## PERIÓDICO TCHÊ QUÍMICA

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### ISOLAMENTO E IDENTIFICAÇÃO DE FUNGOS DE DETRITOS DE PLANTAS SUBMERGIDAS EM HABITATS AQUÁTICOS NA PROVÍNCIA DE MISAN

# ISOLATION AND IDENTIFICATION OF FUNGI FROM SUBMERGED PLANTS DEBRIS IN AQUATIC HABITATS IN MISAN PROVINCE

عزل وتشخيص الفطريات من البقايا النباتية المغمورة في البيئات المائية في محافظة ميسان

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#### **RESUMO**

Quarenta e oito táxons de fungos saprófitos foram isolados e identificados a partir de amostras de restos de plantas submersas coletadas em diferentes locais na província de Misan, sul do Iraque, que são Maymouna, Al-Salam, Majar Al-Kabir e Amara. Dentre elas, 24 espécies pertenciam a Ascomycota (seis delas em estado sexual), 19 espécies a hifomicetos (fungos anamórficos), 4 a Zygomycota e uma a Oomycetes. Seis espécies foram isoladas e recentemente registradas no Iraque, que são Aniptodera margaration, Cirrenalia iberica, Cordana lignicola, Cordana verruculosa, Pseudoacrodictys appendiculata e Scytalidium thermophilum. No entanto, na avaliação dos métodos de câmara úmida e cultura direta, 34 espécies foram isoladas pelo primeiro método e 27 espécies pelo segundo método, entretanto, 13 espécies (9 pertenciam a Ascomycota, 2 a hifomicetos e 2 a zigomicetos) foram recuperado por ambos os métodos. Aspergillus terrus apareceu com maior frequência e ocorrência (11,76%, 42,55%, respectivamente), seguido por Aspergillus horti (10%, 36,17%, respectivamente) e, em seguida, A. niger com uma frequência e ocorrência de 5,29% e 19,14%, respectivamente, enquanto 17 espécies apresentaram menor frequência e ocorrência, atingindo 0,58%, 2,12%, respectivamente, para todas. Cento e sete isolados foram recuperados de todos os locais de estudo. Enquanto isso, sessenta e quatro isolados foram relatados de Majar Al-Kabir, em comparação com outros locais, seguido por Amara (43 isolados), enquanto 39 isolados foram isolados de Maymouna e 24 isolados de Al-Salam. A biodiversidade de fungos isolados de restos de plantas submersos foi comparada com estudos anteriores. Breves descrições dos novos fungos registrados foram fornecidas.

Palavras-chave: lignocelulose, restos de plantas submersas, Ascomycota, Hyphomycetes, Iraque.

#### **ABSTRACT**

Forty-eight taxa of saprophytes fungi were isolated and identified from submerged plant debris samples collected from different sites in Misan province, southern Iraq, which are Maymouna, Al-Salam, Majar Al-Kabir, and Amara. Among them, 24 species belonged to Ascomycota (six of which are sexual state), 19 species to hyphomycetes (anamorphic fungi), 4 to Zygomycota, and one to Oomycetes. Six species were isolated and newly recorded from Iraq, which are Aniptodera margaration, Cirrenalia iberica, Cordana lignicola, Cordana verruculosa, Pseudoacrodictys appendiculata, and Scytalidium thermophilum. However, in the evaluation of both moist chamber and direct culture methods, 34 species were isolated by the first method and 27 species by the second method, meantime, 13 species (9 belonged to Ascomycota, 2 to hyphomycetes, and 2 to Zygomycetes) were recovered by both methods. Aspergillus terrus was appeared in highest frequency and occurrence (11.76%, 42.55%, respectively), followed by Aspergillus horti (10%, 36.17%, respectively), and then A. niger with a frequency and occurrence of 5.29% and 19.14%, respectively, while 17 species were appeared lowest frequency and occurrence to reach 0.58%, 2.12%, respectively, for all. One hundred and seven isolates have been recovered from all study sites. Meantime, sixty-four isolates have been reported from Majar Al-Kabir, as compared with other sites, followed by Amara (43 isolates), while 39 isolates have been isolated from the Maymouna, and 24 isolates from Al-Salam. The biodiversity of fungi isolated from submerged plant debris was compared with previous studies. Brief descriptions of the new recorded fungi were given.

