BIODIVERSITAS
Volume 23, Number 6, June 2022
Pages: 3099-3107

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230637

New records of mycobiota associated with stored wheat and its by-products in Iraq

WASAN F. FADHIL[1], ABDULLAH H. AL-SAADOON[2,♥], FALEH M. AL-MOUSSAWI[3]

[1]Department of Biology, College of Science, University of Basrah. Basrah, Iraq
[2]Department of Pathological Analyses, College of Science, University of Basrah. Basrah, Iraq. ♥email: abdullah.hamood@uobasrah.edu.iq
[3]Quality Control Department, Food Science, Grain Board of Iraq. Baghdad, Iraq

Manuscript received: 26 April 2022. Revision accepted: 27 May 2022.

Abstract. *Fadhil WF, Al-Saadoon AH, Al-Moussawi FM. 2022. New records of mycobiota associated with stored wheat and its by-products in Iraq. Biodiversitas 23: 3099-3107.* Wheat is a staple food for the Iraqi population and is an important feed source due to its high nutritional value. Consequently, wheat and wheat by-products are susceptible to various species of fungi during processing, transportation, or storage. In this paper, thirty-two selected fungal isolates were subjected to morphological and molecular analysis for identification through combined sequences of ITS regions, actin, calmodulin, glyceraldehyde-3-phosphate dehydrogenase, and β-tubulin genes according to the target fungal genus. The results showed that eighteen fungal species were identified from wheat grains, flour, and bran samples collected from different silos and mills in three provinces in southern Iraq. In addition, nine species were recorded for the first time from Iraq, i.e., *Alternaria consortialis, A. japonica, A. lolii, A. multiformis, A. ventricosa, Aspergillus montevidensis, Cladosporium halotolerans, C. versiforme,* and *Staganosporopsis tanaceti.* Brief descriptions of the new records are presented. This study represents an important addition to the mycobiota of Iraq.

Keywords: Bran, flour, fungi, Iraq, wheat grains

INTRODUCTION

Iraq is a Middle Eastern country with an area of 438.317 km^2 and is located at longitude (38° 48°E) and latitude (29° 37°N). Various crops were cultivated in Iraq that depend on rain-fed, especially in the northern regions, and on irrigation from the Tigris and Euphrates rivers and their tributaries in the plain lands. Wheat is the main cultivated cereal in Iraq, followed by barley (Soppe and Saleh 2012).

Bread wheat (*Triticum aestivum* L.) is an annual plant belonging to the family Poaceae, with long culms, 70 cm high, flat leaves, and spike inflorescence (Al-Mayah et al. 2016). Globally, it is the most produced food among the cereal crops after rice (Shiju 2010). It is a staple food for the Iraqi people (Iraqi CSO 2019). Bread flour is also an important feed source for livestock and poultry due to its high nutritional value (Shewry 2009). It is the most important winter crop, usually sown in November, and the harvest season starts approximately from mid-April until late May (DEAT 2019; FAO 2021). Its production accounts for about 70% of total cereal production in Iraq (UNESCO 2019; Ewaid et al. 2020). Most of the harvested wheat is stored in silos and bunkers (FAO 2021) for varying periods until it is transported to mills distributed around the country (FAO 2021). The milling companies transform wheat grains into flour and bran. Bran is an important by-product of the wheat process, not only for human and animal consumption but also for biotechnological applications (Katileviciute et al. 2019; FAO 2021). Foods and feeds, including wheat grains and their derivatives products (flour and bran), are susceptible to various fungal mycobiota during processing, transportation, or storage (Riba et al. 2008). Wheat crops are susceptible to various diseases, such as root rot, Fusarium head blight, and crown rot diseases (Mohammed-Ameen et al. 2021). These fungi are acquired from the field or post-harvest and pose a great threat to the wheat crop, both quantitatively and qualitatively (Magan et al. 2003). In addition, there is a threat to human and animal health represented by toxin-producing fungi (Gregori et al. 2013), especially the genera *Alternaria, Aspergillus*, *Fusarium*, and *Penicillium* (Magan et al. 2003). The genera *Alternaria* and *Fusarium* represent field fungi, while *Aspergillus* and *Penicillium* are storage fungi, and the population structure depends mainly on surrounding conditions and the harvesting process (Magan and Lacey 1986; Lacey and Magan 1991). In Iraq, many studies have been conducted on the fungi associated with cereals, particularly wheat grains and their derivatives (Sulaiman and Husain 1985; Juber and Al-Salahi 2006; Abdullah and Atroshi 2014; Al-Defiery 2015; Abdullah and Atroshi 2016; Ghadban et al. 2017; Hussein and Saddullah 2018; Saido et al. 2020; Thalij et al. 2021). Since wheat is of great importance crop mainly related to the sustenance of people and animals, it is necessary to periodically investigate the safety of this crop and its by-products, starting from the field, passing through the silos, until reaching the mills, and before reaching the consumer. Therefore, in continuation to previous studies, several fungal species were identified from wheat grains and their derivatives (Flour and bran) as a new addition to the wheat mycobiota of Iraq.

MATERIAL AND METHODS

Sample collection

Forty-five local wheat samples were collected from silos and warehouse yards in the southernmost governorates of Iraq, namely Basrah, Dhi-Qar, and Maysan, from November 2020 to February 2021, with an average of 15 samples from each governorate. In addition, 45 flour samples (15 from each governorate) and 45 bran samples (15 from each governorate) from which grain samples were previously collected. Five sites were chosen randomly to collect each type of sample, as much as five kilograms in each area, then mixed, and 500g was taken to represent the sample from each area (Saleemi et al. 2016). It was placed in sterilized plastic bags, then transferred to the laboratory, and kept in the refrigerator at 4◦c for further analysis.

Isolation of fungi

Fungi were isolated from wheat grains by taking 50 grains from each sample and sterilizing them with 1.5% sodium hypochlorite solution for 2 minutes. Then, they were washed three times with sterile distilled water. Next, the grains were dried between two sterile filter papers. Finally, ten sterile grains were cultured on a Petri dish containing PDA medium (Himedia, India), amended with 100 mg/L chloramphenicol (Wareing 1997; Belkacem-Hanfi et al. 2013). The dishes were incubated at 25°C for a week and periodically monitored for any fungal growth. As for the flour and bran samples, the dilution method was followed by taking 10g of each sample, placing it in 90 mL of sterile distilled water, agitating for about 15 minutes, and then preparing a series of dilutions. One mL was withdrawn from each dilution and placed in a Petri dish containing a PDA medium and 1 mL onto an MEA medium. Three replicates were carried out for each dilution; then, the dishes were incubated at 25°C for 10 days with periodic growth follow-up.

Morphological identification of fungi

All fungi were identified to genus and species level based on macro and micromorphological characteristics according to the following references, Ellis (1971, 1976); Samson et al. (2004); Klich (2002); Watanabe (2002); Pitt and Hocking (2009); Aveskamp et al. (2010); Guarro et al. (2012); Woudenberg et al. (2013); Bensch et al. (2018); Moral et al. (2018).

