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AFLATOXIN PRODUCED BY TOXIGENIC FUNGI ISOLATED FROM FEEDSTUFF OF ANIMAL DIETS

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Abstract

Our study was aimed out to identify the common fungi associated to feeds stuff of cows. Ten samples of feedstuff (wheat, bran) were collected from farms in which cows are feeds and analyzed for detecting associiated fungi, 4 predominant fungi such as, *Aspergillus flavus, Aspergillus niger Altrrnaria* spp., *cladosporium* spp. and *Rhizopus* spp were isolated. Among them, *Aspergillus flavus,* was the most incidence fungi at100% and frequency of 45%.Samples were under gone of production Mycotoxin (Aflatoxin B1) which cause harm hazards to animals and human,. Mycotoxins such as AfB1 considered carcinogenic there is a need for astimate and detection the toxin. different screening methods for investigating of mycotoxin production have been reported. These methods rely on using varial types of media, as PDA andused test of ammonium solution at 20%. also supports our results by using TLC to investigate ability of *Aspergillus flavus* tproduce mycotoxin AfB1.

Keywords **:** Feedstuff, Mycotoxin (AFB1), fungitoxic, TLC

Introduction

The main components of animal diets, such ascorn, wheat, barley and sunflower seeds, have been exposed to fungal infections during storage, especially the species belonging to the genus *Aspergillus, Penicillium, Fusarium* (Bothast *et al*., 1974). The infection of these fungi leads to the emergence of bad cases in the production of poultry and dairy cattle and agricultural products or their remnants and these damage is proportional to the severity of the injury to increase the probability of the secretion of these fungi to mycotoxins (Lynne, 1995)

The losses caused by aflatoxins are the loss of poultry when exposed to high doses of toxins and indirectly in the production of eggs. For meat and weight loss as well as for cows when fed on fodder fed with aflatoxins by 1.5 mg/kg of feed for two weeks leads to reduced milk production, diarrhea, liver damage, intestinal bleeding (Morehouse, 1979) and sheep.

Mycotoxins are Secondary metabolites important economically to humans. They are useful as antibiotics and harmful such as Mycotoxins and found More than 300 mycotoxin is produced by 350 known fungal species. Mycotoxin currently known are produced by the genus *Aspergillus, Fusarium, Penicillium* (Eskola, 2002; Abdou, Kh. *et al.,* 2017). Scientists have discovered that the most dangerous toxins to human and animal health are aflatoxins, ochratoxin, tericothicines, zeralenone, Fumonisin, and Ergot alkaloids. The oldest fungal toxin known in humans is Ergotism caused by *Clavicepis purpurea* in 943 CE in some European continental regions due to people consuming contaminated rye.

Aflatoxin

Aflatoxins are polyketide group and these toxins produce by *Aspergillus flavus and A. parasiticus.* The main types of aflatoxin found in agricultural products are aflatoxins B1, B2, G1 and G2 (lee and Hagler , 1991).. The International Organization for Research on Cancer (IARC) identified Aflatoxin B1 as being among the first group of compounds Chemical carcinogen is one of the causes of cancerous tumors of the human liver (IARC, 2002).

The toxic effects of aflatoxins in human and animals

Toxicity of Afb1 have been very important because of their effective effect on humans and animals. aflatoxin are one of the most important causes of liver cancer in humans (Groopman *et al*., 1991). It also has inhibitory effects on the immune system as well as its mutagenic effects and its congenital malformations (Massey *et al*., 1995).

Asynergistic relationship was found between some diseases and aflatoxins, including hepatitis B virus. Hepatitis B virus is found to be carcinogenic and has increased risk of liver cancer. The case was found to be the result of Hepatitis in India in 1974, which resulted in the injury of 400 people and the deaths of 100 of them, which was confirmed by the presence of aflatoxin (Krishnamachari *et al*., 1975). There was a positive relationship between the amount of aflatoxinsand effect, (Bhatnagar *et al*., 1999).

