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Studies on Mycoflora, Aflatoxigenic Fungi and Aflatoxin in Poultry Feeds.

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ABSTRACT

Poultry feed samples were collected from the Jalgaon district in Maharashtra, and the mycoflora associated with the samples was isolated using agar-plate techniques. Every Aspergillus flavus isolate that was isolated from the samples that were collected was examined for aflatoxigenic potential in SMKY liquid medium. Poultry feeds were used to isolate seventeen distinct fungus. The most prevalent fungus that infest feeds were Aspergillus flavus, A. niger, A. ochraceus, Aspergillus sp., Fusarium sp., and Penicillium sp. Aspergillus flavus dominated all fungi, and 76% of its strains were aflatoxigenic. Crude poultry feed had the highest amount of aflatoxigenic fungus (86.2%). Aflatoxin B1 was estimated in all the samples by extracting the aflatoxin and spotted in an activated thin layer chromatography (TLC) plate with standards and ascertained the concentration by visual comparison method in a UV viewing cabinet. When the natural aflatoxin contamination of poultry feeds was analyzed, 26.67% of the samples had aflatoxin contamination. Maximum concentration of aflatoxin B1 was detected in Local poultry feed (93.67 ppb) followed by Crude poultry feed (92.89 ppb) and Commercial poultry feed (84.38 ppb). poultry feeds contaminated with aflatoxin has poses a potential threat for the life of poultry animals. Hence the regular screening of toxins in every lot of feed prior to feeding the animals or poultry needs to be regularized.

Keywords: Poultry feeds, Mycoflora, Aspergillus flavus, aflatoxin.

INTRODUCTION

One of the most significant cereal crops in the world, maize (Zea mays L.) helps ensure food security in the majority of developing nations (Ranum et al., 2014). After wheat and rice, maize is becoming the third most significant crop in India. It contributes roughly 9% of the nation's total food grain production. Feed utilization, mostly for poultry feed, accounts for almost half of total production. Around the world, one of the biggest issues is aflatoxin contamination in maize kernels. Some strains of Aspergillus flavus Link ex Fries and Aspergillus parasiticus Speare create a class of secondary metabolites called aflatoxins, which are structurally similar polyketides. These substances are immunosuppressive, mutagenic, carcinogenic, teratogenic, and acutely poisonous. Aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) are common aflatoxins. Of all aflatoxins, AFB_1 is the most effective (Lee et al, 2004). The strongest carcinogen in nature is this poison (Castegnaro and McGregor, 1998). While other Aspergillus species can infect maize in the field, A. flavus is the predominant fungus that produces aflatoxin, particularly in tropical areas (Calvert et al., 1978, Setamou et al., 1997). When the temperature is between 18°C and 33°C and the relative humidity is higher than 50%, the fungus thrives. India's current climate encourages the fungus's growth and, in turn, the formation of aflatoxin in kernels. Any time before and after harvest, as well as during drying, storage, and processing, maize can get contaminated with aflatoxin. Poultry birds may consume potentially dangerous levels of aflatoxin when tainted maize kernels are utilized as an element in poultry feed. The primary sources of poultry feed are primarily soybeans, sunflower seed, canola, rapeseed, safflower, flaxseed, mustard seed, peanuts and cottonseed and their cake, Jowar, Bajra, Wheat and Maize. (Kolhe and Chaudhari,2022) AFB1 metabolism produces a number of metabolites that are transferred to edible animal products, such as liver, muscle, and eggs (Bintvihok and Davitiyananda, 2002). These metabolites have teratogenic, immunosuppressive, and poisonous effects on animal with humans. The majority of developed nations have strict regulations on the amount of aflatoxins allowed in imported and sold

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commodities in order to prevent aflatoxins from entering the food chain. (Van Egmond, 1989). For instance, the Food and Drug Administration (FDA) in the United States has set an action level of 20 parts per billion (ppb) for the overall amount of aflatoxins in human food (Park and Liang, 1993). Poultry companies suffer large financial losses as a result of aflatoxin contamination in their diets (Awad et al., 2006). When chickens consume such aflatoxin-contaminated feed, they get aflatoxicosis. According to Choudary and Rao (1982), there was a 100% fatality rate from an aflatoxicosis outbreak in commercial poultry farms in the Chittoor area of Andhra Pradesh state, India. Hence It is necessary to know the mycoflora, incidence of aflatoxigenic fungi and aflatoxin contamination. The present investigation is an attempt in that direction.