Keywords: lignocellulose, submerged plants debris, Ascomycota, Hyphomycetes, Iraq.

تم عزل وتشخيص 48 نوعا من الفطريات المترممة والمتواجدة على البقايا النباتية المغمورة من عينات جمعت من مناطق مختلفة في محافظة ميسان/ جنوب العراق وهي الميمونة والسلام والمجر الكبير والعمارة. وجد ان من بين الأنواع المعزولة، 24 نوعا تعود إلى الفطريات الكيسية (ستة أنواع منها hyphomycetes و 19 نوع تعود إلى الفطريات اللاقحية ونوع واحد من بالطور الجنسي) و 4 أنواع تعود إلى الفطريات اللاقحية ونوع واحد من الفطريات البيضية. تم عزل وتسجيل ستة أنواع لأول مرة في العراق هي Aniptodera margaration و Cortana e Cirrenalia iberica و Scytalidium thermophilum و Pseudoacrodictys appendiculata و Cordana verruculosa الفطريات الغرفة الأولى و 10 بالطريقة الأولى و 10 بالطريقة الثانية، ولوحظ ان 13 نوع قد تم عزلها بالطريقتين معا (9 أنواع معارطية والزرع المباشر لعزل الفطريات، فتم عزل 34 نوع بالطريقة الأولى و 11.76 الظهر الفطريات الكيسية ونوعين من كل من الفطريات اللاقحية والبيضية). اظهر الفطر على الفطريات الكيسية ونوعين من كل من الفطريات اللاقحية والبيضية). ومن ثم الفطر المبائل الفطريات الكيسية تردد وظهور و 25.5% على التوالى)، تلاه الفطر المبائل الفطريات اللاسبة تردد وظهور بلغت 5.2% و 19.18% على التوالي لكل فطر. تم عزل 9 مناطق الدراسة، ووجد ان أعلى عدد من العزلات قد تم عزلها من موقع المجر الكبير مقارنة المتنوع الاحيائي حيث بلغت 64 عزلة من موقع المعزولة من مقا النباتات المغمورة بالدراسات السابقة. واعطى وصف مختصر الفطريات التى سجلت لأول مرة في العراق.

الكلمات المفتاحية: lignocellulose، بقايا النباتات المغمورة، Hyphomycetes ،Ascomycota، العراق.

#### 1. INTRODUCTION:

Dead plant substrates (lignocellulose) are colonized and degraded by a wide variety of biological agents such as fungi, bacteria, and insects if the appropriate environmental conditions are available (Goodell et al., 2003), and are essential components in the terrestrial and freshwater ecosystems (Tsui et al., 2001). Fungi evolutionarily and functionally ubiquitous and found in the vast majority of ecosystems (Grossart et al., 2019). Fungi are characterized by tremendous environmental diversity, the majority belong to terrestrial fungi, and a few are isolated from aquatic habitats and woody submerged remains (Shearer, 1993). Nearly 3,000 fungal species and 138 non-fungal oomycetes have been reported from aquatic habitats (Ittner et al., 2018). Furthermore, the majority of these fungi have occurred in temperate regions and Asian tropical areas. Moreover, most of them belonged to the phyla ascomycetes, basidiomycetes, chytridiomycetes, zygomycetes, and oomycetes (Shearer et al., 2007; Raghukumar, 2012). The freshwater (sometimes referred hyphomycetes Amphbions or Ingoldian fungi) mainly belong to the ascomycetes with a small percentage in the basidiomycetes (Ittner et al., 2018).

Aquatic fungi have a vitally important role in ecosystems because they are among crucial decomposer microorganisms that fall within the food web (Rani and Panneerselvam, 2009; Daniel, 2016), and participate in the decomposition of detritus and the energy stream to the higher trophic levels (Bärlocher, 2009). Fungi from the different groups under suitable conditions will often colonize and degrade the wood material and even attack the same wood cells (Daniel, 2016). It can break down the

organic materials (Raja *et al.*, 2018), so they play an essential functional role in the recycling of carbon, nitrogen, and other nutrients (Cai *et al.*, 2006; Kantharaj *et al.*, 2017) because it can produce various wood decay enzymes (Sonigo *et al.*, 2011; Andlar *et al.*, 2018).

