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Indoor Fungal Load in Broiler Flocks Environment at Different Stages of Production Cycle

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Abstract: A longitudinal study was developed in order to determine the fungal contamination level (mould and yeast) in air, settled dust and litter of three broiler flocks at different stages of production cycle at 10 day fixed interval. Air samples were obtained from the examined flocks using impingement and the environmental pooled samples were collected from settled dust and litter. The concentrations of mould and yeast colonies count in air, settled dust and litter in a broiler flocks have been found to rise with bird age reaching maximum at end of production cycle. The most predominant genera of mould were among *Aspergillus*, *Penicillium*, *Mucor* and *Alternaria* spp. while, *Cryptococcus*, *Geotrichum* and *Candida* spp. were the dominant yeast genus. The examined samples contained group two risk species of *Aspergillus fumigatus* and *Candida albicans*. The high contamination of broiler facilities with fungi in summer season indicating importance of proper biosecurity measures and good ventilation. Furthermore, presence of pathogenic fungi may provoke adverse effects for animal and workers health beside the surrounding environment.

Key words: Mould, yeast, broiler, AGI-30, litter, settled dust

INTRODUCTION

During the last decade, poultry industry continues to grow to meet the demand for poultry products in world markets. Poultry production provides poultry meat of high quality proteins and eggs rich in proteins and vitamins. Therefore, poultry farms are the operations that can fulfil the growing demand for meat and eggs. Such production that deals with large numbers of animals in small areas considering poultry houses environment primarily air (bioaerosol) as a significant source of biological microorganisms (Seedorf *et al.*, 1998). Bioaerosol releases from settled dust which originates mainly from feed, manure, litter and microorganisms (Just *et al.*, 2011). Poultry farms bioaerosol consist mainly of bacteria with a level reached to 10^9 CFU/m³, but fungi present in a significant amount in bioaerosol ranged between 10^4 and 10^6 CFU/m³ (Radon *et al.*, 2002). The fungal aerosol in poultry house contains mainly molds from the genera: *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhizopus*, *Scopulariopsis* and *Trichophyton* (Jo and Kang, 2005) and yeast from genera *Candida*, *Cryptococcus*, *Rhodotorula*, *Trichosporon*, *Geotrichum* and *Sporotrichum* (Lonc and Plewa, 2010). Furthermore, litter is one of the most important factors to fungal contamination in poultry farms (Just *et al.*, 2009). The health hazard effects of fungal contamination are on animals as well as on workers and persons who live in close proximity to the farms (Lonc and Plewa, 2010; Rimac *et al.*, 2010). Moreover, these contaminated

environments may also transfer different microorganisms from one livestock building to another allergenic and adverse health effects (Lugauskas *et al.*, 2004). The bioaerosol sampling can be detected with several sampling methods. Impingement samplers are one of most effective sampling methods as it collect a wide range of bioaerosol concentrations basing on hypothesis that the bioaerosol trapped in a liquid (Grinshpun *et al.*, 2007). Although, some studies examined poultry farms microbial contaminants at various stages in poultry production cycle (Radon *et al.*, 2002; Bakutis *et al.*, 2004; Lawniczek-Walczyk *et al.*, 2013), but there have been fewer reports on fungal contamination of poultry farm environments (Hameed *et al.*, 2010; Nichita *et al.*, 2010). The maximum concentration of fungal spores in poultry farms occurs mainly in summer or early autumn (Springorum and Hartung, 2012). Bearing this in mind, the goal of this study was to evaluate the mould and yeast levels and identify the predominant genera in air, settled dust and litter of different broiler flocks at different stages of the production cycle during summer season.

