
Mycobiota associated with grains of soft wheat (*Triticum aestivum* L.) cultivars grown in Duhok Province, Kurdistan Region, Iraq

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The study dealt with the survey and identification of fungi associated with grains of several cultivars of soft wheat (*Triticum aestivum* L.) growing in Iraq by using agar plate, blotter paper and deep freezing methods. A total of 21 species representing 11 fungal genera were recovered and identified. Among the common genera were *Penicillium* (4 species), *Alternaria* and *Aspergillus* (3 species each), *Chaetomium*, *Cladosporium* and *Ulocladium* (2 species each). The genera *Arthrinium*, *Bipolaris*, *Coniocessia*, *Curvularia*, *Emericella* were represented by a single species. Nineteen species were recorded using the agar plate method, 11 species were also isolated by the deep freezing method and 8 species were isolated by blotter paper method. Cultivars Azadi (sample 6), IPA99 (sample 1) and unknown cultivar (Shekhan) (sample 11) and showed the highest mean percentage contamination (42.0%, 39.0% and 35.0%) respectively. The study revealed that there is a variation in the fungal composition and the total number of detected species as well as in their percentage of occurrence and percentage of contamination according to cultivar type and detection method. *Coniocessia annandra* was reported in one occasion and represented a new record for Iraq. A brief description along with photographs is provided for the species.

Key words: Mycobiota, *Coniocessia*, soft wheat grains, Iraq

Introduction

The cultivation of wheat (*Triticum* sp.) reaches far back into history. The most archaeological records show that agriculture began around 10,000 B.C. where Iraqi people settled into villages near the Tigris and in the regions known as the Fertile Crescent (Lev-Yadunet *al.*, 2000; Anonymous, 2007).

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Wheat was one of the first domesticated food crops and for 8000 years has been the basic staple food of the major civilization of the world (Tanno and Wilcox, 2006). Its production leads all crops, including rice, maize and potatoes and is grown in almost all the temperate and subtropical regions of the world (Agrawal and Sinclair, 1996).

The actual planted areas with wheat in Kurdistan region of Iraq are 1.321.250 donum, yielded 325.938 metric tons in 2012 (USDA,2012). The total cultivated areas of wheat in Iraq are 6.159 million donum and the total production is 2.180 million metric tons. However, Iraqi wheat consumption is currently estimated at 5 million tons a year (USDA, 2012).

Wheat plants are largely susceptible to various pests and diseases (Prescott *et al.*2006).Seeds from the time of their inception at flowering of the parent plants until they germinate and develop into seedlings, are prone to attack by various microbial agents (Harman,1983).

Fungi associated with seeds prove to be hazardous for the seed itself, or the new plant created from it. The associated fungi may be pathogenic, weak parasites or saprophytes.The study of seed-borne fungi is very important to determine the health of grains and to protect them from seed-borne pathogens (Health Grain Crops Association, HGCA, 2012).

Wheat is affected by a range of fungal seed-borne pathogens including wheat bunt (*Tilletia* spp.), Septoria seedling blight (*Phaeospheria nodorum*),*Fusarium* seedling blight (*Microdochium nivale*), loose smut (*Ustilago tritici*), foliar diseases such as leaf blotch (*Septoria tritici*,Teleomorph: *Mycosphaerella graminicola*), yellow rust (*Puccinia striiformis*), root rots (*Fusarium culmorum*, *F.graminearum*) and Fusarium root crown and foot rots (*Fusarium* spp.), black stem rust (*Puccinia graminis*f.sp.*tritici*),Fusarium head blight (*Fusarium* spp.) and root rot and black point of wheat caused by *Bipolaris sorokiniana*,teleomorph:*Cochliobolus sativus*(Richardson,1983;Wiese, 1998; Acharya *et al.*,2011; HGCA,2012).

