Investigation on mycoflora of poultry breeding houses' air and studying the efficacy of spraying and fumigation on inactivating the airspora

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Abstract:

BACKGROUND: A common concern of the poultry industry is the presence of fungal pathogens in the birds' environment, which may constitute a considerable health hazard to the birds, farmers, and those living in proximity of the farm. **OBJECTIVES:** The aims of this study were to assess the mycoflora in the indoor and outdoor environments of poultry breeding houses and studying the efficacy of disinfection methods, including spraying and fumigation, on reducing airspora concentration. **METHODS:** Indoor air of 12 poultry houses were sampled by exposing Petri dishes containing Sabouraud's glucose agar after removal of old litter, spraying with disinfectant solutions, and fumigation with formalin plus permanganate. The plates were incubated at 30 °C for seven days and fungi were counted and identified microscopically and macroscopically according to standard mycological methods. **RESULTS:** A total of 182 and 181 fungal colonies were recovered from indoor and outdoor air of poultry houses, respectively. *Candida* (30.2%) and *Aspergillus* (26.9%) species were the most common yeast and mold in the indoor, respectively, whereas *Alternaria* (37.6%) and *Candida* (19.3%) species were the most predominant fungi in the outdoor air of poultry houses. Disinfection of the poultry houses using spraying and fumigation methods led to a 38.1% and 75% reduction in airspora concentration ($p<0.05$), respectively. **CONCLUSIONS:** Based on the findings of the present study, *Candida* spp and *Alternaria* spp had the highest indoor and outdoor concentrations in poultry breeding houses' air, respectively, and fumigation was the most efficient method in reducing airspora.

Introduction

Intensive poultry production, implying large densities of animals in small areas, is a significant source of air pollution which may constitute a considerable health hazard to the birds, farmers, and those living in proximity of the farm (Lonc and Plewa, 2009). On the other hand, spread of bio aerosols outside of animal houses may result in local or even more extensive environmental pollution (Bakutis et al., 2004). The indoor air of poultry houses is usually contaminated with high concentrations of microorganisms (Jo et al., 2005). According to

the existing studies, bacteria are the dominant microorganisms found in the poultry house bio aerosols, whose concentrations reach as much as 10^9 Colony Forming Unit (CFU)/m^3 (Radon et al., 2002), but fungi constitute a significant part of the airborne microflora in this sector as well. Their concentrations in stationary measurements usually range from 10^2 to 10^4 CFU/m^3 (Rimac et al, 2010), whereas in personal measurements for poultry farm workers- they are contained within 10^4-10^8 CFU/m^3 (Lee et al., 2006).

In general, outdoor atmosphere is dominated by representatives of the genera *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria* and by yeasts and *Mycelia sterilia*. In relation to outdoor environments, indoor atmospheres typically display lower diversity (Araujo and Cabral, 2010). The fungal flora in the indoor air of breeding houses often contains molds from the genera: *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhizopus*, *Scopulariopsis*, and *Trichophyton* (Lugauskas et al, 2004). Both viable forms of these fungi and their metabolites (mycotoxins) may cause a number of disorders in birds and poultry breeding workers, concerning mainly the respiratory tract (mucous membrane irritation, invasive mycoses of lungs, allergic rhinitis, allergic pulmonary alveolitis, asthma) and the skin (dermatomycoses and onychomycosis) (Dutkiewicz et al., 1999). Currently, fungal infections caused by *Microsporum gallinae* (Taghavi et al., 2014), A. fumigatus (Khosravi et al, 2007), and *Candida* species (Hashempour et al., 2014) are among the most frequent causes of fungal problems in poultry birds in Iran.

Poultry house sanitation plays a crucial role in controlling and preventing pathogenic infectious diseases. Removal of old litter, followed by cleaning and disinfecting poultry houses, can help reduce pathogen loads and break disease cycles. Methods for the application of disinfectants include spraying, misting, fogging, or fumigation (Eckman, 1994). A good sanitation program can benefit the grower by optimizing bird performance while lowering the incidence of contaminated flocks.

Mazandaran is a province in the North of Iran. It is located on the southern coast of the Caspian Sea. In the coastal plains - where we conducted our study - the humidity is high and the climate is temperate, which favors fungal growth; they are found through air. The present study was aimed to evaluate airspora concentration in the indoor and outdoor of poultry houses and studying the efficacy of disinfection methods including spraying and fumigation in inactivating a variety of common fungi in poultry houses.

