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Isolation of Aspergillus flavus From Poultry Feed in Khartoum State

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Abstract:

Aspergillus flavus is an important fungal species which mayoccurs in foods and feed, producing a number of toxins including aflatoxins.There are several diseases that affect poultry and cause severe losses; among which is mycotoxicosis caused by contaminated poultry feed with Aflatoxin-producing by strains of *Aspergillus-flavus*; consequently the research problem concerned the isolationof *Aspergillus flavus* from poultry feed.Aflatoxin-producing strains of *Aspergillus flavus* and these toxins affected poultry, animal and human. Poultry farms are suitable environments for growth of fungi. Most poultry feeds are prone to fungal growth during different stages of the manufacturing process, production, transportation and storage. Poultry are source of meat and eggs for human consumption.This study was carried out in Khartoum State, from different localities for identification of *Aspergillus-flavus* from food poultry cakes (Ombaz) and detection of Aflatoxins. Identification of the *Aspergillus-flavus*based on the morphological characteristics of the colonies and microscopic examinations.Thirty samples (Cake) were collectedfrom different places in Khartoum State.(Omdurman, Bahri and Sharg-elnile). All samples cultured in Sabouraud's Dextrose Agar (SDA),plates put in incubator for seven days in temperature 25 – 30 C. After the fungus growth,it was sub cultured in potato dextrose agar(PDA, *Aspergillus-flavus* had green colour colonies, the old colony appeared as dark green

Introduction:

The genus *Aspergillus* was first described by Florentine priest and mycologist P. A. Micheli in1729(Ross,1951,et al). During 20th century.and named based on the structural similarity of its conidiophore structure to the aspergillum, a liturgical implement used to sprinkle holy water (Bennett 1992). This common genus has been classified based on morphology manytimes (Samson 1992) and currently contains over 200species. One of the most important ubiquitous fungal species in tropical environments .*Aspergillus flavus*

that can be found in soil and other substrates (Powell et al. 1994) *it* was described by Linkin 1809 and has been known as an asexual species that only produces asexual spores, conidia. Recently the sexual stage of *A. flavus* has been reported and classified as *Petromyces flavus*(Horn BW 2009). The extent of contamination of food commodities by aflatoxin also varies with different geographical locations among the country according to United Nations Food and Agriculture Organization, 25% of world's agriculture commodities are contaminated with fungal toxins, which leads to severe economic and health loss to the affected country (FAO, 2003).

Animals can also be infected by *A. flavus*(and other Aspergilli). In particular, rabbits, domestic chickens, geese, and turkey are quite susceptible to aspergillosis diseases caused by *A. flavus*(Hedayati 2007).

The first description of aflatoxin arose from investigations of the Turkey X disease in England in1962, where thousands of poultry died upon eating aflatoxin-contaminated peanuts (Forgac 1962).

Poultry feed ingredients are derived from a variety of raw materials that originate from plants and animals. It is usually a mixture of cereals (mostly maize) that serves as energy source, animal protein sources (fish meal, meat, and bone meal), and plant protein sources (soybean meal and peanut). Maize, the predominant grain used in poultry feeds, can be contaminated by mycotoxins from Aspergillus.

Aflatoxin causes aflatoxicosis, resulting from inhaling or ingesting high levels of aflatoxincontaminated food and feed. In addition, it isa major problem in developing countries, especially Asia and Africa, and contaminated maizekilled hundreds of people in Kenya in recentyears (Lewis , Onsongo et al.2005 ,Nierman et al. 2008).

The occurrence of mycotoxins in feed ingredients depends on several factors that include climatic conditions, diversity of fungi contaminating the crops, harvesting methods of the individual crops, storage practices, and seasonal variations, while the types and levels of mycotoxins in the feed largely depend on the mycotoxins in the individual feed ingredients, the mix proportion of feed ingredients, feed processing techniques, and storage practices (Warth et al. 2012; Ezekiel et al. 2014)

Aflatoxicosis outbreaks due to aflatoxin-contaminated maize in commercial dry dog foods killed dogs in the United States in1998 and 2005–2006 (Dereszynski , et al. 2008)(Garland . 2001)(Stenske and, Smith 2006).

The protein cake (oilcake meal) residue from oil processing is used as an animal feed and as a soil fertilizer. Groundnut cake is a livestock feed, mostly used by cattle as protein supplements(Deshpande, . 2000)). Poor storage of the cake may sometimes result in its contamination by aflatoxin, a naturally occurring mycotoxin that is produced by *Aspergillus flavus*.and*Aspergillus parasiticus*.The bone marrow of poultry feeds is Peanut seeds(cakes) have a high protein content, up to 50%, and are valued as a cheap protein sourcein animals and poultry feeds (Feul 1966).

Peanuts are an excellent source of plant-based protein, offering 25.8 g per 100 g of peanuts, or around half of a person's daily protein needs.

Through processing often requires dehulling.the hulls generated in large amounts by the peanut industries can then be used to feed livestock, particularly ruminants.

