Fungal Contaminant of Poultry Feed in Basrah, Iraq

Fungal Contaminant of Poultry Feed in Basrah, Iraq

R. N. Alkhursan1, M. H. Khudor2 and B. A. Abbas2*

1Department of Biology, College of Sciences, University of Basrah, Basrah, Iraq
2Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Email: basil.abbas@uobasrah.edu.iq

Abstract: A total of 180 samples of concentrated poultry feed were collected from different broilers, broiler breeders, layers farms and local market of poultry in Basrah province. Feed samples were collected during the period from Sep. 2014 to Apr. 2015. About 10 - 30 representative samples of 1 kg were collected from several locations. They were cultured on Potato dextrose agar (PDA) and malt extract agar (MEA) and then sub cultured on Sabouraud dextrose agar (SDA). Seven genera were recovered from 180 samples of poultry feed .The most genera which recovered were Aspergillus spp. frequency (Fr) 62.77% - Relative density (RD) 52.03%), followed by Penicillium spp. (Fr 47.77% - RD 17.01%) and they were the predominant genera isolated from poultry feed. Fusarium spp. isolates were less frequency and relative density (Fr.1.66%, RD 2.11%). The most frequently isolated Aspergillus spp. was Aspergillys flavus (Fr 65.48%) and had the most RD (27.55%), followed by Aspergillus niger (Fr. 58.40%, RD14.23%), the less occurrence of Aspergillus spp. was Aspergillus paraciticus (Fr.1.76%, RD0.89%).

1. Introduction

A large quantity of animal feeds purchased from abroad and with the increased emphasis on animal resources, this amount is expected to increase substantially in the coming years. It is necessary to have quality control of the feed because the feed can have a great effect on the weight and mortality of birds which prepared as starter and finisher diets for poultry [1; 2]. Diets for poultry consist largely from grain, nutritional supplements such as concentrated protein, soybean meal, vitamin and mineral [3; 4].

The storage conditions are necessary for safe feed, so weather extremes, unsuitable storage practices ,and improper of feed condition can cause feed contamination, especially with mycotoxins [5]. Poultry feed is frequently contaminated by mycotoxins [6;7]. Poultry feed it is more susceptible to fungal growth during processing, therefore identification of fungi with the ability to produce mycotoxins is essential [8]. There are many clinical signs as a result of mycotoxins effected with low concentration were always observed in different broiler, broiler breeder , it is appeared reduced weight gain, anorexia, feed conversion efficiency, egg production and egg shell quality [9]. Molds can grow and produce mycotoxins in preharvest or during storage, transport, processing or feeding and during these periods, humidity and temperature play an important role in the fungal growth and mycotoxins contamination [10]. In wet feeds, higher moisture levels allow mold growth if oxygen is available [11]. Feeds containing more than 12-15% moisture suitable to grow fungi and Because most molds are aerobic, high moisture concentrations can eliminate air and prevent mold growth [12]. The conditions are most suitable for mold growth and for mycotoxin production are not necessarily the same [13]. More than 100.000 fungal species are considered as natural contaminants of agricultural and food products.

The present study was aim to investigate the occurrence ,distribution and frequency of fungal contaminant of poultry feed.

2. Materials and methods

2.1. Collection of samples

A total of 180 samples of poultry feed pellet were collected from different breeders broiler farms and local markets in Basrah province. Feed samples were collected during Sep. 2014 to Apr. 2015, and stored for 2-3 days in sterile containers at room temperature (22-25ºC).Then, they were prepared for fungal isolation and identification [2].

2.2. Isolation and identification of fungi

Twenty gram poultry feed samples were suspended with 180 ml of saline solution (0.85% Sodium Chloride) in addition to 0.05% Tween 80 (polyoxyethylene sorbitan monooleate) on a horizontal shaker for 30 min. to liberate the spores from fruiting bodies and to break the spore clumps [4]. 0.1 ml of suspension was inoculated on PDA and MEA media [15; 16]. The distinct colonies were stained on a slide using lactophenol cotton blue and lacto-fuchsin, then morphological characteristics of fungal isolates were described by microscope [17; 18]. The colony color and conidia morphology were investigated. Each colony type was counted for individual cfu/g counts and was recorded [19].