MATERIALS AND METHODS

Broiler flocks and sampling strategy: Three broiler flocks in three different farms were investigated four times during one production cycle in the summer of 2015 at Dakahlia province, Egypt. All three flocks were housed on deep litter system with natural ventilation supported with

electrical fans during summer days. The numbers of birds in tested flocks ranged from 8000 till 12000 birds. The litter materials were straw in flock 1, meanwhile, in flock 2 and 3 were saw dust. The drinking system was automatic with pendulous drinkers. The samples were obtained during 4 different stages of the chicken production cycle. The sampling time were carried out biweekly between 10:00-12:00. The initial sampling session (-1 d) was conducted one day before 1 day old chicks entered the poultry houses. The second (10 d), third (20 d) and fourth (30 d) sampling session were performed when the chickens were 10, 20 and 30 days old, respectively.

Sampling: The following sampling designs were carried out inside each flock.

Air sampling was done by impingement using All-Glass-Impingers (AGI-30, Ace Glass Inc., Vineland, NJ). For each measurement there were two impingers running parallel. The samples were collected approximately 1.5 m above ground level in the middle way of the building away from possible ventilation. Each AGI-30 was filled with 30 ml phosphate-buffered saline (PBS). The collection times were 30 min. with airflow 12.5 l/min. for environmental samples, in each flock, pooled sample of about 2.5 g of settled dust was collected in a sterile zip lock bag using a sterile brush from different locations in bird house. Furthermore, pooled sample of about 250 g of litter was obtained.

During sampling, temperatures and relative humidity % (RH) were recorded with a thermo-hygrometer (China).

Laboratory analysis: All samples were shipped directly under cool conditions and analyzed within 2 h.

Estimation of airborne fungi (mould and yeast) was done by plate count method on Sabouraud Dextrose Agar medium (SDA, Oxoid, UK). Air samples were analyzed quantitatively for the presence total mould and total yeast count. Impingers were shaken for 30s at full speed with a vortex (El-Nasr, Egypt) then 1 ml was aspirated to 9 ml 0.1% BPW for ten-fold-serial dilution (up to 10^{-4}). Triplicate spreading of aliquots (0.1 ml) from dilutions were plated on SDA supplemented with chloramphenicol at a concentration of 100 ppm. After incubation at 25°C for 3 to 5 days, the average numbers of colony forming units per cubic meter of air (CFU/m³) of one dilution step were used for calculating the total cultivable airborne fungi.

For settled dust 0.1 g was dissolved in 10 ml 0.1% BPW and vortex at high speed for 1 min. Furthermore, 25 g of pooled litter samples were added to 225 ml 0.1% BPW each and homogenized by vortex (El-Nasr, Egypt). All the above prepared solutions were considered as first 10-fold dilution step to prepare serial dilutions (up to 10^{-4}) for counting of total mould and total yeast. For each sample 0.1 ml of suitable dilutions were spread on SDA in triplicate. The plates were incubated at stated before, after which the resulting colonies were counted and the concentrations were expressed as CFU per g for settled dust and litter.

From each sample at least one colony of each visually apparently different type of colony was selected for subculture on Czapek agar media (Oxoid, UK). The

recovered fungal colonies were identified on the bases of macroscopic features as color, topography of the surface of the culture, color of the reverse of the colony and the presence of diffuse color pigment. As well as, microscopic features of fungal colonies was identified as indicated in the literature to identify their species according to the key described by Hoog *et al.* (2000).

RESULTS

Mould contamination increases simultaneously in air, settled dust and litter toward the end of fattening period. The mould colonies isolated from air in different broiler flocks ranged between $9 \times 10^1 \pm 5$ to $1.3 \times 10^4 \pm 2.4 \times 10^3$, $1.2 \times 10^2 \pm 8$ to $4 \times 10^3 \pm 8.5 \times 10^2$ and $1 \times 10^2 \pm 1.7 \times 10^1$ to $1 \times 10^5 \pm 2.6 \times 10^4$ CFU/m³ in flocks 1, 2 and 3, respectively (Fig. 1). Furthermore, the mould detected in the settled dust of broiler flocks varied from $5 \times 10^1 \pm 2.5$ to $8 \times 10^3 \pm 9.8 \times 10^2$, $1 \times 10^2 \pm 8$ to $2 \times 10^4 \pm 2.1 \times 10^3$ and $4 \times 10^2 \pm 3.1 \times 10^1$ to $2 \times 10^5 \pm 9 \times 10^2$ CFU/g in flocks 1, 2 and 3, respectively. Additionally, Fig. 1 showed that the mould recovered from litter in different broiler flocks varied from $1 \times 10^2 \pm 9$ to $2.3 \times 10^4 \pm 1.45 \times 10^2$, $1 \times 10^2 \pm 1.1 \times 10^1$ to $1.03 \times 10^4 \pm 6.14 \times 10^3$ and $1.2 \times 10^2 \pm 6.24$ to $3.6 \times 10^5 \pm 2.84 \times 10^3$ CFU/g in flocks 1, 2 and 3, respectively.