Although peanuts are rich in essential nutrientsIn a 100 g serving, peanuts provide 570 calories and are an excellent source(defined as more than 20% of the Daily Value, DV)of several B vitamins, vitamin E, several dietary minerals, such as manganese (95% DV),

magnesium (52% DV) and phosphorus (48% DV), and dietary fiber. They also contain about 25 g protein per 100 g serving, a higher proportion than in many tree nuts.
The fungus grows on a wide range of agricultural commodities that includepeanuts, dried corn, millet, tree nuts and cotton seeds (Michael and Ensley, 2007) and left over foods such as rice. It is also found in water damaged carpets.
In general *Aspergillus* species can be adapted to conditions without free water and can grow at lower relative humidity (70%) and reported that *Aspergillus* species is more of a problem in tropical countries.
*Aspergillus flavus*is widely distributed in nature and is largely found at cereal and grains.Before harvest or during storage, *A. flavus*grows on agricultural crops (Saini, S. S. and Kaur, A. (2012)). Its growth affected by the environmental condition such as temperature and relative humidity (Giorni, P.; Leggrieri,et al 2012)
This fungus has a worldwide distribution due to its numerous conidia production, which easily disperses by air movements and possibly by insects. *Aspergillus flavus*is mainly a saprophyte in the soil, where it plays a major role as nutrient recycler, supported by plant and animal debris and contaminates a wide variety of agricultural products in the field, storage areas, processing plants, and during distribution. The ability of *A. flavus* to survive in unfavorable conditions allows it to easily out-compete other organisms for substrates in the soil or plant.
Thestarchy foods and feeds are especially susceptible ofcolonization by *Aspergillus* species where they may produce aflatoxins along several stages of the food chain, either atpre-harvest, processing, transportation or storage (Ellis *et al*.,1991).
Aflatoxinseffect in poultry, animals and human which causes diseases.It is serious problem that Poultry Feed contaminated with *aspergillus-flavus*. Which can be an obstacle to the poultry economy. Aflatoxicoses , the disease caused by exposure to aflatoxin have made severe economic losses in the poultry industry, affecting ducklings, broilers,layers, quail and turkeys to cause clinical signs include anorexia, decreased weight gain,decreased egg production, hemorrhage, embryotoxicity, and increased susceptibility to environmental and microbial stressors (CAST. (2003)).
Several incidences of aflatoxicosis in humans have been reported in many countries includingSoutheast Asia and Africa. Furthermore, it is estimated that **4.5** billion people in the developing nations are chronically exposed to aflatoxins in their food, hence putting them at arisk of cancer related diseases (Li et al., 2001). As if that isnot enough, Africa loses approximately US$450 through aflatoxins contaminated grain.
Aflatoxins are potent toxic, carcinogenic, mutagenic, immune suppressive and teratogenicagents produced as secondary metabolites by *A. flavus*and*A. parasiticus*(Krishnamurthyand Shashikala, etal,.2006). These toxins are named after the fungus producing them, e.g. "A"from the genus name *Aspergillus*, "fla" from the species name *flavus* added to toxin to givethe name aflatoxin.
Aflatoxin is extremely durable under most conditions of storage, handling and processingof seeds or in foods or feeds made from contaminated seeds. It is very heat stable with stand temperatures up to boiling. Aflatoxin levels in maize may decline in storage, but may still be present after 7 years (Abbas, 2005).
The geographical distribution of AF follows that of its producer, which is the fungusAspergillus. This mold is common and widespread in nature. It occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and it

invades all types of organic substrates whenever the conditions (high moisture content and hightemperature) are favorable for its growth (Udoh*et al*., 2000).However, A.Fs are most prevalent in latitudes between 40° N and 40° S of the equator, and thegreatest health risk lies within developing countries in tropical regions, which relyon commodities susceptible to contamination by these toxins as their staple food source (IITA,2011).

Materials and Methods:

Sample collection

Thirty Samples of Cakes (Ombaz)grain and solid collected from different places in Khartoum State.From different localities such as Omdurman (Seed markets,peanut oil factory), Bahri(peanut oil factory)and Sharg-elnile(seed market – hillat koko). Some of them solid and other are grain.

Preparing Media:

6.5 gram from SabouraudDextroseAgar Dissolved in 100ml distill water and put in autoclave for half an hour,Let the flask sit at room temperature until you can touch your fingers to the side of the flask for 2-3 seconds without removing them. At this point the agar should be warm enough that it won't soon solidify, but cool enough that it won't inactive the antibiotics .adding 0.02 chloramphenicol antibiotics (vet services).Pour the plates and let solidify in Refrigerate

Preparing samples.

Grain the solid samples, one gram from each sample were suspended in 10 ml normal saline, after half an hour diluted2-3 times of each samples using 5m normal saline. few amount from each sampleculture in plates. The plates were incubated at25- 30ºC for 6-7 days, thenisolates and Identification

Subculturesonother media, potato dextrose agar until get pure AF.to confirm the pure of *Aspergillus-flavus* fungus culture 5-6 time. Identification of *Aspergillus* sp. was made as(per Raper and Fennell).

