SOME NEW RECORDS OF FUNGI FROM WEST PAKISTAN

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During the screening of soil fungi from Karachi soils some new records, *Aspergillus sclerotiorum* Huber, *Penicillium lilacinum* Thom, *Penicillium ehrlichii* Kleb, *Dactylium fusarioides* Frag. & Cif. *Myrothecium verrucaria* Ditm. ex Fr., *Microascus trigonosporus* Emmons & Dodge, and *Pseudoarachniotus hyalinosporus* Kuehn, Orr & Ghosh were isolated for the first time from West Pakistan. The first five belong to Imperfect Fungi while the latter two are members of Ascomycetes.

These organisms were grown on Czapek's dox agar and corn meal agar media under similar conditions. The difference in respect of growth and formation of fruiting bodies was noted. The Czapek's medium was more favourable than the corn meal medium. The controversy regarding *Penicillium lilacinum* and *Spicaria violacea* Abbot was discussed. In the author's opinion the organism should be determined as *Penicillium lilacinum* in view of its having more affinities towards the same.

Introduction

For the last two decades soil fungi and its relationship with other micro-organisms have been of great interest to mycologists and plant scientists. Their manifold investigations on various problems of soil fungi have enlarged the vision of agricultural science. Many new and interesting organisms were encountered during the investigations. Some interesting fungi were isolated by the authors during the screening of soil samples from different localities of Karachi.[1] These fungi are reported for the first time from West Pakistan. It was, therefore, considered proper to describe these new records in a separate paper. Detailed taxonomic studies were carried out to observe morphological characters, difference in growth on different media and formation of fruiting bodies. Their position in the toxanomic classification was also ascertained. Different investigators have adopted different techniques for isolating fungi from the soil. The useful technique (the soil plate method) devised by Warcup[2] is adopted in the present studies. In the present investigation fifty eight species belonging to twenty-five genera of fungi were isolated. Out of these four genera and seven species were recorded for the first time from West Pakistan. Seven of these new records are described and discussed in the present paper.

Material and Method

The soil samples were collected randomly from different localities of Karachi, namely, Bizerta lines, Mohammed Ali Housing Society, Karachi Airport, Malir, Azizabad and North Nazimabad. A hole 2 feet deep and 1 foot in diameter was dug to expose the soil profile. The samples were collected at various depths by means of sterilized iron tubes. The soil samples were inoculated on Czapek's dox agar medium using soil plate method. The petridishes were incubated at 28-30°C and examined after one week. The emerging colonies were isolated into pure cultures by repeated inoculations on Czapek's slants. The fungal colonies were microscopically examined and organisms were identified with the help of various taxonomic literature[3-12] and were confirmed from Commonwealth Mycological Institute. The colonies were grown on different media, pH and temperature. Slides were prepared and various taxonomic characters like, size, shape and septation of mycelium and size of conidia and conidiophores were considered. Measurements were taken and camera lucida drawings were made in order to determine the specific identity of various fungi under investigations.

Results

Aspergillus sclerotiorum Huber (Plate I). Isolated from uncultivated barren soil of Malir, colonies depressed, growing slow on Czapek's dox agar medium. Mycelium white, in tufts, irregular. Sclerotia developing after three days covering entire surface of the medium. Two days after inoculation mycelium shows characterstic irregularly shaped balls which later form sclerotia. Sclerotia developing into concentric rings on Czapek's slants (Plate I-A) while on corn meal agar few sclerotia are formed. Sclerotia white in the beginning turning yellow at maturity. Reverse of colony cream to yellow. Conidial heads (Plate-I C,D) abundant on corn meal than on Czapek's, mostly columnar rarely oval, with chains of conidia upto 270μ long, tapering downward. Conidiophores (Plate-I,D) smooth walled, septate, yellow, 200-400μ long, 7-10μ in diameter, arising from the distinct foot cell (Plate 1, B). Vesicle globose, 13-18μ. Sterigmata in two series primary phialides 8-10 μ × 2.8-4.5μ, secondary phialides 6.8-9.1 × 1.5-2 μ. Conidia globose, smooth, 2.2-2.8μ. Sclerotia (Plate I, E) 1.2-1.8 mm in diameter, cream to yellowish pink, irregularly shaped, hard, arranged in well defined rings on Czapek's dox agar. In corn meal sclerotia are scanty and small.

