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INDOOR AND OUTDOOR AIR QUALITY OF POULTRY FARM AT BANGALORE

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ABSTRACT

The present study was to determine the concentration, in terms of monthly and seasonal distribution and in relation to meteorological factors, of indoor and outdoor airborne fungal spores in poultry farm at Bangalore. Samples were collected at fortnightly for a period of one year in duplicates between January 2011 to December 2011 by using Andersen two stage viable air sampler petri plates containing Malt Extract Agar media to the air for 5 minutes. A total of 16912.23 CFU/m^3 fungal species was isolated from the indoor (80662.52 CFU/m^3) and outdoor (8849.71 CFU/m^3) of the poultry farm. In indoor 22 species belonging to 12 genera and 18 species belonging to 11 genera from outdoor of the poultry farm. The dominant fungal species were *Cladosporium, Penicillium* and *Alternaria* from indoor and outdoor respectively. Correlation between seasonal and meteorological factors were carried out. An attempt has been made to forecast atmospheric fungal spores concentration in poultry farm.

KEYWORDS: Poultry farm, Fungal spores, Andersen, Seasonal and Meteorological

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INTRODUCTION

Aerobiology is a scientific discipline focusing on the study of the passive transport of organisms and particles of biological origin in the atmosphere [1]. Bioaerosols or organic dust may consist of pathogenic or non-pathogenic, live or dead bacterial and fungal spores, viruses, high molecular weight allergens, pollen and plant fibers, etc. The fungal spores are the pre-dominant allergens in indoor environment and have been regarded as detrimental in causing health hazards [2]. Fungal spores owing to their smaller size can penetrate into the lower respiratory tract resulting in allergies [3]. Fungi can produce allergic reactions in hypersensitive subjects such as eczema, allergic rhinitis, allergic asthma, atopic dermatitis etc. The manifestation of a fungal allergy ranges from the common conjunctivitis, rhinitis and rhinoconjunctivitis to the more detrimental in ascending order of severity, sinusitis, asthma, bronchopulmonary mycoses, hypersensitivity pneumonitis and allergic alveolitis [4,5]. Respiratory infection or damage may occur in caretakers as well as livestock and poultry with prolonged exposure to the environment at high microorganism levels [6]. Microbial aerosols of high levels are also associated with allergy and asthma in caretakers [7]. Air fungi have much attention from medical researchers as well as environmentalists [8].Airborne microbial quantity and quality vary with time of day, year and location [9]. PCR and probe hybridization techniques have been demonstrated to be sensitive and accurate for detection of fungi in clinical specimens in indoor environments and in other environmental samples [10,11]. Molecular approaches have been developed for the assessment of microbial diversity in complex communities [12]. Methods based on DNA analysis reveal fungal diversity in ecosystems and offer the potential benefits of highly sensitive and rapid detection [13]. In recent years public interest has increasingly focused on released particulate matter from animal production facilities. This assumption is mainly based on experiences cited in occupational health studies, in which persons have been exposed to bioaerosols with subsequent deterioration of their health status [14]. Bangalore is considered to be one of the fastest growing cities in the world. Due to urbanization activities the Bangalore climate is not suitable for allergic prone patients. During the present investigation in poultry farm the determination of monthly, seasonal variation in fungal spore concentration in relation to meteorological parameters and PCR-based detection and quantification of fungal spores in dust extracts.

MATERIALS AND METHODS

(i) Sampling site and period

The poultry farm selected for fungal sampling at Hessaraghatta village, situated 10 km away from northwest of Bangalore in the State of Karnataka, India. The Hessaraghatta village during winter rarely drops below 12^0C, summer temperature seldom exceeds 30^0C to 32^0C; annual rainfall is around 253 mm. This study was conducted from January 2011 to December 2011. Indoor and outdoor airborne fungal samples were collected fortnightly for a period of one year in duplicates.