Materials and Methods

Sampling sites: Twelve poultry breeding houses (broilers; Ross 308) were randomly selected from across the coastal plains of the Mazandaran province. Indoor and outdoor air samples were collected in the winter of 2014. The flock population in buildings was between 7000 and there were 35.000 birds. The rearing period was between 45 to 56 days for birds.

Air sampling: Indoor air samples for determining concentrations of fungi were collected by exposing Petri dishes containing Sabouraud's glucose agar (Merck Co., Darmastadt, Germany) in three steps as follows: Step 1: after the removal of old litter; Step 2: after spraying with disinfectant solutions (dispatag, chloracid 2000, nanosil, savlon, and formalin); Step 3: after fumigation using formalin along with permanganate. Petri dishes were placed at the height of 1.5 meters above the floor. Sampling took 30 minutes. During indoor mycoflora sampling at the same point, outdoor air sampling was performed as well (Shokri et al., 2010).

Laboratory analysis: Samples were incubated at 30 °C for seven days, after which the resulting colonies were counted as Colony Forming Units (CFU/plate/30 min). The cul-

ture plates were discarded after 4 weeks if no signs of fungal growth were seen. At least one colony of each apparently different types of colonies from indoor and outdoor air samples was selected for subculture and identification. The isolated fungi were identified to the genus level on the basis of colonial morphology on diagnostic media and on microscopic morphology by keys to identification and also using biochemical tests with API 20C AUX kit (bio-Merieux-Vitek, Inc., Hazelwood, USA) (Pitt, 2000; Larone, 2002; Samson et al., 2004).

Statistics: For statistical analysis, a chi-square test was performed to reveal the differences among different poultry breeding houses; with respect to the concentration of isolated airborne fungi. A p value less than 0.05 was considered statistically significant.

Results

Forty-eight plates were collected, of which 36 were indoor air samples and 12 outdoor. Thirty-eight plates turned out positive. The types and concentrations of airborne culturable fungi determined in poultry breeding houses and outside of those houses were presented in Table 1. The analysis of airspora showed that the indoor concentration of fungi (no. 182 CFU) was to some extent higher than that of outdoor concentration (no. 181 CFU) at a distance of 20 meters from the poultry houses. No significant differences were observed between airspora in the indoor and outdoor air of poultry breeding houses.

In this study, a total of 13 genera of fungi from the indoor air samples were identified from poultry houses. As shown in Table 2, the most predominant yeasts were *Candida* spp (30.2%), *Trichosporon* spp (6%), *Rhodotorula* spp (1.6%), and *Geotrichum* spp (0.5%), as well as nine genera of molds which were identified as follows: *Aspergillus* spp (26.9%), *Alternaria* spp (11.5%), *Mycelia sterilia* (9.9%), *Penicillium* spp (8.8%), *Chrysosporium* spp

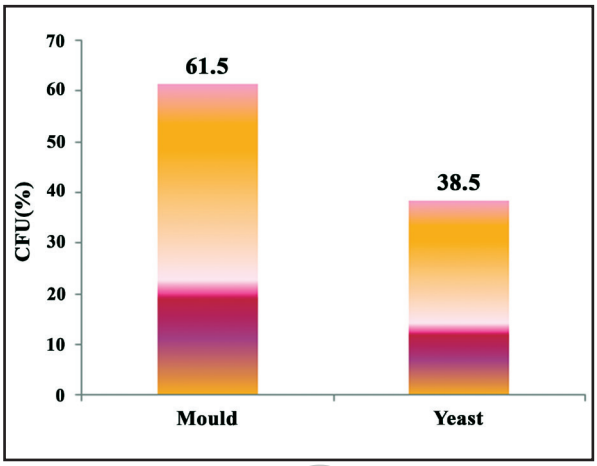

Figure 1. Comparison of the concentrations of moulds and yeasts in the inside air of poultry breeding houses (CFU/plate/30 min).

Table 1. Concentrations of airspora in the indoor and outdoor environments of the poultry breeding houses (CFU/plate/30 min). (*) Step 1: After removal of old litter; Step 2: After spraying with disinfectant solutions (dispatag, chloracid 2000, nanosil, savlon and formalin); Step 3: After fumigation using formalin along with permanganate.

