



Short Communication

Airborne fungi in vegetable growing areas of Edirne, Turkey

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Abstract

We aimed at the investigation of the airborne fungi and their outdoor incidence in five vegetable growing areas in Edirne province (Turkey) by exposing a petri dish with potato dextrose agar medium to air for 15 minutes and then counting the number of growing colonies. Sampling procedure for fungi was performed 6 times in research stations at an interval of one month between April–September 1996. From the 90 petri dishes obtained fungi were isolated and 1166 colonies were counted. 12 genera (*Absidia*, *Alternaria*, *Aspergillus*, *Botryotrichum*, *Clamydomyces*, *Cladosporium*, *Endocochlus*, *Fusarium*, *Nematochtonus*, *Penicillium*, *Trichoderma* and *Torula*) and 25 species were identified. Among them, *Aspergillus clavato-nanica* and *Penicillium estinogenum* are very likely to be new records for Turkey. *Cladosporium carpophilum* and *Alternaria alternata* were the most abundant species in the study area. Correlation analyses were applied to the data.

Introduction

Concentration of airborne microorganisms are correlated with the presence of dusts from soil and human activities. As a result of air pollution, increasing CO₂ concentrations in the atmosphere may induce sporulation of common soil fungi (Klironomos et al., 1997). In addition, concentrations of fungal spores in the air is linked to the geographical region considered and seasonal variations; also meteorological parameters such as wind, humidity, temperature, rainfall, altitude and vegetation affects numbers and types of airborne fungi (Agarwal and Shivpuri 1969; Bandyopadhyay et al., 1991; Pasanen, 1992; Di Giorgio et al., 1996). Various factors, such as the type of collection medium, the identification process, the period of the day in which the collection of fungi takes place, the sampling frequency and duration, influence the airborne fungi monitoring (Takahashi, 1997). Furthermore, viable microorganisms can become airborne due to the presence of sewage treatment plants, stock – farming, fermentation processes, agricultural and

industrial activities (Di Giorgio et al., 1996). Also, fungal spores, mycelial fragments and sclerotia can be dispersed in the air by insects (Kerssies, 1993).

The determination of airborne fungal concentration in an area is important because some of them may be aeroallergens (Agarwal and Shivpuri, 1969; Beaumont et al., 1985a, b; Rosas et al., 1993; Shivpuri and Agarwal, 1969; Chih-Shan et al., 1995; Hasnain et al., 1995).

We aimed at the determination of fungal concentration and identification in the study area because Edirne, Turkey is the centre for vegetable growing and is partially a residential district.

Materials and methods

Sampling sites

The samples were placed into five vegetable growing areas of Edirne (Figure 1), 4 km to the west of the city. Vegetables such as bean, onion, romaine lettuce, pepper, tomato, cabbage, maize and marrow

Table 1. Some features of the selected research stations and number of the samples taken

Number of sampling stations	Number of taking samples	Number of petri dish in one sampling	Number of total petri dish	Some features of the sampling station
1	6	3	18	Exposed to wind from south direction, bean growing
2	6	3	18	The banks of Arda river, exposed to wind from north direction bean growing
3	6	3	18	Not exposed to wind; bean and maize growing
4	6	3	18	Residential area, exposed to wind; bean, onion, romaine lettuce, corn, pepper, tomato and cabbage growing
5	6	3	18	Residential area, exposed to wind; bean, onion, romaine lettuce, corn, pepper, tomato and cabbage growing
Total	30	15	90	

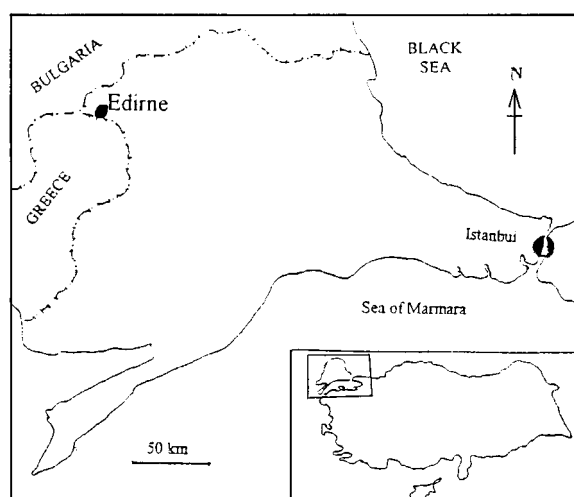


Figure 1. Thrace (European part of Turkey) map showing Edirne province.

are cultivated in the region. The natural trees found in the surroundings are *Populus x canadensis* Moench, *P. alba* L., *P. nigra* L., *Prunus domestica* L., *Salix alba* L., *Juglans regia* L., *Ulmus minör* Miller, *Robinia pseudoacacia* L., *Rosa canina* L., *Morus alba* L., *Persica vulgaris* Miller, *Malus silvestris* L., *Cerasus avium* (L.) Moench, *Acer negundo* L., *Vitis vinifera* L. and *Pyrus communis* L.

