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AFLATOXINS AND AFLATOXIGENIC FUNGI OCCURRENCE IN ANIMAL AND POULTRY FEED OF ASSIUT GOVERNORATE FARMS, EGYPT

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ABSTRACT

Sixty representative pelleted animal and poultry feed samples were collected from Assiut governorate farms for aflatoxins (B_1, B_2, G_1, and G_2) analysis by thin layer chromatography (TLC) and ultra-performance liquid chromatography (UPLC) in the EGAC-accredited central lab. of the Faculty of Veterinary Medicine, Assiut University, as well as for their aflatoxigenic fungi prevalence assessment. TLC results showed that 35% of the analyzed feed samples were positive for AFs. UPLC results showed that means ± SE of AFB_1, AFB_2, AFG_1, AFG_2 and total AFs were 23.36±13.12, 2.88±1.07, 1.33±0.64, 0.52±0.25 and 28.08±13.98 µg kg^{-1} in animal feed and 34.88±25.18, 2.55±0.78, 1.80±1.51, 1.30±0.52 and 40.83±25.59 µg kg^{-1} in poultry feed, respectively. The prevalence of aflatoxins was 70% and 100% in animal feed and poultry feed samples, respectively. *Aspergillus flavus* isolates from the analyzed feed samples showed different degrees of aflatoxigenic ability on coconut agar medium, and the poultry feed samples were more contaminated with AFs and aflatoxigenic fungi than animal feed samples.

Key words: Aflatoxins; animal feed; poultry feed; TLC; UPLC

INTRODUCTION

Fungi not only cause the deterioration of food and feed but also can produce mycotoxins as secondary toxic metabolites. According to the Food and Agriculture Organization (FAO), mycotoxins contaminate 25% of the world's crops (Pankaj *et al.*, 2018). Mycotoxins have threatened the poultry industry all over the world (Diaz and Murcia, 2011), especially aflatoxins (AFs), which act as the most toxic category among various types of mycotoxins and are produced mainly by *Aspergillus falvus* and *Aspergillus parasiticus* (Nakai *et al.*, 2008). In food, aflatoxins B_1, B_2, G_1, G_2, M_1, and M_2 are ubiquitous (Sumit *et al.*, 2010), but in animal and poultry feed stuffs, only AFB_1, B_2, G_1, and G_2 are the major naturally occurring aflatoxins. Aflatoxin B_1 is the most potent and toxic one, followed by G_1, B_2, and G_2 (Bryden, 2007).

Aflatoxin is the most potent mycotoxin due to its acute toxicological impacts and chronic hepatocellular carcinoma. The International Agency for Research on Cancer (IARC) classified aflatoxins as human carcinogens in "group 1," except AFM_1, which was classified as possibly carcinogenic to humans

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in "group 2B" (IARC, 2010). In addition to their carcinogenic effects, AFs have genotoxic, cytotoxic, mutagenic, and teratogenic effects (Deabes *et al.,* 2012). Poultry are the most vulnerable to aflatoxicosis, which can impair their production and reproduction parameters and result in significant economic loss (Hussain *et al.,* 2010).

More than 100 countries have established regulations for AFs on food and feed imports due to their serious impacts on humans, animals, and the worldwide economy. Contamination of food and feed by aflatoxins threatens food security for humans and animals (Udomkun *et al.,* 2017)**.** According to the European Food Safety Authority (EFSA), no tolerable daily intake (TDI) for aflatoxins was established due to the highly toxic effects of this group of mycotoxins (EFSA, 2020).

The current Egyptian regulatory limits for total AFs and AFB1 in animal feed are 20 µg kg^{-1} and 10 µg kg^{-1}, respectively (Egyption Standards, 2010). According to the European Union, the maximum tolerable levels for total AF and AFB_1 in cereals for human consumption are 4 and 2 µg kg^{-1}, respectively, and 20 µg kg^{-1} for total AF in poultry feeds (EFSA 2010).

These regulatory limits obstruct crops' export and import from different parts of the world; thus, our study aimed to evaluate the AFs level in animal and poultry feed in Upper Egypt with TLC and UPLC and detect its validity according to national and international permitted limits. Animal and poultry feed was intended to be chosen in the current study due to the further residues of AFs in animal and poultry products, especially milk, meat, and eggs.