In particular, ascomycete fungi and fresh aquatic hyphomycetes occur on a variety of submerged substrates (Michel *et al.*, 2005; Sati and Pathak, 2016) and are osmoorganotrophs, absorbing nutrients via their cell wall. Moreover, the majority of them have a filamentous growth phase during their life cycle. This morphology helps them invade deep into the substance and directly decompose particles of organic matter to obtain nutrients for growth and reproduction (Christian *et al.*, 2011).

Freshwater lignicolous hyphomycetes are a predominant and variety group, which live on submerged plant debris and decaying tree leaves (Shearer *et al.* 2007; Hyde *et al.* 2016), and are traditionally identified based on the morphology conidia (Sridhar, 2009). In addition, these fungi grow abundantly and develop large quantities of conidia (Bärlocher, 2009) and mycelia formation that develop within and outside remains of the plant (Wurzbacher and Grossart, 2012).

Several researchers have studied the occurrence and distribution of fungi from submerged wood in aquatic habitats in different sites of Iraq. Forty-two species have been isolated from Basrah in southern of Iraq (Muhsin and Kalaf, 2002). On the other hand, eight species were recorded from different sites of Iraq (Al-Saadoon and Al-Dossary, 2010). Al-Saadoon and Al-Dossary (2014) reported 67 species in several locations in southern Iraq. Al-Nasrawi (2014) Isolated tow species from Al-Huwaizah marshes in Southern Iraq. Al-Huwaizah marshes

in Southern Iraq. It is known that the water bodies, including marshes, swamps, ponds, and rivers, represent broad areas of the Misan province, so these environments are rich in aquatic plants and submerged plant debris.

Consequently, it is rich in aquatic fungi and fungi associated with plant debris. Therefore, due to the lack of studies on these fungi in such environments. Accordingly, the study aimed to isolate and identify fungi from submerged plant debris present in some sites.

#### 2. MATERIALS AND METHODS:

#### 2.1 Sample collection

Forty-seven samples from submerged and floating plant debris such as small branches and stem were collected from many locations in Misan province (12 samples from Maymouna, 11 samples from Al-Salam, 12 from Majar Al-Kabir, and 12 from Amara) during September 2019 to February 2020. The samples were placed in snap lock plastic bags and sent to the laboratory.

#### 2.2 Methods for isolating fungi

#### 2.2.1 Moist Chamber Method

The samples were washed gently with tap water and then with sterile distilled water several times, 5 pieces (1-2 cm) were put in a glass chamber contain moist filter papers, incubated at 25 °C for 8 weeks with the addition of sterile distilled water whenever needed to keep it from drying out. The samples were examined daily after 5 days of incubation for any fungal growth. Upon observing the growth of mycelium on the samples, a portion of the mycelium was transferred with a sterile loop to the Petri dishes containing Czapek-Doxe Agar and incubated at 25 °C for 7 days to for obtaining pure cultures. For ascomycetes, once the ascocarps emerged on samples, the fruit bodies were squashed on a sterile slide mounted with water to release asci and ascospores (to remove the gelatinous coats and appendages on, or around the ascospores, Indian ink (Himedia, CAS No. 0) was added), and then covered with cover slides for an initial examination, water was replaced with cotton blue lactophenol for measuring (ascocarps, asci, ascospores, conidiophores, conidia, etc.) and photography (microscope camera system, Scope Image Dynamic Pro Co.China).

#### 2.2.2 Direct culture method

The samples were washed as the previous paragraph and cut into pieces of 1 cm. Five pieces were planted on Malt Extract Agar (MEA) and Potato Carrot Agar (PCA) plates and incubated at 25 °C for 7 to 10 days. Then, different fungal colonies were isolated and cultured separately in MEA and PCA.

To examine the fungal mycelium that appeared in both methods, slides were prepared by cutting a portion of the mycelium using a sterile surgical blade and put on a slide and stain with a drop of cotton blue lactophenol or lactophenol according to the color of the specimen. The slides were examined under a light microscope. The identification of the isolated fungi is made according to the available taxonomic keys. Permanent slides, dried samples, and pure cultures were preserved at the biology department from the College of Sciences of the University of Misan.

