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ISOLATION AND IDENTIFICATION OF THE AIR FUNGI PRESENT INSIDE THE SCHOOLS BUILDINGS IN BABYLON PROVINCE

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ABSTRACT : The present study included 660 samples were collected from inside schools buildings. These samples represented 310 from primary schools and 350 from middle and secondary schools for the period from January / 2020 to March / 2021. The results of the current study showed that 530 fungi isolates were obtained included (280, 130, 120) isolates from the primary, secondary and middle school, respectively. The highest percentage was recorded in primary schools as it reached (52.80%), followed by secondary schools as the percentage was (24.53%), while in middle schools the percentage was (22.64%). The results showed that *Aspergillus* spp. recorded the highest percentage (23, 102.9, 20.68) in appearance, frequency and distribution density, respectively. While, the fungus *Cladosporium* spp, the proportion of appearance, frequency and density of distribution was less than the previous fungi, the ratios were (21, 18.96, 18.96), respectively. The *Fusarium oxysporum* and *Penicillium* spp, recorded the highest rates, reaching 19% and 17% in the percentage of occurrence, respectively. Whereas, *Alternaria alternate* and *Trichophyton* spp recorded (11%, 9%) respectively and Steril mycelium recorded 11% and this percentage surpassed yeast's appearance rate of 8%.

Key words : Air fungi, schools air, *Aspergillus* spp, *Alternaria alternate*.

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INTRODUCTION

Fungi are eukaryotic organisms that differ in terms of behavior and composition, as well as in the way of feeding, from other organisms, as they are characterized by being heterotrophic, that is they cannot make their food because their cells do not contain chlorophyll. By parasitizing other organisms or recovering from organic materials, so the fungi possess special ingredients that make them able to carry out a wide range of activities and actions that can enter the joints of human life (Deepake, 2009; Nazish and Jaitly, 2021). Fungi have the ability to produce toxins of various kinds, in addition to the presence of a highly efficient enzyme system, and thus constitute a source of danger to humans directly through dealing with fungi, and it is possible to contract some types of allergies that result from inhaling pores, or indirectly. By inhaling or eating foodstuffs that are contaminated with mycotoxins (El-Gali *et al*, 2014).

Interior air quality affects health either directly or indirectly. For this reason, the health effects of interior air filled with pollutants emitted from biological or non-biological sources have been a growing concern within the scientific community (Shelton *et al*, 2002). Over the past few years, important progress has been observed in the study on interior airborne fungi, as well as the biomedical and pathological consequences of the plants caused by the fungus reproduction. Fungi species often appear during the seasons, despite fluctuations related to meteorology and weather conditions (Basilico *et al*, 2007).

Most scientific studies indicate that inhaling fungi spores or parts of fungal strings that may cause diseases, infection, poisoning and allergies, and this depends on two factors, the type of fungus and the period of exposure to it. Also allergic responses (asthma, nasal allergies, hypersensitivity and pneumonia) are among the most common and most common natural problems related to inhaling fungi and air fungi are active in summer, autumn and spring and the least in winter (Zhiguo *et al*, 2005;

Epstein and Fan, 2001).

Air fungi can also spread by air currents in the form of pieces of fungal strings and spores and can enter buildings through the entry of outside air and ventilation and heating in the air-conditioning system or through windows and doors as well as building materials and other contents as their pollutants, if the percentage increases sufficient moisture (Shelton *et al*, 2002). The increasing importance of air fungi and its potential for harmful effects on health, in terms of exposure to it causes allergies, irritants and other toxic effects. Measuring the interior air quality continues to reduce the occurrence of any kind of damage (Hedayati *et al*, 2005; Shelton *et al*, 2002; Basilico *et al*, 2007).

Compelling epidemiological links can be formed between mold allergy and diseases such as asthma and allergic rhinitis. Where, it was found that there is a widespread fungal spread in areas with high concentrations of fungal spores. Epidemiological studies have failed to prove a direct relationship between fungal allergy and allergic rhinitis, either through external or internal exposure (Hamilos, 2010).