Molecular identification of fungi

Genomic DNA was extracted using the Presto mini gDNA yeast kit (Geneaid Biotech Ltd) according to the manufacturer's instructions. The ITS region was amplified using the universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC – 3') as described by White et al. (1990). Several gene sequences were selected for molecular identification of different genera to the species level. A fragment of the 5' portion of the β-tubulin gene was amplified using the primers Bt2a (5' GGT AAC CAA ATC GGT GCT GCT TTC -3') and Bt2b (5'- ACC CTC AGT GTA GTG ACC CTT GGC -3') according to Glass and Donaldson (1995); a segment of the calmodulin gene was amplified using primers CMD5 (5'- CCG AGT ACA AGG AGG CCT TC – 3') and CMD6 (5' – CCG ATA GAG GTC ATA ACG TGG -3') as described by Hong et al. (2006); a region of the glyceraldehyde -3- phosphate dehydrogenase gene was amplified using the primers GPD1 (5'- CAA GGG CTT CGG TCG CAT TG -3') and GPD2 (5' – GCC AAG CAG TTG GTT GTG C -3') as described by Berbee et al. (1999); and finally, the primer pair ACT–512F (5' – ATG TGC AAG GCC GGT TTC GC -3') and ACT-783R (5' – TAC GAG TCC TTC TGG CCC AT -3') was used to amplify the actin gene according to Bensch etal. (2012)

The amplification of all genes was conducted according to PCR conditions described in Table 1. PCR products of about 32 selected isolates were purified and sequenced at Macrogen Inc. (South Korea). The sequences obtained were compared with sequences deposited in the GenBank database through the BLASTn program, available at the NCBI.

RESULTS AND DISCUSSION

Out of 32 isolates subjected to phenotypic and molecular identification, 26 strains were identified to the species level. Accordingly, based on the morphological characteristics and the analysis of the sequences of the ITS region and the specific genes, which are actin, calmodulin, GPD, and β-tubulin, it appeared that these strains belong to 18 species. To our knowledge, 9 species are new records for the Iraqi mycobiota (Table 2). All newly recorded species are given a short description with brief and specific comments, especially about their basic ecology.

Table 1. Details of the polymerase chain reaction conditions used to amplify different genes.

PCR fragment	Denaturation		Anneling		Extention		Number of cycles
Bt2a Bt2b	95°C	1 min	59°C	30 sec	72°C	1 min	35
ITS1 ITS4	95°C	1 min	56°C	45 sec	72°C	1 min	35
ACT-512F ACT-783R	95°C	1 min	58°C	30 sec	72°C	1 min	35
GPD1 GPD2	95°C	1 min	58°C	30 sec	72°C	1 min	35
CMD5 CMD6	95°C	1min	58°C	30 sec	72°C	1min	35

Table 2. Summary of morphological and molecular identification of fungi isolated from wheat grains and their products in some governorates of southern Iraq

Strain no.	Source	Identification			Final	NCBI acc. no.
		Morphological	ITS primer	Specific primers		
ISW1	Wheat grain	*Didymella* sp.	*Didymella* sp. MW723759.1(96.83%) *Coniothyrium aleuritis* KY318503.1(98.14%)	*Didymella glomerata* (99.55%) (Actin)	*Didymella glomerata*	LC682581.1
ISW2	Wheat grain	*Alternaria*	uncultured fungus KY823599.1(98.72%)	*Alternaria botrytis* (80.79%) (Actin)	*Alternaria botrytis*	LC682582.1
ISW3	Wheat bran	*Cladosporium halotolerans*	*C.halotolerans* MN555569.1(95.86%)	*C. halotolerans*(98.95%) (Actin)	*C. halotolerans*	LC682583.1
ISW4	Wheat bran	*Cladosporium* sp.	*C. uredinicola* JN088229.1(92.27%)	*Cladosporium* sp.(98.94%) (Actin)	*Cladosporium* sp.	Not registered
ISW5	Wheat bran	A. japonica	*Alternaria japonica* MN610564.1(99.11%)	*Alternaria japonica* (98.93%) (GPD)	*A. japonica*	LC682584.1
ISW6	Wheat flour	No identified	*Alternaria* sp. KT268806.1(100%) *Alernaria lolii* FJ357313.1(99.60%)	*Alternaria lolii (98.11%)* (GPD)	*A.lolii*	LC682585.1
ISW7	Wheat grain	*Alternaria ventricosa*	*A. ventricosa* MT196809.1(100%) *A.infectoria* MT883449.1(100%) *A. alternata* MF141014.1(100%)	*Alternaria sp.(98.63%) (GPD)*	*A. ventricosa*	LC682580.1
ISW8	Wheat grain	*Alternaria multiformis*	*A. consortialis* MK907943.1(99.62%) *A.cucurbitae* EU330457.1(99.62%) *A.multiformis* KP117291.1(99.62%)	*A.multiformis(99.65%) (GPD)*	*A.multiformis*	LC682858.1
ISW9	Wheat bran	*Alternaria consortialis*	*A. consortialis* KM977759.1(99.44%) *Alternaria* sp. MK713358.1(99.25%) *Ulocladium* MT735246.1(99.25%)	*A. consortialis (100%) (GPD)*	*A. consortialis*	LC682859.1
ISW10	Wheat bran	Cladosporium versiforme	*C. allicinum* MG946767.1(99.60%) *C.herbarum* MN486496.1(99.60%)	*C. versiforme (98.91%)* (Actin)	*C.versiforme*	LC682860.1
ISW11	Wheat grain	*Penicillium grisefulvum*	*P. polonicum* KF597019.1(90.22%) *P. grisefulvum* MF034654.1(92.80%)	*P. griseofulvum (99.79%) (*B-tubulin and calmodulin)	*P.griseofulvum*	LC682861.1
ISW12	Wheat grain	*Aspergillus pseudogloucus*	*A. medius* KT832076.1(91.89%) *A. amstelodami* KT232081.1(88%) *A. pseudogloucus* KX69362.1(98.83 %)	*A. pseudoglaucus (99.81%) (B-tubulin and calmodulin)*	*A. pseudogloucus*	LC682862.1
ISW13	Wheat bran	*Aspergillus amstelodami*	*A. medius* KT832076.1(91.72%) *A. amstelodami* KT232081.1(87.99%)	*A. amstelodami* (100%) (B-tubulin)	*A. amstelodami*	LC682863.1
ISW14	Wheat flour	*Aspergillus montevidensis*	*A. medius* KT832076.1(92.83%) *A. amstelodami* KT 232081.1(88.83%)	*A. montevidensis (99.82%) (calmodulin)*	*A. montevidensis*	LC682864.1
ISW15	Wheat flour	*Alternaria consortialis*	*Ulocladium* sp. KJ361489.1(99.44%)	*A. consortialis(100%) (GPD)*	*A.consortialis*	LC682865.1
ISW16	Wheat grain	*Alternaria triticina*	*A. ventricosa* MT196809.1(99.64%) *A.infectoria* MT883449.1(99.82%)	*A. triticina(98.44%) (GPD)*	*A. triticina*	LC683188.1
ISW17	Wheat grain	*Penicillium digitatum*	*P. polonicum* KF597019.1(91.64%) *P.echinulatum* MK256746.1(94.01%) *P. digitatum* MK450692.1(99.63%)	*P. digitatum (99.09%) (B-tubulin)*	*P.digitatum*	LC683189.1
ISW18	Wheat grain	*Alternaria consortialis*	*A. consortialis* KM977759.1(99.25%) *Ulocladium* sp. KM977761.1(99.06%)	*A. consortialis(100%) (GPD)*	*A.consortialis*	LC683190.1
ISW19	Wheat grain	*Alternaria consortialis*	*A. alternata* JQ080319.1(91.99%) *A. consortialis* KM977766.1(99.08%)	*A. consortialis(100%) (GPD)*	*A.consortialis*	LC683191.1
ISW20	Wheat bran	*Alternaria consortialis*	*Ulocladium* sp. KM977761.1(99.25%) *Alternaria consortialis* KM977759.1(99.25%)	*A. consortialis (100%) (GPD)*	*A.consortialis*	LC683192.1