Sampling methods

The samples were taken in the afternoon (12:00–15:00) at the height of 1.5 m above the ground, at one month intervals between April and September, 1996. The Petri Plate Gravitational Method (Verhoeff et al., 1990; Savino and Caretta, 1992; Rosas et al., 1993) was employed for the isolation of fungi. Potato Dextrose Agar (PDA) adopted as collection and the medium petri dishes were exposed to the air for 15 minutes. Three petri dishes were used for each sampling.

The features of research stations and the number of samples are shown in Table 1.

The petri plates containing the samples were incubated for 3–5 days at room temperature (25 °C–27 °C). Subsequently, colonies were transferred to tubes containing Malt Extract Agar (MEA), Czapek – Dox Agar (CDA) and PDA media for identification. Furthermore, we used CDA media with 20, 40 and 60% sucrose for identification of osmophilic *Aspergillus pseudoglaucus* Blochwitz. Stereomicroscope and light microscopes were used to determine the colonial features and the morphological structures of the fungi. The determination of the morphological structures of fungi was carried out on material mounted in lactophenol.

Table 2. Monthly meteorological parameters recorded from 1986–1996 (Source: Meteorology Directorate of Edirne Province, Turkey)

Months	Average monthly temperature (°C)		Total monthly rainfall (mm)		Average monthly relative humidity (%)		Average monthly wind velocity (m/sec)	
	1986–96	1996	1986–96	1996	1986–96	1996	1986–96	1996
January	2.45	1.2	25.61	23.4	80.77	77.6	1.62	1.9
February	4.30	2.1	31.11	121.0	73.35	78.9	1.88	2.3
March	7.62	3.5	58.88	52.9	73.16	76.1	1.96	2.1
April	12.40	11.5	45.35	75.0	69.66	66.8	1.85	2.0
May	17.13	20.6	56.11	32.3	65.68	62.9	1.69	1.8
June	22.22	23.3	39.73	1.4	61.65	51.1	1.58	1.8
July	24.47	25.3	20.76	0.0	56.84	48.0	1.84	1.8
August	24.05	23.8	27.05	38.1	59.07	60.6	1.56	1.6
September	19.52	18.1	23.37	65.2	63.27	68.9	1.45	1.6
October	14.01	13.2	59.13	23	72.52	71.3	1.55	1.4
November	7.82	10.1	85.89	129.1	79.24	76.5	1.63	1.7
December	4.13	6.0	74.67	94.6	81.60	81.6	1.79	2.2
Total (for rainfall)			547.66	656				

Identification

Fungi were identified to genus level using Barnett's work (1972). 'A Manual of the *Penicillia*' (Raper and Thom, 1949) and 'Manual and atlas of the *Penicillia*' (Ramirez, 1982) were followed for the identification of *Penicillium* Link species, 'The genus *Aspergillus*' (Raper and Fennell, 1965) was used for identification of *Aspergillus* species. 'Dematiaceous Hyphomycetes' (Ellis, 1971) was also used for the identification of *Alternaria* Nees ex Fr. and *Cladosporium* Link ex Fr. species. *Fusarium* Link ex Fr. species were identified by Dr. Helgard I. Nirenberg [BBA, Institute for Plant Virology, Microbiology and Biological Safety Konigin-Luise-Straße 19 D-14195 Berlin (Dahlem) – GERMANY]. 'The Genus *Fusarium* – A Pictorial Atlas' (Gerlach and Nirenberg, 1982) and '*Fusarium* species – An Illustrated Manual for Identification' (Nelson et al., 1983) are used for identification of *Fusarium acuminatum* Ellis & Everh.

Citation of the author names presented in this paper are standardized according to the '*Authors of Fungal Names*' (Kirk and Ansell, 1992).

Meteorological measurements

Records of monthly average temperature, monthly total rainfall, monthly average relative humidity and monthly average wind speeds for ten years and 1996 year (Table 2) are obtained from *Meteorology Direct-*

orate of Edirne Province. The meteorological station was located 4 km from the sampling sites.