This study aims to know the extent of the presence of air fungi in school buildings and the type of fungi most present in them and their impact on the environment and human health.

MATERIALS AND METHODS

Sample collection

During the study, 660 sample were collected from inside school buildings. These sample consisted of 310 sample from primary schools and 350 sample of air fungi from middle and secondary schools for the period from January / 2020 to March / 2021 from inside the buildings of Babil governorate schools by the open plat method and on the medium of Potato dextrose agar (PDA) (Uzochukwu and Nkpouto, 2013). The study included primary, middle and secondary schools and with four schools for each stage and 3 dishes for each site for the period from 9 to 12 am.

Preparation of the culture media

Sabouraud's dextrose agar medium : This medium was prepared according to the instructions of the supplier company by dissolving 65 g of Sabourauds dextrose agar powder in 1000 ml of distilled water.

Potato Dextrose Agar Medium (PDA) : Prepare the (PDA) medium according to the manufacturer's instructions by dissolving 39 g of the medium powder in 1000 ml of water. Before sterilization the antibiotic Amoxicillin was added at a concentration of 100 mg/L.

The two mediums were sterilized in a autoclave at 121°C at a pressure of 15 lb/inch^2 for 15 minutes.

Collection and isolation of fungi and their diagnosis

The plates containing the medium of the PDA were exposed to the air of the schools (primary, middle and secondary) for 5 minutes and at a height of 2 meters from the surface of the ground, after which they were recovered and placed in nylon bags and placed in the incubator at 25-27^{0}C for 5-7 days. After that the appearance diagnosis was made depending on the appearance of the colony in the plate (morphology features) in terms of color and colony shape, and the plates were examined in a preliminary examination to count the colonies growing on the cultural media.

The fungal species were purified by transferring parts of the growth colonies isolated primarily by a sterile transfer needle into dishes containing Sabouraud's Dextrose Agar (SDA) with the antibiotic Amoxicillin at a concentration of 100 mg/L and they were incubated at a temperature 25ºC for 5-7 days to obtain pure colonies. The fungi were diagnosed with a microscope features, in terms of the shape and size of the conidia carriers and spores by taking part of the fungal colony and adding a drop of lactophenol dye to a glass slide and then placing the slide cover and examined under a light microscope. The fungi were also re-cultured on potato Dextrose Agar medium and the fungi were diagnosed according to their morphology and colony colors are in addition to the diagnostic characteristics of each fungus. Fungi were diagnosed according to the taxonomic keys referred to in the classification (Ellis, 1971; Pitt and Hocking, 1997; Watanabe, 2002).

The following were calculated:

1. The total number of isolates and the isolated fungal species

2. The frequency percentage was calculated from the following law :

$$\text{Frequency } \% = \frac{\text{The number of single-sex isolates}}{\text{The total number of all isolates}} \times \% \, 100$$

The percentage of appearances was calculated from the following law:

$$\text{Occurrence } \% = \frac{\text{The number of samples in which the gender or species appeared}}{\text{The total number of samples during the study}} \times 100\%$$

DII (Distribution Intensity Index) :

The density index of the distribution for all isolated

fungi is calculated from the following law:

DII = $\sqrt{\%\ \text{Occurrence} \times \text{Frequency}\ \%}$ (Booth *et al*, 1988).

RESULTS AND DISCUSSION

Isolation and diagnosis of fungi

Public and crowded places such as hospitals, schools and Institutions represent the growth and multiplication of fungi, which leads to a high percentage of clicks resulting from them. Some of the fungi in the study in some School air in Babylon governorate, where six genera of fungi were discovered, as well as Sterile fungi and yeasts. During this study 660 samples were collected from inside school buildings. These sample represented 310 from primary schools and 350 sample from middle and secondary schools. The results revealed that 530 fungi isolates were obtained, where the highest percentage of the total numbers of primary schools was 280 (52.83%), followed by secondary schools classes with 130 (24.53%) and the lowest percentage 120 (22.64%) appeared in the middle schools, as shown in Table 1.

