DIVERSITY OF MICROFUNGI IN LITTER OF PINE FORESTS IN DUHOK

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(Accepted for publication: June 9, 2013)

ABSTRACT

The litter microfungi of Pine forests located in Zawita and Atrush were studied with objective to compare the composition, frequency of occurrence and diversity of fungi in forest habitats from Duhok, Kurdistan region of Iraq. A total of 11 species in addition to sterile mycelia were identified from L1 litter layer from the two sites, whereas, 9 species were identified from L2 layer at both sites. In general, the detected species from both litter layers (L1,L2) at the two sites included: *Aspergillus flavus, A.fumigatus, A.niger, Alternaria alternata, Aurobasidium pullulans, Cladosporium cladosporoides, C.gallicola, Fusarium sp., Papulaspora pallidulla, Penicillium glabrum, Rhizopus* sp. *Scytalidium lignicola,Ulocladium atrum* in addition to non-sporulating mycelia. Of these *A.pullulans* was the most frequent species on freshly and decomposed pine needles litter at the two sites. The least (SI) index for fungal community inhabiting Pine litter was recorded between L1 and L2 layers at Zawita. Whereas, the highest (SI) was found between L1 layer at Atrush and L2 layer at Zawita site. *Papulaspora pallidulla* represents a first record for Iraqi mycobiota. Brief description along with photographs are provided for the newly reported species.

KEYWORDS: *Litter fungi. Forests. Iraq.*

INTRODUCTION

It is now well established that the decomposition of plant litter on the soil surface is brought about by a variety of microorganisms including bacteria, fungi, and actinomycetes (Hattenschwiler *et al*. , 2005). Among the microbes, fungi are regarded as efficient decomposes of the organic matter, especially plant litter (Dickinson and Pugh, 1974; Shanthi and Vital, 2010).

A major part of the total litter input in forest soils are non-woody plant residues such as leaves, fruits and reproductive structures . The total litter fall in the pure forest was estimated to 1-1.5 tones, the larger of which the needles accounted for 69-87% in the total amount (Pausas, 1997). Complete structural disintegration of conifers needles litter takes about 7 years compared to most deciduous litter which takes between 9 months to 3 years to be fully disintegrated (Body and Watkinson, 1995)

Colonization of pine needles by fungi begins on the tree. Some of the spores germinate immediately and grow on the leaf surface and form the phylloplane or leaf-surface fungi. These grow as saprophytes on leaf surface living on simple organic substances such as sugars, amino acids and in-organic ions, which are exuded or diffuse out of the leaf. They persist as surface inhabitants until after leaf-fall. With leaf senescence any facultative parasites within the leaves may persist and spread. Spores of leaf saprophytes present on the leaf surfaces germinate and rapidly colonize. The majority of these are Fungi Imperfect, the commonest of which are *Cladosprium* spp .*Epicoccum* spp., *Aureobasidium pullulans* and *Alternaria* spp. These all are very common air-borne fungi (Hudson, 1977).

Litter decomposition in temperate and boreal forests is mainly driven by fungal activity. Dobranik (1999) found that litter decomposition was faster at sites with high fungal diversity.

A lot of studies were carried out worldwide on the diversity of microfungi in litter of coniferous forests (Kendrick and Burges, 1962; Hayes, 1965; Tokumasu, 1980; Tokumasa and Aoiki, 2002: Botela*et al*., 2010; Koukol, 2011). Most of these studies have demonstrated that a few initial colonizers are present on the tree, and these are replaced by a larger number of secondary saprobic species that colonize litter in the ground after needle fall (Koukol, 2011).

In Iraq, however, so far there is no previous work on fungi inhabiting litter in forests except the work by Rattan and Abdullah(1976) and Rattan *et al*.(1978) on fungi causing decay of *Pinus bruitia* logs in Duhok forests.

The present study was carried out on litter fungi present in Atrush-zawita natural pine forests in Duhok governorate to study species diversity and taxonomy.

MATERIALS AND METHOD

Site description

The study was conducted in the unique natural pine forest restricted to Zawita-Atrosh

locality (36°52¯- 36°90¯N latitude and 43° 17¯-43° 58¯ E longitude).

