

# Original Article

# Fungi Associated with Medicinal Juniper Tree Roots Juniperus Phoenicea L. at Al-Jebal-AL-Akhdar-Libya

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#### ARTICLE INFO

https://doi.org/10.5281/zenodo.4603943

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**Received:** 28-2-2021 **Accepted:** 13-3-2021 **Published:** 14-3-2021

**Keywords:** Medicinal, Juniper Tree, *Juniperus Phoenicea L.*, Fungi, Root.

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#### **ABSTRACT**

Objectives. The present study conducted during April 2016 in area at Al-Jebal Al-Akhdar-Libya. The goal of this study was to identify fungal species isolated from roots of Juniperus phoenicea L. Methods. Fungal growth was assessed after seven days of incubation at 28°C. Colony diameter, culture characteristics (texture, surface, reverse coloration, zonation and sporulation of fungi) were observed. Identification and characterization of the fungi were made with the help of authentic manuals of soil fungi and confirmed by the Region Center for Mycology and Biotechnology in Al-Azhar University. Results. The current results reported the presence of 24 fungal species belonged to 14 genera. The isolated fungi were identified as Microphaeropsis olivacea, Trichoderma viride, Rhizopus azygosporus, Alternaria alternata, Fusarium oxysporum, F. poae, F. tabacinum, F.dimerum, Emericella nidulans, Aspergillus niger, A. niveus, A. candidus, A. terreus var. aureus, Penicillium crustaceum, P. simplicissium, P. steckii, P. wortmanniim, Plectosporium tabacinum, Cunninghamella **Trichophytom** bertholletiae, verrucosum, Memnoniella echinata, Pseudallescheria boydii *Talaromyces* assiutensis and T. trachyspermus were prevalent in the roots samples. Result shows the highest frequency and occurrence of roots fungi were A. niger 75%. Conclusion. The present study shows that presence of 24 fungal species belonged to 14 genera were isolated from roots of J. phoenicea L. They include 2 Zygomycetes, 7 Ascomycetes, 5 Deuteromycetes. therefore, the highest frequency and occurrence of roots fungi were A. niger 75%.

Cite this article: Abubaker N, Ali A. Health Information System and Their Impact on The Quality of Health Care at Benghazi Medical Center. Alq J Med App Sci. 2021;4(1):132-145.

## INTRODUCTION

The ecosystem processes are influenced by the biological interactions among plant roots and fungi, these interactions can be of a mutuality, neutral or parasitic depending on the identities of the host plant and fungus [1]. The total number of fungi species is estimated at around 1.5 million [2,3]. Fungi are microscopic cells with special features (spores, mycelium, fruiting bodies) [4]. Most fungi are non-

motile [5,6] reported that fungi are group of organisms that are playing important roles in organic matter decomposition, plant growth promotion and in disease development and control. Besides, Soil is the main reservoir of fungi living in the rhizosphere [7] demonstrated that an environment that is rich in root exudates and decayed organic matter, are hast to many fungi and their activities. Despite, the ecosystem and the ability of plants to obtain nitrogen



and other nutrients was affected by the composition and quantity of microbes in the soil [8].

The Juniperus phoenicea is a conifer species occurring naturally in Libya, it distributes in the east part of Libya at El-Gabel El-Akhdar (Cyrenica) and it constitutes about 80% of the total vegetation of this area, in southern Europe, south Asia and northern Africa [9]. the most common number of species Fungi associated with roots and twigs of Juniper trees in Asir region were Acremoniun sp., Alternaria sp., A. alternata., Botryodiplodia juniperina, Cercospora sp., Cladosporium sp., Cylindrocarpon sp., Fusarium spp., F. avenaceum, F. chlamydosporium, F. equiseti, F. moniliforme, F. oxysporum, F. semitichtum, F. solani, Phoma sp., Phoma eupyrena, Pythium sp., Rhizoctonia solani, Stigmina juniperina, Theilaviopsis sp. [10]. The present study aimed to isolation and identification of fungal species from roots of Juniperus phoenicea tree and determine the frequency and distribution isolates fungi.

#### **METHODS**

## Study Settings and Data Collection

The samples were collected from Belhaded district in Al-Jebal AL Akhdar Libya. The samples were collected during April 2016, for studying the distribution and identification of fungi in roots of *Juniperus phoenicea*. The area of studying site was (100 mx50m= 5000m<sup>2</sup>) was divided into (6) sectors. Each one around (83 m<sup>2</sup>). From each sector three of *Juniperus phoenicea* plants were determined to collect roots samples for each part in line by zigzag fashion. Root samples from *Juniperus phoenicea* plants were collected [11].