ISW21	Wheat bran	*Aspergillus montevidensis*	*A. medius* KT832076.1(91.50%) *Aspergillus* sp. KP702151.1(89.40%) *A. montevidensis* MT487826.1(99.80%)	*A. montevidensis (99.82%) (B-tubulin and calmodulin)*	*A. montevidensis*	LC683193.1
ISW22	Wheat grain	*Aspergillus amstelodami*	*A. medius* KT832076.1(91.92%) *Aspergillus* sp. KP702151.1(89.92%) *A. amstelodami* KT232081.1(89.11%)	*A. amstelodami (100%) (B-tubulin)*	*A.amstelodami*	LC683194.1
ISW23	Wheat grain	*Phoma* sp.	*Phoma costaricensis* KT881552.1(91.02%) *Didymella* sp.MW723759.1(93.81%)	*Stagonosporopsis tanaceti (92.01%) (GPD)*	*S.tanaceti*	LC683195.1
ISW24	Wheat bran	*Curvularia lunata*	*Curvularia* sp. JQ765410.1(95.90%) *Curvularia lunata* JK396064.1(95.90%)	*Curvularia lunata (90.99%) (Actin)*	*Curvularia lunata*	
ISW25	Wheat flour	*Alternaria atra*	*A. alternata* JQ080319.1(91.37%)	*A. atra(100%) (GPD)*	*A. atra*	
ISW26	Wheat grain	*Alternaria atra*	*A. atra* MH864091.1(100%) *Alternaria* sp. MH029120.1(100%)	*A. atra(100%) (GPD)*	*A. atra*	
ISW27	Wheat grain	*Alternaria alternata*	*Alternaria* sp. KP027305.1(91.84%)	*A. alternata(100%) (GPD)*	*A. alternate*	
ISW28	Wheat bran	*Bipolaris* sp.	*Bipolaris cactivora* MW246248.1(99.30%) *Curvularia nicotiae* MT735236.1(99.12%) *C. neergaardii* KJ909784.1(99.64%)	*C. lunata (87.12%) Exserohilum protrudens(91.10%) (Actin)*	*Scytalidium* sp.	
ISW29	Wheat grain	*Alternaria alternata*	*Alternaria alternata* MG711600.1	*A.alternaria*(99%)	*A. alternate*	
ISW30	Wheat grain	*Alternaria* sp.	*A. ventricosa* MT196809.1 (100%) *A. Alternata* MF141014.1 (100%) *A.infectoria* MT883452.1 (100%)	Not amplified	*Alternaria* sp.	
ISW31	Wheat bran	*Alternaria* sp.	Fungal endophyte FJ 450014.1(93.9%) *Alternaria ventricosa* MT 196809.1(99.6%)	*Alternaria* sp. (99.60%) (GPD)	*Alternaria* sp.	
ISW32	Wheat grain	*Alternaria* sp.	*A. infectoria* MT635276.1(100%) *Lewia* sp. MN833932.1(100%)	Not amplified	Not identified	

Alternaria consortialis (Thüm) J.W. Groves & S. Hyghes, Canad. J. Bot. 31: 636. 1953. Figure 1.

Colony on PDA was dark brown to black; reverse black, reaching 6 cm in 7 days at 25°C. Conidiophores 30-75 µm long, 3.5-6 µm thick, pale golden brown. Conidia are obovoid to ellipsoidal, smooth or slightly rough, with 1-5 cross septa and 1-6 longitudinal septa.

Specimen examined: *A. consortialis* has been isolated from wheat grains, Basrah silo, 26 January 2020; wheat grains, Maysan silo, 7 January 2021; bran, Uzair mill, Maysan, 7 January 2021; bran, Sumer mill, Al-Nasiriyah; 8 February 2021. In addition, pure cultures of all strains were deposited at the Mycology lab, College of Science, University of Basrah.

Notes: *Alternaria consortialis* was isolated from soil, seeds of *Brassica* and *Coriandrum*, on paper, and from many plant substrates such as *Lycopersicon, Phaseolus*, and *Morus* in Asia, Africa, Europe, and North America under the name *Ulocladium consirtiale* (Ellis 1976). This species was considered a recurrence name and placed in the new section *Ulocladioide* Woudenb & Crous (Woudenberg et al. 2013). It is the first record for the species from wheat grains and bran in Iraq.

Alternaria japonica Yoshii, J. PI. Protect. 28:17. 1941. Figure 2.

Colony on PDA was pale gray to green-gray; reverse dark green to black reaching 6 cm in 7 days at 25°C. Conidiophores are short, solitary, 18-80 µm long, and 4-6 µm thick. Conidia are singly or in small chains of 1-2, ovate to obclavate or ellipsoid, with 2-7 transversed septa and 0-1longitudinal septa. Chlamydospores are intercalary within aerial hyphae and immersed in agar either singly or in chains.

Specimen examined: *A. japonica* has been isolated from wheat bran, Uzair Mill, Maysan, 7 January 2021. Living culture has been deposited in the Mycology lab, College of Science, University of Basrah.

Notes: *Alternaria japonica* is a type species in Section *Japonicae* created by Woudenberg & Crous, and this species is common in Brassicaceae (Woudenbery et al. 2013). It was isolated from the leaves and seeds of *Raphanus sativus* and leaves of *Brassica rapa* in Japan, as well as it was isolated from other families such as *Carya* (Juglandaceae), *Kalanchoe* (Crassulaceae), *Oryza* (Poaceae), *Sesamum* (Pedaliaceae) and *Viga* (Fabaceae) (Huang and Hanlin 1975; Farr and Rossman 2018; Nishikawa and Nakashima 2020). *A. japonica* is a worldwide fungal species, and it has been recorded in many countries in Asia, Africa, Europe, North and South America, Australia, and New Zealand (Simmons 2007; Gannibal and Gasich 2009; Ren et al. 2012; Bassimba et al. 2013; Siciliano et al. 2017; Farr and Rossman 2018). *In addition, A. japonica* has been associated with a black point of wheat in Tunisia (Bensassi et al. 2009).

