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Prevalence of Mycoflora and *Fusarium graminearum* Chemotype DON in Wheat in Bechar Province of South-Western Algeria

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Wheat and its derivatives are a main staple food for the Algerian populations. The objective of this study aims to analyze local and imported wheat grains for fungi particularly *Fusarium graminearum* chemotype DON and evaluate deoxynivalenol (DON) contaminated wheat collected from Bechar region, in south-western Algeria. A total of 64 of wheat samples were examined for fungal contamination and fungal load was determined by means of standard microbiological method. DON was detected using the ELISA technique. The results revealed that 98.44% of analyzed samples showed positive results regarding fungal contamination. More precisely, local wheat was dominated by *Aspergillus* and *Penicillium* and imported wheat was dominated by *Fusarium*, *Penicillium* and *Aspergillus* species. Results showed that 62.5% of *F. graminearum* strains produced DON. Contamination levels of wheat with DON ranging from < 0.04 ppm to > 5ppm for soft wheat and from < 0.12ppm to > 15ppm for durum wheat. So, 62.96% of soft wheat grains and 55.56% of durum wheat imported from France, and also 18.18% of local durum wheat exceed the permissible limit by far. This study provides basic grounds in assessing the degree of fungal and potential DON contamination in Algerian wheat.

Keywords: Deoxynivalenol (DON), *Fusarium graminearum*, fungal load, wheat, Bechar district (Algeria).

Moulds play a significant role in wheat grains spoilage worldwide causing important economic losses and lowering their quality (Pitt and Hocking, 2009; Saladino et al., 2016; Luz et al., 2017; Kaktcham et al., 2018; Guimarães et al., 2018a). Moreover, their secondary metabolites are highly toxic chemical products. These compounds cause carcinogenic, mutagenic, teratogenic, neurotoxic, nephrotoxic, immunosuppressive and estrogenic diseases (Bennett and Klich, 2003; Pawlowska et al., 2012; Guimarães et al., 2018b).Thus, they are considered as sources of severe health risk for humans and animals.

Fusarium graminearum Schwab (teleomorph *Gibberella zeae*) (Kim et al., 2003) is the primary phyto-agent of Fusarium head blight (FHB) or scab of small grain cereals

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around the world (Hornok, 2007; Yli-Mattila et al, 2009). Indeed, several researchers have revealed that *F. graminearum* is the most prevalent species in North America (USA and Canada), Australia, central southern Europe and China (Chen et al., 1982; Burgess et al., 1987; Gilbert and Tekauz, 2000; Tan and Niessen, 2003). In France, *F. graminearum* is the most common species associated with FHB on wheat and durum wheat causing serious losses in crop revenues, bad quality of grains and toxins contamination such as deoxynivalenol, nivalenol and fumonisin (Chehri et al., 2011; Korn et al., 2011; Orlando et al., 2019). Moreover, this devastating agent can be found in fields and persists during the long years of storage (Zhang et al., 2007). Deoxynivalenol or vomitoxin belongs to trichothecenes type B (Malachova et al., 2015; Chilaka et al., 2018; James and Zikankuba, 2018) which can cause several toxic effects including infertility and reproductive problems, mutagenic and carcinogenic (Nelson et al., 1991; Thiel et al., 1991; Shephard et al., 2005), diarrhoea, vomiting, leukopenia, haemorrhage and shock (Foroud and Eudes, 2009).

In Algeria, wheat is the most widespread staple crop which is consumed in the form of couscous, pasta, macaroni, spaghetti, bread, and *frik* is a cultural tradition (Riba et al., 2010). In spite of the production of more than a million and a half hectares of wheat on average since 1961, grain yields stay below the demand of the national market. Consequently, Algeria remains dependent on the importation of wheat grains. However, massive importation and long-term storage promote fungal contamination, with damage ranging from the loss of quality and sanitation of the grain (Djaaboub et al., 2018). Although many studies about aflatoxigenic and ochratoxigenic contamination were carried out (Khalef et al., 1993; Riba et al., 2008, 2010), there is no data either on the frequency of *Fusarium graminearum* chemotype DON or about levels of DON in wheat grains. The aim of the present study was to assess the safety of wheat consumed in Algeria, especially in Bechar province with respect to fungal species particularly *Fusarium graminearum* chemotype DON, and DON contamination.

Materials and Methods

Sampling

Local and imported wheat grains were collected from Bechar area, southwestern Algeria (31°37’00’’ north, 2°13’00’’ south), from the local grain cooperative and dried vegetable silos and the farmers (for local durum wheat) in sterile conditions. A total of 64 samples of 1kg were brought to the laboratory to be analyzed for fungal contamination, especially for *Fusarium graminearum* chemotype deoxynivalenol (DON).The details of each sample are provided in (Table 1).