# Isolation of Juniperus Phoenicea Roots Fungi

Endophytic fungi were isolated according the protocols described by [12] the roots of *Juniperus phoenicea* tree taken from the field were washed twice in distilled water. Then surface sterilized by immersion for 2 mins in 70% ethanol and then

washed three times in sterilized distilled water for 1 minute each time. After surface sterilization, root samples were cut into 1cm pieces aseptically transferred to plates contain potato dextrose agar (PDA) triplicate of each plant. And then were incubated at 28°C. Each fungus was isolated, purified and then maintained at 4°C on PDA for further identification.

## Identification of Fungal Species

Fungal colony was first grown on the Potato Dextrose Agar medium and its morphology was studied using standard cover-slip technique and lacto phenol cotton blue staining procedure. The cover slip was inserted in tilted position in the petriplate itself and the culture was allowed to grow for seven to ten days. Then the cover slip was taken out with the help of forceps and put inverted on slide containing a drop of lactophenol cotton blue stain and visualized under microscope (Lica microsystem cms gmbh dm 1000 Led) at 10X, 40 X, 100X magnification. The fungi were identified on the basis of mycelia and spore characteristics, and the identification of fungal genera and species was made based on previous studies [13-18].

## Cultural Characteristics of Isolated Fungi

The most important taxonomic criteria used for classification of fungi were: fungal spores are formed either asexually or sexual reproducyon. and Mycelium, coenocitic hyphae, conidiophore branching, shape of conidia, sporangia, oogonium, antheridia, radial growth colony, morphology of fruiting structures).

## Determine Frequency of Isolated Fungal Species

The frequency of isolated fungal species in the studied area was calculated by using the following formula [19].

% Contribution = Total No. of CFU of an individual species / Total No. of CFU of all species X 100 \*CFU-Colony forming Unit.



#### **RESULTS**

# Fungi Isolated from Roots

The fungal genera and species were identified on the basis of mycelia and spore characteristics, by using the light microscope. The results show that morphological and microscopic examination for fungi were isolated from the roots Fig 1-24.

# Rhizopus Azygosporus

Cultural characteristics on (PDA), colonies hairy, reaching 6cm colony diameter in 4 days, grayish-brown ochraceous becoming dark grey with age, reverse yellowish brown. Microscopic examination revealed the following characteristics. Sporangia globose, 75.5µm in diameter. Collumella subglobose to conical 80% of sporangium. Sporangiophores single or in small groups 14.0µm in diuameter. Sporangiospores spherical to ovoidal, 7µm in diameter. Chlamydospores abundant (Fig. 1).

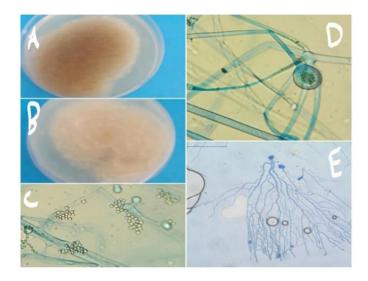


Fig. (1) Rhizopus azygosporus A- Growth colonies on PDA at 28°C, B- Reverse, C- Sporangiospores, D- Sporangium and Sporangiophores, E- Rhizoids

## Cunninghamella Bertholletiae

Cultural characteristics on (PDA), colonies white to tannish grey, reverse colorless, reaching 6 cm colony diameter in 4 days. *Microscopic* shows.: Sporangia globose, 28x25µm in diameter. Sporangiophores erect with a whorl of short lateral branches, each branch ending in a swollen vesicle 10.0µm in diameter.

Sporangiospores spherical to ovoidal 9.0µm, with finely echinulate (Fig. 2).

