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Full Length Research Paper

Survey of the mycobiota of freshly harvested wheat grains in the main production areas of Tunisia

Fatma Bensassi[1,2]*, Chennaoui Mahdi[1], Hassen Bacha[2] and Mohamed Rabeh Hajlaoui[1]

[1]Laboratory of Plant Protection, The National Institute for Agricultural Research, INRA Tunisia, Rue Hedi Karray, 2049 Ariana, Tunisia.
[2]Laboratory of Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, 5019 Monastir, Tunisia.

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Wheat is one of the most important cereals produced and consumed in Mediterranean countries. A mycological survey was carried out on freshly harvested wheat grains from the main production regions at the Northern part of Tunisia, namely Jendouba, Beja and Bizerte, during the crop year of 2009. A total of 88 samples were analysed. The incidences, isolation frequencies and relative densities of fungi were determined. The results showed the presence of several genera among them, in which both of the field fungi, *Fusarium* and *Alternaria*, were present in the Tunisian wheat with dominance for the genus *Alternaria*. The most predominant mycotoxigenic species were *Fusarium culmorum* among *Fusarium* species and *Alternaria alternata* among *Alternaria* species. They were detected in about 66 and 95.5% of fields, respectively. These species are known to decrease the food quality indirectly by the biosynthesis of several hazardous compounds.

Key words: Wheat, Tunisia, mycobiota, *Fusarium, Alternaria,* quality.

INTRODUCTION

Wheat (*Triticum turgidum* ssp. *durum*) is one of the most important cereal crops in the world and the main staple food in Tunisian homes. Indeed, it represents about 61% of the cultivated cereal area and the most important income for the Tunisian economy. Most of the production is for human consumption, chiefly related with pasta-making and many other traditional foods, such as “Couscous” and “Borghol”. In this regard, a high quality of wheat and its products is needed.

In Tunisia, the quality assessment, usually performed as a routine analysis by the cereal collecting centers, is based mostly on visual examination of the overall appearance of the entire grain sample such as discoloration, weighing and shrivelling of kernels: an analysis that is essentially in relation with the price range assessment. Nevertheless, until now, there are no available data about fungal contamination of wheat which can lead directly to economic losses (Nganje et al., 2004; Windels, 2000). Hence, the identification of wheat mycoflora is becoming essential in order to control food contamination by fungi, be it pathogenic or saprophytic species.

Mould contamination of grains can occur during the harvest and the post-harvest periods under suitable conditions of temperature and humidity (Doohan et al., 2003), which play an important role in the growth and geographical distribution of fungi (Kosiak et al., 2004). A number of fungal species have been associated with wheat, belonging mainly to the genera *Fusarium* and *Alternaria*, known as field fungi and the so-called storage fungi, such as *Penicillium, Aspergillus* and *Rhizopus* (Bottalico and Perrone, 2002; Gohari et al., 2007; Juan et al., 2008). Among these genera, some species are of

*Corresponding author. E-mail: bensassifatma83@yahoo.fr. Tel: +216.71.23.53.17. Fax: +216.71.23.53.17.

major concern because of their toxigenic properties. They have the potential to produce a range of toxic secondary metabolites known as mycotoxins (Betina, 1984; Saberi et al., 2004). Such contamination can lead to reducing and downgrading of the grain quality, making it unsafe for human and livestock consumption (Goswami and Kistler, 2004; Osborne and Stein, 2007). Mycotoxins are often unavoidable and of a worldwide preoccupation (Bennett and Klich, 2003).

These hazardous compounds that occur simultaneously in food or unprocessed materials can cause acute or chronic intoxication to humans and animals (Wild and Hall, 1996; Placinta et al., 1999). Mainly due to the diversity of their chemical structures, several classes of mycotoxins have been identified as important contaminants in foodstuffs (Steyn, 1995; Meky et al., 2001), including Aflatoxins and Ochratoxins from *Aspergillus* spp. and *Penicillium* spp. (Payne, 1998; Frisvad and Samson, 2004), Trichothecenes, Zearalenone and fumonisins from *Fusarium* spp. (Langseth and Rundberget, 1999; Bakan et al., 2002 ; Edwards, 2004), *Alternaria* toxins from *Alternaria* spp. (Woody and Chu, 1992) and many other toxic compounds. The ingestion of mycotoxin contaminated grains may lead to a wide array of biological effects being genotoxic, carcinogenic, embryotoxic and teratogenic (IARC, 1993; Smith et al., 1995; Bennett and Klich, 2003).

In this paper, the significance of the internal mycoflora of freshly harvested wheat grains intended for human consumption is considered. The aims of this survey are to identify the fungi associated with wheat grains harvested in the Northern part of Tunisia during the crop year of 2009, to determine the species distribution of the genera of mycotoxicological interest, and to compare the mycobiota at different cropping areas in Tunisia.