	Indoor air (CFU)			Outdoor air (CFU)
	Step 1	Step 2	Step 3	
Poultry house 1	54	4	9	44
Poultry house 2	6	17	2	12
Poultry house 3	23	18	0	33
Poultry house 4	5	5	0	6
Poultry house 5	10	4	0	12
Poultry house 6	47	0	0	17
Poultry house 7	6	11	10	3
Poultry house 8	4	0	0	5
Poultry house 9	6	32	0	1
Poultry house 10	5	7	7	20
Poultry house 11	6	5	0	6
Poultry house 12	9	9	0	22

(1%), *Mucor* spp (1%), *Cladosporium* spp (1%), *Rhizopus* spp (0.5%), and *Dematiaceous fungi* (0.5%). There was a statistically significant difference between the concentrations of molds (61.5%) and yeasts (38.5%) ($p<0.05$) (Fig. 1). A total of 11 fungal genera were identified from the outdoor air samples of poultry houses. As shown in Table 2, *Alternaria* spp were the most frequent organisms and formed about 37.6% of the total fungal community.

Table 2. The composition of fungal genera in outside and inside air of poultry breeding houses (CFU/plate/30 min). (*) Step 1: After removal of old litter; Step 2: After spraying with disinfectant solutions (dispatag, chloracid 2000, nanosil, savlon and formalin); Step 3: After fumigation using formalin along with permanganate.

Poultry houses	**Indoor air (CFU)**			**Outdoor air (CFU)**
	Step 1	**Step 2**	**Step 3**	
Poultry house 1	*Aspergillus* (5), *Penicillium* (1), *Alternaria* (4), *Mycelia sterilia* (1), *Candida* (30), *Rhodotorula* (1), *Geotrichum* (1), *Trichosporon beigelii* (11)	*Cladosporium* (2), *Trichosporon beigelii* (2)	*Alternaria* (1), *Cladosporium* (1), *Mycelia sterilia* (7)	*Aspergillus* (1), *Penicillium* (7), *Alternaria* (2), *Mycelia sterilia* (13), *Candida* (21)
Poultry house 2	*Alternaria* (2), *Mycelia sterilia* (2), *Chrysosporium* (2)	*Drechslera* (1), *Aspergillus* (1), *Penicillium* (3), *Fusarium* (1), *Alternaria* (4), *Cladosporium* (1), *Mycelia sterilia* (4), *Rhodotorula* (1), *Candida* (1)	*Rhizopus* (1), *Mucor* (1)	*Alternaria* (3), *Mycelia sterilia* (1), *Chrysosporium* (2), *Penicillium* (4), *Candida* (2)
Poultry house 3	*Aspergillus* (17), *Penicillium* (5), *Candida* (1)	*Aspergillus* (1), *Cladosporium* (1), *Alternaria* (7), *Rhodotorula* (1), *Candida* (8)	0	*Aspergillus* (2), *Penicillium* (1), *Alternaria* (30)
Poultry house 4	*Aspergillus* (2), *Alternaria* (3)	*Mycelia sterilia* (1), *Candida* (1)	0	*Fusarium* (3), *Mycelia sterilia* (1), *Alternaria* (1), *Candida* (1)
Poultry house 5	*Aspergillus* (5), *Penicillium* (3), *Rhizopus* (1), *Alternaria* (1)	*Penicillium* (1), *Mycelia sterilia* (3)	0	*Penicillium* (1), *Mycelia sterilia* (1), *Alternaria* (4), *Dematiaceous fungi* (3), *Rhodotorula* (3)
Poultry house 6	*Aspergillus* (20), *Penicillium* (4), *Alternaria* (3), *Candida* (20),	-	0	*Penicillium* (1), *Mycelia sterilia* (3), *Alternaria* (5), *Fusarium* (1), *Candida* (7)
Poultry house 7	*Alternaria* (2), *Mucor* (1), *Mycelia sterilia* (3)	*Cladosporium* (8), *Alternaria* (2), *Trichosporon beigelii* (1)	*Cladosporium* (8), *Alternaria* (1), *Penicillium* (1)	*Aspergillus* (1), *Alternaria* (1), *Mycelia sterilia* (1)
Poultry house 8	*Alternaria* (1), *Mucor* (1), *Penicillium* (2)	-	0	*Aspergillus* (1), *Alternaria* (1), *Penicillium* (3)
Poultry house 9	*Mycelia sterilia* (6)	*Cladosporium* (10), *Alternaria* (1), *Mycelia sterilia* (1), *Rhodotorula* (20)	0	*Mucor* (1)
Poultry house 10	*Mycelia sterilia* (2), *Cladosporium* (1), *Dematiaceous fungi* (1), *Rhodotorula* (1)	*Alternaria* (3), *Cladosporium* (3), *Penicillium* (1)	*Stemphylium* (1), *Cladosporium* (2), *Alternaria* (1), *Penicillium* (2), *Mycelia sterilia* (1)	*Aspergillus* (7), *Cladosporium* (2), *Alternaria* (3), *Penicillium* (2), *Mycelia sterilia* (2), *Candida* (4)
Poultry house 11	*Cladosporium* (1), *Alternaria* (1), *Penicillium* (1), *Mycelia sterilia* (2), *Rhodotorula* (1)	*Cladosporium* (3), *Dematiaceous fungi* (1), *Rhodotorula* (1)	0	*Mycelia sterilia* (3), *Cladosporium* (1), *Dematiaceous fungi* (1), *Alternaria* (1)
Poultry house 12	*Alternaria* (4), *Mycelia sterilia* (2), *Candida* (3)	*Cladosporium* (8), *Mucor* (1),	0	*Alternaria* (20), *Mucor* (1), *Rhodotorula* (1)

