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Effect of Some Ecological Factors on Thegrowth of *Aspergillus niger* and *Cladosporium sphaerospermum*

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ABSTRACT

Indoor airborne fungi have been implicated in human health problems, damage to building materials, books, clothes and stored foods, which effected by different ecological factors. *Aspergillus niger* and *Cladosporium sphaerospermum* are the most dominant indoor airborne fungi were obtained from our previous study. The effect of normal indoor conditions (Temperature and Relative Humidity) on their growth was studied. Including their nutrients growth requirements. The PDA medium was the appropriate growth medium for *Cl. sphaerospermum*, with a significant difference at ($p<0.05$); where as GYA medium was the appropriate growth medium for *A. niger*, with no significant difference. The temperatures 25 and 30°C favored colony diameter growth for *Cl. sphaerospermum* and *A. niger*, respectively with a significant difference at ($p<0.05$). The growth of *Cl. sphaerospermum* increased to reach its maximum at 100% RH. Whereas, *A. niger* prefers to grow at lower RH comparing to *Cl. sphaerospermum* to reach its maximum at 75% RH with a significant difference at ($p<0.05$). Controlling the normal indoor conditions can play a significant role in reducing the growth of indoor airborne fungi. *Cl. sphaerospermum* can be used as indicator fungi for the high humidity level in residences. The result has proved that keeping the humidity low enough can prevent fungi growth.

Keywords: Relative Humidity (RH), Glucose Yeast Extract Agar (GYA), Prevent Fungi Growth, Culture Media, Temperature, Airborne Fungi

1. INTRODUCTION

The importance of indoor airborne fungi has been emphasized in recent decades. They have been implicated in human health problems, damage to building materials, books, clothes and stored foods (Burshtein *et al*., 2011; Rahoma, 2011; Chadeganipour *et al*., 2010; Haleem *et al*., 2009; Tasic and Miladinovic-Tasic, 2007; McClenny, 2005; Unlu *et al*., 2003; Yano *et al*., 2003).

Most people in developed countries spend more than 90% of their time indoors, in locations such as homes, offices and factories (Brasche and Bischof, 2005; Li and Kendrick, 1995). Concern regarding the health effects of indoor air quality has grown. In Saudi Arabia that has along, hot summer, the significance of indoor air quality is obvious.

Environmental factors such as humidity and temperature plays an important role in dispersing fungi spores in air for short and long distances and when spores deposited a solid or liquid surface and if conditions of moisture and food are appropriate, they germinate (Bennett, 2010; Goncalves *et al*., 2010). Normal indoor conditions such as humidity and temperature provide a suitable environment for the growth of a wide of fungal spores (Ababutain, 2011; Li and Kendrick, 1995).

Few researchers on airborne fungi reported that an increase in fungi spore concentration was seen at air temperatures between 15 and 25°C and relative humidity at 60-70% (Segvic and Pepeljnjak, 2006). Relative humidity and temperature extremities may result in decreased airborne fungi spore concentration (Levetin and Horowitz, 1978). *Aspergillus*, *Penicillium* and *Cladosporium* were

considered to be closely related to local microenvironments and urbanization (Awad, 2005).

In order to understand the significance of indoor airborne fungi and to be able to predict the occurrence of indoor airborne fungi, it is critical to indicate the relationships of indoor airborne fungi concentration to normal indoor conditions. Thus, the influence of three culture media, temperature and relative humidity on the growth of most dominant indoor airborne fungi *A. niger* and *Cl. sphaerospermum* were studied.

2. MATERIALS AND METHODS

2.1. Fungi Isolates

A. niger and *Cl. sphaerospermum* were obtained from my pervious study (Ababutain, 2011).

2.2. Environmental Studies

The effect of some cultural conditions such as nutrient media, incubation temperature and Relative Humidity (RH) on the linear growth of two dominant indoor fungi (*Aspergillus niger* and *Cladosporium sphaerospermum*), was carried out. The inoculums were in the form of disks, prepared using a sterile cork poorer (5 mm). The disks were obtained from homogenous growth of 5 days old cultures grown in Potato Dextrose Agar (PDA) medium at 28°C. All experiments were performed in triplicate.