MATERIALS AND METHODS

Chemicals and reagents

The components of the culture medium were purchased from Biomatik Corporation Company (Cambridge, Ontario, Canada), while the sodium hypochlorite solution (NaOCl), the sterile water and Petri dishes were supplied by Sigma Aldrich Company (St. Louis, MO).

Sampling

Wheat grains for human consumption without visible signs of moulds contamination were collected during the 2009 crop year from the major cropping regions in Tunisia, including Jendouba, Beja and Bizerte. A total of 88 grain samples were taken directly from farmers fields immediately after the harvest. The number of samples collected from each area depends on the importance of the region in the national production of wheat: 35, 29 and 24 samples were harvested from Jendouba, Beja and Bizerte, respectively. The total weight of the composite sample was 3 kg. After homogenisation, a subsample of approximately 1 kg was used prior to mycoflora identification. The harvested grain samples were at a moisture content that is lower than 16%. Samples were kept refrigerated in sterile dry containers till analysis.

Mycological analysis

Following homogenization, 100 grains were taken from each subsample. They were surface-disinfected with 1% NaOCl solution for 10 min followed by rinsing twice with sterile water then dried over a filter paper in a sterile laminar flow cabinet. Grains were plated on potato dextrose agar (PDA), ten kernels per plate and incubated at 25°C for 7 days in darkness. The experiment was carried out three times separately. The developing fungal colonies were subcultured onto PDA and identified based on their macro- and microscopic features (Nelson et al., 1983; Simmons, 1986, 2000; Pitt and Hocking, 1997). Isolates not fitting the description were recorded as *Alternaria* and *Fusarium* sp.

The fungi of primary interest in this study were *Fusarium* and *Alternaria* species because of their known ability to alter food quality firstly in the field and to produce mycotoxins. All isolated fungi were recorded according to Gonzalez et al. (1995). The isolation frequency (Fr), relative density (Rd) and incidence (In) of species were recorded respectively, as follows:

$$Fr\ (\%) = (ns/N) \times 100$$
$$Rd\ (\%) = (ni/Ni) \times 100$$
$$In\ (\%) = (ng/Ng) \times 100$$

where ns = the number of samples in which a genus/species occurred; N = the total number of samples; ni = the number of isolates of a genus/species; Ni = the total number of fungal isolates obtained; ng = the number of grains infected by a genus/species; Ng = the total number of grains.

Statistical analysis

The statistical analysis was performed with the SPSS 13.0 program. The different data (Rd, Fr and In) were expressed as percentage, while significant differences were compared using non-parametric χ^2 test. Differences were considered to be significant at $p< 0.05$.

RESULTS AND DISCUSSION

Wheat is mainly cultivated in Northern Tunisia, and this is related chiefly with climatic conditions suitable for the development of cereals. Nevertheless, these same conditions failed, almost all the time, in offering a compromise between a high productivity and a good quality. Indeed, Northern Tunisia is an ecosystem favourable for fungal invasion in the field which results generally in yield loss and quality reduction.

The data presented in this study gave detailed information on the mycobiota of wheat from a Mediterranean country. The results indicated that the grains harvested in the main cropping regions in Tunisia were heavily contaminated with micromycetes as presented in Figure 1. Indeed, the percentage of uncontaminated grains was lower for about 4 times than the percentage of infected grains in the prospected regions ($p< 0.01$). The most recovered genera, in terms of frequency from wheat samples were Alternaria *and Fusarium*. Many other moulds