Generally, the concentrations of yeast colonies count in air, settled dust and litter in a poultry flock have been found to rise with bird age. The yeast colonies isolated from air in broiler flocks ranged between $4 \times 10^1 \pm 4.36$ to $1.3 \times 10^4 \pm 5.77 \times 10^2$, $1.2 \times 10^2 \pm 1.78 \times 10^1$ to $3.3 \times 10^5 \pm 8.68 \times 10^1$ and $2.9 \times 10^2 \pm 3.74 \times 10^1$ to $3 \times 10^5 \pm 8.67 \times 10^1$ CFU/m³ in flocks 1, 2 and 3, respectively. Figure 2 showed that the yeast detected in settled dust of broiler flocks varied from $3 \times 10^1 \pm 1.73$ to $4.9 \times 10^5 \pm 3.48 \times 10^2$, $1 \times 10^2 \pm 8.72$ to $2 \times 10^4 \pm 8.91 \times 10^1$ and $4 \times 10^2 \pm 3.1 \times 10^1$ to $2 \times 10^5 \pm 9 \times 10^2$ CFU/g in flocks 1, 2 and 3, respectively. Additionally, the yeast recovered in litter material from different broiler flocks varied from $7 \times 10^1 \pm 6.08$ to $2.3 \times 10^5 \pm 8.9 \times 10^1$, $4.2 \times 10^2 \pm 1.76 \times 10^1$ to $7 \times 10^5 \pm 8.72 \times 10^1$ and $2.3 \times 10^2 \pm 9.34 \times 10^1$ to $3.3 \times 10^6 \pm 1 \times 10^2$ CFU/g in flocks 1, 2 and 3, respectively Fig. 2.

In this study, a total of 6 different genera of mould from the air, settled dust and litter samples were identified from broiler flocks (*Aspergillus*, *Alternaria*, *Penicillium*, *Mucor*, *Fusarium* and *Cladosporium* spp). As shown in Table 1, the predominant mould genera recovered from air were *Mucor mucedo* (30 and 29%) and *Aspergillus fumigatus* (25%) in flocks 1, 2 and 3, respectively. Regarding the settled dust, the dominating genera were *Aspergillus niger* (55%), *Mucor mucedo* (20%) and *Aspergillus fumigatus* (21%) in flocks 1, 2 and 3, respectively. In case of litter samples the highly isolated mould were *Aspergillus niger* (30%), *Aspergillus fumigatus* (35%) and *Mucor mucedo* (32%) in flocks 1, 2 and 3, respectively. The different mould genera during microscopical examination (*Penicillium* spp., *Aspergillus*, *Alternaria alternata* and *Cladosporium* spp are shown in Fig. 3.

