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Airborne and Dustborne Fungi in the Atmospheric Air of El-Beida City, Libya.

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Abstract: *The aim of this study was assess the level of contamination in different samples from indoor, outdoor and dust fungal aerosols in domestic homes in El-Beida, Libya. Air samples and dust samples were examined by used Open Petri Dish and Dilution methods. Colonies grown on Potato dextrose agar (PDA) and Malt extract agar (MEA) were counted and identified. Cladosporium cladosporioides, Penicillium digitatum and Pencillium chrysogenum were the dominant genus in indoor, outdoor and dust samples respectively. Other fungi such as Rhizopus nigicans, Alternaria alternata, Fusarium solani and Trichothecium roseum were also isolated from all samples. The influence of culture media on the isolated colonies was not significant when the total number of isolated colonies were considered on a monthly basis, but in reviewing a few of the fungal genera there were marked differences between the two media, especially with Curvularia sp. The Atmospheric conditions are factors that affect the development of fungi. From the results obtained, there is need for proper attention to the quality of the indoor, outdoor and dust environments.*

Keywords: *Fungi, Indoor, Outdoor, dust, Environment, Libya*.

1. INTRODUCTION

There are multi-thousands of recognized species of fungi. They are found in soil, in water, on animals, on vegetation, in humans, and in almost every part of the environment. Anemophilous fungi are spread by the atmospheric air. Fungal spores are always present in the air, with rain and snow washing down most if not all spores from the air, and sunshine and wind cause an increase in the atmospheric distribution of such spores [1]. Humans requires clean air in their dwellings especially in indoor environments where about 90% of their time is spent working or resting [2,3]. Indoor environments are potential sources of fungal spores, mycelia and harmful organic compounds such as mycotoxins which can be harmful to human health [4, 5]. Fungal spores gradually settle out and the process of settling out and becoming airborne may be repeated for long periods of time, because the fungal spores can survive for months in suitable conditions. Low humidity, physical activity and the wind speed inside the buildings are effective in the release and distribution of spores [7]. According to Chandeganipour *et al*. [8], all atmospheric air, whether indoor or outdoor, contains certain varieties and some fungal spores. Previous studies have shown that airborne fungal spores are very important sensitizing agents in allergic respiratory diseases such as asthma and rhino conjunctivitis [9, 10]. Unfortunately; there are strong indications that in many parts of the world, our homes, schools and work places are heavily contaminated with airborne moulds and other biological contaminants [11, 12]. This study is aimed at determining the prevalence of airborne fungi in the indoor, outdoor and dust environments of El-Beida city, Libya.

2. MATERIALS AND METHODS

2.1.Medium Culture

Two media, Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), were used for collecting samples to observe any possible effect of media on the collection of the fungal spores. 40 U of streptomycin per ml were added to each of the media to suppress bacterial growth.

2.2. Atmospheric Conditions

During sampling the mean maximum and minimum temperatures, relative humidity, rainfall and wind speed for each month of sampling were made and variability of these environmental factors was observed during all year days.

2.3. Air Sample Collection

Air samples were taken inside and outside of domestic homes the research team travelled to the homes to collect samples during four seasons through the year, from the spring of 2013 until the winter of 2014. Samples were taken in the first day in all month. We used the same hour of the day (9:00 a.m. to 12:00 noon). At the same time, samples from the immediate outdoor environment of the home (window, balcony or terrace) were collected using the same method as for indoors. The sampling period was 15-min. After sampling, they were covered, labelled and transported to the laboratory. For each sample, the number of homes 15 and the number of exposed plates was 3 indoors and 3 outdoors in one home.

2.4. Dust Sample Collection

In this technique, the dust collecting in watch glass (60 mm) after 10 days. These samples were supplied in sealed. Three subsamples of approximately 50 mg (the actual mass was recorded) were added individually to 10 ml of sterile distilled water and suspended to made three dilution. Three Petri plates were set up in this manner for each of the three stock suspensions. The medium was mixed with the dilution aliquot by gently swirling the Petri plates prior to solidification.

2.5. Laboratory Studies

The exposed plates were incubated initially for 3 days at 25ºC, the colonies were counted, and the plates were left for further incubation, with daily colony counts made for up to 7 days. This was found necessary from preliminary studies, which indicated a need for continued incubation up to 14 days at 25ºC for the development of slow-growing organisms.

