Original Article **Asian J Agri Biol, 2013, 1(3):105-117.**

MORPHOLOGICAL IDENTIFICATION OF *ASPERGILLUS* SPECIES FROM THE SOIL OF LARKANA DISTRICT (SINDH, PAKISTAN)

Hina Afzal[1*], Saleem Shazad[2] and Syeda Qamar Un Nisa[3]
[1] *Department of Botany, University of Karachi, Karachi, Pakistan*
[2] *Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Pakistan*
[3]*The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan*

ABSTRACT

Aspergillus is a large genus of anamorphic fungi. Aspergilli have great importance in many fields like plant, animals, and human health etc. The present study was conducted to identify *Aspergillus* isolates from district Larkana Sindh Pakistan. There are no reports that cover the whole mycoflora of Sindh province. In this study two differential media, Czapek Solution Agar (CZA) and Malt Exract Agar (MEA) were used for the identification of *Aspergillus* species using macroscopic characteristics such as colony growth, conidial color, colony reverse, and microscopic characteristics including conidiophore, vesicle, matulae, phialides and conidia. All the eight *Aspergillus* species viz., *Aspergillus ficcum, Aspergillus flavus, Aspergillus flavus var. columnaris, Aspergillus terreus* var. *aureus, Aspergillus fumigatus, Emericella nidulans, Emericella rugulosa and Apergillus terricola* var. *americana* have been reported for the first time from Larkana whereas, *Aspergillus terricola* var. *americana* appeared to be a new records from Pakistan.

Keywords: *Apergillus*, *Aspergillus ficcum, Aspergillus terricola* var. *americana, Emericella rugulosa*, Morphological Observations, Czapek Solution Agar, Malt Extract Agar.

INTRODUCTION

Aspergillus is one of the oldest genera of fungi described by Micheli in 1729 (Ross, 1951). During 20th century Clark (Clark, 1966), Thom and Raper (Thom & Raper, 1945) and Raper and Fennell (Raper & Fennell, 1965) divided *Aspergillus* into eighteen groups viz., *Aspergillus clavatus, Aspergillus glaucus, Aspergillus ornatus, Aspergillus cervinus, Aspergillus restrictus, Aspergillus fumigatus, Aspergillus ochraceous, Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Aspergillus wentii, Aspergillus cremeus, Aspergillus sparsus, Aspergillus versicolor, Aspergillus nidulans, Aspergillus ustus, Aspergillus flavipes and Aspergillus terreus*. Gams et al., (Gams, Christensen, Onions, & Samson, 1985) divided these groups into six subgenera and eighteen sections. They introduced a new subgenus section called circumdati (instead of *Aspergillus ochraceous* group). The genus *Aspergillus* encompasses organisms whose characteristics are of high pathological, agricultural, industrial, pharmaceutical, scientific and cultural importance and play a important role in the degradation of organic substrate, particularly plant material (Bignell, 2010; Goldman & Osmani, 2008; Samson & Varga, 2009). Aspergilli are known for their ability to secrete a variety of biologically active chemical compounds including antibiotics, mycotoxins, immune-suppressants, and cholesterol lowering agents (Goldman & Osmani, 2008). Some species of subgenus Circumdati are also used in industry especially in biotransformations, Section *Aspergillus flavi* (*Aspergillus oryzae, Aspergillus sojae* and *Aspergillus tamarii*) are used in oriental food fermentation processes (Samson, Hong, & Frisvad, 2006; Varga, Juhasz, Kevei, & Kozakiewicz, 2004). More than 250 Aspergillus species have been reported from different parts of the world (Samson & Pitt, 2000), only 79 species have been reported from Pakistan so far, including 34 species from Sindh province (J. H. Mirza, 2007; J. Mirza & Qureshi, 1978; Nazir, Mirza, Akhtar, Bajwa, & Nasim, 2007). In Sindh species of Aspergillus have been recorded only from Kotri Barrage (Suhail, Irum, Jatt, Korejo, & Abro, 2007) and Karachi (Ahmed & Rizvi, 1969), whereas no report is available from

*Corresponding author: e-mail: hina.afzal.siddiqui@gmail.com

other district.

Generally basic and essential tool for identification of Aspergillus species are macroscopic characteristics such as colony diameter, conidial color, exudates, colony reverse and microscopic characteristics including conidiophore, vesicle, metulae, phialides and conidia (Raper & Fennell, 1965; McClenny, 2005; Diba, Kordbacheh, Mirhendi, Rezaie, & Mahmoudi, 2007; Domsch, Gams, Anderson, & Heidi, 1980). Although molecular methods continue to improve and become more rapidly available, microscopy and culture remain commonly used and essential tools for identification of the fungi like *Aspergillus* species (Diba et al., 2007).

A survey by the American society for Microbiology documented that 89% of laboratories performing mycological examination (morphology based), 16% of them use serological tests and fewer than 5% use molecular test for identification of microbial pathogens (ASM, 2004 Washington). Only 3% of reporting laboratories use home brew molecular testing for microbial pathogens (Warris, Voss, & Verweij, 2001). The purpose of this study was to contribute to the checklist of *Aspergillus* species of Sindh. This is the first report of *Aspergillus* species from Larkana district. The species were identified on the basis of morphology which comprises both macroscopic and microscopic characters. The fungi herein appeared to the first record of *Aspergillus* species from Larkana including *Aspergillus terricola* var.*americana* that has been reported for the first time from Pakistan.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from different locations of district Larkana viz., Larkano, Ratodero, Dokri, and Bakrani in since 2011 to 2012. Map of investigation area is shown in Fig. 9.

