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ORIGINAL PAPER

Atmospheric concentrations and intradiurnal pattern of *Alternaria* and *Cladosporium* conidia in Tétouan (NW of Morocco)

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Abstract Fungal spores of *Alternaria* and *Cladosporium* are ubiquitous components of both indoor and outdoor air samples and are the main causes of human respiratory allergies. Monitoring these airborne fungal spores during 2009–2014 was carried out by means of Hirst-type spore trap to investigate their airborne spore concentrations with respect to annual load, seasonality and overall intradiurnal pattern. *Alternaria* and *Cladosporium* spores are present throughout the year in the atmosphere of Tétouan, although they show seasonal variations. Despite important differences between years, their highest levels presented a first peak during spring and a higher second peak in summer or autumn depending on the year. The spore concentrations were homogeneously distributed throughout the day with slight increase of 7.6 and 3.7% on average between 12–14 and 14–16 h for *Alternaria* and *Cladosporium*, respectively. The borderline of 3000 sp/m^3 of *Cladosporium* linked to the occurrence of allergic diseases was exceeded between 13 and 31 days. Airborne spores of *Alternaria* overcame the threshold value of 100 sp/m^3 up to 95 days, suggesting that *Cladosporium* and *Alternaria* could be clinically significant aeroallergens for atopic patients.

Keywords Aerobiology · Fungal spores · *Alternaria* · *Cladosporium* · Intradiurnal variation · Tétouan

1 Introduction

Fungal spores are ubiquitous in the indoor and outdoor environment and the most diverse and numerous airborne particles with 100–1000 times higher than those of atmospheric pollen (Adhikari et al. 2004; Jones and Harrison 2004; Zahlane et al. 2008). *Alternaria* and *Cladosporium* conidia appeared the most frequently genera in most aerobiological studies as these genera comprise many species and tolerate a wide range of environmental conditions (Domsch et al. 1993; Filali Ben Sidel et al. 2015).

Species of *Alternaria* are pathogenic to a number of crop plants and may develop on a variety of organic substrate. Their large multicellular conidia with distinct beak are potentially allergenic and cause various respiratory problems (Hasnain et al. 2004; Jacob et al. 2002; Stark et al. 2005). *Cladosporium* is characterized by a huge production of small conidia which can be dispersed or transported by large air masses, and

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there have been some reports of *Cladosporium* species infections in immune-comprised individuals (Levetin 2004). A range of allergens between 16 and 88 kDa was identified in *Cladosporium cladosporioides* extracts by electroblotting immunochemical technique using sera from *Cladosporium* allergic patients (Bouziane et al. 2006). Recently in Tétouan, 5% of patients consulting for respiratory allergy were sensitized to *Cladosporium* extracts (Bardei et al. 2016).

Recent aerobiological study was carried out in the city of Tétouan to analyze the seasonal variation of fungal spores that reach some years 600,000 spores (Bardei et al. 2013). The majority of aerobiological investigations has focused on seasonal spores' variation in a particular area (Adhikari et al. 2004; Aira et al. 2008; Oliveira et al. 2010; Gargouri et al. 2010; Recio et al. 2012), but studies on intradiurnal variation are becoming more interesting and some papers have shown that their concentrations vary considerably between 3 and 30% over a 24-h period (Peternel et al. 2004; Rodriguez-Rajo et al. 2005; Thibaudon and Lachasse 2006). As *Cladosporium* spores were also detected in rainy days (Bardei et al. 2013), it should be interesting to include these days in intradiurnal studies.

This study was conducted to investigate a 6-year record of daily and bihourly *Alternaria* and *Cladosporium* spores concentration analyses from Tétouan with respect to overall annual, seasonal and intradiurnal pattern.

