

Effect of seasonal variation on aeromycoflora of Bilaspur, Chhattisgarh, involved in allergic reactions

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Abstract The biotic (bacteria, fungi, pollen grains, algae) and abiotic (dust mites) factors present in the atmosphere are responsible for the common allergic reactions in humans. Fungi are one of the major biotic factors resulting in allergic conditions and sometimes lead to fatal diseases affecting vital organs. A detailed survey was carried out for 2 years (from May 2014 to April 2016) to assess the seasonal distribution and comparative diversity of aeromycoflora present in the urban and rural areas of Bilaspur, Chhattisgarh (India). During the survey, a marked variation in the types of fungal isolates and their colonies were observed in the samples collected at study sites. The maximum number of fungal colonies was obtained in the month of October and November during the first and second year, respectively. Among the isolates, *Aspergillus* and *Curvularia* sp. were found with the highest percent frequency and percent contribution. The predominant fungal species observed during the survey were *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Alternaria alternata* which are known to cause different types of diseases including allergy in human beings, suggesting the possibility of occurrence of fungal allergy in the human population of Bilaspur.

Keywords Aeromycoflora · Allergy · Allergens · Seasonal variation · Fungal diversity

1 Introduction

Aerobiology (Meier 1930), a multidisciplinary science, is principally concerned with sources, dispersion, diversity and the effect of airborne particles such as pollen, spores and other components of biological origin (Bajwa et al. 1997). The atmosphere contains various biotic (bacteria, fungi, pollen) and abiotic components (dust mites, etc.) which affect the air quality (Khan et al. 2009; Chelak and Sharma 2012; Hayleeyesus and Manaye 2014). The airborne fungi (both indoor and outdoor) are considered to be the indicator of atmospheric bio-pollution (Kakde et al. 2001). The fungal allergic reactions are mainly due to contact with fungi or inhalation of spores (Horner et al. 1995). The airborne fungal spores are among the major factors capable of causing various diseases like asthma, allergic rhinitis, aspergillosis, allergic sinusitis, mycoses, atopic dermatitis (Anand and Tiwary 2010; Anand et al. 2013, 2015). The conidia-producing anamorphs of ascomycetes such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium* and *Penicillium* species are the most commonly studied allergenic fungal genera (Knutsen et al. 2012). The study of seasonal variation of aeromycoflora may

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provide insight about the fungal diversity and fungal load in the environment having allergic potential.

2 Materials and methods

2.1 Study site

Bilaspur is situated at 22.08° north latitude, 82.15° east longitude and 264 m elevation above the sea level. The areas around Agrasen Chowk (an urban area, 22.08° north latitude, 82.08° east longitude) and Koni (a rural area, 22.07° north latitude, 82.08° east longitude) of Bilaspur, Chhattisgarh, were selected as the sampling sites for the survey.

2.2 Sampling of aeromycoflora

Aeromycological surveys were conducted on a monthly basis, for a period of 2 years (May 2014–April 2016). Sampling was made by a gravitation method using a Petri dish at sampling sites. The culture plate exposure method was applied for the sampling of aeromycoflora. Different nutrient media like potato dextrose agar (PDA), Czapek Dox agar (CPZ) and Sabouraud dextrose agar (SDA) were utilized for the growth and isolation of the fungi. Petri dishes containing media were exposed for 10 min at the selected sampling sites and incubated at 30 ± 1 °C. Colonies appearing after 48–72 h were

department, Bilaspur. The fungal colonies were enumerated for each season on a monthly basis.

2.3 Identification of fungal isolates

Identification of fungal strains was performed on the basis of both macroscopic and microscopic features following the keys and description given by Tsuneo (2002).

2.4 Fungal diversity

Diversity of fungi was calculated by Shannon's diversity index using the following formula:

$$H (\text{Diversity index}) = \text{SUM} [(p_i) * \ln(p_i)]$$

$$E (\text{evenness}) = H/H_{\max}$$

where SUM = summation, P_i = number of individuals of each species/total number of samples, S = total number of species, $H_{\max}$ = maximum diversity possible, E = Evenness.

