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Incidence of Mycoflora and Mycotoxigenic Fungi in Poultry Feeds in Warangal (T.S.), India

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A B S T R A C T

Extensive investigations carried out on the incidence of common and toxigenic fungi revealed that all types of broiler feeds were found to be infested by common as well as toxigenic fungi and none of the samples was free from contamination. *Aspergillus* species were dominant in all the samples, followed by species of *Penicillium*, *Fusarium*, *Alternaria* and *Chaetomium*. Highest number of fungal genera (11) were recorded in broiler concentrate feed amended and broiler concentrate feed with coarse of maize flour. Layer feeds showed association of different fungal species. *Absidia*, *Aspergillus*, *Brevilegnia*, *Curvularia*, *Fusarium*, *Penicillium* and *Mucor* were the major fungal genera isolated from layer feed samples of this region. Toxigenic studies on fungal isolates of poultry feeds revealed that aflatoxin, sterigmatocystin, ochratoxin A, penicillic acid, cyclopiazonic acid, fumonisin B1, HT-2 toxin, moniliformin, deoxynivalenol, nivalenol, T-2 toxin, zearalenone, diacetoxyscirpenol, citrinin, patulin, islanditoxin, roridin E, penicillic acid were the common mycotoxins produced by them. But the percentage of toxigenic isolates varied with the sample. In all the feed samples, highest number of *Aspergillus flavus* isolates were found to be toxigenic followed by *A. ochraceus* for ochratoxin, *Penicillium citrinum* for citrinin. In sunflower meal more number of *Aspergillus parasiticus* strains were positive for aflatoxin. It is concluded that high incidence of toxigenic *Aspergillus flavus* and *A. ochraceus* strains for aflatoxin and ochratoxin A indicates the probability of presence of these toxins in the feed samples.

Keywords

Poultry feeds, Molds, Mycotoxins, Layers, Broilers.

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Introduction

Poultry is one of the fastest growing segments of the agricultural sector in India today. About 3 million farmers directly and 15 million agrarian farmers indirectly are employed in the poultry industry which contributes to the tune of Rs.26,000 crore to the national income. Both Telangana and Andhra Pradesh account for production of 3.5 crore live birds every month, which constitutes one-third of the country's production, apart from six crore eggs per a day. In revenue terms, poultry's contribution to the state's annual GDP is estimated to be about Rs.20,000 crore with employment to close to 20 lakh people. Poultry industry mainly located around Hyderabad city, Vishakapatnam, Chittoor and Warangal districts. According to a survey conducted by our laboratory, in Warangal district annually 26 lakh layer birds are grown and average egg production is 20,00,000 per day and the number of broiler birds grown and consumed in this district are 15,00,000 per month. More than six commercial poultry feed brands are being used in this district especially for broiler. Feed is the key input for successful

poultry production and it accounts for the major cost of poultry production constituting up to 70 percent of the total cost. Poultry diets are formulated from a mixture of ingredients that include cereal grains, cereal by-products, fats, plant protein sources, animal byproducts, vitamin mineral supplements, crystalline amino acids and feed additives.

Two types of feeds *i.e*. broiler feed and layers feed are formulated. Since poultry feeds are rich in all types of nutrients easily to prone to microbial growth at appropriate conditions. Defects in formulations, faulty storage conditions and unseasonal rains promote the infestation of feeds by microorganisms. Fungal contamination of poultry feeds is a regular occurrence on a worldwide scale and detrimental to the poultry industry. Against this background, investigations were taken up for one year duration (June, 2014 to May, 2015) to assess the qualitative and quantitative incidence of mycoflora of different types of poultry feeds. An attempt was made to assess the incidence of mycotoxigenic fungi

Materials and Methods

Studies on mycoflora

Isolation and enumeration of mycotoxigenic fungi associated with poultry feed, feed ingredients

Study region

Samples of commercial poultry feeds, feed ingredients were collected for one year at monthly intervals from poultry farms located in Warangal district, Telangana state of India and the respective feed manufacturers located in other places also. In case of farm mixed poultry feeds, individual feed ingredients along with mixed feeds were also collected.

