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Full Paper

Optimization of HPLC with Fluorescence Detection for the assay of Aflatoxins from *Aspergillus* Strains of Section *Flavi* Originated from Grains in Upper Egypt

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Abstract

The aflatoxigenic ability of strains related to *Aspergillus* section *Flavi* isolated from peanut seeds, corn and wheat grains collected from Sohag and Assiut Governorates, Egypt was assessed. A simple and rapid high performance liquid chromatographic method with fluorescence detection is optimized and employed for the determination of the aflatoxins in peanuts, corn and wheat. Sample were extracted and the analytes were separated within 7 min. Validation of the proposed method was assessed to take into account the effect of possible components. The recoveries were in the range 89–103% and RSDs were < 3.9%. Analysis of the extracts of *A. flavus* strains confirmed the results obtained on CAM agar plates as blue color fluorescence at 365 nm. All *A. flavus* strains isolated from peanut (14), corn (11) and wheat (12) produced aflatoxin(s) B_1, B_2 and/or G_2. Among the 11 strains analyzed from corn, one strain (AUMC 9793) gave the highest concentration for the three types of toxins (B_1, B_2 and G_2). However, aflatoxin G_2 was detected from strains originated only from corn (6 strains) and wheat (9 strains), but was missing in all the 14 strains analyzed from peanut samples. Among peanut strains, AUMC 9779 gave the highest concentration for both aflatoxins detected (B_1 & B_2). From the 12 *A. flavus* strains from wheat grains, 8 produced the three aflatoxins (B_1, B_2 and G_2) of which the most potent strains yielded high concentration of aflatoxins B_1 & B_2 (one strain) or the three toxins (3 strains).

Keywords: Peanut, Corn, Wheat, Aflatoxins, AFPA, DRBC, CAM, High performance liquid chromatography (HPLC).

1. Introduction

The aflatoxins were initially isolated and identified as the cause of the Turkey X disease (i.e hepatic necrosis) in 1960s in England [1-4]. In the word aflatoxin, the first syllable (a) was derived from the genus *Aspergillus*, the fla) from the species *flavus* and the term (toxin) came from the adjective poison [5,6].

Aflatoxins (AFs) are a group of heterocyclic, oxygen-containing mycotoxins that possess the bisdifuran ring system, these secondary metabolities are produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus* [7-12] and rarely by *A. nomius* [13, 14]. *A. bombycis*, *A. parvisclerotigenous*, *A. ochraceoroseus*, *A. rambellii* and *A. pseudotamari*, *Emericella astellata* and *E. venezuelensis* are also reported as AFs producers [15].

Aflatoxins contaminate different types of food and feed commodities, especially in hot and humid regions of the world [14]. The major AFs are characterized as B_1, B_2, G_1 and G_2, among these four, B_1 is best known because of its hepatocarcinogenic nature [16]. The AFs M_1 and M_2 are produced by biological metabolism of AFB_1 and AFB_2 from contaminated feed used by animals and excreted in milk and dairy products [10, 17].

Natural occurrence of mycotoxins are present in a large part of the world food supply and bear potential threat to food safety and food security [18- 23]. From global prospective of food safety and food security, mycotoxins contamination of foods has gained much attention as potential health hazards for humans and animals [24].

Cereals and other crops are exposed to fungal attack in the field or during storage and this attack may results in mycotoxins contamination of the crop [25]. Ochratoxin A (OTA) and Aflatoxin B_1 (AFB_1) are among the most frequent observed combination of mycotoxins in different plant products [26]. Moreover, seeds could support the growth of aflatoxigenic and ochratoxigenic molds and the AFB_1 and OTA production [27].

The objective of this study is to isolated and evaluate the aflatoxins originated from *Aspergillus* strains in peanut seeds and corn and wheat grains using HPLC analysis.

2. Materials and Methods

2.1 Aspergillus flavus strains

Aspergillus flavus strains**,** previously isolated from samples of peanut seeds, corn and wheat grains collected from Assiut and Sohag Governorates, Egypt [28] were used. The 43 selected strains were checked for their aflatoxin-producing abilities on coconut agar medium, CAM [29] and all proved positive for aflatoxin production [28]. All of these strains except 5 from those originating from corn and one from peanut were subjected for HPLC analysis.