Statistical methods

Correlation analyses were carried out on PC using '*Bio-mathematical and Statistical Methods*' book (Cengiz, 1984).

Results

We enumerated a total of 1166 fungal colonies in 90 petri dishes during six months (April–September 1996) and identified 25 species belonging 12 genera (Table 3). *Cladosporium* generally occurred in maximum percentage (43.22%), followed by *Alternaria* (23.67%) and *Penicillium* (18.69%) (Table 3). *Cladosporium* was the dominant type in all sampling sites.

We performed correlation analysis between kinds and concentration of fungi and some meteorological parameters such as temperature, wind velocity, rainfall and relative humidity. Correlation coefficients are given in Table 4. Some species correlated positively with the above meteorological factors (Table 4).

Table 3. Colony numbers, percentage (%), sampling sites of fungal species and the months during which the samples were found (April–September 1996)

Fungi	Number of colony	%	Site in which the samples were taken	Months during which the samples were found
Absidia Tiegh.	1	0.08	5	U: 1 (0.87)
Alternaria	276	23.67	1, 2, 3, 4, 5	U: 14 (8.8), M: 3 (1.27), J: 99 (53.51), L: 23 (7.82), U: 100 (87.71), P: 37 (20.55)
<i>A. alternata</i> (Fr.) Keissl.	261	22.38	1, 2, 3, 4, 5	M: 2 (0.93), J: 99 (55.61), L: 23 (8.21), U: 100 (93.45), P: 37 (20.67)
<i>A. citri</i> Ellis & N. Pierce apud N. Pierce	15	1.28	1, 5	U: 14 (9.85), M: 1 (0.46)
Aspergillus	22	1.88	1, 2, 3, 4, 5	M: 3 (1.27), J: 5 (2.70), L: 4 (1.36), U: 7 (6.14), P: 3 (1.66)
<i>A. clavato-nanica</i> Bat., H. Maia & Alecrim*	3	0.25	2, 5	J: 2 (1.12), L: 1 (0.35)
<i>A. niger</i> Tiegh.	7	0.60	2, 4, 5	M: 1 (0.46), L: 3 (1.07), U: 1 (0.91), P: 2 (1.11)
<i>A. parvulus</i> G. Sm.	2	0.17	2	J: 1 (0.56), U: 1 (0.91)
<i>A. pseudoglaucus</i> Blochwitz	3	0.25	2	U: 3 (2.80)
<i>A. versicolor</i> (Vuill.) Tirab.	2	0.17	2, 5	M: 1 (0.46), J: 1 (0.56)
<i>A. wentii</i> Wehmer	5	0.42	1, 2, 3	M: 1 (0.46), J: 1 (0.56), U: 2 (1.86), P: 1 (0.55)
Botryotrichum Sacc. & Marchal	2	0.17	1, 2	J: 2 (1.08)
Chlamydomyces Bainier	13	1.11	3, 4, 5	U: 5 (3.1), M: 5 (2.12), U: 2 (1.75), P: 1 (0.55)
Cladosporium Link ex Fr.	511	43.82	1, 2, 3, 4, 5	U: 3 (1.8), M: 100 (42.55), J: 57 (30.81), L: 222 (75.51), P: 129 (71.66)
<i>C. carpophilum</i> Thüm. (<i>Cladosporium</i> state of <i>Venturia carpophila</i> Fisher)	441	37.82	1, 2, 3, 4, 5	M: 90 (42.25), L: 222 (79.28), P: 129 (72.06)
<i>C. herbarum</i> (Pers.) Link ex Gray	70	6.00	1, 2, 3, 4, 5	U: 3 (2.11), M: 10 (4.69), J: 57 (32.02)
Endocochlus Drechsler	1	0.08	1	U: 1 (0.87)
Fusarium Link ex Fr.	76	6.51	1, 2, 3, 4, 5	U: 35 (22.1), M: 14 (5.95), J: 16 (8.64), L: 8 (2.72), P: 3 (1.66)
<i>F. acuminatum</i> Ellis & Everh.	2	0.17	2	J: 2 (1.12)
<i>F. equiseti</i> (Corda) Sacc.	27	2.31	1, 2, 3, 4, 5	M: 14 (6.57), J: 13 (7.30)
<i>F. semitectum</i> Berk. & Ravenel var. <i>semitectum</i> ²	18	1.54	1, 2, 3, 4	U: 18 (12.67)
<i>F. solani</i> (Mart.) Appel & Wollenw. emend. W.C. Snyder & H.N. Hansen ³	28	2.40	1, 2, 3, 4, 5	U: 17 (11.97), J: 1 (0.56), L: 7 (2.50), P: 3 (1.67)
<i>F. solani</i> var. <i>martii</i> (Appel & Wollenw.) Wollenw. ⁴	1	0.08	4	L: 1 (0.35)
Nematochtonus Drechsler	11	0.94	2, 3	U: 11 (6.9)
Penicillium Link	218	18.69	1, 2, 3, 4, 5	U: 90 (56.9), M: 93 (39.57), J: 2 (1.08), L: 24 (8.16), U: 2 (1.75), P: 7 (3.88)
<i>P. brevicompactum</i> Dierckx	4	0.34	2	L: 4 (1.42)
<i>P. camemberti</i> Thom	54	4.63	1, 2, 3, 4, 5	U: 54 (38.02)
<i>P. cyclopium</i> var. <i>echinulatum</i> Raper & Thom	18	1.54	1, 2, 3, 4	U: 16 (11.26), P: 2 (1.11)
<i>P. estinogenum</i> A. Komatsu & S. Abe ex G. Sm.*	3	0.25	2, 4	J: 1 (0.56), L: 2 (0.71)
<i>P. funiculosum</i> Thom	5	0.42	2, 4	P: 5 (2.79)
<i>P. griseum</i> (Sopp) Biourge	4	0.34	2	U: 4 (2.81)
<i>P. implicatum</i> Biourge	16	1.37	1, 3, 4, 5	U: 16 (11.26)
<i>P. lanosum</i> Westling	91	7.80	1, 2, 3, 4, 5	M: 91 (42.72)
<i>P. restrictum</i> J.C. Gilman & E.V. Abbott	6	0.51	1, 2, 3, 4	M: 2 (0.93), L: 4 (1.42)
<i>P. rugulosum</i> Thom	13	1.11	4	L: 13 (4.64)

