)
SALTY			
Attock	18.22 ± 5.31	14-24	3 (50%)
Chakwal	40.85 ± 32.33	15-85	4 (67%)
Jhelum	34.98 ± 25.47	20-64	3 (50%)
Rawalpindi	59.77 ± 19.69	32-75	4 (67%)
UNRIPE			
Attock	42.46 ± 29.21	22-85	4 (67%)
Chakwal	34.05 ± 13.55	15-45	4 (67%)
Jhelum	47.42 ± 25.87	21-80	4 (67%)
Rawalpindi	46.76 ± 15.38	33-63	3 (50%)

Similarly, mean concentration (µg/kg) of aflatoxin B₁ in peanuts of Chakwal was 45.85 ± 32.13 µg/kg (roasted), 40.85 ± 32.33 µg/kg (salty) and 34.05 ± 13.55 µg/kg (unripe). And average concentration of aflatoxin B₁ in roasted, salty & unripe peanuts were 71.28 ± 14.86, 59.77 ± 19.69 & 46.76 ± 15.38 µg/kg, respectively. Overall, 82 % samples showed the positive results for aflatoxin B₁ in all type of samples, in which 86% samples were above the toxic limit of aflatoxin B₁ (15 µg/kg) defined by FDA. Aflatoxin G₁, B₂ & G₂ were also observed in all samples, results of positive samples were shown as graph (Fig. 2).

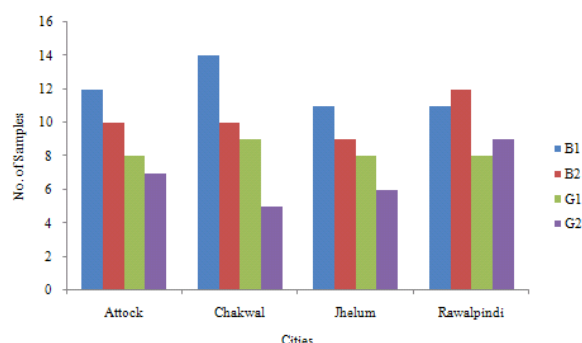


Fig. 2 Incidence of Aflatoxin (B₁, B₂, G₁ & G₂) in different cities

IV. DISCUSSION

Several studies have been conducted previously for aflatoxin contamination, particularly Aflatoxin B₁ observed by Brudzynski [11] in maize, peanuts and cassava ranged from 12-1000 mg/kg. Another study reported the occurrence of aflatoxin up to 2000 mg/kg in Nigerian peanut [5] and 12 to 329 mg/kg in peanut samples collected from Botswana [12]. Mutege [14] also explored the aflatoxin contamination of peanuts in western Kenya and found aflatoxin upto 7525 mg/kg and Kamika and Takoy [15] stated the 70% contamination of aflatoxin in peanuts.

Occurrence of aflatoxin (B₁ ranged from 10-1099 mg/kg) contamination (12-85%) in developing countries were observed in groundnuts, tree nuts, dried fruit, spices, rice, maize, soybeans, and wheat specially in peanuts and peanut products [16]-[21].

Pothohar is the well-known area for peanut production in Pakistan. Due to its rain-fed area moisture contents are mostly upto 30%, thus it favors the growth of aflatoxin. This study showed that peanuts available in these areas were highly contaminated (82%) ranged from 14.25 to 98.80 µg/kg, and 86% samples were above the toxic limit (15 µg/kg) decided by Codex Alimentarius commission [9]. Rawalpindi division is the most affected area of aflatoxin contamination (59 %) as compared to others. All type of aflatoxin was observed in the current study but aflatoxin B₁ mostly occurs. This study also showed that roasted peanut were most contaminated (40%) with aflatoxin as compared to salty and unripe.

It was also observed that peanut were mostly contaminated by Aflatoxin B₁, other types of aflatoxin (G₁, B₂ & G₂) were also present but in less quantity, shown in Fig 2.

The current study was also in agreement with other studies on aflatoxin contamination in peanuts conducted in different countries, because of the vulnerability of peanuts to be contaminated by aflatoxins in favorable climatic conditions. This study illustrated that the high risk of human disclosure to aflatoxin from utilization of peanuts.

V. CONCLUSION

The current study exposed that peanuts collected from the linked area of Pothohar plateau (Attock, Chakwal, Jhelum & Rawalpindi) were highly contaminated with aflatoxin. Approximately 82% peanut samples were found contaminated and 86% samples exceeded the maximum limit of 15 µg/kg set by WHO/FAO jointly.

This indicated the alarming sign for exposure to human health as well as export. Therefore, it should be highlighted that continuous study from other sites, should be executed to assess the natural incidence of aflatoxins countrywide.

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