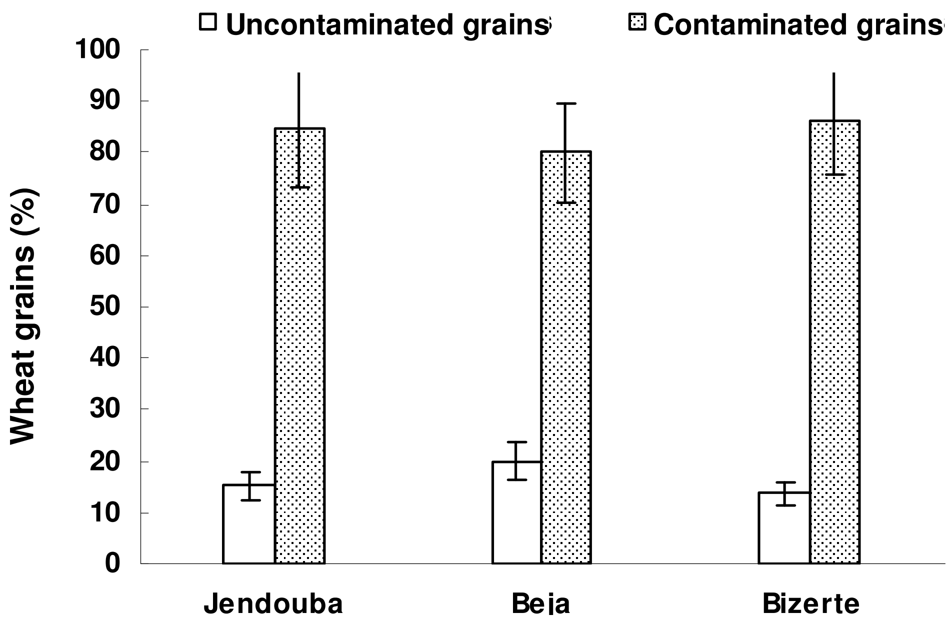

Figure 1. Fungal invasion of wheat grains in the main cropping regions in Northern Tunisia. Data are expressed as percentage ± S.D.

Table 1. Frequency, relative density and incidence of fungal species in wheat from the major cropping regions in Northern Tunisia.

Fungal isolates	Jendouba			Beja			Bizerte		
	Fr (%)	Rd (%)	In (%)	Fr (%)	Rd (%)	In (%)	Fr (%)	Rd (%)	In (%)
Fusarium culmorum	62.86	2.7	2.29	62.07	2.41	1.93	75	3.38	2.92
F. pseudograminearum	34.29	0.84	0.69	20.69	0.43	0.34	45.83	0.63	0.54
F. avenaceum	31.43	0.78	0.66	27.59	0.82	0.66	37.5	1.4	1.21
Fusarium sp.	97.14	9.67	8.2	82.76	8.23	6.59	87.5	8.2	7.08
Michrodochium nivale	100	26.15	22.17	100	29.87	23.9	100	20.42	17.63
Alternaria alternata	94.29	21.27	18.03	93.1	19.7	15.76	100	25.82	22.29
A. tenuissima	91.43	13.41	11.37	89.66	9.7	7.76	91.67	15.06	13
Alternaria sp.	97.14	9.06	7.69	72.41	4.91	3.93	95.83	9.02	7.79
Epicoccum nigrum	31.43	1.35	1.14	17.24	0.52	0.41	29.17	1.11	0.96
Trichotecium spp.	25.7	2.39	2.03	37.93	3.8	3.04	33.33	2.8	2.42
Rhizopus spp.	14.29	4.45	3.77	24.14	6.85	5.48	29.17	7	6.04
Penicillium spp.	8.57	0.54	0.46	13.79	0.91	0.72	12.5	0.58	0.5
Aspergillus spp.	5.71	0.81	0.69	17.24	1.38	1.1	8.33	1.4	1.2
Other fungi	82.86	6.58	5.58	82.75	10.47	8.38	79.16	3.18	2.75
Total samples		35			29			24	

Fr = frequency; Rd = relative density; In = incidence.

moulds were also isolated in 100% of fields with a density of approximately 19%, including mainly *Rhizopus* and *Trichothecium* and in low levels for *Aspergillus, Penicillium, Epiccocum nigrum* and other unknown species (Table 1).

As shown in Figure 2a, the most frequent fungus was *Alternaria*, which was higher about 3 times than the genus *Fusarium*. A similar study conducted in Argentina showed that *Alternaria* was present in 100% of samples with a density of 85% (Patriarca et al., 2007). The same situation was observed in China (Li and Yoshizawa, 2000). Species of the genus *Alternaria* are common field fungi contaminating cereal grains including both plant pathogenic and saprophytic species (Logrieco et al., 1990; Saberi et al., 2004). Besides, some *Alternaria* species have been reported as mycodeteriogens in some

