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## Fungal Diversity of Winter Wheat Parts, Seed and Field Soil in Iraq, Basra Province.

Mohammed Hussein Minati<sup>1, a</sup>, Mohanad Khalaf Mohammed-Ameen<sup>2</sup>

1. Dept. Biology, College of Education/ Qurna, Basra University, Basra, Iraq.

2. Dept. Biology, College of Science, Basra University, Basra, Iraq.

<sup>a)</sup> Corresponding author: [abo\\_azher70@yahoo.com](mailto:abo_azher70@yahoo.com); Tel.: +9647710803047

### Abstract

This study was conducted to survey the fungal microflora in winter wheat (*Triticum aestivum* L.), ear, stem, root, seed and field soil in 17 field distributed on Basra province, Iraq. The mycoflora of plant parts, seeds and soil were determined morphologically and molecularly. In total 46 genera with 66 species of different fungal groups were found. The prevalent fungal group on all examined sources was the anamorphic Ascomycota (85.34%), followed by Zygomycota (5.46%). Telemorphic Ascomycota was only at (3.64%) and Basidiomycota was in less than (1%). *Aspergillus*, *Penicillium*, and *Fusarium* were the dominant genera in soil and rhizosphere region. The same genera were found in seed but *Alternaria* was instead of *Fusarium*. The fungal diversity in plant parts was different, as *Alternaria*, *Fusarium*, *Helminthosporium*, *Cladosporium*, *Penicillium* and *Aspergillus* genera were more occurrence. The highest total fungal population was observed in rhizosphere region and soil, and the lowest was in wheat head. The occurrence of collective fungal community was almost identical in wheat seed, root and stem.

**Keywords:** anamorph, morphological identification, molecular, rhizosphere, telemorph.

### Introduction:

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world [1]. Since 2013, globally wheat production has been increased reaching more than 700 million metric tons per year. The production is mainly concentrated in China, India, the United States and Russia, representing more than a quarter of the world's wheat production. In Iraq, bread wheat is the first cultured crop and the uppermost cereal consumed by population. In 2017, the Iraqi production of wheat was 2,974,136 million metric tons [2].

Fungi are common plant pathogens that cause significant economic losses in crop production [3], even though some fungi are plant parasites but their nature can vary depending on environmental



conditions [4]. Wheat crop is exposed to many fungal diseases that reduce the quantity of production, such as brown rust, black rust, yellow rust, loose smut, powdery mildew, scab, ergot and downy mildew that caused by *Puccinia graminis*, *Puccinia striiformis*, *Ustilago tritici*, *Fusarium spp.*, *Claviceps purpurea* and *Sclerophthora macrospora* respectively [5].

Fungi is the second largest living group after insects, with almost only 80000-12000 fungal species have been identified thus far, although the total number of fungal species is estimated at 1.5 million, and this makes fungi one of the most important sources of biodiversity with less discovery [6]. Many plants have been found to be a good place for many microorganisms that grow inside their tissues, which called endophytes [7], they are mostly microscopic fungi that colonize plant hosts in all or part of their life cycle, without causing any damage or disease symptoms [8]. The existence of these fungi inside the plant tissues may last for many years without any symptoms, but may become pathogenic parasites when their hosts are exposed being stressed [9]. Most of these endophytic fungi are belonging to Ascomycota, Basidiomycota or Fungi Imperfecti groups and some of them may include other groups such as Zygomycota and Oomycota fungi [10].

Endophytic fungi can produce effective secondary metabolic compounds, which may play an important role in the relationship between fungi and plants [8]. Such fungi may transfer from a season to another through seeds [11], where fungal mycelia grow inside stem or leaf tissues until they reach the flowering buds to be entered into the seeds and then transfer to the next generation [12].

Many strategies have been followed to produce authorized, certificated and healthy seeds, one of them is the determination of seed-borne pathogenic sources in wheat fields. Where infected grain is seeded, not only crop production would be reduced but also the harvested seeds will be inoculum sources of diseases due to the seed-borne pathogens [13]. Numerous endophytic fungi have been isolated from different parts of plants such as leaves, stems, roots and seeds of many plants, for example many vascular plants including the species of Poaceae (Gramineae) family such as wheat and barley [14-17].

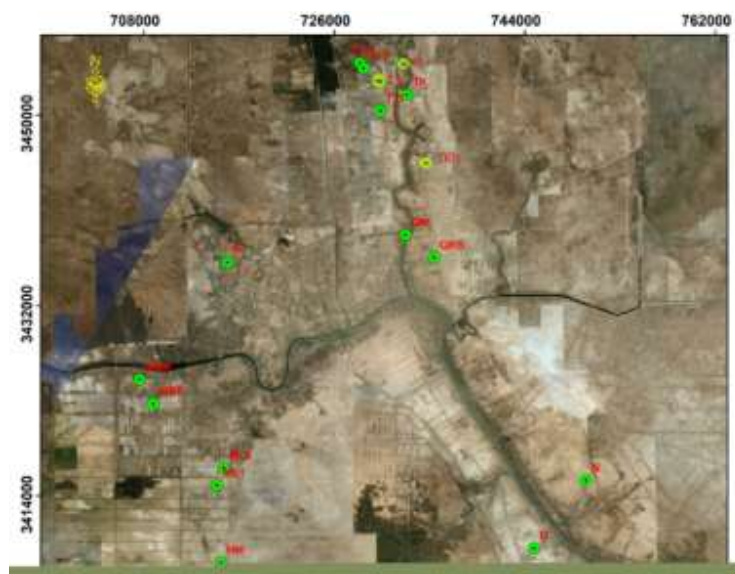
In Iraq, there is no data on the comprehensive mycoflora accompanying with entire wheat plant parts, seed, rhizosphere region and field soil, as the mostly used up cereal crop, except some published studies focused on determination of endophytic fungi associated with wheat parts at

