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SEASONAL VARIATION OF AEROMYCOFLORA IN VEGETABLE MARKET OF KARWAN BAZAR, DHAKA, BANGLADESH

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ABSTRACT

Aeromycoflora in relation to occurrence and prevalence, their seasonal variation and distribution of fungal spores in air was studied from October, 2010 to September, 2011. During the study a total of 31 fungal species belonging to 18 genera of fungi were isolated and identified. Total fungal colony count was 2308. The highest occurrence and prevalence of fungi was contributed by *Aspergillus niger* 1075 colonies, *Penicillium* spp. 582 colonies, *Aspergillus fumigatus* 167, *A. flavus* 112 colonies, *Rhizopus* sp. 121 colonies, *Candida* spp. 39 colonies, *Chladosporium* spp. 36 colonies, *Syncephalestrum* sp. 25 colonies, and *Fusarium* spp. 14 colonies. Lowest colony count was recorded one, in *Aspergillus ustus*, *Drechslera* sp., *Nigrospora* sp. and *Ulocladium* sp. Maximum fungal pollutants were found 339 in July, 2010 at temperature 27.12°C and moisture 84.2% during the wet and rainy days and declined in January, 2011 when temperature and moisture label decreased to 20.26°C and 59.5%, respectively. Highest colony density was recorded in July, 2011, it was moderate in February and March, 2011. Lowest colony density was recorded in January and May, 2011.

Key words: Seasonal variation, Aeromycoflora, Vegetable market

INTRODUCTION

Karwan bazar is one of the largest hole sale markets of Dhaka city located in the centre. It is an important area for accumulation of vegetables. All the sellers collect their goods directly from agricultural producing regions and exhibit them for sale in an open spaces and tin-shade area. Vegetables in the market place are exposed directly to the rainfall and climate hazards. The neighboring areas of the market are highly populated and unhygienic due to disposable items in open dust bins.

Environmental aeromycology constitutes one of the major aspects mainly because of the dominance of fungal spores in the airspora. The spores are often liberated in the air in massive concentration and remain airborne for a long time. The fungal spore constitutes a

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major component of airspora. Some spores of the fungi are responsible for allergy, since the spores are inhaled and deposited on sensitive mucosa. Many allergic human diseases such as asthma, rhinitis and a range of cardio-respiratory diseases are attributed to inhalation of airborne fungal spores and pollen grains. In the vegetable markets of metropolitan cities, rotten vegetables and fruits, gunny bags, paper bags, packing materials, straw, discarded leaves and stems are the main substrates for the growth of airborne fungi, hence were conducted in Pimpri vegetable market area for a period of one year (Ahire and Sangale 2012).

Intensive research has been done on aeromycoflora home and abroad but very little information is available on aeromycoflora of vegetable markets (Bagwan 2010, Saadabi 2011, Kumer *et al*. 2013, Kakde and Kakde 2012). Aeromycological research from Bangladesh is limited and scattered (Begum *et al.* 2007, 2009, Pasha and Hossain, 2009, 2011).

Present study was undertaken to find out the (i) occurrence, prevalence and frequency of aeromycoflora, (ii) characterization and identification of the isolates fungi, (iii) seasonal variation of the fungi, and (iv) spatial distribution of fungal spores in air.

MATERIALS AND METHODS

For the sampling five corners of Karwan bazaar vegetable market were selected of which four were exposed to natural environment and a tin-shade building. Common vegetables of the market were *Allium cepa* L., *A. sativum* and *Zingiber officinale* L. *Capsicum fruticans* L. (Chili), *Cucurbita maxima* Duch. (Pumkin), *Lablab purpureus* L., (Sweet bean), *Lycopersicon esculentus* L., *Solanum nigram* L. (Brinjal), *Solanum tuberosum* L., *Trichosanthes anguina* L. (Snake gourd) and *Trichogenthes diocica* Roxb. (Pointed gourd).

Present study provides a mix of quantitative data (spatial and attributed information) with a specimen collection survey providing breadth of coverage. The research design was composed mainly of problem formulation, specimen collection procedures, laboratory analysis of collected specimen, data manipulation, analysis and interpretation, and geographical analysis with a GIS (Geographic Information System).