2.5 Ecological studies

At the end of the incubation period, the percentage frequency and the percentage contribution of fungal microflora were calculated with the help of the following formulae (Sharma et al. 2011):

$$\text{Percentage frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total no. of observations}} \times 100$$

$$\text{Percentage contribution} = \frac{\text{Total no. of colonies of a species in all observations taken together}}{\text{Total no. of colonies}} \times 100$$

point inoculated separately on fresh culture plates and subsequently purified by the single colony isolation method. The isolated pure colonies were maintained on CPZ slants. The meteorological data like temperature, average rainfall and percent humidity were obtained from the Government meteorology

3 Results and discussion

3.1 Sampling of the aeromycoflora

An aeromycological survey was carried out for two consecutive years to analyze the diversity of fungi. During the survey, isolates belonging to a number of

Table 1 Occurrence of different fungal isolates in the urban site of Bilaspur, from May 2014 to April 2015

S. no.	Fungal strains	Occurrence of fungal strains												Total
		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
1.	<i>Aspergillus niger</i>	2	1	0	1	0	2	4	0	2	0	0	1	13
2.	<i>Curvularia sp.</i>	1	0	0	0	4	5	0	0	0	²	1	0	13
3.	<i>Fusarium sp.</i>	0	0	0	0	0	2	1	1	0	0	0	0	4
4.	<i>Alternaria alternata</i>	0	2	0	0	0	0	0	0	3	0	0	1	6
5.	<i>Alternaria sp</i>	0	2	0	0	0	0	0	0	0	0	1	0	3
6.	<i>Aspergillus glaucus</i>	0	0	2	0	1	0	0	0	0	0	0	0	3
7.	<i>Aspergillus flavus</i>	2	0	0	0	0	1	0	0	0	2	0	0	5
8.	<i>Bipolaris sp.</i>	0	2	0	0	0	0	2	0	0	0	0	0	4
9.	<i>Aspergillus nidulans</i>	1	0	0	0	0	2	0	0	1	0	0	0	4
10.	<i>Beauveria sp.</i>	1	0	1	0	1	0	0	0	0	0	1	0	4
11.	<i>Penicillium sp.</i>	0	1	0	0	0	1	2	2	1	0	0	0	7
12.	Unidentified (UIId) fungi	10	6	7	4	5	10	8	9	9	5	8	2	83
	Total	17	14	10	5	11	23	17	12	16	9	11	4	149

Table 2 Occurrence of different fungal isolates in the urban site of Bilaspur, from May 2015 to April 2016

S. no.	Fungal strains	Occurrence of fungal strains												Total
		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
1.	<i>Aspergillus niger</i>	2	0	1	0	0	0	3	2	1	0	0	0	9
2.	<i>Curvularia sp.</i>	2	1	0	2	1	1	0	0	0	1	0	1	9
3.	<i>Fusarium sp.</i>	0	0	0	0	0	1	2	0	1	0	0	0	4
4.	<i>Alternaria alternata</i>	2	0	0	0	2	0	2	2	1	0	0	0	9
5.	<i>Aspergillus fumigatus</i>	0	0	1	1	0	2	0	0	0	0	0	0	4
6.	<i>Aspergillus flavus</i>	0	1	0	0	0	1	2	0	1	0	0	1	6
7.	<i>Penicillium sp.</i>	0	1	0	1	0	0	2	1	0	0	0	0	5
8.	Unidentified(UIId) fungi	2	3	2	5	3	5	5	5	4	4	4	2	44
	Total	8	6	4	9	6	10	16	10	8	5	4	4	90