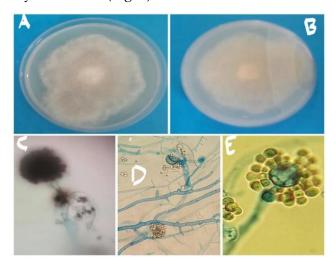


Fig. (2) Cunninghamella bertholletiae: A. Growth colonies on PDA at 28 °C, B. Reverse, C. Sporangiophores, D. Sporangia, E. Collumella and Sporangiospores

# Aspergillus Niger

Cultural characteristics colonies attaining 5-6cm in diameter after 7 days on (PDA) at 28°C, margin white, conidial heads black, exudate lacking, reverse colorless or yellow. Microscopic examination revealed the following characteristics: Conidiophores up to 184.25µm long x6.14µm wide, smooth-walled, hyaline, mycelium septate, 2.7µm in diameter. Conidial heads radial, vesicles globose to subglobose up to 40µm. Primary sterigmata 9.90x2.9µm. Secandary sterigmata 4.4x3.9µm. Conidia brown, roughened, globose to sub globose, 3.2µm in diameter (Fig. 3).

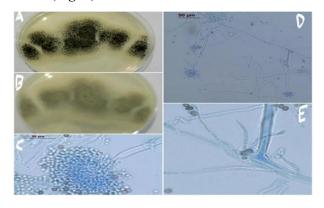


Fig. (3) Aspergillus niger: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidia and Conidial heads, D. Conidiophore, E. Foot cell



# Aspergillus Terreus Var. Aureus

Cultural characteristics colonies on (PDA), at 28°C, attaining a diameter of 3.0-3.5cm within 7days gives buff to yellow brown, reverse deep brown pigment observed. Microscopic examination revealed the following characteristics: Conidial heads columnar. Vesicle **diam.** sub-globose 12.0µm in diameter, primary sterigmata 5.0x2.0µm, secandary sterigmata 4.2x1.6µm. Conidiophore 4.0µm in diameter. Conidia globose, smooth, 2.0µm in diameter (Fig.4).

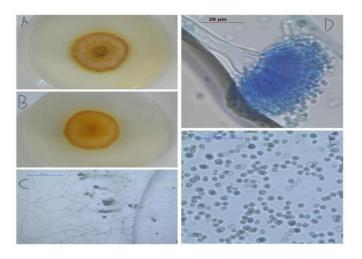


Fig. (4) Aspergillus terreus var. aureus: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiospores, D. Conidial heads, E. Conidia

## Aspergillus Candidus

Cultural characteristics colonies on (PDA) at  $28^{\circ}$ C, attaining a diameter of 3.0-3.5cm within 7days gives white to cream conidial heads, brown reverse observed. Microscopic examination revealed the following characteristics: Conidial heads radial, vesicle diameter subglobose  $25.0\mu m$  in diameter, primary sterigmata  $6.0x2.2\mu m$ , secandary sterigmata  $3.6x1.5\mu m$ . Conidiaphore diameter  $3.5\mu m$ . Conidia globose, smooth  $2.5\mu m$  in diameter (Fig. 5).

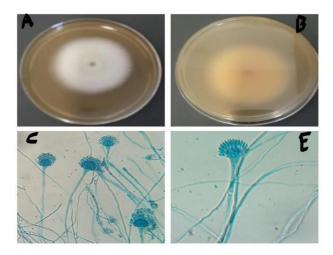


Fig. (5) Aspergillus candidus: A. Growth colonies on PDA at 28 °C, B. Reverse, C. Conidiophore and Conidia, D. Conidial heads

# Aspergillus Niveus

Cultural characteristics colonies on (PDA), reaching 3-4cm diameter in 7 days at 28°C, white became yellowish buff with brown reverse. Microscopic shows: Conidial heads radiate, conidiophore 6.0µm in diameter. Vesicle globose- subglobose, 14.0µm, first sterigmata 7.5x3.0µm, second sterigmata 5.3x2.0µm. Conidia globose, 2.5µm (Fig. 6).

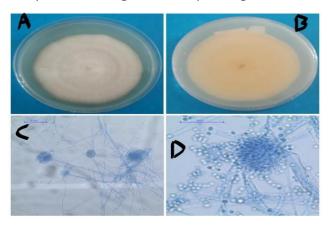


Fig. (6) Aspergillus niveus: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophore, D. Conidial heads and Conidia

#### Emericella Nidulans

Cultural characteristics colonies on (PDA), reaching 3-4cm diameter in 7 days at 28°C, White-Green with white margin, reverse brown age. Microscopic examination revealed the following characteristics: Conidial heads radiate. Conidiophore 5.0µm in



diameter. Vesicle globose 10.0µm. First sterigmata 9.0x3.0µm, second sterigmata 6.0x2.2µm. Conidia sub-spherical 4.0µm, hűll cells were observed (Fig. 7).

