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# STUDIES ON AEROMYCOFLORA OF POULTRY HOUSES WITH REFERENCE TO OCCURRENCE OF MYCOTOXIGENIC FUNGI

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## ABSTRACT

*Investigations on the occurrence of aeromycoflora of poultry houses were carried with an objective of assessing its impact on poultry workers and poultry birds and also mycotoxin production potential of components of aeromycoflora. These studies revealed the incidence of 33 fungal species representing 11 genera. Most of the species belonged to Aspergillus. The incidence of spores of fungal species varied with the season. Different Aspergillus species isolated from the air of the poultry shed were found to produce aflatoxins, sterigmatocystin, ochratoxin A and cyclopiazonic acid. Fusarium species produced deoxynivalenol, zearalenone, moniliformin and T-2 toxin. High temperature, humidity and organic materials favored proliferation of fungi and release of spores that contributed to the aeromycoflora.*

## KEY WORDS

*Aeromycoflora, poultry houses, mycotoxins, Aspergillus, Fusarium*

## Introduction

The aeromycoflora of the indoor and outdoor environments is considered to be important in the fields like plant pathology, environment, biodeterioration, allergic diseases etc. Several workers have investigated aeromycoflora of fruits and vegetable markets [1,2], store markets [3,4],

indoor air of poultry houses can be an important source of fungi [5,6,7] and involve high risk of occupational exposure. High level relative humidity, litter accumulation in the floor promote the growth of certain moulds. These moulds produce spores which are released into the air of poultry shed. Though the aeromycoflora has been studied from different perspectives, few attempts have been made to study the aeromycoflora in relation to poultry diseases. Further, there are very few studies on aeromycoflora of poultry shed in relation to mycotoxin problem [8,9,10]. Against this background, investigations were carried out to screen the aeromycoflora of different poultry houses and also to assess the extent of incidence of mycotoxigenic fungi.

## Materials and Methods

**Isolation of aeromycoflora:** Aeromycoflora of poultry houses was investigated for one year with monthly intervals (June 2014 to May 2015). Petriplates containing sterilized Asthana and Hawker's medium A were exposed for 2 min at the 6 ft height. Petriplates thus exposed were kept for incubation for 7 days at room temperature (27 ± 2º C) [8]. The fungal colonies developing from the plates were isolated and later on purified by repeated subculturing. Microscopic slides were prepared on lacto phenol cotton blue from individual colonies and the fungi were identified based on morphological features. The percentage of incidence, frequency and abundance of individual fungi were calculated with the help of following formulae.