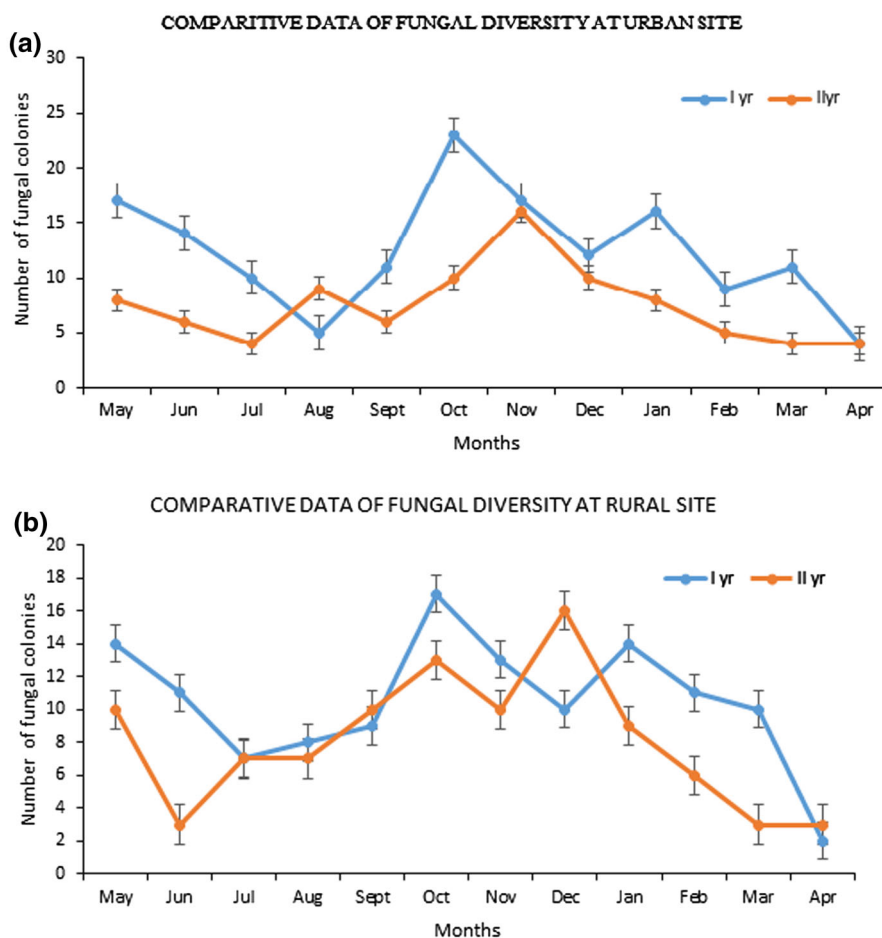
fungal genera were observed. Among the nutrient media used for isolation, best results were obtained with potato dextrose agar (PDA). Further Czapek Dox agar (CPZ) complete medium was found to be the most suitable for the maintenance of fungal cultures. From the urban site, a total of 149 and 90 fungal colonies were isolated in the first and second year, respectively (Tables 1, 2, Fig. 1a). Similarly, in the rural area, altogether 126 fungal colonies were obtained during the first year, whereas 97 fungal colonies were isolated during the second year (Tables 3, 4, Fig. 1b). The variation in the number of colonies at two different sites was likely due to different environmental conditions which affect the

growth and occurrence of fungal isolates in a particular area.

3.2 Identification of fungal isolates

All the fungal isolates were identified macroscopically as well as microscopically. Altogether 65 isolates were collected from urban site samples during the first year and were assigned to 11 genera, while during the second year 90 fungal isolates were categorized into 7 different genera. Similarly, from the rural site, 51 fungal isolates were taxonomically classified to 8 different genera and 40 isolates collected during second year of survey could be categorized to 8 genera.

Fig. 1 **a** Two years (2014–2016) of comparative data of fungal diversity from the urban site of Bilaspur and **b** two years (2014–2016) of comparative data of fungal diversity from the rural site of Bilaspur



3.3 Effect of seasonal variation

3.3.1 Monthly variation of aeromycoflora

In urban area, maximum number of aeromycoflora was observed in the month of October and November during the first and second year, respectively. The minimum number of colonies from urban site was obtained in the month of April during the first year and in the month of March and April during the second year.

Similarly, from the rural site, largest number of fungal colonies was found in the month of October and December during the two successive years, while the least number was observed in the month of April during the first year and in the month of April, June and March during the second year.

3.3.2 Seasonal variation of aeromycoflora

The results of the survey confirmed a seasonal variation in the occurrence of the airborne molds. In both the years, maximum number of fungal isolates was obtained in winter, irrespective of the site of the rural or urban area. Further, the minimum number of isolates at both the sites was recorded during the summer season of both the successive years. Similar results were obtained by Sharma (2012) and Luka et al. (2013) with a maximum number of fungal colonies in the winter and minimum in the summer. Although the environmental conditions during the rainy seasons may favor the growth and sporulation of fungi by providing moisture and suitable temperature, a possible reason behind getting a lower number of fungal colonies during the rainy season in our study could be due to settling down of conidia with rain spills.

