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Micromycete diversity associated with the rhizospheres of plants from different polluted soils of Lahore, Pakistan

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Abstract

Micromycetes have been beneficial as well as harmful in the history of mankind. Their correct identification is the first step for their use in further research (i.e., utilization in medicine, myco-remediation or biodegradation processes, and various industries). The present study provided a baseline regarding identification and their systematic position of these fungi. During the study, micromycetes were isolated and identified from the rhizosphere of different plants of Lahore city. A total of thirty-three different species belonging to eight genera were isolated, including *Alternaria* (3 spp.), *Aspergillus* (13 spp.), *Mucor* (4 spp.), *Penicillium* (2 spp.), *Trichoderma* (4 spp.), *Exserohilum*, *Fusarium* and *Rhizopus* (1 sp. each), along with 4 unidentified species.

Key words – Biodegradation – Decomposition – Colony – Species

Introduction

Study of fungi from a taxonomic point of view is of considerable practical value and important due to their beneficial effects and increasing role in the industrial area as well as serving as nutrient recyclers in ecosystems by decomposition and increasing soil fertility (Gupta & Soni 2000, Bergquist et al. 2003, Nevalainen & Te'o 2003) apart from their disease-causing nature (Wainwright 1995). Most micromycetes are producers of mycotoxins. Some micromycetes produce allergens, contribute to asthma progression, and initiate alveolitis of a nontypical allergic character. Micromycetes such as *Aspergillus fumigatus* Fresen, *A. candidus* Link and others can be cause of deep mycoses (Lugauskas et al. 2011).

Their diversity in soil depends on their competitive saprophytic ability and tolerance to antibiotics, heavy metals, chemicals and temperature (Garrett 1963, Malik & Sandhu 1973, Moustafa 1975, Ortuno et al. 1977, Robinson & Morris 1984, Wainwright 1995, Fenice et al. 1997, Taiwo & Oso 1997, Brazauskiene 1998, Lebedeva et al. 1999, Redman et al. 1999, El-Said 2001, Fenice et al. 1998, Robinson 2001). Micromycetes represent an extensive group of microorganisms in the soil. They are ubiquitous and important because of their presence in all climates and on all substrates. The present study was carried out to assess micromycete diversity associated with the

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rhizosphere of several different plants. The results will be of help in further research to find which micromycetes are best for detoxification and bioremediation because resistant species can be used by researchers as bioremediation agents in reducing soil contamination due to the presence of different hazardous chemicals. The ability of micromycetes to transform a wide variety of hazardous chemicals has aroused a lot of interest in using micromycetes for the process of bioremediation. They are also used for preparation of medicines (e.g., antibiotics such as penicillin).

Materials and Methods

Sampling

Soil samples were taken from the top 20 cm of the soil making up the rhizosphere of a number of different. These samples were placed in separate sterile polythene bags and were brought to the laboratory for further research work.

Preparation of medium

For the isolation of micromycetes from soil samples, a 2% Malt Extract Agar (MEA) medium was used. For the preparation of a 500 ml solution, ten grams of Malt Extract was dissolved in 500 ml of distilled water and the pH was adjusted to 6.5. Then 10 g agar was added to the latter. The medium was autoclaved at 121 °C at 15lbs/inch^2 for 15 minutes. A pinch of streptomycin was added to the autoclaved medium to check bacterial growth. The medium was poured in Petri plates when temperature reached up to 45 °C.

Growth of colonies

By means of the soil dilution plate method, the soil samples were mixed with sterile distilled water. From the dilutions, a pipette was used to add 1 ml volumes onto the medium in Petri plates. Three Petri plates were set up for each replicate of soil sample. Petri plates were labeled. Sealing of Petri plates was done with paraffin film in order to avoid contamination, and these plates placed at room temperature for 4–7 days. Fungal cultures were examined when they were 5–10 days old.

Macroscopic and microscopic characterization of colonies and identification

In macroscopic characterization of fungal colonies, such features as color, appearance on medium, texture, growth and growth rate were observed, and colonies were photographed.

For microscopic characterization, a small part of medium along with growing fungal colony was taken. It was cut longitudinally into thin slices. A thin slice was mounted in a KOH solution on a slide, and the latter examined under a microscope. Microscopic morphology of micromycetes (i.e., hyphae, conidiophores, and conidia) was observed and compared with available literature. Images of slides were obtained and some hand drawings of microscopic structures were made.