In Iraq, however, information on wheat seed-borne mycoflora is fragmentary. Among these few studies, Sulaiman and Husain (1985) carried out a survey on fungi associated with stored food grain in silos in Iraq, Juber and Al-Salahi (2006) worked on the fungi associated with wheat grains imported to Iraq. More concern was focused on wheat smut diseases caused by *Tilletias* pp, their dissemination and control (Al-Maarroof *et al.*, 2005; 2006; Al-Taae and Al-Ameery, 2010 a, b; Hassan, 2006; Hassan and Shams-Allah (2010); Hassan *et al.*2010b; Rust disease caused by *Puccinia recondita* was also investigated (Hassan *et al.*2010a). *Fusarium* species (*F.graminearum* and *F.pseudograminearum*) causing crown rot disease was also reported by (Khalifah and Matny, 2013). Root rot disease in wheat caused by

Helminthosporium sativum (current name: *Bipolaris sorokiniana*) and its control was also investigated by Sarhan (2013). Recently, Abdullah and Atroshi (2014) reported for the first time 8 fungal species on durum and soft wheat grains from Iraq.

Objectives: The objective of this study was to identify the fungi associated with grains of softwheat cultivars samples from Duhok province Kurdistan Region of Iraq using different detecting methods and to assess the percentage of seed contamination with fungi in each cultivar.

Materials and methods

Samples collection

A total of 14 seed samples of wheat were obtained during the year 2013-2014 from different sources in Duhok governorate as shown in Table 1.

Isolation of fungi

The mycoflora associated with seed samples were determined using three different detection standard methods (agar plate, blotter paper and deep freezing techniques) according to International Rules of seed Testing (ISTA 2009). A total of 200 seeds in four replicates were tested from each sample.

Agar plate method: Two isolation media were used [Potato dextrose agar (PDA) (Himedia laboratories, India) and oat meal agar (OTA) (30 g oat flakes, 15 g agar and 1L distilled water)]. All types of media were amended with 250 mg/L chloramphenicol.

Surface disinfected grains with 1% Sodium hypochlorite solution for 2 minutes and then washed with distilled sterilized water were aseptically placed on each of PDA and OTA media, 10 grains for Petri dish. The plated grains were incubated for 5-7 days at 25 °C under 12 h alternating cycles of light (white cool fluorescent light) and darkness. Colonies growing out from the grains on each medium were isolated to newly fresh appropriate media for identification.

Standard paper blotter method: Surface disinfected grains with 1% Sodium hypochlorite solution for 2 minutes and then washed with distilled sterilized water were aseptically placed on moistened three layers of blotter papers in 9 cm sterilized Petri dishes. 10 seeds were placed in each Petri dish and the dishes were incubated for 7-10 days at 25 °C under 12 h alternating cycle of fluorescent light and darkness. The plates were moistened with sterilized distilled water when it is necessary. Grains were examined individually under a

dissecting microscope. The fungi growing from the grains were either identified directly from the plates or were sub cultured onto other appropriate media used for identification.

Deep freezing method: The procedure is similar to paper blotter method except that firstly, disinfected grains in plates were incubated for 24h at 25 °C and then transferred to -20 °C in a freezer for 8 h and finally was incubated for 7-10 days at 25 °C with (regime 12 h of darkness and 12 h of cool white fluorescence light). Grains were examined individually under a dissecting microscope. The fungi growing from the grains were either identified directly from the plates or were sub cultured onto other appropriate media used for identification.

Identification of fungi: Culture media used for identification of fungi included Czapeck's agar (CZ), malt extract agar (MEA), oat meal agar (OTA) and potato dextrose agar (PDA). The media were prepared according to Pitt and Hocking (1977). The isolated fungi were identified according to keys and descriptions provided by Ellis (1971, 1976); Klich (2002); Watanabe (2002); Prescott, 2006; Samson *et al.*, (2010); Asgari and Zari (2011); Guarro *et al.* (2012).

Data analysis

The percentage frequency of occurrence (FO) of fungi in the seeds of each cultivar and percentage contamination (PC) was calculated.

$$FO\% = \frac{\text{Number of grains on which a fungal species was identified}}{\text{Total number of tested grains}} \times 100$$

$$PC\% = \frac{\text{Number of infected grains from each cultivar}}{\text{Total number of grains in each cultivar}} \times 100$$

A factorial experiment (14×4) was conducted by completely randomized design. The data has been analyzed by using computer through the SAS program (2000), and the means comparison was done by Duncan's Multiple Ranges Test under 5% which was claimed by (SAS, 2000).