MATERIALS AND METHODS

1. Chemicals

- Chemicals used for aflatoxins analysis were of analytical reagent grade and included dichloromethane, chloroform, acetone, methanol, acetonitrile, sodium sulphate anhydrous, acetic acid, and deionized water. Thin-layer chromatography aluminate silica gel plates were obtained from Merck (Germany) and aflatoxins standards (B_1, B_2, G_1, and G_2) from Wesel (Germany).
- *Aspergillus flavus* and *parasiticus* agar media were prepared in the lab. according to Pitt and Hocking (2009) used as a selective media for *Aspergillus* spp. and coconut agar media also was prepared in the lab. according to Yazdani *et al.* (2010) and they were used as a screening method for the aflatoxigenic ability of *Aspergillus* spp.

2. Sampling and sample preparation

Sixty representative pelleted feed samples (30 animal feed and 30 poultry feed samples) were collected in proper paper bags from twenty Assiut governorate farms through 2022. During the analysis, each of the three samples per farm was mixed and represented with one sample. Each sample was ground in a laboratory mill and used for aflatoxins analysis and mycological examination.

3. Detection of aflatoxins in animal and poultry feeds

3.1. Using Thin Layer Chromatography

Finely ground feed samples were prepared for immediate aflatoxins analysis on TLC, according to Braicu *et al.* (2008). Then the plates were viewed under long-wavelength UV light (365 nm) for AF fluorescence detection. Aflatoxins were identified by comparing them to aflatoxin standards on a TLC plate. The retention factor (RF) on TLC was estimated according to Abd-Elaah and Samya (2005) as the following equation:

$$Retention\ factor = \frac{\text{Distance moved by substance (spot)}}{\text{Distance moved by the solvent (solvent front)}}$$

3.2. Using Ultra-Performance Liquid Chromatography

Thin layer chromatography was followed with an ACQUITY® UPLC H-class system equipped with an ACQUITY BEH C18 column (2.1 x 100 mm, particle size 1.7 µm) with a fluorescence detector (excitation 365 nm and emission 429 nm), 64 water, 18 methanol, and 18 acetonitrile (HPLC grade) mobile phases, and a 0.4 ml/min flow rate, 1 µl injection volume, and a 4-minute running time according to the method described by Benvenuti and Burgess (2010) in the central laboratory of the Faculty of Veterinary Medicine at Assiut University.

The LOQ was 0.02, 0.03, 0.16, and 0.20 ng ml^{-1}; the LOD was 0.0061, 0.0100, 0,0539, and 0.0659 ng ml^{-1}; recovery % was 90.39, 94.47, 87.82, and 87.14; and repeatability% was 85.35 ± 3.83, 86.84 ± 6.87, 93.77 ± 7.96, and 89.77 ± 8.2 for AFB_1, AFB_2, AFG_1, and AFG_2, respectively.

3.3. Standard preparation

According to Benvenuti & Burgess (2010), standard working solutions were immediately prepared by diluting an appropriate amount of Mycotoxin Mix 1 (Aflatoxin) standard with 100% methanol, where it is light sensitive and storage occurs at -8 to -22ºC or below. Mycotoxin Mix 1 (Aflatoxins) standard included AFB_1, AFG_1, AFB_2, and AFG_2 with 2.00 µg/ml, 2.01 µg/ml, 0.500 µg/ml, and 0.503 µg/ml concentrations, respectively.

4. Isolation of *Aspergillus* species from feed samples on *Aspergillus flavus parasiticus* agar (AFPA) medium

Ten grams of each milled feed sample were mixed with 90 ml of distilled water and shaken for 20 minutes, followed by the culturing of 1 ml on AFPA medium as a selective medium for *Aspergillus flavus* and *Aspergillus parasiticus* (dissolve dichloran 2 mg, ferric ammonium citrate 0.5 g, peptone 10 g, yeast extract 20 g, agar 15 g, and 100 mg chloramphenicol in one liter of distilled water by heating till boiling on a hot plate, then autoclave for 15 minutes at 121ºC), followed by cooling, pouring in Petri dishes, culturing the samples, and incubating for 7 days in the dark at 25–28ºC.

5. Determination of the aflatoxigenic ability of *Aspergillus* spp. on coconut agar medium (CAM):

Fungi were identified by their colonial morphology and microscopic characteristics. After that, their aflatoxigenic ability in CAM was determined (100 g of shredded coconut was homogenized for 5 minutes with 300 ml hot distilled water, then the homogenate was filtrated through four layers of cheesecloth, the pH of the clear filtrate was adjusted to 7, then completed to 1000 ml distilled water and add 20 g agar, the medium was autoclaved for 15 minutes at 121ºC, cooled to about 40–45ºC, poured into Petri dishes, and the isolates were cultured).