Table 3. Continued

Fungi	Number of colony	%	Site in which the samples were taken	Months during which the samples were found
<i>Torula</i> Pers.	24	2.05	1, 2, 3, 4, 5	M: 6 (2.55), J: 4 (2.16), L: 13 (4.42), U: 1 (0.87)
<i>Trichoderma</i> Pers.	11	0.94	2, 5	M: 11 (4.68)
Total	1166	100		

*It is likely to be a new record for Turkey.

Letters indicate: **A**: April; **M**: May; **J**: June; **L**: July; **U**: August; **P**: September; **First number in the last column**: Colony number; **Number placed in parenthesis**: Percentage.

²This strain is preserved in BBA culture collection (BBA 70944) (BBA= *Biologische Bundesanstalt für Landund Forstwirtschaft, Berlin, Germany*).

³ This strain is preserved in BBA culture collection (BBA 70942).

⁴ This strain is preserved in BBA culture collection (BBA 70943).

Table 4. Correlation coefficients (r) between colony numbers and meteorological factors

Genus	Correlations with meteorological factors			
	Temperature (°C) r	Wind velocity (m/sec) r	Total monthly rainfall (mm) r	Average relative humidity (%) r
<i>Absidia</i>	0.325	-0.542	0.043	0.051
<i>Alternaria</i>	0.479	-0.477	-0.335	-0.323
<i>Aspergillus</i>	0.792	-0.217	-0.678	-0.674
<i>Botryotrichum</i>	0.277	0.108	-0.532	-0.500
<i>Chlamydomyces</i>	-0.672	0.478	0.604	0.636
<i>Cladosporium</i>	0.436	-0.128	-0.463	-0.439
<i>Endocochlus</i>	0.325	-0.542	0.043	0.051
<i>Fusarium</i>	-0.715	0.951	0.292	0.148
<i>Nematochtonus</i>	-0.863	0.759	0.622	0.411
<i>Penicillium</i>	-0.615	0.744	0.371	0.359
<i>Torula</i>	0.622	0.159	-0.778	-0.787
<i>Trichoderma</i>	0.016	0.108	-0.048	0.185

Discussion

It is well known that microfungi can live in extreme conditions in almost all regions and all climates. They usually live in soil and can be dispersed into the atmosphere owing to various factors. In recent years, aerobiologists have shown a great interest in airborne fungi due to both their constant existence in the air and the increase of allergies caused by them (Larsen and Gravesen, 1991; Pasanen, 1992). *Aspergillus* and *Penicillium* spores are the most widespread aeroallergens in the world. According to qualitative and quantitative reports, the former is the dominant species in tropical regions whilst the latter is dominant all over the world (Rosas et al., 1992).