Table 1. List of wheat cultivars and their sources

Sample	Cultivar	Source
1	IPA 99	Directorate of agricultural research, Duhok
2	IPA 99	Department of field crops, Duhok University
3	AbuGraib	Department of field crops, Duhok University
4	AbuGraib	Directorate of agricultural research, Duhok
5	Azadi	Department of field crops, Duhok University
6	Azadi	Directorate of agricultural research, Duhok
7	Rezgary	Department of field crops, Duhok University
8	Rezgary	Directorate of agricultural research, Duhok
9	Tamouz 2	Department of field crops, Duhok University
10	Tamouz 2	Directorate of agricultural research, Duhok
11	Unidentified cultivar	Shekhan silo
12	Unidentified cultivar	Shekhan silo
13	Unidentified cultivar	Shekhan silo
14	Unidentified cultivar	Zakho silo

Results and Discussion

Contamination of wheat grains by fungi

The results of contamination percentage of soft wheat cultivars by fungi as detected by different isolation methods are presented in Table 2. The degree of fungal contamination ranged from 2.0 to 40.0% as detected in blotter method, from 0.0 to 54.0% by deep freezing, from 10.0 to 46.0% on PDA and from 0.0 to 74.0% on Oat Meal Agar. The mean values of the contamination percentages were significantly different according to detection method (Table 2). Cultivars Azadi (sample 6)), IPA99 (sample 1) and unknown cultivar (shekhan silo)(sample 11) showed the highest mean percentage contamination by fungi (42.0%, 39.0% and 35.0%) respectively as detected by the four detection methods. The mean value of contamination percentages of each cultivar was significantly different by using different isolation method (Table 2). Abu Ghraib (sample 4) showed the lowest mean percentage contamination by fungi (4.0%) followed by Azadi (sample 5) (9.0%) and unknown cultivar, Zakho silos (14) (16.0%).

The degree of percentage of fungal contamination varied among wheat cultivars. Such variations may be attributed to the differences in geographical locality of cultivations, storage conditions or to differences in physico-chemical nature of different wheat genotypes. Moreover, such variation may be due to

isolation techniques used in the present study. This is in line with results obtained by Christensen (1955), Habib *et al.*, (2011) and Singh *et al.* (2011).

Table 2 .Contamination Percentage of soft wheat grains of different cultivars by fungi as detected by different isolation methods.

Cultivar	%Contamination by fungi				
	Blotter method	Deep freezing	PDA	Oatmeal agar	Mean
1-IPA 99	16.0 b*	54.0 a	38.0 a	50.0 ac	39 ab
2- IPA 99	8.0 b	8.0 ce	38.0 a	48.0 ac	25 ae
3-Abu-gharaib	2.0 b	24.0 bc	46.0 a	40.0 ac	28 ad
4-Abu gharaib	4.0 b	0.0 e	10.0 a	2.0 c	4.0 f
5-Azadi	8.0 b	4.0 de	24.0 a	2.0 c	9.0 ef
6-Azadi	40.0 a	10.0 ce	46.0 a	74.0 a	42.0 a
7- Rezgary	14.0 b	18.0 cd	22.0 a	62.0 ab	29.0 ad
8-Rezgary	12.0 b	38.0 ab	24.0 a	12.0 bc	21.5 bf
9-Tamouz 2	8.0 b	16.0 ce	32.0 a	34.0 ac	22.5 be
10-Tamouz 2	8.0 b	24.0 bc	38.0 a	0.0 c	17.5 cf
11-Unknown(shekhan silo)	12.0 b	40.0 ab	26.0 a	62.0 ab	35.0 ac
12-Unknown(shekhan silo)	2.0 b	24.0 bc	14.0 a	40.0 ac	20.0 cf
13-Unknown(shekhan silo)	4.0 b	22.0 bc	36.0 a	26.0 ac	22.0 be
14-Unknown(Zakho silo)	8.0 b	24.0 bc	12.0 a	20.0 bc	16.0 df
Mean	10.3 c	21.9 b	29.0 ab	33.7 a	—

*Means followed by different letters in the same column are significantly different based on Duncan's Multiple Range Test (P=0.05).

