# NEW RECORDS OF FUNGI ON WHEAT GRAINS FROM IRAQ

Samir Khalaf Abdullah<sup>1</sup> and Halben Ismat Mohammad Atroshi<sup>2</sup>

<sup>1</sup>Biology Department, Faculty of Science, University of Zakho, Kurdistan Region – Iraq. <sup>2</sup> Department of Plant Protection, Faculty of Agriculture and Forestry, University of Duhok, Kurdistan Region - Iraq.

(Accepted for publication: December 30, 2014)

## ABSTRACT

The present study recorded eight fungal species on wheat grains for the first time in Iraq. These included Arthrinium phaeospermum, Bipolaris sorokiniana, B.spicifera, Chaetomium elatum, Emericella rugulosa, Eurotium herbariorum Nigrospora state of Khuskia oryzae and Ulocladium alternariae. Brief descriptions with photographs are provided for the newly recorded species.

### **INTRODUCTION**

 $\mathbf{F}^{\mathrm{ungi}}$  associated with seeds are responsible for both pre-and postemergence damping-off of grains thus causing a reduction in germination. They can also causing mycotoxicoses in live-stock, poultry and humans (Agrawal and Sinclair, 1987). Seed -borne pathogens may also causes seed abortion, seed rot, seed necrosis and production of metabolites (such as toxins) which may alter grain composition or metabolism or render it unfit for human or animal consumption (Christensen and Kaufman, 1969, 1974; Naraiah et al., 1986). Moreover. close association with seeds facilitates the long term survival, introduction into a new areas and widespread dissemination of pathogens (Agrawal and Sinclair, 1987).

Therefore, the study of seed-borne fungi is very important to determine the health of grains and to protect them from seed-borne pathogens (HGCA, 2012).

During our continuous survey on seed-borne fungi from different economic crops growing in Iraq (Abdullah and kadhum, 1987; Abdullah and Al-Mosawi, 2006, 2009, 2010; Haleem *et al.*,2013), several interesting fungal species newly recorded on wheat grains from Iraq have been identified and briefly described along with photographs.

### MATERIALS AND METHODS

#### **Sample sources**

Twenty six samples of wheat grains were obtained from official sources in Duhok provine (Department of field crops, Faculty of Agriculture and Forestry,Duhok University and from directorate of agricultural research,Duhok) and from silos at Shikhan and Zakho. Fourteen samples were belonging to soft wheat (*Triticum*) *aestivum* L.) and twelve samples of durum wheat (*T. durum* Desf.).

# **Detection of seed-borne fungi**

Fifty grains were taken randomly from each sample (total of 1300 grains) and were surface disinfected with 1% sodium hypochlorite for 5 minutes, then washed twice with sterilized distilled water. The seeds were dried on sterilized filter paper and placed on two media dextrose (Potato agar (PDA) (Himedia laboratories, India) and Oat meal agar (OTA):30 g oat (Quiker oat),15 g agar 1L water in five replicates for each medium, each replicate contains 10 grains . Chloramphenicol (50 mg/L) was added to each medium to inhibit bacterial growth. Plates were incubated at 25°C for 6-10 days under near ultraviolet (NUV) light at 12 hours interval of alternation with darkness (Mathur and kongsdal, 2003). Pure cultures from growing colonies were obtained by transferring fungal colonies individually on different fresh media plates (MEA (Himedia laboratories, India), PDA and OTA) for identification.

#### **Fungal Identification**

All species identifications were according to the keys and descriptions provided by Ellis (1971, 1976); Arx *et al*.1986; Sivanesan, 1987; Klich(2002); Watanabe(2002) and Guarro *et al*.,(2012).

## **RESULTS AND DISCUSSION**

Five mitosporic fungi and three teleomorphic ascomycetes have been identified, briefly described and discussed as below:

## Arthrinium phaeosprmum (Corda)M.B.Ellis

Mycol.Pap.103:8(1965). Figure 1.

Colonies on PDA are at first hyaline, becoming brown to dark brown when conidia are produced, reaching 44 mm diam in 2 weeks at 25°C. Mycelium are mostly superficial, septate.Conidiophores are micronematous, unbranched, subhyline to pale brown, varying in length up to 60 um long and 1-5 um wide. Conidiogenous cells are discrete, 5-9×3-5 um. Conidia are often found in tight clusters along а narrow conidiophore, lenticellular, and smooth, pale brown to brown,  $9-11\times5-7$ um in size with longitudinal germ slit.