The forest supports pure stand of *Pinus bruitia* Tern trees covering a mountainous area of 100 Km square, 10 Km N.W of Duhok city. The forest is treated by many others as represent, the southern limits of the species distribution in the eastern Mediterranean (Townsend and Guest, 1966; Shahbaz, 2007).

Both sites were about 8 Km apart from each other and have similar climatic conditions, the average minimum temperature is 8.97C° and average maximum temperature is 22.34 C°. The total rainfall average is 578.5mm. The monthly average relative humidity (RH%) is ranging between 18.5% to 68% with a total average 44.18% (Anonumous 2005**).**

Collection of Pine Leaf Litter

A total of 20 *Pinusbruitia* leaf litter samples were collected from the Litter horizon in the two sites (10 samples from each site). In the laboratory litter samples were sorted into two categories, freshly fallen indicated as L1-layer (litter layer) and partly degraded leaves (L2-layer).

Isolation of Fungi from Litter Layers

A set of 20 needles taken from each layer in each sample was surface sterilized by sodium hypochlorite (2%)for 1 minute as described by Kendrik and Burges (1962). The surface disinfected needles were placed in Petri plates of malt extract agar (MEA) (powder malt extract 20g, peptone 100g, glucose 20.0g, agar 20.0g, 1L distilled water). The medium was supplemented with 50mg/L chloramphenicol.

The plates were incubated at 25C°and examined periodically for one month. Fungi sporulating on and around each of the needle, were recorded . Pure cultures were obtained by cutting hyphal tips from each colony or transferring conidia from sporulating fungi into a new fresh plates containing appropriate media for identification.

Identification of Fungi

Identification of fungal isolates was based on morphological and cultural characteristics. General and specific taxonomic literature was used for identification of fungal species. Domsch*et al*., (1980); Klich (2002), Ellis (1976), Watanabe (2002.

DATA ANALYSIS

Frequency of occurrence of litter fungi was calculated based on the following formula:

$$\%FO = \frac{number\ of\ needles\ that\ a\ particular\ \ fungal\ species\ occur\ on}{total\ number\ of\ needles\ exa\ mi\ nd} \times 100$$

Comparing the similarity of fungal species composition between different habitats, Sorensen´s index (SI) was applied (Sorenson, 1948). The index was calculated with formula

$$\mathbf{SI} = \frac{2c}{a+b}$$

Where

a = total number of species at site 1

b = total number of species at site 2

c = number of species common to both sites.

Similarity is expressed with values between 0 (no similarity) and I (absolute similarity).

RESULTS AND DISCUSSION

Data for the fungi associated with freshly fallen pine needle at both sites were presented in table (1).

Total of 8 species assigned in 7 genera in addition to non-sporulating mycelia were isolated from Zawita site, whereas, seven species representing seven genera in addition to sterile mycelium were detected from Atrush site. *Aspergillus niger*, *Alternaria alternata*, *Aurobasidiumpullaluns*, *Mucor* sp., and white sterile mycilium were common to both sites. Two species of *Cldosporium*, *Papullaspora pallidula* and *Rhizopus* sp., were detected only from Zawita site, while *Fusarium* sp., *P.glabrum*, *Ulocladium atrum*, and Yellow sterile mycelium were found in Atrush site. The most frequent species of freshly fallen litter needles in both sites was *A. pullulans*.

Table (1). % Occurrence of Fungi in Fresh Fallen Litter (L1 layer)

Fungal species	Zawita	Atrush
*Aspergillus niger*Tiegh	20%	30%
Alternaria alternata(Fr.)keissl.	40%	20%
Aurobasidium pullulans (de Bary)Arnaud	100%	90%
*Cladosporium gallicola*Sutton	20%	-
C.cladosporioides(Fresen) de Vries	20%	-
Fusarium sp.	-	10%
Mucor sp.	30%	10%
*Papulaspora pallidula*Hotson	10%	-
Penicillium glabrum (Wehmer)Westling	-	10%
Rhizopus sp.	50%	-
Sterile mycelium(brown)	10%	-
Sterile mycelium(white)	100%	90%
Sterile mycelium(yellow)	-	10%
*Ulocladium atrum*Preuss	-	30%

Data for fungi detected from decomposed pine litter (L2 layer) at both sites were presented in table (2).