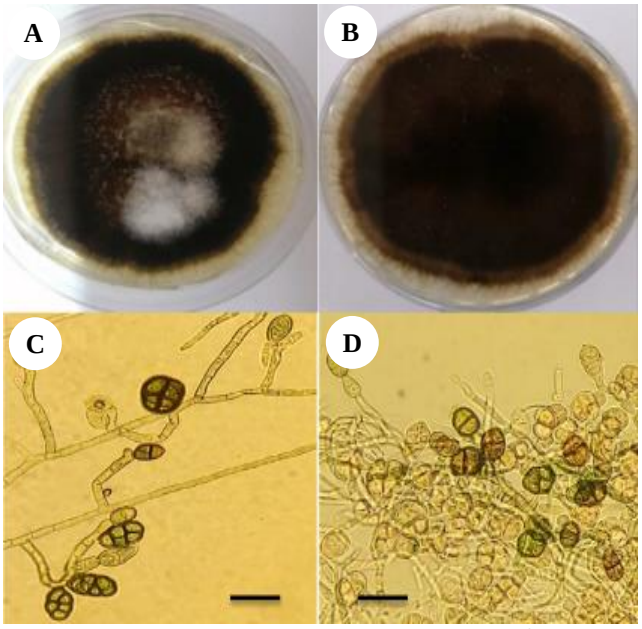

Figure 1. *Alternaria consortialis.* A. obverse view of the colony on PDA. B. Reverse view of the colony. C. Conidiophores and conidia. D. Conidia. Scale bars = 30 µm

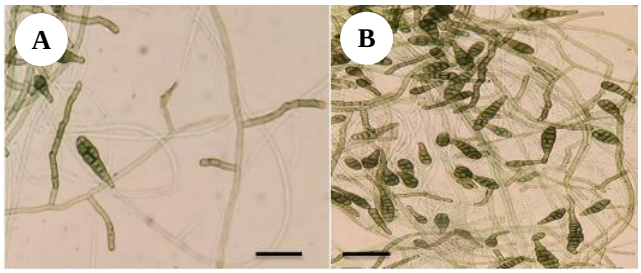

Figure 2. *Alternaria japonica.* A. Conidiophores. B. Conidia. Scale bars = 40 µm

Alternaria lolii (E.G. Simmons & C. F. Hill) Woudenb. & Crous, Studies in Mycology 75:193.2013. Figure 3.

Colony on PDA was dark brown to black; reverse dark brown, growing rapidly, reaching 8cm in 7 days at 25°C. Mycelium brown to dark brown, dense, branched. Neither conidiophores nor conidia were borne on the culture. Chlamydospores occur abundantly.

Specimen examined: The species has been recovered from flour, Sumer Mill, Al-Nasiriyah, 8 February 2021. The pure culture was deposited at the Mycology lab, College of Science, University of Basrah.

Notes: This species has been isolated from perennial ryegrass (*Lolium perenne*) in New Zealand as new species under the generic name *Embellisia* (Simmons 2004), but Woudenberg & Crous transferred this species to the genus *Alternaria* based on molecular analysis as a new combination and placed in the new section *Embellisioides* (Woudenberg et al. 2013). Remarkably, blast results for the 15w6 strain hit *Alternaria* sp. (ITS, 100% similarity), *Alternaria lolii* (ITS, 99.60% similarity), and *Alternaria lolii* (GPD, 98.11% similarity) (Table 2); however, the morphological characteristics of 15w6 strain did not match those of the GenBank strain, particularly in sporulation. Thus, we believe that this strain needs further genetic analysis.

Alternaria multiformis (E.G. Simmons) Woudenb. & Crous, Studies in Mycology 75:204. 2013. Figure 4.

Colony on PDA was yellowish-brown; reverse dark brown to black, reaching 6 cm in 7 days at 25°C. Conidiophores are undulate and geniculate, 35-50 µm long. Conidia 20-35 X 3-5 µm, ellipsoidal to oval, with 3-5 transverse septa and longitudinal septa, smooth to slightly rough, lobed.

Specimen examined: This species has been detected from wheat grains, Maysan Silo, 7 January 2021. Living culture has been deposited at the Mycology lab, College of Science, University of Basrah.

Notes: The first isolation of this species was from sandy soil in Manitoba-Canada and was under the name *Ulocladium*, but it was transferred to the genus *Alternaria* as a new combination in the Section *Ulocladioides* Woudenb. & Crous (Woudenberg et al. 2013). *A. multiformis* was isolated from some parts of *Lycium* and *Salsola* in Babylon Province, Iraq, under the synonym *Ulocladium multiformis* (Imran 2011). Finally, this species was recovered as an endophytic fungus based on the phenotypic sequences of the ITS region. It was isolated from sour cherry trees (*Prunus cerasus*) in some Iranian provinces (Aghdam and Fotouhifar 2017). It is the first isolate of the species from wheat in Iraq.

Alternaria ventricosa R.G. Roberts, Mycotaxon 100:164. 2007. Figure 5.

The colony on PDA was olive-brown to dark brown, dark gray in the center, reaching 7 cm in 7 days at 25°C. Conidiophores are simple, 32.5-55 µm long, and 3-6 µm thick. Conidia are globose, oval, or ellipsoidal, 15-42.5 X 5-17.5 µm, with 7-9 transverse septa, 1-2 longitudinal septa.

Specimen examined: The species has been isolated from wheat grains, Al-Basrah Silo, 26 Nov 2020. Living culture has been deposited at the Mycology lab, College of Science, University of Basrah.

Notes: *Alternaria ventricosa* was discovered for the first time as new species on imported Ya Li pear fruit from China (Roberts 2007). It was isolated from wheat grains stored for about six months at 14% moisture content and 5 g/ton phosphine concentration in Egypt (Al-Bedak et al. 2020).

Aspergillus montevidensis Talice & Mackinnon, C.R. Séance. Soc. Biol. Argentina: 107. 1931. Figure 6.

The colony on PDA was yellow to gray-black in the center, with a margin of light yellow; reverse pale brown. Conidiophores 125-175 X 3-6 µm. Conidia globose to subglobose, 3-5 X 2.5-3 µm. Cleistothecia yellow, globose. Ascospores 2-3 X 1.5-2.7 µm.

Specimen examined: The species has been isolated from wheat grains, Maysan Silo, 7 January 2021. Living culture has been deposited at the Mycology lab, College of Science, University of Basrah.

Notes: *A. montevidensis* is an endophytic fungus that was isolated from *Medicago sativa* grew in the hypersaline region, and it was found that in these environmental conditions, the colony morphology changed drastically, and even conidia production compared to the environments free of salts (Liu et al. 2017; Ding et al. 2019). It was isolated from honey as a xerophilic fungus (Rodrigues – Andrade et al. 2019).