Table 2 shows the percentage of 5 different yeast genera were isolated and identified from air, settled dust and litter of different broiler flocks investigated (*Candida*, *Cryptococcus*, *Rhodotorula*, *Trichosporon* and

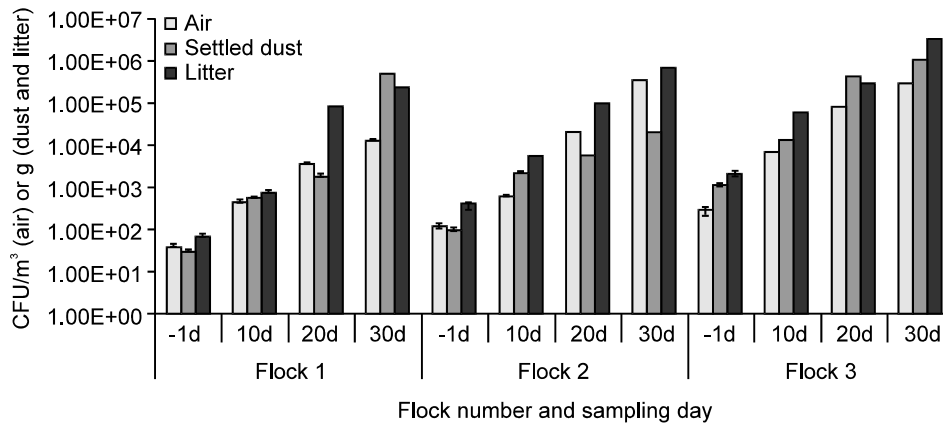


Fig. 1: Average concentration±standard deviation of mould (CFU/m³ or g) isolated from air, settled dust and litter of three broiler flocks during study period (one day before stocking until 30th day of production cycle)

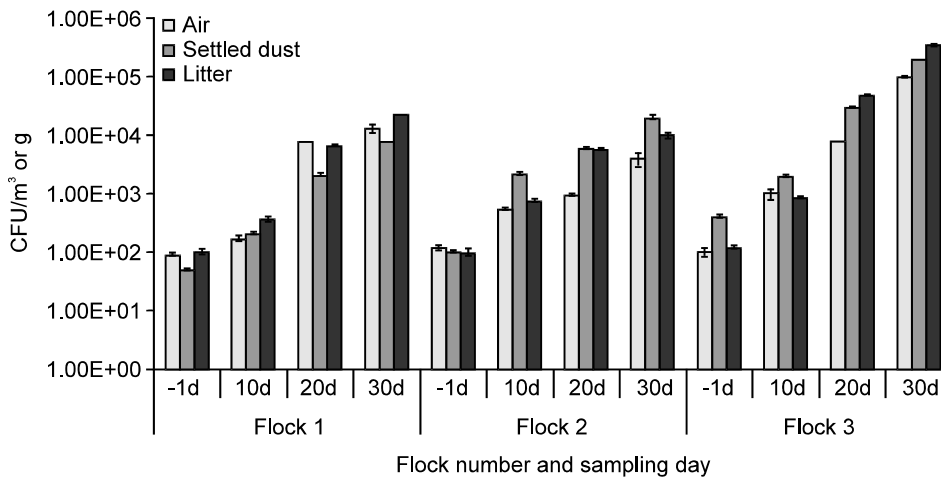


Fig. 2: Average concentration±standard deviation of yeast (CFU/m³ or g) isolated from air, settled dust and litter of three broiler flocks during study period (one day before stocking until 30th day of production cycle)

Geotrichum spp). The predominant yeast genera recovered from air were *Cryptococcus laurentii* (36 and 30%) and *Geotrichum* spp (32%) in flocks 1, 2 and 3, respectively. For settled dust the dominating genera was *Geotrichum* spp (30%), *Cryptococcus laurentii* (35 and 42%) in flocks 1, 2 and 3, respectively. In case of litter the highly isolated yeast were *Geotrichum* spp (34%), *Cryptococcus laurentii* (29 and 39%) in flocks 1, 2 and 3, respectively.

Within the identified fungi *Aspergillus fumigatus* (group 2 hazardous biological agents) was detected in air, settled dust and litter of flocks 2 and 3 (Table 1 and 2).

Furthermore, *Candida albicans* (group 2 hazardous biological agents) was also identified from all tested samples from three investigated flocks (Table 2).