Results and Discussion

Isolated micromycetes belonged to eight different genera, which include *Alternaria* Nees, *Aspergillus* P. Micheli, *Exserohilum* K.J. Leonard & Suggs, *Fusarium* Link, *Mucor* P. Micheli ex L., *Penicillium* Link, *Rhizopus* Ehrenb., and *Trichoderma* Pers. During the present study related to the biodiversity of micromycetes, *Aspergillus* was found as dominant genus, with maximum species number as reported from most different soil samples. For proper identification various morphological characters were used as key identifying factors used by other researchers (e.g., Alwakeel 2007, Morya et al. 2009, Bandh et al. 2012). Species of *Aspergillus* are highly aerobic and are found in almost all oxygen-rich environments but many species of *Aspergillus* demonstrate oligotrophy, where they are capable of growing in nutrient-depleted environments or environments in which there is a complete lack of key nutrients. This genus can tolerate temperature up to 60 °C (Bourgeaois et al. 1996).

Other commonly distributed species belongs to the genus *Alternaria* (*A. alternata* [Fr.] Keissl., *A. porii* [Ellis] Cif. and *A. solani* Sorauer). Four species of *Mucor* were identified. *Mucor* is

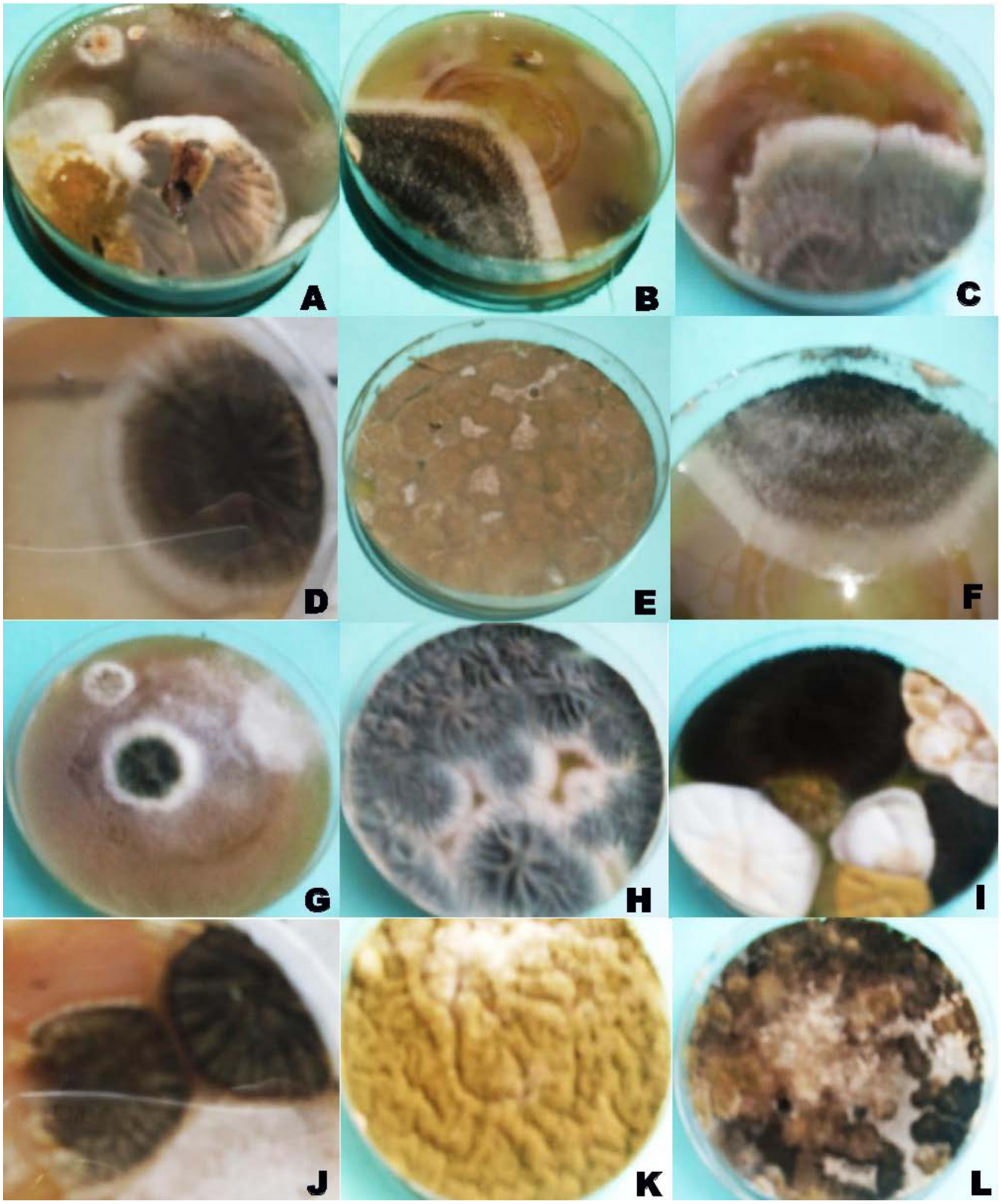

Fig. 1 – Colonies of different micromycetes. (A) *Alterneria alternata*. (B) *A. porri*. (C) *A. solani*. (D) *Aspergillus alliaceus*. (E) *A. caespitosus*. (F) *A. carbonarius*. (G) *A. flavus*. (H) *A. fumigatus*. (I) *A. nidulans*. (J) *A. niger*. (K) *A. ochraceus*. (L) *A. parasiticus.*