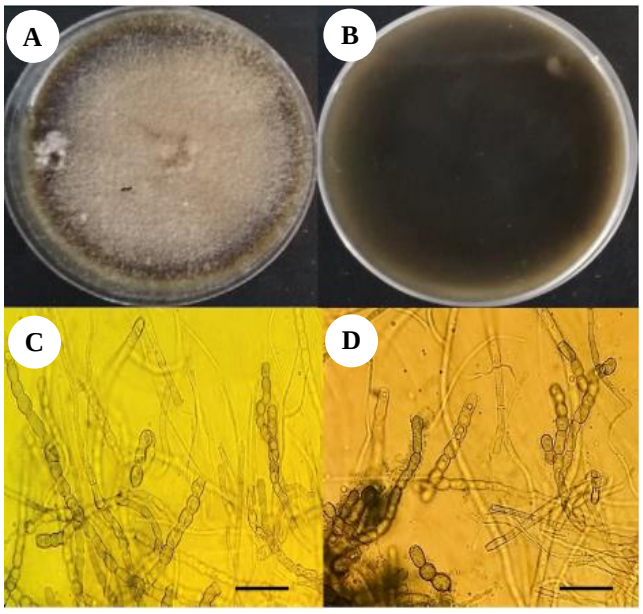

Figure 3. *Alternaria lolii.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C and D. Chlamydospores. Scale bar = 20 µm

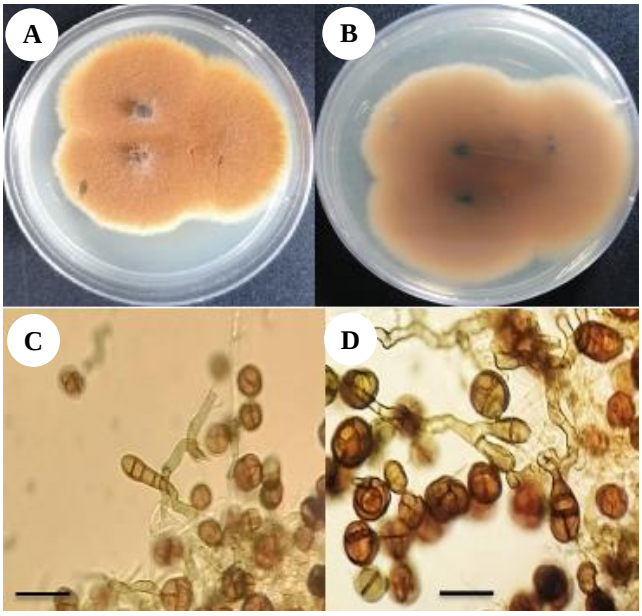

Figure 4. *Alternaria multiformis.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C and D. Conidiophores and conidia. Scale bars: C= 20 µm, D= 40 µm

Cladosporium halotolerans Zalar, de Hoog & Gunde – Cimeman, Studies in Mycology 58: 172. 2007. Figure 7.

Colony on PDA was olivaceous; reverse pale green to black, reaching 4 cm in 7 days at 25°C. Conidiophores are erect, straight, smooth to slightly roughened, 20-70 X 2.5-3 µm. Ramoconidia is rarely formed. Conidia are verruculose, brown to dark brown, aseptate, globose to subglobose, 2.5-6 X 2.5-3 µm. Secondary ramoconidia are cylindrical to globose 0-1 septate, 8-12.5 X 2.5-3 µm, with scars.

Specimen examined: The species has been detected from bran, Sumer Mill, Al-Nasiriyah, 8 February 2021. The living culture was deposited at the Mycology lab, College of Science, University of Basrah.

Notes: *Cladosporium halotolerans* was described from hypersaline water in Namibia, South Africa, and has been isolated from various environments, including indoor habitats, plant phyllosphere, the stem of *Hypericum perforatum*, peanut cell suspension culture, clinical samples, laboratory air, bathrooms, arctic ice, soil, mycorrhizal roots, and dolphin skin; around the world (Haubold et al. 1998; Meklin et al. 2004; Zalar et al. 2007; Bensch et al. 2018). Bran may be a new substrate for the species.

Cladosporium versiforme Bensch, Crous & U. Braun, Studies in Mycology 82:68, 2015. Figure 8.

Colony on PDA olivaceous brown; reverse olivaceous gray to black, reaching 7 cm in two weeks at 25°C. Conidiophores are solitary, rough, polymorphic, 75-100 X 5-6 µm. Ramoconidia 15-22.5 X 3-6 µm, septate. Conidia are variable shaped, olivaceous brown, globose to subglobose, 0-3 septate, verrucose, 7.5-12.5 X 3-6 µm.

Specimen examined: This species has been recovered from bran, Al-Mithaq Mill, Basrah, 28 Nov 2020. Living culture has been deposited at the Mycology lab, College of Science, University of Basrah.

Notes: The species was originally isolated from *Hordeum* sp. (Poaceae) in Iran and belong to the *herbarum* species complex (Bensch et al. 2015). *Cladosporium herbaroides* appear similar but differ from *Cladosporium versiforme* in having longer and wider conidiophores and narrower conidia (Bensch et al. 2012).

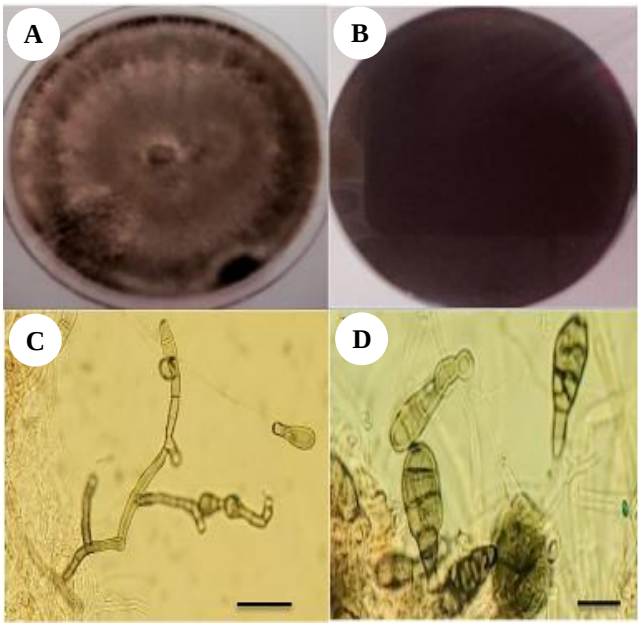

Figure 5. *Alternaria ventricosa.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C. Conidiophores. D. Conidia. Scale bars: C= 30 µm, D= 15 µm

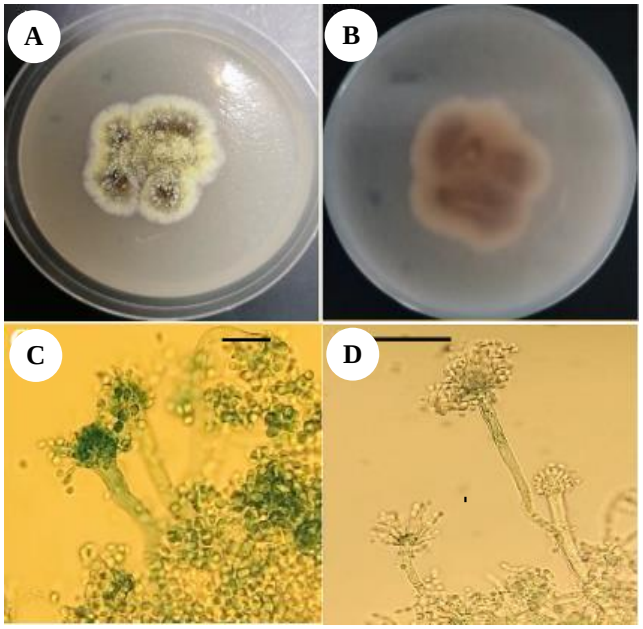

Figure 6. *Aspergillus montevidensis.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C and D. Conidiophores and conidia. Scale bars: C= 50 µm, D= 20 µm