**Table 1 : Month-wise qualitative and quantitative analysis of incidence of aeromycoflora of poultry houses (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* | 16.0 | 7.3 | - | 7.8 | 5.4 | 3.2 | 1.4 | - | - | 1.9 | - | 7.7 | 66.66 | 4.6 |
| *A. solani* | 5.3 | 3.6 | 5.7 | 6.9 | 2.2 | 1.1 | - | 11.3 | 1.8 | 3.8 | - | 2.6 | 83.33 | 3.9 |
| *Aspergillus candidus* | 2.7 | - | - | 1.0 | - | 2.1 | - | - | 3.6 | - | 3.4 | - | 41.66 | 0.9 |
| *A. chevalieri* | - | - | 2.9 | - | 3.3 | - | - | - | - | - | - | - | 16.66 | 0.7 |
| *A. clavatus* | - | 2.7 | 1.9 | 1.0 | - | 2.1 | 2.7 | - | 10.9 | 1.9 | - | 7.7 | 66.66 | 2.2 |
| *A. flavipes* | 2.7 | - | 1.9 | - | 4.3 | - | - | 3.2 | - | - | 6.9 | - | 41.66 | 1.3 |
| *A. flavus* | 10.7 | 11.8 | 4.8 | 5.9 | 2.2 | 9.6 | 13.7 | 3.2 | 5.5 | - | 3.4 | - | 83.33 | 6.6 |
| *A. fumigatus* | - | 1.8 | 3.8 | - | - | 3.2 | 5.5 | 9.7 | 14.5 | - | - | - | 50.00 | 3.0 |
| *A. nidulans* | 4.0 | 7.3 | - | 8.8 | - | 12.8 | 4.1 | - | - | 5.7 | - | 2.6 | 58.33 | 4.4 |
| *A. niger* | - | - | 2.9 | 8.8 | 3.3 | 8.5 | 8.2 | 6.5 | 3.6 | - | 3.4 | - | 66.66 | 4.0 |
| *A. ochraceus* | 5.3 | - | 7.6 | - | 3.3 | - | - | 3.2 | - | 3.8 | - | 2.6 | 50.00 | 2.2 |
| *A. ornatus* | - | 3.6 | - | - | 7.6 | - | 4.1 | - | - | 1.9 | - | - | 33.33 | 1.7 |
| *A. terreus* | - | 5.5 | - | 2.0 | - | 1.1 | - | - | 3.6 | - | 10.3 | 15.4 | 50.00 | 2.2 |
| *A. versicolor* | - | - | 2.9 | - | - | 3.2 | - | 8.1 | - | 11.3 | - | - | 33.33 | 1.9 |
| *Chaetomium globosum* | 1.3 | 1.8 | - | 2.0 | - | 4.3 | 4.1 | - | 5.5 | - | 3.4 | - | 58.33 | 1.8 |
| *C. indicum* | - | - | - | - | 4.3 | - | - | 9.7 | - | - | - | - | 16.66 | 1.1 |
| *Circinella spinosa* | - | - | 3.8 | 3.9 | - | - | - | - | - | - | - | - | 16.66 | 0.9 |
| *Cladosporium epiphyllum* | 14.7 | 13.6 | 25.7 | 10.8 | 8.7 | 7.4 | 4.1 | 8.1 | 10.9 | 15.1 | 44.8 | 25.6 | 100.00 | 13.9 |
| *C. herbarum* | 10.7 | 7.3 | 10.5 | 3.9 | - | 2.1 | - | 6.5 | 3.6 | - | 3.4 | - | 66.66 | 4.5 |
| *Curvularia subulata* | - | 7.3 | - | 10.8 | - | - | 4.1 | - | 9.1 | - | - | 7.7 | 41.66 | 3.4 |
| *C. lunata* | 4.0 | 0.9 | 4.8 | - | 4.3 | 2.1 | - | 1.6 | - | 9.4 | - | 5.1 | 66.66 | 2.6 |
| *Drechslera halodes* | - | - | - | - | 1.1 | - | 1.4 | - | - | - | - | - | 16.66 | 0.2 |
| *Fusarium graminearum* | 4.0 | 4.5 | - | 8.8 | 13.0 | 10.6 | 8.2 | 1.6 | - | 7.5 | - | 5.1 | 75.00 | 5.8 |
| *F. oxysporum* | - | 3.6 | 1.0 | - | 3.3 | 6.4 | 4.1 | - | 10.9 | 5.7 | - | - | 58.33 | 2.9 |
| *F. poae* | - | - | 3.8 | 5.9 | 7.6 | 9.6 | 4.1 | 1.6 | - | 3.8 | - | - | 58.33 | 3.6 |
| *F. solani* | - | - | 1.9 | 2.9 | 5.4 | - | 1.4 | - | 3.6 | - | 3.4 | - | 50.00 | 1.6 |
| *Mucor globosus* | 16.0 | 7.3 | 6.7 | - | 7.6 | - | 12.3 | - | - | 11.3 | - | 10.3 | 58.33 | 6.0 |
| *M. mucedo* | - | 8.2 | 2.9 | 2.9 | 5.4 | 2.1 | 1.4 | 4.8 | 3.6 | - | 3.4 | - | 75.00 | 3.3 |
| *Penicillium chrysogenum* | - | - | - | 2.9 | - | 4.3 | - | 9.7 | - | - | - | - | 25.00 | 1.5 |
| *P. citrinum* | - | 1.8 | - | 2.0 | 3.3 | 3.2 | 6.8 | 6.5 | - | 11.3 | - | - | 58.33 | 2.8 |
| *P. islandicum* | - | - | 2.9 | - | 3.3 | 1.1 | 1.4 | 4.8 | - | - | 10.3 | - | 50.00 | 1.6 |
| *P. italicum* | - | - | - | - | - | - | - | - | 7.3 | - | - | - | 8.33 | 0.4 |
| *Trichothecium roseum* | - | - | 1.9 | - | - | - | 6.8 | - | - | 5.7 | - | 7.7 | 33.33 | 1.5 |
| Sterile mycelia | 2.7 | - | - | 1.0 | 1.1 | - | - | - | 1.8 | - | 3.4 | - | 41.66 | 0.7 |