The percentage of frequently (Eq. 1) and the occurrence (Eq. 2) of fungal species were measured using Equations 1 and 2 (Krebs, 1978).

#### 3. RESULTS AND DISCUSSION:

#### 3.1 Taxonomic study

Forty-eight species of saprophytes fungal were isolated during this study from submerged plant debris from different sites (Maymouna, Al-Salam, Mgr Al-kabeer, and Amara) in the Province of Misan, comprising 24 species belonged to Ascomycota (six of which are sexual state), 19 hyphomycetes, four Zygomycota, and one Oomycota. Six species were recorded for the first time in Iraq. These are Aniptodera margaration, Cirrenalia iberica, Cordana lignicola, C. verruculosa, Pseudoacrodictys appendiculate, and Scytalidium thermophilum. The taxonomic notes on these taxa are described below.

#### 1- Aniptodera margaration Shearer, (1989).

Isolate examined: from submerged stem wood, Maymonia. Isolation No. Ma3570, Figure 1.

Ascocarps hyaline, globose or subglobose, immersed or superficial, with ostiole,  $145-162~\mu m$  diam. Asci hyaline, subglobose to pyriform ovoid, rounded at the apex, 20-35~X~45

-70 μm. Containing eight ascospores. Ascospores ellipsoidal or oblong-ellipsoidal, hyaline, 1-septum, not constricted at the septum, 7.5 - 10 X 20 - 25 μm.

This feature of the present isolate conforms with Shearer (1989). Jones *et al.* (2009) indicated the ability of some species of *Aniptodera* to occur in fresh and saltwater. The most important characteristic of this species is its formation of hyaline ascomate and the growth as saprobes on submerged wood.

2- Cirrenalia iberica Hernandez-Restrepo et al., (2017).

Isolated examined: on submerged wood, Mgr Al-kabeer. Isolation No. Mg0512, Figure 2.

The colony is superficial. Mycelium hyaline, branched, septated, 2.5-5 $\mu$ m wide. Conidiophores micronematous, pale brown, 7.5-10 x 12.5-15  $\mu$ m. Conidia straight or slightly curved, with 1-4 septa with an almost globose cell, the distal cells are much bigger and dark than others. Basal cell 7.5-12.5  $\mu$ m, middle cell 10-15  $\mu$ m, apical cell 25-30  $\mu$ m, one conidium only produced on the apex of conidiophore

The present isolates are were precisely similar to those described by Hernandez-Restrepo et al., (2017). There are 13 species of *Cirrenalia*, isolated from terrestrial and marine habitats. Based on molecular phylogenetic analysis, many species of *Cirrenalia* have been transferred to *Halazoon*, *Hiogispora*, *Hydea*, and *Matsusporium* belonging to the Lulworthiales (Abdel-Wahab et al., 2010).

3- Cordana lignicola Luo, et al., (2019).

Isolated examined: submerged wood, Maymona. Isolation No. Ma1245, Figure 3.

The colonies were superficial, hairy like, brown to dark brown, and mycelium branched, septate. Conidiophore macronematous 2.5-5 x 100 - 120 µm unbranched, containing septa and nodes, dark intercalary brown, acropleurogenous. Conidia elongated-ellipsoid, 1 - 2 septa. 1-septate conidia had slightly constricted at the septum, septate near the apex, globose or subglobose at the apex. 2-septate conidia not constricted at the septum, elongate Conidia hyaline cylindrically. when young, becoming brown when aged. 2.5 -5 x 7.5 -12.5

This present isolate feature conforms with the designated species *C. lignicola* (Luo *et al.,* 2019). This species was similar to *C. mercadiana* except that the ellipsoidal or cylindrical conidia, 0-1 septum, and conidiophores were longer in the letter species compared to *C. lignicola* the conidia elongated-ellipsoid, 1-2 septa. Phylogenetic analysis showed a difference between them

(Hernández-Restrepo et al., 2014; Luo et al., 2019).

4- C. verruculosa Hernández-Restrepo et al., (2014).

Specimen examined: submerged wood, Amara. Isolation No. Am40121, Figure 4.