Penicillium ehrlichii Kelb (Plate II). Colonies dirty brown to pale buff showing restricted growth on Czapek's attaining a diameter of 2-2.5 cm in 20 days at 28°C. Mycelium consists of aerial cottony sterile hyphae in the centre. Margins are deeply furrowed. Cleistothecia (Plate II, B) engulfed in sterile mycelium, pale to brown in colour. Conidial heads few and not very distinct in the colony. Reverse of colony light yellow, conidial heads few, very irregular sterigmata rarely present, phialides never a true verticil, often single or in groups of 2 or 3, strongly divergent, usually arising at different levels, variable in form and dimension, 10-15 × 2.5-3.5μ with narrow tip which bears conidia. Conidia elliptical, 3.5-4.8×3.2-3.9μ, ends pointed, smooth walled. Cleistothecia abundantly produced and dominate the colony, oblong to globose, 100-150μ in diameter, composed of pseudoparenchymatous tissue. Asci (Plate II, A and C) developing after 15 to 20 days on the lateral branches of fertile hyphae. Spherical to oblong, at maturity, 7.5-9.5μ in diameter, 8 spored. Ascospores round to oval 3.5-3.9×2.5-3μ with distinctly spinulosed wall and one equatorial furrow. Colonies on Corn meal smooth and good in growth. Very thin layer of cleistothecia entangled into sterile hypae, brown to buff in colour. Penicilii very fragmentary, limited in number, cleistothecia ripening quickly, producing abundant asci in 12 to 14 days.

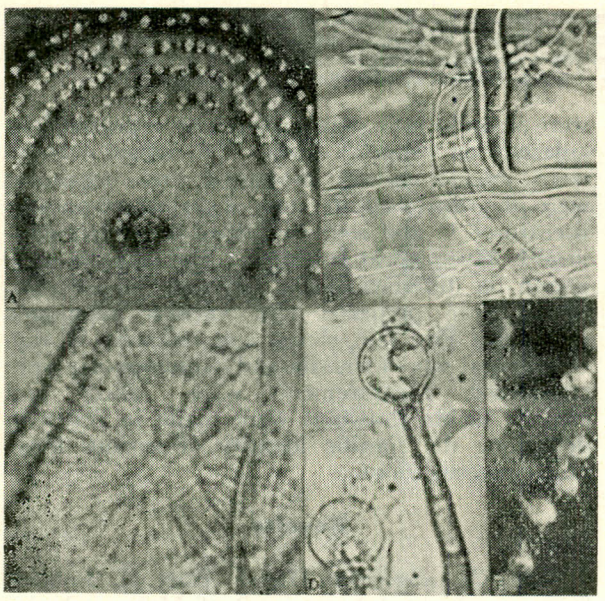

Plate 1.—*Aspergillus sclerotiorum*.—A. Colony on Czapek's dox showing concentric rings of sclerotia. B. Foot cell. C. Conidial heads showing globose vesicles, primary and secondary phialides with chains of conidia. D. An enlarged conidiophore showing vesicle (conidia are detached). E. Irregular and hard sclerotia.

Penicillium lilacinum Thom (*Spicaria violacea* Abbot) (Plate III). This organism was isolated from Airport soil at 4 inch depth. Colonies (Plate III, A) on Czapek's dox agar medium floccose, loosely textured and deep seated. Centre of colony raised with wedged margins. Furrows present 5-6 in a colony of 2 cm. Mycelium at first white later gradually turning into lilac to vinaceous shades. Reverse of colony at first white becoming vinaceous at maturity. Conidiophore vary in size and dimension, arising either from the colony margin or from the centre. Centrally situated conidiophores are shorter than the marginal conidiophores. Conidiophore wall smooth, rarely rough, colourless. Penicilli varying in size and complexity, larger penicilli consists of whorls of metulae arising at different levels, smaller penicilli consists of 1-2 verticil of metulae (Plate III, B,C)