MATERIALS AND METHODS

Samples of poultry feeds (crude poultry feed, local poultry feed, commercial poultry feed) were collected from March to May 2024 from five different marketing centers in Jalgaon district, Maharashtra (Jalgaon city, Bhusawal, Chalisgaon, Pachora, and Jamner). At each location, four samples of each feed type were collected from different vendors, yielding a total of 20 samples per feed type (5 locations × 4 samples = 20), and 60 samples overall. All samples were collected in sterile polyethylene bags, sealed immediately, transported to the laboratory within 6 hours of collection, and stored at 4°C until analysis. Samples were collected during the pre-monsoon season when temperature (28-38°C) and relative humidity (60-75%) are conducive to fungal growth, representing a worst-case scenario for contamination. Isolation of mycoflora was done by agar plate methods using peptone, glucose, rose bengal agar medium containing streptomycin. (Booth,1971). Fungal colonies formed were identified and percent incidence of each fungus was calculated.

The isolates of Aspergillus flavus were screened for their aflatoxin producing potentials in SMKY liquid medium (Diener and Davis, 1966). Ten days old culture filtrates were extracted with chloroform (v/v) and qualitatively analyzed for different types of aflatoxins on TLC plates (Reddy et. al., 1970).

For analysis of aflatoxin contamination in poultry feeds. Powdered feed sample were macerate and extracted with methanol: water (6:4 v/v) and sodium chloride (Anon, 1975). The aqueous methanolic extract was defatted using n-hexane followed by its extraction for aflatoxin with chloroform which was processed for qualitative analysis of aflatoxin on TLC plates (Reddy et. al., 1970). The TLC plates were air-dried and observed under long-wave UV light (360nm) for aflatoxins (B_1, B_2, G_1 & G_2). The aflatoxins were also chemically confirmed by spraying trifluoroacetic acid and 25%sulfuric acid. Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric measurement at 360 nm using a temperature controlled using Shimadzu UV160A Spectrophotometer (Nabney and Nesbitt, 1965).

RESULTS AND DISSCUSION

Isolation of mycoflora: The fungus (in percentage) isolated from poultry feeds are listed in Table 1. Aspergilli clearly outnumbered other genera, i.e. Aspergillus flavus was shown to be predominant on all kinds of poultry feeds, together with Aureobasidium, Alternaria, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium, and Rhizopus sp. The following decreasing sequences can be used to arrange different poultry feed according to the percentage occurrence. Commercial poultry feed comes after local poultry feed and crude poultry feed.

Table 1: Mycoflora associated with Poultry feed and their percentage incidence

Mycoflora	Poultry feed		
	Crude	Local	Commercial
Aspergillus flavus	58	55	46
A. niger	11	20	20
A. candidus	1	--	--
A. terreus	2	--	--

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A. ochraceus	**10**	**5**	
A. parasiticus	**2**	**__**	
Aspergillus sp. (Unidentified sp.)	**3**	**6**	**10**
Alternaria sp.	**1**	**1**	**2**
Aureobasidum pullulans	**_ -----**	**2**	**----**
Cladosporium sp.	**1**	**---**	**1**
Curvularia sp.	**2**	**---**	**1**
Fusarium sp.	**4**	**7**	**10**
Mucor sp.	**2**	**----**	**2**
Penicillium sp.	**2**	**4**	**5**
Rhizopus sp.	**2**	**---**	**3**

The most frequent fungi that infest the test oil seeds are Aspergillus flavus, Aspergillus niger, and Aspergillus ochraceus. In contrast, Aspergillus candidus, A. terreus, and A. parasiticus were exclusively found in crude poultry feeds. Following Aspergilli, the most prevalent infestants were Alternaria sp., Fusarium sp., and Penicillium sp. (isolated from all poultry feed), followed by Aureobasidium pullulans (isolated from local poultry feed) and Cladosporium, Curvularia, Mucor and Rhizopus sp. (isolated from crude poultry feeds and commercial poultry feed).

Table 2: Ability of Aflatoxin producing potentials of Aspergillus flavus obtained from Poultry feeds.