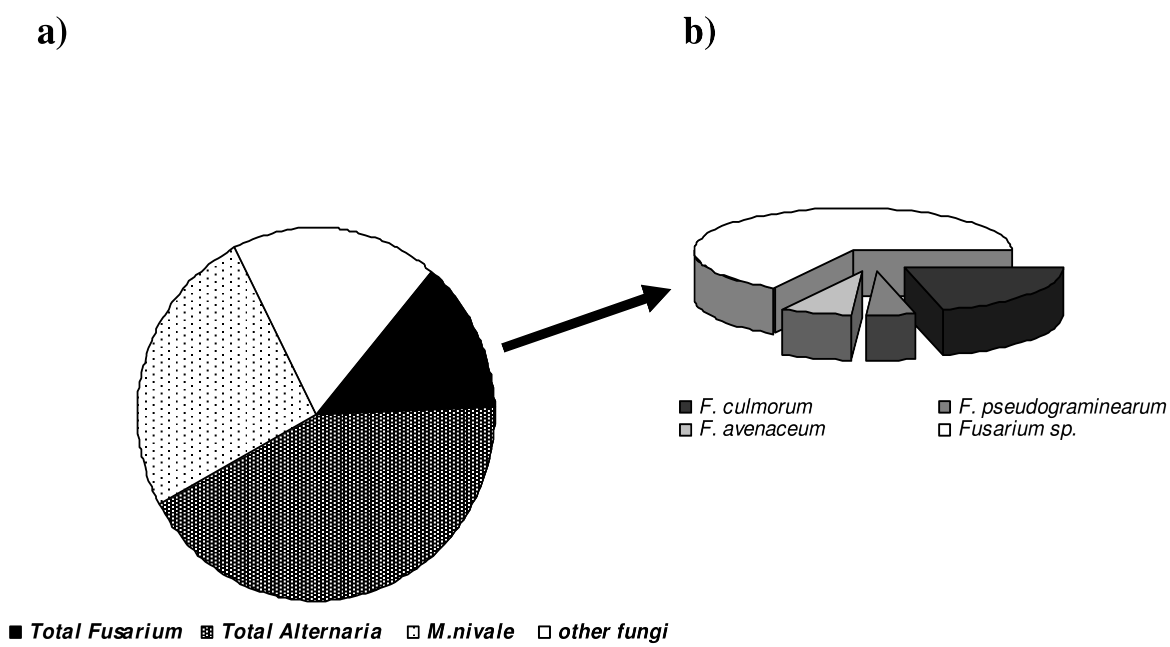

Figure 2. Occurrence of the most dominant fungi in Northern Tunisia; (a) with a specific concern for the genus *Fusarium* in (b).

cereals in the field (Ilhan and Asan, 2001). *Alternaria* species can cause serious problems in agriculture by reducing crop yield in the field and causing spoilage in storage (Andersen et al., 2001). The majority of *Alternaria* isolates belonged to *A. alternata* and *A. tenuissima* with *A. alternata* being the most predominant (Rd = 22% in northern Tunisia) (Table 1). *A. alternata* is known to be the causative agent of black point in wheat grains (Rana and Gupta, 1982), and also the most frequent species in Argentinean and Iranian wheat (Gonzalez et al., 1999; Gohari et al., 2007), as well as in Tunisian wheat (Bensassi et al., 2009).

In this survey, we noted also the dominance of *Microdochium nivale,* formerly known as *Fusarium nivale* detected in all prospected wheat samples with a density of about 26% (Figure 2a). *M. nivale* is one of the predominant causal organisms associated with *Fusarium* Head Blight (FHB) (Parry et al., 1995), a devastating disease of wheat and other small-grain cereals, representing a sporadic threat to wheat production throughout the world (Bai and Shaner, 1994; McMullen et al., 1997; Windels, 2000). Several studies have shown the pathogenicity of *Microdochium nivale* and *Fusarium* species in cereals (Simpson et al., 2004; Brennan et al., 2005). In fact, a competitive interaction has been usually suggested between *M. nivale* and species of *Fusarium* (Doohan et al., 1998; Simpson et al., 2001). The most frequently isolated *Fusarium* species were: *F. culmorum* (66%), *F. pseudograminearum* (31%) and *F. avenaceum* (28%). These species were commonly associated with FHB (Bottalico, 1998; Osborne and Stein, 2007). In France, a recent study showed that FHB symptoms on durum wheat were associated in the field with *F. graminearum*, *F. culmorum* and *M. nivale* (Ioos et al., 2004). In Tunisia, Kammoun et al. (2010) reported the occurrence of FHB disease on harvested durum wheat caused chiefly by *F. culmorum*, even though *F. pseudograminearum* and *M. nivale* contributed well in the spread of the disease. The results of this study clearly showed the dominance of *F. culmorum* among *Fusarium* species, it represents 2.8% of the total obtained isolates (Figure 2b). This finding is in agreement with the results of *F. culmorum* occurrence in North European countries (Bottalico, 1998; Kosiak et al., 2003). The prevalence of *F. culmorum* on wheat kernels under Tunisian climate may be explained by the suitable climatic conditions occurring during the most susceptible stage to FHB infection, that is, the flowering. Previous investigations reported that *Fusarium* infection was appreciably influenced by environmental conditions, especially temperature, rainfall and moisture during heading and flowering periods of cereals (Doohan et al., 2003; Xu, 2003). In the crop year 2009, a homogenous situation was observed concerning fungal infection in the 3 prospected regions under study. Wheat grains, from these regions, were invaded to different degrees with moulds. This difference can be chiefly attributed to weather conditions, throughout the growing season, which influence the distribution of the infecting fungal species and lead to geographical variation in the species distribution. Table 2 represents the total rainfall and temperatures registered in the three major cropping

Table 2. Average meteorological data[a] for the period of March to May 2009 in the main cropping regions in Northern Tunisia. [a] data provided by the National Institute of Meteorology (Tunisia).