Specimens examined:

The species is very common on both soft wheat and durum wheat grains. Representative

dried and living cultures have been deposited at mycology bank, Plant Protection Department,Faculty of Agriculture and Forestry, Duhok university.

This is the first report for the species in Iraq. However, the species has been reported from wheat grains in Scotland (Flannigan, 1970) and from freshly harvested wheat from Argentina (Broggi *et al.*, 2007).An *Arthrinium* sp was also reported common on soft white winter wheat grains from Ontario, Canada (Clear and Patrick, 1993). The species was also detected from spelt wheat (*T.spelta* L.) grains in Poland (Kurowski and Wysocka, 2009).



**Figure 1:** *Arthrinium phaeosprmum* conidia and conidiophores. Scale bar = 10 um.

*Bipolaris sorokiniana* (Sacc.) Shoem . Can . J. Bot . 37: 884 (1959) Teleomorph : *Cochliobolus sativus* (Ito& kurib) Drechsler ex Daster . Ind .J . Agric . Sci . 12: 733 (1942). Figure 2

Colonies on PDA are dark brown growing rapidly reaching 65 mm in 2 weeks at 25 °C. Mycelium in forming a velvety layer, dark brown, smooth on veruculose .Conidiophores are erect, pale to dark brown up to 200 um long and 6-10 um wide and bearing 1-6 conidia at short distances in the upper half. Conidia are curved to straight smooth , olivaceos brown , fusoid to broadly ellipsoidal, terminal partly each cell subhyaline, 3-12(mostly 6-10) septate, 40- $120 \times 20-28$ um. Specimen examined: The species has been isolated in one occasion for each of soft wheat and durum wheat; dried and living cultures have been deposited at mycology bank, at Plant Protection Department, Faculty of Agriculture and Forestry, Duhok University.

This is the first record for the species on wheat grains in Iraq. However, the fungus was recently isolated from a naturally infected roots of a winter wheat plants collected from commercial field in Diwaniya governorate, middle Iraq (Sarhan, 2013).

*Bipolaris spicifera* (Bainier) Subram, Hyphomycetes 756 (1971).

Teleomoraph : CochliobolusspiciferNelson , Mycologia56 : 198 (1964 ). Figure 3

Colonies on PDA are dark brown to black , rapidly growing reaching 70 mm and 2 weeks at 25 C°. Conidiophores are pale to med brown with obvious and numerous scars , up to 250 um long at 4-8 um wide . Conidia are straight, cylindrical, rounded at ends, smooth, 3- septate, golden brown,  $20-38 \times 9 - 13$  um.

Specimen examined: The species has been detected in one occasion from durum wheat.

Dried and living cultures have been deposited at mycology bank, at Plant Protection Department, Faculty of Agriculture and Forestry, Duhok University.

This is the first report for the species on wheat grain in Iraq. However, the species was previously detected from wheat grain important to Iraq from Hungary and India (Juber and AL-Salahi, 2006).



Figure 2: Bipolaris sorokiniana conidia. Scale bar= 25 um.



Figure 3: Bipolaris spicifera conidia. Scale bar= 10 um.

## Chaetomium elatum

Kunze, Mycol. Hefte. 1:16(1817). Figure 4 (A, B).

Colonies on PDA are white with pale aerial mycelium reaching 60 mm diam in 2 weeks at 25 °C and often with yellow exudates. Ascomata are spherical or ovate maturing within 2 weeks with brown wall, ostiolate 170-350um size. Ascomata hairs are numerous, long, dichotomously branched mainly in the upper part, dark brown, regulose or warty, septate,4-5um thick at base . Asci are clavate, 8-spored, evanescnt,  $30-40 \times 14-18$  um. Ascospores are liminiform, bilaterally flattened, brown at maturity  $8-11 \times 7-9$  um with an apical germ pore.