(ii) Sampling Instrument [15]

Andersen two stage air sampler, a multi-orifice cascade impactor was used and it is constructed of aluminum with two stages, each stage contains 200 tapered orifices. The diameter of the orifices on the first stage is 1.5 mm and 0.4 mm on the second stage. Standard 90 mm petridish with Malt Extract Agar was used as sampling media are used as collecting surfaces on each stage. Sampler is placed in the center of the cow shed and 1.5 meters above the ground level. Air flow was 28.3 L/min during the sampling and the sampling time is limited to 5 minutes.

(iii)Treatment of Samples [16,17]

The air sampled plates were incubated for 5 to 7 days at room temperature between 25^0C to 30^0C and colony morphological characteristics were observed microscopically with strain determination by using manuals and references slides. The number of fungal colonies on each

plate are counted and totaled. They are expressed as number of colony forming unit per cubic meter (CFU/m^3) of air.

(iv)Meteorological Data

The meteorological data such as temperature, relative humidity, wind speed and rainfall were collected from the Department of Statistics, Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore for the present of air exposure.

(v) Statistical analysis

The statistical analysis was performed using SPSS-16, 2007 version software. Two-way ANOVA, month and season of indoor and outdoor poultry farm. There is no significant difference in mean scores of indoor and outdoor poultry farm.

RESULTS

Air sampling studies in poultry farm at Hessaraghatta village, Bangalore recorded a total number of 16912.23 CFU/m^3 was isolated from both indoor and outdoor of the poultry farm period from January 2011 to December 2011 of which indoor contributed to 80662.52 CFU/m^3 and the outdoor contributed 8849.71 CFU/m^3 (Table 1 and 2). The qualitative analysis showed altogether 22 fungal species belonging to 12 genera with other unidentified fungal form were isolated from indoor poultry farm, when compared to 18 fungal species belonging to 11 genera with other unidentified from outdoor poultry farm. Among the total number of isolated fungal species from indoor poultry farm *Aspergillus* (5.80 %) was represented by 4 species *viz., A. flavus*, *A. fumigatus, A. niger* and *A. terreus* followed by 2 species of *Fusarium* (14.87 %) *viz., F. moniliforme* and *F. oxysporum,* 2 species of *Penicillium* (17.54 %) *viz., P. chrysogenum* and *P. griseofulvum*, 1 species of *Cladosporium* (36.68 %) *viz., C. cladosporioides,* 1 species of *Curvularia* (2.88 %) *viz., C. lunata* along with *Alternaria* sp., (6.52 %), *Helminthosporium* sp., (1.79 %), *Mucor* sp., (0.96 %), *Neurospora* sp., (0.87 %), *Phoma* sp., (1.79 %), *Rhizopus* sp., (1.4 %) and *Trichoderma* sp., (5.21 %) as shown in Table 1. When these findings were compared with outdoor poultry farm it was observed that all the fungal genera and species remain the same, with variation in their percentage occurrence. Few of the fungal species like *A. fumigatus, C. lunata, P. chrysogenum* and *Phoma* which found in indoor poultry farm are presented in Table 2.Based on comparative analysis the dominant fungal species in indoor poultry farm were *Cladosporium* sp., (20.88 %)*, C. cladosporioides* (15.8 %) and *Penicillium* sp., (11.99 %) but *Aspergillus* spp., (0.74 %), *A. flavus* (0.74 %), *Neurospora* spp., (0.87 %) and *Mucor* spp., (0.96 %) were least recorded. Whereas in outdoor poultry farm the dominant fungal species recorded were *Cladosporium* sp., (18.86 %), *Alternaria* spp., (13.32 %) and *C. cladosporioides* (12.92 %) but *F. moniliforme* (0.39 %), *Helminthosporium* sp., (0.39 %) and *Rhizopus* sp., (0.63 %) were least recorded. Monthly incidence in the indoor poultry farm showed maximum spore distribution in May (886.03 CFU/m^3) followed by February (741.3 CFU/m^3) and November (702.47 CFU/m^3) when compared to other months of year, whereas the monthly incidence of fungal spores in outdoor poultry farm showed maximum spore distribution during August (967.22 CFU/m^3) followed by July (921.33 CFU/m^3) and April (833.08 CFU/m^3). The seasonal occurrence of fungal spores indoor and outdoor air of the poultry farm, which shows that the winter season contributed the maximum 2746.34 CFU/m^3 followed by summer 2679.27 CFU/m^3 and rainy 2636.91 CFU/m^3 in indoor. In outdoor the rainy season 3116.99 CFU/m^3 contributed maximum, followed by summer 2944.02 CFU/m^3 and winter 2788.7 CFU/m^3.