Poultry feed	**Number of isolates**		**Number of Isolates producing aflatoxin**				**Amount of aflatoxin B_1 (mean) ppm**
	Screened	**Toxigenic (percent)**	**B_1**	**B_1+B_2**	**B_1+B_2+G_1**	**B_1+B_2+G_1+G_2**	
Crude	**29**	**25 (86.2 %)**	**13**	**7**	**3**	**2**	**24.38**
Local	**27**	**22 (81.5 %)**	**8**	**10**	**3**	**1**	**21.92**
Commercial	**19**	**10 (52.6 %)**	**7**	**2**	**1**	**--**	**21.63**
Total	**75**	**57 (76 %)**	**28**	**19**	**7**	**3**	**------**

Ability of Aflatoxigenic potentials of Aspergillus flavus isolates obtained from Poultry feeds:

Altogether 75 isolates of Aspergillus flavus obtained from Poultry feeds and screened for their aflatoxin producing potentials in SMKY liquid medium (table 2), only 57 isolates were aflatoxin producers. The incidence of toxigenic isolates varied with the commodities from which they were isolated. Depending upon the presence of toxigenic A. flavus isolates of Poultry feeds could be arranged in the following decreasing sequence:

Crude Poultry feeds > Local Poultry feed > Commercial Poultry feed.

Aflatoxin components were produced in a liquid media by toxic isolates of A. flavus from poultry diets. All of the toxic isolates of A. flavus produced aflatoxin B_1 (57). In the absence of AFB_1, none of the isolates produced aflatoxin B_2, G_1, or G_2; 28 isolates were able to create AFB_1 alone, 19 isolates were able to elaborate both AFB1 and B2, and 7 isolates were able to make AFG_1 in addition to AFB_1 and B_2. All four aflatoxin types (B_1, B_2, G_1, and G_2) could only be produced by three isolates. Additionally, Table 2 shows that the ability of toxic A. flavus isolates to produce AFB_1 differed depending on the kind of poultry feed; isolates from commercial poultry feed

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were the least aflatoxigenic, whereas isolates from crude poultry feeds were highly aflatoxigenic. Furthermore, as reported by Raper and Fennell (1965), none of the isolates of A. flavus are toxic. Hiscocks observed in 1965 that while the majority of A. flavus isolates produced both B and G toxins, certain isolates produced just one. In the absence of B1, none of the isolates generated B_2, G_1, or G_2 (Lillehoj et al., 1977). It has been proposed that the isolates' genetic composition may control their toxicity. Ciegler (1977). A temperature of about 25% and a substrate moisture level of more than 14% are necessary for mold growth and toxin production. Aflatoxin production is reduced when there is less oxygen present (Diener et al., 1987).

Aflatoxin contamination in Poultry feeds:

Only 42 of the 60 samples that were evaluated for aflatoxin contamination tested positive for the BGYF test (table 3). However, just 16 samples tested positive for aflatoxins, according to extraction studies. The results indicate that approximately 35% of samples of crude poultry feed had aflatoxin contamination, followed by samples of local poultry feed (25%) and commercial poultry feed (20%). All four forms of aflatoxins were found in both local and crude poultry feed; commercial poultry feed contained AFB1+B2. Samples of poultry diets could be grouped in the following decreasing order based on the reported amounts of AFB1 (ppb):

Crude poultry feed > Commercial poultry feed > Local poultry feed

Table 3: Aflatoxin contamination in Poultry feeds.

Sample of Poultry feeds	Number of samples						Aflatoxin B_1 Concentration (mean) ppb
	Screened	Positive (Percent)	Samples Positive For				
			B_1	B_1+B_2	$B_1+B_2+G_1$	$B_1+B_2+G_1+G_2$	
Crude	20	6 (30.0)	2	4	1	1	92.89
Local	20	7 (35.0)	4	3	1	1	93.67
Commercial	20	3 (15.0)	1	2	-	-	84.38
Total	60	16 (26.66)	7	9	2	2	---

Indian climatic conditions coupled with socio-economic backwardness offer excellent conditions for mycotoxin production. Hot and humid climate, which is ideal for mould growth, is prevalent in most parts or India, particularly during the monsoon season. The consumption of mycotoxin contaminated food often becomes indispensable due to acute food shortage and poverty.