Results of this study indicated that disinfection by spraying method of the poultry houses led to a 38.1% reduction in airspora concentration, while the application of fumigation, especially formaldehyde along with potassium permanganate, led to a 75% reduction in airspora concentration in the indoor air of poultry breeding houses, representing significant efficacy of fumigation method in reducing the airspora concentration ($p<0.05$).

Discussion

The presence of fungi in the indoor air of poultry house is a natural phenomenon. Microorganisms are constituents of saprophytic and pathogenic fungi, which were found in the poultry houses air. Aerial count of fungal elements in the indoor air of poultry house and monitoring of its emission from this building to the adjacent environment are important parameters for the assessment of the influence of poultry houses on the animals' health and environmental pollution (Lonc and Plewa, 2009). In the present study, fungal contamination in the indoor air was determined at 12 poultry breeding houses in Mazandaran province, Iran.

In the current study, a total of 48 impacted plates were collected, of which 36 were indoor air samples and 12 outdoor. Thirty-eight plates turned out positive. As shown in Table 1, the indoor concentration of fungi (no. 182 CFU) was to some extent higher than that of outdoor concentration (no. 181 CFU) at a distance of 20 meters from the poultry houses. No significant differences were observed between airspora in the indoor and outdoor air of poultry breeding houses. Baykov and Stoyanov (1999) also reported higher fungal levels inside poultry houses than in nearby areas and the average values were similar to our results.

The present study indicated 13 genera of fungi from the indoor air samples of poultry houses. The most predominant yeasts were *Candida* spp (30.2%), *Trichosporon* spp (6%), *Rhodotorula* spp (1.6%), and *Geotrichum* spp (0.5%), as well as nine genera of molds which were identified as follows: *Aspergillus* spp (26.9%), *Alternaria* spp (11.5%), *Mycelia sterilia* (9.9%), *Penicillium* spp (8.8%), *Chrysosporium* spp (1%), *Mucor* spp (1%), *Cladosporium* spp (1%), *Rhizopus* spp (0.5%), and *Dematiaceous fungi* (0.5%). While the share of yeasts in the entire pool of the determined fungi reached only 38.5%, molds constituted 61.5% in that pool, representing significant difference between molds and yeasts ($p<0.05$; Fig. 1). The fungal mycoflora isolated in these houses was largely diversified. A similar diversity was observed by other researchers, who surveyed poultry houses (Radon et al., 2002; Lugauskas et al., 2004; Karwowska, 2005). Furthermore, consistent with the data presented in their studies, the molds' population prevailed in the indoor air of breeding houses. According to the data obtained by Soliman et al. (2009), fungi, e.g. *Candida albicans*, *A. niger*, *A. nidulans*, *Penicilium* spp, and *Mucor* spp were prevalent in poultry farms in Egypt. However, Romanowska-Słomka and Mirosławski (2009) described the occurrence of the molds and yeasts such as, *Aspergillus*, *Penicillium*, *Candida* and *Cryptococcus* species, in the poultry houses.