Since Karwan Bazar is the one of the largest hole sale markets of Dhaka city, five sites were chosen there in the vegetable market for specimen collection. Geographical coordinates (WGS84) of those sites are A (X = 90.393644258, Y = 23.7525262415), B (X = 90.3939710222, Y = 23.7525374762), C (X = 90.3940232814, Y = 23.7527854377), D (X = 90.3940698908, Y = 23.753018574) and E (X = 90.3937952214, Y = 23.7528543833), respectively. Specimens were collected from three vertical heights (0, 1 and 2 meters) of each site for one year with temporal interval of one month.

The data for spatial analysis with GIS operation were categorised as: (a) vector spatial data and (b) descriptive attribute data. The vector spatial data assigned to sampling sites and distribution of fungi species or colony are allocated by means of X, Y coordinates; while the attribute data are stored as records (rows) in a relational database. The attribute data of different map features (i.e., sampling height, month, numbers of colonies and numbers of species) were collected from primary sources and laboratory analysis.

Three dimensional thematic maps were developed to define the pattern of fungi distribution and its spatial variation by using spatial interpolation methods. Interpolation is the process of estimating the value of parameters at unsampled points from a surrounding set of measurements (Burrough and McDonnel 1998). When the local variance of sample values is controlled by the relative spatial distribution of these samples, geostatistics can be used for spatial interpolation (Oliver and Khayrat 2001). Geostatistical approaches rely on both statistical and mathematical methods, which can be used to create surfaces and assess the uncertainty of the predictions (Johnston *et al.* 2001) Geostatistics represent one of the most powerful procedures for producing contour maps for regionalized variables (Beliaeff and Cochard 1995, Badr *et al.* 1993).

The spatial pattern of fungi distribution was analyzed and interpolated in a GIS environment (Surfer-10) by using the Kriging method. This is a stochastic and optimal point interpolation method for the unbiased estimation of field variables (Burrough and McDonnel 1998, Oliver and Khayrat 2001, Rizzo and Dougherty 1994, Patgiri and Baruah 1995, Isaaks and Srivastava 1989, Phillips and Marks 1996, Tsanis and Gad 2001). The method is based on the theory of regionalized variables (Davis 2002, Wang *et al*. 2001, Kalabokidis and Omi 1995, Petkov *et al.* 1996). Kriging is a means of local estimation in which each estimate is an average of the observed values in the neighbourhood (Patgiri and Baruah 1995, Soderstrom and Magnusson 1995). It is a distance weighing estimation method that takes into account the spatial characteristics of the local structure through a variogram function (Davis 2002, Seron *et al.* 2001). The advantage of Kriging is that the estimated values at observation sites are equal to the actual measurements (Rizzo and Dougherty 1994).

The fungi distribution map produced by Kriging is constrained by the spherical semivariogram fits. The experimental variogram was computed from the laboratory data and a mathematical model (Persicani 1995, Brooker *et al.* 1995, Mapa and Kumaragamage 1996) was fitted to the fungi distribution values by weighted least-squares approximation, using ArcGIS. The parameters of the variogram model for fungi concentrations were used with their values for estimating their concentrations over the area. The semivariogram, $\gamma(h)$, is half the average squared difference between pairs of data $Z(x_1)$ and $Z(x_1 + h)$ separated by a given distance h(lag). An estimate of the semivariogram with $N(h)$ as the number of sampling pairs separated by a distance of h is given by the following equation (Lacaze *et al.* 1994):

$$¥(h) = \frac{1}{2N(h)} \sum_{i+1}^{N(h)} \{Z(x_i + h) - Z(x_i)\}^2$$

The semivariogram for the fungi data illustrates a number of common features (Petkov *et al.* 1996, Gerlach *et al.* 2001), (a) increases from smaller to larger lags but a limiting 'sill' is always found; (b) $\gamma(h)$ approaches the small lags, suggesting a large 'nugget effect'; and (c) the spherical semivariogram model gives good and acceptable fits to $\gamma(h)$. Ordinary Kriging was used in this study since fungi concentrations in vegetable market are high uneven. Ordinary Kriging, which is the most widely used type of Kriging to estimate values when data point values vary or fluctuate around a constant mean value (Seron *et al.* 2001). It is applied for an unbiased estimate of the spatial variation of a component (Wang *et al.* 2001).

Temperature and humidity of air was measured using a thermometer and hygrometer device in the study site.