Sampling criteria

Samples of commercially prepared poultry feeds, farm mixed poultry feeds and feed ingredients were collected through systematic random sampling. For each sample, three initial samples of three kg each were collected from different places. After thorough mixing a composite sample of 1 Kg placed in a sterile polythene bag was brought to the laboratory for inoculation on the culture media. Conditions of the sample at the collection time were recorded.

Isolation and assessment of fungi

Dilution plate technique (Waksman, 1922)

10g of each sample was taken in 250 ml conical flask containing 100 ml of 0.1% sterilized peptone water and subjected to horizontal shaking for 30 minutes and dilutions were made as desired. 0.5 ml of this suspension was poured aseptically into sterilized Petriplates containing cooled Asthana and Hawker's medium A (KNO_3 3.5g, KH_2PO_4 1.75g, $MgSO_4$. $7H_2O$ 0.75g, glucose 5g, Agar-agar 16g and distilled water 1000ml).The medium was poured by making gentle rotational movement so as to ensure uniform spreading of the sample.

Petri plates thus prepared were incubated in an inverted position at 27 ± 2º C under dark condition. To suppress the bacterial growth and to restrict the fungal colonies streptomycin and rose Bengal were added. The fungal colonies developing from the plates were isolated and later on purified by repeated sub culturing. Slides were made on lactophenol cotton blue from individual colonies and the fungi were identified based on morphological features. The percentage of incidence, frequency and abundance of individual fungus were calculated with the help of following formulae.

$$\% \text{ of incidence} = \frac{\text{Number of colonies of a species in all plates}}{\text{Total number of colonies of all the species in all plates}} \times 100$$

$$\% \text{ of frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

$$\% \text{ of abundance} = \frac{\text{Total number of colonies of a species in all observations}}{\text{Total number of colonies in all observations}} \times 100$$

Identification of fungi

Identification of fungi was made on the basis of their colony characters on different culturing media (macroscopic) and microscopic characters (Klich and Pitt, 1988; Singh *et al.,* 1991; Barnett and Hunter, 1998).

Different macroscopic characters used to identify included colony form, size, elevation, margin/border, surface, color (pigmentation), opacity, texture and margins (rim) of colony.

Screening of fungi for mycotoxin elaboration

Different mycotoxins produced by species of *Aspergillus, Fusarium and Penicillium* were detected by standard methods. Aflatoxins (Stack and Pohland, 1975); ochrotoxin-A (Gimeno, 1979); sterigmatocystine (Adey and Mateles, 1964); penicillic acid and citrinin (Gorst-Allman and Steyn, 1979); diacetoxy scirpenol, deoxynivalenol and nivalenol (Ramakrishna and Bhat, 1987); moniliformin fumonisin B1, zearalenone, HT-2 toxin and T-2 toxin (Kamimura *et al.,* 1981); Cyclopiazonic acid (Rathinavelu and Shanmugasundaram, 1983); patulin (Subramanian, 1982) and islanditoxin (Takeda *et al.,* 1973).