Specimen examined: The species is very common on both soft wheat and durum wheat

grains. Representative dried and living cultures have been deposited at mycology bank at Plant Protection Department, Faculty of Agriculture Forestry, Duhok University. and This is the first report for the species on wheat grains in Iraq. However, the fungus was previously reported from maize(zea mays L.) grains and sunflower (Helianthus annuas ) seeds in Iraq (Abddullah and Al-Mousawi,2006,2010) .from leaves of sugarcane (Sacharum officinarum L.) cultivars in Iraq (Abdullah and Saleh,2010) and from soil at date palm plantations and from surface sediments of Shatt Al-Arab river and its creeks in Basrah, Iraq (Abdullah and Zora,1993,Abbullah and Abbas,2008).



**Figure 4:** *Chaetomium elatum*. A: Ascomata with dichotomously branched hairs. Scale bar = 20um. B: Asci and ascospores. Scale bar =10um.

*Emericella rugulosa* (Thom&Raper) E.R.Beng. Mycologia 47:680 (1955). Figure 5 (A, B).

Anamorph :*Aspergillus rugulovavus* Samson&W.Gams. in Samson and Pitt (eds.) Advances in *Penicillium* and *Aspergillus* systematics ,New York .49.1984.

Colonies on MEA are green reaching 55 mm diam. in 2 weeks at  $25^{\circ}$ C with light yellow reverse. Cleistothecia are abundant developed within 2 weeks, 250-300 um diameter. Hule cells are globose, 18-20 um in diameter. Ascospores are grayish red, 3.5-4.5×3-3.5 um with rugulose convex wall and two equatorial crests. Conidial heads are short columnar. Conidiophores are smooth, 60-80×3-4 um. Vesicles are ovate to

flask-shaped 8-12 um wide, biserriate, strigmata  $4-7 \times 3-3.5$  um . philides are 6-7.5 um long and 3-3.5 um wide. Conidia are green in mass, spherical to subspherical 2.5-3.5 um diam.

Specimen examined: The species has been detected in one occasions for each of soft and durum wheat. Dried and living culture has been deposited at mycology bank, Department of Plant Protection, Faculty of Agriculture and Forestry, Duhok University.

This is the first report for the species on wheat grains. However, the species was previously reported from soil in Kurdistan region, Iraq (Abdullah and Abdullah, 2009; Abdullah and Saadullah, 2013).



Figure 5. *Emericella rugulosa*. A: Asci and ascospores. Scale bar =5um. B: Ha anamorph. Scale bar = 10um.

.B: Hule cells and Aspergillus

*Eurotiom herbariorum* (WH.Wigg.) Link.Mag.Gesell.Naturf.Freunde,Berlin

3:31(1809). Figure 6 (A- C).

Anamorph: *Aspergillus glaucus* (L.)Link .Mag, Gesell.Naturf.Frunde, Berlin 3:82(1809).

Colonies on MEA are cream to orange buff reaching 50mm diam. in 2 weeks at 25°C .Cleistothecia are abundant, 100-160um diam, yellow to orange yellow encrusted with red hyphae. Peridium composed of a single layer of thin-walled pseudoparenchymatous cells of texture angulrta. Asci are 8-spored, globose to sub globose 11-14 um in diam. Ascospores are white to yellow,  $6-7 \times 5-5.5$ um with pronounced furrow and 2 equatorial crests with convex surface finely roughened.Conidial heads sparse, radiate to loosely columnar, pale blue green with clavate to ellipsoidal uniseriate vesicle, 15-35um in diameter.Conidia are globose to ellipsoidal, dull green, thick walled, spinose to spinulose 5-8mm in diameter.







**Figure 6:** *Eurotiom herbariorum*. A: *Aspergillus* anamorph. Scale bar =5um. B: Cleistothecia. Scale bar = 50 um. C: Asci and ascospores. Scale bar =5um.

This is the first report of the species from wheat grains in Iraq. However, the species has been recently isolated from soil at grapevine plantations in Duhok (Abdullah and Saadullah, 2013).

*Nigrospora state of Khuskia oryzae* Hudson. Trans.Br.mycol.Soc.46:355-360(1963). Figure 7. Anamorph : *Nigrospora oryzae* (Berk.&Br.) Petch, J.Indian Bot.Soc.4:24(1924).