2.6. Identification

Starting at 72 hours and during the following 7 days the plates were observed and the isolated colonies were counted and identified. Most of the fungal colonies were identified by genera only, but some were fully identified by species. Identification was based on the macroscopic and microscopic morphology of the colonies according to the criteria published in the specialized literature on the [13, 14, 15, 16, 17, 18, 19, 20, 21]. Also research articles and other related literature fungal Identification and illustrations were made up to the Genera and Species level.

3. RESULTS

A total of 11,525 colonies were isolated from a total of 270 Petri dishes/month. A total twenty one species belonging to sixteen genera were isolated from indoor, outdoor and dust samples. All isolated fungi were grouped in different classes depending to taxonomy (Table 1). Ascomycetes was the most dominant fungi followed by Deuteromycetes. Zygomycetes represented in two genus and Bazediomycetes in one genus alone. Non sporulating strains were grouped in *Mycelia sterilia*.

Table 1. *Different classes of fungi isolated from airborne and dustborne samples*

Fungal genera and species	Class	Fungal genera and species	Class
Alternaria alternata	Deuteromycetes	Penicillum chrysogenum	Ascomycetes
Aspergillus flavus	Ascomycetes	Penicillum digitatum	Ascomycetes
Aspergillus fumigatus	Ascomycetes	Penicillum sp	Ascomycetes
Aspergillus niger	Ascomycetes	Phoma sp	Ascomycetes
Aspergillus terrus	Ascomycetes	Rhizopus nigricans	Zygomycetes
Cheatomium murorum	Ascomycetes	Rhizoctonia solani	Bazediomycetes
Cladosporium cladosporioides	Deuteromycetes	Trichoderma harzianum	Deuteromycetes
Curvularia sp	Deuteromycetes	Trichothecium roseum	Deuteromycetes
Fusarium oxysporum	Deuteromycetes	Ulocladium botrytis	Deuteromycetes
Fusarium solani	Deuteromycetes	Mycelia sterilia	Non
Mucor sp	Zygomycetes		

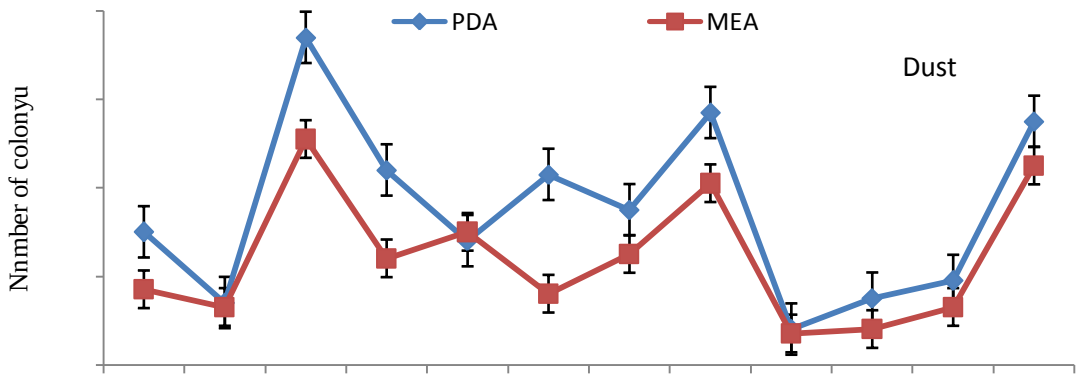
The genera of isolated airborne and dusborne fungi depending on frequency in number of colony counts were classified as predominant and less frequent isolates. The dominant species were members of the genera *Penicillium* (54.4%), *Cladosporium* (37.5%), *Rhizopus* (29.8%) *Alternaria* (28.7%), *Fusarium solani* (25.4%) and *Trichothecium*. The less prevalent was *Rhizoctonia* (0.08%) (Table 2).