2.3. Statistics

The frequency (Fr.) and relative density (RD) of isolation genus and species were calculated as follows [20; 21 ; 22]:

$$\text{Fr. (\%)} = \frac{\text{samples number with a genus or species}}{\text{samples total number}} \times 100$$

$$\text{RD(\%)} = \frac{\text{isolates number of a genus or species}}{\text{Total number of fungal isolates}} \times 100$$

3. Results

3.1. Fungal isolation

In the present study, about seven fungal genera and forty species were identified from 180 samples of poultry feed. Several species were identified in each sample. The morphological characteristics of fungal isolates were described under the microscope. The colony color and conidial morphology were investigated. The most important recovered genera of fungi were Aspergillu sspp., Penicillium spp., Rhizopus spp., Cladosporium spp., Muco rspp., Alternaria spp. ,and Fusarium spp. (figure 1 and 2). There were 9 Aspergillus spp. Recovered; A. flavus, A. niger, A. fumigatus, A. terreus , A. flavipes, A. carbonarius, A. ochraceus, A. candidus and A. parasiticus.

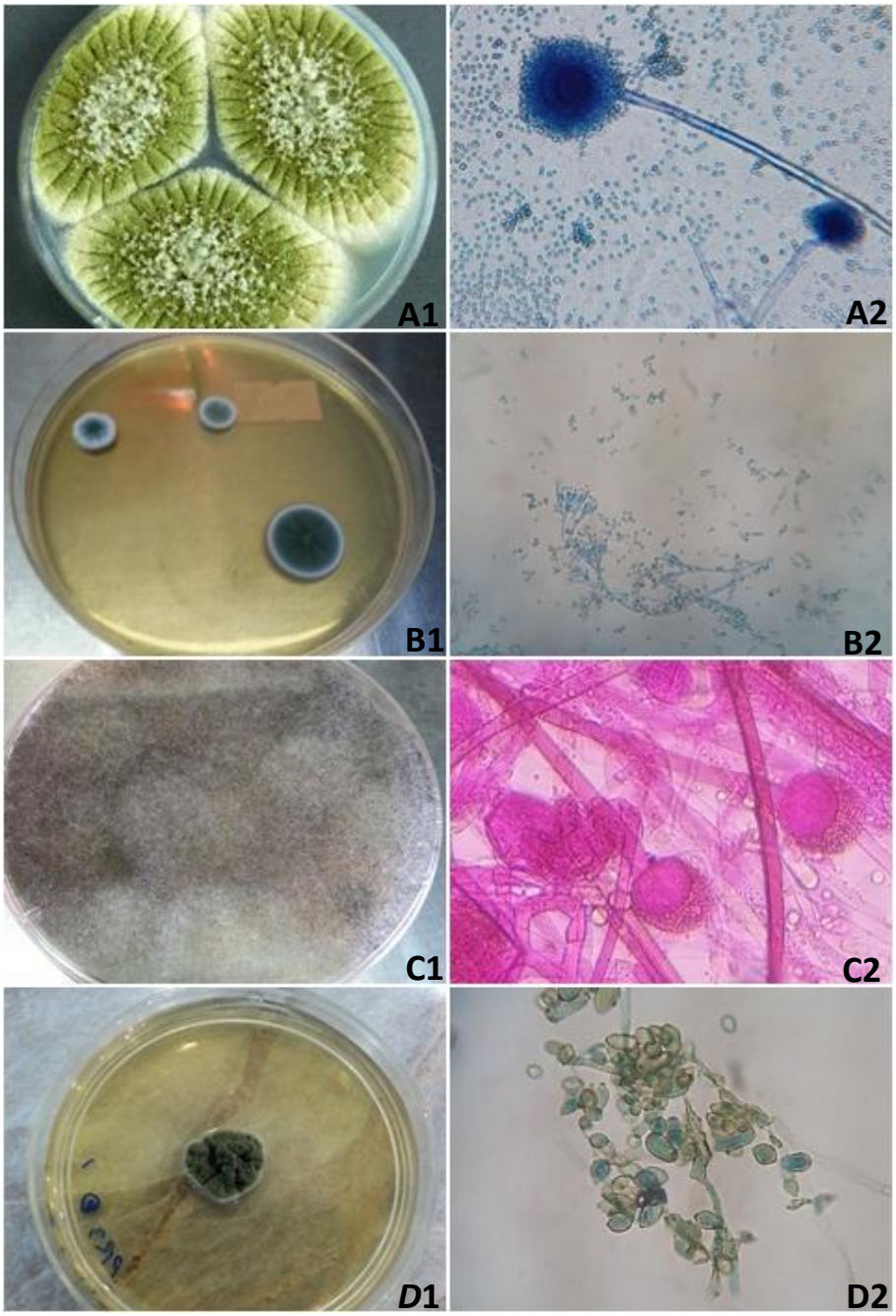

Figure 1: The isolated molds genera from poultry feed on PDA medium. A: *Aspergillus* sp., B: *Penicillium* sp., C: *Rhizopus sp.*, D: *Cladosporium* sp. (1): In culture, (2): Microscopically, 40X.