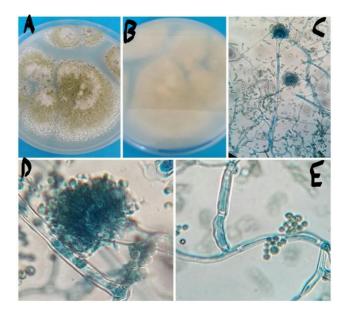


Fig. (7) Emericella nidulans: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophore, D. Conidial heads, E. Conidia

#### Memnoniella Echinata

Cultural characteristics colonies attaining 6.2-7cm in diameter after 7days on (PDA) at 28°C, margin white, changing into greyish or black towards the centre, exudate in the form of numerous colourless droplets, reverse yellow. Microscopic examination revealed the following characteristics: Conidiophores erect up to 191.9x4.9µm, 2-3 septate, mycelium septate, 4.4µm in diameter. Phialides cylindrical or elliptical 7.7x2.5µm. Conidia in persistent chains, spherical, darkly, echinate 3.6µm in diameter (Fig. 8).

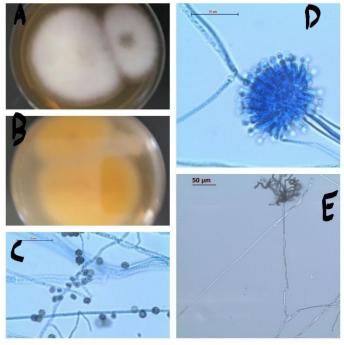


Fig. (8) Memnoniella echinata: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidia, D. Conidial heads, E. Conidiophore

#### Penicillium Steckii:

Cultural characteristics: colonies attaining 4.8-5.0cm in diameter, after 7 days on (PDA) at 28°C, surface lanose, margin white, conidial areas Grayed-Green, exudates in the form of few colourless droplets or lacking, reverse brown. Microscopic examination revealed the following characteristics: Conidiophores smooth-walled, up to 140.8x3.5μm, usually not ramified, mycelium septate, 2.13μm in diameter. Penicilli typically biverticillate and asymmetrical, metulae in verticils of 2-4 more 14.4x3.17μm. Phialides ampulliform, 6.9x1.7μm. Conidia spherical to subspherical, smooth-walled to roughened, 2.0μm in diameter (Fig. 9).



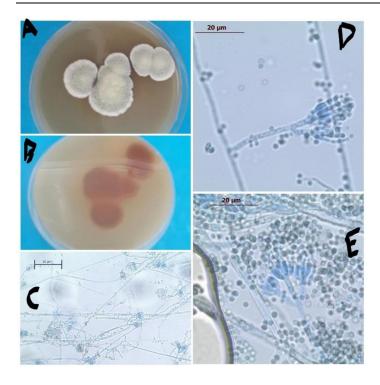
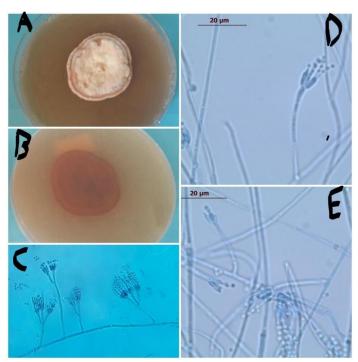


Fig. (9) Penicillium steckii: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophore, D-E Metulae, Phialides and Conidia

## Penicillium Simplicissium

Cultural characteristics colonies on (PDA), attending 4-6 cm diameter at  $28^{\circ}$ C, white, grayish, buff, pale yellow mycelium to green, reverse reddish brown. *Microscopic* Microscopic examination revealed the following characteristics: Penicillus type biverticillate, conidiophore diameter  $3.0\mu m$ , metulae  $12.2x3.0\mu m$ , phialides  $7.0x2.0\mu m$ . Conidia ellipsoidal  $3.5x~2.6\mu m$  (Fig. 10).



**Fig. (10)** *Penicillium simplicissium:* **A.** Growth colonies on PDA at 28°C, **B.** Reverse, **C.** Conidiophore, **D-E.** Phialides and Conidia

## Penicillium wortmannii

Cultural characteristics colonies on (PDA), attending 3-5cm diameter at 28°C, radially, white, grayish, buff to green, yellow soluble pigment produced and yellowish-brown reverse. *Microscopic.*: Penicillus type bi-verticillate and sometime terverticillate. Conidiophore diameter 2.8µm. Rami 21.0x3.7µm, metulae 12x3.0µm. Phialides 9.0x2.2µm. Conidia elliposoidal 2.5x2.0µm (Fig. 11).