The toxicity of aflatoxins was identified by giving experimental animals animals diet contaminated with aflatoxins and observing the effects of these animals after their dissection, such as hemorrhagic and death of tissue in the liver after kidney congestion and rapid proliferation of epithelial cells found in the Bile (Yabe and Hamasaki, 1993)

AFB1 is the most dangerous, followed by AFG1but. Aflatoxin B2 and G2 are significantly less dangerous than AFB1. Aflatoxin M1 is a serious and dangerous cancer cause (Smith *et al*., 1994). The toxicity level of) for aflatoxin B1 can be determined as Lethyl Dose 50 (LD_{50}) on different types of animals

Fodder mixed for commercial feed production

The feed is usually mixed by large farmers with special equipment mixing the different grains and ingredients to form a high-protein whey, giving a mixture of energy and protein that is digestible and suitable for use, and usually the components of food concentrates and blended feeds are based on the animal's nutritional requirements and their price (Wood head publishing Limited, 2004)

Transmission of mycotoxins from animal feed to animals products in milk products

The most common incidence of aflatoxin in cattle milk and of high importance (Petterson 1997) its of AFB1, which is consumed by the animal through contaminated feed. A section of it is destroyed in the dairy cows' stomach and a section of them is re-absorbed by the cows and is rapidly metabolised from AFB1toAF M1 in the liver.

A number of studies have focused on or demonstrated the transfer of aflatoxins from feed to milk in the first study by (Applemen, 1982 and, Sieber, 1978). There is 0.18-3.44% less amounts of milk produced from cows infected with Aflatoxins than an infected cows and studies have been carried out.

The Afltoxin M1:

1. AFM1 is the metabolic product and AFB1 is produced in mammals
2. Found in milk and dairy products
3. Record in human breast milk especially in developed countries (South Asia, Africa, Middle East) (Mwanza *et al*., 2013).

Material and Methods

Samples preparing

All 10 samples of feedstuffs (wheat and bran) mixed well, which collected from farms of cows feeding, The samples were collected putted in polyethylene bags and kept in refrigerator at 4ºC until testing.

100gm of each samples mixed to homogenising, 10gm diet from every one kept in sterile small bag.

Isolation and Identification of fungi

To Isolate Fungi Associated with Wheat and Bran mixture by using potato dextrose agar medium (P.D.A) supplemented with cloramphenicol, after pouring the media in (9 cm) plates and left to hardening at room temperature, 1gm of the homogenized diet for each sample spread on the surface of plates, Then plates transferred to the incubator and incubated at 25 °C for 3-10 days and the fungal growth was monitored for 48 hours from the date of the treatment (Rapper and Fennel, 1966; Barnett and Hunter 1977). After the incubation period, the isolates of the developing fungi were purified and then classified according to the taxonomic characteristics mentioned by (Rapper and Fennell, 1965; Domsch *et al*., 1980; Hoching and Pitt, 1997).

The incidence and frequency percentage of fungi were calculated according to the (Ghiasian *et al*., 2004).

Aflatoxin Detection by Ammonia solution method

The ability of *Aspergillus flaves* isolates to produce aflatoxins was detected by using 20% ammonia solution by placing filter paper saturated with ammonia solution in the lid of the dish containing the growing fungi isolates of the *A. flavus*. The plates were incubated upside down for two days at 25 °C. A change in colour of the culture medium was used as indicator of the toxicity of isolates (Machida and saito1999). After 24hr, the under side of aflatoxin producing isolates changed in to pink to red colour. But no colour change happened indicate non-toxic isolates (Kumar *et al*., 2007; Dianese and Lin 1976).

Standard toxin Aflatoxin

The standard Aflatoxin B1 was obtained in an amorphous glass container from Hi-media with a weight of 1 mg, and in 5 ml of chloroform solution, the concentration was 200 µg / mL. The solution was considered as stock solution and kept at (-18ºC) until use.