2.3. Effect of Different Culture Media

The tested fungi were cultivated on three different media of: (PDA), Glucose Yeast extract Agar (GYA) and Czapek's agar (Cz). Petri Plates were prepared with the different media and inoculated with the tests fungi. Petri Plates were incubated in darkness at 28°C for one week. After the end of incubation period, the linear growth (cm) was determined.

2.4. Effect of Temperature

The tested fungi *Cl. sphaerospermum* and *A. niger* were incubated in PDA and GYA media respectively and incubated in darkness at 5, 10, 15, 20, 25, 30, 35, 40 and 45°C for one week. After the end of incubation period, the linear growth (cm) was determined.

2.5. Effect of Relative Humidity

Seven levels of RH were maintained by mixtures of appropriate combinations of concentrated sulphoric acid and distilled water (**Table 1**) as described by Ayyasamy and Baskaran (2005). Mixtures were taken in the desiccators for each level of RH.

Table 1. Preparation of solutions for maintenance of different Relative Humidity (RH) levels

Treatment No.	Distilled water (mL)	Sulphoric acid (mL)	RH (%)
1	100.0	0.0	100
2	88.50	11.5	95
3	80.00	20.0	90
4	70.00	30.0	75
5	62.00	38.0	65
6	56.00	44.0	50
7	49.00	51.0	35

Petri plates of PDA and GYA media were inoculated at the center with a 5mm diameter disc of fungi *Cl. sphaerospermum* and *A. niger* respectively, kept in the desiccators and covered with lids and sealed off with cellophane tape. Desiccators were incubated in darkness at 25°C for *Cl. sphaerospermum* and 30°C for *A. niger* for one week. After the end of incubation period, the linear growth (cm) was determined.

2.6. Statistical Analysis

Data were analyzed using SPSS Version 15.0 (SPSS. Inc., Standard Version).

3. RESULTS

3.1. Effect of Different Culture Media

The PDA medium was the appropriate growth medium for *Cl. sphaerospermum* with a significant difference at ($p<0.05$); where as GYA medium was the appropriate growth medium for *A. niger*, with no significant difference (**Fig. 1 and Table 2**). The colony diameter was 5.33±0.3 cm and 2.7±0.15 cm for *A. niger* and *Cl. sphaerospermum* respectively.

3.2. Effect of Temperature

The growth of *A. niger* and *Cl. sphaerospermum* on different temperatures is shown in **Fig. 2 and Table 3**. The temperatures 25 and 30°C favored colony diameter growth for *Cl. sphaerospermum* and *A. niger*, respectively so they considered as the optimum growth temperature. The temperatures range for *Cl. sphaerospermum* was narrow from 15-30°C whereas the temperature range for *A. niger* was wide from 10-40°C. At the temperature of 10°C, *A. niger* was unable to form spores and only the mycelium growth has appeared.

3.3. Effect of Relative Humidity

The growth of *A. niger* and *Cl. sphaerospermum* on different RH is shown in **Fig. 3 and Table 4**.