seedling stage [18], wheat root [19]. Other studies focused on isolation of specific fungi from individual wheat parts or soil, such as *Fusarium graminearum* from soil of wheat field in Amara province [20], *F. pseudograminearum* from wheat stem in Basra province [21], *F. graminearum*, *F. pseudograminearum* from stem and *Alternaria triticina* from leaves [22], *F. culmorum* from stem in Baghdad [23] and nine *Fusarium* species associated with wheat heads and stems in Basra province [24-27].

Taking into account these considerations, the aim of this study was to determine the most common fungal genera (Fungi community) associated with whole wheat plant as well as seed, rhizosphere and soil in the North of Iraq, Basra province, using traditional methods based on morphological and molecular identification.

### Materials and Methods:

In 2017/2018, samples of wheat tillers were collected at harvesting time, while soil and rhizosphere samples were collected two weeks after planting time and at flowering stage from 17 wheat fields distributed on 7 districts in Basra province as shown in (Fig. 1). The selected fields were chosen to study (isolate and identify) most communities of fungi associated with wheat tillers, rhizosphere and soil.



**Fig. 1** Map of the 17 selected wheat fields in the South of Iraq, Basra province, where plant and soil samples were collected in 2017/ 2018.

### Soil sample collection:

A 6-10 samples of soil were collected from a depth of 25 cm from each field, due to the different size of field areas, placed separately in non-transparent plastic bags, transferred directly to the laboratory, kept at 4°C in the fridge until dilution methods.

#### **Soil Dilution Method:**

Potato Dextrose Agar (PDA) for soil dilution were used in this study. Sterile distill water was carried out for serial dilution of the soil samples. Dilutions were ranged from ( $10^{-4}$  -  $10^{-7}$ ), a 1ml of every particular dilution was transferred and distributed onto the surface of PDA media. Plates were incubated at 25 ıC for 4-7 days for fungal growth. Each different colony was sub cultured onto new PDA media. These purified colonies were again incubated at 25 ıC until obvious mycelial growth and at times fungal sporulation were noticed. The identification was performed morphologically.

#### **Plant Sample Collection:**

Samples of wheat tillers were collected and sited in non-transparent plastic bags and sent directly to the laboratory, washed by running water, dried up, and reserved at 4°C until being cultured. Seeds were also collected at a harvest time from each field and then were separately placed in paper bags and stored at 4°C until being cultured for fungal isolation.

#### **Isolation of Fungal Species:**

The collected wheat tillers were cut and divided to individual plant part (ear, crown, basal stem and root), washed for 8-12 minutes in running water for surface decontamination, drenched in 10% sodium hypochlorite for 1-2 minutes, soaked in sterile distilled water 2-3 times and then dried up on a sterilized filter paper. Then, they were positioned onto PDA plates with (9 cm), three replicates for each, and 5 pieces of plant part per plate. The plates were incubated at 25°C for 5-7 days and checked daily for fungal growth. All different colonies were sub-cultured onto PDA, with three replicates for each, incubated again under the same conditions, and identified morphologically.

Seeds were disinfected by soaking in 70% ethanol for 2-3 minutes, saturated in 2% sodium hypochlorite for 1-2 minute, washing in sterile distilled water three times and then dried up on a sterilized filter paper. Then, they were sited in plates of PDA with three replicates for each, 5 seeds per plate. The plates were incubated at 25°C for 7-10 days. All different colonies were isolated and purified onto PDA media for morphological identification.

### **Identification of fungi:**

As mentioned in the introduction section that this study was planned to isolate, purify and identify (morphologically and molecularly) all fungi community occurring on plant parts (ear, stem, crown and root), rhizosphere, soil, seeds of wheat plant in the 17 examined fields. The majority of isolated fungi were identified based on morphological identification (Table 1). However, only 25 fungal taxa regardless of *Fusarium* spp. were identified molecularly due to some different features on their morphology, by using the technique of PCR, and the sequence of ITS region was amplified using the universal primer pairs ITS1 and ITS4. According to the closest identification of BLAST search in NCBI, the DNA sequencing of these 25 isolates were matched to the GenBank databases at > 97% as global similarity (Table 2). The molecular identification of these 25 fungal taxa was combined with all other isolated fungi identified to species level morphologically in Table 1.

No specific strategy was used for isolating the fungal isolates during this study to determine obligate pathogens such as rusts, due to unsuitable climate conditions for their development in our regions.

### **Molecular Identification**

#### **DNA Extraction**

Fungal genomic DNA prepared from a colony of each isolates was extracted using the Plant Genomic DNA Mini Kit (GP100) Geneaid protocol according to the manufacturer's instructions.

