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Incidence of the Most Common Toxigenic *Aspergillus* Species in Broiler Feeds in Kermanshah Province, West of Iran

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Abstract: The objectives of this study were to investigate the occurrence and identification of *Aspergillus* species in broiler feeds in Kermanshah province, west of Iran. From April 2008 to March 2009, a total of 50 samples of broiler feeds were collected from different broiler farms located in Kermanshah province. All samples were aseptically transported to the laboratory, homogenized, quartered to obtain a 1 kg laboratory sample and were stored at 4°C for fungal analyses. Ten grams of each feed sample were homogenized in 90 ml sterile physiological saline for 30 minutes to obtain a concentration of 10^{-1} (dilution 1). This mixture was then serially diluted to 10^{-2} (dilution 2), 10^{-3} (dilution 3) and 10^{-4} (dilution 4). From each dilution, 25 µL of mixture was deeply inoculated on dichloran rose-bengal-chloranphenicol agar (DRBC) and incubated at 30°C for 15 days. During incubation period, gross and microscopic features of fungal colonies were studied. Out of 50 feed samples, 46 samples (92%) in dilution 1, 38 samples (76%) in dilution 2, 28 samples (56%) in dilution 3 and 20 samples (40%) in dilution 4, were contaminated to *Aspergillus* species. The most prevalent species was *Aspergillus flavus* followed by *Aspergillus niger* and *Aspergillus fumigatus*. These results showed that a potential exists for the production of mycotoxins by the *Aspergillus* species. They suggest an association of mycotoxicosis with poultry feeds in western parts of Iran.

Key words: *Aspergillus* · Poultry feed · Broiler farms · Kermanshah province

INTRODUCTION

Mold and mycotoxin contamination of feed and feed ingredients occurs worldwide and because of the ubiquitous nature of these micro-organisms they cannot be totally eliminated from feeds and ingredients [1]. The presence of mold and mycotoxins in poultry feeds result from the raw material used in their production. Mold and mycotoxins contamination of the raw materials occur during the pre-harvest and/or the post-harvest periods. During these periods, temperature and humidity, as well as processing and handling of animal feed play an important role in the growth of fungi and mycotoxins contamination [2,3]. In general, the mixed feeds of poultry constitute corn and soybean as major ingredients, which represent an excellent substrate for growth and reproduction of numerous fungi, under favorable conditions such as high moisture and increased temperature [4]. When long-term physiological and environmental conditions for fungal growth are provided, mycotoxins are produced, which can not be removed from the feed completely [4]. Fungal contamination is undesirable because of the potential for mycotoxin production [5]. Fungal toxins can be stored in meat, milk and egg and finally transferred to human beings [6].

Most species of *Aspergillus* and *Penicillium* are able to grow on a wide range of organic substrates. They are essentially saprophytic and are particularly associated with stored moldy plant products [7, 8]. *Aspergillus* genera is the most important toxigenic fungi [9, 10].

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Poultry are highly susceptible to mycotoxicoses and mycosis [10-12]. Aspergillosis is an increasingly common ubiquitous fungal infection of birds and occasionally other animals including man. *Aspergillus fumigatus* is the most commonly isolated species from the cases of aspergillosis, followed by *Aspergillus flavus* and *Aspergillus niger* [13].

At present, aflatoxins are considered to be one of the most toxic, carcinogenic compounds produced by several members of the *Aspergillus flavus* in foods and feeds [14, 15]. Therefore, throughout the world great attention is paid to investigation on *Aspergillus* species and elaborating means for controlling them [17]. Data on the microbiota and mycotoxins from poultry feeds in Iran are scarce. For this reason, our aim in this study was to isolate and identify the *Aspergillus* species found as contamination in broiler feeds.

