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RESEARCH ARTICLE

CONCENTRATION OF MYCOTOXIN SPECIES IN POULTRY FARM AREAS OF DAVANGERE CITY, KARNATAKA, INDIA

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ABSTRACT

Concentrations of airborne fungi and its Endotoxin were studied in a poultry farm area of Davanagere district of Karnataka. Samples were collected three different locations for a period of one year from February 2012 to January 2013 by Petri plate exposure method containing czapeck Dox agar media. The present investigation shows the total number of 1056 CFU of fungal colonies were identified thought out the year. The station I and II show maximum fungal diversity while minimum diversity recorded in station III. The present study it shows that diversity of airborne fungi and its Endotoxin were found maximum in poultry farm area of Davanagere city.

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INTRODUCTION

Today poultry production is usually contaminated with heavy amounts of airborne fungal spores and Endotoxin. They are suspended as the indoor and outdoor bioaerosols that may be generated either as liquid droplets or as dry particles and transit in air individually or as clusters (Millner, 2009). The microorganisms are strongly influenced by environmental conditions such as temperature and humidity of the air and other factors, including radiation and sunlight (Hartung and Schulz, 2008). Numerous fungal sporeshave attached themselves in bioaerosol particles and they are always observed in the atmosphere, the concentration changes depending on environmental factors in the atmosphere (Kasprzyk, 2008). According to Crook, long-term or repeated exposure to high concentrations of airborne fungal spores in a range of agricultural environments is recognized as contributing to a decline in lung function and allergic reactions such as asthma and allergic alveolitis known asfarmer's lung disease (Wang *et al*., 2007). Fungal spores are especially important in the tropics, in which climate conditions are very favorable to the growth of fungi and may result in a high concentration of spores in the air, which in turn causes an increased incidence of allergic respiratory diseases (Siddalingappa Thirumala *et al*., 2013). The air in poultry houses is usually heavily contaminated by great amounts of debris particles of biological and non-biological origin, toxic gases (NH3, CO2, H_2S), and odour (Cambra-Lopez *et al*., 2010), (Hayes *et al*., 2006), (Kocaman *et al*., 2006), (Pavan and Manjunath, 2004), (Schierl *et al*., 2007). For poultry workers, the main health risk is most likely posed by biological aerosols. Bioaerosol in poultry houses contains particles released chiefly from settled dust, which originates from feed, manure, litter, feather fragments and animal skin, as well as microorganisms (bacteria, fungi, viruses), their bio- products and fragments (Bakutis *et al*., 2004; Golbabaei and Islami, 2000; Just *et al*., 2011). Fungal composition of the atmosphere of a given area depends on many biological and environmental factors such as precipitation, wind movement, humidity and atmospheric

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temperature have influences on the production, dispersal and deposition of airborne fungal spores. Besides seasonal differences are also daily concentration differences among and within taxa (Thirumala *et al*., 2012). Many fungal spore produce airborne toxins that can cause serious breathing difficulties, dizziness, flu-like symptoms, and bleeding in the lungs (Thirumala *et al*., 2013). The growth of fungi in particular among the solid waste generated areas is found to be more consistent due to the amiable environment such as moisture and the decaying matter causing health hazards and impact on environment. Especially the airborne fungal particles are a major cause of respiratory ailments of humans causing allergies, asthma and pathogenic infections of the respiratory tract (Thirumala *et al*., 2012). The aims of this survey were to investigate the exposure of airborne fungi and endotoxin in a poultry house environment and potential evaluation of the possible risk of respiratory diseases in the occupants.

MATERIALS AND METHODS

Study area

The Davanagere city were geographically situated in the centre of the Karnataka state. The geographical location of the city is 14.4666° N, 75.9242° E and its height is 602.5 meter above the Mean sea level (MSL). The number of poultry farm industries has causes the increasing the level of air borne fungal pollution in the city. Present investigation Three different sampling stations in the city were selected for study purposes which include villages, urban areas, and semi urban areas. The Belavanuru is village area, Hadadi city is covers urban area and Bada city come under semi urban area of Davanagere city. Present research work were carried out for a period of one year from February 2012 to January 2013.