Isolation of fungi via Serial Dilution Technique

One gram of soil was added into the tube containing 9mL of sterile distilled water to obtain 1/10 dilution (stock solution) and a series of 1/00, 1/1000, 1/10,000, and 1/100,000 dilutions was prepared by adding 1mL of solution to 9 ml of sterile distilled water respectively (Waksman & Fred., 1922). One mL suspension from each dilution was transferred onto Water Agar (WA) (Johnston & Booth, 1983) and Potato Dextrose Agar (PDA) (Johnston & Booth, 1983) media. Plates were incubated at room temperature for 7 to 15 days (15 days for the production of sclerotia and ascospores) at 25 ◦ C.

Morphological Identification of *Aspergillus* species

Aspergillus species were identified using manual about the genus aspergilli (Raper & Fennell, 1965; McClenny, 2005; Diba et al., 2007; Domsch et al., 1980; Samson & Pitt, 2000) and Gams etal., classification system (Gams etal., 1985). Aspergillus species were cultured on two differential media ie. Czapek Solution Agar (CZA) (Raper & Fennell, 1965) and Malt Extract Agar (MEA) (Johnston & Booth, 1983). After seven days of incubation, plates (in triplicates) were observed for macroscopic characteristics such as colony diameter, exudates, colony reverse and microscopic characteristics including conidiophore, vesicle, metulae, phialides and conidia. For microscopic characteristics slides were stained with cotton blue and mounted in lectophenol. Photographs were taken with digital camera Canon Power Shot A550, 7.1 mega pixels. A morphological examination of species was first made with naked eye and at low magnification power of microscope after that detailed examination were done according to Raper and Fennell (Raper & Fennell, 1965) and Gams etal., (Gams etal., 1985) by measuring the dimensions of the microscopic structures, photographing the microscopic structures and using relevant literature as reference.

Color chart

RHS Mini Color Chart was used in this study to record the colony (Royal Horticultural Society (RHS) Mini Color Chart, 2005).

RESULTS

Eight species out of fifty isolated from ten soils samples collected from different locations of Larkana district were identified as Aspergillus species by using two differential media CZA and MEA. It included *Aspergillus ficcum, Aspergillus flavus, Aspergillus flavus* var. *columnaris, Aspergillus terreus* var. *aureus, Aspergillus fumigatus, Emericella nidulans, Emericella rugulosa* and *Apergillus terricola* var. *americana.* Effects of CZA and MEA medium on colony growth of different species of *Aspergillus* were observed.

Morphological Characters of *Aspergillus ficcum*

Aspergillus ficcum (Reich) Hennings, in Hedwigia 34 :86 (1895), Synonym Ustilago ficcum Reichardt, in Verhandl. K

Macroscopic characters:
Colonies on Czapek solution agar attaining 55 mm after seven days (Fig. 1a), colony color black (RHSN186B), reverse mostly hyaline to light yellow (RHS4D), Colonies on MEA 66 mm in diameter after seven days at 25 ◦C (Fig. 1b). Colony color black (186A), reverse uncolored.

Microscopic characters:
Conidial heads on CZA radiate, 300 – 400µ conidiophores 1000 – 1400µ long, 8 – 12µ wide with 1.5 – 2.5(3)µ thick wall. Vesicle globose, 40 – 55µ in diameter. Sterigmata biseriate. Phialides ampuliform, 15 – 25(35)µ long by 4 – 6µ wide. Matulae club shaped, 7 – 10µ long by 2 – 3µ wide (Fig. 1c). Conidia globose, 3.5 – 4µ in diameter.
Conidial heads on MEA radiate, 300 – 500µ in diameter. Conidiophores 1000 – 1450µ long, 8 – 12µ wide with 2 – 2.8µ thick wall. Vesicle globose, 40 – 60µ in diameter. Sterigmata biseriate. Phialides ampuliform, 15 – 25µ long by 3 – 4µ wide. Matulae club shaped. 5 – 7µ long by 2 – 3µ wide. Conidia globose, 3.5 – 4µ in diameter.

Morphological Characters of *Aspergillus flavus*

Aspergillus flavus Link, in observation, p. 16 (1809); also cited by Link in species Plantarum vI, p. 66 (1864), Synonym Eurotium Aspergillus flavus De Bary and Woronin, in Beitrage Zur Morphologic and Physiologic der Pilze, III Reihe, p. 380 (1870).

Macroscopic characters:
Colonies on CZ agar 45 mm in diameter after seven days at 25 ◦C (Fig. 2a). Colony color on CZA showed variation in different strains, yellow (RHS12A) to green (RHS137C), or dark green (RHS137A), reverse hyaline. Sclerotia white to wood brown, globose-subglobose in shape and less than 1mm (400 – 750µ) in diameter. Exudates transparent to red brown droplets in heavily sclerotial strain. Colonies on MEA 57 mm in diameter after seven days at 25 ◦ C (Fig. 2b), Colony color on MEA dark green (RHS137A), reverse hyaline.