Frequency of occurrence of fungal species on the soft wheat cultivars

The most common species on soft wheat grains were *A.alternata*, *A.flavus*, *C.herbarum*, *Penicillium* spp, *A.niger*, *A.teunissima* and *U.atrum* (Table 3).

Prevalence of these species was in agreement with studies from Pakistan (Rajput *et al.* 2005, Habib *et al.* 2011, Hussain *et al.* 2013), from Iran (Seberi *et al.* , 2004; Gohari *et al.* 2007), from Syria (Alkadri *et al.* ,2013) and from Argentina (Broggi *et al.* 2005). *Alternaria alternata*, *A.flavus*, *C.herbarium* and *Penicillium spp.*, showed the highest percentage frequency of occurrence with a frequency of occurrence 0.5-10%, 0.5-4.0%, 1-4.0% and 0.5-3.5% respectively (Table 3). Juber and Al-salahi (2006) detected *A.alternata*, *A.flavus* and *Penicillium spp.* as the most commonly encountered species from wheat grains imported to Iraq.

Among all the three species of *Alternaria*, *A.alternata* and *A.tenuissima* were the most common. *A.chlamydozoora* was isolated in one occasion. The former two species were reported among the most prevalent wheat seed-borne fungi in Pakistan (Rehman *et al.* 2011). Species in the genus *Alternaria* are including plant pathogenic and saprophytic fungi (Logrieco *et al.*, 1990) and are common field fungi infesting cereal grains (Ilhan and Asan, 2001). Rehman *et al.* , (2010) demonstrated that the growth of *A. alternata* on stored wheat grains had resulted in low nutritional contents due to the decreasing in the carbohydrates, fats and ash contents as compared to freshly harvested grains. *A.alternata* is known as one of the causative agent of black point disease in wheat (Dey *et al.* 1992), and causing 82% reduction in germination (Mahmuda *et al.*, 1987).

Among the species of *Aspergillus*, *A.flavus* and *A.niger* were the predominant on soft wheat cultivars with a frequency of occurrence 0.5-4.0% and 0.5-3.0% respectively (Table 2). Husain *et al.* (2013) reported high incidence of *A.flavus* and *A.niger* on commercial wheat cultivars in Punjab, Pakistan, while Rajput *et al.* (2005) reported *A.niger* among the predominant fungi on wheat grains collected from Sind province of Pakistan. In central Iran, Hajihassani *et al.* (2012) reported high incidence of *A.niger* on wheat grains from irrigated wheat fields in Markazi province. *A.flavus* and *A.niger* have been shown responsible for poor seed germination of wheat (Sulaiman and Hussein, 1984; Ijaz *et al.* 2001).

Arthrinium phaeospermum was detected in four cultivars of soft wheat with a percentage frequency of 0.5-2.5% (Table 3). The species was recently reported on wheat grain in Iraq (Abdullah and Atroshi, 2014). However, the species was frequently isolated from wheat grains from Scotland (Flannigan, 1970), from Ontario, Canada (Clear and Patrick, 1993), and from Argentina (Broggi *et al.* 2007). A species identified as *A.sacchari* was reported as the causal pathogen of damping-off of durum wheat in Canada (Mavragani *et al.* 2007).

Table 3. Percentage frequency of occurrence of fungi on soft wheat (*Triticum aestivum*) grains and their sources.