2.*2 Aflatoxins Analysis*

2.2.1 Growing of Aspergillus Flavus Strains on Liquid Medium

All *Aspergillus flavus* strains (37 strains: 14 from peanut seeds, 11 from corn and 12 from wheat grains) were cultivated on Czapek liquid medium of the following composition (g/l distilled water): sucrose 30, $NaNO_3$ 3, K_2HPO_4 1, KCl 0.5,

$MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.01, pH 6.2±0.2 (refer to Samson *et al.* 2004) fortified by yeast extract (2 g/l) and peptone (10 g/l) [30]. Fifty ml of the liquid medium were added in each of 250 ml sterile Erleynmyer flasks. After sterilization, each flask was inoculated with 3 agar discs made from 7-day-old cultures on CAM agar plates. The flasks were then incubated at 28 °C for 10 days.

2.2.2 Extraction and Identification of Aflatoxins

At the end of the incubation period, each flask-containing *A. flavus* culture was extracted with 100 ml chloroform for 24 h at room temperature (~ 20°C). The chloroform extract was dried over anhydrous sodium sulphate, filtered and distilled to near dryness. The residue was diluted with chloroform to one ml.

2.2.3 Aflatoxins Analysis Using HPLC

2.2.3.1 Chemicals

Aflatoxins standards B1, B2, G1 and G2 were purchased from Sigma Aldrich Chemical Company. Standard solutions were prepared in methanol and stored at – 20°C for up to three months.

2.3 Assessment of aflatoxins produced by HPLC

2.3.1 Extraction and determination of aflatoxins by HPLC

The presence of aflatoxins in culture media was confirmed by HPLC method. HPLC is an automated Agilent Technologies 1200 Series managed by computer with the chemstation software. It is equipped with an auto-sampler (100 µl, injector loop), Zorbax Eclipse plus C18, Analytical 4.6 mm x 250 mm, 5 µ column and a UVE LC-Tech, Photochemical post column Derivatizer UVC 254 nm as Post column) and a Fluorescence Detector G1321A**,** The detector was set at 365 nm (excitation) and at 455 nm (emission). Fifteen (15) ml of the final filtrate were diluted with 15 ml of phosphate buffered saline (PBS) (for 1 liter of PBS pH 7.4: (KCl, 200 mg; KH_2PO4, 200 mg; $NaH_2PO_4H_2O$, 116 mg; NaCl, 8.0 g, make up to volume with distilled water, The mobile phase was Water/AN/MeOH 55%/30%/15% (v/v/v) Injection volume: 30 µl, the flow rate was set at 1.5 ml/min at 30ºC.

Calibration curves for all the aflatoxin were determined, using a series of standard solutions prepared in methanol. Linear calibration graphs were obtained by plotting the peak area against the aflatoxin amount injected. Each experiment was conducted in duplicate and quantification of aflatoxins was performed by comparing the peaks areas with the calibration curves. With regard to the limits of determination, limits of detection (LOD) and limits of quantification (LOQ) were calculated for each type of aflatoxin as follows:

$$LOD = [b_0+3\ S(b_0)] / b1 \quad \text{and}$$

$$LOQ= [b_0+10\ S(b_0)]/b_1$$

Where b_0 is the intercept of the calibration curve, $S(b_0)$ is the standard deviation of the blank and b_1 is sensitivity (calibration curve slope).

2.4 Statistical analysis

All experiments were conducted in duplicates and the data were presented as Mean ± SD. Statistical significance was ascertained by using the single linear method. Statistical relation between variables was analyzed by correlation

analysis which is highly significant.

3. Results and discussion

3.1 Optimization of the proposed HPLC Method

The HPLC method was optimized by considering the mobile phase to have optimal parameters of the retention time, peak response, sensitivity, peak sharpness as well broadening and separation efficiencies. Acetonitrile– water 60:30 v/v failed to separate the AFs while Methanol- water was able to separate them but with poor sensitivity. The best mobile phase for AF standards separation was found to be water– acetonitrile-methanol in the combination of 55: 30: 15% (v/v/v) (Fig. 1).

Calibration curves for various concentrations of AFs were constructed by plotting the response against aflatoxins' concentration. A linear relationship was obtained for each aflatoxin with correlation coefficients ranged from 0.9995 to 0.9998 (Table 1).