Colonies were superficial, brown to light brown. Mycelium growing superficial and partially immersed in the wood, branched, light brown, 2.5–5  $\mu$ m diam. Conidiophore mononematous or macronematous, straight, unbranched, and distinguish present intercalary nodes and spherical basal cell, 2.5-5 x 115-120  $\mu$ m. Conidia ellipsoidal, obovoid or verruculosa, light brown to brown, acropleurogenous, non-septate, 2.5-5x 2.5-7.5  $\mu$ m.

The reason for naming *C. verruculosa* was due to the verruculosa shape of conidia. This species can be easily recognized from *C. solitaria* and *C. semaniae* (which are produced black, lacking septa conidia) by morphological characters. However, the conidia size is smaller and pale brown (Hernández-Restrepo *et al.*, 2014).

5- Pseudoacrodictys appendiculata (Ellis) Baker and Morgan-Jones, (2003).

Specimen examined: submerged wood, Maymonia. Isolation No. Ma0510, Figure 5.

Colonies were effuse, black. Mycelium branched, superficial,  $2.5-5~\mu m$  diam. Conidiophore macro-nematous, single or in a group of two or three, bearing a single conidium, straight, black or dark brown,  $2.5-5~x~15-35~\mu m$ . Conidia turbinate to pyriform, black, bearing 1-4 black appendages (muriform),  $25-37.5~x~30-57.5~\mu m$ .

The characters of the examined isolate match well with Ellis (1965) description, which was erected as *Acrodictys appendiculata* and transferred into *P. appendiculata* by Baker and Morgan-Jones (2003) due to the dark color, large size, irregular shape, and septa of conidia.

6- Scytalidium thermophilum (Cooney and Emerson) Austwick, (1976).

Specimen examined: submerged wood, Amara. Isolation No. Am1334, Figure 6.

Colonies white. Mycelium hyaline, branched, septated, 2-5  $\mu$ m diam. Conidia as arthroconidia ellipsoidal or subglobose, 5- 7.5 x 7.5 - 17.5  $\mu$ m. Chlamydospores present, 7.5 - 12.5  $\mu$ m.

Our isolate matches the original description of

Austwick (1976).

#### 3.2 Population dynamic of isolated fungi

A total of 48 fungal taxa were isolated from submerged plant debris, comprising 24 Ascomycota (50%), 19 hyphomycetes (39.58%), four species belonged to Zygomycota (8.33%), and one Oomycota (2.08%) were recorded. 34 species were isolated using the moist chamber method, and 27 by direct culture method, 13 species (9 belonged to Ascomycota, 4 to hyphomycetes) were recovered by both methods (Table 1). In the habitat of submerged plant debris, A. terrus was appeared in the highest frequency and occurrence (11.76%, 42.55%, respectively) followed by A. horti (10%, 36.17%). In comparison, the frequency and occurrence of A. margiration and A. oryzae were found in less amount reaching 0.58%, 2.12%, respectively.

Thirty-four species were isolated using the moist chamber method, and 27 by direct culture method, 13 species (9 belonged to Ascomycota, 2 to hyphomycetes, and 2 to Zygomycetes) were recovered by both methods (Table 1). In the habitat of submerged plant debris, A. terrus has in the highest frequency appeared occurrence (11.76%, 42.55%, respectively), followed by A. horti (10%, 36.17%), and then A. niger with a frequency and occurrence of 5.29 and 19.14, respectively. In comparison, the frequency and occurrence of 17 species were found in less amount reaching 0.58%, 2.12%, respectively (Table 1).

This study revealed that 107 isolates had been isolated from all study sites, 64 isolates were recorded from Majar Al-Kabir, 43 isolates were reported from Amara, 39 isolates from Maymouna and, 24 isolates from Al-Salam.

Many studies have also investigated the biodiversity of fungi found on submerged plant debris (Shearer et al., 2007; Daniel, 2016). The most frequent fungi identified were ascomycetes and hyphomycetes (Hu et al., 2010; Sati and Pathak, 2016). Anamorphic fungi were more frequent in submerged plant debris (Samson, et al., 2011, Lepère et al., 2019). However this due to rapid growth, the ability to produce a large number of spores (conidia) that are long and branched or filamentous; this assist their attachment to substrates in running water (Ghate and Sridhar, 2015; Raja et al., 2018) and its adaptation to different ecological environments (Domsch et al., 1980; Daniel, 2016).