#### **Polymerase Chain Reaction (PCR) Amplification:**

All isolates of various fungal species from wheat plants, their seeds, rhizosphere and soil in the south of Basrah were identified to genus or species based on the morphology, while 25 isolates were identified to species molecularly by means of the technique of PCR, and the sequence of Internal Transcribed Spacer (ITS) region was amplified using the universal primer pair

**ITS1 (F) (5'-TCC GTA GGT GAA CCT GCG G-3')** and

**ITS4 (R) (5'-TCC TCC GCT TAT TGA TAT GC-3').**

Amplification of genomic DNA was executed in a total volume of 25  $\mu$ l consist of 5  $\mu$ l Master Mix (Bioneer, Korea), 1.5  $\mu$ l of each primer, 5  $\mu$ l of the genomic DNA as a template and 12  $\mu$ l deionized sterile distilled water.

PCR reactions were accomplished in a Thermo-cycler (MyGenie96 Thermal Block, Bioneer, Korea) and the amplification programme initiated with denaturation at 95  $^{\circ}$ C for 5 min, followed by 35 cycles of 30 seconds denaturation at 95  $^{\circ}$ C, 30 seconds of annealing at 60  $^{\circ}$ C and 45 seconds of extension at 72  $^{\circ}$ C with final extension at 72  $^{\circ}$ C for 5 minutes. In the last part 1.5 % agarose gel electrophoresis was dissolved in 1x Tris Borate EDTA (TBE) buffer, stained with ethidium bromide, visualized under UV transilluminator and photographed by GeneSnap photo imaging system (SynGene).

#### **DNA Sequencing:**

The sequences of ITS1- 5.8S rDNA and the purification for the amplified PCR products of 25 isolates were conducted commercially, (MACROGEN Company, South Korea) “<http://dna.macrogen.com>”.

#### **Density Estimation of Isolated Fungal population:**

The seven varieties of winter wheat plant grown in the 17 fields were populated by pathogenic and saprophytic fungi throughout the growing season in 2017/2018. Based on morphological and molecular identification, broad-spectrum isolated genera of microscopic fungi were encountered during this study, 46 genera of fungi were identified with 66 species, as well as white and black sterile mycelia. Fungi belonging to Anamorphic Ascomycota (formerly Deuteromycetes) were in a dominant position with percentage of occurrence reached 85.34%, followed by Zygomycotina with percentage of occurrence 5.46%. Sterile mycelia (white and black) occurred with 4.81%, and Teleomorphic Ascomycota (Pezizomycotina) were occurred with a percentage of (3.64%). Finally, Basidiomycota (Agaricomycotina) were present with very low percentage of 0.75% (Fig. 2).

**Table 1:** General isolated species of microscopic fungi (66) within (46) Genus that isolated from symptomatic **Plant Parts** (ear, stem, crown and root), **Rhizosphere, Soil, Seeds** during this study based on morphological and molecular identification.