The relative standard deviations (RSDs) of precision of AF mixtures were 2.04 – 4.09 % (n=7). Excellent retention times for AFB1 (6.3±0.02), AFB2 (5.3±0.015), and AFG2 (4.4±0.02) were observed for corresponding mixtures of AFs (Table 1). The slope, intercept and regression coefficient of the calibration curves for each AF is cited in Table 1.

Accuracy analysis was performed by testing samples at three different concentrations 1µg L^{-1}, 5 µgL^{-1} and 10 µgL^{-1}. The extract was spiked with high, medium and low levels of AF standards. It is found that the accuracy is ranged from 86.7% to 108.1%, indicating good applicability of the method. The LOD based on signal-to-noise ratio (S: N) of 3 are cited in Table 1.

The method was then applied for the quantitation of aflatoxins produced from different strains from peanuts, corn and wheat (Fig. 2).

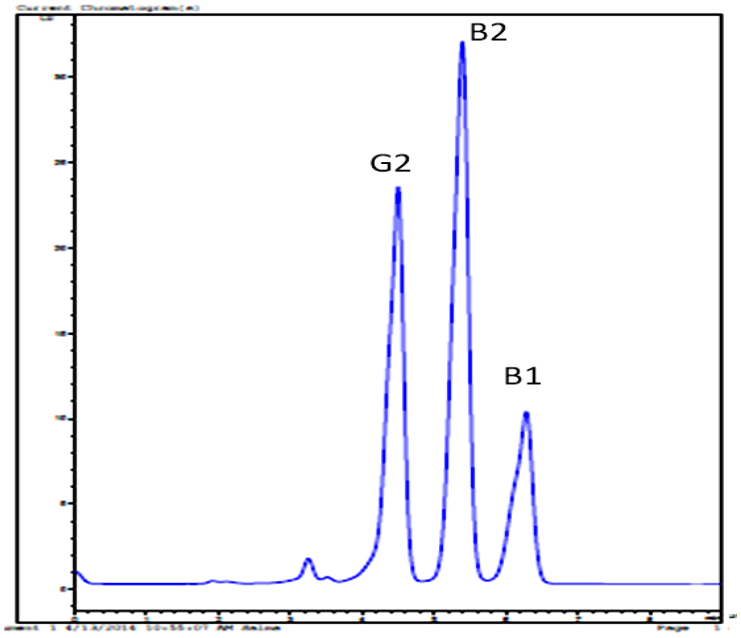

Fig. 1: HPLC chromatograms of aflatoxins' standards (B1, B 2 and G2)

Table 1. Parameters of linear regression and the accuracy of the method for the determination of B1, B2 and G2.

Aflatoxin	Ret. time (min)	Slope	Intercept	R^2	LOD, ngL^{-1}	LOQ, ngL^{-1}
B1	6.30	99.15	0.20	0.9996	0.0023	0.0182
B2	5.30	165.08	0.01	0.9995	0.0096	0.0032
G2	4.40	43.12	0.11	0.9998	0.0040	0.0132