Regions	Total rainfall (mm)	Minimum temperature (°C)	Maximum temperature (°C)
Jendouba	80.4	8.9	21.9
Beja	126.9	9.1	22.6
Bizerte	106	11.2	21.3

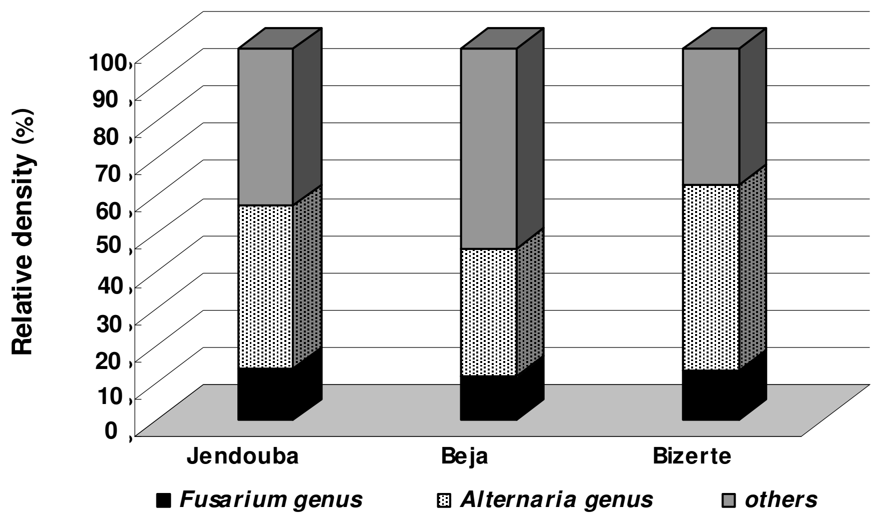

Figure 3. Relative densities of the dominant toxigenic genera of wheat grains from the major cropping regions in Tunisia.

regions in Tunisia during the flowering period and kernel development of wheat which is also a critical period for fungal infestation. The grains obtained from Bizerte were more contaminated by *F. culmorum* and *F. avenaceum* than the other regions, but those isolated from Jendouba were the most infested with *F. pseudograminearum* (Table 1). Although considerable deviations were seen between regions on average, *F. culmorum* contamination of wheat was the highest and this observation agreed with those from other cereals producing regions worldwide (Andersen et al., 1996; Eskola et al., 2001; Kosiak et al., 2003). Wheat grains, from the growing region of Bizerte, were also the most susceptible to *A. alternaria* and *A. tenuissima* infection (Table 1).

A specific concern was attributed to mycotoxigenic contaminating field fungi as *Fusarium* and *Alternaria* in this survey. Figure 3 summarized the distribution of *Fusarium* and *Alternaria* among the isolated fungi in wheat grains of the 3 growing regions. Slight differences were observed between the prospected regions mainly between Bizerte and Jendouba ($p < 0.05$). Concerning wheat, from Beja, it appeared to be the least contaminated either with *Alternaria* or *Fusarium*; it was more invaded with other fungi including *M. nivale*, *Trichothecium* spp., *Penicillium* spp. and many other unidentified moulds (Table 1). There is no doubt that the climate is the principal factor triggering the fungal attacks, but several other factors may intervene and consequently affect the incidence of the fungal infection in cereal crops over the regions such as the impact of location, cultivar properties and agricultural practices (crop rotation and management) (Langseth et al., 1995; Dill-Macky and Jones, 2000; Guerif et al., 2001; Krstanovic´ et al., 2005). Moreover, the spread of fungal infections in Tunisian cereal crops may be favored by the relatively low use of fungicides. Indeed, only fungicides anti-septoria and anti-rust are used by Tunisian farmers (Fakhfakh et al., 1999; ATPP, 2009).