Table 1

Fungal spores (CFU/m^3) recorded from January 2011 to December 2011 in the indoor air of poultry farm

Sl. No.	Genera and Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	%
1	*Aspergillus* spp.	10.59	7.06	0	0	0	0	3.53	0	0	0	21.18	17.65	60.01	0.74
2	*A. flavus*	7.06	3.53	0	0	0	0	7.06	0	7.06	17.65	10.59	7.06	60.01	0.74
3	*A. fumigates*	0	0	14.12	10.59	7.06	21.18	14.12	7.06	7.06	7.06	0	0	88.25	1.09
4	*A. niger*	7.06	10.59	17.65	24.71	17.65	10.59	14.12	3.53	28.24	14.12	7.06	7.06	162.38	2.01
5	*A. terreus*	10.59	14.12	0	0	0	0	17.65	17.65	14.12	3.53	14.12	7.06	98.84	1.22
6	*Alternaria* sp.	56.48	21.18	24.71	52.95	63.54	35.3	17.65	63.54	45.89	74.13	42.36	28.24	525.97	6.52
7	*Cladosporium* spp.	88.25	144.73	144.73	151.79	211.8	151.79	144.73	123.55	102.37	155.32	109.43	155.32	1683.81	20.88
8	*C. cladosporioides*	112.96	137.67	116.49	81.19	137.67	109.43	56.48	63.54	120.02	84.72	130.61	123.55	1274.33	15.8
9	*Curvularia* spp.	3.53	14.12	0	0	14.12	28.24	3.53	3.53	10.59	21.18	10.59	24.71	134.14	1.66
10	*C. lunata*	14.12	10.59	3.53	3.53	17.65	7.06	0	0	0	0	14.12	28.24	98.84	1.22
11	*Fusarium* spp.	127.08	98.84	137.67	52.95	91.78	35.3	70.6	45.89	24.71	14.12	28.24	10.59	737.77	9.15
12	*F. moniliforme*	14.12	3.53	0	7.06	45.89	21.18	38.83	3.53	10.59	24.71	21.18	10.59	201.21	2.49
13	*F. oxysporum*	3.53	7.06	21.18	3.53	17.65	7.06	14.12	74.13	56.48	21.18	28.24	7.06	261.22	3.23
14	*Helminthosporium* sp.	0	3.53	7.06	7.06	24.71	21.18	35.3	17.65	10.59	3.53	3.53	10.59	144.73	1.79
15	*Mucor* sp.	0	10.59	3.53	0	21.18	10.59	0	14.12	10.59	7.06	0	0	77.66	0.96
16	*Neurospora* sp.	17.65	7.06	0	0	0	0	17.65	7.06	17.65	3.53	0	0	70.6	0.87
17	*Penicillium* spp.	95.31	98.84	91.78	95.31	84.72	112.96	91.78	70.6	21.18	63.54	63.54	77.66	967.22	11.99
18	*P. chrysogenum*	21.18	0	28.24	3.53	45.89	14.12	49.42	14.12	31.77	24.71	28.24	7.06	268.28	3.32
19	*P. griseofulvum*	0	24.71	3.53	24.71	21.18	7.06	0	31.77	35.3	7.06	24.71	0	180.03	2.23
20	*Phoma* sp.	3.53	7.06	0	0	0	0	31.77	14.12	14.12	35.3	17.65	21.18	144.73	1.79
21	*Rhizopus* sp.	7.06	14.12	0	24.71	0	17.65	7.06	0	21.18	7.06	10.59	3.53	112.96	1.4
22	*Trichoderma* sp.	35.3	52.95	0	0	31.77	0	10.59	56.48	35.3	35.3	88.25	74.13	420.07	5.21
23	Unidentified	21.18	49.42	7.06	10.59	31.77	7.06	0	3.53	38.83	67.07	28.24	24.71	289.46	3.59
Total		656.58	741.3	621.28	554.21	886.03	617.75	645.99	635.4	663.64	691.88	702.47	645.99	8062.52	100