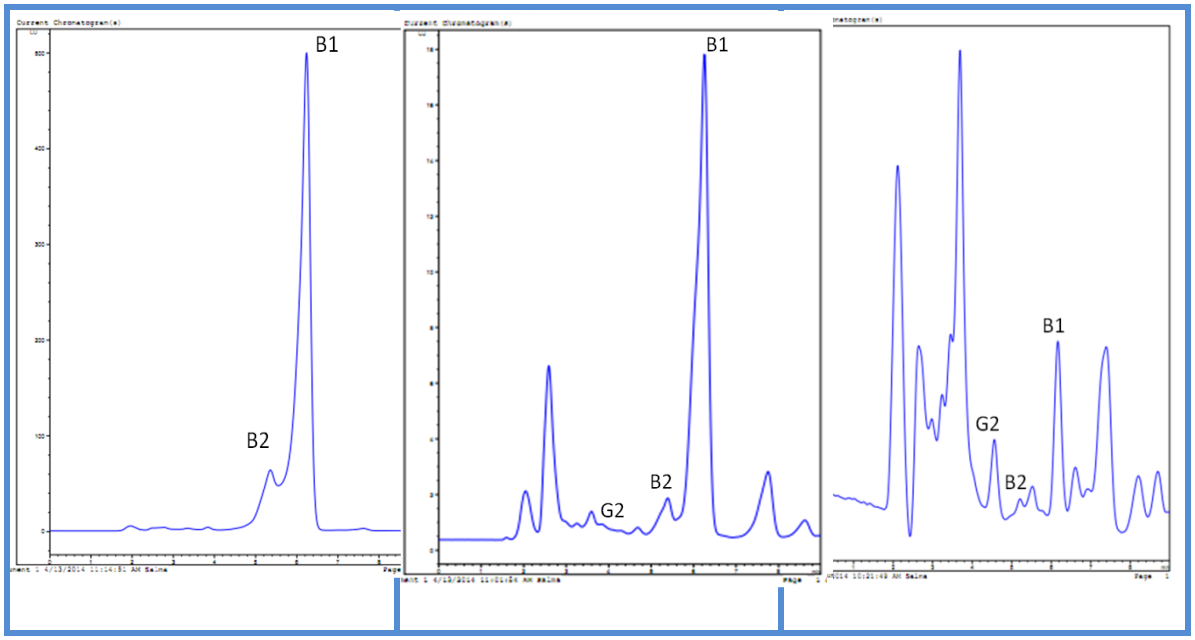

Fig. 2: HPLC chromatograms of aflatoxins B_1 & B_2 produced from AUMC strain no.9779 originated from peanut (left), aflatoxins B_1, B_2 and G_2 produced from AUMC 9793 originated from corn (middle) and those produced from AUMC 9816 originated from wheat (right).

3.2 Quantitative analysis of aflatoxins in A. flavus strains originated from peanut seeds using HPLC

After method optimization, the 14 strains have been analysed. Aflatoxins B_1 and B_2 were detected (**Table 2**). Among the 14 strains analyzed, the strain No. 9779 gave the highest concentrations for the two types of toxins (1746.14 and 33.48 ppm). Strain AUMC 9771 yielded the lowest concentrations of both types (16.63 and 0.10 ppm) (**Table 2**). These 14 strains were also positive for aflatoxin production in the previous work of Ismail *et al*. [28]. It is noted that all strains gave higher concentrations of AFB_1 than AFB_2. In this respect, and in agreement with the current results [31] detected aflatoxins B_1 and B_2 in 4 out 7 strains of *Aspergillus flavus* and *A. parasiticus* (3 of them were isolated from groundnut) using coconut agar medium and HPLC analysis with B_1 concentration being higher than that of B_2.

Sultan and Magan [32] Found also that among 88 isolates originated from Egyptian peanuts from five governorates and tested for their producing ability of AFB_1 using HPLC, only 5 gave false negative results. The concentrations of AFB_1 in the positive isolates ranged from 10 to 100,000 ng g^{-1} however 2 isolates were able to produce AFB_1 > 100.000 ng g^{-1}. All the aflatoxigenic isolates produced AFB_1 and B_2 but none produced AFG_1 and G_2 on YES medium. Also they noticed that among these 88 isolates of *Aspergillus* section *Flavi*

tested on coconut agar medium, 74 were aflatoxigenic producing a blue fluorescence under ultraviolet light at 365 nm. However, Afsah-Hejri *et al.* [33] on their screening for aflatoxigenic ability of *Aspergillus* strains from raw unshelled peanut brands marketed in Malaysia on CYA, DG18, MEA and PDA and checked for UV fluorescence under UV light found that the results were not reliable. Amonium vapour method proposed by Saito and Machida [34] was also used for screening in which a drop of ammonium solution (25%) was placed into the lid of the petri dish and immediately covered by the bottom of PDA Petri dish and developing pink/red colour at the reverse of the colonies indicates aflatoxin-producing abilities. Results revealed that only 42 out of 46 plates were aflatoxin producers. On the other hand, all isolates which showed pink/red colour at their reverse had strong signal on AFB_1 followed by AFB_2 and AFG_1 in their HPLC chromatogram Abdel-Hadi *et al.* [35] estimated the aflatoxin-producing abilities of *Aspergillus flavus* strains isolated from Egyptian peanut by qualitative fluorescence using coconut cream agar medium (CAM) and on a conductive yeast extract sucrose (YES) medium using HPLC. Overall, 13/18 strains producing aflatoxin B_1 and aflatoxin B_2 in the range 1.27 – 213.35 µg/g medium. The results on CAM showed a good correlation between fluorescence and levels of aflatoxin as measured by HPLC which gave similar results.