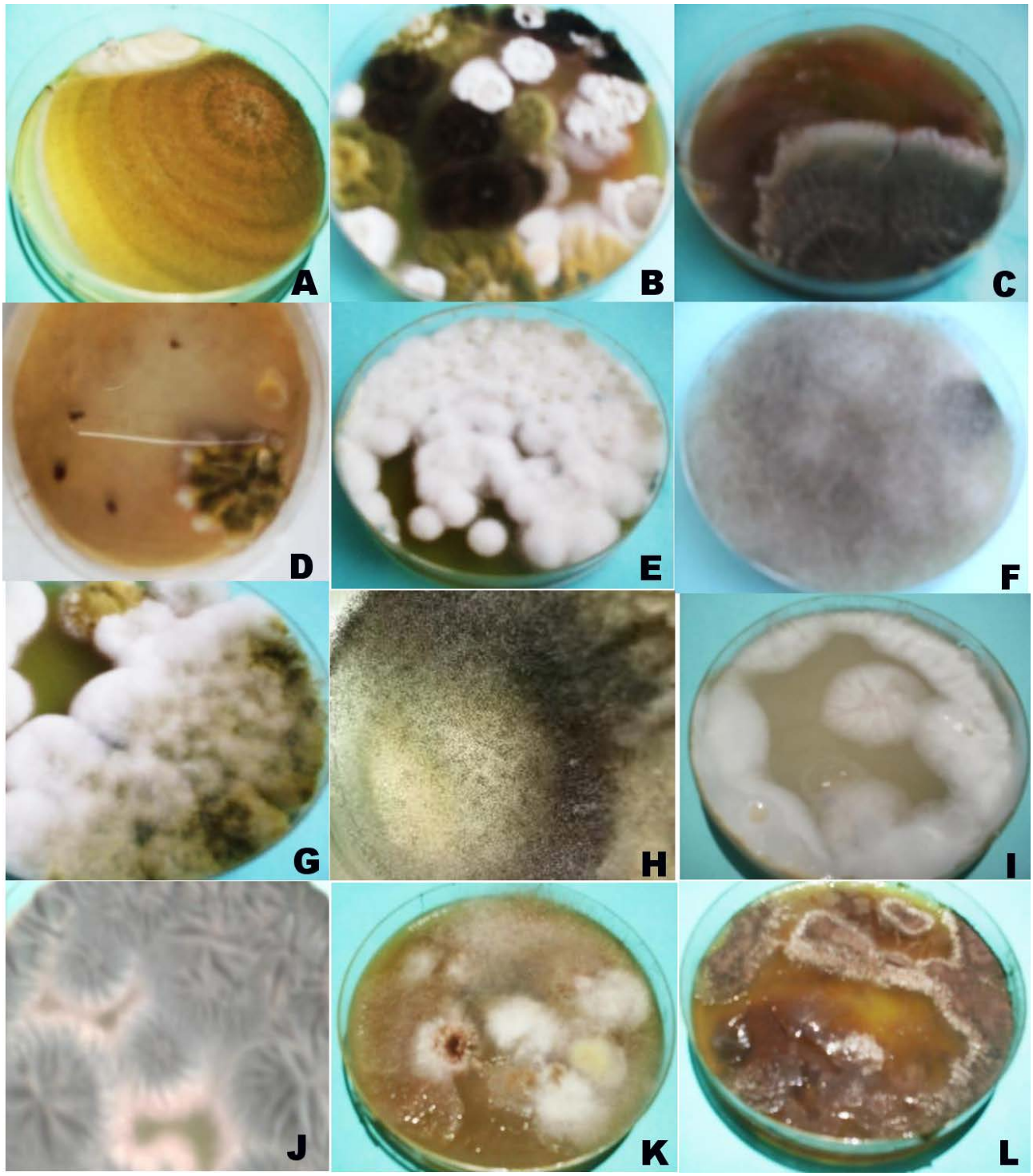

Fig. 2 – Colonies of different micromycetes. (A) *Aspergillus sydowii*. (B) *Aspergillus tamari*. (C) *Aspergillus terreus*. (D) *Aspergillus versicolor*. (E) *Fusarium solani*. (F) *Mucor mucedo*. (G) *Mucor racemosus*. (H) *Mucor* sp. II. (I) *Penicillium commune*. (J) *Penicillium roqueforti*. (K) *Rizopus stolonifer*. (L) *Trichoderma viride*.