Microscopic characters:
Conidial heads typically radiate, 250 – 350µ in diameter. Conidiophore uncolored, coarsely roughend, less than 1mm long by 8 – 12µ wide with 1 – 2µ thick wall. Vesicle subglobose-globose, 25 – 30µ in diameter. Sterigmata biseriates. Matulae 5 – 8µ long by 3 – 4.5µ. Phialides ampuliform, 6 – 10µ long by 3 – 4µ wide. Conidia globose or subglobose, 3 – 4.5µ in diameter (Fig. 2c), conspicuously echinulate.
Conidial heads on MEA typically radiate, 250 – 350µ in diameter. Conidiophores uncolored, coarsely roughened, less than 1mm long by 8 – 14µ wide with 1 – 1.5µ thick wall. Vesicle subglobose-globose, 25 – 30µ in diameter. Sterigmata biseriates. Matulae 5 – 8µ long by 3 – 4µ. Phialides ampuliform 6 – 10µ by 3 – 4µ. Conidia globose to subglobose, 3.5 – 4.5µ in diameter.

Morphological characters of *Aspergillus flavus* variety *columnaris*

Aspergillus flavus var. columnaris Raper and Fennell (1965) p (366-367)

Macroscopic characters:
Colonies on Czapek solution agar attaining 56 mm after seven days at 25 ◦C (Fig. 3a), colony color green (RHS146C), reverse hyaline, Colonies on MEA attaining 64mm at 25 ◦C after seven days (Fig. 3b). Colony color dark green (RHS137A), reverse hyaline.

Microscopic characters:
Conidial heads on CZA both columnar and radiate, columnar heads 350 – 450µ long by 30 – 50µ in diameter and radiate heads 300 – 400µ in diameter. Conidiophore smooth,

hyaline, 400 – 500µ long by 6 – 10µ wide with 0.5 – 1.0µ thick wall. Vesicle globose to subglobose, 15 – 25µ in diameter. Sterigmata mostly uniseriates, phialides ampuliform, 7 – 12µ long by 3 – 4µ, but some biseriate heads also present. Matulae club shaped, 6 – 8µ long by 2 – 3µ wide. Phialides ampuliform, 7 – 12µ long by 2 – 3µ wide. Conidia elliptical, 6 – 8u or globose, 4 – 5µ in diameter (Fig. 3c). Conidial heads on MEA typically columnar, 500 – 700µ long by 150 – 200µ in diameter. Conidiophore smooth, hyaline, 400 – 550µ long by 7 – 10µ in diameter. Vesicle globose to subglobose, 15 – 25µ in diameter. Sterigmata mostly uniseriates, phialides ampuliform, 6 – 15µ long by 3 – 4µ, but some biseriate heads also present. Matulae club shaped, 5 – 7µ long by 2 – 3µ wide. Phialides ampuliform, 6 – 12µ long by 2 – 3µ wide. Conidia elliptical 6 – 7 u or globose, 4 – 5µ in diameter.

Morphological characters of *Aspergillus terricola* variety *Americana*

Aspergillus terricola var. *americana Marchal, in Thom and Church, Am. J. Botany 8: 125 (1921). See also Thom and Church, pp. 192-193 (1926); Thom and Raper, pp. 253-254, Fig. 68-D-F (mislabeled A. terricola (1945).*

Macroscopic characters:

Colonies on Czapek solution agar attaining 45 mm after seven days, Colony color on CZA brown (RHSN199A to RHSN199C) in shade, reverse uncolored (Fig. 4a). Hyaline to light honey color droplet type exudates present, Colonies on MEA 56 mm after seven days, Colony color on MEA green (RHS146C), reverse uncolored (Fig. 4b).

Microscopic characters:

Conidial heads on CZA radiate, 150 – 250µ in diameter. Conidiophore brown, smooth, 300 – 700µ long and 5 – 8µ wide.Vesicle brown, subglobose, 25 – 30µ in diameter. Sterigmata biseriate, Matulae brown, club shaped, 6 – 10µ long and 5 – 6µ wide, Phialides brown, pear shaped, 8 – 10µ long by 4 – 5µ (fig. 4c), Conidia prolate to globose, brown, 4.5 – 5.6µ in diameter (Fig. 4d).

Conidial heads on MEA, radiate 150 – 300µ in diameter. Conidiophore brown, smooth,400 – 650µ long and 5 – 8µ wide, Vesicle brown, subglobose, 25 – 35µ in diameter, sterigmata biseriate, Matulae brown, club shaped, 8 – 12µ long and 5 – 6µwide, Phialides brown, pear shaped, 6 – 11µ long by 4 – 5µ, Conidia prolate to globose, brown, 5 – 6µ in diameter having warted ornamentation.

Morphological Characters of *Aspergillus terreus* variety *aureus*

Aspergillus terreus var. aureus Thom and Raper, in A Manual of Aspergilli, pp. 198-200, Fig. 57B (1945).

Macroscopic characters:

Colonies on CZA attaining 31 mm in diameter after seven days at 25 ◦C, Colony color light yellow (RHS 2A) to dark orange yellow(RHS21B), reverse light yellow (RHS20A), exudates transparent (Fig. 5a).

Colonies on MEA attaining 45 mm in diameter after seven days at 25 ◦C. Colony color orange brown (RHS173B), reverse yellow brown (RHS167B), exudates transparent (Fig. 5b).