The experiment was carried out during October, 2010 to September, 2011 to assess the aeromycoflora of the largest vegetable market of Dhaka city. Five location of the Karwan bazar vegetable market was selected for sampling representing east, west, north, south and central areas. The air samples were collected by following exposed Petri plate culture methods (Ismail *et al*. 2002) using potato dextrose agar media. At each location five Petri dishes (90 cm diameter) containing potato dextrose agar (PDA) medium were exposed in the atmosphere of the market. To avoid bacterial contamination 5% lactic acid was added the media. The exposure time was 10 minutes in the morning (6.00 - 7.00 a.m.) when the market was highly active, at the height of 0, 1, and 2 m above the vegetable baskets, respectively at one month intervals in second day of each month. After the completion of exposure period the Petri plates were placed in clean air tight plastic basket and brought into the laboratory and placed in incubator at 25 ± 1°C for 5 - 7 days. The fungal colonies grown for each species were counted and the total colony also recorded. The incidence, abundance and percentage contribution of fungi were recorded. The percentage contribution of each genus was calculated on the basis of the number of colonies of the genus against total number of colonies of all recorded genera during the entire twelve months sampling period.

Identities of the isolates were determined following the standard literatures (Ellis 1971, 1976, Benoit and Mathur 1970, Booth 1971, Barnet and Hunter 1972). All the specimens were preserved in the Herbarium of Mycology and Plant Pathology section, Department of Botany, University of Dhaka.

RESULTS AND DISCUSSION

Analysis for a period of one year revealed as many as 18 genera of fungal colonies distributed over 31 species and total 2308 colonies were recorded by exposed Petri plate methods in air of Karwan bazaar vegetable market. *Aspergillus* spp. (1422 colonies, 11 species) was most frequent and predominant genus detected followed by *Penicillium* spp. (582 colonies, 2 species), *Rhizopus* sp. (124 colonies), *Candida* spp. (37 colonies) and *Cladosporium* spp. (36 colonies), and lowest colony found in *A. ustus*, *Drechslera* sp, *Nigrospora* sp., and *Ulocladium* sp. one colony, respectively. Spores were also abundant, which are well-known allergenic and pathogenic. High concentration of airborne spores was recorded in January followed by December, February and November. Maximum contribution of the fungal flora was observed by *Aspergillus niger* followed by, *Penicillium* spp., *Aspergillus fumigatus*, *Rhizopus* sp., *Aspergillus flavus, Aspergillus tereus, Candida* spp., *Cladosporium* spp., *Syncephalastrum* sp., *Fusarium* spp., *Trichoderma* sp., *Aspergillus versecolor, Pestalotia* sp., *Aspergillus aculeatinus, Aspergillus nidulance*, *Paecilomyces* sp., *Alternaria* spp., *Arthrinium* sp., *Alternaria alternata , Aspergillus flavus st sclerotia, Aspergillus glaucus, Aspergillus sclerotioniger, Monilia* sp., *Aspergillus ustus, Curvularia* spp., *Drechslera* sp., *Nigrospora* sp., *Ulocladium* sp., and unidentified 24 colonies. List of fungi, total number of colonies, year-wise frequency of particular fungus in air and their month wise distribution on air are presented in Tables 1 and 2. Speices of *Apergillus* and *Penicillium* are well-known allergenic and pathogenic only the *A. niger* occurred throughout the 12 months of survey. *A. flavus* and *A. fumigatus* was absent in January and *Penicillium* spp. was absent in April. *Aspergillus ustus* recorded in May, *Drechslera* sp. and *Nigrospora* were recorded in the month of February. *Ulocladium* sp. was recorded in January (Tables 1 and 2).

Maximum fungal pollutants were found 339 in June, 2010 at temperature 27.12°C and moisture 84.2% during the wet and rainy days and declined in January, 2011, when moisture and temperature label decreased (Fig. 1). Metrological parameters like temperature and humidity seem to play vital role on the occurrence of mycoflora in the air of vegetable market.

Highest colony density was recorded in July, 2011, it was moderate in February and March, 2011. Lowest colony density was recorded in January and May, 2011 (Fig. 2).

Spatial distribution of fungi by digital elevation model of semi-variogram Kriging was first time used in study of aero-mycoflora.