Results and Discussion

Broiler feeds

The mycoflora isolated from broiler concentrate feeds are shown in the Table 1. It is evident from the table that a variety of fungi was associated with the broiler concentrate feed samples of Warangal district. 27 fungal species representing 11 genera were recovered from feed samples. Of these, most of the species belonged to *Aspergillus*, *Fusarium* and *Penicillium*. Genus *Aspergillus* represented with 8 species, similarly *Fusarium* with 6 speceis and *Penicillium* with 3 species. Highest total number of 20 fungal species was isolated in June 2014 and January 2015, whereas least number of fungal species appeared in the month of April 2015. A total of 8 species of *Aspergillus* appeared in the month of July 2014, similarly 6 species of *Fusarium* appeared in the month of October, 2014 and 3 speceis of *Penicillium* appeared in 3 months September, 2014, December 2014 and Febraury 2015. None of the *Fusarium* species appeared in April 2015, and similarly no *Penicillium* species were recovered in May 2015. *Aspergillus humicola*, *A. candidus*, *A. flavus* and *Cladosporium cladosporioides* showed highest percentage of frequency appeared for 10 months of the study out of 12 months. *Chaetomium cupreum* recorded with lowest percentage of frequency appeared only in 2 months of the study. Rests of the fungal species studied are recorded with intermediate frequency. *A. flavus* stands for highest incidence for 9 months, while *F. moniliforme* showed highest incidence in October 2014 to January 2015. Highest abundance was recorded with *A. flavus*, whereas lowest abundance was recorded with *Absida repens*.

Toxigenic potential of different species of mycoflora isolated from broiler concentrate feed were screened and the results are

presented in the Table 2. A critical perusal of the table reveals that many fungi colonizing broiler concentrate feeds are toxigenic. However, the degree of toxigenicity varied with the fungus. Out of 126 isolates of *Aspergillus flavus*, 87 isolates elaborated aflatoxin and sterigmatocystin, only 4 isolates of *A. nidulans* were positive for sterigmatocystin, out of 25 isolates screened. Out of 58 isolates of *A. ochraceus* 22, 11 and 2 isolates were positive for ochratoxin A, penicillic acid and cyclopiazonic acid respectively. Only 2 isolates of *A. versicolar* were able to produce cyclopiazonic acid out of 18 isolates screened.

Out of 26 isolates of *Fusarium avenaceum* 8, 3, 4, 2 and 5 isolates respectively were found to be positive for fumonisin B_1, HT-2 toxin, moniliformin, T-2 toxin and zearalenone production. On the other hand, screening of 32 isoltes of *F. equiseti* revealed that 6, 8, 9 and 11 isolates were positive for production of moniliformin, nivalenol, T-2 toxin and zearalenone, respectively. Out of 43 isolates of *F. moniliforme* 11, 13, 3, 6, 8 and 1 isolates were found to produce diacetoxyscirpenol, fumonisin B_1, moniliformin, nivalenol, T-2 toxin and zearalenone respectively. Out of 14 isolates of *F. oxysporum* screened 3, 3, and 4 isolates were positive for production of moniliformin, nivalenol and zearalenone respectively. Only one isolate of *F. solani* proved to be positive for T-2 toxin out of 14 isolates screened.

Nineteen isolates of *Penicillium citrinum* elaborated citrinin when 56 isolates were screened for toxin production. Nine isolates of *P. griseofulvum* elaborated patulin when 27 isolates were screened for their toxigenic potentials. Only 3 isolates of *P. islandicum* elaborated island toxin when14 isolates were screened. It was observed that *A. versicolar* failed to produce sterigmatocystin. Similarly *F. avenaceum, F. equiseti, F. moniliforme* have failed to produce nivalenol, diacetoxyscirpenol, HT-2 toxin respectively in culture filtrates.

Layer feeds

Layer chick feed

Mycoflora isolated form layer chick feed is presented in the Table 3. An overall 12 fungal species representing 8 genera were isolated during 12 months of the study. Highest number of fungal species appeared in October 2014, whereas least number of fungal species appeared for 4 months i.e. June to August 2014 and in April 2015 during entire study. Most of the fungal species belonged to *Aspergillus* and *Alternaria* represented by 4 and 3 species respectively. All the *Aspergillus* spp. appeared in the month of May 2015 whereas all the *Alternaria* spp. appeared in September 2014. *Aspergillus flavipes* showed highest incidence for 5 months during entire study. In the month of November 2009, *Alternaria humicola*, *Aspergillus flavus*, *A. ochraceus*, *Curvularia geniculata* and *Mucor globosus* have showed highest incidence. *Curvularia geniculata* was recorded with highest incidence in June 2009. *A. ochraceus* showed highest frequency appeared in nine months of the study. *Alternaria solani* with least frequency traced for 2 months in the study. *Alternaria flavipes* showed highest abundance with highest number of colonies.