A total of five species distributed in five genera in addition to non-sporulating mycelium were found in Zawita site, whereas a nine species assigned to seven genera in addition to sterile mycelium were detected from Atrush site. *P.pullulans*, *Fusarium* sp., *Rhizopus sp*., white sterile mycelium and *U. atrum* were found common to both sites, *Aspergillus flavus*, *A. fumigatus*, *A.niger*, *Alternaria alternata*, *Penecilium glabrum*, and sterile mycelium (yellow) were detected from Atrush site. *Scytilidium lignicola* was isolated only from Zawita site. *A. pullulans* and sterile mycelium (white) were the most common frequent species followed by *Fusarium* sp., *U. atrum*, on decomposed litter in both sites. Most of the fungi recorded in present study have been also described previously from conifer (Zamora*et al*., 2008; Botella*et al*., 2010).

Similarity index (SI) for fungal community on litter (L1, L2 layers) is presented in table(3) .The highest similarity index (0.705) was found between L1 layer at Atrush with L2 layer at zawita. The least similarity index was recorded for L1 and L2 litter layers at Zawita (0.5).

Species such as *Alternaria alternate*, *Cladosporium cladosporioides*, *C.gallicola* are known as epiphytes but under appropriate conditions penetrate the plant tissue and are able to colonize the interior part of the needles at the onset in the senescence process (Petrini, 1991; Virza De Santo, *et al*., 2002).

Species in the genera *Aspergillus*, *Fusarium*, *Pinicillium*, *Rhizopus*, and*Ulocladium* detected in this study are typical soil fungi (Domsch*et al*., 1980). Previous studies on fungal succession on fallen pine needle have demonstrated that a few initial colonizers are present on the tree, and these are replaced by a large number of secondary saprobic species that colonized litter on the ground after needle fall (Hayes, 1965; Tokumasu and Aoki, 2002).

Table (2) % Occurrence of Fungi in Decomposed Litter (L2 layer)

Fungal species	Zawita	Atrush
*Aspergillus flavus*Link	-	10%
*A.fumigatus*Fresen	-	10%
*A.niger*Tiegh	-	40%
Alternaria alternate (Fr.)Keissl.)		20%
Aurobasidium pullulans (de Bary)Arnaud	80%	80%
Fusarium sp.	40%	60%
Penicillium glabrum (Wehmer)Westling	-	30%
Rhizopus sp.	20%	50%
Sterile mycelium(white)	100%	50%
Sterile mycelium(yellow)	-	30%
*Scytalidium lignicola*Pesante	10%	-
*Ulocladium atrum*Preuss	50%	30%

*Papulaspora pallidula*Hotsonisolatedfrom freshly fallen pine needles collected from Zawita site is newly recorded from Iraq. Brief description with photographs is provided.

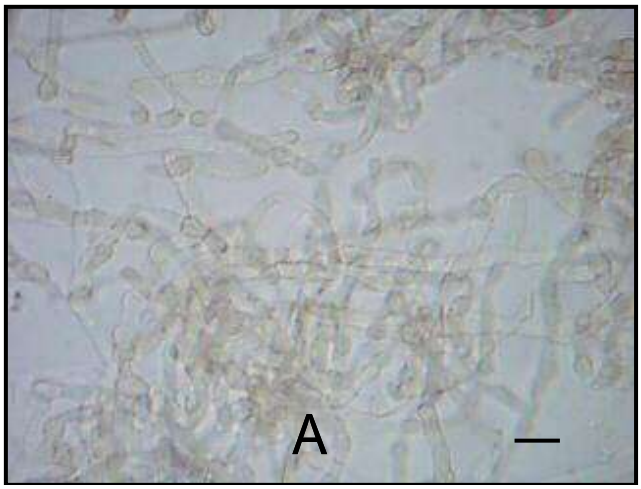
***Papulaspoa pallidula*Hotson**Bot.Gaz.64:264 (1917). Fig. 1 (A-C).