Aspergillus spp. isolates were inoculated centrally in a petri dish containing 10–15 ml of CAM and incubated for 7 days in the dark at 25ºC. Cultures were observed for fluorescence under long-wave UV light (365 nm) after 3, 5, and 7 days. The positive results were shown as a blue fluorescence zone around colonies of *A. flavus* with different intensities (Yazdani *et al.*, 2010)**.**

6. Statistical analysis:

Results were expressed as a mean ± SE or SD. Aflatoxins level results were analyzed statistically using the computer program SPSS for Windows, version 16.0, through analyses of variance (Green & Salkind, 2010).

RESULTS

1. Detection of aflatoxins in animal and poultry feeds with TLC and UPLC

Animal and poultry feed samples were analyzed for aflatoxins with TLC, followed by UPLC. Poultry feed samples were more contaminated with AFs than animal feed samples **(Figure 1)**. TLC results showed that 35% of the analyzed feed samples were positive for AFs, compared to the standard by UV lamp **(Tables 1, 2, and Figure 2)**.

UPLC results showed that aflatoxin B_1, B_2, G_1, G_2, and total AFs prevalence were 70%, 70%, 40%, 40%, and 70% in animal feed and 100%, 100%, 20%, 60%, and 100% in poultry feed samples, respectively. Mean ± SE of AFB_1, AFB_2, AFG_1, AFG_2, and total AFs were 23.36 ± 13.12, 2.88 ± 1.07, 1.33 ± 0.64, 0.52 ± 0.25, and 28.08 ± 13.98 µg kg^{-1} in animal feed samples and 34.88 ± 25.18, 2.55 ± 0.78, 1.80 ± 1.51, 1.30 ± 0.52, and 40.83 ± 25.59 µg kg^{-1} in poultry feed samples, respectively. A comparison of the previous results with AF's regulatory permissible limits showed that about 35% and 53% of positive samples exceeded EU and Egyptian regulatory permissible limits, respectively **Table (1 and 2).**

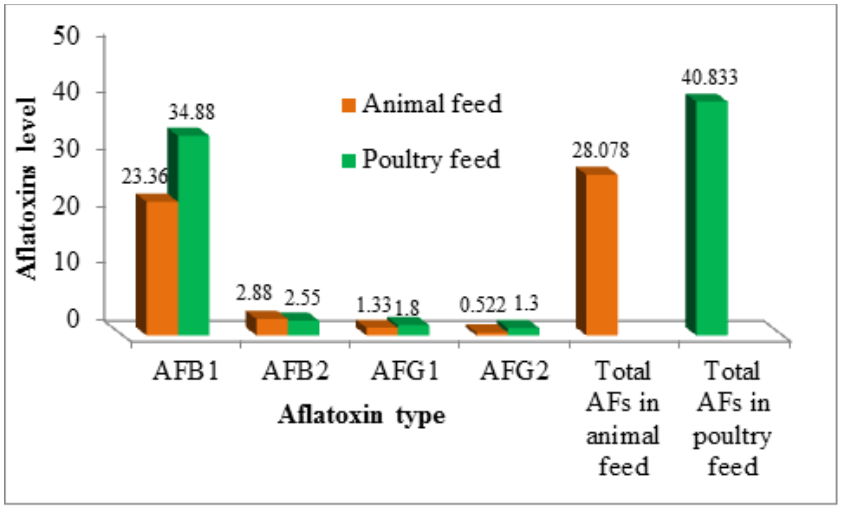

Figure 1: Comparison between AFs level (µg kg^{-1}) in animal and poultry feed samples.

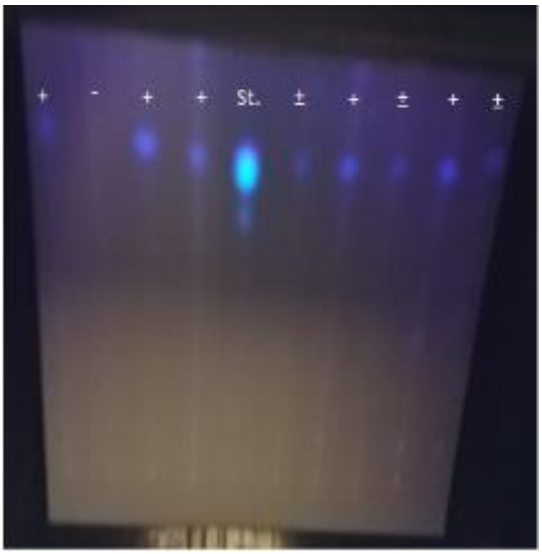

Figure 2: Thin layer chromatography results of feed samples were either negative (-), suspect (±), or positive (+) for AFs compared with standard (St.).