Microscopic characters:

Conidial heads on CZA compact short columnar, 80 – 150µ long by 40 – 60µ in diameter. Conidiophore hyaline, smooth, 100 – 200µ long and 4 – 5µ wide with 1µ thick wall. Vesicle globose to hemispherical, 10 – 18µ in diameter. Sterigmata biseriates, Matulae ampuliform, 5 – 8µ by 1.5 – 2µ wide. Phialides ampuliform, 5 – 7µ by 1.5 – 2µ wide. Conidia globose, 1.5 – 2.5µ in diameter (Fig. 5c).

Conidial heads on MEA compact columnar, 80 – 160µ long by 40 – 50µ in diameter. Conidiophore hyaline, smooth, 100 – 225µ long and 4 – 5µ wide with 1µ thick wall. Vesicle globose to hemispherical, 10 – 20µ in diameter. Sterigmata biseriates. Matulae ampuliform, 5 – 7µ by 1.5 – 2µ wide. Phialides ampuliform, 5 – 8µ by 1.5 – 2µ wide. Conidia globose, 1.6 – 2.5µ in diameter.

Morphological Characters of *Aspergillus fumigatus*

Aspergillus fumigatus Fresenius, in Beitrage Zur Mycologie, p. 81, plate 10, figs. 1-11, Frankfurt (1863). Thom and Church, The Aspergilli, p. 129 (1926). Thom and Raper, Manual of the Aspergilli, pp. 148 - 151, plate IV, Fig. 37 (1945).

Macroscopic characters:
Colonies on CZA attaining 60 mm after seven days at 25 ◦ C. Colony color grey (RHSN200C), reverse hyaline (Fig. 6a).
Colonies on MEA 75 mm after seven days at 25 ◦C. Colony color blue grey (RHSN189B), colony reverse hyaline (Fig. 6b).

Microscopic characters:
Conidial heads on CZA columnar, 100 – 200µ long and 50 – 60µ wide. Conidiophore hyaline, 150 – 300µ long and 3 – 5µ wide. Vesicle ovate to flask shape 15 – 25µ in diameter. Sterigmata uniseriates. Phialides ampuliform, 4 – 6µ long by 2 – 3µ wide. Conidia globose to prolate, 1.5 – 2.5µ in diameter (Fig. 6c).
Conidial heads on MEA columnar, 200 – 300µ long and 50 – 60µ wide, conidiophore hyaline, 200 – 325µ long and 3 – 5µ wide, vesicle ovate to flask shape 15 – 20µ in diameter, sterigmata uniseriates, phialides ampuliform, 4 – 7µ long by 2 – 3µ wide, conidia globose to prolate, 2 – 3µ in diameter.

Morphological Characters of *Emericella nidulans*
Aspergillus nidulans (Eidam) Wint. In Rab. Krypt. - F1. 1(2) : 62 (1884).

Macroscopic characters:
Colonies on CZA 35mm after seven days at 25 ◦C. Colony color on CZA green (RHS 16C to 137C), Reverse color orange red (RHS37A) to purple red (RHS55A) or purple (RHSN79C) with age (Fig. 7a).
Colonies on MEA 41mm after seven days at 25 ◦ C. Colony color on MEA green (RHS146C), reverse color orange brown (RHS166C) (Fig. 7b).

Microscopic characters:
Conidial heads on CZA short columnar, 55 – 80µ long by 30 – 55µ wide. Conidiophore smooth, some shade of brown, 100 – 130 by 2.5 – 3µ in diameter. Vesicle ovate to flask shape 10 – 15µ in diameter. Sterigmata biseriate, matulae 5 – 6µ long by 2 – 3µ wide. Phialides 5 – 6µ long by 2 – 3µ wide. Conidia globose, small 3 – 3.5µ in diameter (Fig. 7c). Perfect state developed within 10 – 15 days. Cleistothecia 100 – 150µ in diamerter. Ascospores orange (RHS32A) to orange red (RHS41B) in color, having two 0.5 – 0.8µ thick equatoraial crests, surface smooth, 5 – 6µ long by 4 – 4.3µ wide (Fig. 7d), Hu¨lle cells globose, 15 – 20µ in diameter.
Conidial heads on MEA short columnar, 55 – 80µ long by 30 – 55µ wide. Conidiophore smooth, some shade of brown, 100 – 130 by 2.5 – 3µ in diameter, Vesicle ovate to flask shape 10 – 15µ in diameter. Sterigmata biseriate, matulae 5 – 6µ long by 2 – 3µ wide. Phialides 5 – 6µ long by 2– 3µ wide. Conidia globose, small 3 – 3.5µ in diameter. Perfect state developed within 10 – 15 days. Cleistothecia 100 – 150µ in diamerter. Ascospores orange (RHS32A) to orange red (RHS41B) in color, having two 0.5 – 0.8µ thick equatoraial crests, surface smooth, 5 – 6µ long, by 4 – 4.3µ wide, Hu¨lle cells globose, 15 – 20µ in diameter.

Morphological Characters of *Emericella regulosa*
Aspergillus regulosus Thom and Raper, in Mycologia 31:660 - 663, Fig. 4 (1939)

Macroscopic characters:
Colonies on CZA attaining 26 mm after seven days at 25C. Colony color on CZA dark green (RHS144A), reverse Brown orange (RHS34D) to dark purple brown (RHSN77A) (Fig. 8a).