Table 2

Fungal spores (CFU/m^3) recorded from January 2011 to December 2011 in the outdoor air of the poultry farm

Sl. No.	Genera and Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	%
1	*Aspergillus* spp.	42.36	10.59	49.42	52.95	35.3	60.01	74.13	63.54	21.18	24.71	7.06	28.24	469.49	5.3
2	*A. flavus*	3.53	14.12	0	14.12	24.71	0	21.18	17.65	31.77	21.18	14.12	21.18	183.56	2.07
3	*A. niger*	14.12	24.71	21.18	0	0	0	21.18	42.36	24.71	10.59	31.77	28.24	218.86	2.47
4	*A. terreus*	35.3	74.13	0	0	35.3	42.36	67.07	35.3	42.36	84.72	45.89	35.3	497.73	5.62
5	*Alternaria* sp.	137.67	102.37	137.67	134.14	144.73	77.66	95.31	137.67	0	0	123.55	88.25	1179.02	13.32
6	*Cladosporium* spp.	158.85	109.43	116.49	180.03	141.2	123.55	148.26	120.02	155.32	81.19	137.67	197.68	1669.69	18.86
7	*C. cladosporioides*	77.66	42.36	165.91	134.14	63.54	105.9	84.72	165.91	0	151.79	52.95	98.84	1143.72	12.92
8	*Curvularia* spp.	49.42	60.01	0	35.3	88.25	3.53	84.72	130.61	63.54	60.01	0	42.36	617.75	6.98
9	*Fusarium* spp.	141.2	0	91.78	137.67	112.96	88.25	144.73	77.66	0	81.19	24.71	70.6	970.75	10.96
10	*F. moniliforme*	0	0	0	21.18	0	0	14.12	0	0	0	0	0	35.3	0.39
11	*F. oxysporum*	28.24	14.12	3.53	14.12	0	0	17.65	35.3	35.3	0	10.59	3.53	162.38	1.83
12	*Helminthosporium* sp.	0	0	7.06	0	0	0	0	10.59	0	17.65	0	0	35.3	0.39
13	*Mucor* sp.	14.12	0	21.18	0	3.53	0	10.59	7.06	7.06	14.12	10.59	14.12	102.37	1.15
14	*Neurospora* sp.	0	0	10.59	3.53	21.18	3.53	10.59	17.65	21.18	0	7.06	10.59	105.9	1.19
15	*Penicillium* spp.	84.72	98.84	84.72	67.07	77.66	56.48	74.13	63.54	98.84	63.54	81.19	70.6	921.33	10.41
16	*P. griseofulvum*	0	0	0	0	21.18	0	3.53	10.59	0	21.18	24.71	14.12	95.31	1.07
17	*Rhizopus* sp.	14.12	7.06	0	0	0	0	7.06	7.06	0	10.59	0	10.59	56.48	0.63
18	*Trichoderma* sp.	0	21.18	0	28.24	3.53	10.59	14.12	7.06	3.53	14.12	7.06	7.06	116.49	1.31
19	Unidentified	14.12	35.3	21.18	10.59	0	35.3	28.24	17.65	31.77	35.3	17.65	21.18	268.28	3.03
Total		815.43	614.22	730.71	833.08	773.07	607.16	921.33	967.22	536.56	691.88	596.57	762.48	8849.71	100

Table 3
Meteorological data recorded at Hesaraghatta village, Bangalore

Monthly Average				
Year 2011	Temperature ^{0}C	Relative Humidity %	Wind speed km/h	Rainfall mm
Jan	27.8	89.9	4.8	Nil
Feb	29.5	87.0	4.8	16.6
Mar	32.9	85.0	5.1	Nil
Apr	32.6	89.3	4.5	57.4
May	32.1	90.0	5.0	126.0
Jun	29.1	94.0	9.0	30.0
Jul	27.8	94.0	7.6	95.8
Aug	27.3	94.0	6.4	253.2
Sep	28.0	94.0	5.8	59.7
Oct	28.6	93.0	2.9	122.6
Nov	26.6	89.0	4.8	38.0
Dec	26.9	91.0	4.5	5.2