Ravi Babu et al. [36] estimated the toxin yields in *A. flavus* strain isolated from groundnut in India, grown on rice and extracted for aflatoxin analysis using HPLC. Four aflatoxins (AFB_1, AFB_2, AFG_1 & AFG_2) were detected of which AFB_1 predominated over other aflatoxins. AFB_1 levels ranged from 634 to 950, AFB_2 from 125 to 167, AFG_1 from 160 to 458 and AFG_2 from 22 to 62 with total ranging from 964 to 1511 µg/g of substrate. The higher percentage of B1 produced is of great interest. Also, Rajarajan *et al.* [37] detected the afaltoxins, in *A. flavus*-infected stored peanuts in India, by HPLC and found different levels of toxin yield were recorded higher percentage of B1 toxin was produced which is great interest for aflatoxin studies.

3.3 Quantitative analysis of aflatoxins in A. flavus strains originated from corn grains using HPLC

Aflatoxin(s) B_1, B_2 and/or G_2 was (were) detected in the 11 strains analysed. However, AFB_1 concentration was higher in all strains than both AFB_2 and AFG_2. Only one strain (AUMC 9783) yielded only AFB_1 and 4 strains (AUMC nos. 9786, 9790, 9796 & 9797) yielded both AFB_1 and AFB_2. On the other hand, only 2 yielded both AFB_1 and AFG_2 while 4 gave the three types of toxins (B_1, B_2 and G_2) (**Table 3**). Among the 11 strains analyzed, AUMC strain no. 9793 gave the highest aflatoxin concentrations for the three types of toxins (279 mgL^{-1} AFB_1, 4.58 mgL^{-1} AFB_2 and 1.69 mgL^{-1} AFG_2). Strains no. 9785 and 9794 yielded the lowest concentrations of toxins (**Table 3**). In this respect, Perrone *et al.* [38] estimated aflatoxin production using HPLC in 135 strains of *Aspergillus* sect. *Flavi* (110 *A. flavus*, 20 *A. tamarii*, 2 *A. wentii*, 2 *A. flavofurcatus* and 1 *A. parvisclerotigenus*) from maize in Nigeria and Ghana and found that only

25 strains of the 110 *A. flavus* strains were the only strains that produced aflatoxin B1, but none produced any G series aflatoxins, and 16 of the 25 B_1 producing strains produced both B_1 and B_2. Neither the 20 *A. tamarii* strains nor the 2 *A. flavofurcatus* and the 2 *A. wentii* strains produced any aflatoxins. The single strain of *A. parvisclerotigenus* produced all four aflatoxins (B_1, B_2, G_1 & G_2).

In full agreement with our results, Fakruddin *et al.* [39] found that the 15 *A. flavus* strains isolated from poultry feed and grains in Bangladesh and tested for their aflatoxin-producing ability on Harra *et al.* [40] medium and observed as zones of diffusible aflatoxin around the fungal colony under long wave (365) UV light were positive. Also toxin-producing ability of cultures grown on Czapek-yeast extract agar medium extracted and estimated by HPLC showed that 11 isolates were capable to produce both types of toxins (AFB_1 and AFB_2) and isolates originated from grains showed more aflatoxin-producing capability of producing than those from feed [39].

Table 2: Aflatoxin concentrations of *A. flavus* strains recovered from peanut as detected by HPLC**.**

Species	**AUMC No.**	**Source**	**Isolation medium**	**Concentration of aflatoxins in 50 ml medium (**mg/L**)**		
				B_1	B_2	Total
A. flavus	9768	Assiut	AFPA	52.297	1.452	53.749
A. flavus	9769	Assiut	AFPA	464.451	15.095	479.546
A. flavus	9770	Assiut	AFPA	292.175	5.462	297.637
A. flavus	9771	Assiut	AFPA	16.626	0.103	16.729
A. flavus	10135	Assiut	DRBC	72.459	1.496	73.955
A. flavus	9801	Assiut	DRBC	167.988	2.683	170.671
A. flavus	9772	Assiut	DRBC	31.447	4.098	35.545
A. flavus	9773	Assiut	DRBC	121.341	1.290	122.631
A. flavus	9778	Sohag	AFPA	78.3537	1.833	80.1867
A. flavus	9779	Sohag	AFPA	1746.138	33.482	1779.62
A. flavus	9780	Sohag	DRBC	283.065	7.229	290.294
A. flavus	10134	Sohag	DRBC	NT	NT	---
A. flavus	9781	Sohag	DRBC	972.805	16.400	989.205
A. flavus	9782	Sohag	DRBC	102.175	1.364	103.539
A. flavus	9803	Sohag	DRBC	145.823	1.598	147.421