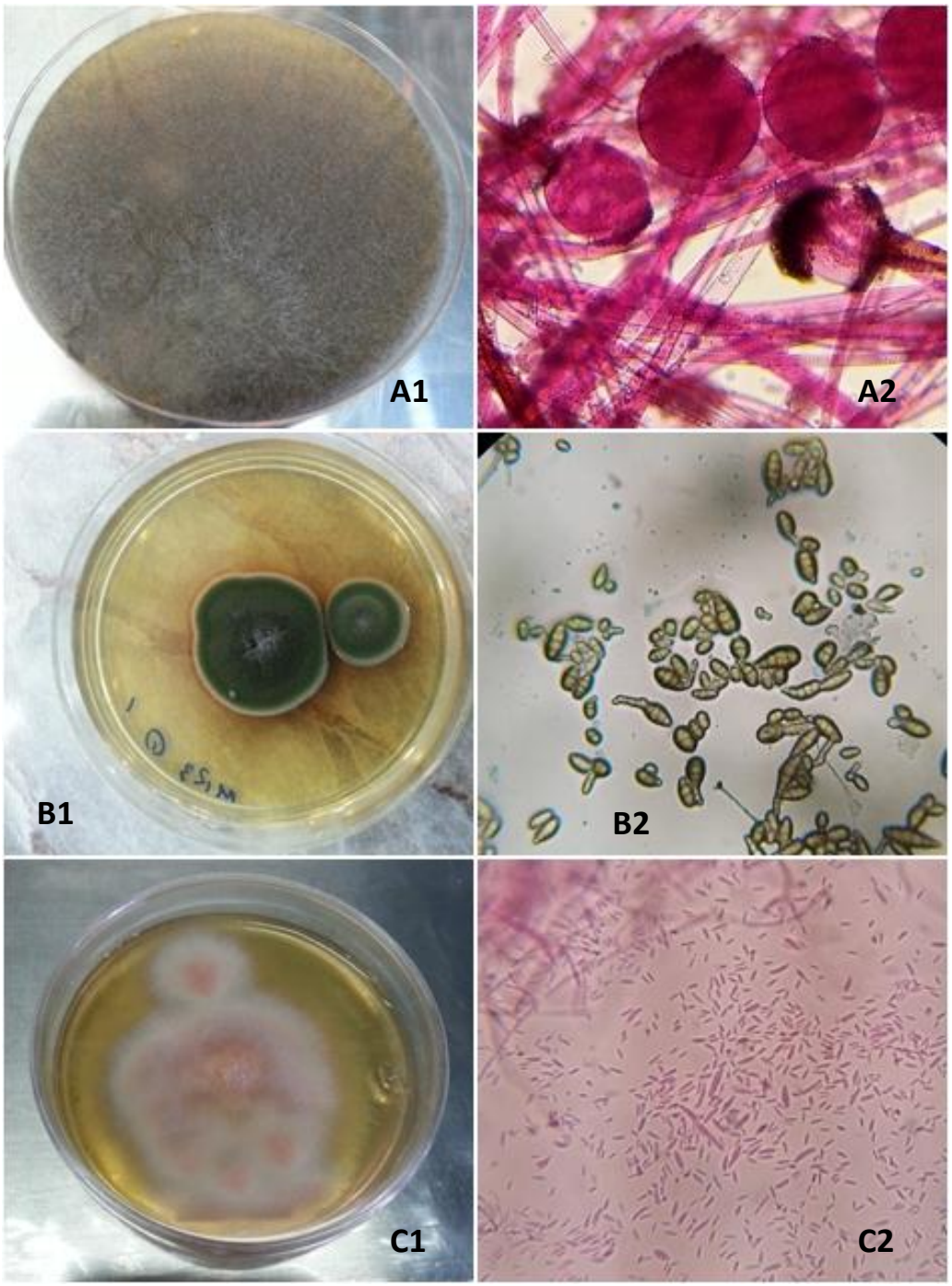

Figure 2: The isolated molds genera from poultry feed on PDA medium. A: *Mucor* sp., B: *Alternaria* sp.*l*, C: *Fusarium* sp.. (1): In culture ,(2): Microscopically , 40X .

The total fungal counts cfu/g were ranged from $5X10^{1}$- $2.1X10^{6}$ of feed sample, with an average of $1.5X\ 10^{5}$ cfu/g of a sample (tables 1) .

Table 1: Range and average count cfu/g of recovered molds genera from poultry diet samples.

Genus	Total mold count (CFU/g)	
	Range	Mean values
Aspergillus spp.	$2.2X10^5$ - $2.1X10^6$	$1.1X10^6$
Penicillium spp.	$7.1X10^3$ - $8.0X10^4$	$4.3X10^4$
Rhizopus spp.	$4.2X10^3$ - $2.1X10^4$	$1.2X10^4$
Cladosporium spp.	$2.4X10^3$ -$2.5X10^3$	$2.4X10^3$
Mucor spp.	$1.5X10^3$ -$2.0X10^3$	$1.7X10^3$
Alternaria spp.	$6.0X10^1$ - $6.3X10^1$	$6.1X10^1$
Fusarium spp.	$5.0X10^1$- $5.6X10^1$	$5.3X10^1$

The frequency (Fr.) and relative density (RD) recorded the highest value which belonged to *Aspergillus* spp., while *Fusarium* sp. was identified as a low value of Fr. and RD (Table 2). The most frequent mycotoxigenic fungi from 180 samples were those from the genus *Aspergillus* spp.. This genus recovered from113 samples (Fr. 62.77%) also with most RD= 52.03% with a range of $2.2X10^5$ - $2.1X10^6$ cfu/g, and a mean value of $1.7X10^6$ cfu/g, followed by *Penicillium spp.* which recovered from 90 samples (Fr.47.77%), also the RD17.01% with a range of $7.1X10^3$ - $8.0X10^4$ cfu/g, and a mean value $4.1X10^4$ cfu/g , *Rhizopus* was recovered from 86 samples with Fr. 50% and RD10.01% , had a range 4.2 X 10^3 - $2.1X10^4$ cfu/g , with mean value $1.6X10^4$ cfu/g . *Fusarium* spp., was found as a low ratio, it was recovered from 7 samples (Fr. 1.66%) RD 2.11% with a range of $5X10^1$- $5.6X10^1$ cfu/g and of a mean value of $7.8X10^1$ cfu/g.

Table 2: Frequency and relative density of recovered mold genera from poultry feed samples.