Table 1. Sampling station in Davanagere city

S. No	Name of the study site	Site Number
1	Belavanuru village	S I
2	Hadadi city area	S II
3	Bada city area	S III

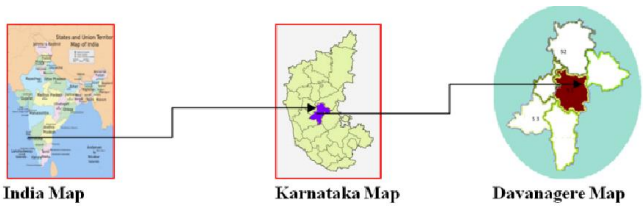
Sampling method

Samples were taken from study area in selected sampling stations. The air were sampled by using petri plate exposure method. (Gravitational Petri plate method for monitoring of air borne microorganisms in air, Sen and Asan). For sampling of air contaminants a set of sterilized petriplates with czapeck Dox agar were exposed at a time to capture and cultivate the available fungi in air. The petriplate with czapeck Dox agar was exposed in the selected location at height of 3m (Breathingheight) from the ground for the period of 10 minute. The fungal culture were incubated at 25^0 C for 5 days.

Identification methods

Species concentrations were studied by colony counting, and identification of fungal species is done by colony morphology and direct microscopic method.

Location map



RESULT AND DISCUSSION

The increases human population and exploitation of atmospheric air is playing an important role to motivate the fungal species in the atmosphere. Certain genera of airborne fungi are commonly present in urban and industrial environment, those that are specific in cities or regions and they significant in terms of therapeutics and epidemiology. For the finding of allergy and hyposensitizing treatments it is necessary to know the airborne fungal species. The present paper represents the colony morphological feature and their numbers are discussed. During investigation total 1056 CFU number of colonies were identified throughout the year. The maximum growth occurred in the monsoon season of the month June, (126CFU), July (144 CFU), August (128 CFU) and lower concentration were recorded in the pre monsoon month of March (66 CFU). The station I and station II shows the maximum occurrence of fungal growth were recorded. The present investigation Aspergillus fumigates colony on czapek Dox agar shows typical smoky gray and green with a slight yellow. Matured colonies are turn slate gray, Conidiophores are smooth-walled, uncolored and terminate in a dome-shaped vesicle.

The average contribution 34 % in station I, 37.2% in Station II and with 28.44 % in Station III. The yearly average contribution of Aspergillus flavus is 46.4% in Station I, 25% in Station II and with 28.57 % in Station III were recorded. Colonies of Aspergillus flavusare olive to lime green with a cream turn round. Conidial heads are radiate to loosely columnar with age; Conidiophores are coarsely roughened, uncolored, wide vesicles. Aspergillus niger Colonies are white, quickly becoming black with conidial production. overturn is pale yellow and growth may produce radial fissures in the agar. Hyphae are septate and hyaline. The total yearly average contribution of species were23.3% in Station I,33.3% in Station II and with 43.3 % in Station III were recorded. Colonies of Aspergillus oryzaeyearly contribution of 20 % in station I, 35% in Station II and with 45 % in Station III, they are grayish yellow to olive and rapid growth Conidial heads radiate to loosely columnar, vesicles pyriform to subglobose, uniseriate often biseriate, conidia smooth to finely roughened. Species of Aspergillus terreusshow yearly 31.5 % in the station I, 31.5% in Station II and with 36.8% in Station III with Colonies are buff to cinnamon color, Reverse is yellow and yellow soluble pigments are present. Alternaria alternate fungal species colonies are greenish white, flat, short, aerial hyphae, and brown to black at Reverse side, Septate is present, Conidiophores are also septate and brown in colour The average contribution of species is 38.8 % in Station I, 26.3% in Station II and with 34.7% in Station III.

Table 2. Total number of common fungal species colony and their percentage contribution Poultry farm area of Davanagere, during Feb 2012-Jan 2013