Table 3 Occurrence of different fungal isolates in the rural site of Bilaspur, from May 2014 to April 2015

S. no.	Fungal strains	Occurrence of fungal strains												Total
		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
1.	<i>Aspergillus niger</i>	2	1	1	0	0	0	2	3	0	1	0	0	10
2.	<i>Curvularia</i> sp.	0	2	0	1	1	0	2	0	0	2	1	0	9
3.	<i>Fusarium</i> sp.	0	0	0	0	0	1	2	0	0	0	1	0	4
4.	<i>Alternaria alternata</i>	1	0	0	2	1	0	0	0	3	0	0	0	7
5.	<i>Aspergillus fumigatus</i>	0	0	2	0	1	3	0	0	0	1	0	0	7
6.	<i>Aspergillus flavus</i>	2	0	0	0	0	1	0	0	0	2	0	0	5
7.	<i>Aspergillus nidulans</i>	1	0	0	0	0	2	0	0	1	0	0	0	4
8.	<i>Penicillium</i> sp.	0	1	0	0	0	0	1	2	1	0	0	0	5
9.	Unidentified (UId) fungi	8	7	4	5	6	10	6	5	9	5	8	2	75
10.	Total	14	11	7	8	9	17	13	10	14	11	10	2	126

Table 4 Occurrence of different fungal isolates in the rural site of Bilaspur, from May 2015 to April 2016

S. no.	Fungal strains	Occurrence of fungal strains												Total
		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
1.	<i>Aspergillus niger</i>	1	0	1	0	0	0	2	0	1	1	0	0	6
2.	<i>Curvularia</i> sp.	0	1	0	2	0	1	0	2	0	0	0	1	7
3.	<i>Fusarium</i> sp.	0	0	1	0	0	2	1	0	0	1	0	0	5
4.	<i>Alternaria alternata</i>	2	0	0	0	1	0	0	2	1	0	0	0	6
5.	<i>Aspergillus fumigatus</i>	0	0	0	0	0	1	0	0	0	1	0	0	2
6.	<i>Aspergillus flavus</i>	1	0	0	0	2	0	0	1	0	1	0	0	5
7.	<i>Aspergillus nidulans</i>	2	0	0	0	1	0	0	1	0	0	0	0	4
8.	<i>Penicillium</i> sp.	0	0	2	0	0	1	0	1	1	0	0	0	5
9.	Unidentified (UId) fungi	4	2	3	5	6	8	7	9	6	2	3	2	57
	Total	10	3	7	7	10	13	10	16	9	6	3	3	97

3.3.3 Fungal diversity

The fungal diversity at two different sites was determined by calculating a Shannon's diversity index that accounts for both species abundance and evenness of the species present.

The diversity in the rural area was found to be less compared to the urban area ($H_{\text{rural}} = 0.64$ V/s $H_{\text{urban}} = 0.90$) during the first year. During the second year, similar trends were visible with higher biodiversity in the urban area ($H_{\text{rural}} = 0.65$ V/s $H_{\text{urban}} = 0.82$) compared to the rural area. One possible explanation behind higher diversity in an urban area could be the availability of the favorable

environmental conditions like temperature, relative humidity, rainfall and suitable substrates for the growth of fungi.