*All *A. flavus* strains fluoresced blue at 365 nm on CAM, except AUMC 9769 gave greenish yellow flourscence in the work of Ismail *et al.* (2016). AFPA= Aspergillus flavus/parasiticus agar medium, DRBC= Dichloran rose Bengal chloramphenicol agar medium.

Table 3: Aflatoxin concentrations of *A. flavus* strains recovered from corn as detected by HPLC.

Species	AUMC No.	Source	Isolation medium	Concentration of aflatoxins in 50 ml medium (μgL^{-1})			
				B1	B2	G2	Total
A. flavus	9783	Assiut	AFPA	60	-	-	60.0000
A. flavus	9784	Assiut	AFPA	147.5	1.385	0.6150	149.5000
A. flavus	9785	Assiut	AFPA	14.8	0.126	0.0353	14.9613
A. flavus	9786	Assiut	DRBC	67.45	0.4915	-	67.9415
A. flavus	9787	Assiut	DRBC	116.85	1.383	0.20975	118.4427
A. flavus	9788	Assiut	DRBC	67.5	-	0.0987	67.5987
A. flavus	9790	Sohag	AFPA	173.95	3.135	-	177.0850
A. flavus	9793	Sohag	AFPA	279	4.5795	1.685	285.2645
A. flavus	9794	Sohag	DRBC	14.335	-	1.11	15.4450
A. flavus	9796	Sohag	DRBC	227.3	0.3688	-	227.6688
A. flavus	9797	Sohag	DRBC	106.4	1.014	-	107.414

* Legends as below **Table 2**.

3.4 Quantitative analysis of aflatoxins in A. flavus strains originated from wheat grains using HPLC

Quantitative analysis of aflatoxins produced by *A. flavus* strains recovered from wheat grain samples revealed that aflatoxin(s) B_1, B_2 and/or G_2 was (were) detected in the 12 strains analyzed. However, 8 out of the 12 produced the three toxins (aflatoxins B_1, B_2 and G_2) (**Table 4**). AFB_1 concentration was higher in all strains than AFB_2 and/or AFG_2. Two strains (AUMC 9816 & AUMC 9848) produced aflatoxins B_1 and B_2 but only one strain (AUMC 9850) produced aflatoxins B_1 & G_2 and one (AUMC 9854) produced only aflatoxin B_1. The promising strains which yielded high concentrations of aflatoxins were strains AUMC 9816 (produced both aflatoxins B_1 & B_2), AUMC 9806, AUMC 9810 & AUMC 9813 (the three toxins) (**Table 4**). In this respect, Al-Wadai *et al.* [41] screened 19 isolates of *Aspergillus flavus* originated from wheat grains collected from 3 main regions in Saudi Arabia for their ability to produce aflatoxins using HPLC. Thirteen out of the 19 isolates produced aflatoxins ranging from 0.5 to 2.6 $\mu g\ kg^{-1}$.

However, isolates originated from food or feed were of low capability of producing aflatoxins than those originated from peanuts, corn or wheat as reported by Fente *et al*.[42] who detected aflatoxins after 3 days using HPLC of 7 chloroform YES extracts (out of 11 extracts) from strains of *Aspergillus parasiticus*, *A. flavus*, *A. flavus* group, however extracts from strains from *A. flavus* (4 strains), *A. ochraceus* (1), *A. versicolor* (2) were non-producers. At the same time these results confirmed those obtained on aflatoxin-producing ability (APA) medium after 10 days. Also, Alinezhad *et al.* [43] reported that among 37 *A. flavus* isolates obtained from trout pellet feed and feed ingredients and tested on aflatoxin-producing ability medium (APA) supplemented with

0.3% *β*-cyclodextrin under UV light (365 nm), only 12 isolates produced blue fluorescence under UV light.