Genus	Fr. of Positive samples	Fr.%	RD%
Aspergillus spp.	113	62.77	52.03
Penicillium spp.	90	47.77	17.01
Rhizopus spp.	86	50	10.01
Cladosporium spp.	21	11.66	6.23
Mucor spp.	21	4.44	2.55
Alternaria spp.	8	3.88	2.24
Fusarium spp.	7	1.66	2.11

The total *Aspergillus* spp. counts cfu/g were ranged from $0.3X10^2$ - $1.5X10^5$ of feed samples, with an average $7.5X10^4$ cfu/g sample (table 3).

Table 3: Range and average count cfu/g of recovered *Aspergillus* spp. from poultry feed samples

Aspergillus spp	Total mold count CFU/g	
	Range	Mean values
A. flavus	$1.1X10^4$-$1.5X10^5$	8.0X10
A. niger	$5.8X10^3$-$7.4X10^3$	$6.6X10^3$
A. fumigatus	$1.7X10^3$-$3.0X10^3$	$2.3X10^3$
A. terreus	$1.2X10^3$ - $2.4X10^3$	$1.8X10^3$
A. flavipes	$0.5X10^2$ - $1.0X10^2$	$7.5X10^2$
A. carbonarius	$0.4X10^2$ - $0.7X10^2$	$5.5X10^1$
A. ochraceus	$0.4X10^2$ - $0.7X10^2$	$5.5X10^1$
A. candidus	$0.3X10^2$ - $0.4X10^2$	$3.5X10^1$
A. parasiticus	$0.2X10^2$ - $0.4X10^2$	$0.3X10^1$

The frequency and RD recorded the highest value which belonged to A. flavus, while A. parasiticus was identified as a low value of Fr. and RD (Table 4). The most predominant Aspergillus species recovered from 113 samples were A. flavus, recovered from 74 (Fr. 65.48 %) with RD 27.55 % and with range, 1.1X104 -1.5X105 and had a mean value of 8.6X104 cfu/g , followed by A.niger , recovered from 66 samples (Fr. 58.40%) and RD14.23%, with a range 5.8X103-7.4X103 and a mean value 9.5X103 cfu/g, A. fumigatus, recovered from 19 samples (Fr.16.81%) with RD(7.60%) , recorded with range 1.7X103-3.0X103 cfu/g, and mean value 3.2X103 cfu/g, A. parasiticus , were recovered as low percentage and cfu/g, it was recovered from 2 samples with Fr. 1.76 % and with RD 0.89%, which recorded a range 0.2X102 - 0.4X102 cfu/g, with mean value 0.4X101 cfu/g.

Table 4: Frequency and relative density of recovered *Aspergillus* spp. from poultry feed samples

Aspergillus spp.	Fr. of Positive samples	Fr.%	RD%
A.flavus	74	65.48	27.55
A.niger	66	58.40	14.23
A.fumigatus	19	16.81	7.60
A. terreus	12	10.61	0.50
A.flavipes	9	7.96	2.13
A. carbonarius	8	7.07	2.40
A. ochraceus	6	5.30	2.12
A. candidus	3	2.65	1.34
A. parasiticus	2	1.76	0.89

4. Discussion

Total fungal counts in this study were ranged from 5X101- 2.1X106 of feed sample, with an average 1.5X 105 cfu/g sample and were considered as a high contaminant to poultry feed as compared with GMP [23] which demonstrated that the fungal propagules were useful indicators to determine a quality of feeds hygienic, which should not over a value of 1X104 cfu/g. These results agree with those reported by Bragulat et al., [24] from Spain, in Argentina [25] , in Brazil [8] ,and similar to the study in Iraq [2].