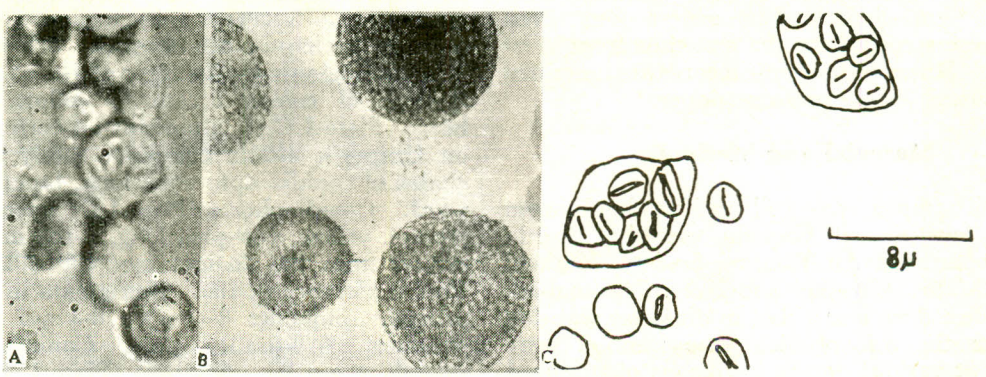

Plate 2.—*Penicillium rehrilichii*.—A. Spherical to oblong asci showing ascospores inside. B. Oblong to globose cleistothecia showing pseudoparenchymatous tissue. C. Camera lucida drawing of asci showing round to oval ascospores with one equatorial furrow.

usually 8-10×2-2.3μ. Sterigmata 5.5-7μ in length, flask shaped, acuminate and bearing chains of conidia. Conidia elliptical 2-3.4×1.5-2.5μ, smooth walled, pale lilac in colour.

Colonies on corn meal agar produce very thin mycelium, not floccose as on Czapek's dox agar medium. Lilac to vinaceous in colour, reverse colourless, sporulation not luxurious but velvety

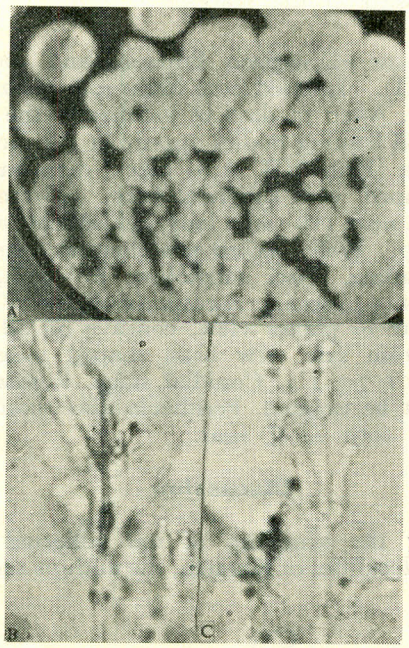

Plate 3.—*Penicillium lilacinum*. A. Floccose colony on Czapek's dox agar, central area of mycelium raised with wedged margin. B. Larger penicilli showing whorl of metulae and primary and secondary phialides. C. Small penicilli with 1-3 verticils.

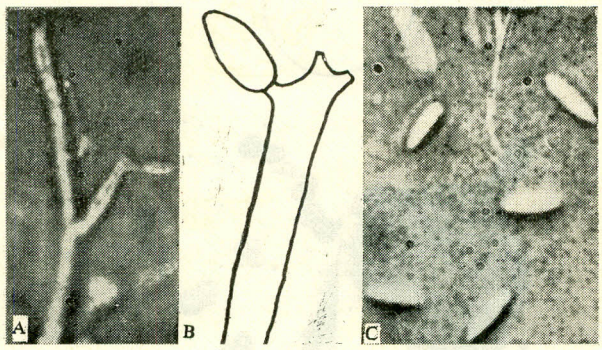

Plate 4.—*Dactylium usarioides*.—A. Conidiophore with flask shaped verticil bearing conidia at the tip B. Vertical showing three notches at the tip. C. Oblong to cylindrical conidia.

spore masses dominate the surface. Margin is not furrowed and the centre of colony is not raised as in Czapek's dox agar.