Toxigenic potential of different fungi isolated from layer chick feed were screened and the results obtained are presented in Table 4. From the table it is clear that fungi colonizing layer chick feed were having toxin producing potentials. Eight isolates of *A. clavatus* elaborated patulin out of 12 strains were isolated. Similarly, 6 and 1 isolate of *A. flavus* were positive for aflatoxin and sterigmatocystin when 25 isolates were screened for their toxigenic capabilities.

Table.1 Month-wise qualitative and quantitative analysis of incidence of mycoflora of broiler concentrate feed (June, 2014 to May, 2015)

Name of the fungus	Percentage of incidence												Percentage of frequency	Percentage of abundance
	A	B	C	D	E	F	G	H	I	J	K	L		
Absidia glauca	1.14	0.65	-	-	1.10	0.62	-	3.94	-	-	5.50	-	50.00	1.07
A. repens	-	-	1.56	0.54	-	-	1.08	0.49	-	1.60	-	-	41.66	0.43
Alternaria fasciculata	3.41	1.96	1.56	-	-	-	2.16	2.46	-	2.40	-	2.70	66.66	1.34
Aspergillus humicola	2.27	1.31	-	3.23	1.66	1.24	0.54	3.94	2.21	-	2.75	1.35	83.33	1.83
A. candidus	1.14	7.19	2.34	0.54	7.73	4.97	-	1.97	7.18	-	5.50	6.76	83.33	3.60
A. flavus	18.18	18.30	31.25	32.26	-	-	15.14	18.72	19.89	33.60	29.36	40.54	83.33	19.66
A. glaucus	6.25	5.23	-	-	-	2.48	-	-	-	4.80	4.59	4.05	50.00	1.99
A. nidulans	1.14	1.96	-	-	-	3.73	2.16	5.42	1.10	8.80	-	-	58.33	2.09
A. ochraceus	12.50	11.76	8.59	-	-	1.24	-	0.99	-	12.00	22.94	25.68	66.66	6.12
A. orantus	-	3.92	2.34	-	2.21	5.59	5.95	5.91	6.08	-	-	2.70	66.66	3.11
A. versicolor	2.27	8.50	-	-	11.60	7.45	5.95	2.96	4.42	11.20	10.09	-	75.00	5.37
Chaetomium cupreum	-	-	-	-	1.66	-	-	0.99	-	-	-	-	16.66	0.27
C. globosum	-	-	-	2.15	-	-	1.08	-	-	-	5.50	-	25.00	0.64
Cladosporium cladosporioides	4.55	3.92	9.38	5.91	8.29	8.70	5.41	0.99	-	2.40	-	1.35	83.33	4.40
Curvularia geniculata	-	5.23	-	-	2.21	1.86	-	0.99	-	0.80	-	1.35	50.00	1.02
C. lunata	3.41	-	7.03	-	6.08	2.48	3.24	1.48	0.55	1.60	-	-	66.66	2.26
Eurotium chevalieri	1.14	-	-	1.08	0.55	1.86	-	-	1.10	-	0.92	1.35	58.33	0.64
Fusarium avenaceum	4.55	3.92	3.91	-	2.21	-	4.86	7.39	6.08	3.20	-	1.35	75.00	3.38
F. equiseti	1.14	1.96	1.56	-	4.42	6.21	3.24	-	-	1.60	-	-	58.33	1.77
F. moniliforme	15.91	-	-	11.83	14.36	16.15	15.68	18.72	11.05	11.20	-	-	66.66	10.90
F. oxysporum	-	11.76	8.59	8.06	8.84	-	14.05	7.88	9.94	-	-	-	58.33	6.44
F. poae	6.82	-	-	5.91	9.94	12.42	-	7.39	6.08	-	-	-	50.00	4.67
F. solani	7.39	7.19	7.81	6.45	7.73	6.83	-	-	5.52	-	-	10.81	66.66	4.78
Penicillium citrinum	4.55	4.58	11.72	5.91	-	-	8.65	-	11.05	-	11.01	-	58.33	4.78
P. griseofulvum	-	-	-	6.45	6.08	9.94	8.11	6.40	6.63	-	-	-	50.00	4.24
P. islandicum	1.14	-	-	9.68	3.31	4.97	2.16	-	1.10	4.80	-	-	58.33	2.47
Rhizopus stolonifer	1.14	0.65	2.34	-	-	1.24	0.54	0.99	-	-	1.83	-	58.33	0.70