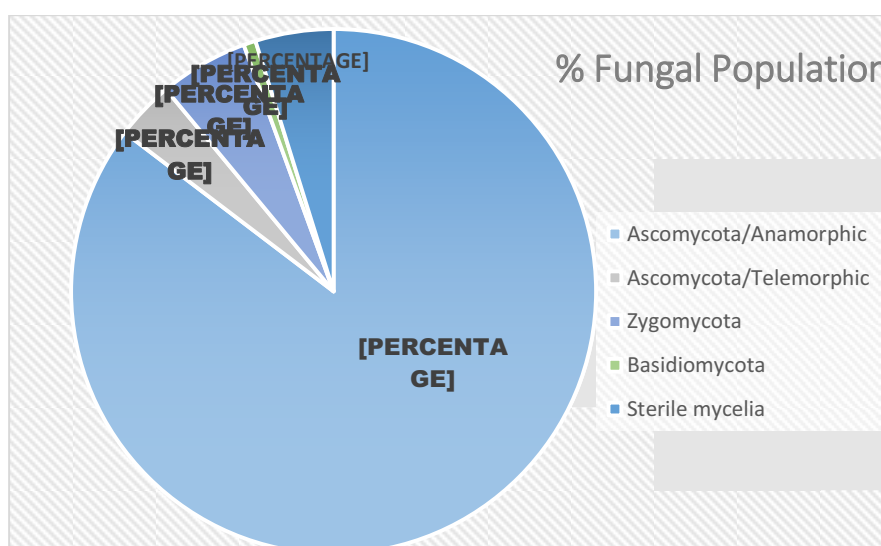
No.	Isolated species of microscopic fungi	No.	Isolated species of microscopic fungi
1	<i>Acremonium zonatum</i> (Swada) W. Gams	35	<i>Epicoccum nigrum</i> . Link
2	<i>A. kiliense</i> Grutz	36	<i>Eurotium spp.</i> Micheli
3	<i>A. egyptiacum</i> (J.F.H. Beyma) W. Gams	37	<i>Exserohilum spp.</i> K.J.Leonard & Suggs
4	<i>A. potronii</i> Vuill.	38	<i>Fusarium spp.</i>
5	<i>Alternaria alternata</i> (Fr.) Keissl	39	<i>Geotrichum candida</i> Link : Fr
6	<i>A. citri</i> (Penz.) Mussat	40	<i>Gymnascella spp.</i> Peck
7	<i>A. ventricosa</i> R.G. Roberts	41	<i>Gymnoascus dankaliensis</i> . (Castell.) Arx
8	<i>A. chlamydospora</i> Mouch.	42	<i>Helminthosporium spp.</i> Link
9	<i>A. tellustris</i> (E.G. Simmons) Woudenberg & Crous	43	<i>Monodictys cerebriform</i> Etayo
10	<i>Aspergillus flavus</i> Link & Gray	44	<i>M. putredinis</i> (Wallr.) S. Hughes
11	<i>A. fumigatus</i> Fres	45	<i>Monosporascus ibericus</i> Collado, Ant. González, Stchigel, Guarro & Peláez
12	<i>A. niger</i> Van Tieghem	46	<i>Mortierella alpine</i> Peyronel
13	<i>A. terreus</i> Thom	47	<i>Mucor spp.</i> Fresenius
14	<i>A. niveus</i> Blochwitz	48	<i>Naganishia diffluens</i> (Zach) X.Z. Liu, F.Y. Bai, M. Groenewald & Boekhout
15	<i>Aureobasidium pullulans</i> Bary G. Arnaud	49	<i>Neocosmospora sp.</i> E.F. Smith
16	<i>Baudoinia compniacensis</i> (Richon) J.A.Scott & Unter.	50	<i>Neonectria macrodidyma</i> Halleen, Schroers & Crous
17	<i>Beauveria bassiana</i> (Bals.) Vuill	51	<i>Neoscytalidium dimidiatum</i> Penz.) Crous & Slippers
18	<i>Bipolaris spp.</i> Shoemaker	52	<i>Neotyphodium coenophialum</i> Glenn <i>et al.</i>
19	<i>Botryotrichum piluliferum</i> Sacc. & Marchal	53	<i>Paecilomyces farinosum</i> (Holmskjold) A.H.S. Brrown & G. Smith
20	<i>Cercosporidium scariolae</i> (Syd.) Deighton	54	<i>Penicillium spp.</i> Link
21	<i>Chaetomium cochliodes</i> Palliser	55	<i>Phaeosclera dematioides</i> Sigler
22	<i>C. globosum</i> Kunze	56	<i>Rhizoctonia solani</i> J.G. Kühn
23	<i>C. Piluliferum</i> J. Daniels	57	<i>Rhizopus spp.</i> Ehrenb
24	<i>Cladosporium tenellum</i> K. Schub., Zalar, Crous & U.Braun	58	<i>Sarocladium strictum</i> (W. Gams) Summerbell
25	<i>C. aphidis</i> Thumen	59	<i>Scytalidium lignicola</i> Pesante
26	<i>Coprinopsis urticicola</i> (Berk. & Broome) Buller	60	<i>Stachybotrys echinata</i> (Rivolta) Galloway
27	<i>Cryptococcus spp.</i>	61	<i>S. cylindrospora</i> D.W. Li
28	<i>Curvularia ovoidea</i> (Hiroë & N. Watan.) Munt.-Cvetk.	62	<i>Stemphylium sarciniforme</i> (Cavara) Wiltshire
29	<i>C. lunata</i> (Wakker) Boedijn	63	<i>Thielavia hyalocarpa</i> Arx
30	<i>Drechslera nodulosa</i> (Berk. & M.A. Curtis ex Sacc.) Subram. & B.L. Jain	64	<i>Trichoderma hamatum</i> (Bonord.) Bainier
31	<i>D. globulifera</i> Mouchacca	65	<i>Verticillium longisporum</i> (C. Stark) Karapapa, Bainbr. & Heale
32	<i>D. neergaardii</i> (Danquah) Alcorn	66	<i>Ulocladium spp.</i> Preuss
33	<i>D. biseptata</i> (Sacc. & Roum.) Richardson & Fraser	67	Sterile mycelium (white)
34	<i>Emericellopsis spp.</i>	68	Sterile mycelium (Black)



**Table 2:** Identification of 25 fungal taxa isolated from the 14 selected wheat fields based on morphological analysis and sequencing of rDNA ITS1-5.8S-ITS4 region during this study.