Also, when these 37 isolates were tested by HPLC, only 19 (51.35%) were able to produce AFB_1 on YES broth in the range of 10.2 to 612.8 $\mu g g^{-1}$ fungal dry weight.

At the time, HPLC analyses of trout feed showed that pellet feed and all feed ingredients tested except gluten were contaminated with different levels of AFB1 in the range of 1.83 to 67.35 µg kg^{-1}, the range of AFB1 produced in wheat was 2.06 – 23.99 µg kg^{-1}.

Table 4: Aflatoxin concentrations of *A. flavus* strains recovered from wheat as detected by HPLC.

Species	**AUMC No.**	**S urc e**	**Isolation medium**	**Concentration of aflatoxins in 50 ml medium (µg L^{-1})**			
				B1	B2	G2	total
A. flavus	9806	Assiut	AFPA	737.4	25.2	78.8	841.4
A. flavus	9808	Assiut	AFPA	NT	NT	NT	--
A. flavus	9810	Assiut	AFPA	614.4	15.05	84.35	713.8
A. flavus	9813	Assiut	AFPA	563.45	15.6	87.05	666.1
A. flavus	10133	Assiut	DRBC	NT	NT	NT	--
A. flavus	9816	Assiut	DRBC	3468.25	28.85	-	3497.1
A. flavus	9817	Assiut	DRBC	3.05	0.05	0.6	3.70
A. flavus	9843	Assiut	AFPA	4.3	0.05	0.55	4.90
A. flavus	9848	Sohag	AFPA	12.2	0.25	-	12.45
A. flavus	9849	Sohag	AFPA	NT	NT	NT	--
A. flavus	9850	Sohag	AFPA	0.45	-	0.35	0.80
A. flavus	9851	Sohag	AFPA	NT	NT	NT	--
A. flavus	9852	Sohag	AFPA	NT	NT	NT	--
A. flavus	9853	Sohag	AFPA	309.1	5.05	54.65	368.8
A. flavus	9854	Sohag	DRBC	47	-	-	47
A. flavus	9855	Sohag	DRBC	39.55	0.95	0.1	40.6
A. flavus	9856	Sohag	DRBC	331.8	6.25	32.9	

* Legends as below **Table 2**

Conclusion

It could be concluded that HPLC with fluorescent detection is able to determine the concentration for the toxins (B_1, B_2 and G_2) with good accuracy and precision in the extracts of *A. flavus* strains of Peanuts, corn and wheat grains. Our community is polluted by these toxins which have a dangerous hazardous effect on the human health.

References

[1] G. Perrone, M. Hadukowski, G. Stea, F. Epifani, R. Bandyopadhyay, J. Leslie and A. J.P.F. D'Mello and A.M.C. MacDonald. Anim. Feed Sci. Technol. 69(1997) 155.

[2] H.S. Hussein and J. M. Brasel. Toxicol. 167(2001) 101.

[3] H.N. Mishra and C. Das. Critical Reviews in Food Science and Nutrition 43(2003) 245.

[4] J.P. Jouany. Animal Feed Sci. Technol. 137 (2007) 342.

[5] W.O. Ellis, J.P. Smith, J.P. Simpson and J.H. Oldham. Crit. Rev. Food Sci. Nutr. 30(1991) 404.

[6] H. Gourama and L. Bullerman. A review. J. of Food Protection 58(1995) 1395.

[7] F. Begum and N. Samajpati. Naturwissenschaften 87(2000) 275.

[8] S. A. Bankole, B.M. Ogunsanwo and O.O. Mabekoje. Food Chem. Toxicol. 42(2004)1309.

[9] I. Rasooli and M.R. Abyaneh. Food Contr. 15(2004) 479.

[10] R.R.M. Paterson. Food Cont. 18(2007) 817.

[11] B. Romagnoli, V. Menna, N. Gruppioni and C. Bergamini. Food Contr. 18(2007) 697.

[12] L.R Ferguson. Curr. Opin. Clin. Nutr. Metab. Care 12(2009) 343.

[13] Y. Wen, H. Hatabayashi, H. Arai, H.K. Kitamoto and Y. Kimiko. Appl. Environ. Microbiol. 71 (2005) 3192.

