

# 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.

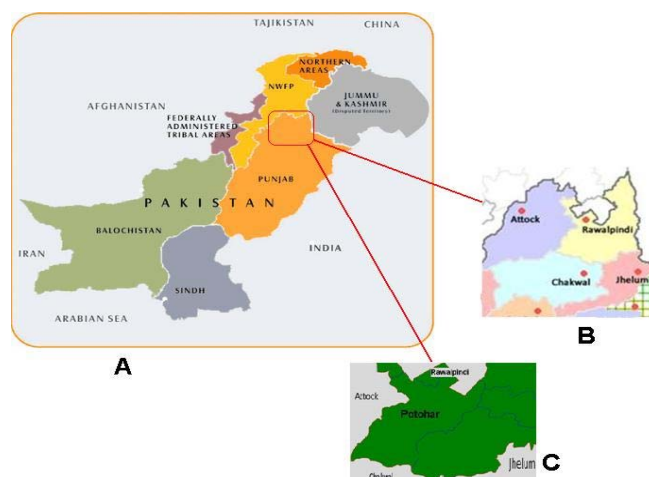


Fig. 1 (a) Pakistan map, (b) Showing sampling cities (Attock, Jhelum, Chakwal and Rawalpindi), (c) Showing Pothohar plateau surrounded by sampling cities

### B. Standard, Chemicals and Columns

Aflatoxin mix standard (Aflatoxin Mix Kit-M) containing B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub> & G<sub>2</sub> 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 B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub> & G<sub>2</sub> 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 un-appropriate 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 B<sub>1</sub> [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 B<sub>1</sub> SPIKED IN PEANUTS

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

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

Table II summarized the results of aflatoxin B<sub>1</sub> in peanut samples (roasted, salty and unripe) collected from different linked areas of Attock, Chakwal, Jhelum and Rawalpindi. Average concentration (μg/kg) of aflatoxin B<sub>1</sub> in peanuts collected from Attock was  $25.93 \pm 13.03$  μg/kg (roasted),  $18.22 \pm 5.31$  μg/kg (salty) and  $42.46 \pm 29.21$  μg/kg (unripe).

TABLE II  
OCCURRENCE OF AFLATOXIN B<sub>1</sub> IN ROASTED, SALTY AND UNRIPE PEANUTS COLLECTED FROM DIFFERENT CITIES

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

Similarly, mean concentration (μg/kg) of aflatoxin B<sub>1</sub> in peanuts of Chakwal was  $45.85 \pm 32.13$  μg/kg (roasted),  $40.85 \pm 32.33$  μg/kg (salty) and  $34.05 \pm 13.55$  μg/kg (unripe). And average concentration of aflatoxin B<sub>1</sub> in roasted, salty & unripe peanuts were  $71.28 \pm 14.86$ ,  $59.77 \pm 19.69$  &  $46.76 \pm 15.38$  μg/kg, respectively. Overall, 82 % samples showed the positive results for aflatoxin B<sub>1</sub> in all type of samples, in which 86% samples were above the toxic limit of aflatoxin B<sub>1</sub> (15 μg/kg) defined by FDA. Aflatoxin G<sub>1</sub>, B<sub>2</sub> & G<sub>2</sub> were also observed in all samples, results of positive samples were shown as graph (Fig. 2).

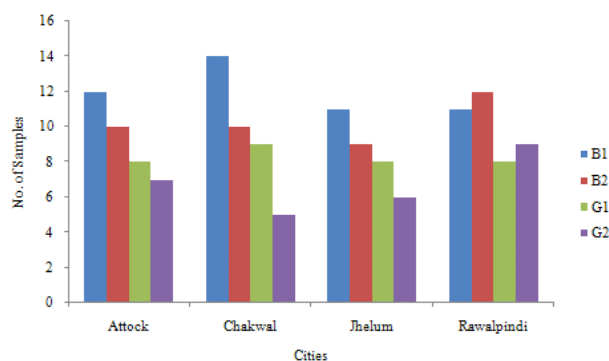


Fig. 2 Incidence of Aflatoxin (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) in different cities

#### IV. DISCUSSION

Several studies have been conducted previously for aflatoxin contamination, particularly Aflatoxin B<sub>1</sub> 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 (B<sub>1</sub> 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 B<sub>1</sub> 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 B<sub>1</sub>, other types of aflatoxin (G<sub>1</sub>, B<sub>2</sub> & G<sub>2</sub>) 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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#### REFERENCES