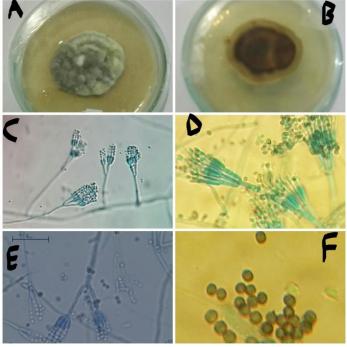


Fig. (11) Penicillium wortmannii: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophore, D.E. Metulae and phialides, F. Conidia

### Penicillium crustaceum

Cultural characteristics colonies on (PDA), attending 2-3cm diameter at 25°C, white, grayish, mycelium to deep green, reverse pale yellow, brown. Microscopic examination revealed the following characteristics: Penicillus type bi-verticillate are present. Conidiophore diameter 2.5 $\mu$ m. Metulae 15x2.3 $\mu$ m. Phialides 5.0 x 2.0 $\mu$ m. Conidia spherical to subspherical 2.5 $\mu$ m (Fig. 12).

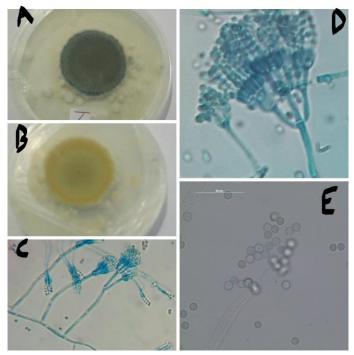


Fig. (12) Penicillium crustaceum: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophores, D. Metulae and Phialides, E. Conidia

# Talaromyces trachyspermus

Cultural characteristics colonies on (PDA), attending 3-4cm diameter at 28°C, white to buff the reverse yellow, to pale brown. Microscopic examination revealed the following characteristics: Penicillus type bito terverticillate are present, conidiophore diameter 2.5µm. Metulae 17x2.3µm. Phialides 11.0x2.0µm, ascomata yellow to green, abundant. Conidia elliposoidal 3.5x1.9µm (Fig. 13).



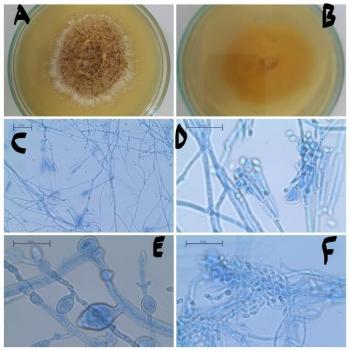


Fig. (13) Talaromyces trachyspermus: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophores, D. Metulae and Phialides, E. Cleistothecia, F. Conidia

# Talaromyces Assiutensis

Cultural characteristics colonies attaining about 3.9-4.2cm after 10 days on (PDA) at 28°C, margin white, white acquiring brown, pink colour towards the exudate colourless, centre, reverse vellow. examination revealed Microscopic the following characteristics: Conidiophores sparse, transparent, smooth, relatively short, septate, 78.64µm long x 4µm wide, mycelium septate, 2.8µm in diameter, penicilli terverticillate to biverticillate symmetrical. Rami in verticils of 2-3, 20.7x 2.8µm. Metulae in verticils of 2-3 more 13.4x2.6µm. Phialides lanceolate, in verticils of 2-4, 17.5x3.8µm. Cleistothecia subglobose to ovoid. Conidia hyaline smooth, ovoid to crescent and spherical, 5.3 X2.4 μm (Fig. 14).

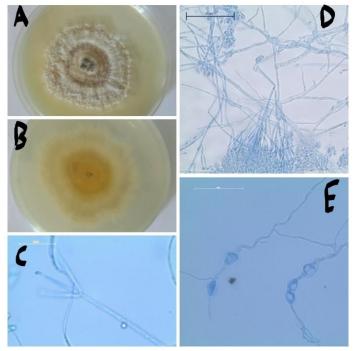


Fig. (14) Talaromyces assiutensis: A. Growth colonies on PDA at 28°C, B. Reverse, C. Phialides, D. Conidiophores and conidia, E. Cleistothecia

## Trichophytom Verrucosum

Cultural characteristics colonies on (PDA), reaching 7-7cm diameter in 7 days at 28°C white with orange to brown reverse. Microscopic examination revealed the following characteristics: Chlamydospores common, in chains or swollen, conidia sometimes observed (Fig. 15).