We detected that the *Cladosporium* was the most common genus in the air (Agarwal and Shivpuri, 1969; Beaumont et al., 1985a; Savino and Caretta, 1992; Takahashi, 1997; Şimşekli et al., 1998), followed by *Alternaria*, *Penicillium* and *Fusarium* genera (Table 3). *Alternaria*, *Penicillium*, *Aspergillus* and *Fusarium* genera were found to be the dominant types in some studies such as Khandelwal (1991), Savino and Caretta (1992).

We used the Petri Plate Gravitational Method for the isolation of airborne fungi because of its practical usage and low cost. This method is useful for the enumeration of fungal spores, but gives only a rough approximation of the kinds and numbers of airborne fungi (Pelczar et al., 1993).

Cladosporium and *Alternaria* were by far the most abundant identified than other fungi in warm summer months and they showed positive correlation with temperature and negative correlation with relative humidity according to the results of correlation analyses (Table 4). *C. carpophilum* was present in May, July and September, with a maximum in July whereas *A. alternata* showed a maximum in August. These results are in agreement with the correlation analysis. *Penicillium* occurred throughout the six months considered but was especially abundant in April. Heavy rainfall, high humidity and high wind velocity may have been the cause. This genus showed negative correlation with temperature, but positive correlation with wind velocity, rainfall and relative humidity. *Fusarium* genus showed negative correlation with temperature, but positive correlation with wind velocity (this result is significant as statistics), rainfall and relative humidity (Table 4). This genus peaked in April. Low temperatures may have been the reason; this case is in agreement with the results of the correlation analysis. Similarly, Bandyopadhyay et al. (1991) indicated positive correlation between heavy rainfall and *Fusarium* concentration. *Aspergillus* genus showed positive correlation with temperature (this result is significant as statistics). A lot of researchers (Agarwal and Shivpuri 1969; Bandyopadhyay et al., 1991; Di Giorgio et al., 1996) reported that various meteorological factors affect the type and concentrations of airborne fungi. Wind velocity, relative humidity and temperature are very important among them. Pasanen et al. (1991) reported that the minimum air velocity at which *Cladosporium* sp. released spores was 1.0 m s^{-1} , however *Aspergillus fumigatus* and *Penicillium* sp. released great numbers of spores at 0.5 m s^{-1} . So, air velocity is significant for the dispersal of fungal spores into the air. Some of our results showed negative correlation with the above mentioned three parameters. Various factors may have affected these results: in particular the number of sampling sites is low (5) and statistical analysis becomes therefore difficult.

We identified two species belonging to the genera *Cladosporium* and *Alternaria*. *A. alternata* occurred in all research stations, whereas *A. citri* occurred only in the 1st and 5th sampling stations. Also 10 species of *Penicillium* were identified. Some *Fusarium* species identified in our study affect various cultivated plants. *F. acuminatum* cause stem rot in maize; *F. equiseti* isolated from bean, cabbage, onion, tomato and maize; *F. solani* cause problem in pea especially in Europe

(Booth, 1971). These *Fusarium* species may cause some problems to various vegetables growing in our study areas. *F. equiseti* and *F. solani* occurred in all research stations. These results highlight the importance of air as a vector for the dispersal of propagules of *Fusarium* species. Similar opinions are reported by Horst et al. (1970).

A. niger, *A. versicolor*, *A. wentii* and *P. funiculosum* identified in our study are widespread in Turkey.

Some species of *Cladosporium*, *Alternaria*, *Penicillium*, *Fusarium*, *Aspergillus*, *Helminthosporium* Link ex Fr., *Phoma* Sacc., *Epicoccum* Link ex Schlecht, *Rhizopus* Ehrenberger and *Mucor* Mich. ex Fr. genera can cause allergy (Shivpuri and Agarwal, 1969; Hellenbarnd and Reade, 1992; Chih-Shan and Yu-Mei, 1994; Chih-Shan et al., 1995). Auger et al. (1994) reported that *P. brevicompactum*, *P. cyclopium* species and *Trichoderma* genus (they were determined in our study) were isolated from residences of individuals showed chronic fatigue syndrome. The airborne fungal species identified in our study may be allergenic to people residing in Edirne. Farmers may be exposed to dust containing mycotoxins derived from fungi associated with vegetables by inhalation or to fungal metabolites by skin contact during farming activities. Therefore, fungal spore monitoring in our region may be useful both from the phytopathological and from the allergological point of view.

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