Isolate	NCBI-Blast Identification	Accession N.	%ID.	Field	Source
S-1	<i>Chaetomium cochliodes</i>	<a href="#">MF992211.1</a>	99%	HM	Rhizo
S-2	<i>Botryotrichum piluliferum</i>	<a href="#">MH873348.1</a>	99%	HM	Rhizo
S-3	<i>Botryotrichum piluliferum</i>	<a href="#">MH873348.1</a>	99%	HM	Rhizo
S-4	<i>Mortierella alpine</i>	<a href="#">KY825117.1</a>	99%	QM	Rhizo
S-5	<i>Naganishia diffluens</i>	<a href="#">MH588282.1</a>	99%	N	Rhizo
S-6	<i>Epicoccum nigrum</i>	<a href="#">KR912314.1</a>	97%	TI1	Head
S-7	<i>Coprinopsis urticicola</i>	<a href="#">MH300678.1</a>	98%	QM	Root
S-8	<i>Botryotrichum piluliferum</i>	<a href="#">MH873348.1</a>	98%	TK	Root
S-9	<i>Acremonium zonatum</i>	<a href="#">KT968535.1</a>	98%	TK	Stem
S-10	<i>Botryotrichum piluliferum</i>	<a href="#">MH858364.1</a>	98%	ML1	Head
S-11	<i>Chaetomium piluliferum</i>	<a href="#">KF915989.1</a>	99%	QRS	Stem
S-12	<i>Neonectria macrodidyma</i>	<a href="#">GU236526.1</a>	98%	H	Stem
S-13	<i>Acremonium zonatum</i>	<a href="#">KT968535.1</a>	98%	TI3	Head
S-14	<i>Acremonium zonatum</i>	<a href="#">KT968535.1</a>	99%	MSR	Seed
S-15	<i>Epicoccum nigrum</i>	<a href="#">MH685171.1</a>	97%	QM	Rhizo
S-16	<i>Sarocladium strictum</i>	<a href="#">KY465763.1</a>	99%	MSP	Head
S-17	<i>Botryotrichum piluliferum</i>	<a href="#">MH873348.1</a>	99%	HM	Rhizo
S-18	<i>Alternaria Chlamydospora</i>	<a href="#">KY402034.1</a>	100%	TI1	Stem
S-19	<i>Gymnoascus dankaliensis</i>	<a href="#">NR_155111.1</a>	100%	TK	Rhizo
S-20	<i>Gymnoascus dankaliensis</i>	<a href="#">NR_155111.1</a>	100%	TK	Rhizo
S-21	<i>Alternaria ventricosa</i>	<a href="#">KM454880.1</a>	99%	TI3	Stem
S-22	<i>Acremonium egyptiacum</i>	<a href="#">MH424670.1</a>	97%	TK	Rhizo
S-23	<i>Aspergillus niveus</i>	<a href="#">MH865978.1</a>	100%	TK	Soil
S-24	<i>Cladosporium tenellum</i>	MK111467.1	100%	TI2	Ear
S-25	<i>Alternaria tellustris</i>	MH864767.1	100%	TK	Ear

\*%ID: percentage of identity.

**Fig. 2** shows the percentage of isolated fungal groups from wheat head, stem, root and seed, as well as rhizosphere region and soil during the growing season in 2017/2018.

### Density Estimation of Fungal population in Soil and Rhizosphere:

The distribution of these percentages of occurrence on plant parts, seeds, soil and rhizosphere were approximately the same with some fluctuations. In terms of soil and rhizosphere, 19 genera of fungi were identified with 32 species, as well as white and black sterile mycelia. anamorphic Ascomycota fungi were occurred in percentage of 88.47%, mainly *Aspergillus* spp. (35.19%), *Penicillium* spp. (28.8%), *Fusarium* spp. (13.23%), *Ulocladium* spp. (4.86%), and *Trichoderma* spp. (2.52%), while *Alternaria* spp., *Cladosporium* spp., *Emericellopsis* spp. *Helminthosporium* spp. and *Verticillium* spp. were found at low frequencies. With regard to Zygomycotina fungi were occurred in the second level, in specific *Rhizopus* spp. and *Mucor* spp. (5.85 and 0.63) % respectively. Teleomorphic Ascomycota (Pezizomycotina) fungi were occurred with percentage of 4.32% mainly *Chaetomium* spp. (3.78%), *Epicoccum* spp. and *Aureobasidium* spp. with minor percentages (0.27 and 0.09) % respectively. While Basidiomycota (Agaricomycotina) fungi were occurred in percentage of 0.81%, mainly *Rhizoctonia solani* and *Coprinopsis* spp. Table 3.

**Table 3.** Identification and occurrence of detected genera of microscopic fungi isolated from **Plant Parts** (ear, stem, crown and root), **Seeds, Soil, & Rhizosphere** during this study based on morphological and molecular identification.

Fungal isolate	Plan tparts <sup>c</sup> (E, S, C &R)		Seed		<sup>d</sup> So &Rh		Total	
	<sup>a</sup> OCN	<sup>b</sup> %	OCN	%	OCN	%	OCN	%
<i>Acromonium</i> sp.	11	2,04	3	1,37	0	0	14	0,74
<i>Alternaria</i> sp.	85	15,76	39	17,88	20	1,80	144	7,70
<i>Aspergillus</i> sp.	31	5,75	84	38,53	391	35,19	506	27,08
<i>Aureobasidium</i> sp.	3	0,55	1	0,45	1	0,09	5	0,26
<i>Baudoinia</i> sp.	3	0,55	0	0	0	0	3	0,16
<i>Beauveria</i> sp.	7	1,29	0	0	0	0	7	0,37
<i>Bipolaris</i> sp.	2	0,37	3	1,37	0	0	5	0,26
<i>Botryotrichum</i> sp.	4	0,74	3	1,37	0	0	7	0,37
<i>Cercosporidium</i> sp.	2	0,37	1	0,45	0	0	3	0,16
<i>Chaetomium</i> sp.	6	1,11	3	1,37	42	3,78	51	2,73
<i>Cladosporium</i> sp.	43	7,97	3	1,37	3	0,27	49	2,62
<i>Coprinopsis</i> sp.	0	0	0	0	1	0,09	1	0,05
<i>Cryptococcus</i> sp.	2	0,37	1	0,45	0	0	3	0,16
<i>Curvularia</i> sp.	2	0,37	0	0	0	0	2	0,10