2 Materials and methods

2.1 Study area

The province of Tétouan is located in the northwest of Morocco in the Martil river valley, over an area of 2574 km^2 covering 0.36% of the total area of the country. In bioclimatic terms, it belongs to the thermomediterranaen stage of the vegetation and Mediterranean climate characterized by the existence of two different seasons: the first humid and fresh, from October to April, and the second, drier and hot, begins in May and lasts until the end of September (El Mrini et al. 2008). The city is located between two mountains ranges; therefore, the prevailing wind is those blowing from NE (known locally as "Chergui") to SW (locally known as "Gharbi").

Along the river Martil where it is located the Faculty of science of Tétouan and the spore trap, the riparian vegetation is formed by *Populus* spp, *Salix* spp and *Tamarix africana* Poir. The Martil river valley is occupied by grass land, crop land (Cereals, Corn, Solanaceae, Brassicaceae and Apiaceae) and herbaceous plants such as Urticaceae (*Urtica membranacea* Poir. and *Parietaria* spp), *Plantago* spp, *Rumex* spp, Euphorbiaceae (*Mercurialis annua* L., *Ricinus communis* L.) and Amaranthaceae. More far and at lower altitudes, the mixed forests include oak trees, pine, but *Tetraclinis articulata* (Vahl) Mast. is the dominant species at low lands. These forests are associated with the typical Mediterranean matorral formation composed by many shrubs' species: *Cistus* spp, *Erica* spp, *Calluna vulgaris* (L.) Hull, *Arbutus unedo* L., *Pistacia lentiscus* L., *Viburnum tinus* L., *Calycotome villosa* (Poir.) LinK, *Chamaerops humilis* L. and *Quercus coccifera* L. *Olea europaea* L. is the most widespread cultivated tree. *Cupressus sempervirens* L. and *Platanus spp* are used as ornamental urban and peri-urban plantations (El Haskouri et al. 2016).

2.2 Aerobiological sampling

Atmospheric fungal spores were sampled over 6 consecutive years (2009–2014) using a Hirst-type volumetric spore trap (1952) located 15 m above ground level on the flat roof of the Biology Department of the Faculty of Sciences. The Spanish Aerobiological network (REA) manual was used for sampling and spore observation (Galán et al. 2007). Bihourly spores' counts were carried out using two longitudinal traverse methods. The daily mean concentrations were expressed as the number of spores per cubic meter of air.

Main spore season (MSS) was determined by the 90% method of Nilsson and Persson (1981) assuming the beginning and the end of MSS as 5 and 95% of the annual spore accumulated sums. Since the number of allergenic spores determines whether or not a risk for atopic individuals exists, the concentrations of 100 sp/m^3 for *Alternaria* and 3000 sp/m^3 for *Cladosporium* were set as allergenicity threshold values according to Peternel et al. (2004) and Rodriguez-Rajo et al. (2005).

2.3 Intradiurnal variation

To know the most concentrated times in terms of intradiurnal fungal spores' emissions, firstly only rain-free days when the mean daily spore concentrations

equaled or exceeded the mean concentration for the MSS were used to establish the intradiurnal variation of *Alternaria* and *Cladosporium* (Galán et al. 1991). Secondly and since rainfall has not shown negative correlations with *Cladosporium* spores (Bardei et al. 2013), rainy days were also included with rain-fee days in the case of *Cladosporium*. The values are expressed as 2-h cumulative %.

In order to evaluate the intradiurnal variation of spores and to compare between the years of the study period, the intradiurnal distribution index (IDI) was calculated by taking into account the maximum and minimum percentage of the total 24-h spore count of the days considered by applying the formula proposed by Trigo et al. (1997). This index is equal to the difference between the maximum and minimum bihourly percentages per 100.

3 Results

For the years 2009–2014, the average mean temperature was 19 °C and mean annual rainfall was 658.7 mm. The mean annual maximum temperature varied between 17.1 and 30.2 °C, whereas in the case of minimum temperatures, the averages ranged from 9.9 to 22.1 °C. The coldest months are January, February and December. The warmest month corresponds to July or August.