[14] P. A. Murphy, S. Hendrich, C. Landgren and C.M. Bryant. An Update. J. Fd. Sci. 71(2006) 51.

[15] J.C. Frisvad, U. Thrane, R.A. Samson and J.I. Pitt. Adv. Exp. Med. Biol. 571(2006) 30.

[16] D. Bhatnager, G.Payne, J.E. Linz, and I.E. Clevland. Inform. 6(1995) 262.

[17] M.E. Zain. J. Saudi Chem. Soc. 15(2) (2010) 129.

[18] D.L. Park and T.C. Troxell. Adv. Exp. Med. Biol. 504(2002) 277.

[19] W.J. De Koe. Arh. Hig. Rada. Toksikol. 50(1999) 37.

[20] E.M. Binder, L.M. Tan, L.J. Chin, J. Handl and J. Richard. Animal Feed Sci. Technol. 137(2007) 265.

[21] J.W. Bennett and M. Klich. Clin. Microbiol. Rev. 16(2003) 497.

[22] H.K. Abbas, R.D. Cartwright, W.

Xie and W.T. Shier. Crop Prot. 25(2006) 1.

[23] M. Castells, S. Marín, V. Sanchis and A.J. Ramos. Int. J. Food Microbiol. 123(2008) 81.

[24] S. Udagawa. Nippon Ishinkin Gakkai Zasshi. 46(2005) 11.

[25] J.I. Pitt and A.D. Hocking. 3rd ed., Springer, (2009) pp.519.

[26] G.J. Speijers and M.H. Speijers. Toxicol. Lett. 153(1) (2004) 91.

[27] A. Molinie, V. Faucet, M. Castegnaro and A.P. Leszkowicz. Food Chemistry 92(2005) 391.

[28] M.A. Ismail, N.T. Abo El-Maali, G.A. Omran and N.M. Nasser. J of Microb, Biotech and Food Sci. 5 (4) (2016) 314.

[29] N.D. Davis, S.K. Iyer and U.L. Diener. App and Environ Microbio 53(7) (1987) 1593.

[30] I. A. El-kady, S.S.M. El-Maraghy and A.A. Zohri. Mycopatholog. 113(1991) 165.

[31] P.B. Ouattara–Sourabie, P. A. Nikiema, N. Barro, A. Savadogo and A. S. Traore. African J of Microbio Res 6(11) (2012) 2603.

[32] Y. Sultan and N. M. Magan. Mycot Res 6 (2010) 133.

[33] L. Afsah–Hejri, S. Jinap and S. Radu. J of Food, Agri & Environ 11 (3&4) (2013) 228.

[34] M. Saito and S. Machida. Mycoscience 40(2) (1999) 205.

[35] A. Abdel- Hadi, D. Carter and N. Magan. World Mycotoxin J 4(2011) 69.

[36] G. Ravi Babu, M.G. Prasad and T.N.V.K.V. Prasad. Intrernational Journal of Pure and App Sci and Tech 5(1) (2011) 16.

[37] P. Rajarajan, K.M. Rajasekaran and N.K. Asha Devi. Indian J of Pharma and Biolog Res 1(4) (2013) 76.

[38] G. Perrone, M, Hadukowski, G. Stea, F. Epifani, R. Bandyopadhyay, J. Leslie and A. Logrieco. Food Microbio 41(2014) 52.

[39] M. Fakruddin, A. Choudhury, M.N. Hossain and M.M. Ahmed. SpringerPlus 4(2015) 159.

[40] S. Hara, D.I. Fennell and C.W. Hesseltine. Appl Microbio 27(6) (1974) 1118.

[41] A.S. Al-Wadai, M.R. Al-Othman, M.A. Mahmoud and A.R.M. Abd El-Aziz. Gen and Mol Res 12(3) (2013) 3335.

[42] C.A. Fente, J. Jaimez Ordaz, B.I. Vazquez, C.M. Franco and A. Cepeda. Applied and Environ Microbio 67(10) (2001) 4858.

[43] S. Alinezhad, M. Tolouee, A. Kamalzadeh, A.A. Motalebi, M. Nazeri, M. Yasemi, S.A. Bankole, B.M. Ogunsanwo and O.O. Mabekoje. Food Chem. Toxicol. 42(2004) 1309.