Colonies on MEA attaining 36 mm after seven days at 25 ◦ C. Colony color on MEA green (RHS146C), reverse light yellow (RHS20A) (Fig. 8b).

Microscopic characters:
Conidial heads on CZA short columnar, 40 – 70µ long by 40 – 50µ wide. Conidiophore smooth, some shade of brown, 50 – 80 by 3 – 4µ in diameter. Vesicle ovate to flask shape 8 – 10µ in diameter. Sterigmata biseriate, Matulae 6 – 7µ long by 3 – 4µ wide, Phialides 6 – 7µ long by 3 – 4µ wide. Conidia globose, small 3 – 4µ in diameter (Fig. 8c). Perfect state developed within 10 – 15 days. Cleistothecia 200 – 325µ in diamerter. Ascospores purple brown (RHSN77A) in color, having two 0.5 – 0.8µ thick equatoraial crests, rugulose surface, spores body having double crests 5 – 6µ long, excluding crest 4 – 4.4µ long by 3.6 – 3.8µ wide (Fig. 8d), Hu¨lle cells globose, 15 – 20µ in diameter.

Conidial heads on MEA short columnar, 40 – 80µ long by 45 – 55µ wide. Conidiophore

smooth, some shade of brown, 40 – 80 by 3 – 4µ in diameter. Vesicle ovate to flask shape 7 – 10µ in diameter. Sterigmata biseriate, matulae 5 – 7µ long by 3 – 4µ wide. Phialides 6 – 7µ long by 3 – 4µ wide. Conidia globose, small 3 – 4µ in diameter. Perfect state developed within 10 – 15 days. Cleistothecia 250 – 350µ in diamerter. Ascospores purple brown (RHSN77A) in color, having two 0.5 – 0.7µ thick equatoraial crest , rugulose surface, spores body having double crests 4 – 4.4µ long by 3.5 – 3.8µ wide, Hu¨ell cells globose, 18 – 20µ in diameter.

Macroscopic characters of all the above Aspergillus species are shown in Table 1, while microscopic characters of same species are shown in Table 2.

Table 1: Macroscopic Characters of Aspergillus Species

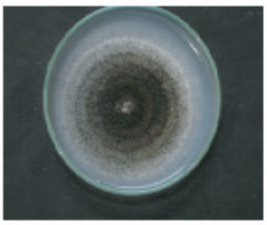
SR	Name of Spp	Medium	Colony Color	Colony reverse color	Sclerotia shape	Sclerotia diameter
1	*A.ficuum*	CZA	black	hyaline to light yellow	-	-
		MEA	black	hyaline	-	-
2	*A.flavus*	CZA	yellow to green	hyaline	globose	400 - 700
		MEA	dark green	hyaline	-	-
3	*A. flavus* var. *columnaris*	CZA	green	hyaline	-	-
		MEA	dark green	hyaline	-	-
4	*A.terricola* var. *americana*	CZA	brown green	hyaline	-	-
		MEA	green	hyaline	-	-
5	*A.terreus* var. *aureus*	CZA	light yellow to dark orange yellow	Light yellow	-	-
		MEA	light yellow	Yellow brown	-	-
6	*A.fumigatus*	CZA	grey	hyaline	-	-
		MEA	blue grey	hyaline	-	-
7	*Emericella nidulans*	CZA	green	purple red	-	-
		MEA	green	yellow	-	-
8	*E. regulosa*	CZA	green	brown orange to dark purple brown	-	-
		MEA	green	yellow brown	-	-

absent characters are shown by –

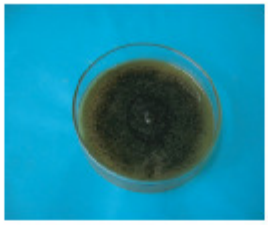
Table 2: Microscopic Characters of Aspergillus Species