Results:

Growth fungus

Different species of *Aspergillus* were grown in Sabouraud Dextrose Agar with different colour. Seven sample were positive**(***Aspergillus-flavus* green color).

The fungus rapidly growth in Sabouraud Dextrose Agar, the colonies began seen after 3 days as granular flat, with radial grooves, yellow at first but quickly turned to bright and dark yellow green.After 7 days the mature colonies cover the all plate with green color.

Sub culture in potato dextrose agar until it grown pure *Aspergillus- flavus*

Morphologicalexamination:

Visual examination

Primary identification of *Aspergillus-flavus* with necked eye as green colour colonies.based on media select.

Microscopiccharacteristics.

For microscopic characteristics slides were stained withlacto phenol cotton blue (wet smear).The morphological characteristics of *A.flavus*isolates were described microscopically according to Domsch and Gams (1980) and Klich (2002).A morphological examination of species was first made with low magnification power (lens 10)of microscope after foxing using oil lens. Characteristics including conidiophore,septate hyphae, vesicle, and conidia

Discussion:

These fungi can cause food spoilage,biodeterioration and are capable of producing different mycotoxins*Aspergillus flavus*strains can cause disease in plants, animals and poultry,during the contamination of food because *A.flavus* were aflatoxigenic and can isolated and identification.

Carlos A. F. Oliveira mansion the factors responsible for the high incidence of aflatoxin contamination of peanuts in Brazilianagricultural practices during planting, harvesting, drying, transportation and storage of the product, from this factors can get control points for aflatoxin.

Chepsergon Jane(2012) and Nancy Keller(2014) discuss control measure, management strategy control currently being studied in maize crops in Thailand. It is important that the strains of *Aspergillus flavus* used in biocontrol be non-toxigenic and be incapable of reversion to toxigenicity (J. M. Misihairabgwiet al.2017). The utilization of nontoxigenic *A. flavus* strains is developing as a major biological controlstory in the field of plant pathology.Positive influences of atoxigenic strain applications carry over between crops provide benefits to plants for severalyears. That is, a single use of atoxigenic strains may benefit.

The advantage of that study not only the treated crop but also rotation crops and second season crops that miss a treatment (Bandyopadhyay et al.,2005).

According to this With domestic and international collaborators including farmers and industry organizations in Arizona, Texas, and California, were seek development of practical methods to utilize atoxigenic-strain technologies to reduce contamination.In the United States, Afla-Guard and AF36, are two commercial biocontrol products containing non-aflatoxigenic strains of *A. flavus* that have been approved by the U.S. Environmental Protection Agency for biocontrol of aflatoxin accumulation in peanut, maize, and cotton seed.

Poultry feed are suitable environment for fungal contamination during production, storage and poultry feeding, also high dose of antibiotics in poultry feeds increase the probability of fungal contamination.

This study is agreement with K.H.M. Nazmul Hussain Nazir(2014) isolation and identification of *Aspergillus flavus*from poultry feed .*A. flavus*was the predominant species found in poultryfeed. In the present study *Aspergillus flavus*had the highestfrequency 79(27%) This is in agreement with Abdel-Rahman (1995)in his studies on seed–borne fungi associated with peanut seeds inGezira&Rahad schemes of the Sudan. These findings are also in agreement with the studies from Senegal and Egypt(Waliyar, 1979& EI-Maghrabi, 1987).Using conventional diagnoses. The infection of *Aspergillus flavus* had bad economic effect ,losses of poultry production and effect in human health specially young children.The solution is to control the infection of agriculture crops preharvest, postharvest, during storage, and during transit to stop contamination of feed by *Aspergillus flavus*.

The Aspergillus spp. isolated from poultry feed samples, A. *flavus* was found in poultry feed. This finding is similar to the finding of Muhammed.,K.S (2010).

Other similar study in isolation of *Aspergillusflavus* from drinking water from poultry farm, by Wisal G. Abdalla (2017). The isolation of fungi from water has demonstrated a common presence of fungi in water distribution systems of poultry farms. Isolation of Aspergillus flavus from tanks in poultry farms was in accordance with Paterson et

al.(1997)who detected aflatoxins, produced by A. flavus in water from a cold water storage tank.

Prevalence of airborne *Aspergillus flavus* in Khartoum this study using horizontal gravitational settling method by exposing potato dextrose agar (PDA) plates to the air for 2 min at a distance of 1.75 m below the air source. This simple method simulates the condition under which the human body and respiratory tract contaminants gain access to infection sites(Raynor GS.).This study is agreement with my study for isolation *Aspergillus flavus*.from dusty air.these mean *Aspergillus flavus*can spread for long distance through wend and air. So strong control method must use. In conclusion *Aspergillus flavus* contamination food is a serious risk for public health having long-term health effects in Human, Animals and poultry because it produce aflatoxin.There are high percentage of *ApergillusSp, A.flavus* isolated frompoultry feed product.

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