Table 2. *Frequency of isolates fungi from indoor, outdoor and dust samples.*

Fungal genera and species	Indoor	Outdoor	Dust	Total frequency
Alternaria alternata	9.7	15.2	3.8	28.7
Aspergillus flavus	0.31	2.8	-	3.11
Aspergillus fumigatus	0.74	-	-	0.74
Aspergillus niger	4.71	1.0	3.6	9.31
Aspergillus terrus	0.44	-	-	0.44
Cheatomium murorum	0.48	0.17	-	0.65
Cladosporium cladosporioides	16.5	20.6	0.4	37.5
Curvularia sp	10.1	0.71	-	10.8
Fusarium oxysporum	0.07	0.71	1.0	1.78
Fusarium solani	5.63	9.1	10.7	25.4
Mucor sp	1.00	0.86	0.28	2.14
Mycelia sterilia	0.46	0.1	-	0.56
Penicillum chrysogenum	13.4	11.9	29.1	54.4
Penicillum digitatum	11.5	22.9	20.2	54.6
Penicillum sp	6.22	-	3.1	9.32
Phoma sp	1.00	2.5	-	3.5
Rhizopus nigricans	8.7	4.7	16.4	29.8
Rhizoctonia solani	-	0.08	-	0.08
Trichoderma harzianum	0.15	0.1	0.27	0.52
Trichothecium roseum	6.46	4.14	9.9	20.5
Ulocladium botrytis	2.45	4.5	1.25	8.2

Regarding density of fungi we noticed indoor samples had the maximum number of isolates fungi (20) genera followed by outdoor samples (18) genera. While the lowest number of isolates fungi (13 genera) was observed in dust samples.

The numbers of colonies growing from plates exposed and plates diluted of airborne and dustborne during each month of sampling are indicated in Fig. 1 the variations of the two media, PDA and MEA, are indicated. The graphs in Fig. 1 show values including those of April, 2013 till March 2014; there appears to be no significant difference. During the entire year of 2013 - 2014, different peaks of monthly colony count were obtained on two media. The seasonal fluctuations of total fungal colony count is best described as a tri peak phenomenon, the first was in June, the second in November and the third in March.



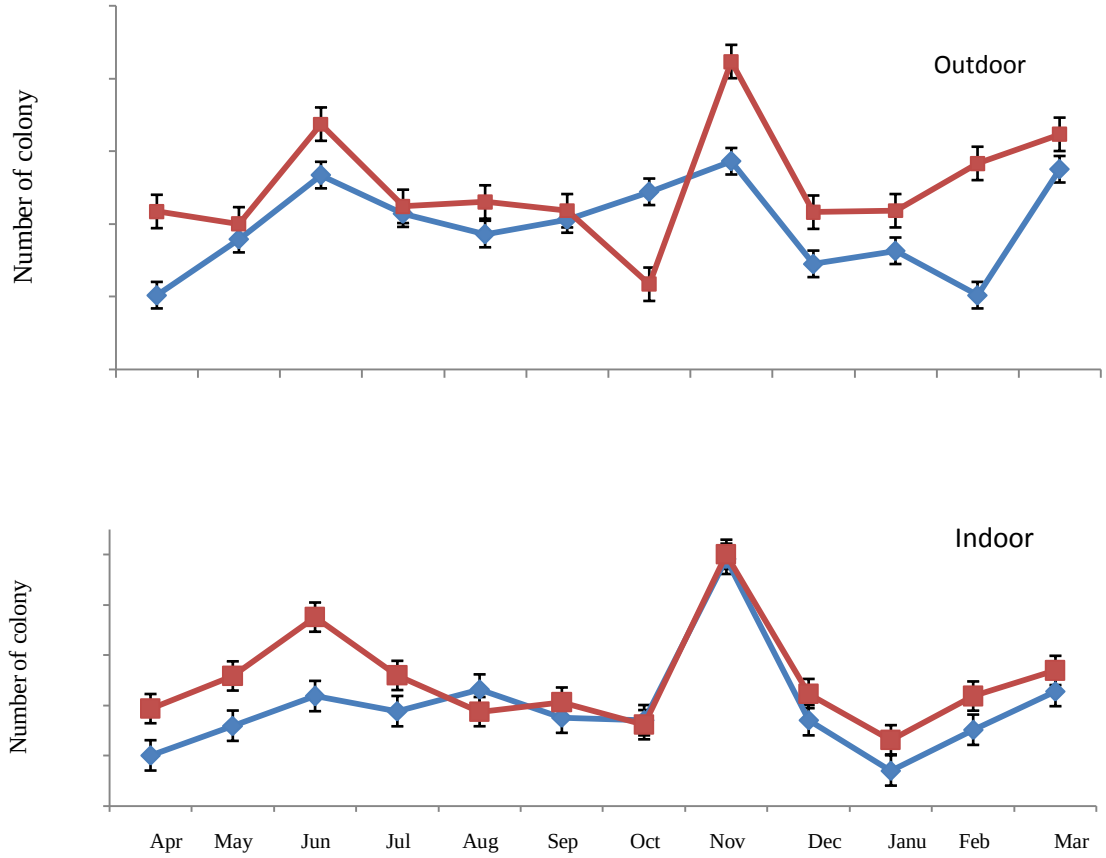
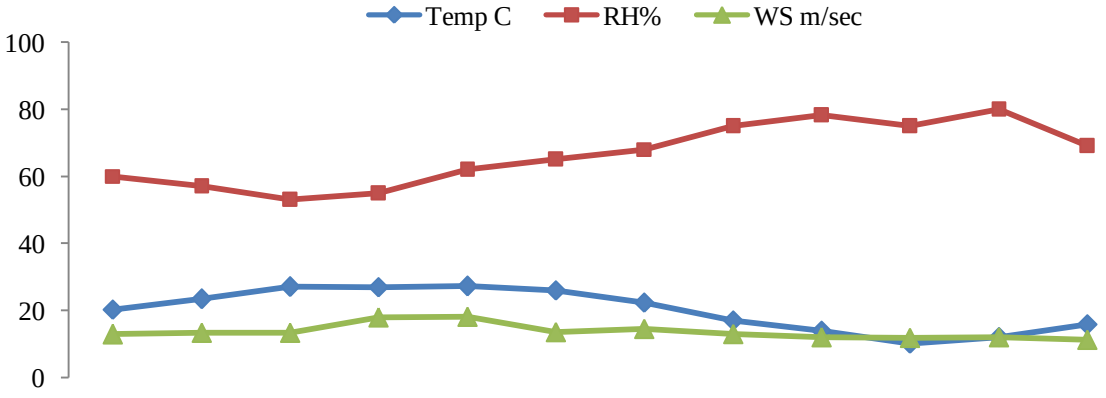