Species Name	Stations	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Total	% age
Aspergillus fumigates	Station I	26	19	15	12	16	26	23	16	11	10	12	6	192	34.3
	Station II	13	6	11	12	20	39	37	15	15	19	11	10	208	37.2
	Station III	5	12	10	13	28	24	16	7	8	11	15	10	159	28.4
	Total	44	37	36	37	64	89	76	38	34	40	38	26	559	
Aspergillus flavus	Station I	1	0	1	0	3	4	2	0	1	0	1	0	13	46.4
	Station II	0	1	2	0	0	0	2	0	0	1	0	1	7	25
	Station III	1	2	1	0	1	0	0	1	0	1	0	1	8	28.5
	Total	2	3	4	0	4	4	4	1	1	2	1	2	28	
Aspergillus niger	Station I	0	0	0	4	0	2	0	1	0	0	0	0	7	23.3
	Station II	1	0	1	0	0	1	2	0	4	1	0	0	10	33.3
	Station III	2	1	0	3	4	1	0	1	0	0	1	0	13	43.3
	Total	3	1	1	7	4	4	2	2	4	1	1	0	30	
Aspergillus oryzae	Station I	0	1	0	1	0	0	1	0	0	1	0	0	4	20.0
	Station II	0	0	1	2	1	0	1	0	1	0	1	0	7	35.0
	Station III	1	1	0	1	0	0	0	1	2	1	2	0	9	45.0
	Total	1	2	1	4	1	0	2	1	3	2	3	0	20	
Aspergillus terreus	Station I	0	2	1	0	0	1	0	1	0	0	1	0	6	31.5
	Station II	0	0	0	1	1	0	1	1	1	0	1	0	6	31.5
	Station III	1	0	1	2	1	0	0	0	0	0	2	0	7	36.8
	Total	1	2	2	3	2	1	1	2	1	0	4	0	19	
Alternariaalternate	Station I	3	2	1	1	5	4	1	4	0	4	1	2	28	38.8
	Station II	1	0	1	0	1	4	2	3	3	2	1	1	19	26.3
	Station III	0	0	1	3	6	3	3	2	0	3	2	2	25	34.7
	Total	4	2	3	4	12	11	6	9	3	9	4	5	72	
Epicocoum	Station I	1	0	0	1	2	2	0	2	1	1	1	1	12	21.0
	Station II	2	4	3	1	0	3	1	0	2	0	2	1	19	33.3
	Station III	3	0	2	3	4	2	3	2	2	4	0	1	26	45.6
	Total	6	4	5	5	6	7	4	4	5	5	3	3	57	
Cladosporium	Station I	0	1	1	0	4	1	4	3	0	3	0	1	18	40.9
	Station II	0	2	2	0	0	2	2	1	1	1	1	1	13	29.5
	Station III	1	1	0	2	1	2	3	1	0	1	1	0	13	29.5
	Total	1	4	3	2	5	5	9	5	1	5	2	2	44	
Fusarium	Station I	4	2	1	0	2	3	4	1	0	5	3	3	28	59.5
	Station II	0	0	3	1	1	1	0	0	0	2	0	0	8	17.0
	Station III	0	1	2	0	2	1	0	0	1	0	3	1	11	23.4
	Total	4	3	6	1	5	5	4	1	1	7	6	4	47	
Microsporium	Station I	5	1	2	0	2	4	3	2	0	0	1	4	24	42.1
	Station II	0	2	2	0	2	3	1	2	4	4	3	0	23	40.3
	Station III	0	2	0	1	1	0	0	0	1	0	3	2	10	17.5
	Total	5	5	4	1	5	7	4	4	5	4	7	6	57	
Mucorglobosus	Station I	0	0	1	1	0	2	4	0	1	1	0	5	15	33.3
	Station II	2	0	0	1	4	0	0	3	1	0	1	1	13	28.8
	Station III	3	0	1	2	0	0	3	0	1	3	2	2	17	37.7
	Total	5	0	2	4	4	2	7	3	3	4	3	8	45	
Pencillium	Station I	0	1	2	0	2	1	0	1	1	2	0	0	10	25.0
	Station II	1	0	1	0	5	1	2	1	1	1	0	2	15	37.5
	Station III	1	0	0	3	2	3	1	2	3	0	0	0	15	37.5
	Total	2	1	3	3	9	5	3	4	5	3	0	2	40	
Rhizopusstolonifer	Station I	0	1	0	1	1	0	4	2	0	1	0	2	12	31.5
	Station II	0	1	0	1	3	2	1	3	2	0	2	1	16	42.1
	Station III	1	0	1	1	1	2	1	0	0	2	1	0	10	26.3
	Total	1	2	1	3	5	4	6	5	2	3	3	3	38	
	Grand Total	79	66	71	74	126	144	128	79	68	85	75	61	1056	
	Station I	40	30	25	21	37	50	46	33	15	28	20	24	369	
	Station II	20	16	27	19	38	56	52	29	35	31	23	18	364	
	Station III	19	20	19	34	51	38	30	17	18	26	32	19	323	