3.1 Aerobiological data of *Alternaria*

The highest annual spore of *Alternaria* was recorded in 2010 with 27,688 spores. On the contrary, the lowest level was registered in 2009 (Table 1). On seasonal basis, concentrations were low in winter and start to increase in early spring. *Alternaria* showed a first peak from April to June depending on the year. The highest *Alternaria* concentrations were observed in August for 2010 and 2011, in September for 2013 or in October for 2009, 2012 and 2014 (Fig. 1). Taking into account the data from yearly records for the whole period, the monthly concentrations varied between 428 and 3155 spores.

The duration of MSS of *Alternaria* varied between years with an average of 242 days. Its mean daily concentration oscillated between 39 and 80 sp/m^3. Start dates of the MSS of *Alternaria* were detected usually during March; however, the beginning of MSS was earlier in 2014 and occurred on the third decade of February and registered later in 2010 on April. End dates of the MSS occurred during November with a maximal difference of 18 days between 2010 and 2013. The intensity and timing of the maximum daily concentration varied each year and reached the highest value of 767 sp/m^3 on 26 April 2010. In the present study, *Alternaria* spores exceeded the allergenicity threshold between 22 and 95 days in 2009 and 2010, respectively (Table 1).

3.2 Intradiurnal variation of *Alternaria*

The intradiurnal variation and its mean showed a gradual increase in spores since the morning reaching a peak in midday toward 12–14 h. In the evening and night, lower percentages occurred and lowest values were recorded generally around 4–6 h. However,

Table 1 Annual total and data parameters of *Alternaria* main spore season (MSS) in Tétouan, 2009–2014

Alternaria	2009	2010	2011	2012	2013	2014
MSS (90%)						
Starting dates	17 Mar.	12 Apr.	15 Mar.	7 Mar.	31 Mar.	24 Feb.
End dates	25 Nov	08 Nov	14 Nov.	12 Nov.	26 Nov.	12 Nov.
Total MSS	12,952	25,028	21,653	18,271	20,878	17,403
Duration (days)	254	208	242	245	241	262
Annual total	14,312	27,688	24,049	20,264	23,092	19,336
Daily maximum	385	767	471	399	511	405
Daily minimum	0	0	0	1	1	0
Mean	39	80	66	57	63	55
Peak date	08 Oct	26 Apr	19 May	10 Oct	25 Oct	24 May
Number of days exceeding the threshold for allergenicity	22	95	75	51	67	55