A=June, 2014; B= July, 2014; C=August, 2014; D = September, 2014; E = October, 2014; F = November, 2014; G = December, 2014; H = January, 2015; I = February, 2015; J=March, 2015, K=April, 2015; L=May, 2015.

Table.2 Screening of toxigenic fungi and respective toxins produced in broiler concentrate feed

Name of the fungus	Number of strains screened	Number of toxin producing strains	% of positive strains	Toxin produced
Aspergillus flavus	126	87	69.1	Aflatoxin
		17	13.5	Sterigmatocystin
A. nidulans	25	4	16.0	Sterigmatocystin
A. ochraceus	58	22	37.9	Ochratoxin-A
	58	11	19.0	Penicillic acid
A. versicolor	18	2	11.1	Cyclopiazonic acid
		ND	--	Sterigmatocystin
Fusarium avenaceum	26	8	30.8	Fumonisin B1
		3	11.5	HT-2 toxin
		4	15.4	Moniliformin
		ND	--	Nivalenol
		2	7.7	T-2 toxin
		5	19.2	Zearalenone
F. equiseti	32	ND	--	Diacetoxyscirpenol
		6	18.8	Moniliformin
		8	25.0	Nivalenol
		9	28.1	T-2 toxin
		11	34.4	Zearalenone
F. moniliforme	43	11	25.6	Diacetoxyscirpenol
		13	30.2	Fumonisin B1
		ND	--	HT-2 toxin
		3	7.0	Moniliformin
		6	14.0	Nivalenol
		8	18.6	T-2 toxin
		1	2.3	Zearalenone
F. oxysporum	14	3	21.4	Moniliformin
		3	21.4	Nivalenol
		4	28.6	Zearalenone
F. solani	14	1	7.1	T-2 toxin
Penicillium citrinum	56	19	33.9	Citrinin
P. griseofulvum	27	9	33.3	Patulin
P. islandicum	14	3	21.4	Islanditoxin

ND = Not detected

Table.3 Month-wise qualitative and quantitative analysis of incidence of mycoflora of layer chick feed (June, 2014 to May, 2015)