Table 3 shows the range of microclimatic parameters (temperature and RH %) in three investigated flocks. In all flocks, the temperature ranged from 23.6 to 37.5°C. While the RH varied from 61.3 to 71.2%.

DISCUSSION

Places of high relative humidity are suitable environment for growth of fungi including mould and yeast and release of its spores. Poultry farms in summer are exactly that places. The fungi present in the air in form of saprophytic and pathogenic fungi. The determination of fungal presence in poultry house is important parameters for the assessment of the influence of poultry production on environmental pollution, bird and human health (Lonc and Plewa, 2010).

The fungal load of air, settled dust and litter in the investigated broiler flocks in this study rise with increasing bird age. Similar result has been reported by Vucemilo *et al.* (2005 and 2007) as they have found that the concentrations of airborne fungi during the first fattening week were 9.8×10^1 CFU/m³. However, this amount increased (3×10^3 CFU/m³) during the fifth fattening week.

The number of fungi in air of poultry facility varied from $5.8 \times 10^3 \pm 123.6$ to $8.2 \times 10^3 \pm 116.4$ (Nichita and Tirziu, 2008).

Table 1: Percentage of mould species isolated from air, settled dust and litter from the broiler flocks 1, 2 and 3 during study period (one day before stocking until 30th day of production cycle)

Genus	Species	Flock 1			Flock 2			Flock 3		
		Air	Settled dust	Litter	Air	Settled dust	Litter	Air	Settled dust	Litter
<i>Aspergillus</i>	<i>Niger</i>	19.1	55	30	18			17	15	3.5
	<i>Fumigatus</i>				15	15	35	25	21	2.5
	<i>Flavus</i>			13		5			12	6
	<i>Versicolor</i>					6	14		3	2
<i>Penicillium</i>	<i>Chrysogenum</i>	7.1	23.2		24	17	25	13	6	7
	<i>Solium</i>					15	9	8	12	5
<i>Alternaria</i>	<i>Alternata</i>	25.8	3.4	22	10			9		11
	<i>Tenuissima</i>	15	2	10				5		
<i>Fusarium</i>	<i>Oxysporum</i>		5	7						5
	<i>Cladosporium</i>			3		17	7		12	26
<i>Mucor</i>	<i>Mucedo</i>	30	11.4	12	29	20	10	12	19	32
Unknown spp		3		3	4	5		11		
Total		100	100	100	100	100	100	100	100	100

Table 2: Percent of yeast species isolated from air, settled dust and litter from three broiler flocks during study period (one day before stocking until 30th day of production cycle)

Genus	Species	Flock 1			Flock 2			Flock 3		
		Air	Settled dust	Litter	Air	Settled dust	Litter	Air	Settled dust	Litter
<i>Candida</i>	<i>Albicans</i>	34	18	16	12	14	11	8	16	14
	<i>Inconspicua</i>		13	8	2	8		5	7	10
	<i>Lambica</i>				2					
<i>Cryptococcus</i>	<i>Laurentii</i>	36	24	32	30	35	29	30	42	39
<i>Rhodotorula</i>	<i>Rubrum</i>				17	9	14			
<i>Trichosporon</i>	<i>Viridae</i>		15	10	9	5	22	25		10
<i>Geotrichum</i>	Spp	30	30	34	28	29	24	32	35	27
Total		100	100	100	100	100	100	100	100	100

Table 3: Temperature°C and relative humidity % measured during different sampling times (one day before stocking until 30th day of production cycle) inside the broiler barn

	Flock 1				Flock 2				Flock 3			
	-1d	10d	20d	30d	-1d	10d	20d	30d	-1d	10d	20d	30d
Temperature°C	37.1	32.2	27.3	23.6	37.3	32.5	28.3	24.9	37.5	32.1	29.4	25.6
Relative humidity %	65.7	66.2	61.3	56.5	67.4	66.5	67.3	62.7	65.4	62.9	71.2	67.1