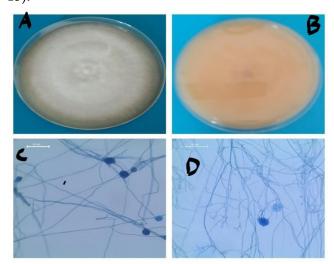


Fig. (15) Trichophytom verrucosum: A. Growth colonies on PDA at 28°C, B. Reverse, C.D. Chlamydospores



# Pseudallescheria Boydii

Cultural characteristics colonies growing rapidly, on (PDA), velvety white became grey to brown with brown reverse. Microscopic examination revealed the following characteristics: Conidia 1 celled, lemon shape 6.5x4.0µm (Fig, 16).

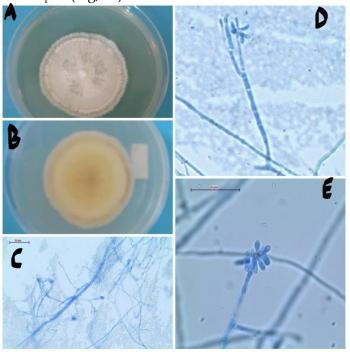
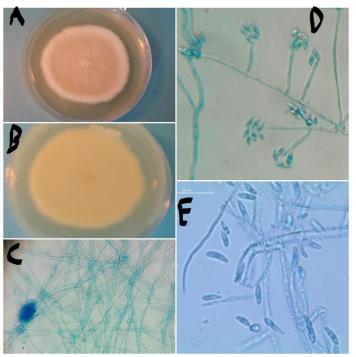


Fig. (16) Pseudallescheria boydii: A: Growth colonies on PDA at 28 °C, B- Reverse, C- Conidiophores, D- Metulae and Phialides, E- Conidia

## Fusarium Tabacinum

Cultural characteristics colonies on (PDA) attaining a diameter of 3.0cm in 4 days. Mycelium cottony whitish to yellowish, reverse whitish to pale brown. Microscopic examination revealed the following characteristics: Conidiophores monophialidic 3.5  $\mu$ m in diam., micro-conidia Absent, macroconidia 1-3 septa, one or two cells is common, 13.0x6.0 $\mu$ m. Chlamydospores found (Fig, 17).



**Fig. (17)** *Fusarium tabacinum:* **A.** Growth colonies on PDA at 28°C, **B.** Reverse, **C.** Chlamydospores, **D.** Conidiophores, **E.** Micro-conidia and Macro-conidia

#### Fusarium Poae

Cultural characteristics colonies on (PDA), attaining a diameter of 3.0cm in 4 days, mycelium cottony in peach or pinkish colour, reverse in riddish shades. Microscopic examination revealed the following characteristics: Micro-conidia, abundantly produced one celled, pyriform, 7.5x4.0µm, macroconidia 2-5 septata, 20.0x5.0µm. Chlamydospores not produced, but swollen hyphal portions can be observed (Fig, 18).



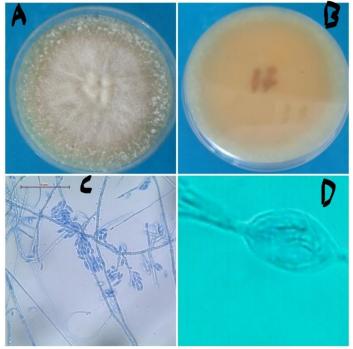


Fig. (18) Fusarium poae: A. Colonies on PDA at 28°C, B. Reverse, C. Micro-conidia, Macro-conidia and Conidiophore D. swollen hyphal portions

#### Fusarium Dimerum

Cultural characteristics colonies on (PDA), attaining a diameter of 3.0cm in 4 days, mycelium white, reverse pale brown. Microscopic shows.: Conidiophores monophialidic 3.0µm in diam., microconidia Absent, macro-conidia 1-3 septa, one or two cells is common, 15.0x4.5µm. Chlamydospores found (Fig, 19).