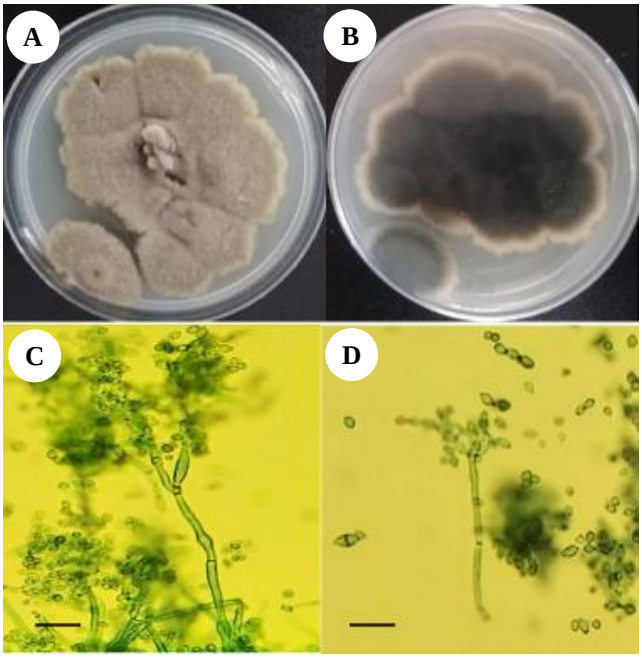

Figure 7. *Cladosporium halotolerans.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C and D. Conidiophores and conidia. Scale bars: C,D= 20 µm

Stagonosporopsis tanaceti Vaghefi, S. J. Pethybridge, Crous & P.W. J. Taylor, Australasian Plant Pathology 41:682. 2012. Figure 9.

Colony on PDA orange to pale olivaceous; reverse orange to black in the colony center, reaching 7 cm in 7 days at 25°C. Pycnidia are unilocular, solitary, or aggregate on the agar surface or immersed, globose to subglobose, glabrous, with 1-3 ostioles, 250-400 µm in diameter. Conidia are ellipsoidal to oblong, often aseptate, scarcely 1- septate, hyaline with two polar guttules, 3.7-7 X 2-3 µm. Chlamydospores are brown, globose, intercalary, chains or clusters, and non-septate.

Specimen examined: the species has been isolated from wheat grains, Al-Basrah Silo, 26 Nov 2020. Living culture has been deposited at the Mycology lab, College of Science, University of Basrah.

Notes: *Stagonosporopsis tanaceti* was reported to cause ray blight in pyrethrum (*Tanacetum cineraiifolium)* in Australia (Vaghefi et al. 2012). It was also found that this species can infect other members of Asteraceae under conditions of artificial inoculation (Pethybridge et al. 2008). It is worth noting that this pathogen was previously identified as *Phoma ligulicola* var. *inoxydabilis* but later transferred to the genus *Stagonosporopsis* as a new species under the name *S. tanaceti* on the morphological characteristics and five gene phylogeny sequences (Vaghefi et al. 2012). It is the first record of the genus *Stagonosporopsis* in Iraq, and wheat grains are perhaps a new substrate.

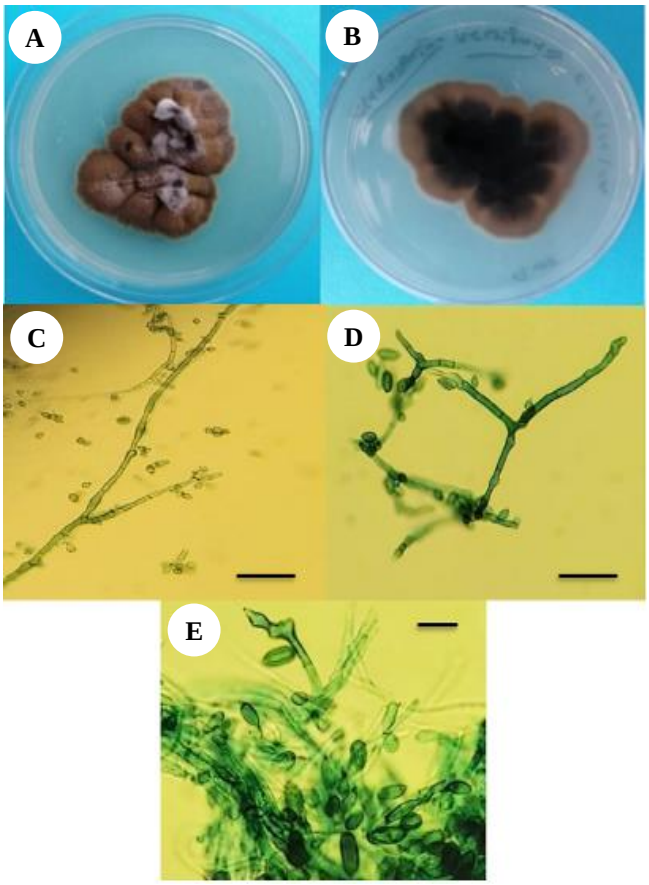

Figure 8. *Cladosporium versiforme.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C and D. Conidiophores. E. Conidia. Scale bars: C,D = 25 µm, E= 10 µm

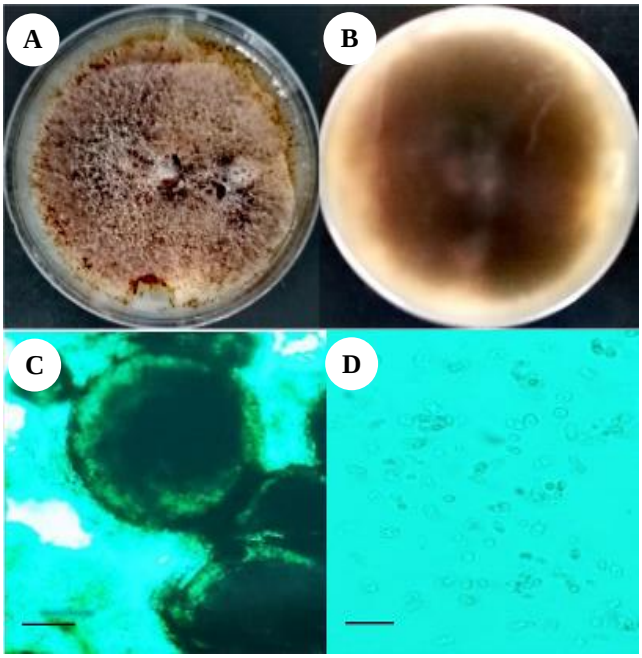

Figure 9. *Stagonosporopsis tanaceti.* A. Obverse view of the colony on PDA. B. Reverse view of the colony. C. Pycnidia. D. Conidia. Scale bars: C = 100 µm, D= 15 µm

REFERENCES

Abdullah SK, Atroshi HIM. 2014. New records of fungi on wheat grains from Iraq. J Univ Zakho 2: 256-265. DOI: 10.25271/2014.2.2.200.