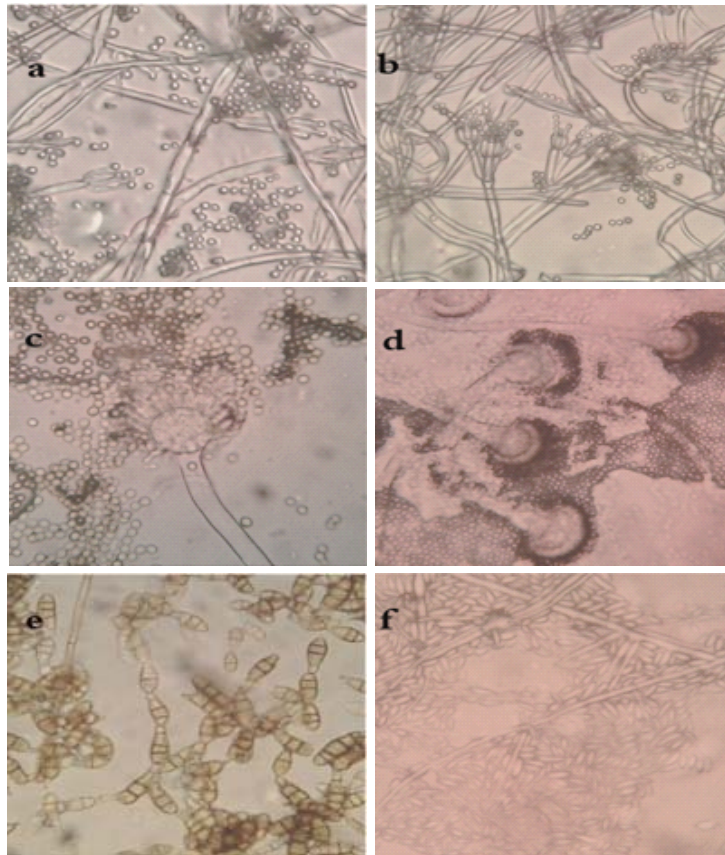


Fig. 3: Mould genera (a, b) *Penicillium* spp. heads, uniseriate (a) biseriate (b) x 1000; (c, d) *Aspergillus* different heads spherical (globose) vesicles (c) hemispherical *A. niger* (d); (e) *Alternaria alternate* conidiophores and muriform conidia, pyriform brown colored have 3-4 transverse septa, 1-2 longitudinal septa x 600 lense; (f) *Cladosporium* spp. branched conidiophores and chains of conidia x 1000

Higher fungal aerosols concentrations (ranged from 1.8×10^2 CFU/ m^3 to 1.8×10^5 CFU/ m^3) was detected in research conducted by Lawniczek-Walczuk and co-workers (Lawniczek-Walczuk *et al.*, 2013). Furthermore, fungal load in air of poultry confinements were found to be $5-119 \times 10^5$ CFU/ m^3 (Okiki and Ogbimi, 2011). All the previous findings matched with our study in which from all investigated farms (during all sampling time) the mould aerosol was ranged from 9×10^1 CFU/ m^3 (d-1 in flock 1) to 1×10^5 CFU/ m^3 (d 30 in flock 3) and the yeast aerosol level was varied from 4×10^1 CFU/ m^3 (d-1 in flock 1) to 3.3×10^5 CFU/ m^3 (d 30 in flock 2). These variations may be due to usage of different techniques in air sampling, ventilation system, density of birds per m^2 and cultural medium used.

Okiki and Ogbimi (2011) reported that fungal load in settled dust of poultry confinements were found to be $3.5-42 \times 10^6$ CFU/g. This finding is similar to our observation that the maximum levels of mould and yeast were 5×10^5 CFU/g and 1×10^6 CFU/g at d 30 in flock 3, respectively. Viegas *et al.* (2012) stated that the fungal contamination of poultry litter ranged from 1.6×10^5 to 8.3×10^5 CFU/g. According to Okiki and Ogbimi (2011) the fungal contamination varied from 1.8×10^5 to 3.7×10^5 CFU/g.