Dactylium fusarioides Frag. & Cif. (Plate IV). Isolated from the soil collected from Mohammed Ali Housing Society at 8 inch depth. Colonies on Czapek's dox agar growing very fast attaining a diameter of about 2-3 cm in 2 days at 28°C. Mycelium floccose, forming a thick turf of sterile hyphae. Prostrate branching, septate and hyaline. The colony is of brick red colour, central portion pale yellow. Reverse dark pink. Conidiophore (Plate IV, A,B) ascending, mostly branched, single or in whorls of 2-3 verticils arising at definite intervals from the conidiophore. The verticil is flask shaped (Plate IV, A) having three notches at the tip (Plate IV, B) bearing conidia (Plate IV, C) hyaline, 1-3 celled oblong to cylindrical, pointed at the base.

The colonies on corn meal agar show very depressed submerged and restricted growth of mycelium attaining a diameter of 2-3 cm in 7 days at 28°C. The thick turf and sterile hyphae are lacking as in Czapek's dox agar medium, surface covered with snowy white masses of conidia, reverse light pink.

Myrothecium verrucaria Ditm. ex Fr. (Plate V). Isolated from Malir soil at 4 inch depth. Colonies on Czapek's dox agar show very luxurious, fluffy thick growth of mycelium (Plate V, A) attaining a diameter of 2-3 cm in 2 days at 28°C. Mycelium in younger colonies white turning whitish yellow

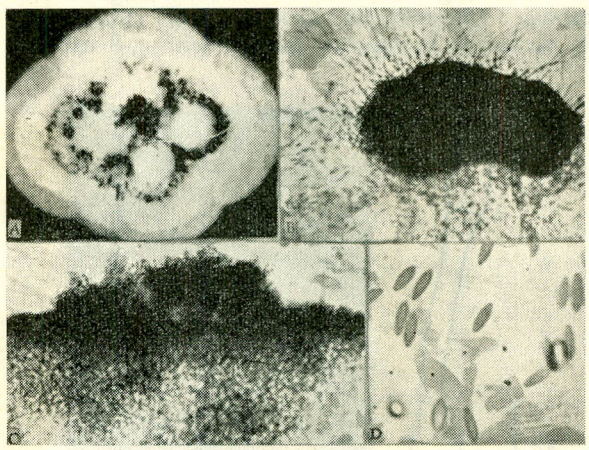

Plate 5.—*Myrothecium verrucaria*.—A. Colony on Czapek's dox agar showing fluffy growth of mycelium with regular zones of sporodochia. B. A sporodochium. C. Sporodochium showing palisade layer of conidiophores bearing conidia. D. Ovate pointed Conidia.

at maturity. Mycelium septate, branched, prostrate, hyaline, reverse pale buff. Sporodochia (Plate V, B) irregular, cushion like, large, dusky olive green to olvaceous black, surface shining, arising from central portion of the colony in irregular zones and gradually dominating all over the mycelial surface. Marginal sporodochia are short and meshed in the mycelium.

Conidiophores compact, subhyaline, short, erect, smooth, septate, forming a palisade layer on which olive green conidia are borne on finger like phialides (Plate V, C). Conidia in chains when young forming a compact ball of conidia at maturity. Ovate, pointed at both ends, smooth, dusky olive green measuring 6-9.2 × 1.7-3.3μ (Plate V, D).

Colonies on Corn meal agar show very different growth of mycelium and sporodochia. Mycelial mat thin, suppresed, growth superficial, white, creeping over the surface, hyaline, prostrately branched, septate, sporodochia cushion like, irregular, scattered, few in number and entangled into the mycelium and never forming zones as in Czapek's dox agar medium.