Other investigators (Gigli et al., 2005; Nieguitsila et al., 2011) isolated and identified many fungal strains, including *Cladosporium* spp, *Aspergillus* spp, *Penicillium* spp, *Scopulariopsis* spp, *Fusarium* spp, *Epicoccum* spp, *Mucor* spp, *Trichophyton* spp, *Alternaria* spp, *Ulocladium* spp, *Basidiospores* spp, *Acremonium* spp, *Aureobasidium* spp, *Drechslera* spp, *Pithomyces* spp, *Chrysosporium* spp, *Geomyces* spp, and *Rhizomucor* spp from breeding houses. The presence of such fungi in poultry houses was proved by the results of our study.

The present study exhibited *Aspergillus* and *Candida* species as the predominant genera in the indoor air of poultry breeding houses,

comprising over 50% of all the determined genera with a significant difference with other fungi ($p<0.05$). The high prevalence of these fungi could be related to humid environment of Mazandaran province. These results are in consistent with most studies, which mentioned *Aspergillus* species as the dominant mycoflora in the poultry house air (Jo et al., 2005; Vučemilo et al., 2007). This genus comprises many saprophytic species, as well as pathogens. *Aspergillus* species may induce aspergillosis and allergic symptoms in both human and birds (Flannigan et al., 2001; Libudzisz et al., 2007). According to the literature, *Candida* species were also isolated from the indoor air of poultry houses, but they do not always make the dominant fungal mycoflora (Radon et al., 2002; Lonc and Plewa, 2009; Rimac et al., 2010). Yeasts of genus *Candida* are ranked among opportunistic pathogens, which only in specific conditions (e.g. deficient immunity system) may induce various types of candidiasis in birds (Larone, 2002).

A total of 11 fungal genera were identified from the outdoor air samples of poultry houses. As shown in Table 2, *Alternaria* spp were the most frequent organisms with the frequency of 37.6% of the total fungal community. *Alternaria* spp are one of the most common fungi associated with asthma. Not only the presence of asthma, but also the persistence and severity of asthma is strongly associated with sensitization and exposure to *Alternaria* spp (Salo et al., 2006). In this study, other commonly occurring fungi from outdoor air were associated with *Candida* (19.3%), *Mycelia sterilia* (13.8%), *Penicillium* (10.5%), and *Aspergillus* (6.6%) species. In a study conducted by Shokri et al. (2010), *Alternaria*, *Cladophialophora*, and *Mucor* species were the most predominant fungi isolated from the air of different locations of Mazandaran province, Iran.

A good sanitation program of poultry breeding houses is the removal of old litter, cleaning, washing, and disinfecting, which are very important in controlling the accumulation and spread of disease-causing microorganisms (Lugauskas et al., 2004). This study was continued to determine whether different disinfection methods including spraying and fumigation are effective in reducing fungal populations. Results of this study indicated that disinfection by spraying method of the poultry houses led to a 38.1% reduction in airspora concentration, while the application of fumigation, especially formaldehyde along with potassium permanganate, led to a 75% reduction in airspora concentration in the indoor air of poultry breeding houses, representing significant efficacy of fumigation method in reducing the airspora concentration ($p<0.05$).

Finally, our study indicated that fumigation appears to be more effective for the inactivation of airspora as compared to routine chemical disinfectant solutions. A total of 100% reduction in the concentration of airspora was achieved in poultry breeding houses 3, 4, 5, 6, 8, 9, 11, and 12 after 48 hours of fumigation. In addition, an 83.3% and 66.7% reduction in airspora concentration was observed in poultry houses 1 and 2, respectively (Table 1). In contrast, airspora concentration increased in poultry houses 7 and 10 after disinfection by spraying and fumigation methods. Poultry houses 7 and 10 were disinfected only using one disinfectant solution (dispatag), while other houses used dispatag along with one or two other antiseptic solutions (chloracid 2000, nanosil, savlon, and formalin). Another probable explanation for this may be the fact that, in the houses 7 and 10, the litter bed system was not correctly removed. In fact, if disinfectants are used without properly cleaning the house prior to application, then the effectiveness of the disinfectants may be compromised. Many disinfectants are ineffective in the presence of organic matter, such as soil or litter (Witkowska et al, 2010). The application of disinfectants, level of organic charge, synergy, temperature, dilution rate, and examination methods influ-