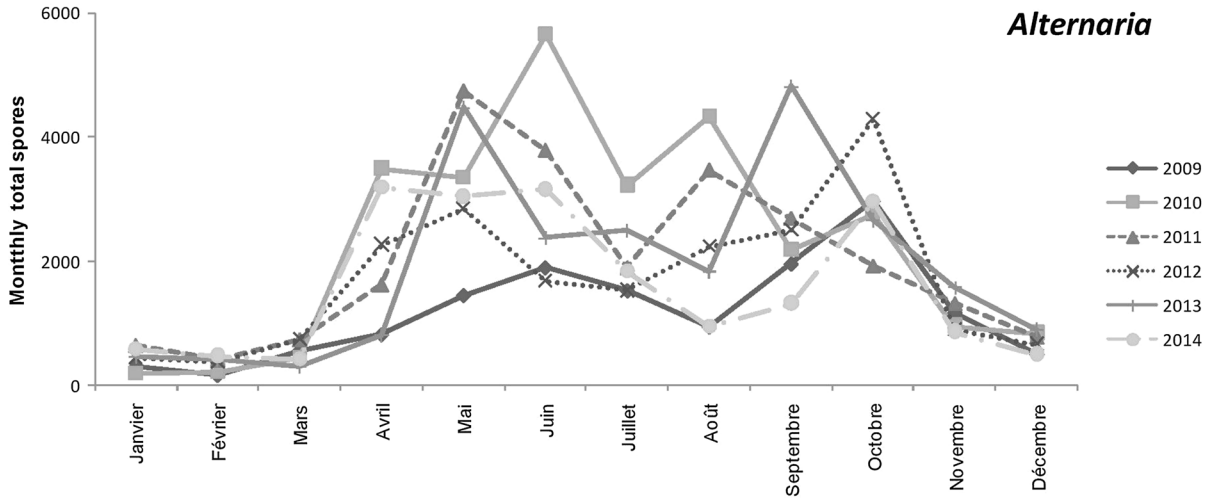

Fig. 1 Monthly variation of *Alternaria* during study period

constant peak daily concentrations were recorded between 10 and 18 h in 2012 and 2014, the 2 years showing the same curve (Fig. 2). Intradiurnal variation presented a similar pattern in all 6 years with minor variation and registered an IDI of 0.08 on average ranging from 0.06 to 0.11. This demonstrates that spores' concentrations were distributed homogeneously throughout the day (Fig. 2).

3.3 Aerobiological data of *Cladosporium*

The annual index of the spore concentrations showed the higher value in 2012 and the lowest one in 2010. The seasonal variations of daily spores' concentrations of *Cladosporium* presented two periods of maximum level, the first in spring (from April to June) and the second, more higher, in August for 2010 and 2011 or in September for 2009 and 2013 or in October for 2012 and 2014 (Fig. 3). The monthly spore concentrations ranged from 7780 to 66,765 spores as average monthly values for the whole of the study period.

The MSS of *Cladosporium* lasted 258 days on average ranging from 231 days in 2009 to 296 days in 2013. Start days of the spore season varied from 1 year to another between the third decade of January and the beginning of the second half of April. End dates