Fungi have important roles in cycling of organic matter and food net (Grossart *et al.,* 2019). So, this study demonstrated that the most

Isola ted fungi belong to the genus Aspergillus (8 species), A. terrus, A. horti, A. fumigatus isolated from all study sites. The reason for its diverse species of Aspergillus and has a worldwide distribution because it can adapt and grow in different ecological environments and secrete many enzymes for breaking down organic matters (Sohail et al., 2009; Wurzbacher et al., 2010).

C. lignicola and C. verruculosa were isolated from submerged plant debris samples from some sites. Anyway, Hernández-Restrepo et al., (2014) indicated that the species of Cordana are distributed in most parts of the world and frequently found on different material, such as soil, plant debris.

pullulans, C. alternate. Α. macrocephalya, M. varius, S. lignicola, E. rostratum, C. globosum, and other sexual ascomycota were isolated by Al-Saadoon and Al-Dossary (2014) from submerged plant debris in Basrah province (southern Iraq). However, C. globosum was isolated from environments such as soil, water, and plant debris (Fallah and Shearer, 2001).

N. Inornate was equivalent to N. Aquatic but different in the way ascospores were released from Ascomate, and ascospores of A. quatica carrying appendices at one time (Hyde, 1992). Furthermore, Pang et al. (2003) demonstrated that the DNA sequence of N. inornata proved that it was closely related to genus Aniptodera. Anyway, N. inornate was isolated from marine habitats (Dethoup and Manoch, 2009) and from submerged dead plants (Muhsin and Kalaf, 2002; Al-Saadoon and Al-Dossary, 2014).

lignicola was isolated from freshwater, brackish, saltwater, and can tolerate high salinity (Hyde, 1994). Moreover, Al-Saadoon and Abdullah (2001) isolated S. lignicola from dead stem submerged from freshwater in Nineveh province (Northern Iraq). S. lignicola is similar to S. fusiformis and S. Longispora and can be distinguished by the size of ascospore as well as by genotypes. (Ho et al., 1997; Boonyuen et al., 2011). Furthermore, the ascospore of S. longispora are longer and thinner (Abdel-Wahab and Jones, 2000), and differs from S. aguatic with the wide of ascospore (Jones and Hyde, 1992).

Chaverri et al. (2011) showed that *Trichoderma* a widespre genus capable of grow in broader habitats. Therefore, tow isolates of *T. harzianum* have been isolated from plant remains from Majar Al-Kabi and Amara because it able to

produce many enzymes such as cellulase, amylases, and pectinase (Sandhya *et al.,* 2004; Schuster and Schmoll, 2010; Li *et al.,* 2013)

Some species of Aspergillus, Pencillium, Fusarium, and Rhizopus appeared high-frequency value, and this result corresponded to many studies (Frisvad and Samson, 2004; Al-Saadoon and Al-Dossary, 2014; Refai et al., 2015).

## 4. CONCLUSIONS:

In the present study, saprophytic fungi that grow on submerged plant debris were recovered. The abundance of plant remains due to the diversity of plants in aquatic environments which are a suitable source for the growth of these fungi, especially ascomycetes and hyphomycetes. The water bodies in Misan province still need further study to identify more new species and new record fungi, especially when using molecular studies.

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Percentage of occurrence = 
$$\frac{\text{The number of samples that appeared to show one type}}{\text{The total Number of samples}} \times 100$$
 (Eq. 1)

Percentage of frequency = 
$$\frac{\text{The number of isolates of the same species}}{\text{The total number of isolates of all kinds}} \times 100$$
 (Eq. 2)

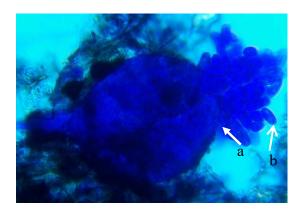


Figure 1: Ascocarp of Aniptodera margaration (arrows a: Ascus b: Ascospore).

