Mycopath (2019) 17(2): 89-92 **Research Article**

Air-borne mycoflora from Lahore to Sahiwal

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Abstract

This study was carried out to identify the air-borne fungal flora on the way from Lahore to Sahiwal. Test tubes containing malt extract agar (MEA) medium in slanted position were exposed from the window of bus at Thokar Niaz Beig Lahore, Manga Mandi, Patoki, Gallen Nursery, Okara, Okara Bypass and Yousafwala while traveling on N-5. Fungal species appeared on agar slants were sub-cultured on MEA containing Petri plates and after sufficient growth, identified on the basis of morphological characters with reference to literature. Five fungal species namely *Penicillium expansum*, *Penicillium* sp., *Aspergillus flavus*, *Alternaria citri* and *Alternaria tenuissima* were identified.

Keywords: Aeromycoflora, *Alternaria,* Lahore, *Penicillium,* Sahiwal.

Introduction

Air is the most important medium for the spread of hyphal fragments and fungal spores. It is seldom free of fungal contaminants (Kumari *et al*., 2016). The concentration and composition of air-borne mycoflora is largely determined by the human activities, vegetation, meteorological factors and geographical location (Roy and Bhattacharya, 2020). Concentration of spores in the atmosphere is influenced by the activities involved in their deposition, release and production. Their accumulation and resuspension are mainly affected by the shape and size of spores as well as environmental conditions (Temperini *et al*., 2019). The relationship between fungi and climate is quite complex and should be explored in different perspectives (Wu *et al*., 2019). Changing meteorological conditions such as wind speed, wind direction, rain, temperature, humidity, and flora and fauna in the survey areas have clearly influenced the dispersal and population of fungi (Pyrri and Kapsanaki-Gotsi, 2017). The influence of environmental factors is interrelated and it is often difficult to know which one is the most significant. A better understanding on the interrelationships of these factors would be helpful in determining the relationship of allergies in humans caused by the fungal spores (Hashimoto and Kawakami, 2018). In outdoor air, the spores are ubiquitous and constitute a biologically dominant component of air (Odebode *et al*., 2020). The diameter of most of the *Penicillium* spp., *Aspergillus* spp., and *Alternaria* spp., falls in the range of 2-9 µm, allowing the easy penetration to the human respiratory tract, from where the infection by the pathogens is tremendously easy (Gonçalves *et al*., 2018). The pathogenic and allergic potential of a wide range of fungi is well established such as the spores of *Penicillium* spp., *Aspergillus* spp., and *Alternaria* spp., are responsible for causing asthma, rhinitis and adverse respiratory conditions (Rostami *et al*., 2017). The objective of the present study was to determine the air-borne fungal mycoflora from Lahore to Sahiwal.

Materials and Methods

A 2% malt extract agar (MEA) was prepared by autoclaving 10 g malt extract and 10 g agar in 500 mL distilled water. After removing from the autoclave, the media flask was cooled down at room temperature. Antibacterial drug streptomycin was added to the media flask while it was still in molten state, mixed well and poured the media in 10-mL volume glass test tubes. Tubes were put in a slanted position to solidify the media.

Survey was conducted from Lahore to Sahiwal on N-5 to isolate air-borne mycoflora. Test tubes containing MEA media were exposed in outside air from bus window at various places on N-5 from 8 AM to 2 PM during day time and again from 5 to 9 PM during evening and night hours. Tubes were exposed at Thokar Niaz Beig Lahore, Manga Mandi, Patoki, Gallen Nursery, Okara, Okara Bypass and Yousafwala. After exposure for 5 minutes, tubes lids were closed. Tubes were incubated at 25 ºC for 5 days. The fungal colonies appeared on the slanted media were sub-cultured on MEA plates. After 7 to 10 days, the fungal colonies were identified with reference to available literature (Domsch *et al*., 2008).

Results and Discussion

A total of 5 fungal species were counted as air-borne fungi in the studied area. These included *Penicillium expansum*, *Penicillium* sp., *Aspergillus flavus*, *Alternaria citri* and *Alternaria tenuissima*. Shabbir *et al.* (2009) reported 15 fungal species of four genera namely *Alternaria*, *Penicillium*, *Cladosporium* and *Aspergillus* in different months of the year 2006-2007 in Lahore. Earlier, Bajwa *et al.*