Detection of Aflatoxin B1 by TLC method

Ten isolates of *A. flavus* were growing on culure media at seven days age, cut the growing fungus of each isolated by a sterile knife in the form of small pieces. Then transfer the pieces by a sterile needle to an electric mixer containing 20 ml of chloroform. Mix the mixture for 10 minutes. The mixture is filtered through filter paper and then taken into a clean, sterile flask. In an electric oven at a temperature of 40 °C until dry, and then dissolve in 1 ml of chloroform To detect the presence of aflatoxin, B1 using TLC (20x20 cm), where the plates were activated in the oven at 105ºC for one hour before using a light straight line was made on a TLC plate 1.5 cm from the base of the plate, 15 microliters were taken by a capillary tube of the standard AFB1 and placed on the line 2 cm from the left edge of the plate and 2 cm from the spot of the standard toxin, spots of samples were at the same distance and quantity equal to the amount of standard Aflatoxin, then left the spots to dry and then placed in the separation Tank containing the separation system composed of chloroform and methanol mixture, 2:98 size/size and was monitored until the solution to reach about 2 cm from the upper end On the plate, the plates were removed and dried under laboratory conditions For a pre period of 5 minutes and then examined under UV light at a wavelength of 365 nm was detected aflatoxin B1 matching deportation Rf coefficient and sparkle color standard for color (Sobolev and Dorner, 2002).

Result and Discussion

Isolation and diagnosis of fungi associated with feedstuff (wheat, bran) the results of isolating the fungus from feedstuffs showed that there were 4 genera of fungi were recovered from tested samples*: A. flaves, A. niger, alternaria* spp., *Cladosporium* spp. and *Rhizopus* spp.

It was found that there is a dominance of *A. flavus* in the feed of wheat and bran followed by *A. niger* and *Rhizopus* spp. with a frequency of 45, 38, 10% and their incidence were 100,100 and 22% respectively diagnosed and identified according to key taxonomic by (Domsch *et al*., 1980 and Pitt 1988). The reason for the dominance of *Aspergillus* in dates is due to its wide spread in the environment, which comes from its ability to form a large number of reproductive units resistant to the conditions Which form plankton in the air because its diameter is less than 15 nanometers and other openings, as well as its growth in wide ranges of heat and humidity, as some species of *Aspergillus* species grow at temperatures ranging from 5 - 45 °C or higher (Moubasher *et al*., 1982; Hocking and Pitt, 1997). As explained in table (1). Our present study explained a degree of fungal contamination of animal feeds. This renders them incompetent for animal consumption and also less the value of cereals as animals feed.

Among the *Aspergillus* isolated from feed samples, *Aspergillus flavus* were the predominant speciesfollowed by *Aspergillus niger* Tere are similar to some reports describing *Aspergillus flavus* as the most predominant followed by *Aspergillus niger* (Saleemi *et al.,* 2010) Fungal infection percentage was found in Cows feed sample and its rate spread are agreed with (Stefi *et al*., 2016).

Table 1 : Percentage of Frequency and Incidence to isolated fungi from feedstuffs

Percentage %		Assocciation Fungi
Frequency	Incidence	
45	100	*Aspergillus flavus*
38	100	*A. niger*
3	12	*Alternaria* spp.
4	16	*Cladosporium* spp.
10	22	*Rhizopus* spp .