- [1] M. A. Shad, P. Humayun, N. Haq, K. Hyder and A. U. Muhammad, "Evaluation of biochemical and phytochemical Composition of some groundnut varieties Grown in arid zone of Pakistan", *Pak. J. Bot.*, vol. 41, no. 6, pp. 2739-2749, 2009.
- [2] N. Hussain and C. R. Ahmed, "Problems of groundnut cultivation in the representative areas of Punjab," *Planning and Evaluation cell, Agriculture department, Lahore*, 1984, pp. 1-14.
- [3] R. V. Bhat and S. Vashanti, "Occurrence of aflatoxins and its economic impact on human nutrition and animal feed," *The New Regulation, Agricultural Development*, vol. 23, pp. 50-56, 1999.
- [4] H. Njapau, E. M. Muzungaile and R. C. Changa, "The effect of village processing techniques on the content of aflatoxins in corn and peanuts in Zambia," *Journal of the Science of Food and Agriculture*, vol. 76, pp. 450-456, 1998.
- [5] D. McDonald and C. Harkness, "Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts, Part IV," *Tropical Science*, vol. 4, pp. 12-27, 1964.
- [6] T. D. Phillips, "Detection and Decontamination of aflatoxin-contaminated food products," in J. H. Williams, D. G. Cummins, G. Hutto and A. King (Eds.), *Impacts and scientific advances through collaborative research on peanut CRSP*, 1997, pp. 117-130.
- [7] S. A. Bankole and A. Adebajo, "Mycotoxins in food in West Africa: current situation and possibilities of controlling it," *African Journal of Biotechnology*, vol. 2, no. 9, 254-263, 2003.
- [8] N. Magan, V. Sanchis and D. Aldred, "Role of spoilage fungi in seed deterioration," in D. K. Aurora (Ed.), *Fungal biotechnology in agricultural, food and environmental applications*, Chapter 28, pp. 311-323, 2004.
- [9] Codex Alimentarius Commission. "Joint FAO/WHO food standards programme," *Codex committee on food additives and contaminants*. Thirty-third session. Hague, Netherlands: CODEX, 2001.
- [10] R. G. D. Steel, J. H. Torrie and D. A. Dickey, "Principles and procedures of statistics," Mc Graw Hill Book Co., Inc., New York, 1997.
- [11] A. Brudzynski, W. Van Pee and W. Kornazewski, "The occurrence of aflatoxin B<sub>1</sub> in peanuts, corn and dried cassava sold at the local market in Kinshasa, Zaire: its coincidence with high hepatoma morbidity among the population," *Zeszyty Problemowe Postepow Nauk Rolniczych*, vol. 189, pp. 113-115, 1977.
- [12] N. Barro, C. A. Quattara, P. A. Nikiema, A. S. Quattara and A.S. Traore, "Microbial quality assessment of some street food Widely consumed in Ouagadougou, Burkina Faso," *Sante*, vol. 12, pp. 369-374, 2002.
- [13] V. V. Garcia, "An overview of peanut utilization in the Philippines," in T. C. Yam and C. Tan (Eds.), *Trends in food product development*, Singapore: Singapore Institute of Food Science and Technology, pp. 21-26, 1989.
- [14] C. K. Mutege, H. K. Ngugi, S. L. Hendriks and R. B. Jones, "Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya," *International Journal of Food Microbiology*, vol. 130, no. 1, pp. 27-34, 2009.
- [15] I. Kamika and L. L. Takoy, "Natural occurrence of Aflatoxin B<sub>1</sub> in peanut collected from Kinshasa Democratic Republic of Congo," *Food Control*, vol. 22, pp. 1760-1764, 2011.
- [16] S. A. Bankole, A. A. Adenusi, O. S. Lawal and O. O. Adesanya, "Occurrence of aflatoxin B<sub>1</sub> in food products derivable from 'egusi'

- melon seeds consumed in southwestern Nigeria,” *Food control*, vol. 21, pp. 974-976, 2010.
- [17] H. E. Ok, H. L. Kim, W. B. Shim, H. Lee, D. H. Bae and D. H. Chung, “Natural occurrence of Aflatoxin B1 in the marketed foods and risk estimates of dietary exposure in Koreans,” *Journal of Food Protection*, vol. 70, no. 12, pp. 2824-2828, 2007.
- [18] S. Egal, A. Hounsa, Y. Y. Gong, P. C. Turner, C. P. Wild, A. Hall, “Dietary exposure to aflatoxin from maize and groundnut in young children from Benin and Togo, West Africa,” *International Journal of Food microbiology*, vol. 104, pp. 215-234, 2005.
- [19] L. Shundo, S. A. Navas, L. C. A. Lamardo, V. Ruvieri, and M. Sabino, “Estimate of aflatoxinM1exposure in milk and occurrence in Brazil,” *Food Control*, vol. 20, pp. 655-657, 2009.
- [20] E. Razzazi-Fazeli, C. T. Novianti, S. Porasuphatana, A. Agus and J. Bohm, “A survey of aflatoxins and B1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELSA and HPLC,” *Mycotoxin Research*, vol. 20, pp. 51-58, 2004.
- [21] J. H. Williams, T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly and D. Aggarwal, “Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions,” *American Society and Clinical Nutrition*, vol. 80, no.5, pp. 1106-1122, 2004.