This fungus is characterized by developing large yellowish browm, globose to subglobosepapulospores. The dimension of papulospores ranged between 75 um to 200 um. Papulospores are composed of discernible numerous component cells with smooth margin and apparently soft sclerotium-like.

Table (3) Similarity Index (SI) between Litter Fungi at ZawitaandAtrush Site.

	Litter Zawita L1	Litter Zawita L2	Litter Atrush L1	Litter Atrush L2
Litter Zawita L1	----------	0.5	0.631	0.6
Litter Zawita L2	0.5	----------	0.705	0.666
Litter Atrush L1	0.631	0.705	----------	0.666
Litter Atrush L2	0.6	0.666	0.666	----------

This is the first report for the species in Iraq. The genus *Papulaspora* was erected in 1851 to accommodate fungi producing single compound spores on prostrate fertile hyphae (Preuss, 1851) and was redefined by Hotson (1912) as a form genus for species producing bulbils and lacking sexual state. Weresub and Le Claire, (1971) redefined *papulaspora*by excluding fungi with basidiomycetes affinities. Currently *Papulaspora* accommodate ascomycetes producing asexual thallodicpropagules that at some points in their development are heterogenus and differentiated into a core of enlarged, often darkly pigmented central cells that is surrounded by something mostly hyaline sheathing cells (Weresub and Le Claire, 1971; Kirk *et al*,. 2001). The propagules of *Papulaspora*have been referred to as bulbils, small sclerotia, conidia and papulospores. Recently, however, are classified under the generalized term 'gemmae' in reference to their function as multicellular asexual reproductive structure (Davey *et al*., 2008). Species of *Papulaspora* have been isolated fromvarietyof substrates including soil, plant debris, wood, dung, other fungi and animal tissues (Hotson, 1912; Warren, 1948; Shadomy and Dixon, 1989).



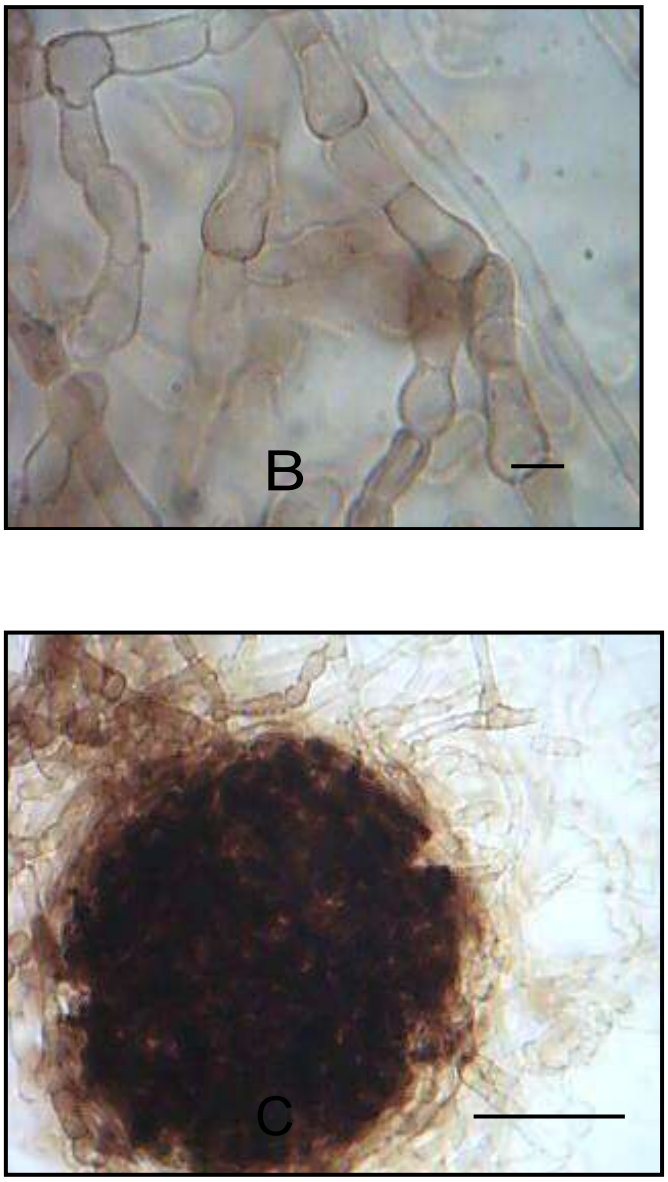


Fig. 1.*Papulaspora pallidula* A, B. Mycelia with thick-walled hyphae. C-Papulospore
Bar A = 5 μm, B = 10 μm, C = 50 μm,