In addition to causing yield losses, many of the fungi isolated from wheat grains are of greater importance because they are known to produce mycotoxins which alter the cereal grain quality (Lessard, 2003). Indeed, the FHB associated pathogens are capable of producing a number of trichothecene mycotoxins, as well as non-trichothecene compounds (Thrane, 2001) as Zearalenone, Beauvericin and Moniliformin (Desjardins, 2006; Leslie and Summerell, 2006; Glenn, 2007). Several trichothecenes are of concern, including Deoxynivalenol, Nivalenol, Diacetoxyscirpenol and T-2 toxin. A recent survey conducted in Tunisia has shown that harvested wheat grains in 2005 were infested chiefly with *F. culmorum* and the majority of the strains were of DON genotype (Kammoun et al., 2010). This result was confirmed by Bensassi et al. (2010), who reported high con-tamination of durum wheat with DON in the 2007 crop year. Unfortunately, DON exposure can lead to acute and long term chronic effects, resulting in terato-genic, neurotoxic, embryotoxic and immunosuppressive effects (Rotter et al., 1996; Desjardins, 2006). In contrast to other FHB pathogens, *M. nivale* does not have any great effect on grain quality (Humphreys et al., 1995; Brennan et al., 2005) and it is thought that *M. nivale* does not produce mycotoxins (Logrieco et al., 1991; Edwards, 2004). In such a crop, it is possible that freshly harvested wheat grains in northern Tunisia might be contaminated by *Alternaria* toxins which are implicated in several human toxicosis as oesophageal cancer and in many digestive complications (Liu et al., 1992; Zureik et al., 2002). Furthermore, in the light of the mycological ana-lysis, there is also a probability of a low ochratoxin and aflatoxins occurrence in the wheat harvested in 2009. Since a low infestation by the associated fungi was no-ticed in the samples, this situation may evolve in storage.

Based on the fungal contamination, the following possible mycotoxins might occur in Tunisian wheat, such as *Alternaria* toxins, Trichothecenes, Zearalenone, Ochratoxins and Aflatoxins, and can negatively affect the quality of the end products based on wheat. In such a case, humans can be exposed to this cocktail of toxins which may lead to significant toxic effects.

Conclusion

In Tunisia, little information is available on the fungal species associated with cereal grains and their distribution in the main production areas. To the best of the authors' knowledge, this is the first survey giving detailed data regarding the mycobiota of freshly harvested wheat in Northern Tunisia. The 2009 crop year seemed to have provided suitable conditions for fungal invasion. Although there was no visible mould damage, the mycological survey indicated high levels of field fungi as *Fusarium* and *Alternaria* that should be of concern because of their toxigenic potential. Such a situation may pose a potential health hazard to consumers. Therefore, further studies should focus on the natural occurrence of mycotoxins in wheat based products. From the current survey, Tunisian authorities should soon monitor fungal infection and mycotoxin contamination at grain laboratories in order to ensure product quality.

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REFERENCES

Andersen B, Kroger E, Roberts RG (2001). Chemical and morphological segregation of *Alternaria alternata*, A. gaisen and A. longipes. Mycol. Res., 105: 291-299.

Andersen B, Thrane U, Svendsen A, Rasmussen IA (1996). Associated field mycobiota on malting barley. Can. J. Bot., 74: 854-858.

Association Tunisienne de la protection des plantes (ATPP) (2009). 4éme édition du guide phytosanitaire de la Tunisie.

Bai G, Shaner G (1994). Scab of wheat: Prospects for control. Plant Dis., 78: 760-766.

Bakan B, Melcion D, Richard-Molard D, Cahagnier B (2002). Fungal growth and *Fusarium* mycotoxin content in isogenic traditional and genetically modified maize grown in France and Spain. J. Agric. Food Chem., 50: 728-731.

Bennett JW, Klich M (2003). Mycotoxins. Clin. Microbiol., 3: 497-516.

Bensassi F, Zaied C, Abid S, Hajlaoui MR, Bacha H (2010). Occurrence of deoxynivalenol in durum wheat in Tunisia. Food Cont., 21: 281-285.

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

Betina V (1984). Biochemical effects of mycotoxins. In: Betina, V. (Ed.), Mycotoxins Production, Isolation, Separation and Purification. Elsevier, Amsterdam, pp. 37-44.

Bottalico A, Perrone G (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol., 108: 611-624.

Bottalico A (1998). *Fusarium* disease of cereals: Species complex and related mycotoxin profiles in Europe. J. Plant Pathol., 80: 85-103.

Brennan JM, Leonard G, Cooke BM, Doohan FM (2005). Analysis of head and leaf reaction towards *Microdochium nivale*. Eur. J. Plant Pathol., 112: 79-83.

Desjardins AE (2006). *Fusarium* mycotoxins. Chemistry, Genetics, and Biology. APS Press, St. Paul, MN.

Dill-Macky R, Jones RK (2000). The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. Plant Dis., 84: 71-76.

Doohan FM, Parry DW, Jenkinson P, Nicholson P (1998). The use of species–specific PCR-based assays to analyse *Fusarium* ear blight of wheat. Plant Pathol., 47: 197-205.

Doohan FM, Brennan J, Cooke, BM (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur. J. Plant Pathol., 109: 755-768.