MATERIALS AND METHODS

From April 2008 to March 2009, a total of 50 feed samples were taken randomly from commercial broiler farms in Kermanshah province. All samples were aseptically transported to the laboratory, homogenized, quartered to obtain a 1 kg laboratory sample and were stored at 4°C for fungal analyses [16]. Ten grams of each feed sample were homogenized in 90 ml sterile physiological saline for 30 minutes and serial dilutions of 10^{-1} (Dilution1), 10^{-2} (dilution 2), 10^{-3} (dilution 3) and 10^{-4} (dilution 4) were made. Then, 25 µL of each dilution was deep point inoculated on dichloran rose-bengal-chloranphenicol agar [17] and incubated at 30°C for 15 days. Fungal colonies were selected for identification, according to the methods proposed for the genus [18]. The distinct colonies were picked, subcultured for purification and characterized using standard techniques [19]. Wet mount smears and slide cultured colonies were stained with lactophenol cotton blue. Taxonomic identification of the fungi was made based on macroscopic and microscopic features in accordance with appropriate keys [17]. Identification of *Aspergillus* species were made as per Raper and Fennell [20]. Statistical analysis of data was performed using SPSS software (Version 16) with 95 percent accuracy. A P-value less than 0.05 was considered significant.

RESULTS

Mycological survey of 50 samples of broiler feed from western parts of Iran showed the presence of potentially toxigenic and infective *Aspergillus* species (Table 1, Figures 1-3). Out of 50 feed samples from each dilution, 46 samples (92%) in dilution 1, 38 samples (76%) in dilution 2, 28 samples (56%) in dilution 3 and 20

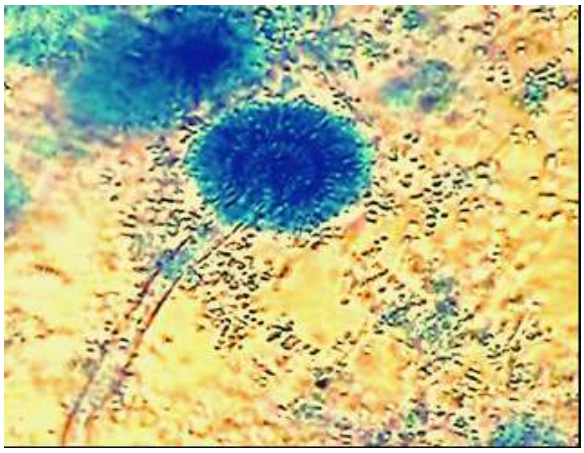

Fig. 1: *Aspergillus flavus*, isolated from compounded broiler feeds in west of Iran

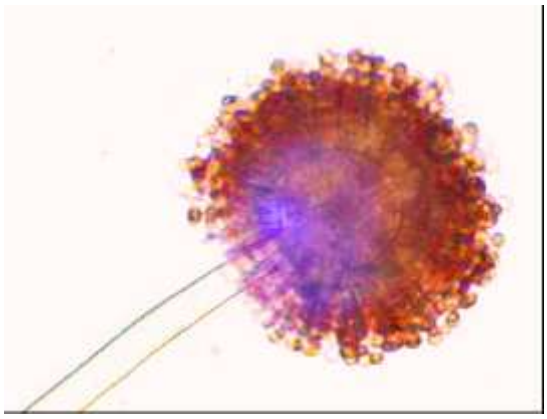

Fig. 2: *Aspergillus niger*, isolated from compounded broiler feeds in west of Iran

Fig. 3: *Aspergillus fumigatus* isolated from compounded broiler feeds in west of Iran

Table 1: The occurrence of *Aspergillus* species isolated from 50 broiler feed samples in west of Iran

	Aspergillus species							
	A. flavus		*A. niger*		*A. fumigatus*		Total	
Dilution	Number	%	Number	%	Number	%	Number	%
1	34	73.9[a]	10	21.7[b]	2	4.3[c]	46	92.0
2	26	68.4[a]	10	26.3[b]	2	5.2[c]	38	76.0
3	22	78.5[a]	4	14.2[b]	2	7.1[b]	28	56.0
4	14	70.0[a]	4	20.0[b]	2	10.0[b]	20	40.0

[a, b, c] Figures with different superscripts within rows are significantly different ($p<0.05$)

samples (40%) in dilution 4, were contaminated to *Aspergillus* species. In dilutions 1, 2, 3 and 4, the highest contamination rate belonged to *Aspergillus flavus* (73.9, 68.4, 78.5 and 70%, respectively), followed by *Aspergillus niger* (20, 14.2, 26.3 and 21.7%, respectovely) and *Aspergillus fumigatus* (4.3%, 5.2%, 7.1% and 10%). There was a significant difference between the contamination rate to *Aspergillus flavus* and the other isolated *Aspergillus s*pecies ($p <0.05$).