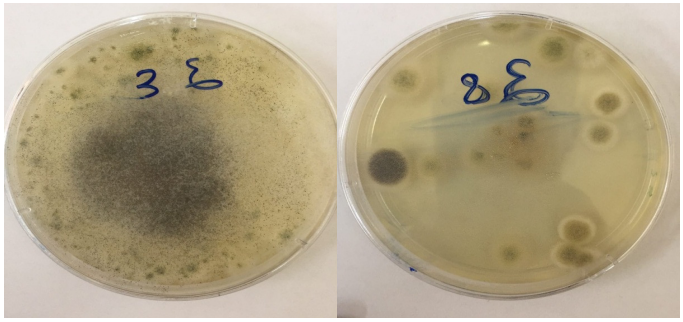

Fig. 1 : Fungi growing on PDA with feedstuff

Ability of *A. flavus* isolates to produce aflatoxin
Ammonia test

The results of this test showed the ability of 7 isolates out of a total of 10 isolates of *A. flavus* to produce aflatoxin B1 by changing the color of the base of the center of the PDA on which the fungus isolates were growing at 70%, whereas only three isolates were given negative test results. The isolates differed in the amount of aflatoxin B1 Due to the intensity of the colony color change. The most 4,5and 10and 2, 7 and 8 have a medium capacity to produce aflatoxins and other isolates (3and6) were negative result of aflatoxin (Table2) and Figures (2, 3). This result is similar to that found by Davis and Diener (1970) reported that 86% of *A. flavus* isolates were capable of infecting peanuts and contaminated with aflatoxin. (Al-Adil *et al*., 1977) noted that 59% of *A. flavus* isolates isolated from some foods in Baghdad are capable of producing aflatoxins. Differences in the ability of isolates to produce aflatoxin may be due to differences in the genetic content of the breeds. This explains the gradient in red color. Dark red isolation indicates its ability to produce large amounts of aflatoxins (section and Machida, 1999). So result agreed with study by (Stefi *et al*., 2016)

Table 2 : Detection of Afla.B1 by ammonia solution test

Aflatoxin B1 production	No. Isolate
+	**1**
+	2
-	3
++	4
++	5
_	6
+	7
+	8
_	9
++	10

(+): The base color has changed to pink, red
(-) : No change in color.

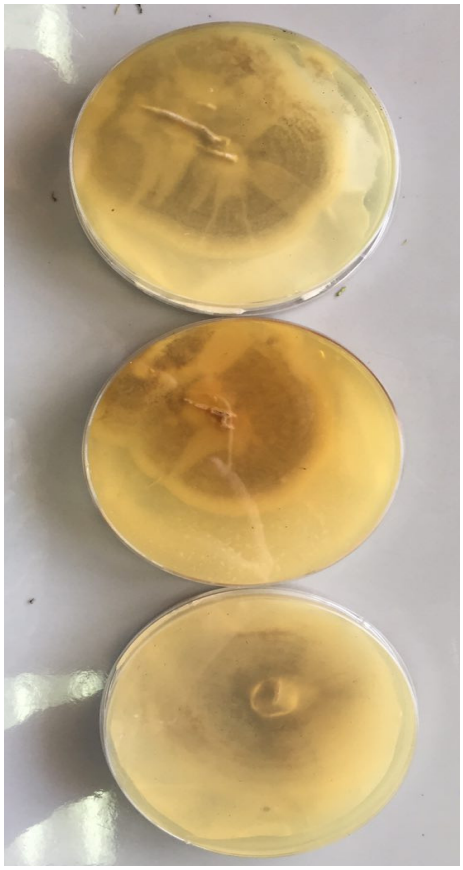

Fig 2 : Aflatoxin Detection on PDA medium by ammonia test
(isolates No. 1, 4 and 3 respectively)

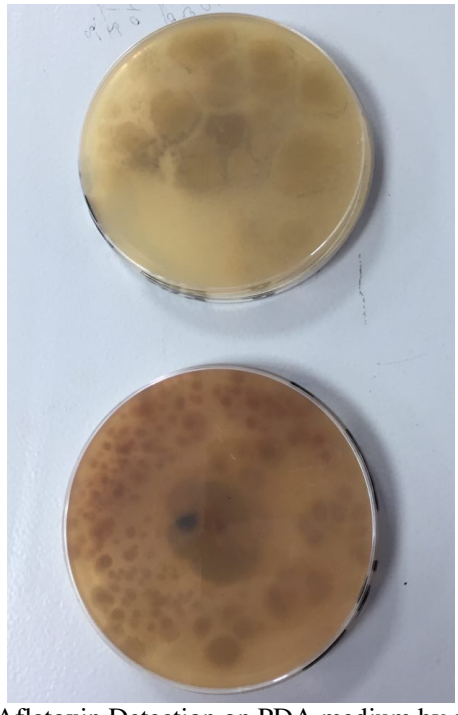

Fig. 3 : Aflatoxin Detection on PDA medium by ammonia test
(isolates No. 8 and 9 respectively)