The highest percentage of aflatoxigenic fungus in maize seeds (86.20%) was found by Kolhe and Chaudhari (2022). Ability of Aspergillus flavus to produce aflatoxin was found in maize (14.38 ppm), followed by wheat (11.63 ppm), jowar (11.11 ppm), and bajra (11.94 ppm). The natural aflatoxin contamination of cereal grain seeds was found in 26.25% of the samples. Maize seeds had the highest concentration of aflatoxin B_1 (27.87 ppb), followed by Jowar seeds (8.38 ppb), Wheat seeds (5.61 ppb), and Bajra seeds (5.25 ppb).

Fungal spores can contaminate feed during processing, especially when grains are pulverized and the feed is pelleted (Embaby et al., 2015). Feed storage and processing techniques, environmental temperatures above 27 °C, humidity levels above 62%, and feed moisture levels above 14% are some of the variables that can promote fungal development in feed, which could lead to the generation of mycotoxin (Mahfouz and Sherif, 2015). Certain isolates that are capable of producing aflatoxin in a culture setting are unable to do so in a natural one. Unfavorable and shifting environment as well as the impact of interactions with other microbes could be the cause of this. Additionally, various strains of A. flavus may produce variable amounts of aflatoxin due to genetic reasons (Maggon et al., 1969; Ciegler, 1977). Aspergillus infection and aflatoxin levels can be significantly increased when cereals are physically damaged or harmed by insects like weevils. The main source of mold in homemade feed concentrates on small-scale farms is protein supplements like cotton seed cakes, sunflower cakes, poultry meal, and other oil seed byproducts that are frequently improperly stored (Lunyasunya et al., 2005).

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According to Bhat et al. (1997), AFBI contamination exceeded the Indian threshold for consumption (30 ug kg) in 26% of maize kernels gathered from various regions of India. According to Waliyar et al. (2003), 43% of maize samples taken from supermarkets or retail stores in Hyderabad, Andhra Pradesh, India, had toxin contamination, with the highest AFB1 level of 806 ng/kg. Kannan et al. (2014) reported that 98% of the poultry feed samples collected from poultry farms and poultry feed dealers of Tamil Nadu, India were contaminated with AFB1 and the levels ranged from 0 to 160.7 ppb and the levels of AFB1 in 29% of the samples exceeded 20 ppb. Kannan and Velazhahan (2015) reported that AFB1 contamination was found in more than 88 % of the poultry feeds samples collected from Tamil Nadu, India and its level ranged from 5.4 to 125.4 µg/kg. The presence of aflatoxins in agricultural commodities poses a serious health threat to both humans and domestic animals. Several studies reported the presence of residual aflatoxins in liver and meat of broilers when fed with aflatoxin contaminated feeds (Oliveira et al., 2000; Hussain et al., 2010; Herzallah, 2013). In the case of laying hens, aflatoxins and their metabolites were detected in the eggs (Trucksess et al., 1983). The aflatoxin B1 concentrations detected in this study (84-94 ppb) exceed not only the Indian regulatory limit of 30 ppb but also the action levels recommended by international bodies such as the FDA (20 ppb for animal feed) and the EU (10-20 ppb depending on animal species). Chronic consumption of feed containing AFB1 at these levels would be expected to cause reduced feed intake, decreased weight gain, impaired immune function, and increased mortality in poultry. For laying hens, carry-over of aflatoxin and its metabolites into eggs has been documented at dietary AFB_1 levels as low as 50 ppb. Economic losses to poultry farmers in the Jalgaon district from reduced productivity, increased veterinary costs, and mortality may be substantial. The higher contamination levels in crude and local feeds (92.89 and 93.67 ppb, respectively) compared to commercial feed (84.38 ppb) suggest that formal feed manufacturing processes offer some quality control advantages. However, even commercial feed exceeded regulatory limits, indicating that all segments of the feed supply chain require improved quality management practices. Therefore, need for regular monitoring of aflatoxin contamination in Poultry feeds for quality control, and to develop method which can reduce the chances of aflatoxin production during storage and transport.

CONCLUSION

The present study indicates high level of contamination of Poultry feed with different fungal species but specially the Aspergillus flavus. were dominant. The co- occurrence of aflatoxin B_1 present a health risk because of their synergistic and /or additive effect. Aflatoxin can be carried over to human food of animal origin; human exposure to aflatoxin may cause health threats. The study show that feed ingredient are important vehicle for contaminating finished Poultry feed as they may be heavily contaminated by aflatoxin. It is necessary to increase the awareness among farmers and traders about the importance of aflatoxins and to adopt improved management practices to minimize aflatoxin contamination in feed ingredients.

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