This study reported different results of that in Argentina [26] , and in Brazil [27] , that which found the level of cfu/g in their study about 103 cfu/g .They deal with samples in different ways from this study in collection place , and the quantity of samples used in homogenizing because they collected monthly during one year from factories directly from the production line (after processing) and used ten grams of each sample for homogenizing also they incubated under cold white and black fluorescent lamps in a 12/12 h photoperiod for 7 days . The processing way could cause a decrease of different microorganisms during storage from New Zealand [28], and most of the fungi are slow-growing under these conditions and observations may be made after 20 days [29] in Ethiopia. In this study, the samples were collected from farms and the local market of poultry, then 20 g of samples were used in homogenizing then incubated in the dark incubator, therefore the mean of cfu/g of this study was higher 105.

The genera of Aspergillus was the predominant isolated from poultry feed in this study with a mean value of(1.7X106)cfu/g (Fr62.77 % - RD 52.03%), because temperature during collection was a range of 37-47°C and considers the optimal for growth of Aspergillus [31] , followed by Penicillium with a mean value of 4.1X104 cfu/g (Fr 47.77% - RD 17.01%) and Rhizopus had mean value of 1.6X104 cfu/g (Fr.50% -RD10.1%). Similar results which were found in Spain [31; 24], in Argentina [25, 26], in Brazil [32], and in Iraq [2], they reported that the Aspergillus genera were the most frequent .While it differs from the study in Serbia [27] who reported the most frequent genus isolated was Penicillium followed by Aspergillus and Fusarium. While two reports in Serbia [10, 26] they confirmed that the Fusarium is the predominant genera followed by Penicillium and Aspergillus, because the location of Serbia is in the moderate continental climate belt, geographical factors are of superior importance for the occurrence of Fusarium and the most frequently isolated fungi contaminating feedstuffs, vegetables, cereals, and fruits are from the Fusarium [33; 34], also the Fusarium was reported as the most genus recovered from poultry feed , followed by Eurotium, Penicillium ,and Aspergillus, they collected their samples from the region in Argentina suitable to permanent of Fusarium growth [26]. The Aspergillus spp. in this study was confirmed there were nine species recovered and the A.flavus was the most frequently and RD isolated Aspergillus with the mean value of 8.6X104 cfu/g and (Fr. 65.48 % , RD 27.55 %), followed by A.niger, was reported with mean value of 9.5X103 cfu/g and with Fr. 58.40% , RD 14.23% ,and A.fumigatus with the mean value of 3.2X103 cfu/g and with Fr. 16.81%, RD 7.60%. The highest dominance of A.flavus in this study agrees with previous studies [26, 27, 35, 36] , but different to that in Pakistan who found that the most frequently isolated Aspergillus were A. niger followed by A. flavus, as a result of high humidity and high temperature might be responsible for a higher frequency of A. niger in poultry feeds compared with other species of Aspergillus [22]. Such climate was found in the area of this study which has been found very rich in different species of fungi [37; 38; 39; 40; 41].

References

[1] Beg, M. U., Al-Mutairi, M., Beg, K. R., Al-Mazeedi, H. M., Ali, L. N. and Saeed, T. 2005. Mycotoxins in poultry feed in Kuwait. Arch. Environ. Contam. *Toxicol*. **50**:594–602.

[2] Shareef, A. M .2010. Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis. *Iraqi Journal of Veterinary Sciences*, **24**(1): 17-25.

[3] Steenfeldt, S. , Kjaer, J. B. and Engberg, R. M. 2007. Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora ,and feather pecking behaviour. *Br. Poult. Sci*., **48** (4): 454-468.

[4] Ravindran, V. 2013. Poultry development review. poultry feed availability and nutrition in

developing countries: main ingredients used in poultry feed formulations viruses in liver cancer aetiology and prevention. *Cancer Letters*, **286**: 22-28.

[5] Dowd, P. 2004. Validation of a mycotoxin predicting computer program for U.S. midwest grown maize in commercial fields. Proc. Aflatoxin & Fungal Genomics Workshop. *Mycopathologia*, **157**:463.

[6] Glenda, R. O., Jessika, M. R., Marcelo, E .F., Lilia, R. C., Gloria, M. D, Kelly, M. K, Ana, M. D and Carlos, A .R .2006. Mycobiota in poultry feeds ,and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia*, **162**:355-362.