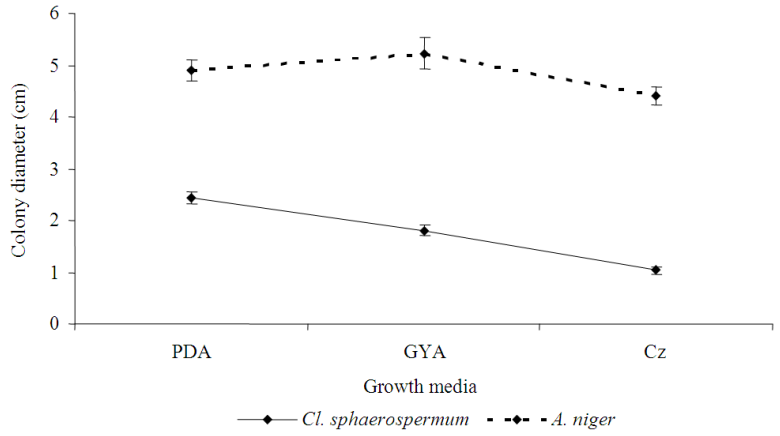

Fig. 1. Effect of different growth media on the linear growth (cm) ± SD of tested fungi

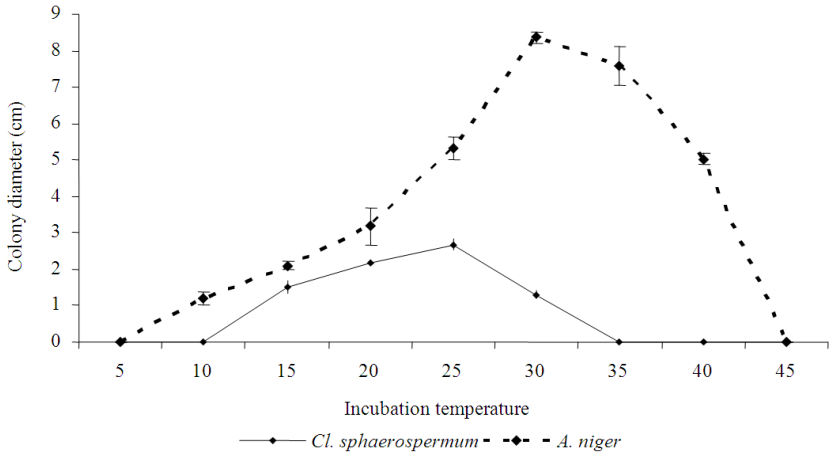

Fig. 2. Effect of incubation temperature on the linear growth (cm) ± SD of tested fungi

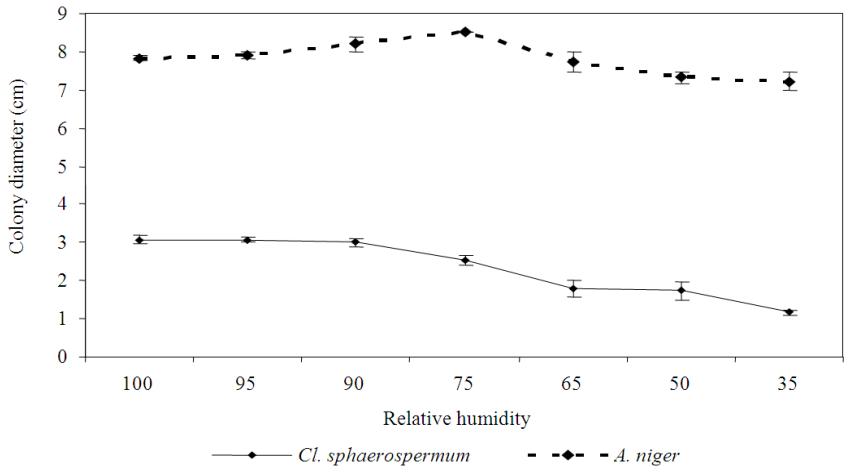

Fig. 3. Effect of Relative Humidity on the linear growth (cm) ± SD of tested fungi

Table 2. Effect of different growth media on the linear growth (cm) ± SD of tested fungi

	Growth media			LSD
Fungi	PDA	GYA	Cz	0.50%
A. *nigar*	4.90±0.20	5.33±0.30	4.4±0.17	0.551
Cl. sphaerospermum	2.43±0.12	1.80±1.00	1.03±0.06	0.471

Table 3. Effect of incubation temperature on the linear growth (cm) ± SD of tested fungi