The above mentioned results was in accordance with our finding as the mould and yeast count reached to 3.3×10^5 CFU/g and 3.6×10^6 CFU/g at d 30 in flock 3, respectively. The results of current study revealed that eighteen different species of fungi were isolated from all examined farms (11 mould species and 7 yeast species).

There were 5 airborne mould genus detected in this study *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium* and *Mucor* spp. This result matched with other studies as Nichita and Tirziu (2008) identified eight fungal genera from the poultry house air including *Aspergillus* and *Penicillium* was the dominating species isolated and other isolated fungi were *Alternaria*, *Rhizopus*, *Mucor*, *Fusarium*, *Cladosporium* and *Scopulariopsis*. Okiki and Ogbimi (2010) found that the fungi isolated from poultry confinement's air were: *Aspergillus*, *Penicillium*, *Mucor*, *Penicillium oxalicum*, *Trichoderma* spp., *Stachybotrys*, *Fusarium*, *Candida*, *Cryptococcus* and *Saccharomyces* spp. In a Polish study, genus *Aspergillus* (*A. niger*, *A. nidulans*, *A. ochraceus*), *Penicillium notatum*, *Penicillium*, *Cladosporium* and *Alternaria* ssp. were isolated from poultry facility air (Karwowska, 2005). Recent research conducted in 2016 concluded that most predominant yeasts in poultry breeding houses air were *Candida*,

Trichosporon, *Rhodotorula* and *Geotrichum* spp, as well as nine genera of molds which were identified as follows: *Aspergillus*, *Alternaria*, *Mycelia sterilia*, *Penicillium*, *Chrysosporium*, *Mucor* and *Cladosporium* spp. (Shokri, 2016).

Twelve different fungal species were detected in fresh litter samples and *Penicillium* was the most frequent genus found (59.9%), followed by *Alternaria* (17.8%), *Cladosporium* (7.1%) and *Aspergillus* (5.7%) in research conducted in Portugal (Viegas *et al.*, 2012). Furthermore, Anbu *et al.* (2004) reported the most prevalent species isolated from litter samples were *Fusarium*, *Aspergillus flavus*, *A. nidulans*, *A. niger*, *Penicillium*, *Scopulariopsis*, *Cladosporium oxysporum* and *Trichoderma viridae*. According to the results of our study, *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Cladosporium* and *Mucor* spp. were detected in litter samples of broiler. This result agree with the findings of Rimac *et al.* (2010) who stated that the most prevalent fungi were *Penicillium*, *Fusarium*, *Aspergillus*, *Mucor* and *Rhizopus* spp.

According to the research reports conducted by Skora *et al.* (2016) and Lee *et al.* (2006), ten different fungal species were detected in settles dust including: *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Trichoderma*, *Stachybotrys*, *Fusarium*, *Candida*, *Cryptococcus* and *Saccharomyces* spp. These results were in accordance with our study finding as *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Fusariu*, *Cladosporium* and *Mucor*, *Candida*, *Trichosporon*, *Rhodotorula*, *Trichosporon* and *Geotrichum* spp, were detected in settled dust of three examined flocks.

Increasing temperature and RH% causing high fungal load as detected in flocks 2 and 3 in comparison to flock 1. These variations in fungal load in 3 investigated flocks may be explained on the bases of variation in temperature and RH in the barn which was also perfectly correlated (Bickert, 2001).

Conclutions: The fungal load of broiler flocks environment rise with increasing bird age during the production cycle. The accumulation of organic matters, dust and litter with moisture in poultry house is considered the specific medium for fungi to grow and sporulate and also produce mycotoxins leading to increase a risk of mycotic infection and allergic conditions for both human and bird. Therefore, the good hygiene practice, biosecurity measures and ventilation of farm are important for both birds and human. Furthermore, presence of opportunistic pathogens from genus of *Aspergillus* and *Candida* poses a risk of invasive aspergillosis for both poultry and workers.

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