Abdullah SK, Atroshi HIM. 2016. Mycobiota associated with grains of soft wheat (*Triticum aestivum* L.) cultivars grown in Duhok province, Kurdistan Region, Iraq. Intl J Agric Technol 12 (1): 91-104.

Aghdam SA, Fotouhifar KB. 2017. Introduction of some endophytic fungi of sour cherry trees (*Prunus cerasus*) in Iran. Rostaniha 18 (1): 77-94.

Al-Bedak OA, Teama EAE, Ali EA, Said MT, Shalaby EM, Moharram ZAM. 2020. Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. J Basic Appl Mycoly 11: 77-97.

Al-Defiery MEJ. 2015. Mycoflora of mold contamination in wheat flour and storage wheat flour. Mesopotamia Environ J 1 (2): 18-25.

Al-Mayah AA, Al-Edani TYM, Al-Asadi WMT. 2016. Ecology and flora of Basrah. Jeekor for Printing & Publishing Ltd., Beirut, Lebanon.

Aveskamp MM, De Gruyter J, Woudenberg JH, Verkley GJ, Crous PW. 2010. Highlights of the Didymellaceae: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65 (1): 1-60. DOI: 10.3114/sim.2010.65.01.

Bassimba DD, Mira JL, Vicent A. 2013. First report of *Alternaria japonica* causing black spot of turnip in Spain. Plant Dis 97: 1505. DOI: 10.1094/PDIS-01-13-0090-PDN.

Belkacem-Hanfi N, Semmar N, Perraud-Gaime I, Guesmi A, Cherni M, Cherif I, Boudabous A, Roussos S. 2013. Spatio-temporal analysis of post-harvest moulds genera distribution on stored durum wheat cultivated in Tunisia. J Stored Prod Res 55: 116-123. DOI: 10.1016/j.jspr.2013.08.008.

Bensassi F, Zid M, Rhouma A, Bacha H, Hajlaoui MR. 2009. First report of *Alternaria* species associated with black point of wheat in Tunisia. Ann Microbiol 59: 465-467. DOI: 10.1007/BF03175132.

Bensch K, Groenewald JZ, Meijer M, Dijksterhuis J, Jurjević Ž, Andersen B, Houbraken J, Crous PW, Samson RA. 2018. *Cladosporium* species in indoor environments. Stud Mycol 89: 177-301. DOI: 10.1016/j.simyco.2018.03.002.

Bensch K, Braun U, Groenewald JZ, Crous PW. 2012. The genus *Cladosporium*. Stud Mycol 72: 1- 401. DOI: 10.3114/sim0003.

Bensch K, Groenewald JZ, Braun U, Dijksterhuis J, de Jesús Yáñez-Morales M, Crous PW. 2015. Common but different: The expanding realm of *Cladosporium*. Stud Mycol 82: 23-74. DOI: 10.1016/j.simyco.2015.10.001.

Berbee ML, Pirseyedi M, Hubbard S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91 (6): 964-977. DOI: 10.1080/00275514.1999.12061106.

DEAT. 2019. Guide to Agricultural Operations in Iraq. Department of Extension and Agricultural Training, Ministry of Agriculture. Baghdad, Iraq.

Ding X, Liu K, Lu, Gong G. 2019. Morphological, transcriptional, and metabolic analyses of osmotic-adapted mechanisms of the halophilic *Aspergillus motevidensis* ZYDH under hypersaline conditions. Appl Microbiol Biotechnol 103: 3829-3846. DOI: 10.1007/s00253-019-09705-2.

Ellis MB. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Ellis MB. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Ewaid SH, Abed SA, Al-Ansari N. 2020. Assessment of main cereal crop trade impacts on water and land security in Iraq. Agronomy 10 (1): 98. DOI: 10.3390/agronomy10010098.

Farr DF, Rossman AY. 2018. Fungal databases, U.S. National Fungus Collections, ARS, USDA.

Food and Agriculture Organization of United Nations (FAO). 2021. Agricultural value chain study in Iraq-Dates, grapes, tomatoes and wheat. Baghdad, Iraq.

Gannibal PB, Gasich EL. 2009. Causal agents of the alternariosis of cruciferous plants in Russia. Species composition, geography and ecology. Mikologiya i Fitopatologiya 43: 447- 456. [Russian]

Ghadban AK, Majeed KR, Al-Moussawi FMS. 2017. Isolation and identification of fungi produced aflatoxin B1 from Iraqi wheat for the agricultural season 2015. Global J Res Anal 6 (8): 58-62.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. Appl Environ Microbiol 61 (4): 1323-1330. DOI: 10.1128/aem.61.4.1323-1330.1995.

Gregori R, Meriggi P, Pietri A, Formenti S, Baccarini G, Battilani P. 2013. Dynamics of fungal and related mycotoxins during cereal storage in silo bags. Food Control 30: 280-287. DOI: 10.1016/j.foodcont.2012.06.033.

Guarro J, Gené J, Stchigel AM, Figueras MJ. 2012. Atlas of soil ascomycetes. CBS Biodiversity, series 10, Utrecht, Netherland.

Haubold EM, Aronson JF, Cowan DF, McGinnis MR, Cooper CR. 1998) Isolation of fungal rDNA from bottlenose dolphin skin infected with *Loboa loboi*. Med Mycol 36: 263-267. DOI: 10.1046/j.1365-280X.1998.00148.x.

Hong SB, Cho HS, Shin HD, Frisvad JC, Samson RA (2006). Novel *Neosartorya* species isolated from soil in Korea. Int J Syst Evol Microbiol 56: 477-486. DOI: 10.1099/ijs.0.63980-0.

Huang LH, Hanlin RT. 1975. Fungi occurring in freshly harvested and in-market pecans. Mycologia 67: 689-700. DOI: 10.1080/00275514.1975.12019798.

Hussein LF, Saadullah AM. 2018. Mycoflora and incidence of aflatoxin in wheat seeds from Duhok province, Kurdistan Region of Iraq. Sci J Univers Zakho 6 (3): 78-81. DOI: 10.25271/sjuoz.2018.6.3.505.

Imran ZK. 2011. Isolation and identification of *Ulocladium* Preuss from different regions in Iraq. Basrah J Agric Sci 24 (2): 27- 47. DOI: 10.33762/bagrs.2011.57206.

Iraqi CSO. 2019. Iraqi Central Statistical Organization Yearly Statistics, Ministry of Planning, Baghdad.

Juber KS, Al-Salahi GM. 2006. Detection of fungi associated with wheat grains imported to Iraq. Iraq J Agric Sci 37:143-148.

Katileviciute A, Plakys G, Budreviciute A, Onder K, Damiatis S, Kodzius R. 2019. A sight to wheat bran: High value-added products. Biomolecules 9 (12): 887-903. DOI: 10.3390/biom9120887.

Klich MA. 2002. Identification of common *Aspergillus* species. CBS, Utrecht, The Netherlands.