Table 1 : The numbers of fungi isolated from school buildings and their isolation rates.

No.	The place where the sample was take n / t	The number of same samples	The percentage of the t total numbers %
1	Primary School	280	52.83
2	Middle school	120	22.64
3	Secondary School	130	24.53
Total		530	100%

Isolated fungal species

Table 2 shows 8 fungal species isolated from inside school buildings during this study, dating back to 6 genera, as well as the isolation of sterile fungal hyphae and yeasts, where *Aspergillus* spp. showed dominance in all samples, followed by the rest of the fungal genera in the presence.

Table 3 shows that *Aspergillus* spp. had the highest percentage in appearance, with 23%, followed by

Table 2 : The fungi isolated from schools and growing at a temperature of 25 ± 2°C and on PDA medium.

No	Names of fungi
1	*Aspergillus* spp
2	*Cladosporium* spp
3	*Pencillium* spp
4	*Fusarium oxysporum*
5	*Alternaria alternate*
6	*Trichophyton* spp
7	*Steril mycelium*
8	Yeast

Table 3 : The percentage of appearance of fungi isolated from schools at a temperature of 25 ± 2°C and on the medium of the PDA.

No	Fungus	Percentage appearing
1	*Alternaria alternate*	11 %
2	*Aspergillus spp*	23%
3	*Cladosporium spp*	21 %
4	*Fusarium oxysporum*	19%
5	*Pencillium spp*	17%
6	*Trichophyton spp*	9 %
7	*Steril mycelium*	10 %
8	Yeast	8 %

Cladosporium spp. with 21% and *Fusarium oxysporum* and *Pencillium* spp. recorded 19% and 17%, respectively. While, *Alternaria alternate* recorded 11% and *Trichophyton* spp. the appearance of 9%, while *Steril mycelium* recorded an appearance rate of 11%, which is more than the appearance rate of yeast, which recorded 8%.

The results obtained in this study and as shown in Table 4 that the fungus *Aspergillus* spp. was higher by a frequency of 20.6% over the fungus *Cladosporium* spp. with a frequency rate of 18.9%. The frequency ratio of 15.5% and then 10.3% of *Fusarium oxysporum* and *Alternaria alternate* was obtained, respectively. Whereas, the lowest frequency ratio obtained by *Trichophyton* spp. was 8.6%. While the frequency ratio of *Steril mycelium* and yeast was 2.2% and 2.6, respectively.

The results are shown in Table 5. As for the density of distribution, the genus *Aspergillus* was the most prevalent 102.9 %, followed by the fungus *Cladosporium* spp 89.6%. As for the fungus *Pencillium spp*. the distribution density was higher than that of *Fusarium oxysporum* (78.5, 66.9%) respectively, whereas *Alternaria alternate* 36.4. While, the fungus *Trichophyton* spp. obtained the lowest density 27.7%. The sterile mycelium and yeasts had a percentage in the density of the distribution where the ratios were 17.3 and 11.9%, respectively.

Table 4 : The frequency ratio of the fungi isolated from schools at a temperature of 25 ± 2°C and on PDA medium.

No	Fungus	Frequency ratio%
1	*Alternaria alternate*	10.3
2	*Aspergillus* spp	20.6
3	*Cladosporium* spp	18.9
4	*Fusarium oxysporum*	15.5
5	*Pencillium* spp	17.2
6	*Trichophyton* spp	8.6
7	*Steril mycelium*	2.6
8	Yeast	2.2

Table 5 : The density of distribution of the fungi isolated from schools at a temperature of 25 ± 2°C and on the medium of the PDA.