Colonies on MEA are black reaching 40mm diam. in 2 weeks at  $25^{\circ}$ C.Aerial mycelium is wooly and hyphae are up to 9mm wide. Conidiophores are short pale brown, bearing

conidia singly and terminally. Conidia are black, globose in end view and ellipsoidal in side view, measuring 13-17 um diam.(mostly 12-14um).

Specimen examined: The fungus has been found in one occasion on durum wheat. Dried and living cultures have been deposited at mycology bank; plant protection Department, Faculty of Agriculture and Forestry, Duhok University.

This is the first record for the species on wheat grains in Iraq. However, Juber and Al-Salahi(2006) reported a *Nigrospora* sp. on wheat grains imported to Iraq.



Figure 7: Nigrospora state of Khuskia oryzae conidia and hyphae. Scale bar = 10 um.

Ulocladium alternariae (Cooke) Simmons, Mycolgia 59:82-83(1967). Figure 8.

Colonies on PDA are reaching a diameter of 50 mm in 2 weeks at  $25^{\circ}$ C.Mycelium is pale brown, smooth 4-5 um. Conidiophores are golden brown up to  $100 \times 4$ -7um. Conidia are golden brown, smooth, obvoid to broadly ellipsoidal,  $20-30 \times 12.5-18$  um, with (1)-3-5 transverse septa and 1 or 2 longitudinal or oblige septa.

Specimen examined: The species has been isolated from both soft wheat and durum wheat grains during this study. Representative dried and living cultures have been deposited at mycology bank at Plant Protection Department, Faculty of Agriculture and Forestry, Duhok University.

This is the first record for the species in Iraq. However, the species has been reported on wheat grains collected from Kerman province, Iran (Gohari *et al* .,2007).



Figure 8. *Ulocladium alternariae* conidia. Scale bar =10 um.

### REFERENCES

- Abdullah, S. K. and Kadhum, S. A. (1987).Seed mycoflora of *Sorghum bicolor* in Iraq. Arab Gulf J.Scient.Res. Agric.Biol.Sci.5:401-410.
- Abdullah, S.K and Abbas, B. A. (2008). Fungi inhabiting surface sediments of Shatt Al-Arab river and its creeks at Basrah, Iraq. Basrah J.Sci.B, 26:68-81.
- Abdullah, S. K. and Al-Mosawi , K. A. (2006). Diversity of fungal species associated with maize (*Zea mays* L.) Cultivars grown in Iraq.Proc.12<sup>th</sup> Cong. Mediterr. Phytopathol. Union, Rhodes Island, Greece, pp. 69-72.
- Abdullah, S. K. and Al-Mosawi , K. A. (2009). Incidence of *Aspergillus* species in seeds of corn and sunflower cultivars grown in Iraq. 1<sup>st</sup>. Scient. Conf. Biol. Sci. 22-32 April, 2009. University of Mosul, pp299-307.
- Abdullah, S. K and Al-Mosawi, K. A. (2010). Fungi associated with seeds of sunflower (*Helianthus* annuus) cultivars grown in Iraq. Phytopathologia 57:11-20.
- Abdullah, S. K and Saadullah , A. A. (2013). Soil mycobiota at grapevine plantations in Duhok, North Iraq. Mesopotamia J. Agric. 41Suppl. (1):437-447.
- Abdullah, S. K. and Salih , Y. A. (2010) Mycobiota associated with sugarcane (*Sacharum officinarum* L.) cultivars in Iraq. JJBS 3:193-202
- Abdullah, S. K and Zora, S. E. (1993) . Soil microfungi from date palm plantations in Iraq. Basrah J. Sci. B,11:45-68.
- Abdullah, W. R. and Abdullah , S. K. (2009) Taxonomic study on Aspergilli and their telomorphs from soil in north Iraq. Proc. 1<sup>st</sup> Scient. Conf.Biol.Sci.22-32 April, 2009, University of Mosul, pp.328-363.
- Agrawal ,V. K and Sinclair , J. B. (1987) Mechanism of seed infection, in Principle of Seed Pathology .CRC Press Inc. 176pp.
- Arx, J. A. Von, Guarro, j and Figueras, J. M. (1986). The ascomycete genus *Chaetomium*, Nova HedwigiaBeih, 841:1-162.
- Broggi, L. E., Gonzalez, .H. H. I and Ana Pecin, S. L. R. (2007). *Alternaria alternate* prevalence in cereal grains and soybean seeds from Entre Rios, Argentina. Rev.Iberoam.Mycol.24:47-51.
- Christensen ,C.M and Kaufman ,H.H.(1969). Grain storage: The role of fungi in quality loss .University of Minnesota press., Minneapolis, M N. 153 pp.
- Clear, R. M and Patrick ,S. K. (1993). Prevalence of some seed -borne fungi on soft white winter wheat seed from Ontario ,Canada. Can. Plant Dis .Surv. 73:143-149.