Fig. 1. *Total number of colonies isolated fungi on each of the media at all months from Indoor, Outdoor and Dust samples*

During the sampling period, the highest number of colony were recorded in November and June followed by March in indoor and outdoor, while in dust samples the highest number of colony was recorded in June followed by November and March. The minimum counts were recorded in the winter months, December, January and February, in both PDA and MEA. There were no marked differences observed with respect to the two media, PDA and MEA in the graph indicating the total number of colonies isolated at all samples (Fig. 1). However fungal group from dust samples which grew better in the MEA medium.

Since the records of the mean temperatures, relative humidifies, rainfalls and wind speed during the 12-month period are shown in Fig. 3, the effects of these factors on the air and dust spora can be evaluated. The months of May till August which were generally dry and which was affected the relative humidity for those months. The decrease total number of colonies was also obtained in the same months for the two media.



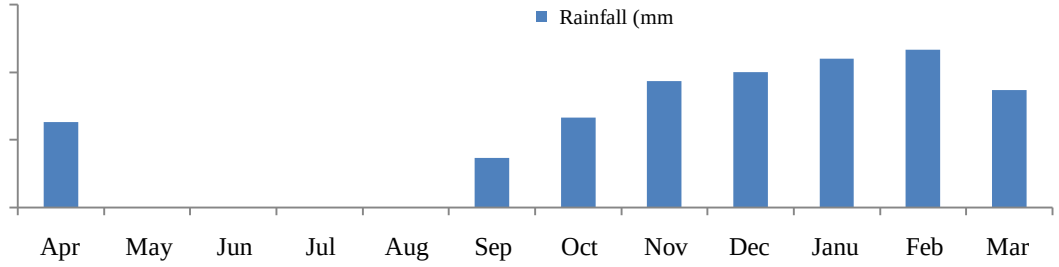


Fig.2. *Mean values of temperatures, Relative humidity, Wind speed and rainfall at El-Beida during each month of sampling.*

In spite of There were no marked differences observed with respect to the two media, PDA and MEA in the graph indicating the total number of colonies isolated at all samples, However there are marked differences shown by a few of fungal genera (Fig. 3), Figure 3 shows the fungi genera found. Twenty one known fungal species were isolated. In indoor studied the dominant fungi was *C. cladosporioides* (575, 322 isolates) on PDA and MEA media respectively. The second most common fungal species found was *Curvularia* sp., which grew better in the MEA medium and it recorded 531 isolates compared with 19 isolates on PDA medium.