SR	Name of Spp	Medium	Conidial head length by width	Conidiophore lengh by width	Conidiophore color	Vesicle diameter	Matulae length by width	Phialide length by width	Conidial diameter	Hulle cell diameter	Cleistothecial diameter	Ascospore color	Ascospore length by width
1	*A.ficuum*	CZA	300-400 by 300-400	1000-1400 by 8-12	hyaline	40-55 (60)	7-10 by 2-3	15 - 25 (35) by 4-6	3.5-4.0	-	-	-	-
		MEA	300-500	1000-1450 by 8-2	hyaline	40-60	5-7 by 2-3	15-25 by 3-4	3.5-4.0	-	-	-	-
2	*A.flavus*	CZA	250-350 by 200-300	500-800 by 8-12	hyaline	20-30	5-8 by 3-4	6-10 by 3-4	3.0-4.5	-	-	-	-
		MEA	250-300 by 250-300	500-900 by 8-14	hyaline	25-30	5-7 by 3-4	6-12 by 3-5	3.5-4.5	-	-	-	-
3	*A. flavus* var. *columnaris*	CZA	350-450 by 30-50	400-500 by 6-10	hyaline	15-25	6-8 by 2-3	7-12 by 3-4	4-5	-	-	-	-
		MEA	500-700 by 150-200	400-550 by 7-10	hyaline	15-25	5-7 by 2-3	6-15 by 3-4	4-5	-	-	-	-
4	*A.terricola* var. *americana*	CZA	150-250 by 200-300	300-700	hyaline	25-30	6-10 by 5-6	8-10 by 4-5	4.5-5.6	-	-	-	-
		MEA	150-300 by 200-300	400-650	hyalin	25-35	8-12 by 5-6	6-11 by 4-5	5-6	-	-	-	-
5	*A.terreus* var. *aureus*	CZA	80-150 by 40-60	100-200 by 4-5	hyaline	10-18	5-8 by 1.5-2	5-7 by 1.5-2	1.5-2.5	-	-	-	-
		MEA	100-160 by 40-50	100-225 by 4-5	hyaline	10-20	5-7 by 2-2.5	5-8 by 2-2.5	1.6-2.5	-	-	-	-
6	*A.fumigatus*	CZA	150-250 by 45-55	150-300 by3-5	hyaline	15-25		4-6 by 2-3	1.5-2.5	-	-	-	-
		MEA	200-300 by 50-60	200-325 by3-5	hyaline	15-20		4-7 by 2-3	2-3	-	-	-	-
7	*Emericella nidulans*	CZA	80-110 by 50-90	100-130 by 3-5	brown	8-12	5-8 by 2-3	4-7 by 2-3	2-3	12-18	200-300	orange to orange red	4.5 - 5.5 by 3.5 - 3.6
		MEA	80-110 by 45-55	100-140 by 3-5	brown	8-10	5-7 by 2-3	4-7 by 2-3	2-3	10-20	150-300	orange to orange red	4.5 - 5.6 by 3.5 - 3.6
8	*E. regulosa*	CZA	40-70 by 50-90	100-130 by 3-5	yellow brown	10-12	5-8 by 2-3	4-7 by 2-3	2.5-3.0	12-18	200-300	purple brown	4.0 - 4.4 by 3.6 - 3.8
		MEA	40-80 by 45-55	100-140 by 3 - 5	yelow brown	8-10	5-7 by 2-3	4-8 by 2-3	2.5-3.0	10-20	150-300	purple brown	4.0 - 4.4 by 3.5 - 3.8

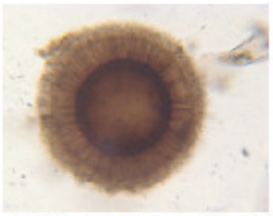
absent characters are shown by –



(a) Colony at 25C after 7 days on CZA

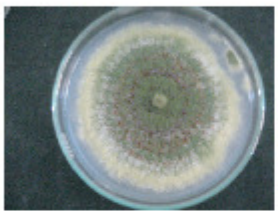


(b) Colony at 25C after 7 days on MEA



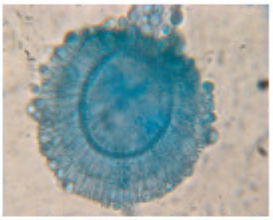
(c) Biseriate head with globose vesicle

Figure 1: Pictures of Aspergillus cuum



(a) Colony at 25C after 7 days on CZA

(b) Colony at 25C after 7 days on MEA

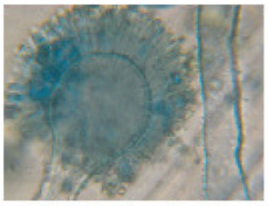


(c) Biseriate head with globose vesicle

Figure 2: Pictures of Aspergillus avus

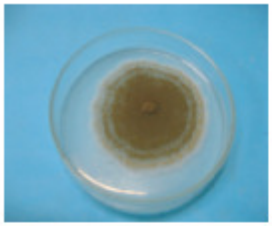
(a) Colony at 25C after 7 days on CZA

(b) Colony at 25C after 7 days on MEA

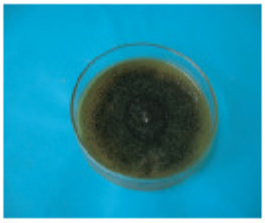


(c) Biseriate head with globose vesicl

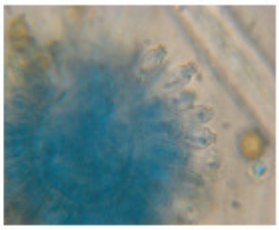
Figure 3: Pictures of Aspergillus avus variety columnaris