No	Fungus	Density distribution %
1	*Alternaria alternate*	36.4
2	*Aspergillus* spp	102.9
3	*Cladosporium* spp	89.6
4	*Fusarium oxysporum*	66.9
5	*Pencillium* spp	78.5
6	*Trichophyton* spp	27.7
7	*Steril mycelium*	17.3
8	Yeast	11.9

DISCUSSION

The reason for the predominance of *Aspergillus* spp in Table 2. Over the rest of the other fungal genera is due to the suitability of different environmental conditions for its growth and reproduction. The fungus can form large numbers of asexual reproductive units, and some of its types have a sexual phase and sclerotia bodies more resistant to inappropriate environmental conditions (Uzoch ukwu, 2013). This is similar to what was mentioned by Menezes *et al* (2004).

Charles *et al* (2012) confirmed that the fungi *Aspergillus* spp. more prevalence followed by *Cladosporium* spp. and *Cladosporium* is one of the fungi that cause allergies and their presence in interior and outside air is apparent.

The fungus *Penicillium* spp. is one of the fungi that exist absolutely in the outside and inside air, and this means the ability of the fungus to adapt and settle in to survive despite the depletion of the basic substance of the food (Park *et al*, 2013).

Alternaria spp. is considered one of the fungi that affect human health (Davis, 2001). In addition to the fungus *Fusarium* ssp., these fungi are considered to be thrush fungi, which spread their spores in the air, and all fungal strains can produce germs, and it is the main part of the air that a person breathes, whether outside air or internal air.

The fungal genera are *Cladosporium* spp, *Aspergillus* ssp, *Penicillium* spp and *Alternaria* spp. one of the fungi that lead to the most common allergens in Kuwait, Iraq, Iran, Turkey and Saudi Arabia (Erkara *et al*, 2009). Also, yeasts are among the predominant group in interior air samples (Su *et al*, 2001). Ismail *et al* (2000) confirmed that yeasts are prevalent in air samples inside and outside buildings.

It was found that the results of the current study in Table 3 agree with current study by El-Gali *et al* (2014) was found that the fungus *Cladosporium* was present in the air of Al-Bayda city in Libya at a rate of 16.5%, followed by the fungus *Penicillium* at a rate of 15.7%, then the fungus *Alternaria* at a rate of 10.8%.

The results in Table 4 are agree with a study conducted by the researcher (Chadeganipour *et al*, 2010), who isolated 31 fungal species from interior air, including the following species: *Alternaria*, *Candida*, *Fusariu* and *Pencillum*. Some studies have shown that the fungi *Pencillum*, *Alternaria* and *Aspergillus* dominate the isolated species from the air in the rest of the species.

The results in Table 5 showed these fungi have a high ability to withstand varying and harsh environmental conditions due to their phenotypic and reproductive adaptations, followed by the genus *Cladosporium* spp. These fungi have special life strategies that enable them to withstand harsh environmental conditions, making them more persistent and widespread than other species and genera (Herbarth *et al*, 2003).

Also the results in Table 5 revealed that the air is not considered an environment for the growth of living organisms, including fungi, but rather they are present in it and are affected by the speed of winds, humidity, temperatures, proximity and distance from agricultural areas and foodstuffs that are consumed in buildings. These accumulated fungi are obligatory and optional and they need simple food and produce reproductive units in large numbers, which causes most of them. Many health problems and irritating diseases such as allergies and asthma (Green *et al*, 2005). Aerobiology, previous studies indicated that the number of deficient fungi exceeds the rest of the taxonomic groups, and this is consistent with the results of the current study, and from the side of other hand, most of the air fungi with an allergic effect to humans are within this group, including the genera that recorded dominance and superiority in its numbers in the current study, namely *Aspergillus*, *Alternaria*, *Penicillium*, *Cladosporium* and *Penicillium* (Begum *et al*, 2009; Shelton *et al*, 2002; Hogaboam *et al*, 2002).

CONCLUSION

The current study revealed the extent of the presence of air fungi in school buildings and the type of fungi most present in them and their impact on the environment and human health.

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