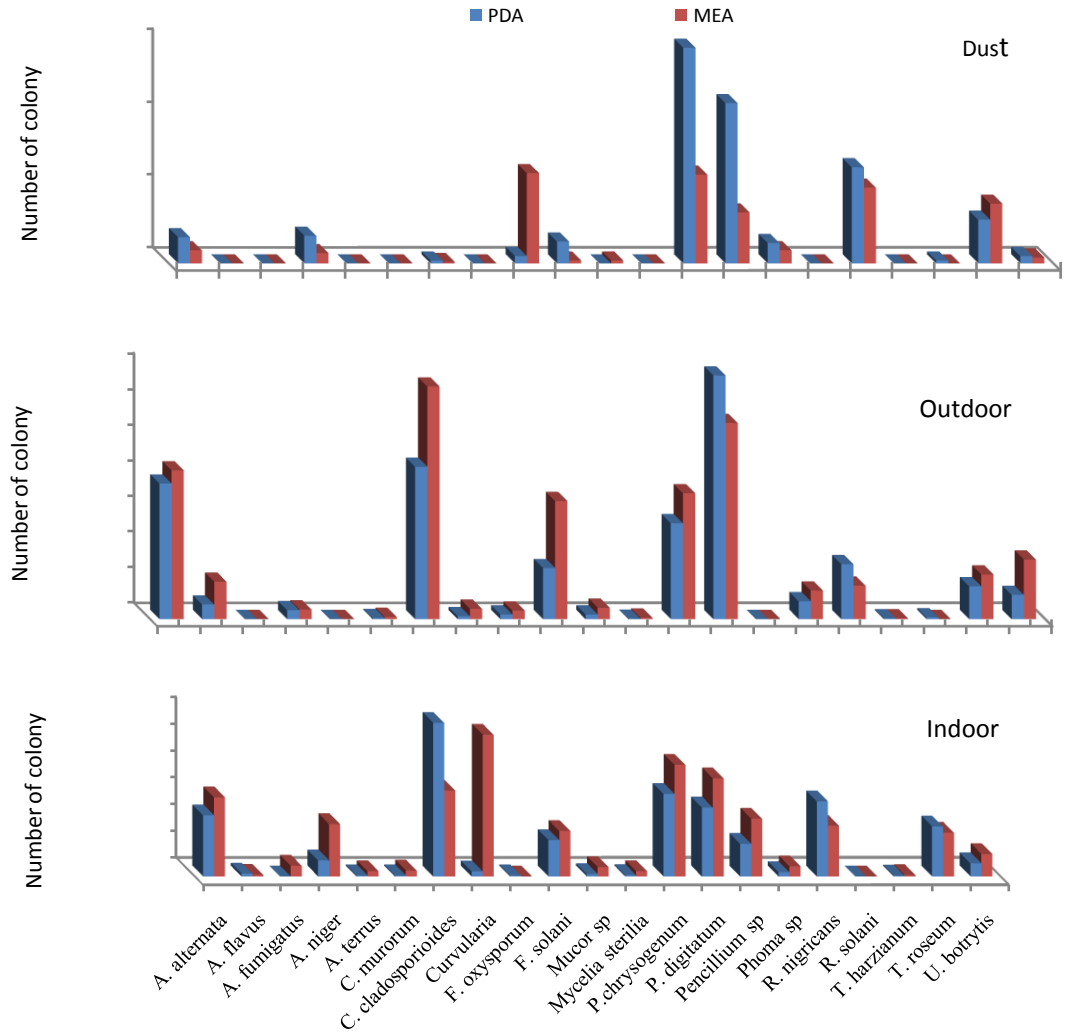


Fig. 3. *Variation in the number of colonies of the prevalent fungal genera isolated on each of the media at each indoor, outdoor and dust samples*