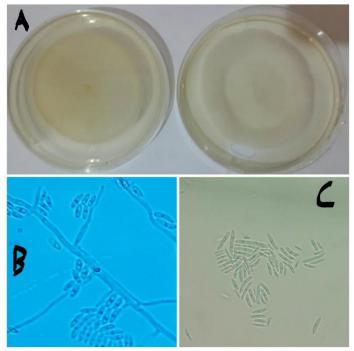


Fig. (19) Fusarium dimerum: A. Growth colonies on PDA at 28°C and reverse, B.C. Micro-conidia, Macro-conidia and Conidiophore

# Fusarium Oxysporum:

Cultural characteristics colonies fast-growing, reaching 7-8cm in diameter after 7 days on (PDA) at 28C°, mycelium abundant and floccose, white, purple or violet, reverse purple. *Microscopic* shows.: Conidiophores transparent, smooth, 22.90 µm, mycelium septate, 3.50 µm in diameter. Micro-conidia abundant single-celled, elliptical, straight to curved 5.1 x 2.61µm, arising formed from phialides simple short, laterally on the hyphae. Macro-conidia abundant, arising formed from phialides or laterally on the hyphae or from short sparsely branched conidiophores, 1-5 septate, slightly sickle-shaped, thin-walled, with a pointed apical cell and a food-shaped basal cell, 11.15x2.8µm. Chlamydospores abundant, formed singly, terminal (Fig. 20).



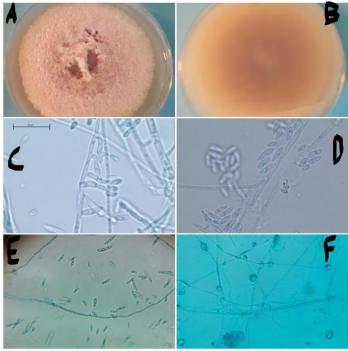


Fig. (20) Fusarium oxysporum: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophores, D.E. Micro-conidia, Macro-conidia, F. Chlamydospores

# Plectosporium Tabacinum

Cultural characteristics colonies on (PDA) whitish to beige with reddish brown reverse Microscopic examination revealed the following characteristics: Conidiophores monophialidic 3.5µm in diamerter. Micro-conidia 1-2 celled 6.0x3.5µm, macroconidia 1-3 septa, slightly curved with more or less pointed apex, one or two cells is common, 12.0x3.0µm (Fig. 21).

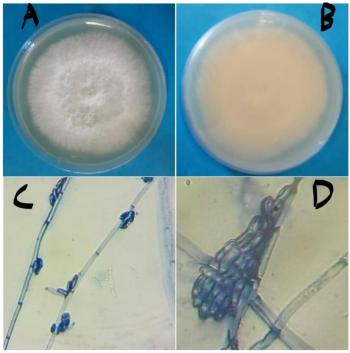


Fig. (21) Plectosporium tabacinum: A. Growth colonies on PDA at 28°C, B. Reverse, C.D. Micro-conidia and Macroconidia

#### Trichoderma Viride:

Cultural characteristics colonies growing rapidly, attaining 8-8cm in diameter after 4 days on (PDA) at 28°C, first thin and translucent, then appearing conidial which at first white becoming greenish later, reverse colorless. Microscopic examination revealed the following characteristics: Conidiophores 6.60µm, not sharply distinct from arise hyphae, sparingly or highly branched lateral branches arising at approximately right angles from the main axis singly or in groups of 2 or 3 branches, all branches terminated with phialides. Mycelium septate, 3.07µm in diameter. Conidia spherical to subspherical, roughened, 2.43µm in diameter. Chlamydospores found, globose (Fig. 22).



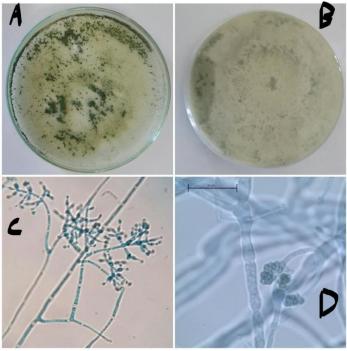


Fig. (22) Trichoderma viride: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophores, D. Conidia

#### Alternaria Alternate

Cultural characteristics colonies attaining about 6.6-6.8cm after 7 days on (PDA) at 28°C, mycelium gray to black or olivaceous black, reverse brown to black. Microscopic examination revealed the following characteristics: Conidiophores simple or branched, straight or curved, pale to mid brown, smooth, up to 77.46 long x 6.24µm wide. Mycelium septate, 3.50µm in diameter. Conidia ovoid or cylindrical beak, 24.07x11.20µm, pale to brown, with 1-8 septa and one or two longitudinal or strongly oblique septa in each of the transverse divisions, wall smooth (Fig, 23).