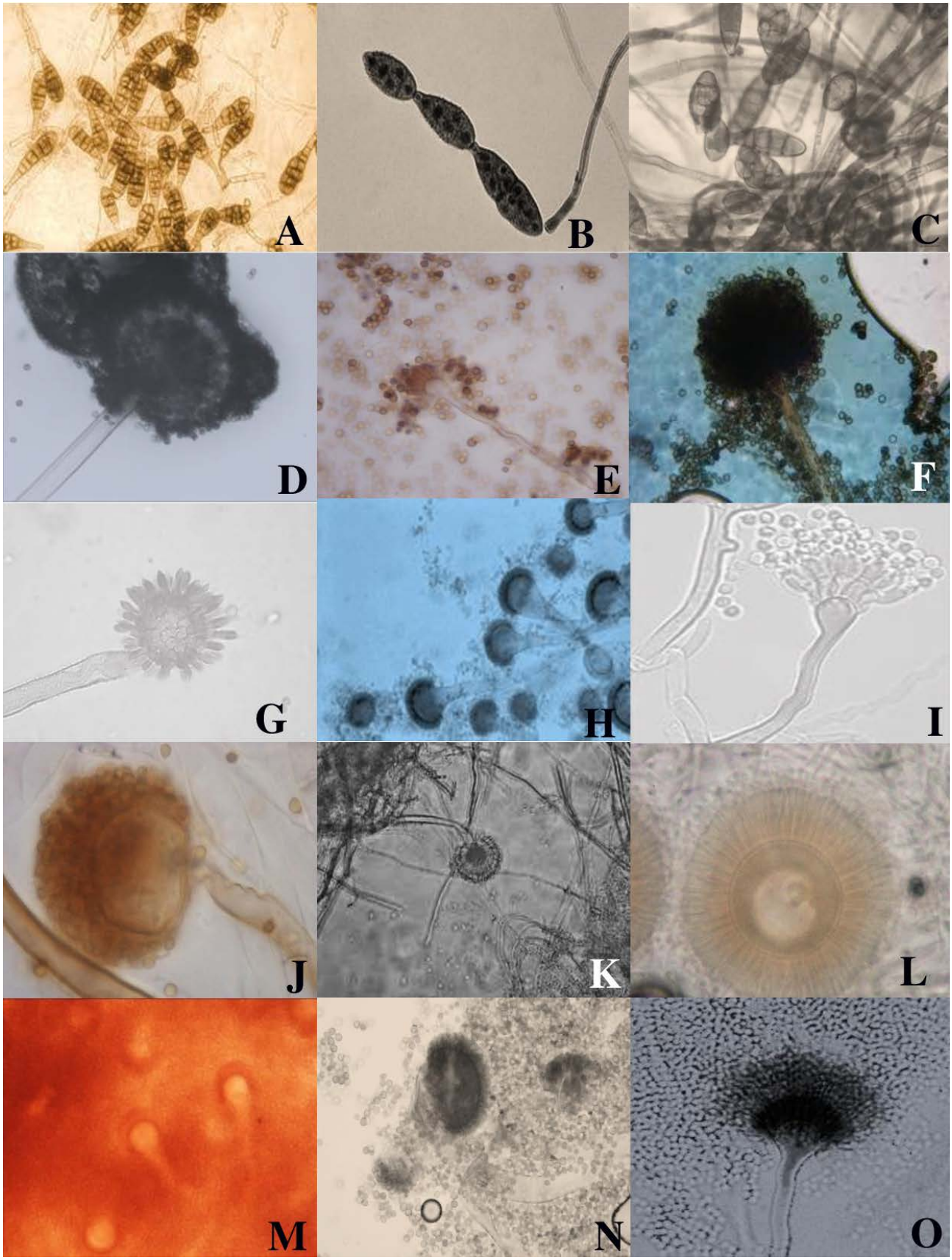

Fig. 3 – Microscopic morphology of isolated micromycetes. (A) *Alterneria alternata*.(B) *Alterneria porri*. (C) *Alterneria solani*. (D) *Aspergillus alliaceus*. (E) *Aspergillus caespitosus*. (F) *Aspergillus carbonarius*. (G) *Aspergillus flavus*. (H) *Aspergillus fumigatus*. (I) *Aspergillus nidulans*. (J) *Aspergillus niger*. (K) *Aspergillus ochraceus*. (L) *Aspergillus parasiticus*. (M) *Aspergillus sydowii*. (N) *Aspergillus tamari*. (O) *Aspergillus terreus.*