Name of the fungus	Percentage of incidence												Percentage of frequency	Percentage of abundance
	A	B	C	D	E	F	G	H	I	J	K	L		
Alternaria flavipes	-	22.22	53.33	37.50	34.78	-	-	32.00	22.22	41.66	-	-	58.33	25.00
A. humicola	-	-	-	10.41	-	13.04	15.38	-	-	-	11.11	-	33.33	4.66
A. solani	-	-	-	4.16	-	-	-	-	-	-	-	14.28	16.66	1.69
Aspergillus clavatus	15.38	33.33	-	-	6.52	8.69	-	-	11.11	-	-	7.14	50.00	5.08
A. flavus	-	-	-	4.16	10.86	13.04	-	16.00	-	-	55.55	42.85	50.00	10.59
A. giganteus	-	-	6.66	-	4.34	-	7.69	12.00	22.22	8.33	-	14.28	58.33	5.08
A. ochraceus	7.69	33.33	33.33	-	-	13.04	15.38	32.00	-	8.33	22.22	7.14	75.00	11.01
Curvularia geniculata	61.53	-	-	12.50	4.34	13.04	15.38	4.00	-	-	-	-	50.00	9.32
Drechslera sp.	-	-	-	4.16	2.17	-	-	4.00	-	25.00	-	14.28	41.66	3.81
Epicoccum nigrum	-	-	6.66	-	4.34	4.34	-	-	11.11	-	-	-	33.33	2.11
Fusarium solani	-	-	-	16.66	21.73	8.69	7.69	-	33.33	-	-	-	41.66	10.16
Mucor globosus	15.38	11.11	-	6.25	8.69	13.04	38.46	-	-	16.66	11.11	-	66.66	8.89
White sterile mycelium	-	-	-	4.16	2.17	13.04	-	-	-	-	-	-	25.00	2.54

A=June, 2014; B= July, 2014; C=August, 2014; D = September, 2014; E = October, 2014; F = November, 2014; G = December, 2014; H = January, 2015; I = February, 2015; J=March, 2015; K=April, 2015; L=May, 2015.

Table.4 Screening of toxigenic fungi and respective toxins produced in layer chick feed

Name of the fungus	Number of strains screened	Number of toxin producing strains	% of positive strains	Toxin produced
Aspergillus clavatus	12	8	33.3	Patulin
A. flavus	25	6	24.0	Aflatoxin
		1	4.0	Sterigmatocystin
A. ochraceus	13	2	15.4	Ochratoxin-A
		ND	--	Penicillic acid

ND = Not detected

Table.5 Month-wise qualitative and quantitative analysis of incidence of mycoflora of layer grower feed (June, 2014 to May, 2015)

Name of the fungus	Percentage of incidence												Percentage of frequency	Percentage of abundance
	A	B	C	D	E	F	G	H	I	J	K	L		
Alternaria alternata	16.67	-	-	5.88	-	-	-	16.67	7.69	-	-	-	33.33	3.48
A. fasciculata	-	16.67	14.29	11.76	-	7.69	-	-	7.69	-	8.33	-	50.00	5.22
Aspergillus flavus	16.67	-	-	5.88	15.79	-	17.65	33.33	15.38	27.78	-	7.69	66.66	12.21
A. ochraceous	16.67	16.67	7.14	-	10.53	-	-	8.33	15.38	16.67	16.67	15.38	75.00	9.88
A. oryzae	-	-	14.29	5.88	-	23.08	11.76	-	-	5.56	-	-	41.66	5.22
A. parasiticus	8.33	8.33	14.29	-	-	-	5.88	-	7.69	-	8.33	-	50.00	4.07
A. repens	-	-	-	-	5.26	-	17.65	-	-	11.11	-	-	25.00	3.48
A. ustus	-	16.67	7.14	5.88	15.79	30.77	-	-	7.69	-	16.67	7.69	66.66	8.72
A. versicolor	8.33	-	7.14	-	5.26	-	-	-	7.69	-	-	-	33.33	2.33
Curvularia affinis	16.67	16.67	14.29	5.88	-	15.38	-	-	7.69	11.11	16.67	15.38	75.00	9.30
C. lunata	-	-	7.14	-	-	-	5.88	-	-	5.56	-	-	25.00	1.74
C. subulata	-	-	-	5.88	-	-	5.88	8.33	-	-	-	7.69	33.33	2.33
Epicoccum nigrum	-	8.33	-	5.88	5.26	-	-	-	7.69	11.11	8.33	-	50.00	4.07
Melanospora damnosa	-	-	-	-	-	-	5.88	-	-	5.56	-	-	16.66	1.16
Memnoniella echinata	-	-	7.14	-	-	-	5.88	16.67	-	5.56	-	15.38	41.66	4.07
Penicillium citrinum	16.67	8.33	-	17.65	26.32	23.08	11.76	8.33	-	-	16.67	15.38	75.00	12.21
P. digitatum	-	-	7.14	11.76	5.26	-	-	-	15.38	-	8.33	7.69	50.00	4.65
P. griseofulvum	-	8.33	-	17.65	10.53	-	11.76	8.33	-	-	-	7.69	50.00	5.81