Table 1: Aflatoxin level (µg kg^{-1}), TLC, CAM results, and acceptance in animal feed samples according to permissible regulations

Animal feed samples	UPLC Aflatoxins level (µg kg^{-1})					TLC	CAM	European Union Acceptance	Egyptian Regulation Acceptance
	AFB_1	AFB_2	AFG_1	AFG_2	Total AFs				
1	4.43	1.7	3.5	1.8	11.43	±	+	√	√
2	6.3	3.2	0.82	ND	10.32	±	+	√	√
3	ND	ND	ND	ND	ND	-	+	√	√
4	28.5	5.4	ND	ND	33.9	+	+	×	×
5	46.7	8.31	3.82	1.96	60.79	+	+	×	×
6	ND	ND	ND	ND	ND	-	+	√	√
7	133.3	8.62	ND	ND	141.7	++	++	×	×
8	ND	ND	ND	ND	ND	-	-	√	√
9	4.3	0.74	ND	0.8	5.84	-	-	√	√
10	10.1	0.88	5.2	0.66	16.8	±	+	√	×
Mean	**23.36**	**2.88**	**1.33**	**0.52**	**28.08**				
SE	**13.12**	**1.07**	**0.64**	**0.25**	**13.98**				
Prevalence	**70%**	**70%**	**40%**	**40%**	**70%**				
Min.	**ND**	**ND**	**ND**	**ND**	**ND**				
Max.	**133.3**	**8.62**	**5.2**	**1.96**	**141.7**				

Ultra Performance Liquid Chromatography aflatoxin levels in animal feed samples (µg kg^{-1}) were represented as means ± SE, (ND): Non-Detected. Thin layer chromatography results in feed samples were represented with negative (-), suspect (±), positive (+), or highly positive (++) for AFs. According to European Union and Egyptian regulations, animal feed samples were either accepted (√) or rejected (×). On CAM, *A. flavus* isolates were highly toxigenic (++), moderately toxigenic (+), or atoxigenic (-)

Table 2: Aflatoxin level (µg kg^{-1}), TLC, CAM results, and acceptance in poultry feed samples according to permissible regulations

Poultry feed samples	UPLC Aflatoxins level (µg kg^{-1})					TLC	CAM	European Union Acceptance	Egyptian Regulation Acceptance
	AFB_1	AFB_2	AFG_1	AFG_2	Total AFs				
1	13.32	1.74	ND	ND	15.06	+	+	√	×
2	6.3	0.8	ND	1.8	8.9	±	+	√	√
3	1.185	0.735	ND	2.185	4.105	-	+	√	√
4	260.669	6.914	ND	ND	267.58	++	++	×	×
5	20.4	3	ND	ND	24.717	+	+	×	×
6	3.5586	4.317	ND	ND	4.335	-	+	√	√
7	6.6	0.776	ND	2.03	10.43	±	-	√	√
8	4.9	1.8	ND	0.2	5.5	-	+	√	√
9	21.778	0.4	15.171	5.101	51.574	+	++	×	×
10	10.13	6.524	2.81	1.66	16.13	±	+	√	×
		1.53							
Mean	**34.88**	**2.55**	**1.80**	**1.30**	**40.83**				
SE	**25.18**	**0.78**	**1.51**	**0.52**	**25.59**				
Prevalence	**100%**	**100%**	**20%**	**60%**	**100%**				
Min.	**1.185**	**0.4**	**ND**	**ND**	**4.105**				
Max.	**260.669**	**6.914**	**15.171**	**5.101**	**267.583**				

Ultra Performance Liquid Chromatography aflatoxin levels in poultry feed samples (µg kg^{-1}) were represented as means ± SE, (ND): Non-Detected. Thin layer chromatography results in feed samples were represented with negative (-), suspect (±), positive (+), or highly positive (++) for AFs. European Union and Egyptian regulations showed that animal feed samples were either accepted (√), or rejected (×). On CAM, *A. flavus* isolates were highly toxigenic (++), moderately toxigenic (+), or atoxigenic (-)