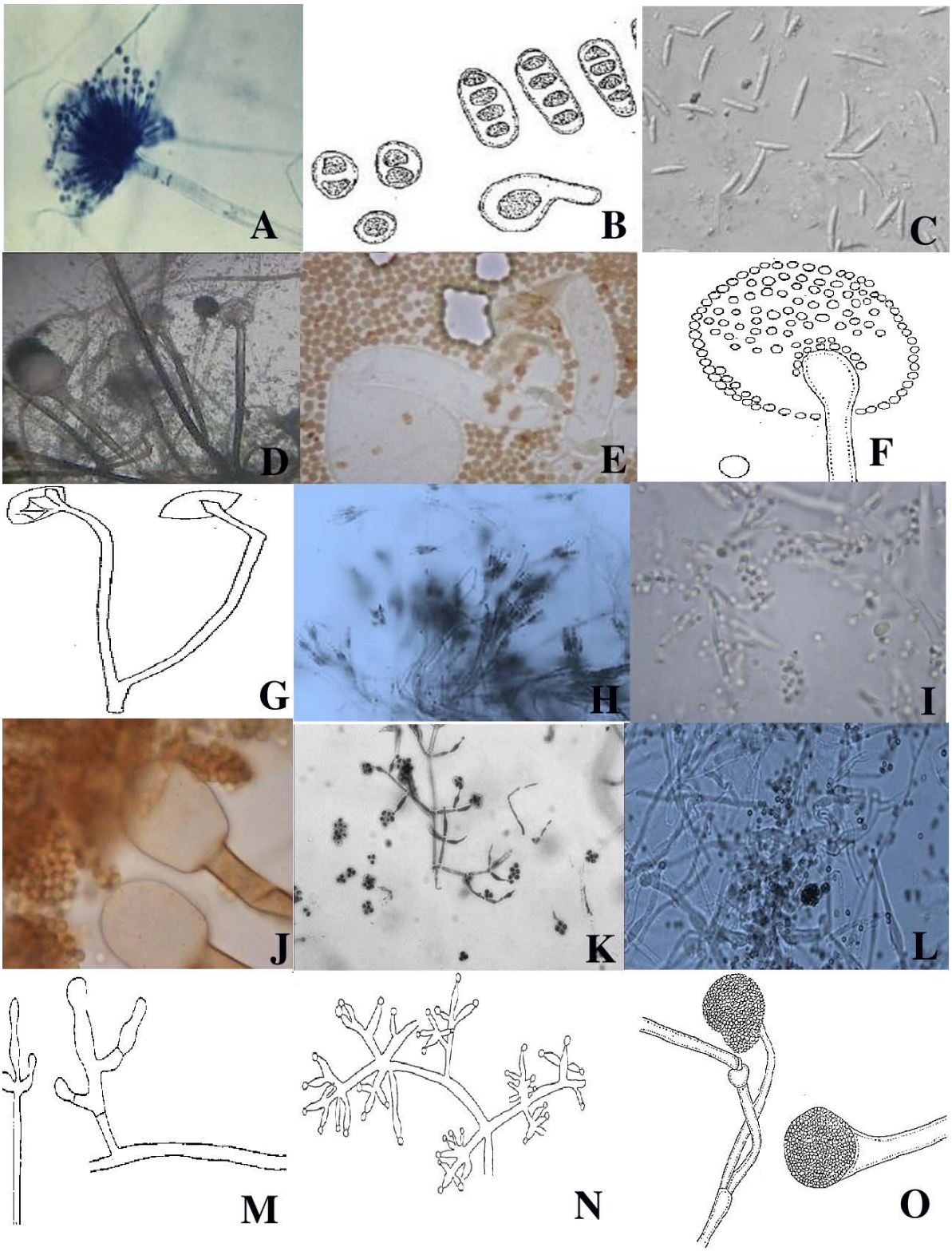

Fig. 4 – Microscopic morphology of isolated micromycetes. (A) *Aspergillus versicolor,* (B) *Exserohilum fusiforme,* (C) *Fusarium solani,*(D) *Mucor mucedo,*(E) *Mucor racemosus,* (F) *Mucor* sp. I, (G) *Mucor* sp. II, (H) *Penicillium commune,* (I) *Penicillium roqueforti,* (J) *Rizopus stolonifer,*(K) *Trichoderma harzianum,*(L) *Trichoderma viride,*(M) *Trichoderma* sp. I, (N) *Trichoderma* sp. II, (O) Species A, (P) Species B, (Q) Species C, (R) Species D.

Table 1 Macroscopic and microscopic characters of different isolated micromycetes

Species	Colony	Growth rate	Hyphae	Conidiophore	Conidia
Alternaria alternata	Greybrown	Rapid	branched, septate	septate	Multicellular, double walled
A. porii	Black dotted appearance	Rapid	branched, septate	septate	Multicellular, septate, double walled
A. solani	Blackish	Rapid	branched, septate	septate	Septate, double walled
Aspergillus alliaceous	Circular green	Slow	hyaline branched, septate	double walled, aseptate, hyaline	globose, unicellular
A. caespitosus	Green	Rapid	smooth	hyaline, double walled	Globose, finely rough
A. carbonarius	Brown to blackish	Medium	long and smooth	Ampulliform with flat base	globose to ellipsoidal
A. flavus	Green with white boundary	Rapid	long and non septate	smooth, double walled, non septate	unicellular, globose, single layered
A. fumigatus	Dark green to bluish green	Rapid	septate, hyaline, smooth greenish grey	short, smooth–walled, long, and erect	globose to sub globose, rough–walled,
A. nidulans	Dark green	Rapid	Biseriate, long, non septate.	70–150um long and 3–6µm wide	spherical, double layered
A. niger	Blackish green	Slow	long, septate, hyaline	unbranched, doubled walled, smooth	smooth, single walled; globose to sub globose
A. ochraceous	Skin to sand brown shade	Rapid	long thick and rough	hyaline, double walled	double walled, circular
A. parasiticus	Dark green	Rapid	Long and rough stipe	Ampulliform with broad neck.	Globose, very rough
A. sydowii	Green, surface growth.	Slow	hyaline, non septate, smooth	hyaline, double walled, smooth	double walled, unicellular
A. tamarii	Dirty green	Rapid	hyaline, aseptate, unbranched	aseptate, double walled, hyaline	circular, unicellular

Table 1 (continued)