The meteorological factors changes (Table 3), at higher temperature more than 32^0C, the total CFU's distribution during January to June 4077.15 CFU/m^3 reached maximum in their CFU's, when the temperature was less than 27^0C, the CFU's reduced between July to December 3985.37 CFU/m^3. The higher relative humidity from June to October (94 %) was less distribution of organism (3254.66 CFU/m^3). The CFU's got gradually increased to 3459.40 CFU/m^3 with lower relative humidity (85 %) between the months of January to April. In wind speed showed less impact on the distribution of organisms with less variation in their CFU's. Almost during the months from June to August with wind speed 9 km/h or higher, the distribution of organisms seems to be less with (1899.14 CFU/m^3) and at less wind speed between the months of October to December (2.9 km/h) the organism number was more with 2040.34 CFU/m^3. The maximum rainfall in the month of August (253.2 mm) distribution of organism was 635.40 CFU/m^3; but during January 656.58 CFU/m^3 though no rainfall but the total organism distribution became higher during these periods. Based on the Two-Way ANOVA for colony and month of the poultry farm, there is no significant difference in growth of CFU's in indoor and outdoor poultry farm over the months, towards late summer there is increase in growth for outdoor poultry farm **(**Table 4). In Two-Way ANOVA for CFU's and season of the poultry farm there is no significant difference in mean scores of indoor and outdoor poultry farm over the seasons **(**Table 5).

Table 4
Two-Way ANOVA for colony and month of the poultry farm

Two-Way ANOVA	Sum of Squares	Degrees of Freedom	Mean Squares	F-Ratio	p-Value
Colony	2.58	1	2.58	1.78	0.21
Month	11.29	11	1.03	0.71	0.71
Error	15.96	11	1.45		
Total	29.84	23			

Table 5
Two-Way ANOVA for CFU and season of the poultry farm

Two-Way ANOVA	Sum of Squares	Degrees of Freedom	Mean Squares	F-Ratio	p-Value
Colony	0.64	1	0.64	4.31	0.17
Season	0.07	2	0.03	0.25	0.79
Error	0.29	2	0.14		
Total	1.02	5			

DNA was isolated from the dust samples in poultry farm and the DNA samples were analyzed on 0.8 % agarose gel and visualized under UV gel Documentation unit. DNA extracted used for PCR amplification using Universal ITS fungal identification primers PCR products were analyzed on 2 % agarose gel (Fig 1). Dust samples gave amplicons of 550bp respectively. The amplicons were purified and was sequenced using ITS primers.