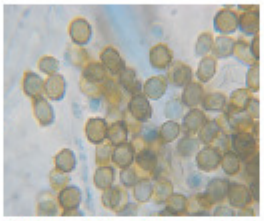
(a) Colony at 25C after 7 days on CZA



(b) Colony at 25C after 7 days on MEA



(c) Biseriate head with with pear shape phialides

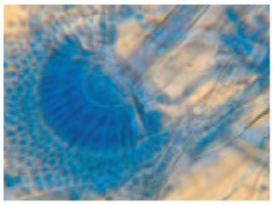


(d) Conidia with prolate to globose shape

Figure 4: Pictures of Aspergillus terricola variety americana

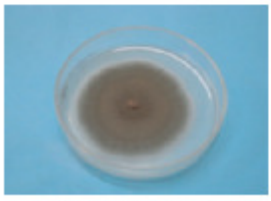
(a) Colony at 25C after 7 days on CZA

(b) Colony at 25C after 7 days on MEA

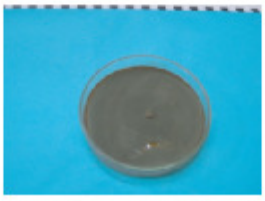


(c) Biseriate head with globose vesicle

Figure 5: Pictures of Aspergillus terreus variety aureus



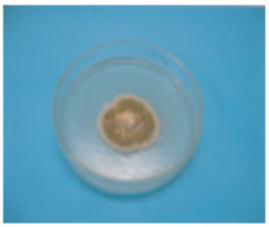
(a) Colony at 25C after 7 days on CZA



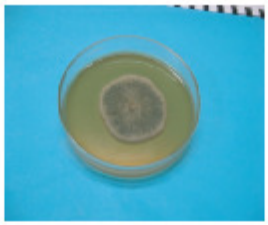
(b) Colony at 25C after 7 days on MEA

(c) Uniseriate head with ovate to ask shape vesicle

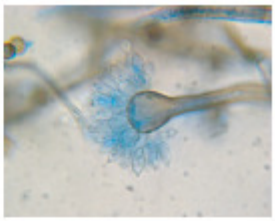
Figure 6: Pictures of Aspergillus fumigatus



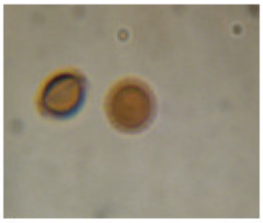
(a) Colony at 25C after 7 days on CZA



(b) Colony at 25C after 7 days on MEA

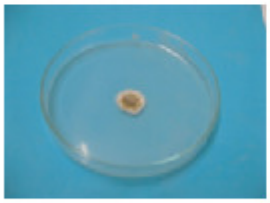


(c) Biseriate head with globose shape vesicle

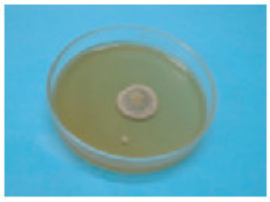


(d) Ascospores with smooth surface

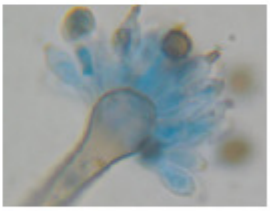
Figure 7: Pictures of Emericella nidulans



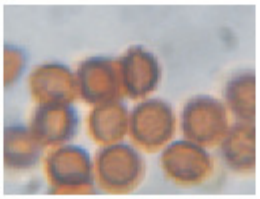
(a) Colony at 25C after 7 days on CZA



(b) Colony at 25C after 7 days on MEA



(c) Biseriate head with globose shape vesicle



(d) Ascospores with rugulose surface

Figure 8: Pictures of Emericella rugulosa

Figure 9: Map of Investigation Area - Larkana

DISCUSSION

This is the first report of mycoflora of Larkana. The results have shown that CZA and MEA media are easy, simple and reliable as also recorded by Raper and Fennell (Raper & Fennell, 1965). Colony diameter was observed more on MEA than on CZA. Various reports have been published that used morphological characters as key identifying factors (Alwakeel, 2007; Morya, Kmal, & Yadav, 2009; Bandh, Kamili, & Ganais, 2012).

Aspergillus fumigatus and *Aspergillus flavus* were dominating species isolated from all areas of Larkana. *Aspergillus terricola* var. *americana* is reported for the first time from Pakistan and *Aspergillus ficcum* and *Emericella rugulosa* first time reported from Sindh, increasing the total number of *Aspergillus* species reported from Pakistan from 79 to 80 and *Aspergillus* species reported from Sindh from34 to 37 (J. Mirza & Qureshi, 1978; J. H. Mirza, 2007; Nazir et al., 2007).

Results have shown that *Aspergillus fumigatus* is a rapidly growing species than *Aspergillus iccum, Aspergillus flavus, Aspergillus flavus* var. *columnaris* and *Aspergillus terricola* var. *americana* followed by *Aspergillus terreus* var. *aureus* and *Emericella rugulosa*. Out of eight species, *Aspergillus fumigatus* showed uniseriates heads, and *Aspergillus flavus* var. *columnaris* showed mostly uniseriates heads but few biseriates heads were also found. The remaining species showed typical biseriates conidial heads.

Distinguishing character of *Aspergillus ficcum* was thickening of conidiophore wall i.e.2 – 3µ as also reported by Raper and Fennell Raper 1965. It was observed that phialides were comparatively smaller 15 – 25µ long4 than described by Raper and Fennell (1965). Identifying character of *Aspergillus flavus* were yellow to green or dark green colony color and radiate heads with rough conidiophore wall. Difference between *Aspergillus flavus* and *Aspergillus flavus* var. *columnaris* was conidial heads; on MEA *Aspergillus flavus* var. *columnaris* showed typically columnar heads whereas *Aspergillus flavus* showed radiate heads. *Aspergillus terreus* var. *aureus* was identified on the basis of light yellow colony color, slow growth and short columnar heads. key characters of *Aspergillus fumigatus* were grey colony color and ovate to flask shape vesicle ¾ fertile with ampuliform phialides. *Emericella nidulans* was identified on the basis of their orange to orange red and smooth wall ascospore. *Emericella rugulosa* was characterized by purple brown ascospores with rugulose ornamentation, *Aspergillus terricola* var. *americana* is characterized by large conidia that is prolate to globose, 4.5 – 5.5(6)µ in diameter.