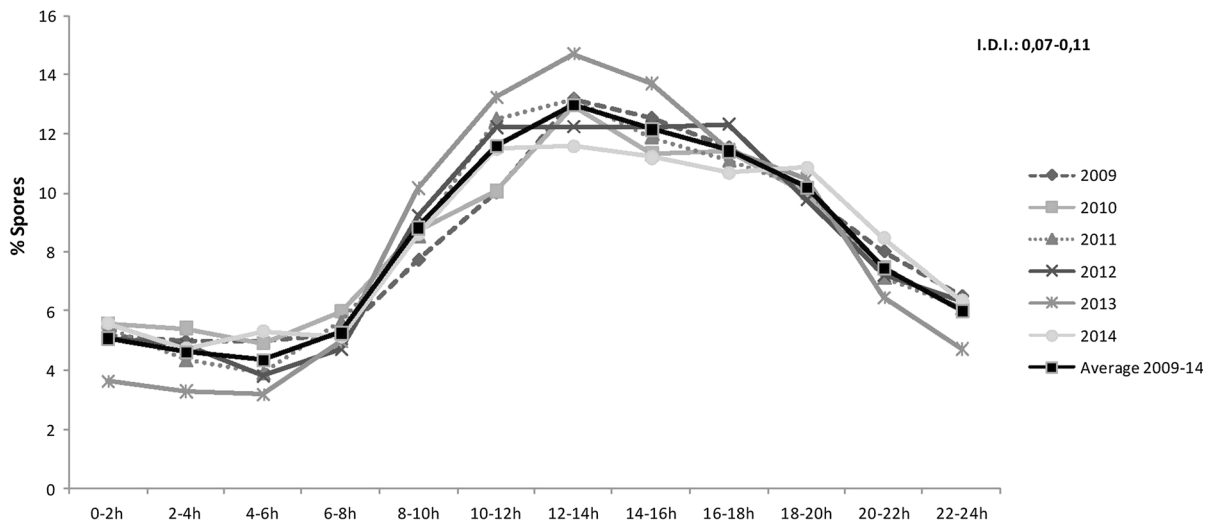

Fig. 2 Intradiurnal distribution pattern of *Alternaria* concentration 2009–2014

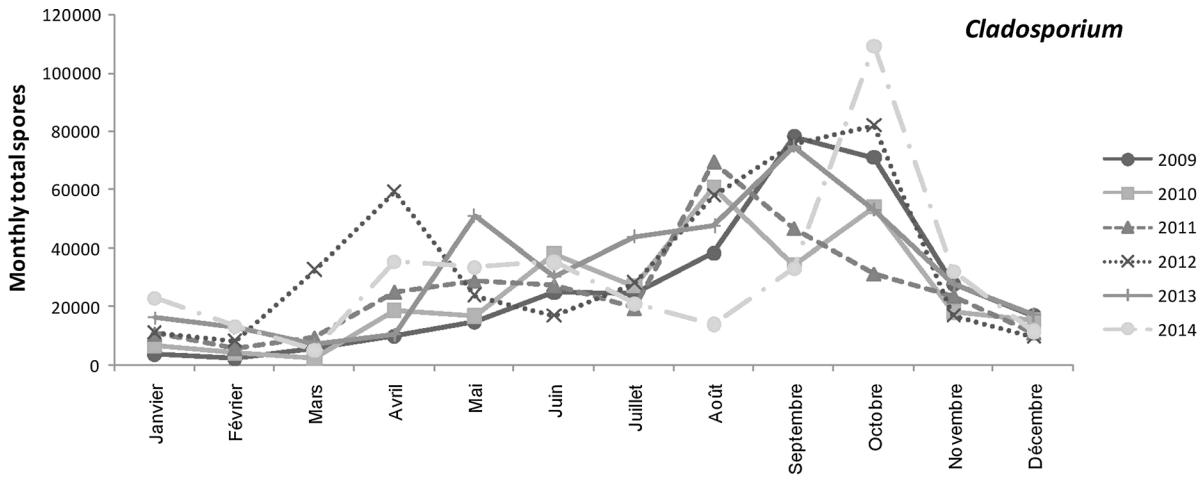

Fig. 3 Monthly variation of *Cladosporium* during study period

ranged from the beginning of November to early December. The recorded daily minimum varied considerably. The peak daily values reached the highest concentration of 13,270 sp/m^3 in 2014. The daily concentrations of *Cladosporium* have exceeded the threshold values for clinical significance between 13 and 31 days in 2011 and 2012, respectively (Table 2).

3.4 Intradiurnal variation of *Cladosporium*

The circadian variation presented very small peaks and smaller differences in the bihourly concentrations recorded (Fig. 4). Peak concentrations occurred between 10 and 20 h varying from 1 year to another, although in some years the maximum concentrations were obtained in the afternoon, progressively decreasing toward night. The graphic curve of the spores of *Cladosporium* showed that they were more evenly distributed throughout the day and the value of the IDI remains below 0.1. As regard to intraday distribution of *Cladosporium* spores during rain-free days as well as when rainy days were considered, the curves were quite concomitant (Fig. 4).

4 Discussion

Cladosporium and *Alternaria* are, respectively, the firth and the fourth most frequent spores in the atmosphere of Tétouan representing around 64 and

Table 2 Annual total and data parameters of *Cladosporium* main spore season (MSS) in Tétouan, 2009–2014

Cladosporium	2009	2010	2011	2012	2013	2014
MSS (90%)						
Starting dates	17 Apr.	9 Apr.	26 Feb.	4 Mar.	5 Feb.	25 Jan.
End dates	3 Dec.	1 Dec.	21 Nov.	4 Nov.	27 Nov.	24 Nov.
Total MSS	288,668	267,291	278,457	376,832	353,189	329,340
Duration (days)	231	234	266	240	296	283
Annual total	320,049	296,707	308,063	417,929	391,861	364,103
Daily maximum	7712	7753	6242	11276	8164	13270
Daily minimum	10	15	59	52	26	111
Mean	877	857	851	1177	1073	1058
Peak date	16 Sep.	30 Oct.	19 Aug	31 Mar.	26 Oct.	11 Oct.
Number of days exceeding the threshold for allergenicity	16	14	13	31	21	15