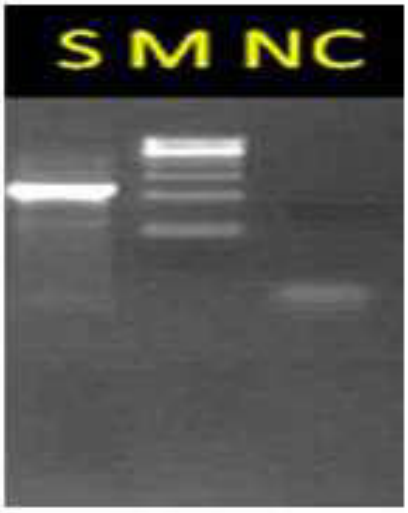
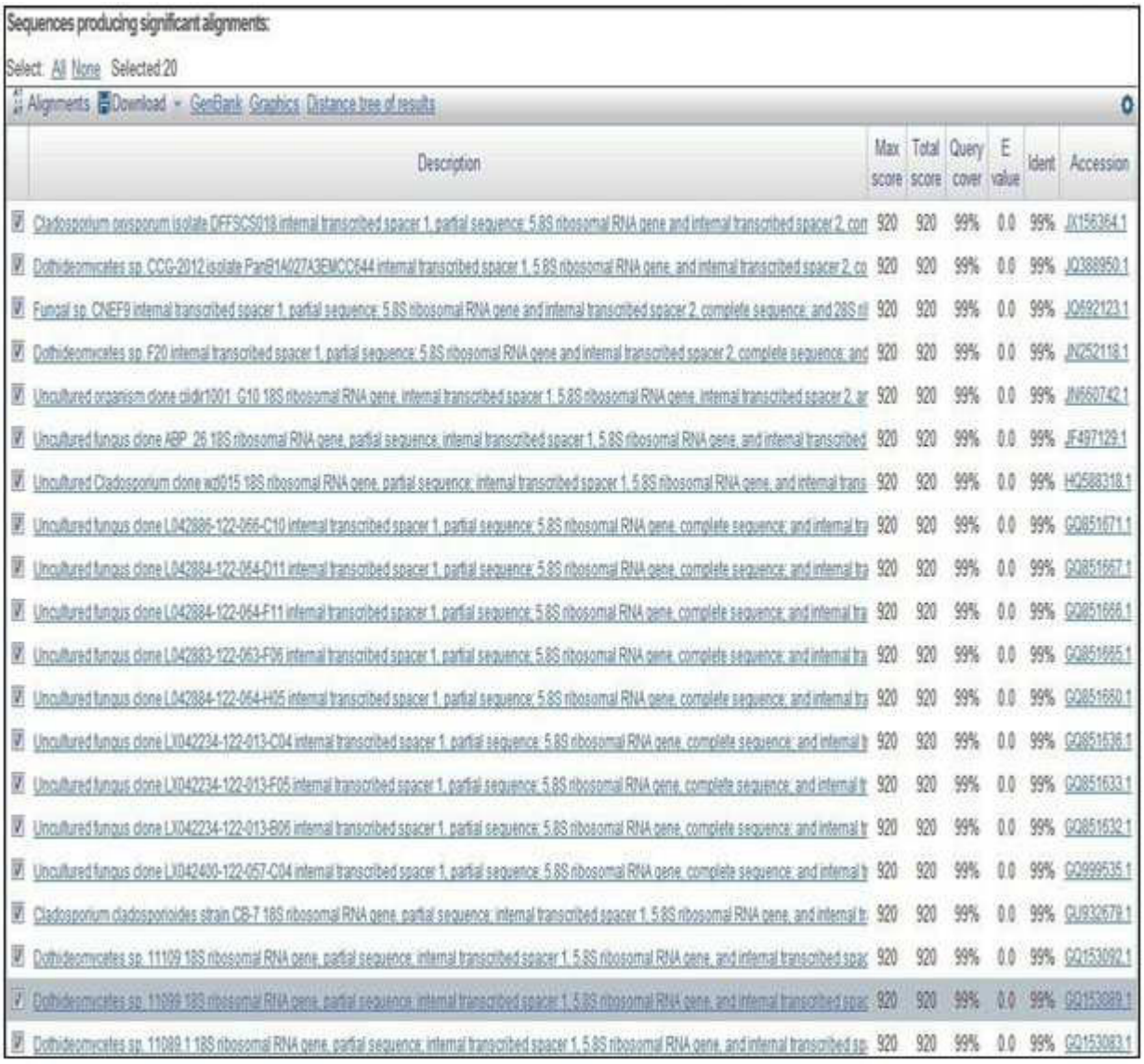


Figure 1
PCR amplicons using fungal ITS primers on 2 % agarose gel. S: Sample, M: 200bp step up DNA Ladder, NC: Negative Control.

The DNA sequencing of poultry farm dust samples sequence was blasted with the NCBI Nucleotide blast database to identify the fungal species 20 closely related species that were produced in BLAST were used to construct the distance tree using Neighbor joining in Newick method (Table 6). *Cladosporium oxysporium* isolate DFFSCS018 ITS region showing 99 % similarity with the poultry dust samples isolate. The result indicates dominating population of the above two species in the poultry farm.