Occurrence of pollen grains, algal fragments, bacteria, trichomes, insect parts and scales, protozoan and cysts were negligible and were not concerned in the present study.

Table 1. Total fungal colonies and their year wise frequency counted in Karwan bazar vegetable market.

Sl. No.	Species	Colony total (12 months)	Year wise frequency
1	*Alternaria* spp.	3	0.129
2	*Alternaria alternata*	2	0.086
3	*Arthrinium* sp.	3	0.129
4	*Aspergillus aculeatinus*	6	0.259
5	*Aspergillus flavus*	112	4.838
6	*Aspergillus flavus st sclerotia*	2	0.086
7	*Aspergillus fumigatus*	167	7.213
8	*Aspergillus glaucus*	2	0.086
9	*Aspergillus nidulance*	5	0.215
10	*Aspergillus niger*	1075	46.436
11	*Aspergillus sclerotioniger*	2	0.086
12	*Aspergillus tereus*	42	1.814
13	*Aspergillus ustus*	1	0.043
14	*Aspergillus versecolor*	8	0.345
15	*Candida* spp.	37	1.598
16	*Cladosporium* spp.	36	1.555
17	*Curvularia* spp.	5	0.215
18	*Drechslera* sp.	1	0.043
19	*Fusarium* spp.	14	0.604
20	*Monilia* sp.	2	0.086
21	*Nigrospora* sp.	1	0.043
22	*Paecilomyces* sp.	4	0.172
23	*Penicillium* spp.	582	25.140
24	*Pestalotia* sp.	7	0.302
25	*Rhizopus* spp.	121	5.226
26	*Rhizopus stolonifer*	3	0.129
27	*Syncephalastrum* sp.	25	1.079
28	*Trichoderma* sp.	10	0.431
29	*Ulocladium* sp.	1	0.043
30	Yeast	5	0.215
31	Unidentified	24	1.036
	Total colonies	2308	

Hoque and Shamsi (2011) reported 16 species of fungi representing 7 genera of Deuteromycetes and one genus of conidial Phycomycetes associated with five selected vegetables namely *Capsicum fruticans* L. (Chili), *Cucurbita maxima* Duch. (Pumkin), *Lablab purpureus* (L.), Sweet bean, *Trichosanthes anguina* L. (Snake gourd) and *Trichogenthes diocica* Roxb. (Pointed gourd). The isolated fungi were *Alternaria pluriseptata*, *Aspergillus niger* van Tiegh., *Aspergillus flavu*s Link ex Fr, *Aspergillus* sp., *Colletotrichum dematium*, *Curvularia brachyspora*, *C. eragrstidis*, *Curvularia fallax* Boedijn, *C. penniseti (*Mitra) Boedijn, *C. prasadii* R.L. & B.L Mathur, *C. stapeliae*, *Fusarium moniliforme* Sheldon, *Penicillium* sp, *Rhizopus stolonifer* (Ehrenb. Ex. Fr.) Lind and *Trichoderma viride* and *Trichoderma* sp.

Table 2. Frequency of air-borne fungal colony of Karwan bazar vegetable market during 2010 - 2011.

Species	Oct.	Nov.	Dec.	Jan.	Feb	Mar.	Apr.	May	June	July	Aug.	Sept.
Alternaria spp.		0.35	0.70							0.29		
A. alternata		1.40										
Arthrinium sp.			0.70		1.19							
Aspergillus aculeatinus	2.08											
Aspergillus flavus	3.08	3.81			1.19	3.68	1.57	2.83	7.69	11.80	9.62	5.05
A. flavus st. sclerotia									1.03			
A. fumigaues	4.2	1.38	4.20		10.12	4.21	3.15	2.83	16.41	13.57	3.85	13.30
A. glaucus					0.60			0.47				
A. nidulance		0.35				1.58					0.96	
A. niger	68.46	46.37	8.39	41.54	39.88	57.89	36.22	31.60	45.64	47.79	50.00	60.09
A. sclerotioniger						1.05						
A. tereus	1.15	3.11	8.39		0.60	0.53	0.79		3.08	1.77	1.92	0.46
A. ustus								0.47				
A. versecolor		0.35			0.60		0.79	1.42			1.92	
Candida spp.	1.54		2.80		0.60		4.72	2.36	1.54		7.69	2.75
Cladosporium spp.			7.69	13.85	2.38	3.16	2.36					
Curvularia spp.					1.79	0.53			0.51			
Drechslera sp.					0.06							
Fusarium spp.			0.70	4.62	0.60			1.42			0.96	
Monilia sp.					1.19							
Nigrospora sp.					0.60							
Paecilomyces sp.		1.38										0.46
Penicillium spp.	9.23	34.60	56.64	38.46	32.14	22.11		32.55	13.33	22.71	18.27	8.26
Pestalotia sp.		2.42										
Rhizopus spp.	8.46	1.73	0.70		2.38	2.11		20.28	5.64	1.47	4.81	9.117
R. stolonifer	1.15											
Syncephalastrum sp.	1.15		5.59					2.36	3.08	0.59		0.46
Trichoderma sp.		0.69	0.70		3.58	0.53						
Ulocladium sp.				1.54								
Yeast						2.63						
Unidentified	1.54	0.35	1.40					1.42	2.05			