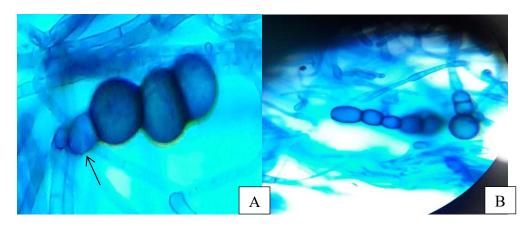
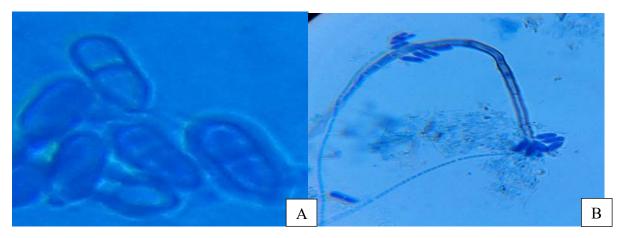


Figure 2: C. iberica, A: Conidia B: straight conidia, the light-colored basal cell (arrow)



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Figure 3: Cordana lignicola A: conidia B: conidiophore and conidia

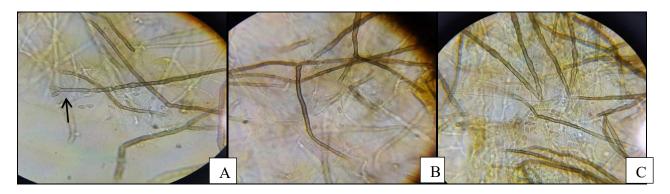


Figure 4: Cordana verruculosa, A: Mycelium and conidia B: conidiophore, C: conidiogenous cells (arrow).

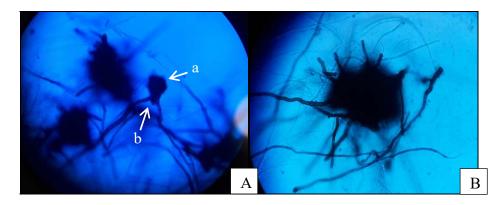


Figure 5: Pseudoacrodictys appendiculata A: a- conidia, b-conidiophore (arrows). B: conidia

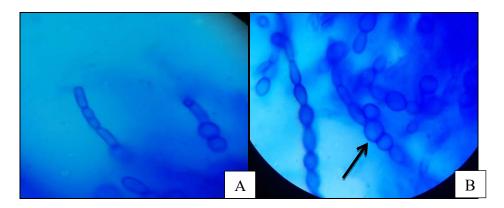


Figure 6: Scytalidium thermophilum A: Arthroconidia B: Clamedospore (arrow) and Arthroconidia

Table 1 List of fungal species isolated from submerged wood in Misan

Fungi species	Maymouna	Al- Salam	Majar Al-Kabir	Amara	Total	Frequency %	Occurrence %	Moist Chamber Method	Direct Culture Method
			Ascomycetes	etes					
Aniptodera margiration Shearer	-	ı	ı	ı	1	0.58	2.12	+	ı
Arthrobotrys dianchiensis (Hao and Zhang) Yu	ı	ı	1	1	1	0.58	2.12	+	ı
Aspergillus flavus	1	2	1	1	4	2.35	8.51	+	+
A. fumigatus Fresen.	1	3	1	3	8	4.70	17.02	+	+
A. horti (Langeron) Dodge	3	9	9	2	17	10	36.17	+	+
A. niger Van Tiegham	2	1	7	1	6	5.29	19.14	+	+
A. oryzae (Ahlburg) Cohn	1	ı	-	ı	1	0.58	2.12	ı	+
Aspergillus sp.	ı	ı	1	1	1	0.58	2.12	+	ı
A. terrus Thom	10	2	3	5	20	11.76	42.55	+	+
A.tubingensis Mosseray	ı	1	-	-	1	0.58	2.12	+	1
Byssochlamys nivea Westling	ı	2	ı	2	4	2.35	8.51	+	+
Candida tropicalis (Castellani) Berkhout	ı	1	ı	1	1	0.58	2.12	+	ı
Chaetomium globosum Kunze	ı	ı	ı	1	-	0.58	2.12	+	1