Table 6

NCBI BLAST results showing distance tree using Neighbour joining in Newick method



Sequences producing significant alignments:

Select: All None Selected 20

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Cladosporium sinosporum isolate DFFSCS019 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, com	920	920	99%	0.0	99%	JX156364.1
Dothideomycetes sp. CCG-2012 isolate PanB1A027A3EMCC544 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, co	920	920	99%	0.0	99%	JQ388950.1
Fungal sp. CNEF9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S rib	920	920	99%	0.0	99%	JQ692123.1
Dothideomycetes sp. F20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and	920	920	99%	0.0	99%	JN252118.1
Uncultured organism clone ckidkr1001_G10 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, pa	920	920	99%	0.0	99%	JN668742.1
Uncultured fungus clone ABP_26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed	920	920	99%	0.0	99%	JF497129.1
Uncultured Cladosporium clone wd015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans	920	920	99%	0.0	99%	HQ588318.1
Uncultured fungus clone L042886-122-066-C10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	920	920	99%	0.0	99%	GQ851671.1
Uncultured fungus clone L042884-122-064-D11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	920	920	99%	0.0	99%	GQ851667.1
Uncultured fungus clone L042884-122-064-F11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	920	920	99%	0.0	99%	GQ851666.1
Uncultured fungus clone L042883-122-063-F06 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	920	920	99%	0.0	99%	GQ851665.1
Uncultured fungus clone L042884-122-064-H05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tra	920	920	99%	0.0	99%	GQ851660.1
Uncultured fungus clone LX042234-122-013-C04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal t	920	920	99%	0.0	99%	GQ851636.1
Uncultured fungus clone LX042234-122-013-F05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	920	920	99%	0.0	99%	GQ851633.1
Uncultured fungus clone LX042234-122-013-B06 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal tr	920	920	99%	0.0	99%	GQ851632.1
Uncultured fungus clone LX042400-122-057-C04 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal t	920	920	99%	0.0	99%	GQ999535.1
Cladosporium cladosporioides strain CB-T 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tr	920	920	99%	0.0	99%	GU932679.1
Dothideomycetes sp. 11109 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	920	920	99%	0.0	99%	GQ153092.1
Dothideomycetes sp. 11099 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	920	920	99%	0.0	99%	GQ153089.1
Dothideomycetes sp. 11089.1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed sp	920	920	99%	0.0	99%	GQ153083.1

DISCUSSION

The study carried out on the indoor and outdoor airborne fungal spores on poultry farm in Hessaraghatta village, Bangalore was analyzed. There are very few reports available on the airborne fungal spores of animal houses conducted in poultry farm [20,21]. Most of the studies were focused on indoor and outdoor airborne fungal spores and few on seasonal studies [22,23,24]. In our study, indoor and outdoor airborne fungi were *Aspergillus flavus*, *A. fumigatus, A. niger, A. terreus, Fusarium moniliforme, F. oxysporum, Penicillium chrysogenum, P. griseofulvum*, *Cladosporium cladosporioides, Curvularia lunata, Alternaria* sp., *Helminthosporium* sp., *Mucor* sp., *Neurospora* sp., *Phoma* sp., *Rhizopus* sp., and *Trichoderma* species were recorded. The involved risk on the health of workers and animals has been addressed by a number of studies from indoor and outdoor airborne fungal investigation reported previously [25,26,27]. *Cladosporium* sp., *Penicillium* sp., and *Fusarium* species were the most dominant fungi in poultry farm. Previous work with similar such work reported the dominance of *Cladosporium* sp., *Penicillium* sp., and *Fusarium* species in

indoor and outdoor air of poultry houses [28,29,30]. Differences between these findings may be due to different sampling methods, different sampling seasons, different geographical conditions and different culture media. Khattab and Levetin [31] have shown that the concentration of airborne fungal spores is also related to sampling height; concentrations of some types of airborne fungal spores were higher at the ground level than at the ceiling level. In our study, total indoor fungi concentration was highest during the winter followed by summer and rainy, whereas outdoor fungi concentration was highest during the rainy followed by summer and winter. Airborne fungal spores are ubiquitous worldwide and their numbers and species are known to vary with the season of the year to weather changes [32,33,34,35]. According to Fang [36] the vigorous growth of plants in summer can allow for the growth of airborne fungi and may induce more favorable growth conditions for fungi due to increased temperatures [37].