Lacey J, Magan N. 1991. Fungi colonizing cereal grain: Their occurrence and water and temperature relationships. In: Chelkowski J (eds) cereal Grain- Mycotoxins, Fungi and Quality in Storage. Elsevier, Amsterdam.

Liu KH, Ding XW, Narsing Rao MP, Zhang B, Zhang YG, Liu FH, Liu BB, Xiao M, Li WJ. 2017. Morphological and transcriptomic analysis reveals the osmoadaptive response of endophytic fungus *Aspergillus*

montevidensis ZYDH to high salt stress. Front Microbiol 8: 1789. DOI: 10.3389/fmicb.2017.01789.

Magan N, Hope R, Cairns V, Aldred D. 2003. Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. Eur J Plant Pathol 109: 723-730. DOI: 10.1007/978-94-017-1452-5_7.

Magan N, Lacey J. 1986. The phylloplane microbial population of wheat and effect of late fungicide applications. Ann Appl Biol 109:117-128. DOI: 10.1111/j.1744-7348.1986.tb03190.x.

Meklin T, Haugland RA, Reponen T, Varma M, Lummus Z, Bernstein D, Wymer LJ, Vesper SJ. 2004. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. J Environ Monit 6: 615-620. DOI: 10.1039/b400250d.

Mohammed-Ameen MK, Minati MH, Abass MH. 2021. Morphogenetic identification, description and pathogenicity of novel pathogens on Iraqi Wheat Plant (*Triticum aestivum*) causing head blight and crown rot diseases. Biodiversitas 22: 2999-3005. DOI: 10.13057/biodiv/d220565.

Moral J, Morgan D, Trapero A, Michailides TJ. 2019. Ecology and epidemiology of diseases of nut crops and olives caused by Botryosphaeriaceae fungi in California and Spain. Plant Dis 103 (8): 1809-1827. DOI: 10.1094/PDIS-03-19-0622-FE.

Nishikawa J, Nakashima C. 2020. Japanese species of *Alternaria* and other species boundaries on host range. Fungal Syst Evol 5: 197-281. DOI: 10.3114/fuse.2020.05.13.

Pethybridge SJ, Hay FS, Clarkson RA, Groom T, Wilson CR. 2008. Host range of Australian *Phoma ligulicola* var. *inoxydablis* isolates from pyrethrum. J Phytopathol 156: 506-508. DOI: 10.1111/j.1439-0434.2008.01401.x.

Pitt JI, Hocking AD. 2009. Fungi and food spoilage, 3rd ed. Springer Science + Business Media, LLC. DOI: 10.1007/978-0-387-92207-2_2.

Ren XX, Zhang GZ, Dai WA. 2012. First report of damping-off caused by *Alternaria japonica* on Chinese cabbage seedlings in China. Plant Dis 96: 1378. DOI: 10.1094/PDIS-04-12-0328-PDN.

Riba A, Mokrane S, Mathiew F, Lebrihi A, Sabaou N. 2008) . Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. Intl J Food Microb 122: 85-92. DOI: 10.1016/j.ijfoodmicro.2007.11.057.

Roberts RG. 2007.Two new species of *Alternaria* from pear fruit. Mycotaxon 100: 159-167.

Rodriguez - Andrade E, Stchigel AM, Terrab A, Guarro J, Cano-Lira JF. 2019. Diversity of xerotolerant and xerophilic fungi in honey. IMA Fungus 10 (1): 20-50. DOI: 10.1186/s43008-019-0021-7.

Saido KA, Mohammed HI, Haleem RA. 2020. Isolation and identification of fungi associated with different species of stored grains. J Univ Duhok 23 (2): 259-266. DOI: 10.26682/ajuod.2020.23.2.29.

Saleemi MK, Khan MZ, Khan A, Hameed MR, Khatoon A, Abadin ul Z, Ul Hassan Z. 2016. Study of fungi and their toxigenic potential isolated from wheat and wheat bran. Toxin Rev 36 (1): 1-9. DOI: 10.1080/15569543.2016.1233890.

Samson RA, Hoekstra ES, Frisvad JC. 2004. Introduction to food and airborne fungi. 7th edition. Centraalbureau voor Schimmelcultures, Utrecht.

Shewry PR. 2009. Wheat. J Exp Bot 60: 1537-1553. DOI: 10.1093/jxb/erp058.

Shiju M. 2010. A review on the effect of fungi on the wheat grain under post harvest storage ecology. Food Environ Safety-J Fac Food Eng 9 (2): 87-96.

Siciliano I, Gilardi G, Ortu G, Gisi U, Gullino ML, Garibaldi A. 2017. Identification and characterization of *Alternaria* species causing leaf spot on cabbage, cauliflower, wild and cultivated rocket by using molecular and morphological features and mycotoxin production. Eur J Plant Pathol 149: 67-92. DOI: 10.1007/s10658-017-1190-0.

Simmons EG. 2004. Novel dematiaceous hyphomycetes. Stud Mycol 50: 109-118.

Simmons EG. 2007. *Alternaria*: An identification manual. CBS Biodiversity Series 6. Centraalbureau voor Schimmelcultures, Utrecht.

Soppe R, Saleh R. 2012. R. Report B2,1 Historical Agricultural Production Data in Iraq. Iraq Salinity Project. The International Center for Agricultural Research in the Dry Areas (ICARDA), Beirut, Lebanon.

Sulaiman ED, Husain SS. 1985. Survey of fungi associated with stored food grains in silos in Iraq. Pak J Sci Ind Res 28: 33-36.

Thalij KM, Sumait SA, Ahmed KN, Badawy AS. 2021. Determination of fungi and aflatoxin contaminating wheat stored in silos of Salah Al-den Governorate –Iraq. Ann Romanian Soc Cell Biol 25 (6): 11715-11724.

United Nations Educational, Scientific and Cultural Organization (UNESCO). 2019. Assessment of the Labour Market and Skills Analysis. Iraq and Kurdistan Region – Iraq, UNESCO, Geneva.

Vaghefi N, Pethybridge SJ, Ford R, Nicolas ME, Crous PW, Taylor PWJ. 2012. *Stagonosporopsis* spp. associated with ray blight disease of Asteraceae. Australas Plant Pathol 41: 675-686. DOI: 10.1007/s13313-012-0161-3.

Wareing PW. 1997. Incidence and detection of thermotolerant and thermophilic fungi from maize with particular reference to *Thermoascus* species. Intl J Food Microbiol 35: 137-145. DOI: 10.1016/S0168-1605(96)01212-3

Watanabe T. 2002. Pictorial atlas of soil and seed fungi. CRC Press, London, UK. DOI: 10.1201/9781420040821.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Snisky J, White T. (eds.). PCR protocols: A guide to methods and applications. New York. DOI: 10.1016/B978-0-12-372180-8.50042-1.

Woudenberg JHC, Groenewald JZ, Binder M, Crous PW. 2013. *Alternaria* redefined. Stud Mycol 75: 171-212. DOI: 10.3114/sim0015.

Zalar PD, De Hoog GS, Schroers HJ, Crous PW, Groenewald JZ, Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Stud Mycol 58: 157-183. DOI: 10.3114/sim.2007.58.06.