Exophiala jeanselmei (Langeron) McGinnis and Padhye	1	1	1	1	1	0.58	2.12	+	
Geotrichum candidum Link ex Leman	1	ı	1	2	3	1.67	6.38	+	+
Kirschstemiothelia maritime (Linder) Hawksworth	7	ı	ı	3	5	2.94	10.63	+	ı
Leptosphaeria agnita (Desm.) Ces. and De Not.		ı	1	1	1	0.58	2.12	+	1
Nais inornata Kohlmeyer	2	ı	2	1	4	2.35	8.51	+	ı
Pencillium chrysogenum Thom	3	1	1	2	7	4.11	14.89	+	+
P.commune Charles and Thom	2	2	-	1	5	2.94	10.63	+	+
Savoryella lignicola Jones and Eaton	ı	ı	2	2	4	2.35	8.51	+	ı
Scedosporium prolificans Hennebert and Desai) Guého and de Hoog	1	ı	-	-	1	85.0	2.12	+	ı
Trichoderma harzianum Rife	-	ı	1	1	2	1.17	4.25	-	+
Zopfiella latipes (Lundquist) Malloch and Cain	1	ı	2	2	5	2.94	10.63	+	1
Total	30	20	26	31	107				
			Hyphomycetes	ycetes					
Alternaria alternate (Fresen) Keissler	1	1	1	1	2	1.17	4.25	+	1
A. chlamedosporia Mouchacca	-	1	1	1	2	1.17	4.25	+	1
Aurobasidium pullulans (de Bary) Arnaud	1	ı	-	1	1	0.58	2.12	ı	+

Cirrenalia iberica HernRestr. and Gene	ı	ı	1	-	1	0.58	2.12	1	+
C. macrocephala (Kohlm.) Meyers and Moore		1	3	1	3	1.76	6.83	1	+
Cladosporium cladosporioides (Fresen.) de Vries	ı	ı	1	1	1	0.58	2.12	1	+
C. cucumerium Ellis and Arthur	ı	1	1	1	2	1.17	4.25	1	+
Cordana lignicola Luo, Hyde and Su		ı	2	ı	3	1.76	6.83	+	ı
C. verruculosa HernRest., Mena., Gene and Guarro	ı	1	1	1	1	0.58	2.12	ı	+
Cuvularia lunata (Wakker) Boedijn	-	1	1	-	2	1.17	4.25	1	+
Fusarium aquiseti (Corde ) Sacc. and Sylloge	1	ı	1	1	1	0.58	2.12	ı	+
F. oxysporium Schlecht	2	-	5	-	L	4.11	14.89	+	+
F. solani (Mart.) Sacc.	1	1	9	-	8	4.70	17.02	+	+
Graphium sp.	1	ı	1	1	2	1.17	4.25	ı	+
Moromyces vrains (Chatmala and somrith.) Abdel-wahab, Pang, Nagahama, Abdel-Aziz and Jones	1	1	2	1	3	1.76	6.83	+	ı
Pseudoacrodictys appendiculate (Ellis)  Baker and Morgan	1	ı	1	-	1	0.58	2.12	ı	+
Ramichloridium schulzeri (Sacc.) de Hoog	1	1		2	2	1.17	4.25	+	-

Scytalidium thermophilum (Conney and Emerson) Austwick	1	1	2	1	3	1.76	6.83	+	1
Tricocladium acrosporium (Meyers and Moore) Dixon	1	1	1	1	2	1.17	4.25	-	+
Total	5	4	28	10	47				
			Zygomycetes	/cetes					
Absidia corymbifera (Cohn.) Sacc. and Trotter	1	ı	3	-	4	2.35	8.51	-	+
Mucor circinelloides Van Tieghem	ı	1	ı	1	1	0.58	2.12	+	ı
M. pseudolamprosporum Nagan. and Hirahara	2	ı	1	-	2	1.17	4.25	+	+
Rhizopus oryza Went and Prinsen Geerligs	1		7	-	8	4.70	17.02	+	+
Total	4	0	10	1	15				
			Oomycetes	etes					
Saprolegina sp.	ı	ı		1	1	0.58	2.12	+	1
Total	39	24	64	43	170	100			

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