The study showed a common representation of many fungi both in the ambient air over the vegetable market and their occurrence in vegetables.

A qualitative and quantitative assessment was carried out to detect air-borne fungi at the largest vegetable market of Khartoum state central Sudan by Saadabi (2011). During the study a total of 23 fungal species belonging to 4 groups of fungi were trapped and identified. The highest occurrence and prevalence of the fungi was contributed by three species of *Aspergillus,* three species of *Penicillium* three species of *Alternaria,* one species of *Rhizopus,* two species of *Curvularia,* and two species of *Fusarium*. Maximum fungal pollutants were found from June to August during the wet and rainy days and declined in hot days in April-May.

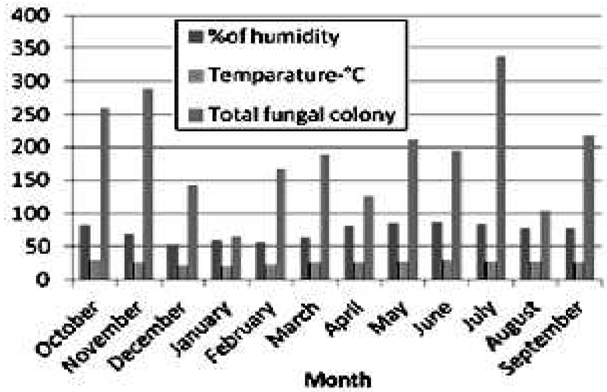

Fig 1. Frequency of fungal colony recorded in 12 months in relation to temperature and humidity.

Kakde and Kakde (2012) reported forty-six fungal species belonging to 26 genera recovered from five varieties of vegetables collected from the same market. Many saprophytic and pathogenic fungi were found to be associated with sampled vegetables from the market. The most dominant forms of fungi were of *Aspergillus* followed by *Cladosporium, Penicillium, Alternaria, Fusarium, Curvularia, Trichoderma*, and *Rhizopus. Aspergillus niger*, A. *flavus, A. fumigatus, Penicillium spp.* and *Cladosporium herbarum,* found to be dominant during the period of investigation. They also observed that the occurrence of fungal spores in the air was markedly seasonal because of these organisms sensitivity to weather changes.

Kumar *et al.* (2013) reported air borne fungi from vegetable market of Uttar prodesh, India. Analysis for a period of one year revealed as many as 16 types of fungal colonies. *Aspergillus* sp. was most frequent and predominant genus detected, *Penicillium* sp. and *Alternaria* sp. spores were also abundant. High concentration of airborne spores was recorded in January followed by December, February and November. Maximum contribution of the fungal flora was observed by *Aspergillus* spp. followed by, *Penicillium* sp. and *Alternaria alternata*..

Present result is very near to observation of Saadabi (2011), Kakde and Kakde (2012) and Kumar *et al.* (2013). In all of the previous and present studies was common future noted was that *Aspergillisus* spp. followed by *Penicillium* spp. are the pre dominating fungi of aero-mycoflora of vegetable markets.