Pseudoarachniotus hyalinosporus Kuehn, Orr and Ghosh[13,14] (Plate VI). Isolated from North Nazimabad at 8 inch depth. Colonies on Czapek's dox agar show very luxurious growth with fluffy tuft of mycelium. Vegetative hyphae hyaline, 1.2-3.2μ in diameter, racquet mycelium (Plate VI, A) often present. Colony is of light yellow shade in the centre while margins are of darker shade. The margins are more fluffy, cottony and are into irregular patches. Asexual stage is unknown. Cleistothecia lacking, asci (Plate VI, B) occurring in exposed groups with 5-8 asci in each group, scattered among the vegetative hyphae. Groups ranging from 25-50μ in diameter. Asci sub globose or obovate, 7.4-10.2 × 6.6-7.9 μ. Ascospores lemon yellow, elliptical, slightly spinulose at the margin, measuring 2.2-3.4 × 2.2-2.8 μ.

Colonies on Corn meal agar show extremely restricted growth, 3-4 cm in 20 days at 28°C. Mycelium depressed, creeping, branched, septate, hyaline, central area of colony light yellow and fluffy, raised at the margins.

Microascus trigonosporus Emmons and Dodge[15] (Plate VII). Isolated from Malir soil on Czapek's dox agar. After 15 days of incubation the black perithecial fruiting bodies appear in the medium. Perithecia (Plate VII, A,B) black, carbonaceous, flask shaped with spherical base, 146-170 μ in diameter, neck 118-126 μ long, cylindrical, swollen at the tip. Well marked protuberances giving a rough outline. Asci subglobose to ovoid, 5.5-8.2 × 8-11.5 μ, sessile. Ascospores (Plate VII, C) triangulate in planer view, concave on all three sides. From side view bean shaped measuring 2.2-3.6 × 1.7-3.1 μ. Conidial stage *Scopulariopsis* could not be isolated. Perithecia also luxuriously grow on corn meal agar.

Discussion

During the sampling of soil collected from different localities of Karachi, the authors succeed-

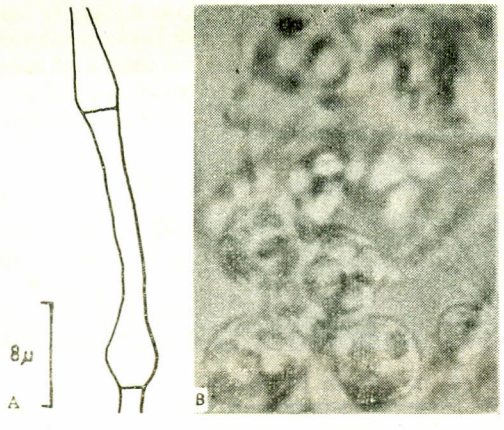

Plate 6. —*Pseudo-arachniotus hyalinosporus*. A. Racquet mycelium. B. Sub globose to obovate asci in exposed groups with ascospores.

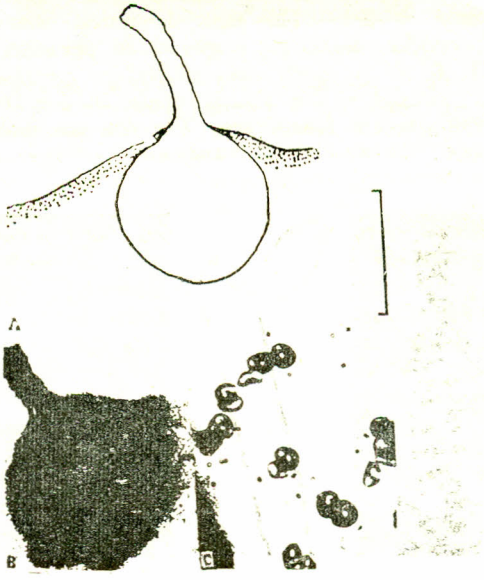

Plate 7.—*Microascus trigonosporus*.—A. Camera lucida drawing of a perithecium showing a spherical base and a long neck. B. Perithecium as seen in culture. C. Ascospores.

ed in isolating some fungi which were not previously reported from West Pakistan. These new records will provide a better picture of fungus flora of Karachi soil to the mycologists of this region. Further studies of these new records on biochemical and physiological aspects would open new horizons in the field of antibiotics and metabolites. These organisms were grown on Czapek's dox agar and corn meal agar media for comparison purposes under identical conditions.