Fungal species	% infected grains of the particular cultivar.													
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Alternaria alternata</i>	7.5	3.0	1.5		1.0	10	1.0	3.0	1.0	1.0	1.0		0.5	
<i>Alternaria chlamydospra</i>							1.0							
<i>Alternaria tenuissima</i>	1.0					1.0				0.5	1.0			
<i>Arthrinium phaeospermum</i>			2.5			0.5					2.5			0.5
<i>Aspergillus flavus</i>		1.5	0.5	3.0	0.5		2.5	2.5	3.0			4.0	3.5	
<i>Aspergillus niger</i>	0.5				1.5		2.5	1.0	3.0	0.5	2.5	3.0		3.0
<i>Aspergillus terreus</i>												2.5		
<i>Bipolaris sorokiniana</i>							2.0							
<i>Chaetomium elatum</i>							2.0	1.0						
<i>Chaetomium globosum</i>								1.0						
<i>Cladosporium herbarum</i>	4.5	4.0				1.5	3.5	2.5		3.5	3.5	2.5	1.5	1.0
<i>Cladosporium sp.</i>									4.5					0.5
<i>Coniocessia anandra</i>								1.0						
<i>Curvularia sp.</i>		0.5						1.0						
<i>Emericella rugulosa</i>									1.0					
<i>Penicillium citrinum</i>									2.5					
<i>Penicillium chrysogenum</i>									2.5					
<i>Penicillium brevicompactum</i>									2.5					
<i>Penicillium spp.</i>	1.0	2.0	1.5			2.5	0.5	1.5	2.5	1.0	3.5	2.0	2.5	1.0
<i>Ulocladium atrum</i>			1.5				1.0				1.0	0.5		
<i>Ulocladium alternariae</i>			1.0											

* 1, 2 –Ipa ; 3,4 Abu Ghraib; 5,6-Azadi; 7,8-Rezgary; 9,10-Tamouz2; 11-14 unknown cultivars. *based on 200 grain of each cultivar.

Bipolaris sorokiniana was detected on Razgary cv. and with a percentage frequency of occurrence of 2.0%. *B.sorokinana* has been reported in several surveys on seed-borne fungi of wheat from different regions of the world (Bhati and Bhutta, 2002; Rehman *et al.* 2011; Hajihassani *et al.* 2012; Hussain *et al.* 2013). The fungus is known as causal pathogen for various diseases like head blight, seedling blight, root rot and black point of wheat (Wiese, 1998; Acharya *et al.* 2011). The species was reported as causal agent of root rot of winter wheat plants grown in middle of Iraq (Sarhan, 2013).

Two species of *Chaetomium* were identified viz. *C.elatum* and *C. globosum*. The former species is detected from 2 cultivars and with frequency of occurrence ranging 1.0-2.0%. *C. globosum* was detected from single cultivar with frequency of occurrence (1.0).

C. globosum was reported earlier by Juber and AL- Salahy (2006) on commercial wheat grains imported to Iraq, whereas, *C.elatum* was detected recently on soft and durum wheat grains in Iraq (Abdullah and Atroshi, 2014). *C.globosum* is very common on stored wheat grains and was reported in several surveys carried out in different regions of the world (Flannigan, 1970, Broggi *et al.* 2007, Habib *et. al.* 2011, Hussain *et.al.* 2013).

Coniocessia annandra was reported from a single cultivar with a percentage frequency of occurrence (1.0). This is the first record for species in Iraq. The species was recently described by Asgari and Zare (2011) from wheat seeds in west Azzebrbijan, Iran. To our best of knowledge, this is also the first report for the species outside its type locality.

The cleistothecial ascomycete *Emericella rugulosa* was detected from a single cultivar. The species was reported recently from wheat grains in Iraq (Abdullah and Atroshi, 2014).

Three species of *Penicillium* have been identified viz. *P. brevicompactum*, *P. citrinum* and *P. chrysogenum*. *Penicillium* spp., are commonly detected from wheat grains (Broggi *et.al.* 2007; Habib *et.al.* 2011; Singh *et.al.* 2011).

Two species of *Ulocladium* viz. *U. atrum* and *U. alternaria* were detected. The first species was more common and isolated from grains of 4 cultivars with a percentage of occurrence between 0.5 – 1.5 % . *Ulocladium alternariae* was isolated on one occasion with a percentage occurrence of 1.0 % . *U. Atrum* was previously reported from Iraq by Juber and AL- Salahy (2006) on wheat grains imported to Iraq. *U. alternariae* was reported on wheat grains in Kerman province, Iran (Ghohari *et.al.* 2007).

Brief description of the newly recorded species

Coniocessia anandra Asgari & Zare Mycol. Progress, 10:205(2011). Figure 1 (A, B).