<i>Drechslera</i> sp.	6	1,11	2	0,91	0	0	8	0,42
<i>Emericellopsis</i> sp.	0	0	0	0	3	0,27	3	0,16
<i>Epicoccum</i> sp.	0	0	0	0	3	0,27	3	0,16
<i>Eurotium</i> sp.	3	0,55	2	0,91	0	0	5	0,26
<i>Exserohilum</i> sp.	2	0,37	0	0	0	0	2	0,10
<i>Fusarium</i> sp.	81	15,02	3	1,37	147	13,23	231	12,36
<i>Geotrichum</i> sp.	1	0,18	0	0	0	0	1	0,05
<i>Gymnascella</i> sp.	1	0,18	0	0	0	0	1	0,05
<i>Gymnoascus</i> sp.	1	0,18	0	0	0	0	1	0,05
<i>Helminthosporium</i> sp.	46	8,53	11	5,04	7	0,63	64	3,42
<i>Monodictys</i> sp.	3	0,55	1	0,45	0	0	4	0,21
<i>Monosporascus</i> sp.	4	0,74	3	1,37	0	0	7	0,37
<i>Mortierella</i> sp.	2	0,37	0	0	0	0	2	0,10
<i>Mucor</i> sp.	7	1,29	0	0	4	0,36	11	0,58
<i>Naganishia</i> sp.	1	0,18	0	0	0	0	1	0,05
<i>Neocosmospora</i> sp.	2	0,37	0	0	0	0	2	0,10
<i>Neonectria</i> sp.	3	0,55	0	0	0	0	3	0,16
<i>Neoscytalidium</i> sp.	2	0,37	2	0,91	0	0	4	0,21
<i>Neotyphodium</i> sp.	7	1,29	4	1,83	0	0	11	0,58
<i>Paecilomyces</i> sp.	3	0,55	0	0	0	0	3	0,16
<i>Penicillium</i> sp.	41	7,60	17	7,79	320	28,80	378	20,23
<i>Phaeosclera</i> sp.	2	0,37	0	0	0	0	2	0,10
<i>Rhizoctonia</i> sp.	0	0	1	0,45	8	0,72	9	0,48
<i>Rhizopus</i> sp.	13	2,41	14	6,42	62	5,58	89	4,76
<i>Sarocladium</i> sp.	2	0,37	0	0	0	0	2	0,10
<i>Scytalidium</i> sp.	5	0,92	1	0,45	0	0	6	0,32
<i>Stachybotrys</i> sp.	4	0,74	0	0	1	0,09	5	0,26
<i>Stemphylium</i> sp.	5	0,92	3	1,37	3	0,27	11	0,58
<i>Thielavia</i> sp.	4	0,74	0	0	0	0	4	0,21
<i>Trichoderma</i> sp.	2	0,37	2	0,91	28	2,52	32	1,71
<i>Verticillium</i> sp.	6	1,11	0	0	2	0,18	8	0,42
<i>Ulocladium</i> sp.	8	1,48	3	1,37	54	4,86	65	3,47
Srericile mycelia	71	13,17	8	3,66	11	0,99	90	4,81
<b>Total</b>	<b>539</b>	<b>100</b>	<b>218</b>	<b>100</b>	<b>1111</b>	<b>100</b>	<b>1868</b>	<b>100</b>

<sup>a)</sup> OCN= Occurrence Number; <sup>b)</sup> % = Occurrence percentage; <sup>c)</sup> (E, S, C &R) = Ear, Stem, Crown and Root; <sup>d)</sup> So & Rh= Soil and Rhizosphere.

Previous studies have shown that fungi belonging to anamorphic Ascomycota are predominant in Iraqi soils, and this was indicated by [28] and [19] for soil samples collected from the Iraqi northern and middle provinces respectively. Ismail and Abdullah [29] also reported that the percentage of anamorphic Ascomycota fungi exceeded 86% of the isolated species from the cultivated and non-cultivated areas in Basra province. This percentage is very close to what was observed in this study (88.47%). In general *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. were constantly in the largest levels in most Iraqi studies as soil mycoflora. The presence of these fungal species in the majority of Iraqi soils may not be only due to the appropriate soil and ecological conditions, but may be attributed to other factors. For example, *Aspergillus* genera is common in warm areas and has a high ability to adapt to high temperatures that rise during the summer season, as well as its capacity to produce masses of conidia [30], and some of its species have enzymatic capacity for consuming different organic substrates [31]. In addition, Abdullah and Zora [32] and Al-Badr [33] indicated that *Aspergillus* spp. are more common in all types of soils in Iraq.

*Penicillium* genera was also in the first levels by its occurrence, as this genera is common in soil and exist in a wide range in nature [34]. As stated in previous studies in Iraq that *Penicillium* spp. occur frequently at high levels, i.e. recorded in the second level in the study of Abdullah and Zora [32] and this is corresponding with the present results.

*Fusarium* sp. are shared soil fungi and have high competitiveness capability against other soil mycoflora. According to [19, 32], *Fusarium* sp. have a good ability to adapt and survive in soil under extreme environmental conditions. The characteristic occurrence of these fungi reveal their ability to produce large numbers of reproductive units in the form of perithecia or sporodochia that survive effectively in hard environmental conditions.