A=June, 2014; B= July, 2014; C=August, 2014; D = September, 2014; E = October, 2014; F = November, 2014; G = December, 2014; H = January, 2015; I = February, 2015; J=March, 2015; K=April, 2015; L=May, 2015.

Table.6 Screening of toxigenic fungi and respective toxins produced in layer grower feed

Name of the fungus	Number of strains screened	Number of toxin producing strains	% of positive strains	Toxin produced
Aspergillus flavus	18	6	33.3	Aflatoxin
		2	11.1	Sterigmatocystin
A. ochraceus	15	3	20.0	Ochratoxin-A
		6	40.0	Penicillic acid
A. parasiticus	7	4	57.1	Aflatoxin
A. versicolor	3	2	66.7	Cyclopiazonic acid
		ND	--	Sterigmatocystin
Penicillium citrinum	11	4	36.4	Citrinin
P. griseofulvum	6	ND	--	Patulin

ND = Not detected

On the other hand, only 2 isolates of *A. ochraceus* reported to produce ochratoxin A out of 13 isolates screened. However, *A. ochraceus* did not produce penicillic acid.

Layer grower feed

The mycoflora isolated from layer grower feed was shown in the Table 5. It is evident from the table that a variety of fungal species are associated with the grower feed samples of Warangal district. Eighteen fungal species representing 7 genera were recorded from feed samples. Of these, most of the species belonged to *Aspergillus*, *Curvularia* and *Penicillium*. Genus *Aspergillus* represented by 7 species similarly *Curvularia* with 3 species and *Penicillium* with 3 species. Highest total number of fungal species appeared in September 2014 whereas least number of fungal species appeared in the month of June 2014 and January 2015. A total of 7 speceis of *Aspergillus* did not appear in any month during 12 months of the study. Total 3 species of *Penicillium* appeared in the months of September, October 2014 and May 2015 and total 3 species of *Curvularia* were not traced out in any one month of the study. *Aspergillus ochraceus*, *Curvularia affinis* and *Penicillium citrinum* showed highest frequency appeared for 9 months of the study out of 12 months. *Melanospora damnosa* recorded with lowest percentage of frequency appeared only in 2 months of the study. Rest of the fungal species was recorded with moderate frequency. *Aspergillus flavus* stands for highest incidence for 3 months. While *Penicillium citrinum* showed highest incidence in September 2014 and October 2014. Highest abundance was found with *A. flavus* and *P. citrinum*, whereas lowest abundance with *Melanospora damnosa*.

Different fungi isolated from layer grower feed were screened for their toxigenic potentials and the results are presented in the Table 6. A critical study of the table reveals that many fungi colonizing layer grower feed were able to produce different mycotoxins. However, the degree of toxigenicity varied with the fungus. Out of 18 isolates of *A. flavus* 6 and 2 isolates respectively were positive for aflatoxin and sterigmatocystin production. Three and 6 isolates of *A. ochraceus* elaborated ochratoxin A and penicillic acid out of 15 isolates screened for their toxigenic potentials. Similarly 4 isolates of *A. parasiticus* elaborated aflatoxin out of 7 isolates screened, on the other hand, out of 3 isolates of *A. versicolor* 2 isolates were positive for production of cyclopiazonic acid. However sterigmatocystin was not produced by *A. versicolor*. Out of 11 isolates of *Penicillium citrinum* only 4 isolates produced citrinin. However, *A. versicolor* failed to produce sterigmatocystin, similarly patulin by *P. griseofulvum*.

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