- Ellis, M. B. (1971). Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, England 608 pp..
- Ellis, M. B. (1976). More Dematiaceous Hyphomycetes, Commonwealth Mycological Institute, Kew, England 507pp.
- Flannigan , R. (1970). Comparison of seed –borne mycoflora of barley ,oats and wheat grains. Trans. Br. Mycol. Soc..55:267-276.
- Gohari , A. M, sedachat, N. , Javan Nikhah, M and Sabeririseh, R. (2007). Mycoflora of wheat grains in the main production area in kerman province, Iran. Int. J. Agric.Biol.9:635-637.
- Guarro, J., Gene, J., Stchigel, A and Figueras, M. J. (2012). Atlas of Soil Ascomycetes. CBS Biodiversity series 10, CBS –KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, 486 pp.
- Haleem, R. A., Saido, K.a, Abdullah, S. K., Aldeen, S. N and Waesi, I. M. (2013). Fungi associated with freshly harvested corn grains in Duhok governorate. J.Univ. Zakho A-Science 1(2):569-574.
- HGCA (2012).Wheat disease management guide. Agriculture and Horticulture Department Board. HGCA publication, Warwickshire, U.K.
- Juber, K. S and Al-Salahi, G. M. (2006). Detection of fungi associated with wheat grains imported to Iraq. Iraqi J. Agric. Sci. 37(1): 143-148.(in Arabic).
- Klich, M. A. (2002). Identification of common Aspergillus Species, CBS, Utrecht, The Netherlands, 116pp. Kurowski,t.p.and Wysocka, U. (2009). Fungi colonizing grains of winter spelt grown under two production systems. Phytopathologia 54:45-52.
- Mathur, S. B and Kongsdal, O (2003).Common laboratort seed health testing methods for detecting fungi. ISTA, Basseradorf.Switzerland 427pp.
- Naraiah,M.I., Rai,P.V and Rajagopor,R.S (1986). Aflatoxin production in wheat flour and its effect on protein and carbohydrate content of flour. J.Food Sci.Tech.23(1):20-24.
- Sarhan, A.R.T.(2013) Biological control of *Heminthosporium sativum* the causal agent of root rot in wheat by some antagonistic fungi. Egypt Acad. J. Biology. Sci. G. Microbiology 5(2)1-8.
- Sivanesan, A. (1987). Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. Mycological papers No.158, C.A.B. International Mycological Institute, U.K.247pp.
- Watanabe, T. (2002). Pictorial Atlas of Soil and Seed Fungi. CRC Press, London, UK. 486 pp.

# پوخــتـه

Arthrinium phaeospermum, Bipolaris sorkiniana, B.spicifera, Chaetomium elatum, Emericella rugulosa, Eurotium herbariorum, Nigrospora state of Khuskia oryzae, JUlocladium alternariae

ههمي جورين بو يكهم جار ل عيراقي هاتينه توماركرن دگهل دياركرني بريكا وينين فوتو گرافي .

الخيلاصيية

أجريت هذه الدراسة لمعرفة واجراء المسح للفطريات المصاحبة لبذور الحنطة في محافظة دهوك.تم خلال هذه الدراسة تسجيل( ٨ ) انواع من الفطريات لاول مرة في العراق مثل:

Arthrinium phaeospermum, Bipolaris sorkiniana, B.spicifera, Chaetomium elatum, Emericella rugulosa, Eurotium herbariorum, Nigrospora state of Khuskia oryzae, JUlocladium alternariae

تم وصف جميع هذه الأنواع التي سجلت لأول مرة في العراق مع التوضيح بالصور الفوتوغرافية.