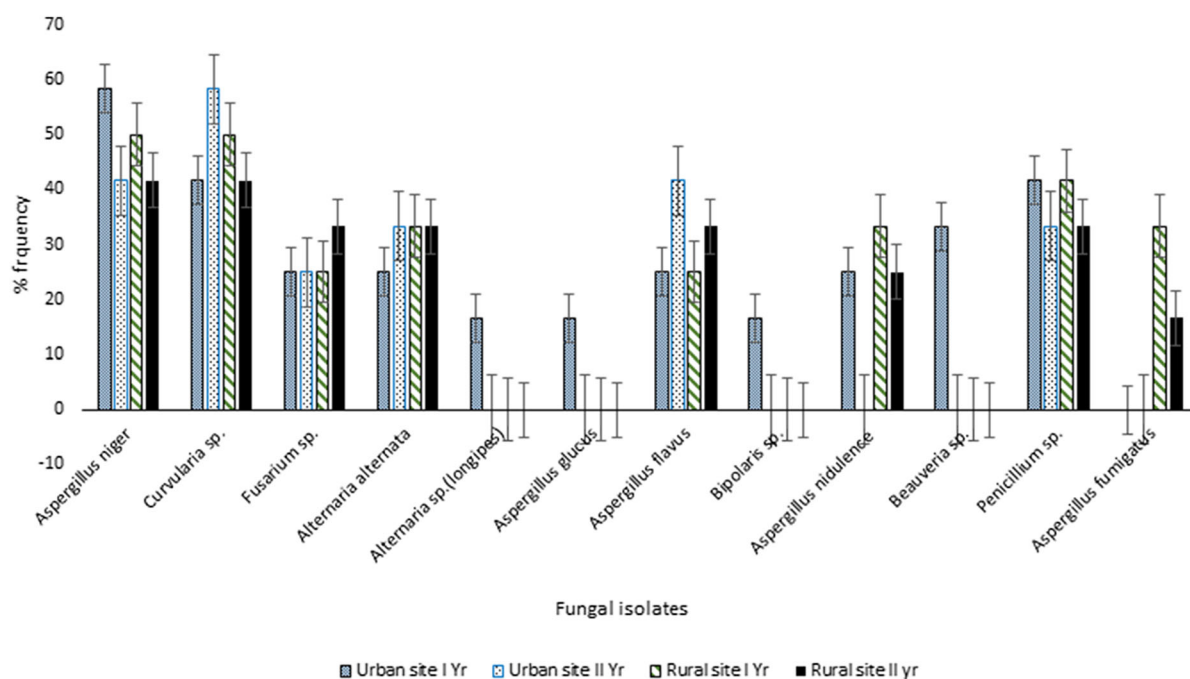
3.4 Ecological studies

3.4.1 Percentage frequency

In the urban area, *Aspergillus niger* (58.3%) and *Curvularia* sp. (41.6%) were the predominant species, during the two consecutive years. The least frequency was observed for *Alternaria longipes* and *Aspergillus glaucus* (16.6%) during the first and second year, respectively. Similar to the urban area, in the rural area

Table 5 Comparison of percentage frequency of fungal isolates in different areas

S. no.	Fungal isolates	Urban site		Rural site	
		I year	II year	I year	II year
1.	<i>Alternaria alternata</i>	25	33.33	33.33	33.33
2.	<i>Aspergillus flavus</i>	25	41.66	25	33.33
3.	<i>Aspergillus fumigatus</i>	0	0	33.33	16.66
4.	<i>Aspergillus glaucus</i>	16.66	0	0	0
5.	<i>Aspergillus nidulans</i>	25	0	33.33	25
6.	<i>Aspergillus niger</i>	58.33	41.66	50	41.66
7.	<i>Alternaria sp.(longipes)</i>	16.66	0	0	0
8.	<i>Beauveria sp.</i>	33.33	0	0	0
9.	<i>Bipolaris sp.</i>	16.66	0	0	0
10.	<i>Curvularia sp.</i>	41.66	58.33	50	41.66
11.	<i>Fusarium sp.</i>	25	25	25	33.33
12.	<i>Penicillium sp.</i>	41.66	33.33	41.66	33.33

**Fig. 2** Comparison of percentage frequency of fungal isolates

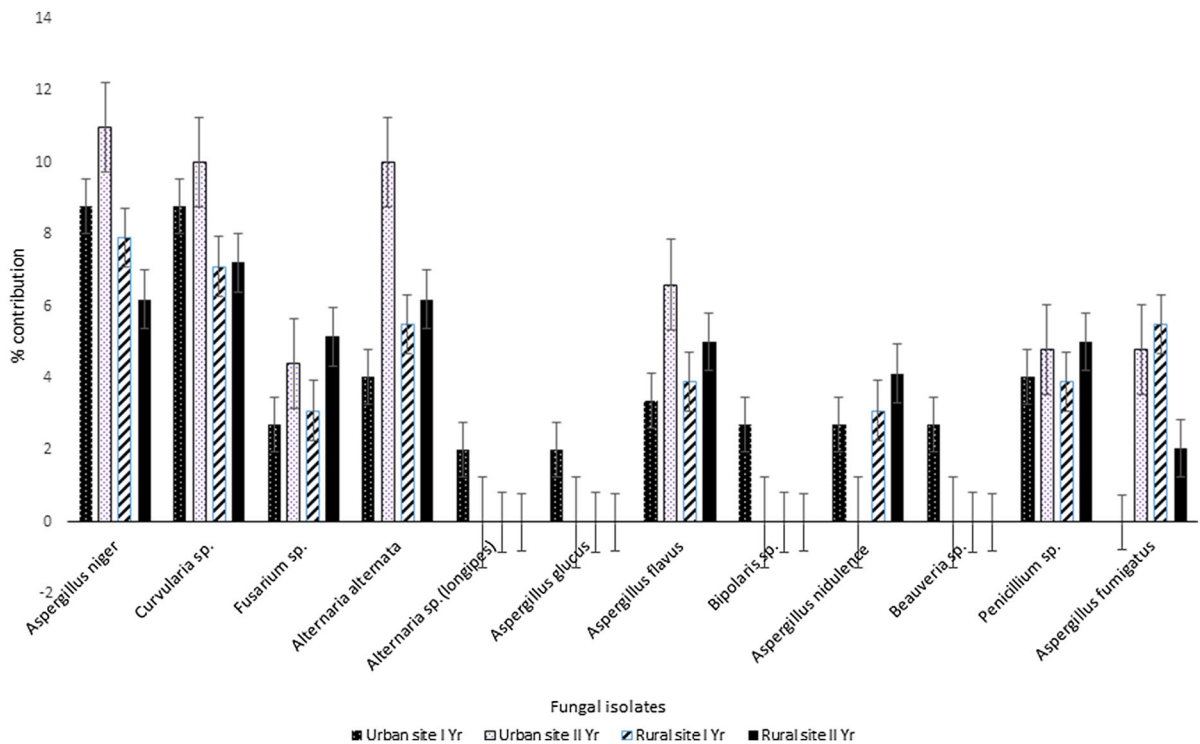
also most frequent fungal isolates were *Aspergillus niger* and *Curvularia sp.* in the first and second year, respectively. In contrary to the urban site, the minimum frequency in the rural area was observed for *Aspergillus fumigatus* (16.6%) during both the years (Table 5, Fig. 2).