Species	Colony	Growth rate	Hyphae	Conidiophore	Conidia
A. terreus	Sand brown to blackish	Rapid	Biseriate, septate, hyaline.	hyaline and smooth–walled	Globose to ellipsoidal
A. versicolor	Light green.	Rapid	Branched, septate, hyaline.	Non septate, hyaline	globose, roughened
Excerohilum fusiforme	Dark green	Medium	branched, septate	Septate, double walled.	Double walled, multicellular
Fusarium solani	White creamy	Fast	White, septate, wide	Multi branched, hyaline, globose	Abundant, cylindrical
Rhizopus stolonifer	Whitish	Fast	prostrate, thick walled	aseptate, thick walled	globose, smooth, unicellular
Mucor mucedo	Dirty white	Fast	Long, smooth, non septate, hyaline	hyaline, single layered, smooth, cylindrical	circular, smooth, single layered
M. racemosus	Greenish white	Moderate	smooth, non septate, hyaline	branched, single walled, hyaline	smooth, unicellular, globose
M. sp. I	Black	Moderate	smooth, septate	cylindrical, double walled	smooth, single walled, rounded
M. sp. II	White threads with black dots.	Rapid	branched, single walled, hyaline	saddle like, transparent	smooth, single walled, globose
Penicillium commune	Rounded white threads.	Rapid	branched, hyaline, single layered	branched, non septate, single walled, hyaline,	rectangular, smooth, single walled
P.roqueforti	Greyish blue.	Fast	smooth, branched, hyaline, single layered	long, smooth, cylindrical, single walled, branched	smooth, green, spherical to sub spherical, unicellular
Trichoderma harzianum	Dirty green	Rapid	Non septate, hyaline, smooth, branched	branches bear extensive phialides	smooth, single layered, non septate
T. viride	Light yellowish brown	Rapid	non septate, branched, hyaline	aseptate, branched, bears phialide	light green, globose, smooth, two layered
T. sp. I	Yellow dots, powdery appearance	Rapid	hyaline, single layered	unbranched, non septate, bearing phialides	single layered, globose to subglobose
T. sp. II	Green, dotted.	Rapid	non septate, branched, single layered, smooth	unbranched, non septate	circular to semicircular, unicellular
Unidentified sp. A	Black, thread like appearance.	Rapid	hyaline, double walled, non septate	branched, two layered	rounded, smooth, unicellular
Unidentified sp. B	White, surface growth	Medium	branched, septate	arise from side of hyphae	smooth, circular to semicircular,
Unidentified sp. C	Black, irregular	Medium	septate, double walled	septate, double walled, hyaline	rounded, unicellular
Unidentified sp. D	Black, thread like appearance.	Medium	hyaline, single layered	hyaline, single walled	Semi globose, two layered,

microbial genus of approximately 3000 species of molds commonly found in soil, digestive systems, on plant surfaces, and in vegetable matter. One species each of *Exserohilum*, *Fusarium* and *Rhizopus* was recorded.

Two species of *Penicillium* (*P. commune* Thom, C. and *P. roqueforti* Thom) were recorded. Species of this genus are ubiquitous soil micromycetes preferring cool and moderate climates and commonly present wherever organic material is available. Most of the soils of study area from which the samples were collected were hot and dry, which probably represents why the small number of species of this genus was found.

Four species of *Trichoderma* were also identified. *Trichoderma* is a genus of fungi that is present in all soils, where they are the most prevalent cultivable micromycetes. *Trichoderma* are biologically active microbial strains which can solve bioremediation problems in region at low cost. Four species were not identified and they were designated as species A, B, C and D.

Conclusions

Our study indicates that polluted soils are a reservoir for a large number of micromycetes. The difference in the prevalence of these micromycetes in different soil samples may be attributed to their tolerance and adaptation to various biotic and abiotic factors such as ecological conditions, soil type, vegetation and soil pH.

The maximum number of strains was isolated from places which were mostly wet, as compared to soils of other places which have more organic material in the form of leaf litter present. This is important because the number of soil micromycetes is dependent on the type and rate of agrochemicals used, on the growing season, and the soil zone. The maximum number isolates belong to genus *Aspergillus*, as it is the most widespread genus in soils and members of the genus have a maximum ability to survive in a nutrient-depleted environment and there is a lack of nutrients in most of the soils of the general study area and they mostly remain rather dry.

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