Existence of *Chaetomium* sp. in soil may be attributed to the availability of cellulose substrates in soil i.e. the residues of agricultural crops, as well as the high capacity of these fungi to colonize cellulose-containing materials [35].

*Rhizoctonia solani* was found to be at low frequency in this study. However, the presence of even a few densities of this species is important for economic terms, because this fungus has a

high ability to reproduce and develop successfully at the availability of susceptible hosts. Thus, it possibly causes significant economic losses [36].

### Density estimation of fungal community in seed

For seeds that were collected at the harvesting time in this research work, 25 genera of fungi were identified with 41 species, along with white sterile mycelia. Anamorphic Ascomycota fungi were occurred in percentage of 85.66%, followed by *Rhizopus* sp. was occurred in percentage of 6.42%. Ascomycota (Pezizomycotina) and Basidiomycota (Agaricomycotina) fungi that were occurred in percentage of (3.19 and 1.8) %.

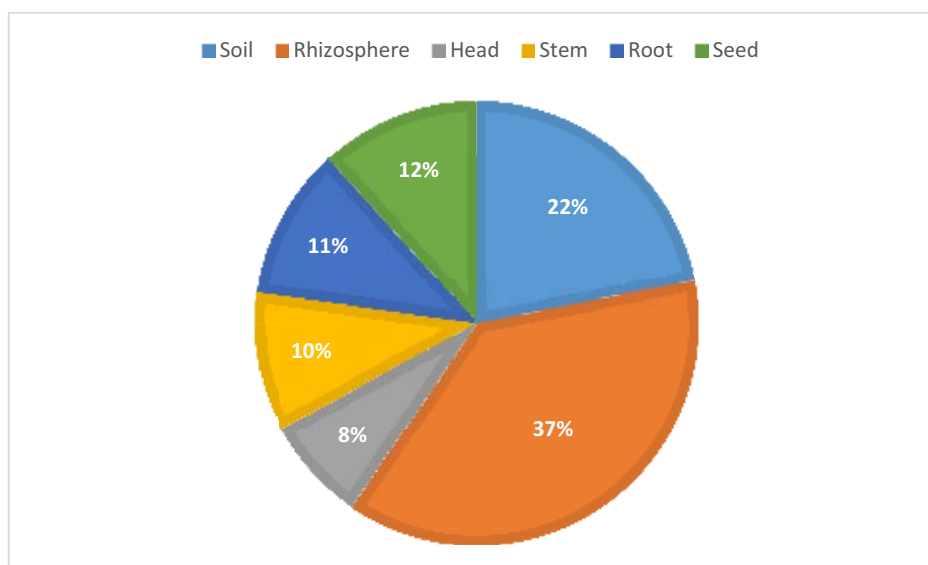
Fungi are the principal spoiling organisms in cereal seeds. Therefore, the majority of fungal wheat crop diseases spread by seed borne pathogenic fungi, which can result in bad seed quality, nutrient content reduction, decreasing the capability of germination as well as damping off and accordingly reducing crop product [37]. In previous years, numerous researches have been detected seed borne fungal community of wheat crop around the world. For instance, as mentioned in Ur-Rahman et al. [38] that in one study in Canada, it was reported that approximately 60 seed borne fungal species with more than 30 genera were isolated from wheat seeds. In Pakistan also, a research found more than 40 species with 17 fungal genera accompanying with wheat seeds [39]. While, another Pakistani study revealed that only 4 genera of seed borne pathogenic fungi were identified and isolated from wheat seeds, including *Alternaria* spp., *Drechslera* spp., *Phytophthora* spp. and *Fusarium* spp. [38]. In the present study, 25 genera with 41 species of fungi were isolated and identified from wheat seeds (Table 2). The mycoflora of winter wheat seeds that isolated most repeatedly were *Aspergillus* spp. (38.53%), *Alternaria* spp. (17.88%), *Penicillium* spp. (7.79%), *Rhizopus* spp. (6.42%), *Helminthosporium* spp. (5.04%) and *Neotyphodium* spp. (1.83%). While, *Fusarium* spp., *Botryotrichum* spp., *Cladosporium* spp., *Chaetomium* spp., *Bipolaris* spp., *Monosparascus* spp., *Stemphylium* spp., *Ulocladium* and *Acremonium* spp. were all occurred with (1.37%). The lowest contamination rate of seed was seen in *Neoscytalidium* spp., *Drechslera* spp., *Aureobasidium* spp., *Eurotium* spp., *Scytalidium* spp., *Monodictys* spp., *Cryptococcus* spp., *Cercosporidium* spp. and *Aureobasidium* spp. was determined in percentages of less than 1%. Along with, [40] indicated in his study that *Alternaria* spp. were on top of fungi frequency associated with the wheat seeds. Furthermore, Habib et al. [41], stated that *Alternaria* spp., *Aspergillus* spp.,

*Cladosporium* spp., *Curvularia* spp., *Helminthosporium* spp., *Penicillium* spp., *Rhizopus* spp. and *Stemphylium* spp. have been isolated and identified as weakly pathogenic or saprophytic seed mycobiota. Additionally, *Ulocladium* spp. was isolated from wheat seeds in Markazi province in Iran by [42].