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 aeromycoflora for mycotoxigenic potential and mycotoxins produced by respective fungi**

| Name of the fungus | Name of the toxin | Number of strains screened | Number of toxin producing strains | % of positive strains |
|---|---|---|---|---|
| *Aspergilus flavus* | Aflatoxin | 80 | 52 | 65.0 |
| | Sterigmatocystin | | 11 | 13.8 |
| *A. nidulans* | Sterigmatocystin | 30 | 12 | 40.0 |
| *A. ochraceus* | Ochratoxin-A | 45 | 28 | 62.2 |
| | Penicillic acid | | ND | -- |
| *A. versicolor* | Cyclopiazonic acid | 25 | 6 | 24.0 |
| | Sterigmatocystin | | 10 | 40.0 |
| *Fusarium graminearum* | Deoxynivalenol | 25 | 8 | 32.0 |
| 22 | Zearalenone | | 6 | 24.0 |
| *F. oxysporum* | Moniliformin | 15 | 2 | 13.3 |
| | Nivalenol | | ND | -- |
| | Zearalenone | | 6 | 40.0 |
| *F. solani* | T-2 toxin | 20 | 9 | 45.0 |
| *Penicillium citrinum* | Citrinin | 38 | 16 | 42.1 |
| *P. islandicum* | Islanditoxin | 15 | 3 | 20.0 |

ND = Not detected

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

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

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

**Identification of fungi**

Identification of fungi was made on the basis of their colony characters on different culturing media (macroscopic) and microscopic characters [11,12,13]. Different macroscopic characters used to identify included colony form, size, elevation, margin/border, surface, color (pigmentation), opacity, texture and margins (rim) of colony.

Different mycotoxins produced by species *Aspergillus, Fusarium and Penicillium* were detected by standard methods. Aflatoxins [14]; ochrotoxin-A [15]; sterigmatocystin [16]; penicillic acid and citrinin [17]; deoxynivalenol and nivalenol [18]; moniliformin, T-2 toxin and zearalenone [19]); cyclopiazonic acid [20]; islanditoxin [21] .

## Results and Discussion

The aeromycoflora encountered during this entire study was listed in the Table 1. The study reveals that air of poultry sheds was heavily loaded with different species of fungi. Thirty-three fungal species representing 11 genera were isolated from poultry sheds of Warangal district. The most dominant fungal species belonged to *Aspergillus*, which was represented by 12 species. The other dominant fungal species belonged to *Fusarium* and *Penicillium*. Most of the fungal species were isolated during the months of August, September and December 2014, whereas least fungal genera were reported in April and May 2015. Interestingly, *Cladosporium epiphyllum* was recorded with highest incidence for 6 months period during this entire study. In June 2014, two species *Alternaria alternata*, *Mucor globosus* and *Aspergillus nidulans* in November 2014, *A. flavus* in December 2014, *Alternaria solani* in January 2015, *Aspergillus clavatus* and *A. fumigatus* in February 2015 showed highest incidence respectively. *Fusarium* spp. appeared in the months of October 2014 and December 2014. *Cladosporium epiphyllum* showed highest frequency appeared in all months during the entire investigation. Least abundance was recorded with *Penicillium italicum* appeared only in one month. *Cladosporium epiphyllum* showed highest abundance whereas *P. italicum* which was with lowest abundance.

Fungal isolated from air of different poultry houses of Warangal district were screened, for their toxigenic potentials and the results are presented in the Table 2. From the table it is evident that out of 80 isolates of *Aspergillus flavus,* 52 and 11 isolates were positive for aflatoxin and sterigmatocystin production respectively. Twelve isolates of *A. nidulans* were found to produce sterigmatocystin out of 30 isolates screened. Similarly, out of 45 isolates of *A. ochraceus,* 28 isolates elaborated ochratoxin A but penicillic acid was not produced by *A. ochraceus*. On the other hand, out of 25 isolates screened 6 and 10 isolates of *A. versicolor* were positive for production of cyclopiazonic acid and sterigmatocystin respectively.

Screening of 25 isolates of *Fusarium graminearum* for their toxigenic potentials revealed that 8 and 6 isolates elaborated deoxynivalenol and zearalenone respectively. Similarly, out of 15 isolates of *F. oxysporum,* 2 and 6 isolates were positive for production of moniliformin and zearalenone respectively, but nivalenol was not produced by *F. oxysporum.* On the other hand, 9 isolates of *F. solani* produced T-2 toxin out of 20 isolates screened.

The results of the present study on aeromycoflora of poultry houses reveal the presence of a wide range of fungi which are in agreement with the findings of Jo and Kang (2005), Ajoudanifar *et at.,* (2011), who reported that *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* are the most prevalent fungal genera isolated from poultry houses. Poultry houses with high

temperature, humidity and organic material levels favour fungal growth and release of spores. These studies also project the health risk of poultry workers due to occurrence of a range of mycotoxigenic fungi. The possible risk of adverse effects on the health of workers and animals has also been addressed by a number studies from different countries [23, 24]. These studies stress the need for maintaining the cleanliness and hygienic conditions in the poultry houses to avoid occupational hazards.

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