Detection of flatoxin B1 by TLC

The results of this test showed the susceptibility of certain isolates of *A. flavus* to the production of Aflatoxin B1. The test showed6 isolates of Aflatoxin B1 out of 10 isolates of *A. flavus* and 60%. (Table 3). The isolates of the fungus varied in their production of the toxin and the isolate No.4was the most productive of the toxin based on the

intensity of its brilliance. These results are agree with a previous study that 75% of *A. flavus* isolates able on the production of aflatoxin B1 (Yu *et al*., 2004). And an approach to what (Gherbawy, 2012) which indicated that 38.88% of *A. flavus* isolates which isolated from dates were able to produce aflatoxin B1. The difference in the ability of isolates to produce aflatoxin B1 may be due to genetic differences between fungal isolates (Lee and Hagler, 1991). It is noted that the percentage of isolates has produced Aflatoxin B1, which was detected by this technique is less than the number of isolates producing toxin afB1 by ammonia solution, so the method of thin Layer chromatography is more accurate in the identification of isolates producing Mycotoxins in general, including aflatoxin B1.

Table 3 : Detection of Afla.B1 by TLC technique

Aflatoxin B1 prod.	No. Isolate
+	1
-	2
-	3
+++	4
+	5
_	6
+	7
_	8
+	9
++	10

(+) : Productive isolate, (-): non product isolate

References

Abdou, Kh.; Atef, A.H.; Nour El-Houda, Y.H. and Houda, R. Abd El-Hamed (2017). Seasonal Variation in Prevalence of Mycotoxins in Feed and Feedstuffs at Beni-Suef Governorate in Egypt., European Journal of Academic Essays, 4(4): 99-109.

AL-Adil, K.M.; Basima, A.A.N.; Sawsan, A.Y. and Kassim, A.D. (1977). Contamination by *Aspergillus flavus* group of some food stuffs in Baghdad. Bull. Biol. Res. Center., 9: 107- 115

Bancroft, J.D. and Stevens, A. (1982). Theory and Practice of histological technique. Churchill Living stone, New York., 117.

Barnett, H.L. and Hunter, B.B. (1972). Illustarated genera of imperfect fungi. 3rd edition, Burgess Publishing company, Minneaposis, Minnesota.

Bothast, R.J.; Rogers, S.F. and Hesseltine, C.W. (1974). Microbiology of corn and dry milled corn products. Cereal Chem., 51: 829-838.

CAST, Council for Agricultural Science and Technology (2003) Mycotoxins Risk in plant, Animal and Human Systems. Report No. 139. Council for Agricultural Science and Technology. Ames Iowa . USA ISBNI – 887383 – 22.0. ISSN 0194 – 4088.

Cole, K.E.; Hsu, I.C. and Trump, B.F. (1986). Comparative ultastural effect of aflatoxin B_1 on mouse, rat and human gepatocytes in primary culture. Cancer Research., 46: 1290-1296.

Davis, N.D. and Diener, U.L. (1970). Limiting temperatures and relative humidity for growth and production of aflatoxin and free fatty acid by *Aspergillus flavus* in sterile peanuts. J. Am. Oli Chem. Int., 78:693-698.

Dianese, J.C. and Lin, M.T. (1976) A coconut agar medium for rapid detection of aflatoxin production by *Aspergillus* spp., Phytopathology., 66: 1416-1418

Domsch, K.H.G. and Anderson, T.H. (1980). Compendium of soil fungi. Academic press, 941.

Eskola, M. (2002). Study on Trichothecenes, Zearalenone and Ochratoxin A in Finnish Cereals: Occurrence and Analytical Techniques. Ph.D. Thesis, Faculty of Agriculture and Forestry, University of Helsinki. Helsinki. Finland. ISSN 1458-6878

Ghiasian, S.A., Kord-Bacheh, P., Rezayat, S.M. Maghsood, A.H. and Taherkhani, H. (2004). Mycoflora of Iranian maized Hrvested in the main production area in 2000. Mycopathology 158(1):113-121.

Gherbawy, A.Y.; Elhariry, H.M. and Bahobial, A.S. (2012). Mycobiota and Mycotoxins (Aflatoxins and Ochratoxins) Associated with some Saudi Date Palm Fruits. Food borne pathogens and Disease. 9(6): 561-567.