Highest number of isolates was accounted for *P. digitatum* (683, 550 isolates) on PDA and MEA media respectively in outdoor studied followed by *C. cladospoiroides* (417 and 652 isolates) and *A. alternata* (380, 417 isolates) on both media respectively. *P. chrysogenum* isolates were greater in counts at PAD than MEA during dust studied. It recorded (148 and 61 isolates, respectively). Nevertheless, the number of isolates of the rest of fungal species. Generally, the indoor air samples collected in this study rendered highest total number of fungal isolates (5431) at each of the medium compared with (5375 isolates) and (719 isolates) in outdoor and dust studies

4. DISCUSSION

This study is unique because it described culturable fungi obtained from indoor air, outdoor air and dust of homes as little or no study have, been carried out on this in this part of the world. *Cladosporium cladospoiroides* (16.5%), *Penicillium digitatum*, (22.9%) and *Penicillium chrysogenum* (29.1%) were the dominant indoor, outdoor and dust samples in this study. Other fungal isolates include *Rhizopus nigicans* (29.8%), *Alternaria alternata* (28.7%), *Fusarium* solani (25.4%), *Trichothecium roseum* (20.5%) and *Curvulara* sp (10.8%). These results are comparable to those from previous study by Shelton *et al*.[22], Ayanbimpe *et al*.[2], Chadeganipour *et al*. [8] and Uzochukwu and Nkpouto [23] who also isolated similar fungi in indoor and outdoor environments. Studies carried out in North, South and Central America has shown that *Cladosporium* sp, *Penicillium* sp, *Aspergillus* sp and *Alternaria* were the prevalent indoor and outdoor airborne fungi [22,23,24]

Far from considering correlations with a certain climatic parameter, the climatic year of the El-Beida area can be divided into two periods which can be repeatedly overlapped: the first is the rainfall temperate period including October to March and the second is the dry high temperature period including May to September (Fig.2). Moreover, the vegetation and mainly the annuals are flourishing between January and March. Therefore, the first peak of spore colony count and species diversity was in June (Fig.1). The sudden rise of fungal count in June due to the country exposure to dust storms in summer 2013 which increasing the contamination by dust borne fungi. This result was harmony with the results was obtained by Fareid [25] and Hjelmros [26].

In July - Septemper the low spore records are associated with the large amounts of drying vegetation in the area due to the high temperatures and the absence of rain, as these are unfavourable conditions for the growth of fungus.

The second peak was in November period (Fig.1). It may be due to an increase in the availability of substrate owing to the seasonal processes of decomposition of plant matter [27]. This peak is also due to the conditions following the rainfall encountered in October and also to the relatively moderate temperature and humid climate. Even though rainfall was too low but still enough to moisten the soil and to cause perennial shrubs and bushes to thrive, and in consequence fungal growth to flourish after the extended dry hot conditions from May to September. Usually rainy months have maximum frequency and concentration of fungal spores due to the favourable growth and sporulation conditions for fungi and the availability of suitable substrates [28].

The increase in March was also recorded. It may be due to favourable temperature and humidity conditions. A significant increase in spore concentration after precipitation was observed by Kramer et al. [29]. This is easily explained as it comes after the 3 months of the highest rainfall and moderate temperature, December till February, so the soil is moistened enough and the vegetation is in its maximum growth and diversity. Basically, soil and vegetation are considered the main sources contributing to airborne fungal spores [30,31,32,33]. In winter there is a decrease in spores due to the low temperatures, which have a negative effect on the content of these particles in the air [34,35], and a high relative humidity which produces an absorption of water by the spores, making them heavier and less transportable by air [36].

5. CONCLUSION

In conclusion, *Cladosporium, Penicillium, Alternaria, Fusarium Curvularia,* and *Trichothecium,* frequently isolated from chronic rhinosinusitis patients [37], are considered to be the most allergenic fungi in environmental air [6]. the present study suggests that the city of El-Beida, as in any of the mountain dwellings in the region, harbors various species of fungi due to its warm and rainy climate and very rich flora. It is of significance that our findings may be of use with regard

to the diagnosis and prophylaxis of allergic diseases thought to be resulting from airborne fungi, and this should be born in mind when using allergic tests the spectrum of the fungal genera examined in this region.

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