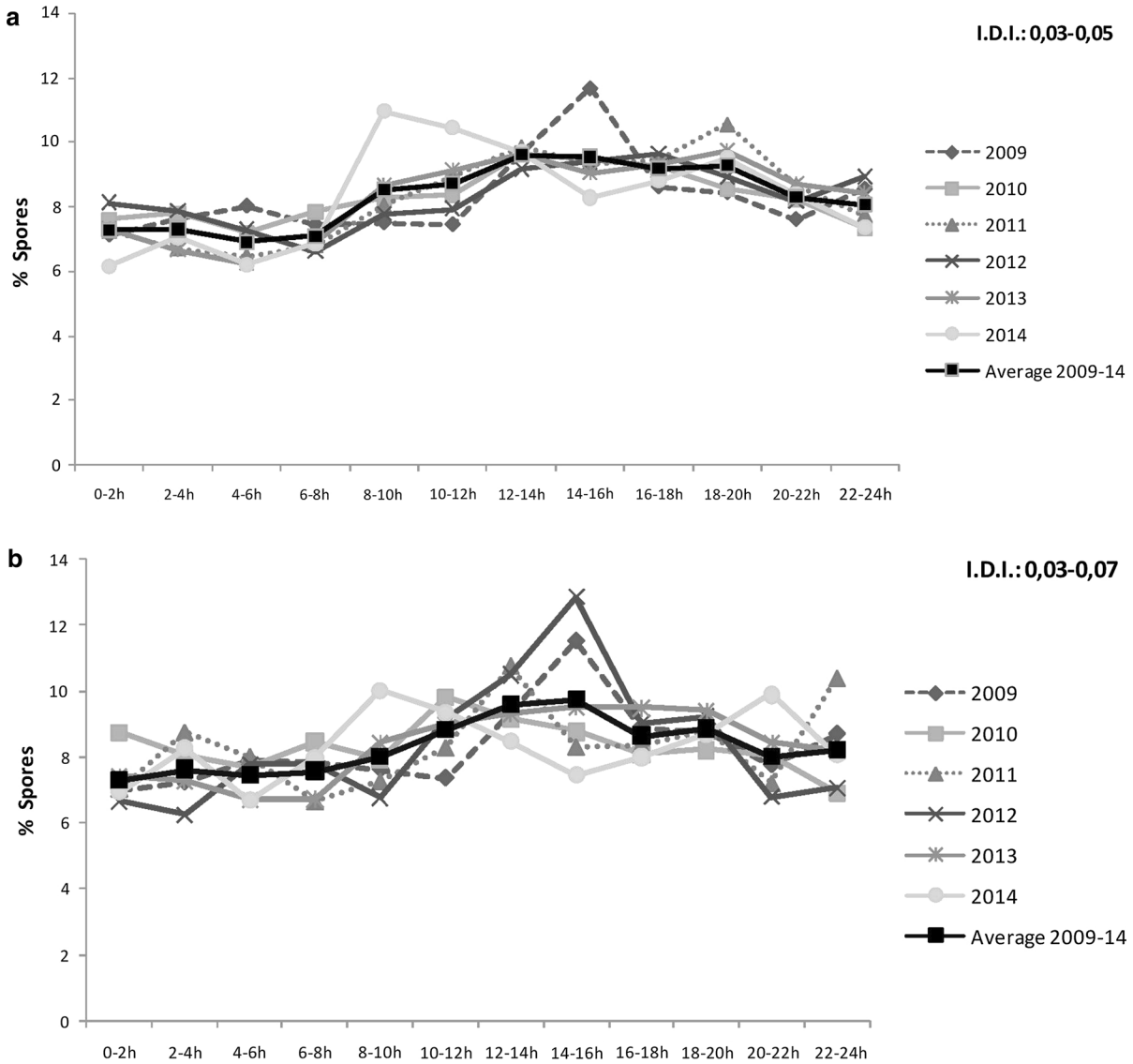

Fig. 4 **a** Intradiurnal distribution pattern of *Cladosporium* concentration 2009–2014. **b** Rainfall included