DISCUSSION

Mycological survey of 50 samples of broiler feed from western parts of Iran showed the presence of potentially toxigenic and infective *Aspergillus* species (Table 1). Many researchers have proved that the majority of feeds have species from *Aspergillus* and *Penicillium* genera as a predominant flora; Bragulat *et at.,* (1995) alreported high frequency of *Aspergillus* species in mixed poultry feeds [21]. Magnoli *et al*. [25] lreported a high frequency of *Aspergillus* group species found in poultry feeds from Argentina [3]. They reported that *Aspergillus flavus* and *A. parasiticus*, which are important aflatoxin producers, were the predominant species isolated. Glenda, *et al*. [11] reported that *Aspergillus* and *Penicillium* species had the highest isolation frequencies followed by *Fusarium spp* in poultry feeds in Brazil. Many studies have shown that most feeds have species from *Aspergillus* and *Penicillium* genera as predominant flora [21, 22].

In the present study, the main contaminating fungus appeared to be *A. flavus*, a potentially toxigenic species for the aflatoxins. Likewise, the species has shown high occurrence frequency in the studies of Labuda and Tancinova [3], Heperkan and Alperden [23], Magnoli *et al.* [24] and Dalcero *et al.* [25] and There was a significant difference between the rate of contamination to *Aspergillus flavus* and other isolated *Aspergillus* species (*A. niger* and *A. fumigatus*) in 4 dilutions ($p <0.05$), similar results obtained from mycological survey on feed ingredients and mixed animal feeds in Ghom province (central parts of Iran) were reported by Khosravi *et al*. [26].

A. flavus was the most prevalent species. This result agrees with Adebajo *et al*. Dalcero *et al*. [27, 28]. Magnoli *et al.* [3] and Accensi *et al.* [29]. Dutta and Das [4] confirmed the predominance of *A. flavus* over *A. parasiticus* in poultry feeds. The majority of these genera representatives such as *A. flavus* are thermophilic and thermo-resistant and distribute abundantly in tropical to subtropical climates [30]. Lacey and Magan [21] showed that the ideal temperature concerning growth and mycotoxin production ranges 25 to 35°C for *A. flavus* strains [7, 8]. The average annual range of temperatures in western parts of Iran varies from 21 to 28°C, but is generally more than 24°C indicating favorable condition for *A. flavus* growth [31, 32].

Among the *Aspergillus* species isolated from broiler feeds, *A. niger* was the second most prevalent species. This is in agreement with Osho *et al*. [29] who reported that out of the 50 samples collected from various commercial poultry farms located in southwest Nigeria, *A. niger* was one of the common fungi found in the feeds [10]. *Rhizopus* spp. had the highest frequency of occurrence (44%), *Fusarium* spp. 42%, *A. flavus* 40%, occurrence and *A. niger*, (38%).

The occurrence of *Aspergillus* species in broilers feed is particularly important because there are known as the most toxigenic among the fungi. Most studies indicate that there is no correlation between the presence of a toxin and the producing fungus in the same substrate, but the presence of toxigenic fungi in feeds may be an indicative of their potentiality to produce mycotoxins. When the storage conditions are not appropriate and the toxigenic fungus is present, this may be able to produce a mycotoxin [33].

The results of this study showed that the broiler feeds in Kermanshah province were highly (92% in dilution 1 and 40% in dilution 4) contaminated with *Aspergillus* species which are the most common toxigenic fungi found in feeds. This study warrant the need for analyzing the samples for *Aspergillus* mycotoxins, especially aflatoxins and also to design effective management strategies to prevent contamination of poultry feed to *Aspergillus species* and *Aflatoxin*. The study highlights a potential risk of poultry feeds getting contaminated with hazardous toxic compound and potentially infective *A. fumigatus*, thus making it for further analysis and continual monitoring and evaluation of feeds.

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