Colonies on oat meal agar (OT) are olivaceous brown with scanty aerial mycelium reaching 40 mm diam in 2 weeks at 25°C. Ascospores are produced abundantly, after 12 days, superficial, solitary, pyriform to ovoid 100-130×140-160µm size, with distinct broad ostiole, 40-60 µm wide, surrounded with hypha-like ostiolar projections, and 10-12 × 2.5-3µm. Peridium is semi-translucent, pseudoparenchymatous consisting of several layers, thin-walled, irregularly-shaped. Asci are 4-spored, cylindrical, with short stipitate, 50-65×13-15µm without an apical ring. Ascospores are one celled, at first hyaline, then becoming dark brown at maturity, globose, smooth-walled with a longitudinal germ slit, inequilaterally flattened, 18-21×12-14µm. Anamorph is not seen.

Specimen examined

Isolated from soft wheat grain (*Triticum aestivum* L.), Rizgary cultivar, on Oat Meal Agar (OTA). Dried and living cultures have been deposited at mycology bank, Department of plant protection, Faculty of Agriculture, University of Duhok., S.K. Abdullah and H.I. Atroshi, Feb. 2014.

This is the first report for the genus *Coniocessia* and the species *C. anandra* from Iraq, and perhaps, the second report for the species in the world outside its type locality (Iran).

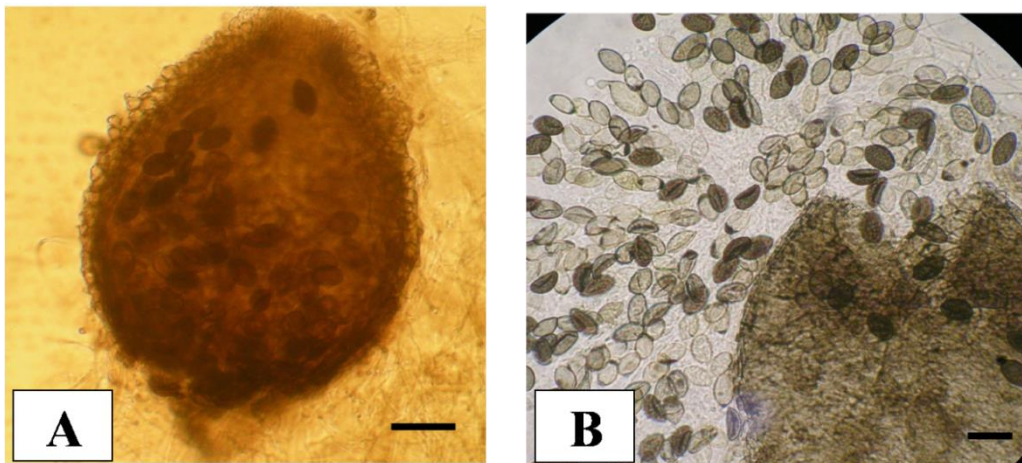


Figure 1. *Coniocessia anandra*. A: Ascogonium. Bar = 50 µm. B: Asci and ascospores. Bar = 10 µm.

The genus *Coniooessia* D. Garcia, Stchigel, D. Hawksw. Guarro was introduced by Garcia *et al* (2006), segregated from *Coniochaeta* (Sacc.) Cooke to accommodate a single species *Coniooessia nodulisporioides* (D. Hawksw.) D. Garcia, Stchigel, D. Hawksw. & Guarro (formerly *Coniochaeta nodulisporioides* D. Hawksw.) mainly on molecular basis. More recently, Asgari and Zare (2011) described four additional new species of *Coniooessia* from Iran, including *C. anandra* Asgari and Zare. The later species was described from wheat grains collected from west Azarbaijan. Our isolate is similar to the type species in cultural and morphological characteristics including the absence of *Nodulisporium* like anamorph. *C. anandra* is the only species in the genus that lacked anamorph and also is easily distinguished by possessing ascospores with broad ostiole.

Conclusion: The present study revealed that grains of soft wheat cultivars grown in Duhok province, Kurdistan region of Iraq were contaminated with a diversity of fungi. The mycological survey revealed the prevalent colonization of the grains with *Alternaria* spp. and storage fungi (*Aspergillus* spp. and *Penicillium* spp.). The detection of *B. sorokiniana* is of particular importance. These results suggest that it may be necessary to test the pathogenicity and toxigenic ability of some isolates.

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