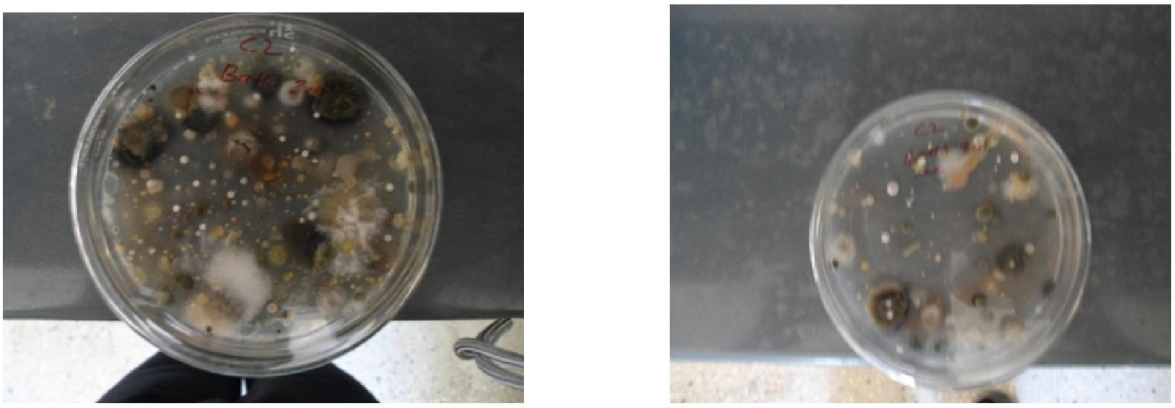


Fig.1. Air borne common fungal species in the petriplates from poultry farm site

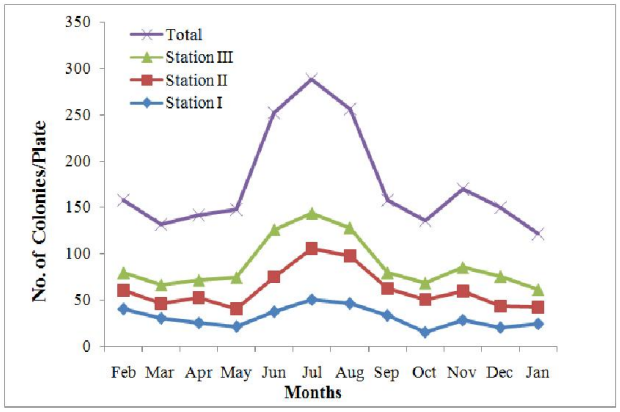


Fig. 2. Showing monthly wise total common fungal species diversity in all station from Feb 2012-Jan 2013

The Epicocum species were found 21.0 % in station I and 33.3% in station II with 45.6% in Station III. The colonies are yellow to orange and same color were observed in reverse. Cladosporium are greenish to black color colonies both front and reverse side of the plate, colony surface is velvety to powdery, Septate is present, brown hyphae, erect and pigmented conidiophores, and conidia. The yearly percentage contribution is 40.9 % in station I**,** 29.5% in station II and with 29.5% in Station III. The Fusarium solani species found yearly 59.5 % in Station I, station II shows 17.0% and Station III 23.4%. Colonies are woolly to cottony with cream to white aerial mycelium and a cream reverse Sporodochia may form and are usually moist and cream-colored. Microsporiumcanis species found throughout the year, the average contribution is 42.1 % in station I, 40.3% in station II and with 17.5% in Station III. The colonies are deep yellow and yellowish orange in reverse.

Mucorglobosus Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia. Average contribution is 33.3 % in Station I, 28.8% in Station II and with 37.7% Station III were recorded. The average percentage contribution of Pencilliummarneffei shows 25 % in the station I, 37.5% in Station II and with 37.5% in Station III. Colonies are bluish-gray-green at centre and white at the periphery, and soluble pigment observed from the reverse is very typical. The Rhizopusschipperae, Colonies on Czapekdox agar are grayish-white, diffuse, thin, floccose. Optimum growth occurs at 30°C, with very restricted growth up to 45 °C. Hyphae are hyaline, broad, ribbon like structure, and irregularly branched. Average contribution of 31.5 % in station I, 42. 1%in station II and with 26.3% in Station III.This investigation shows that bioaerosol particles in the poultry farms have the mediator for the growth of fungal concentration. Increase the level of concentration in the atmosphere depends upon the emission of bioaerosol in the poultry farm. The poorly maintain farm is making an unhygienic situation and may cause fungal growth. The poultry farm workers are inhaled concentration leads to air borne symptoms as a human health hazard.

Conclusion

In this work, the amounts of common airborne mycotoxins detected on poultry farm were higher than the regulatory limits, the occurrence of several common mycotoxins were detected. The atmospheric air at a poultry farm contains odor, dust particles and fungal spores which are discharged to atmosphere. The increased fungal population that may affect the respiratory health of farm worker and living people near the poultry house, particularly compounds like dust, fungal spores and mycotoxins, which are also addressed as bioaerosols, they are supposed to play an important role in the occurrence of respiratory affections in receptive humans as it is known from occupational health reports of farm workers in poultry houses.

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