Temperature	*A. niger*	*Cl. sphaerospermum*
5	0.000000	0.000000
10	1.20±0.17	0.000000
15	2.10±0.10	1.50±0.17
20	3.17±0.49	2.17±0.05
25	5.33±0.30	2.67±0.15
30	8.36±0.15	1.30±0.10
35	7.80±0.53	0.0000000
40	5.03±0.15	0.0000000
45	0.0000000	0.0000000
LSD 0.5%	0.0280000	0.0040000

Table 4. Effect of Relative Humidity on the linear growth (cm) ± SD of tested fungi

Relative humidity	A. *nigar*	*Cl. sphaerospermum*
100	7.83±0.06	3.07±0.12
95	7.90±0.10	3.01±0.06
90	8.20±0.20	3.00±0.10
75	8.50±0.00	2.53±0.12
65	7.73±0.25	1.80±0.21
50	7.33±0.15	1.73±0.25
35	7.23±0.25	1.17±0.06
LSD 0.5%	0.0590000	0.1880000

4. DISCUSSION

The present study revealed that the PDA medium is a general medium for growth due to its nutritional value, which is in agreement with (Trindade, 1994). A similar result was obtained by (Palacios-Cabrera *et al*., 2005) they found that the *A. niger* was able to grow in all culture media, Czapeck Yeast extract Agar (GYA), Dichloran 18% Glycerol Agar and Malt Yeast extract 40% Glucose Agar (MY40G).

The result showed that temperature was clearly the most important growth factor. Upon the present results, *Cl. sphaerospermum* considered as Mesophiles whereas *A. niger* considered as Thermophiles. Many researchers support our results; Subbarao *et al*. (1993); Alwakeel (2008) and Nawar (2008) found that the optimum growth temperature for the *A. niger* was 30°C. Present result do not support the statement of Palacios-Cabrera *et al*. (2005) who showed that *A. niger* prefers to grow at temperatures higher than 30°C. In addition, Al-Garni *et al*. (2007) found that 25°C was the optimum linear growth for *A. niger*. Pitt and Hocking (1997) found the temperature of 8°C was inhibitory for *A. niger*. In addition, Al-Garni *et al*. (2007) were found that *A. niger* failed to grow at 10°C and 55°C. Whereas, Nawar (2008) found that *A. niger* was unable to grow at temperature of 15°C which differs from the result obtained in the present study.

Results of the present study partly agree with the report of Tasic and Miladinovic-Tasic (2007) they noticed that great number of *Cladosporium* spp., does not grow at the temperature above 35°C and does not multiply at the temperature 25°C.

The present study clarified that the two fungi were able to grow at a wide rang of RH. In general, the growth of *Cl. sphaerospermum* increased to reach its maximum at 100% RH, thus *Cl. sphaerospermum* can be used as indicator fungi for the high humidity level in residences. On the other hand, *A. niger* prefers to grow at lower RH to reach its maximum at 75% RH compared to *Cl. sphaerospermum*. Growth of *A. niger* and *Cl. sphaerospermum* decreased by low RH up to 65 and 75%, respectively. In addition, Al-Garni *et al*. (2007) and Nawar (2008) found that the growth of *A. niger* increased regularly with increasing RH up to 100%. The result has proved that keeping the humidity low enough can prevent fungi growth. The present results support the findings of the Ryan (2002) and Ren *et al*. (2001) who both found that there is a significant association between dampness and airborne fungi concentration, confirming that houses experiencing dampness have a greater concentration of airborne fungi than dry houses.

Overall, the differences in the optimum growth temperature and RH level may be attributed to the difference in strains isolated from different regions around the world. My previous study (Ababutain, 2011) revealed that the temperature and RH level were more effective factors than most weather condition. The results of the present study confirmed this finding.

5. CONCLUSION

The importance of studying ecological factors appears through their direct impact on the airborne fungi concentration. Therefore, by controlling these factors airborne fungi concentration will decrease.

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