ence the antimicrobial activity of disinfectants (Russel, 2003). The mechanisms of action of antiseptics and disinfectants on microorganisms include examination of uptake, lysis, and leakage of intracellular constituents, perturbation of cell homeostasis, effects on model membranes, and inhibition of enzymes, electron transport, and oxidative phosphorylation (McDonnell and Russell, 1999; Christopher et al., 2007). Previous studies demonstrated that any number of best management practices, treatments, or disinfectants can comprise a sanitation program. However, if used improperly, sanitation procedures can adversely affect disease prevention, thus, lowering bird performance (Davies et al., 1995). For this reason, it is important to routinely reevaluate the effectiveness of poultry house sanitation programs.

In summary, the dominant fungal mycoflora in the air of poultry breeding houses were molds (61.5%), with the most abundant *Aspergillus* genus. Yeasts constituted another 38.5% of fungal aerosol and were mainly represented by *Candida* spp. In addition, this research has demonstrated the potential for fumigation method to achieve a large percentage reduction in viable spore counts in the indoor air of poultry houses. Results of this study indicated that variables, such as application rate, disinfectant type, and the presence or absence of organic matter, are all important considerations when including a chemical disinfectant application into a sanitation program.

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بررسی فلور قارچی هوای سالن‌های پرورش طیور و مطالعه تأثیر اسپری کردن و دوددهی بر غیرفعال‌سازی اسپورهای هوا

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چکیده

زمینه مطالعه: یک نگرانی شـایع در صنعت مرغداری حضور عوامل بیماریزای قارچی در محیط پرندگان اسـت که خطر قابل ملاحظـه‌ای بـرای پرندگان، کارگران مرغداری و تمام افرادی اسـت که در نزدیکی مرغداری زندگی می‌کننـد. **هدف:** اهداف این مطالعه تشخیص فلور قارچی در محیط‌های داخل و خارج سالن‌های پرورش طیور و مطالعه تأثیر روش‌های ضدعفونی کردن اسپری و دوددهـی روی کاهـش غلظت اسـپورهای هوا بودند. **مواد و روش کار:** هوای داخلی ۱۲ سـالن مرغـداری با روش مواجهه پتری دیش‌های حاوی سابوروگلوکز آگار بعد از برداشت پوشال قدیمی، اسپری کردن با محلول‌های ضدعفونی کننده و دوددهی با فرمالین بعلاوه پرمنگنات نمونه‌برداری شدند. پلیت‌ها در حرارت ۳۰°C بمدت ۷ روز گرمخانه‌گذاری شدند و قارچها به‌صورت میکروسکوپی و ماکروسکوپی مطابق روش‌های استاندارد قارچ‌شناسی شمارش و شناسایی شدند. **نتایج:** در مجموع ۱۸۲ و ۱۸۱ کولونی قارچی بترتیب از هوای داخل و خارج سـالن‌های مرغداری بدسـت آمدند. گونه‌های کاندید\(۳۰/۲٪) و \آسـپرژیلوس (۲۶/۹٪) به‌ترتیب شایع‌ترین قارچ‌های مخمری و رشته‌ای در هوای داخل سالن بودند، در حالیکه گونه‌های \آلترناریا(۳۷/۶٪) و کاندید\(۱۹/۳٪) بعنوان برجسته‌ترین قارچها در هوای خارج سالن‌های مرغداری شناسایی شدند. ضدعفونی سالن‌های مرغداری با استفاده از روش‌های اسپری و دوددهی به‌ترتیب موجب کاهش ۳۸/۱٪ و ۷۵٪ در غلظت اسپورهای هوا شدند (p<۰/۰۵). **نتیجه‌گیری‌نهایی:** در مجموع، گونه‌های کاندید\و \آلترناریا به‌ترتیب بیشترین غلظت را در هوای داخل و خارج سالن‌های مرغداری داشتند و بیشترین تأثیر روی کاهش اسپورهای هوا مربوط به روش دوددهی بود.

واژه های کلیدی: اسپورهای هوا، کاندید\ دوددهی، فلور قارچی، سالن مرغداری

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