3.4.2 Percentage contribution

The results of percentage contribution study of isolates indicated that *Aspergillus niger* contributed maximally (10.9%) in the urban site samples collected during both the years. The minimum percentage

Table 6 Comparison of percentage contributions of fungal isolates in different areas

S. no.	Fungal isolates	Urban site		Rural site	
		I year	II year	I year	II year
1.	<i>Alternaria alternata</i>	4.05	10	5.5	6.18
2.	<i>Aspergillus glaucus</i>	2.02	0	0	0
3.	<i>Aspergillus flavus</i>	3.37	6.6	3.9	5.01
4.	<i>Aspergillus fumigatus</i>	0	4.8	5.5	2.06
5.	<i>Aspergillus nidulans</i>	2.7	0	3.1	4.12
6.	<i>Aspergillus niger</i>	8.78	10.97	7.9	6.18
7.	<i>Alternaria sp.(longipes)</i>	2.02	0	0	0
8.	<i>Beauveria sp.</i>	2.7	0	0	0
9.	<i>Bipolaris sp.</i>	2.7	0	0	0
10.	<i>Curvularia sp.</i>	8.78	10	7.1	7.21
11.	<i>Fusarium sp.</i>	2.7	4.4	3.1	5.15
12.	<i>Penicillium sp.</i>	4.05	4.8	3.9	5.01

**Fig. 3** Comparison of percentage contribution of fungal isolates

contribution was obtained for *Alternaria longipes* and *Aspergillus glaucus* (2.12%) during the first year whereas *Fusarium sp.*, *A. nidulans* and *Bipolaris sp.* (2.7%) during the second year in the samples of the urban area. Similarly, the greatest percentage contribution among the fungal isolates from rural area samples was obtained for *Aspergillus niger* (7.9%),

whereas the least frequent species were *Alternaria sp. (longipes)*, *Beauveria sp.* and *Bipolaris sp.* (2.02%) during both the years (Table 6, Fig. 3).

The highest occurring fungi were *Aspergillus niger*, *Curvularia sp.* followed by *Fusarium sp.*, *Alternaria alternata* and *Penicillium sp.* in both the years of the survey. It was intriguing to note that most of the fungal

isolates identified during the study have been reported to be involved in allergic reactions (Khan et al. 2009; Gupta et al. 2002).

4 Conclusions

The study of the airborne fungal flora of a particular locality is important to determine the quality of air, which in turn affects human life. The present study was therefore undertaken to investigate the occurrence of airborne mycoflora during the different seasons in urban and rural areas of Bilaspur. Our study revealed the existence of a rich population of airborne fungi in the atmosphere of Bilaspur which varies in species composition, abundance and seasonal fluctuations. A number of fungi with allergic potential were observed in the city atmosphere which suggested the possibility of allergy due to fungi in the residents of Bilaspur.

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