It is obvious from the present study that all examined wheat seeds were contaminated by fungi. In order to determine the main involvement of wheat to world food, its production have to be improved to meet the nutritional needs of the growing world population [43]. Importantly, Iraqi farmers should be compelled by Iraqi Ministry of Agriculture to plant certified wheat seeds, which are substantial contribution for crop production and subsequently decrease yield losses due to those associated pathogenic fungi with wheat seeds. Also, preventing agricultural trading companies from importing wheat seeds and limit it to the government and this is a crucial way to make Iraq self-sufficient and also participate to the food security. Additionally, the majority of seed born pathogenic fungi can be basically controlled by treating seeds with fungicides and biological substrates. Furthermore, according to Clark et al. [44], using typical storage services for preserving wheat grains in silos to decrease the contamination level produced by those pathogenic fungi associated with wheat seeds lower than damage threshold is suggested.

#### **Density Estimation of Fungal Community in Wheat Plant Parts:**

Regarding infected plant parts (ear, stem, crown and root), which were collected at the ripening stage, 42 genera with 63 species of fungi were identified. Similarly, anamorphic Ascomycota fungi were settled on top of occurrence in percentage of 78.9%, followed by Ascomycota (Pezizomycotina) fungi 3.21%, Basidiomycota (Agaricomycotina) 3%, Zygomycotina 1.72%, besides sterile mycelia that were occurred in percentage of 13.17% (Table 3). It can be clearly stated that the same fungal groups that were isolated from harvested seed, rhizosphere region and field soil, were also isolated from wheat parts but with different percentages. However, the distribution of the collective fungal groups was varied in percentage of frequency among the examined factors (soil, rhizosphere, seed, head, stem and root). The highest percentage of frequency was occurred in rhizosphere region (37%), followed by soil (22%). The lowest percentage was occurred in wheat head (8%). While, the remaining (seed, root and stem) were almost identical in percentage of frequency with (12, 11 and 10) % respectively (Fig. 3)



**Fig. 3** shows the frequency percentages of collective fungal groups on the plant parts (head, stem, root and seed) as well as soil and rhizosphere region during the growing season in 2017/2018.

To the best of our knowledge, there were not previous publications about the community of environmental mycobiota associated with entire wheat plant parts (ear, crown, stem and root) in Iraq and the Middle East countries. However, there have been some studies about the biology and natural existence of specific fungus on wheat ears, such as the study of Šrobárová [45] that was performed in Slovakia about *Fusarium* spp. during the period 1993-1996. In addition to, numerous studies surveyed various fungal species from stored in silos or new harvested wheat seeds, such as [46-49], [38], and therefore, it can be stated that the recent research is the first survey conducted for that purpose. The prevalence fungi on infected plant parts of winter wheat in the most collected samples in this study were *Alternaria* spp. (15.76%), *Fusarium* spp. (15.02%), *Helminthosporium* spp. (8.53%), *Cladosporium* spp. (7.97%), *Penicillium* spp. (7.6%), *Aspergillus* spp. (5.75%), *Rhizopus* spp. (2.41%) and *Acremonium* spp. (2.04%). The rest of surveyed fungi such as *Aureobasidium* spp., *Baudoinia* spp., *Beauveria* spp., *Bipolaris* spp., *Botryotrichum* spp., *Cercosporidium* spp., *Chaetomium* spp., *Cryptococcus* spp., *Curvularia* spp., *Drechslera* spp., *Eurotium* spp., *Exserohilum* spp., *Geotrichum* spp., *Gymnoascus* spp., *Monodictys* spp., *Mortierella* spp., *Mucor* spp., *Naganishia* spp., *Neocosmospora* spp., *Neonectria* spp., *Neoscytalidium* spp., *Neotyphodium* spp., *Phaeosclera* spp., *Sarocladium* spp., *Scytalidium* spp., *Stachybotrys* spp., *Stemphylium* spp., *Thielavia* spp., *Trichoderma* spp., *Verticillium* spp. and *Ulocladium* spp. were also isolated with less than 2 % of relative

frequency. The most of these environmental mycoflora were determined as fungi producing spores on the infected parts (ear, stem, crown and root) of wheat plant. For example, *Fusarium* spp. were found generating sporodochia as survival forms on the spikelet of wheat ears. The saprophytic fungi such as *Alternaria* spp. and parasitic fungi belonging to the genera *Fusarium* were the most dominant on all infected plant parts in the 14 selected wheat fields. Pastirčák [50] reported that the environmental fungi associated mostly with ears of winter wheat involved mainly of anamorphic Ascomycetes followed by teleomorphic Ascomycetes and Zygomycetes in rare rate of frequency. This result is corresponded to what was achieved in the recent study.

Extensive searches on the population of fungi associated with wheat plant parts (ear, stem, crown and root) collectively should be surveyed determining the potential yield losses, disease incidence, severity and host reaction. Likewise, it is substantial to put more efforts for studying other Iraqi provinces mainly the south and middle of Iraq to classify the distribution of such mycoflora communities on wheat crop and their responses to different weather conditions. An integration of more effective investigation, expansion in crop observation and applicable strategies to provide contributions are required to attain settled economic and social status of the population [51].

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