4% of the annual total (Bardei et al. 2013). *Alternaria* and *Cladosporium* are present throughout the year; the highest levels are detected in spring and also during summer or autumn. However, they show important fluctuations and concentrations may be very low in winter.

In comparison with *Alternaria*, the annual total concentrations of the spores of *Cladosporium* were between 10- and 22-fold higher for the whole of the study period. Similar to Recio et al. (2012), the annual variations were very similar for the two airborne spores, both showing higher monthly concentrations at the same months of the year with a first peak during spring and a second peak in summer or autumn. The total annual value recorded for these two genera spores in Tétouan is higher than the levels that are observed in the NW Iberian Peninsula (Rodriguez-Rajo et al. 2005; Aira et al. 2008) or in Portugal (Oliveira et al. 2010). The annual amount of *Alternaria* registered in Tétouan is generally comparable to the levels that are observed in the Catalonian cities of Spain as Bellaterra, Girona and Manresa, where the annual spore index ranged between 21,511 and 21,770 (De linares et al. 2010).

With regard to daily pattern, in general, the behavior followed by each spore type throughout the 24 h of the day was very similar in the 6 years of study with low spores' percentages during night and early morning, increasing gradually onwards to reach peaks around 12–14 h for *Alternaria* and 14–16 h for *Cladosporium*. A number of studies have shown a peak in late afternoon or late evening and minimum in the night or early morning (Ricci et al. 1995; Rodriguez-Rajo et al. 2005; Thibaudon and Lachasse 2006; Aira et al. 2008; Oliveira et al. 2009; Skjøth et al. 2012). This suggests that the overall load of these two fungal spores is due to local sources and dispersion. The intraday variation obtained in this study was homogenous and practically without variation throughout the day. In the northwest Iberian Peninsula, intradiurnal spores' percentages varied between 2 and 14% for *Cladosporium* and 2 and 7% for *Alternaria* (Rodriguez-Rajo et al. 2005; Aira et al. 2008). According to Peternel et al. (2004), the concentrations of *Alternaria* spores during the night declined to reach 200 sp/m^3 and rose to peak between 10 and 12 h. For *Cladosporium*, minimal and maximal intradiurnal spore concentrations recorded 1200 and 3600 sp/m^3, respectively (Peternel et al. 2004). The fact that the intradiurnal pattern of the spores of *Cladosporium* was quite similar when rain-free days were used as well as when rainy days were added would make possible to establish intradiurnal variation including days with rainfall, at least for spores that no correlate negatively with this parameter.

In clinical significance terms, *Cladosporium* exceeded the threshold value up to 31 days and *Alternaria* did it between 22 and 95 days. Combined with a long spore season and the almost continuous presence of the spores in the atmosphere throughout the 24 h of the day could trigger respiratory allergic reactions for patients sensitized to *Alternaria* or *Cladosporium* spores.

5 Conclusion

Alternaria and *Cladosporium* spores are present in the air of Tétouan throughout the year with important interannual differences. They present maximum values at the same period of the year with a first peak during spring and a second peak, more important in summer or autumn.

Intradiurnal concentrations of *Alternaria* and *Cladosporium* spores were homogeneously distributed over the 24 h of the day increasing slightly in midday or in the afternoon. Fungal spores of *Cladosporium* and *Alternaria* exceeded the threshold value of clinical significance for sensitized patients up to 1 and 3 months, respectively.

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