2. Isolation of *Aspergillus* species on AFPA and determination of their aflatoxigenic ability on CAM

On AFPA medium, *Aspergillus* niger, *Aspergillus flavus*, and *Aspergillus terreus* were the most frequent *Aspergillus* spp., which were 56%, 33%, and 11%, respectively, in animal feed samples, but in poultry feed samples only *Aspergillus niger* (60%) and *Aspergillus flavus* (40%) were observed **(Figure 3)**. *Aspergillus parasiticus* was not isolated in poultry and animal feed samples during the current study.

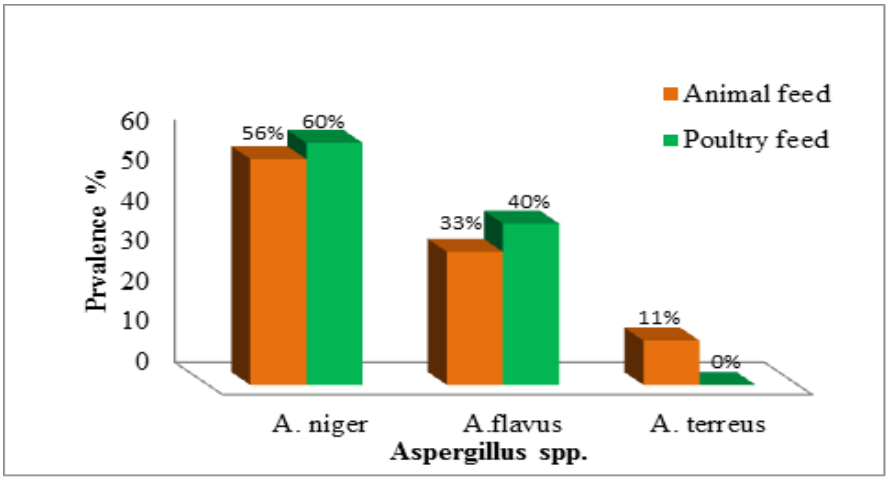

Figure 3: Prevalence % of *Aspergillus* species (*A. niger*, *A. flavus*, and *A. terreus*) in animal and poultry feeds.

Thin-layer chromatography and coconut agar medium are used for aflatoxins and aflatoxigenic fungi presumptive analysis, respectively. On CAM, *Aspergillus flavus* isolates were tested for AFs-producing potential, and results indicated that 85% of *A. flavus* isolates had suitable conditions to produce aflatoxins in feed with different blue fluorescent haloes around the *Aspergillus flavus* colony according to (**Table 1, 2 and Figure 4**).

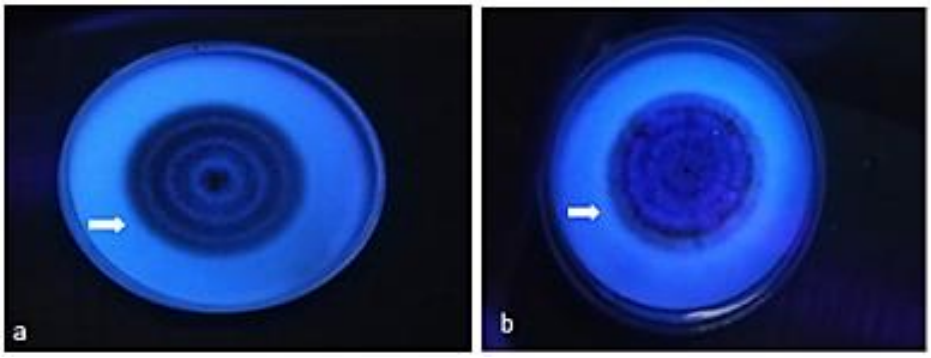

Figure 4: *A. flavus* isolates on coconut agar medium showing (a) moderately toxigenic and (b) highly toxigenic isolates based on the degree of blue fluorescent halo around the *Aspergillus flavus* colony (arrow).

DISCUSSION

A contaminated feed with aflatoxigenic fungi and aflatoxins is considered a health risk for humans and animals, where aflatoxins are the most toxic and potent secondary fungal metabolites.