[7] Hassan, Z .U., Khan, M. Z., Khan, A., Javed, I., Sadiqu, U. and Khatoon, A. 2012. Ochratoxicosis in white leghorn breeder hens: production and breeding performance. *Pak. Vet. J.*, **32**(4):557-561.

[8] Rosa, C. A., Ribeiro, J. M., Fraga, M. J., Gatti, M., Cavaglieri,L. R., Magnoli, C .E., Dalcero, A. M. and Lopes, C .W. 2006. Mycoflora of poultry feed and ochratoxin- producing ability of isolated Aspergillus and Penicillium species. *Vet. Microbiol*., **113**: 89-96.

[9] Cegielska-Radziejewska, R., Stuper, K. and Szablewski, T. 2013. Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. *Annals of Agricul. Environ. Med.*, **20**(1): 30-35.

[10] Krnjaja, V., Stojanović, L. j, Cmiljanić, R., Trenkovski, S.and Tomašević, D .2008. The presence of potentially toxigenic fungi in poultry feed. Biotehnol. *Anim. Husb.*, **24**(5-6):87-93.

[11] Lanyasunya, T. P., Wamae, L .W, Musa, H. H., Olowofeso, O. and Lokwaleput, I. K. 2005. The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. *Pak. J. Nutr*., **4**(3): 162–169.

[12] Whitlow, L .W. and Hagler, J .R .W. 2008. Mold and mycotoxin issues in dairy cattle*:* effects, prevention ,and treatment. *WCDS Advances in Dairy Technol.* , **20**:195-209.

[13] Simpson, D. R., Weston, G. E, Turner, J. A., Jennings, P.,and Nicholson, P. 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European J. Plant Path*. **107**(4):421-431.

[14] Mishra,S., Kumar, P .,and Malik, A. 2013. Evaluation of Beauveria bassiana spore compatibility with surfactants. *World Acad. Sci. Eng. Technol*. **73**: 109-113 .

[15] Pitt, I. and Hocking, A .D. 2009. Fungi and Food Spoilage. 3rd ed., Springer, USA., 519.

[16] Greco, M. , Franchi, M. L., Rico Golba, S .L., Pardo, A .G. and Pose, G .N. 2014. Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals. *Scientific World J.* ,968215.

[17] Domsch, K. H.and Gams, W. 1980. Compendium of soil fungi. Academic Press. London, New York, Toronto, Sydney, San Francisco,566.

[18] Klich, M. A. 2002. Identification of common Aspergillus species, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

[19] Beuchat, L. R.and Cousin, M. A . 2001 .Yeasts and molds. In: Downes, F.P., Ito, K. (Eds.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D. C., 209–215.

[20] Gonzalez, H. H., Resnik, S. L., Boca, R .T. and Marasas, W. F. 1995. Mycoflora of Argentinian corn harvested in the main production area in 1990. *Mycopathol.*, **130**(1): 29–36.

[21] Saleemi, M. K., Khan, M. Z, Khan, A. and Javed, I. 2010. Mycoflora of poultry feeds and mycotoxins producing potential of Aspergillus species. *Pakistan Journal of Botany* **, 42** (1): 427-434.

[22] Pacin, A. M., Gonz´alez, H .H. L., Etcheverry ,M., Resnik, S. L., , L., and Espin S. 2003. Fungi associated with food and feed commodities from Ecuador. *Mycopathol.*, **156**(2): 87–92.

[23] Good Manufacturing Practices, GMP 2005 .Regulations on product standards in the animal feed sector. *GMP***14**: 17-08-05.

[24] Bragualt, M .R., Abarca ,M. L., Castella, G. and Cabanes, F. J. 1995. A mycological survey on mixed poultry feeds and mixed rabbit feeds. *J. Sci. Food Agri*., **67**(2): 215-220.

[25] Dalcero, A., Magnoli, C., Chiacchiera, S., Palacios, G. and Reynoso, M .1997. Mycoflora and incidence of aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina.