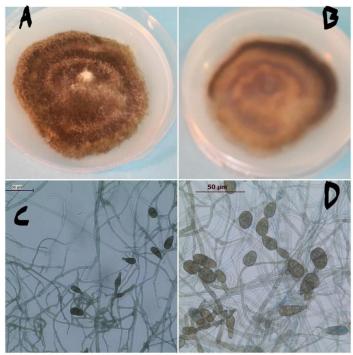


Fig. (23) Alternaria alternate: A. Growth colonies on PDA at 28 °C, B. Reverse, C. Conidiophores, D. Conidia

# Microphaeropsis Olivacea

Cultural characteristics colonies reaching 3-5cm diameter in 7 days at 28°C, on (PDA), velvety white with pale brown reverse. Microscopic shows conidia 1 celled, ellipsoidal 5.5x3.2µm (Fig, 24).

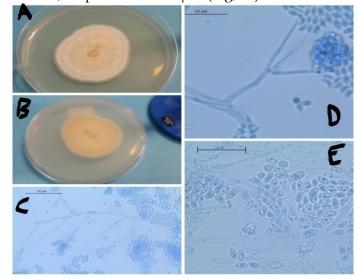


Fig. (24) Microphaeropsis olivacea: A. Growth colonies on PDA at 28°C, B. Reverse, C. Conidiophores, D. E. Conidia



## Determine Frequency of Isolated Fungal Species

A total of 35 colonies were isolated. 24 species belonging to 14 genera were isolated from roots of *J.* phoenicea L. They include 2 Zygomycetes, Ascomycetes, 5 Deuteromycetes. The total species isolated 24 fungi from the roots. The species of the genus Microphaeropsis olivacea (8.33 %), Trichoderma viride (62.5%), Rhizopus azygosporus (54.16 %), Alternaria alternate (32.32 %), Aspergillus niger (% 75.00), A. niveus (8.33 %), A. candidus (4.16 %), A. terreus var. aureus (8.33 %), Emericella nidulans (4.16 %), Penicillium wortmanniim (29.16 %), P. crustaceum (54.16%), P. simplicissium (12.5%), P. steckii (16.66%), Talaromyces assiutensis (29.16 %), T. trachyspermus (41.66 %), Fusarium poae (20.83%), F. tabacinum (47.21%), F. dimerum (66.66%), F. oxysporum Plectosporium (37.5)tabacinum %), Cunninghamella bertholletiae (8.33 %), Trichophytom verrucosum (8.33 %), Memnoniella echinata (4.16 %), and Pseudallescheria boydii (4.16 %) were prevalent in the roots.

#### DISSCUSION AND CONCLUSION

In the present study, 24 fungal floras were isolated from roots of Juniperus phoenicea L. A total of 35 colonies were isolated. About 24 species belonging to 14 genera were isolated from roots of J. Phoenicea L. They include 2 Zygomycetes, 7 Ascomycetes, 5 Deuteromycetes. The results are in agreement with earlier studies reported that Trichoderma koningii, Alternaria alternata, Phoma sp., Acremonium strictum were isolated from maize roots [10,20]. Rajput et al., found that Fusarium solani, F. moniliforme, F. equiseti, F. oxysporum. F. semitectum, Rhizoctonia solani, Alternaria alternata, Curvularia lunata, Aspergillus niger and Penicillium sp. were isolated from infected roots, bark, seed and stem of shisham [21]. Previous studies also reported that Trichoderma sp., Fusarium sp., Acremonium sp., Aspergillus sp., Penicillium sp., and Botryodiplodia sp. were isolation from roots [12,22]. Also, in agreement with results of Srimathi et al., mention that Alternaria, Aspergillus, Curvularia, Fusarium, Nigrospora, Colletotrichum, Papulospora Pestalotiopsis, Phoma, Phomopsis, Penicillium,

Leptosphaerulin, Mycelia and Trichoderma were isolated from the medicinal plants [23]. Shemshura et al., report that Aspergillus candidus was isolated from the root zone and Fusarium dimerum was saprotroph in soil and on plant materials [24]. Moreover, earlier studies revealed that Alternaria spp., Cylindrocladium sp., Fusarium spp., Phoma sp., Pythium spp., Rhizoctonia sp., Thielaviopsis sp. and Verticillium sp, isolate from root and soil-borne [25,26].

#### Disclaimer

The article has not been previously presented or published.

# Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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