In the current study, TLC analysis results for animal and poultry feed samples confirmed the findings of Kotinagu *et al.* (2015) that

30% of livestock feed and feed ingredients were contaminated with AFB_1. However, Ramesh *et al.* (2013) results were higher than the results of our study, where 79.66% of feed samples were positive for aflatoxin B_1 on TLC.

UPLC results showed that aflatoxin B_1, B_2, G_1, G_2, and total AFs prevalence were 70%, 70%, 40%, 40%, and 70% in animal feed and 100%, 100%, 20%, 60%, and 100% in poultry feed samples, respectively. Mean ± SE of AFB_1, AFB_2, AFG_1, AFG_2, and total AFs were 23.36 ±13.12, 2.88 ± 1.07, 1.33± 0.64, 0.52±0.25, and 28.08 ± 13.98 μg kg^{-1} in animal feed samples and 34.88 ± 25.18, 2.55 ± 0.78, 1.80 ± 1.51, 1.30 ± 0.52, and 40.83 ± 25.59 μg kg^{-1} in poultry feed samples, respectively**.** These results agreed with previous studies of poultry feeds where aflatoxins' occurrence was between 64% and 100% (Aboagye-Nuamah *et al.,* 2021; Kana *et al.,* 2013; Mokubedi *et al.,* 2019; Taylor & Ezekiel, 2012)**.**

Taylor and Ezekiel (2012) showed that among the regulatory detected toxins in poultry feed in Nigeria's districts and states, aflatoxins had a prevalence of 62% with levels above 20 μg kg^{-1}. In Cameroon, the prevalence of aflatoxins in mixed poultry feeds was 93.3% and 83% at levels 2 to 52 μg/kg and 2 to 23 μg/kg in broiler and layer feed samples, respectively (Kana *et al.,* 2013).

Mokubedi *et al.* (2019) analyzed different mycotoxins in poultry feed samples from South Africa and observed that the AF incidence was 92%, with generally low concentration levels ranging from 0.1 to 3.7 μg/kg.

In sub-Saharan Africa, Kemboi *et al.* (2020) showed that aflatoxins' occurrence in feed and feed ingredients was 70%, with a range of 0.2-318.5 μg/kg. According to Nakavuma *et al.* (2020)**,** aflatoxins levels in poultry feed produced by feed processors and farmers were 7.5 ± 0.71 to 393.5 ± 19.09 and 19.0 ± 1.41 to 188.5 ± 2.12 μg kg^{-1}, respectively.

Our study results also compared with Abdelhamid (1990) who observed that peanuts have the highest contamination mean of AFB_1 (400 μg kg^{-1}), but the lowest AFB_1 mean was in soybean samples (5 μg kg^{-1}). Salem (2002) showed that total AF levels in feedstuff samples from Assiut province were between 2 and 60 μg kg^{-1}. Njobeh *et al.* (2012) revealed that the incidence of AFs was 30% with a 0.2–71.8 μg kg^{-1} range and a 9.0 μg kg^{-1} mean in South African compound feeds, and Abdallah *et al.* (2017) showed that only AFB_1 was present in commercial feed samples in upper Egypt with a 4% incidence and 11 μg kg^{-1} as the maximum level, and only one of the positive samples was above the maximum Egyptian regulatory limits, which were lower than our total AFs mean.

The maximum permissible level of aflatoxins in food and feed was set by various national and international organizations to provide food security and safety for consumers (Udomkun *et al.,* 2017). A comparison of our results with AF's regulatory permissible limits showed that about 35% and 53% of positive samples exceeded EU and Egyptian regulatory permissible limits, respectively. Kang'ethe and Lang'a (2009) reported that 67% and 58% of the AF-positive samples from farmers and grain millers exceeded the FAO recommended limits, respectively. According to the European regulatory limit for aflatoxins, Gruber-Dorninger *et al.* (2018) showed that 54.4% of different African countries' finished feed samples were unfit for consumption and exceeded permissible limits and Nakavuma *et al.* (2020) revealed that all the analyzed feed samples from feed processing plants were contaminated with aflatoxins, but only 18.8% were within the recommended limit and safe for consumption.

The prevalence of *Aspergillus* spp. and the ability of *Aspergillus flavus* isolates for AFs-production were tested, our results disagree with previous studies, as Aliyu *et al.* (2016) showed that the most prevalent fungal species isolated from poultry feeds in Sokoto

metropolis were *Aspergillus fumigatus* (58.8%), followed by *Aspergillus flavus* (41.2%), but Hassan *et al.* (2021) showed that *A. flavus* (62.5%) was the most predominant *Aspergillus* spp., followed by *A. niger* (25.9%), and *A. fumigatus* (15.8%). Only 57.3% of *A. flavus* isolates could produce AFs.