Some studies with similar results were reported by Klich (Klich, 2002), McClenny (McClenny, 2005) and Curtis (Curtis *et al.*, 2005). Recently Kim (Kim *et al.,* 2009) and Diba (Diba *et al.,* 2007) studies the morphological characters for the identification of *Aspergillus* species. This is the first report that described the mycoflora of district Larkana and includes *Aspergillus terricola* var. *americana* and *Aspergillus ficuum , Emericella rugulosa* new reports from Pakistan and Sindh respectively.

REFERENCES

Ahmed SI and Rizvi YM, 1969. A preliminary survey of aspergillus species from Karachi (West Pakistan). Pak. J. Bot. 1: 59-65.

Alwakeel SS, 2007. Bacterial and *Aspergillus* spp. contamination of domestic kitchens in Riyadh, Saudi Arabia. Saudi J. Biol. Sci. 14(1): 1-6.

American Society for Microbiology (ASM), 2004. *Clinical microbiology workforce issues*, Washington

Bandh SA, Kamili AN and Ganais BA, 2012. Identification of some aspergillus species isolated from dal lake, Kashmir by traditional approach of morphological observation and culture. Afr. J. Microbial. Res. 6(29): 5824-5827.

Bignell E, 2010. *Aspergillus: molecular biology and genomics*. Caister Academic Press.

Clark TF, 1966. *History of myers park presbyterian church 1926-1966*. Myers Park Presbyterian Church, Charlotte, NC.

Curtis L, Cali S, Conroy L, Baker K, Ou CH, Hershow R, Norlock-Cruz F, and Scheff P.,

2005. Aspergillus surveillance project at a large tertiary-care hospital. J. Hosp. Infect. 59:188-196.

Diba K, Kordbacheh P, Mirhendi SH, Rezaie S and Mahmoudi M, 2007. Identification of aspergillus species using morphological characteristics. Pak. J. Med. Sci. 23(6): 867-872.

Domsch KH, Gams W, Anderson and Heidi T, 1980. *Compendium of soil fungi*. Academic Press, London and New York.

Gams W, Christensen M, Onions JP, AHS, and Samson RA, 1985. *Advances in penicillium and aspergillus systematic*, Springer.

Goldman GH and Osmani SA, 2008. The aspergilli. CRC Press.

Johnston A and Booth C, 1983. *Plant pathologist's pocketbook*. Commonwealth Agricultural Bureaux.

Kim DH, Kim SH, Kim SO, Kim SJ and Hong SB, 2009. Reidentification of aspergillus spp. isolated from clinical specimens of patients suspected as pulmonary aspergillosis in korea. Korean J. Med. Mycol. 14(3): 133-144.

Klich MA, 2002. *Identification of common aspergillus species*. Fungal Biodiversity Centre, Netherland.

McClenny N, 2005. Laboratory detection and identification of aspergillus species by microscopic observation and culture: the traditional approach. J. Med. & Vet. Mycol. (Suppl. 1). 43: S125-S128.

Mirza J and Qureshi MSA, 1978. *Fungi of Pakistan*. Dept. of Plant Pathology. University of Agriculture, Faisalabad, Pakistan.

Mirza JH, 2007. *First fungal culture bank of Pakistan performance report*. Institute of Mycology and Plant Pathology. University of the Punjab.

Morya VK, Kmal and Yadav D, 2009. Diversity of indigenously isolated aspergilli from soil of monoculture teak forest. Res. J. Soil Biol. 1(3): 77-83.

Nazir N, Mirza JH, Akhtar N, Bajwa R and Nasim G, 2007. Some studies on thermophilic and thermotolerant fungi from Lahore, Pakistan, J. Myco-phytopathological Soc. Pak. 5(2): 95-100.

Raper KB and Fennell DI, 1965. *The genus aspergillus.* Williams and Wilkins Company.

Ross C F, 1951. A case of pulmonary aspergillosis. J. Pathol. Bacteriol. 63(3): 409-416.

Royal horticultural society (RHS), 2005. *Mini colour chart,* Flower Councle Holland. Samson

Samson RA, Hing SB, and Frisvad JC , 2006. Old and new concepts of species differentiation in Aspergillus, J. Med. & Vet. Mycol. 44(1): 133-148

Samson RA and Pitt JI, 2000. *Integration of modern taxonomic methods for penicillium and aspergillus classification*. CRC Press.

Samson RA and Varga J, 2009. What is a species in Aspergillus? J. Med. & Vet. Mycol. 47: 13-20

Suhail M, Irum F, Jatt T, Korejo F and Abro H., 2007. Aspergillus Mycoflora isolated from soil of Kotri Barrage Sindh, Pakistan. Pak. J. Bot. 39(3): 981-984

Thom C and Raper KB, 1945. *A manual of the aspergilli*. The Williams & Wilkins Company.

Varga J, Juhasz A, Kevei F and Kozakiewicz Z, 2004. Molecular diversity of agriculturally important aspergillus species. Eur. J. Plant Pathol. 110 (5-6): 627-640.

Waksman SA and Fred EB, 1922. A tentative outline of the plate method for determining the number of micro-organisms in the Soil. J. Soil Sci. 14(1):27-28

Warris A, Voss A and Verweij PE, 2001. Hospital sources of Aspergillus species: New routes of transmission? J. Mycol. 18(4):156-62.