Edwards SG (2004). Influence of agricultural practices on *Fusarium* infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicol. Lett., 153: 29-35.

Eskola M, Parikka P, Rizzo A (2001). Trichothecenes, ochratoxin A and zearalenone contamination and *Fusarium* infection in Finnish cereal samples in 1998. Food Addit. Contam., 18: 707-718.

Fakhfakh MM, Sbei R, Nasraoui R, Zouani R, Mahouachi MA, Jarrahi, T, M'Hehbi K (1999). Impact de quelques traitements fongicides sur la septoriose de blé, pp. 551-556. Proccedings du deuxième symposium régional sur les maladies des céréales et des légumineuses alimentaires, Novembre 10-12, 1999, Nabeul, Tunisia, p. 561.

Frisvad JC, Samson RA (2004). Polyphasic taxonomy of *Penicillium* subgenus *Penicillium* A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. Stud. Mycol., 49: 1-173.

Glenn AE (2007). Mycotoxigenic *Fusarium* species in animal feed. Animal Feed Sci. Technol., 137: 213-240.

Gohari AM, Sedaghat N, Nikkhah MJ, Saberi-Riseh R (2007). Mycoflora of Wheat Grains in the Main Production Area in Kerman Province, Iran. Int. J. Agric. Biol., 09: 635-637.

Gonzalez HHL, Martinez EG, Pacin A, Resnik SL (1999). Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurence on Argentinean durum wheat. Mycopathologia, 144: 97-102.

Gonzalez HHL, Resnik SL, Boca RT, Marasas WFO (1995). Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia, 130: 29-36.

Goswami RS, Kistler HC (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol., 5: 515-25.

Guerif J, Richard G, Durr C, Machet JM, Recous S, Roger-Estrade J (2001). A review of tillage effects on crop residue management, seedbed conditions and seedling establishment. Soil Tillage Res., 61: 13-32.

Humphreys J, Cooke B, Man D, Story T (1995). Effects of seedborne *Microdochium nivale* on establishment and grain yield of winter sown wheat. Plant Varieties and Seeds, 8: 107-117.

IARC (1993). IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Vol. 56. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. International Agency for Research on Cancer.

Ilhan S, Asan A (2001). Soilborne fungi in wheat fields of Kirka vicinity (Turkey). Biologia Bratisl., 56: 263-271.

Ioos R, Belhadj A, Menez M (2004). Occurrence and distribution of *Microdochium nivale* and *Fusarium* species isolated from barley, durum and soft wheat grains in France from 2000 to 2002. Mycopathologia, 158: 351-362.

Juan C, Moltó JC, Lino CM, Mañes J (2008). Determination of ochratoxin A in organic and non-organic cereals. Food Chem., 107: 525-530.

Kammoun Gargouri L, Gargouri S, Barreau C, Richard-Forget F, Hajlaoui MR (2010). Trichothecenes chemotypes of *Fusarium culmorum* infecting wheat in Tunisia. Int. J. Food Microbiol., 140: 84-89.

Kosiak B, Torp M, Skjerve E, Andersen B (2004). *Alternaria* and *Fusarium* in Norwegian grains of reduced quality- a matched pair sample study. Int. J. Food Microbiol., 93: 51-62.

Kosiak B, Torp M, Skjerve E, Thrane U (2003). The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. Acta Agric. Scand. Sect. B-Soil Plant Sci., 53: 168-173.

Krstanovic´ V, Klapec T, Velic´ N, Milakovic Z (2005). Contamination of malt barley and wheat by *Fusarium graminearum* and *Fusarium culmorum* from the crop years 2001–2003 in eastern Croatia. Microbiol. Res., 160: 353-359.

Langseth W, Hoie R, Gullord M (1995). The influence of cultivars, location and climate on deoxynivalenol contamination of Norwegian oats 1985–1990, Acta Agric. Scand., 45: 63-67.

Langseth W, Rundberget T (1999). The occurrence of HT-2 toxin and other trichothecenes in Norwegian cereals. Mycopathologia, 147: 157-165.

Leslie JF, Summerell BA (2006). The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, IA.

Lessard F (2003). Implication of mycotoxin producing fungi on cereal grain quality: risk management through a full knowledge about toxinogenesis. Phytoma, 566: 14-18.

Li F, Yoshizawa T (2000). *Alternaria* mycotoxins in weathered wheat from China. J Agric. Food Chem., 48: 2920-2924.

Liu G, Qian Y, Zhang P, Dong W, Qi Y, Guo HT (1992). Etiological role of *Alternaria alternata* in human oesophageal cancer. Chin. Med. J., 105: 394-400.

Logrieco A, Bottalico A, Solfrizzo M, Mule G (1990). Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. Mycologia, 82: 501-505.