Mycopathologia **137**: 179-184.
[26] Magnoli, C., Chiacchiera, S., Miazzo, R., Palacio, G., Angeletti, A., Hallak, C. and Dalcero, A .2002. The mycoflora and toxicity of feedstuffs from a production plant in córdoba, Argentina. *Mycotoxin Res*., **18**(1):7-22.
[27] Oliveira, G. R., Ribeiro, J. M ., Fraga, M. E., Cavaglieri, L .R, Direito, G. M., Keller, K. M., Dalcero, A. M. and Rosa, C. A .2006. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins ,and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia* , **162:** 355–362.
[28] Bakker, G. R. 2004. The effects of disease management practices on phylloplane microorganism populations of apple. M.Sc. thesis. Lincoln University, New Zealand.
[29] Gorfu, D. and Sangchote, S. 2005. Fungi associated with field pea seeds from Ethiopia and seed transmission of Ascochyta pinodes. *Seed Sci. & Technol*. **33**:387-396.
[30] Francisco, F. G. and Usberti, R. 2008 Seed health of common bean stored at constant moisture and temperature. *Sci. Agric*., **65** (6): 613-619.
[31] Abarca, M. L., Bragulat ,M. R, Castella, G. and Cabanes,F.J. 1994. Mycoflora and aflatoxin producing strains in animal mixed feeds. *J. Food Prot*., **57**: 256-258.
[32] Simas, M. M .S. , Botura, M. B, Correa, B., Sabino, M., Mallmann, C .A., Bitencourt, T. C .B .S. C. ,and Batatinha, M.J.M. 2007. Determination of fungal microbiota and mycotoxins in brewers grain used in dairy cattle feeding in the State of Bahia, *Brazil. Food Control*., **18**:404-408.
[33] Lević, J., Stanković , S.,Bočarov-Stančić, A., Škrinjar, M. and Mašić, Z .2004. The overview on toxigenic fungi and mycotoxins in Serbia and Montenegro. In An Overview on Toxigenic Fungi and Mycotoxins in Europe; Logrieco, A., Viskonti, A., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 201−218.
[34] Popovski, S. and Celars, F. A .2013. The impact of environmental factors on the infection of cereals with Fusarium species and mycotoxin production – a review. *Acta. Agricul Slovenica*, **101**(1): 105-116.
[35] Lateef, A., Hussaini, M., and Muhammad, H. 2013. Survey of Mycotoxigenic Fungi in Concentrated Poultry Feed in Niger State, Nigeria. *Journal of Food Research*. **2**(2): 128.
[36] Atehnkeng, .J, Ojiambo, P. S., Donner, M., Ikotun, T., Sikora, R. A. and Cotty, P. J. 2008 .Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agro-ecological zones in Nigeria, *Intl .J. Food Microbiol*. ,**122**(1-2):74-84.
[37] Sivanesan, A., Abdullah ,S. K., and Abbas, B. A. 1993. Exserohilum curvisporum sp. nov., a new hyphomycete from Iraq. *Mycological Research*, **97**(12), 1486-1488.
[38] Abdullah, S .K. and Abbas, B. A .1994. Occurrence of thermophilic and thermotolerant fungi in aquatic sediments of Shatt Al-Arab River and its creeks at Basrah, Iraq. *Marina Mesopotamica*, **9**, 39-47.
[39] Abdullah, S. K. and Abbas, B. A .1994 .Taxonomic study on fungi from Water and surface sediments of Shatt Al-Arab river and its creeks I, Ascomycetes. *Marina Mesopotamica*, **9**, 91-100.
[40] Cannon P. F., Abdullah, S. K., and Abbas, B. A. 1995. Two new species of Monascus from Iraq, with a key to known species of the genus. *Mycological Research*, **6**(99), 659-662.
[41] Abdullah, S. K. and Abbas, B. A .2008. Fungi inhabiting surface sediments of Shatt Al-Arab River and its creeks at Basrah, Iraq. Basrah Journal of Science, 26(1B English), 68-81.