Outdoor air is the main source of airborne fungi in indoor air. Therefore, seasonal variations in climatic conditions are also responsible for changes in the concentrations and types of fungi in the air indoors. Matkovic [27] suggested that the total fungal count in air depends on animal species, housing conditions and animal feeding and grooming. Ventilation system can also play an important role in indoor environment. Investigators from different countries who used sampling methods similar to ours have reported diverse concentration ranges of airborne fungi in poultry farm [38,39,25,26,28]. The species of *Aspergillus* and *Penicillium* are often associated with allergic symptoms in the respiratory system [40]. In recent years, the role of indoor *Penicillium* as a cause of allergies in some people has been proven [41]. Higher concentrations of *Cladosporium* and *Penicillium* indoor could cause allergic diseases [36]. Su and El-Morsy [42,33] pointed out that fungal genera that most commonly cause allergies are *Cladosporium, Alternaria, Aspergillus* and *Fusarium*. Exposure to *Alternaria* spores is an important agent for allergic rhinoconjunctivitis [43]. It was found that spores belonging to the genus *Alternaria* were associated with severe asthma in the study carried out by Burch and Levetin [44]. Harmanci [45] indicated that *Cladosporium* and *Aspergillus* were the most common causes of allergy in a population study. The effects on human health of inhaled fungal spores depend not only on their concentration and composition, but also their size. While fungal spores larger than 10 µm, like *Alternaria* species, are deposited in the nose and pharynx, spores smaller than 5 µm in diameter can penetrate the lungs and might lead to allergies and asthma [36]. Mold contamination in buildings may cause sick building syndrome including symptoms such as headaches; eye, nose and throat irritation, a dry cough, dry or itchy skin, dizziness and nausea, difficulty in concentrating, fatigue and sensitivity to odors [46]. Some members of the genus *Aspergillus* may cause invasive aspergillosis in immune compromised persons. *Penicillium chrysogenum* may cause central nervous system infection, otomycoses, endophthalmitis, keratitis and endocarditis [47]. Mycotoxin producers common in indoor fungi are species of *Aspergillus, Penicillium* and *Fusarium* as we reported in our study [48]. In addition, *Alternaria* and *Aspergillus flavus* isolated in the present study are potential mycotoxin producers.In dust extracts there are few reports on the airborne fungi and fungal aeroallergens of organic dusts including airborne fungi [49,50]. This study gave rough approximation and qualitative information about the types and concentrations of the various airborne fungi in organic dust. A major objective of this study was to determine the effectiveness of using PCR to amplify the ITS region of fungal DNA for the purposes of sequencing and eventual species identification. The technique proved successful as its application led to the identification of unknown fungi in the dust samples to the species level. The dusts and fungal spores concentration could be reduced by way of choosing to open or semi-blocked structure animal house. Adjusting the temperature and humidity could control fungal concentration. However the results obtained give scope for further such studies. The present survey of both qualitative and quantitative information obtained from this study could be useful for Aerobiologists, Veterinarians and Clinicians to forecast fungal spore load to

the atmosphere and for therapeutic studies including allergy diagnosis. Working in the intensive animal rearing houses breeding and production farms poses a risk for workers and requires the use of personal protection measures, safety measures such as fumigation, maintenance of clean environment, avoiding the dumping of wastes, to keep the microbial load to a minimum has to be employed as has been observed [51].

CONCLUSION

The present study was contribute much to the overall understanding of indoor and outdoor airborne fungi and the effect of bioaerosols on the health of poultry farm workers. Fungal spores are ubiquitous and quite dominant in the indoors as against the outdoor environments. The results showed that the airborne fungi size was very closely correlated with the endangerment of airborne fungal particles as well. Aerosol fungal concentrations were influenced by many factors such as animal habit, weather, sanitation condition and illumination. Caretakers at the sampling sites have prolonged exposure to such environment which result in occult infection or develop to chronic nosomycosis and lead to predisposition to other diseases. At present, there are no safe levels of airborne fungi concentration in indoor environments, but high concentration would result in threats to the health of human beings and animals.

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