Original Article

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

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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 *Acremonium 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. semitichum*, *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 m x 50m= 5000 m²) was divided into (6) sectors. Each one around (83 m²). 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 reproductyon. 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].

\[
\% \text{ Contribution} = \frac{\text{Total No. of CFU of an individual species}}{\text{Total No. of CFU of all species}} \times 100
\]

*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 diameter. Sporangiospores spherical to ovoidal, 7μm in diameter. Chlamydospores abundant (Fig. 1).

**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).

**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).

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

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

**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 7 days 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, secondary sterigmata 4.2x1.6μm. Conidiophore 4.0μm in diameter. Conidia globose, smooth, 2.0μm in diameter (Fig. 4).

Aspergillus Candidus
Cultural characteristics colonies on (PDA) at 28°C, attaining a diameter of 3.0-3.5cm within 7 days gives white to cream conidial heads, brown reverse observed. Microscopic examination revealed the following characteristics: Conidial heads radial, vesicle diameter subglobose 25.0μm in diameter, primary sterigmata 6.0x2.2μm, secondary sterigmata 3.6x1.5μm. Conidiophore diameter 3.5μm. Conidia globose, smooth 2.5μm in diameter (Fig. 5).

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).

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, hüll 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 7 days 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).

**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°C, white, grayish, buff, pale yellow mycelium to green, reverse reddish brown. *Microscopic* Microscopic examination revealed the following characteristics: Penicillus type biverticillate, conidiophore diameter 3.0μm, metulae 12.2x3.0μm, phialides 7.0x2.0μm. Conidia ellipsoidal 3.5x 2.6μm (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 ellipsoidal 2.5x2.0μm (Fig. 11).
**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 2.5μm. Metulae 15x2.3μm. Phialides 5.0 x 2.0μm. Conidia spherical to sub-spherical 2.5μm (Fig. 12).

**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 bi- to 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 centre, exudate colourless, reverse yellow. Microscopic examination revealed 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](image)

**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 μm in diam., micro-conidia Absent, macroconidia 1-3 septa, one or two cells is common, 13.0x6.0μm. 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](image)

**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).
**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., micro-conidia Absent, macro-conidia 1-3 septa, one or two cells is common, 15.0x4.5μm. Chlamydospores found (Fig. 19).

**Fusarium Oxysporum:**
Cultural characteristics colonies fast-growing, reaching 7-8cm in diameter after 7 days on (PDA) at 28°C, 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).

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

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

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 diameter. 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).

**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).
Alternaria Alternate

Cultural characteristics colonies attaining about 6.6-6.8 cm 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).

Microphaeropsis Olivacea

Cultural characteristics colonies reaching 3-5 cm 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).
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, 7 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 wortmannii* (29.16 %), *P. crustaceum* (54.16 %), *P. simplicissium* (12.5%), *P. steckii* (16.66%), *Talaromyces assutensis* (29.16 %), *T. trachyspermus* (41.66 %), *Fusarium poae* (20.83 %), *F. tabacinum* (47.21 %), *F. dimerum* (66.66 %), *F. oxysporum* (37.5 %), *Plectosporium tabacinum* (8.33 %), *Cunninghamamella bertholletiae* (8.33 %), *Trichophyton verrucosum* (8.33 %), *Mennoniella echinata* (4.16 %), and *Pseudallescheria boydii* (4.16 %) were prevalent in the roots.

**DISCUSSION 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 alternate*, *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 isolated 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.

**REFERENCES**