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خلاصة

تم دراسة الفطريات الدقيقة المستوطنة للدبال فى ارضية غابات الصنوبر فى زاويته واتروش وذلك لمعرفة تركيب وتنوع المجتمع الفطري في بيئة غابات الصنوبر في دهوك. تم تشخيص أحد عشر نوعا" من الفطريات فضلا"عن الخيوط العقيمة من طبقة الدبال الأولى ، بينما شخصت تسعة انواع من طبقة الدبال الثانية ولكلا الموقعين (زاويته وأتروش) الانواع الفطرية التي عزلت من قبل كلا الموقعين هي *Aspergillus flavus, A.fumigatus, A.niger, Alternaria alternata, Aurobasidium pullulans, Cladosporium cladosporoides, C.gallicola, Fusarium sp., Papulaspora pallidulla, Penicillium glabrum, Rhizopus* sp.,*Scytalidium lignicola,Ulocladium atrum*

A. pullulans كان اكثر الانواع ترددا على اوراق الصنوبر الحديثة والمتفسخة.النوع تم مقارنة معامل التشابه لكلا الموقعين وان اقل معامل تشابه كان بين الفطريات المستعمرة للاوراق في الطبقة الاولى والطبقة الثانية لموقع زاويته بينما كانى اكثر معامل تشابه بين الطبقة الاولى لموقع اتروش مع الطبقة الثانية في زاويته.

*P.pallidulla*تم تسجيل الفطر لاول مرة في العراق وتم وصف النوع المسجل مع توضيح بالصور الفوتوغرافية.

كورتى

ئەڤ ڤەكولینە لسەر كەروەكانى ووردیلەى هاتیەكرن یێن خوجهى ل ئەردێ دارستانین داركاژ ل زاویتەیى و ئەتروشى ئەو ژى ژ بو زانینا پیكهاتى وجورێن د ژینگەها دارستانا كاژادا ل دهوكى. یازده جورێن كەرووا هاتنە دەست نیشانكرن سەرەرایى دافێن بێ بەرهەم یێن بەرگێ (الدبال الاول) بەلى پا نەھ جورییین دى ژ بەرگێ دووى (الدبال الپانیە) بو هەردوو جها (زاویتەو ئەتروشى) ئەو جورییین كەرووا ئەوییین هاتینە جوداكرن ل هەر دوو جها *Aspergillus flavus, A. fumigatus, A.niger, Alternaria alternata, Aurobasidium pullulans, Cladosporium cladosporoides, C.gallicola, Fusarium sp., Papulaspora pallidulla, Penicillium, Penicillium glabrum, Rhizopus sp., Scytalidium lignicola, Ulocladium atrum A. pullulans.*

پیتریا ژڤان جورا یین دووبارە بین ل سەر بەلگێن داركاژا سەردەم ویا كەڤنار جورێ *A.pullulans* **جیاوازى هاتیە كرن بو هوكارێن وەك ئیك بو هەردوو شوێنا و كیممترین هوكارێن هەفپشك دنافبەینا كەرووا**(colony) **دبەلگادا لبەرگێ ئیكێ و دوێ ل زاویتەیى ،بەلێ پا پترین هوكارێن وەكى ئیك دنافبەرا بەرگێ ئیكێ ل ئەتروشێ و لگەل بەرگێ دوێ ل زاویتەیى . ئەف كەرووە**(*P.pallidulla*) **هاتیە تومارکرن بو جارا ئیكێ ل عیراقێ دا ، ئەو جورە هاتیە تومارکرن و شلوڤەكرن بوینەیێ فوتوگرافى .**