It can be concluded that the fungal colony count showed *Aspegillus* spp. followed by *Penicillium* spp., *Rhizopus* sp., *Candida* spp., *Chladosporium* spp., *Syncephalestrum* sp. and *Fusarium* spp. Lowest colony count was recorded in *Aspergillus ustus*, *Drechslera* sp., *Nigrspora* sp. and *Ulocladium* sp. Maximum fungal pollutants were found in July, 2010 when temperature and moisture was highest during the wet and rainy days and declined in January, 2011 when temperature and moisture label was lowest in winter. Highest colony density was recorded in July, 2011, it was moderate in February and March, 2011. Lowest colony density was recorded in January and May, 2011.

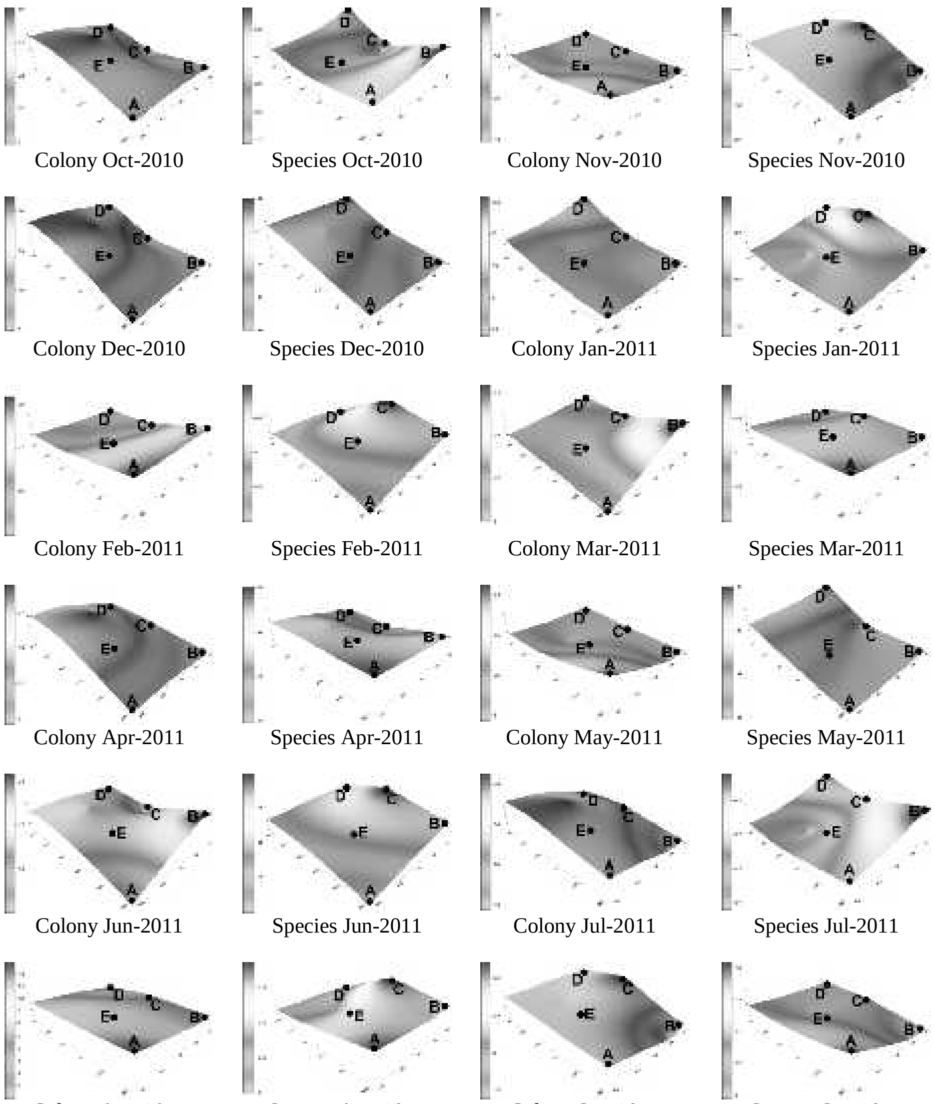

Fig. 2. Spatial distribution of fungi at Karwan Bazar vegetable market (Digital elevation model of Semi-Variogram Kriging).

Spatial distribution graphs of this study clearly indicated that fungal concentration varied in different months. Growth and development of the fungi were found to be dependent on climatic factors, such as air humidity and precipitation (Abu-Dieyeh *et al*. 2010). Therefore, the variation of the spatial distribution of fungi in the survey area may be affected by varying humidity levels in different months.

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