Logrieco A, Vesonder RF, Peterson SW, Bottalico A (1991). Reexamination of the taxonomic disposition of and deoxynivalenol production by *Fusarium nivale* NRRL 3289. Mycologia, 83: 367-370.

McMullen M, Jones R, Gallenberg D (1997). Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis., 81: 1340-1348.

Meky FA, Hardie LJ, Evans SW, Wild CP (2001). Deoxynivalenol-induced immunomodulation of human lymphocyte proliferation and cytokine production. Food Chem. Toxicol., 39: 827-836.

Nelson PE, Tousson TA, Marasas WFO (1983). *Fusarium* Species. An Illustrated Manual for Identification. The Pennsylvania State University: University Park.

Nganje WE, Bangsund DA, Leistritz, FL, Wilson WW, Tiapo NM (2004). Regional economic impacts of *Fusarium* head blight in wheat and barley. Rev. Agric. Econ., 26: 332-347.

Osborne LE, Stein JM (2007). Epidemiology of *Fusarium* head blight on small-grain cereals. Int. J. Food Microbiol., 119: 103-108.

Parry DW, Jenkinson P, McLeod L (1995). *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol., 44: 207-238.

Patriarca A, Azcarate MP, Terminiello L, Fernández Pinto V (2007). Mycotoxin production by *Alternaria* strains isolated from Argentinean wheat. Int. J. Food Microbiol., 119: 219-22.

Payne GA (1998). Process of contamination by aflatoxin-producing fungi and their impact on crops. In: Sinha, K.K.S., Bhatnagar, D. (Eds.) Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, pp. 279-306.

Pitt JI, Hocking AD (1997). Fungi and food spoilage. New York: Blackie Academic and Professional.

Placinta CM, D'Mello JPF, Macdonald AMC (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Animal Feed Sci. Technol., 78: 21-37.

Rana JP, Gupta PKS (1982). Occurrence of black point disease of wheat in West Bangal. Indian Phytopathol., 35: 700-702.

Rotter BA, Prelusky DB, Pestka JJ (1996). Toxicology of deoxynivalenol (vomitoxin). J. Toxicol. Environ. Health, 48: 1-34.

Saberi R, Javan-Nikkhah M, Heidarian R, Hosseni S, Soleimani P (2004). Detection of fungal infectious agent of wheat grains in store – pits of Markazi province, Iran. Agri. Appl. Biol. Sci., 67: 418-423.

Simmons EG (1986). *Alternaria* themes and variations (22–26). Mycotaxon, 25: 287-308.

Simmons EG (2000). *Alternaria* themes and variations (244–286). Mycotaxon, 75: 1-115.

Simpson DR, Thomsett MA, Nicholson P (2004). Competitive interactions between *Microdochium nivale* var. *majus*, *M. nivale* var. *nivale* and *Fusarium culmorum* in planta and *in vitro.* Environ. Microbiol., 6: 79-87.

Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P (2001). Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur. J. Plant Pathol., 107: 421-431.

Smith JE, Solomons G, Lewis C, Anderson JG (1995). Role of mycotoxins in human and animal nutrition and health. Nat. Toxins, 3: 187-192.

Steyn PS (1995). Mycotoxins, general view, chemistry and structure. Toxicol. Lett., 82/83: 843-851.

Thrane U (2001). Developments in the taxonomy of Fusarium species based on secondary metabolites. In: Summerell, B.A., Leslie, J.F., Backhause, D., Bryden, W.L., Burgess, L.W. (Eds.), *Fusarium* Paul E. Nelson Memorial Symposium. APS Press, Minnesota, pp. 27-49.

Wild CP, Hall AJ (1996). Epidemiology of mycotoxin-related disease. In: Howard, D.H., Miller JD (Eds.), The Mycota VI. Human and Animal Relationships. Springer Verlag, Berlin, pp. 213-227.

Windels CE (2000). Economic and social impact of *Fusarium* head blight. Changing farms and rural communities in the northern Great Plains. Phytopathol., 90: 17-21.

Woody MA, Chu FS (1992). Toxicology of *Alternaria* mycotoxins. In: Chelkowsky, J., Visconti, A. (Eds.), *Alternaria* Biology, Plant Diseases and Metabolites. Amsterdam, Netherlands: Elsevier Science Publishers, pp. 409-434.

Xu X (2003). Impact of environmental conditions on the development of *Fusarium* ear blight. Eur. J. Plant Pathol., 109: 683-689.

Zureik M, Neulck C, Leynnart B, Liard R, Bausquet J, Neukirah F (2002). Sanitization to airborne mould and severity of asthma: Cross sectional study from European Community respiratory health survey. Brit. Med. J., 325: 411.