Aspergillus sclerotiorum produces large irregularly shaped sclerotia abundantly produced on Czapek's agar while on corm meal the production of sclerotia is less. This difference may be due to the selective nature of the fungus with regards to the production of sclerotia.

Penicillium ehrlichii although showing restricted growth on Czapek's medium, but the production and distribution of Cleistothecia was deep and luxurious as compared to corn meal agar. Asci form earlier in Czapek's medium than on corn meal. It was noticed that most of the organisms such as *Aspergillus sclerotiorum, Myrothecium verrucaria Penicillium lilacinum, Penicillium ehrlichii and Pseudoarachniotus hyalinosporus* etc. show very luxurious growth of mycelium and fruiting bodies on Czapek's dox agar medium. The colonies mature comparatively late on corn meal than on Czapek's medium.

Penicillium lilacinum shows very divergent characters in the conidial structure. Some penicilli consist of 2-3 verticil arising (Plate III, C) at definite nodes. The tips of verticil bear chains of conidia, although majority of penicilli (Plate III, B) of primary and secondary phialides are very similar to the typical broom shaped structure found in the *Penicillium*. On the conidial difference and mode of arrangement of verticil and phialides on conidiophore, a confusion occurred as to the correct taxonomic position of the fungus. Abbot[16] very firmly advocated that presence of 2-3 verticil (Plate III, C) on the conidiophore bearing conidia on each tip is enough reason to give the organism an independent position equal to *Penicillium lilacinum* and named it *Spicaria violacea*. He further pointed out some other differences as regard to colour, and on these colour differences Abbot emphatically advocated that this organism is not *P. lilacinum*, but *Spicaria violacea*, and should be given an independant position.

Thom[17] is reluctant to believe the transfer of *P. lilacinum* to *Spicaria violacea*. He argues that Abbot's *Spicaria violacea* was considered in relation to *P. lilacinum* but the idea of changing the name of the organism to *Spicaria violacea* was rejected since the conidial structure of Penicillia varies in form and that it is often *Verticillium* like penicillate. He advocates that *P. lilacinum* shows close resemblence and affinities to the typical *Penicillium* rather than to *Spicaria* and therefore this organism must be included into *Penicillium*.

The authors found these two divergent conidial characters in the same culture and there was no marked difference in morphology and colour of the colony. If the organism was grown on two different media, the difference in conidial structure was observed, however, this difference was not enough to place the organism in a separate genus. Thus the authors believe that Abbot has misinterpreted *P. lilacinum* to *Spicaria violacea*. The absence of metulae and primary phialides is a secondary character and may be due to some nutritive deficiency in the medium. The *penicillium* like heads were found many times as compared to *Spicaria* like structures. The organism was also sent to Commonwealth Mycological Institute who also identified it as *P. lilacinum* and not *Spicaria violacea*.

Note.—At the time of sending this manuscript for publication a short communication entitled, "Fungus Flora of Lahore Soils" by Mahboob Alam Qureshi appeared in this Journal, **9**, 90 (1966. In this short communication *Penicillium lilacinum* and *Myrothecium verrucaria* were mentioned to be isolated from Lahore soil. These two fungi have also been described by the authors in their paper. Mr. Qureshi's communication tells about the isolation of these two organisms while the authors have described them in detail. The position of *P. lilacinum* with respect *Spicaria violacea* is discussed in detail. Regarding *Myrothecium*, the authors feel that it should also be included in the paper as it is an important organism, its morphology and taxonomy is discussed and described, also some good photographs of its characteristic mycelium, colony, sporodochia and spores are given. Besides Czapek's dox agar, these organisms have also been grown on corn meal agar and some interesting observations have been made with respect to growth and formation of sporodochia along with other characters.

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