The production of aflatoxin is dependent on the growth of fungi strains and the availability of suitable environmental conditions. Moisture is the first factor where *A. flavus* can produce aflatoxins at a moisture content of 16%–28% (Dorner, 2008)**.** Temperature is the second factor, where; *A. flavus* can grow at 12–47°C, but the optimum temperature for aflatoxin production is 25–32°C (Smalley, 1986). Aflatoxins and aflatoxins-producing fungi have a high occurrence in tropical and subtropical regions where humidity and temperature conditions are optimal for toxin production (Jacobsen *et al.,* 2007) and contaminate different agricultural commodities that will be used in processing rations in the future (Rushing & Selim, 2019; Tajkarimi *et al.*, 2011).

The variation in aflatoxigenic and aflatoxin level incidence in the current study is not only due to differences in atmospheric conditions in Egypt and feed storage conditions, but also due to the ingredients of animal and poultry feed, where raw materials include corn, rice, wheat, soybeans, and other additives. This is based on animal or poultry needs and eating habits. These cereals are highly susceptible to aflatoxigenic and AF contamination, and most industrial processes do not detoxify aflatoxins.

CONCLUSION

This study revealed that pelleted poultry feed is more contaminated with AFs and aflatoxigenic fungi than pelleted animal feed. Thus, the continuous monitoring of AFs in food and feed by TLC and UPLC and strict application of permissible limits are necessary for human security and safety. Predisposing factors for AF production such as temperature, relative humidity, and moisture must be controlled, as in suitable conditions, aflatoxigenic fungi can produce different levels of AFs.

Declarations

Author Contribution

(1) Heba Fawzy Kamaly has contributed substantially to the practical section, the composition of the paper, data analysis, and statistical analysis. (2) Zakaria Mukhtar Zaky has contributed to the practical section and the revision of the manuscript.

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مدي تواجد الأفلاتوكسينات والفطريات المفرزة للأفلاتوكسينات في أعلاف الحيوانات والدواجن بمزارع محافظة أسيوط، مصر

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تعد الأفلاتوكسينات من أخطر السموم الفطرية التي تُهدد صحة الإنسان والحيوان علي مستوي العالم. أوضحت الدراسة الحالية مدي تواجد الأفلاتوكسينات والفطريات المفرزة لها في الأعلاف حيث تم تجميع ٦٠ عينة من أعلاف الحيوانات و الدواجن من مزارع محافظة أسيوط وتم تحليلها باستخدام تقنية كروماتوجرافيا الضغط العالي السائل بالمعمل المركزي بكلية الطب البيطري جامعة أسيوط وكشفت نتائج هذه الدراسة أن أعلاف الدواجن أكثر تلوثا بالأفلاتوكسينات والفطريات المفرزة لها من أعلاف الحيوانات حيث أوضحت نتائج كروماتوجرافيا الضغط العالي السائل أن متوسط مستوي الافلاتوكسينات بي ١، بي ٢، جي ١ وجي ٢ كانت ٢٣,٣٦±١٣,١٢، ٢,٨٨±١,٠٧، ١,٣٣ ± ٠,٦٤ و٠,٥٢±٠,٢٥ ميكروجرام / كيلو جرام في أعلاف الحيوانات و٣٤,٨٨±٢٥,١٨، ٢,٥٥±٠,٧٨، ١,٨٠±١,٥١ و ١,٣٠±٠,٥٢ ميكروجرام / كيلو جرام في أعلاف الدواجن علي الترتيب. كما أوضحت النتائج أيضا أن عزلات الأسبرجلس فلافس التي تم عزلها من هذه الأعلاف تتفاوت في قدرتها علي افراز الأفلاتوكسينات. ولذلك يتضح ضرورة الرصد المستمر لمستوي الأفلاتوكسينات في الأغذية والأعلاف باستخدام تقنية كروماتوجرافيا الضغط العالي السائل وتطبيق الحدود المسموح بها بصرامة من أجل أمن وسلامة الانسان وكذلك ضرورة التحكم في العوامل المساعدة علي انتاج الافلاتوكسينات ونمو الفطريات المفرزة لها ومنها درجة الحرارة والرطوبة والرطوبة النسبية.