(1997) reported 28 fungal species from thickly populated areas of Lahore. These included species of *Alternaria*, *Aspergillus*, *Curvularia*, *Chochliobolus*, *Dematium*, *Fusarium* and *Phoma*. Likewise, Bajwa *et al.* (1995) reported 39 species from non-commercialized areas of Lahore. In other countries, a number of similar studies were carried out and a variety of fungal species were found in the air of those areas. Ianovici and Tudorica (2009) found *Cladosporium* sp. as the most abundant fungal species in the air of Timisoara, Romania followed by species of *Drechslera*, *Alternaria* and *Epicoccum*. Chakraborty *et al.* (2003) identified 26 fungal species in the air of Calcutta, India with species of Basidiomycetes as the frequently occurring species. Ababutain (2011) conducted a study in three eastern provinces of Saudi Arabia and reported 30 air-borne fungal species with *Cladosporium* sp. and *Aspergillus* sp. as the dominating fungi. Low number of fungal species in the present study as compared to previous studies could be attributed to difference in methods of isolation of aeromycoflora. In the present study, narrow-mouth test tubes were exposed while in previous studies, Petri plates or other instruments were used to capture the air-borne fungi. In 1970s, various similar studies were carried out. However, some fungal species which were identified at that time were absent in the present study and other studies which were carried out in recent years. These were species of genera *Acrocylindrium, Beltrania, Nigrospora, Cylindrosporium, Cephalosporium, Humicola, Carcosporella, Lacellina, Trichothecium* and *Torula* (Tasneem, 1971; Samina, 1975).

Two species of the genus *Penicillium* namely *P. expansum* and an unidentified *Penicillium* sp. were found in the air of studied areas. Reboux *et al.* (2019) reported that the genus *Penicillium* is known to produce the most potent mycotoxins that can easily migrate through the air. Hence, deeply understanding the presence of *Penicillium* species in air will lay down the foundation for development of new management strategies against pathogenic fungi. In the present study, both the species were identified on the basis of macroscopic and microscopic characters. The observed features of *P. expansum* were the presence of conidiophores that arise from the superficial mycelium. The penicilli were tervertivillate and terminal with smooth septate stipes. Phialides were ampulliform and smooth. Conidia were borne in columns, globose to subglobose and having smooth structures. The size of conidia from each isolate was in the range of 2.48–4.36 × 2.31–3.72 µm, with an average size of 3.31 × 2.94 µm (Fig. 1 A-C). Previously, Matthei and Roberts (1992) also explained the important features used for the identification of *Penicillium* species under the light microscope are the presence of numerous brush-like closely packed structures that produce spores called penicilli. They possess branching or simple structures that are slightly elongated, end in the shape of flask-like clusters, known as conidiophores or phialides. The conidia are produced in chains and emanate from phialides tips. The youngest spores are found at phialides whereas the oldest spores occupy the phialides apex.

One species of genus *Aspergillus* namely *A. flavus* was identified in the air of studied areas. When viewed under light microscope the observed characters were the presence of smooth colorless spores and conidiophores. The conidiophores were rough whereas the conidial heads were dark brown in color and globose in shape. The spores were in the range of 180–364 mm in size. The stipes color was grey around the apex with a smooth surface. The conidial surface was spinose or either smooth (Fig. 1 D-F). The similar findings were also given by Achar *et al.* (2009). Jaksic *et al.* (2019) reported that Aspergilli can be found throughout in nature with the presence of abundant spores in the air. Being saprophytes, they obtain nutrition from dead decayed organic matter and cause infections in humans and animals (Agbetiameh *et al.*, 2019).

Two species of genus *Alternaria* namely *A. citri* and *A. tenuissima* were recognized in the air above studied areas (Fig. 1 G-L). Apangu *et al.* (2020) found that the changing weather and climatic conditions can results in the wide dispersal of *Alternaria* spores from infected to uninfected areas. The observed characters of *A. citri* showed the presence of septate hyphae that were brown in color. Conidiophores were also pale to dark brown in color with a straight and/or flexuous appearance. They had simple or branched conidia in the range of 8–11 × 26–33 µm with both transverse and longitudinal septations. The conidia were present in acropetal chains or observed singly which might produce germ tubes. They were smooth from surface, brown in color, obclavate, pigmented and smooth or rough with a short beak or having no beak at all (Fig. 1 G-I). Slifkin, (1971) also studied the same features that were quite similar with the present findings. The observed features of *A. tenuissima* were quite different from *A. citri* under light microscope. A wide variation was measured in spore length ranging from 8.4–37.8 µm. The fungal spores were thin-walled, relatively small, smooth, beaked and light in color. Spore chains were either obscured or absent. The mycelium was sterile, composed of relatively thin cells as some of them were having a diameter of 22 µm. The conidial curve was varied in length from 12–27 µm consisting of a series of overlapping and smaller conidiophores (Fig. 1 J-L).