Groopman, J.D.; Sabbion, G. and Wild, C.P. (1991). Molecular dosimetry of aflatoxin exposure of human cancer in epidemilogical, analytical and social consideration (Groopman, J. D. and Skipper, P. Eds. CRC Press, Bocaraton, Fl, pp. 302-324).

Hocking, A.D. and Pitt, J.I. (1997). Fungi and food spoilage (Second ed.) Shampan and Hill .U.K., PP: 989.

IARC (2002). Aflatoxin in Monographs on the evaluation of carcinogenic Risks to Humans, 9-13. IARC Scientific publication No. 82, Lyon, France: IARC pres .

Krishnamachari, K.A.V.; Bhat, R.V.; Nagarajan, V. and Tilak, T.B.G. (1975). Investigations into an outbreak of hepatitis in part of western India. Indian J. Med. Rej., 63: 1036-1048.

Kumar, S.; Shekhar, M.; Ali, K.A. and Sharma, P.A. (2007). Rapid technique for detection of toxigenic and non-toxigenicstrain of *Aspergillus flavus* from maize grain. Indian Phytopathology., 1: 31-34.

Lee, Y.J. and Hagler, W.M.J. (1991). Aflatoxin and Cyclopiazonic Acid produced by *Aspergillus flavus* isolated from contaminated. J. Food Sci., 56: 871-872.

Li, F.Q.; Yoshizawa, T.; Kawamura, S.; Luo, S.Y. and Li, Y.W. (2001). Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. J. Agric. Food Chem., 49:4122-4126.

Lynne, L.C.; Trenholm, H.L.; Prelusky, D.B. and Rosenberg, A. (1995). Economic Losses and decontamination. Natural Toxins. 3:199-203.

Morehouse, L.G. (1979). Mycotoxicosis of the bovine with reference to fungi and toxins associated with disease. The Bovine Practitioner., 14: 175-180.

Massey, T.E.; Stewart, R.K.; Daniels, J.M. and Ling, L. (1995). Biochemical and molecular aspects of mammalian susceptibility to aflaoxin B_1 carcinogencity. Proc. Soc. Exp. Bid-Med., 208: 213-227.

Mwanza, M.; Lubanza, N.; Mathew, N.; Lebohang, M. and Frank, B. (2013). (A Decade of Aflatoxin M1 Surveillance in Milk and Dairy Products in Developing Countries (2001-2011).

Mycotoxin and Food Safety in Developing Countries Ch.2 : 39-60.

Pitt, J.I. (2000). Toxigenic fungi : which are important. Med. Mycol., 38 (Suppl.): 17- 22.

Raper, K.B. and Fennel, D.L. (1966). The genus Aspergillus. The Willims and Wilking Co., Battimore, 686.

Saleemi, M.K.; Khan, M.Z.; Ahrar, K. and Javed, I. (2010). Mycoflora of poultry Feeds and Mycotoxin Producing Potential of Aspergillus Species. Pakistan Journal Bot., 42(1): 427-434.

Satio, M. and Machide, S. (1999). A rapid identification method for aflatoxin producing strains of *Aspergillus flavus* and *Aspergillus parasitica* by ammonia vapor. Mycoscience. 40: 250- 258.

Sobolev, V.S. and Doruer, J.W. (2002). Cleanup procedure for determination of aflatoxin in major agricultural commodities by liquid chromatography. J. of Association of official Analytical chemists International, 85: 642- 45.

Stefi, R.V.; Christo, J.P.; Shenpagam, N.H. (2016). Mycotoxin production by fungi isolated from commercially prepared livestock feed in Kerala International Journal of Applied Research, 2(5): 154-159.

Wood Head Publishing Limited (2004). Mycotoxins in food and control Edited by N. Magan and M. Olsen.

Yu, J.; Erlich, J.W.; Cary, D.; Bhatnagar, T.E.; Cleveland, G.A.; Payne, J.E.; Linz, C.P.; and Bennet, J.W. (2004) Clustered pathway genes in aflatoxin biosynthesis. Contamination of crop. Appl Environ. Microbiol, 70: 1253-1262.