Conclusion

This study showed that the air above Lahore to Sahiwal is enriched with *P. expansum*, *Penicillium* sp., *A. flavus*, *A. citri* and *A. tenuissima.*

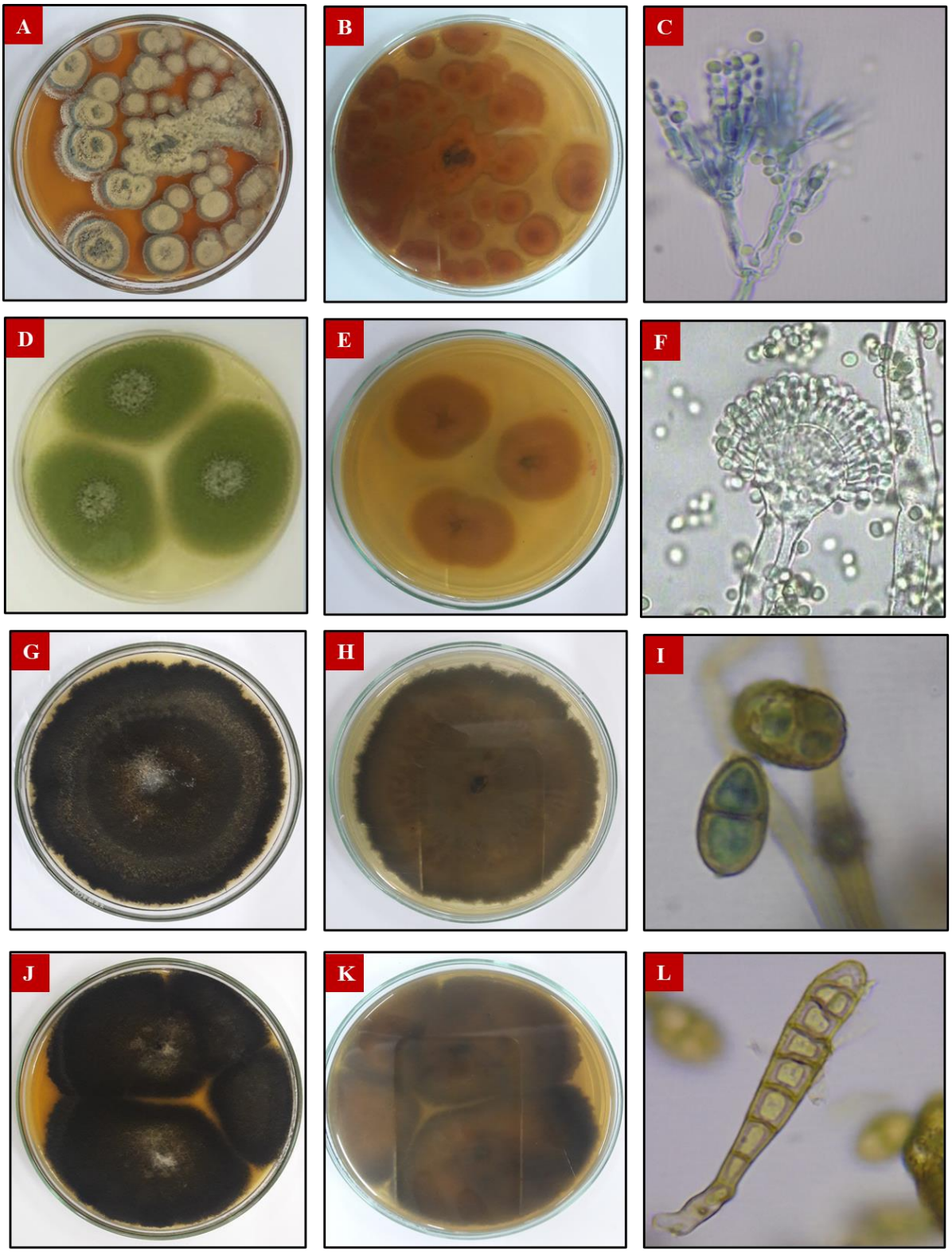

Fig. 1: Morphological characterization of air-borne fungal flora on MEA. Front, reverse and 100X microscopic image of *Penicillium expansum* (**A-C**), *Aspergillus flavus* (**D-F**), *Alternaria citri* (**G-I**) and *Alternaria tenuissima* (**J-L**).

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