Materials

Culture medium used for fungal isolation and enumeration was Potato Dextrose Agar (PDA). Furthermore, various standard agar media have been used for the morphological and toxicological identification of fungi, Spezieller Nahrstof-farmer (SNA for microscopic identification), Czapek Yeast Extract (CYA), Dichloran Chloramphenicol Malt Extract (DCMA for *Alternaria* species), Dichloran Chloramphenicol Pepton (DCPA a se-

Table 1

Informative data on the samples of wheat

Sample	Nbr	Provenance
Imported soft wheat	27	France
Local soft wheat	6	Taghit. Bechar district. Algeria
Imported durum wheat	9	France
Local durum wheat	22	Oum Chegag. Bechar district. Algeria
Total	**64**	

lective medium for *Fusarium spp.*), 25% Glycerol Nitrate (G25N), Malte Extract (MEA) and Glucose Yeast Extract Pepton Broth (GYEP for screening *F. graminearum* chemotype DON). Immunoaffinity columns for cleaning DON (I clean C + DON) and quantitative ELISA kit for the detection DON (CelerDONV3) were procured from tecnalab (Italy) and DON standard from Lbios (France).

Fungal isolation and identification

For fungal isolation, a dilution method was performed. Five grams of ground grains were added to 45 ml sterile 9% physiological water (dilution 10^{-1}) and two drops of Tween 80.1ml of this dilution was diluted in 9 ml sterile 9% physiological water (dilution 10^{-2}). 200 μl of each dilution were inoculated onto PDA medium in duplicate by the spread plate method. The plates were then incubated at 25 ± 2 °C for 7 days. The total fungal load (FL) per genus was calculated as colony forming unit per gram (CFU/g) of the sample (Aiko and Mehta, 2016), percent frequency (Fr %) and percent relative density (RD %) were also, calculated using the following formula (Nagaraja et al., 2016):

$$Frequency\ \% = \frac{Nbr\ of\ samples\ infected\ with\ fungi\ Genus}{Nbr\ of\ samples\ analyzed} \times 100$$

$$Relative\ Density\ \% = \frac{Nbr\ of\ fungi\ species\ isolated\ by\ Genus}{Total\ Nbr\ fungi\ isolated} \times 100$$

After that, each fungal pure culture was identified macroscopically and microscopically based on their cultural and morphological characteristics using fungi identification keys and manuals of Leslie and Summerell, 2006; Pitt and Hocking, 2009. The origin of each purified isolate was given in (Table 2).

Screening Fusarium graminearum *chemotype DON*

Spore suspension (10^{-4} spores/ml) of F. graminearum isolates were inoculated in 8 ml of GYES broth and further incubated at 25 °C for 10 days (Boutigny et al., 2009). Afterwards, 4 ml of the culture medium was removed by centrifugation and added to 8 ml of acetonitril for DON extraction. The extracts were further purified by immunoaffinity columns (I Clean C + DON) as indicated in the instructions provided by the manufacturer. DON was detected by TLC plate.20 μl of each extract and DON standard (100 mg/ml)

Table 2

Origin of purified isolate

Sample	Fungal purified isolate
Imported soft wheat	*Alternaria infectoria, Alternaria alternata, F. graminearum, F. verticillioides, F. poae, F. solani, Aspergillus clavatus, A. niger, A. fumigatus, A. flavus, A. parasiticus, A. niveus, A. nidulans, P. variabile, P. funiculosum, P. janthinellum, Cladosporium cladosporioides, C. herbarum, P. italicum, P. chrysogenum,*
Local soft wheat	*A. flavus, A. parasiticus, A. niger, Alternaria infectoria, Cladosporium cladosporioides, P. Simplicissimum, P. Expansum, A. nidulans,*
Imported durum wheat	*F. equiseti, F. solani, F. subglutinans, P. fellutanum, P. janthinellum, P. oxalicum, A. parasiticus, A. nidulans,*
Local durum wheat	*A. sydowi, A. oryzea, A. fischerianus, A. flavus, A. parasiticus, A. japonicus, A. nidulans, A. terreus, A. ustus, F. oxysporum, P. chrysogenum, P. italicum, P. paxilli, P. janthinellum, P. expansum, P. thomii, P. citreonigrum, P. glandicola, P. digitatum, P. pinophilum, P. oxalicum, P. glabrum, Cladosporium cladosporioides, C. herbarum, Alternaria alternata, Ulocladium botrytis*.

were spotted on the TLC plate and reveled chemically by aluminum chloride after a heating at 120 °C for 10 mn (Balzer et al., 2004). The TLC plates were visualized under ultra violet (UV) light at 365 nm and blue spots appeared.

Screening wheat grains for DON contamination

Quantitative analysis of DON from wheat grains was carried on, according to manufacturer instructions of ELISA Kit for detection of DON (Celer DON V3. Code MD 101).

Statistical analysis

Three independent experiments were performed for all assays of fungal load and mean values ± standard deviation (SD) were calculated.

Results

Fungal contamination

In this study, fungal load in the samples was enumerated and given in (Table 3). The results show that 98.44% of analyzed samples were positive for fungal contamination. Imported soft wheat samples had the highest total fungal contamination (4342×10^2±cfu/g) which oscillated between a maximum of $1199.5 \times 10^2 \pm 89.44$ cfu/g (S13) and a minimum of $1 \times 10^2 \pm 1.41$ cfu/g (S31), followed by local soft wheat (1066.5×10^2 cfu/g in the range of $315 \times 10^2 \pm 190.92$ cfu/g for S6 to $69.5 \times 10^2 \pm 95.46$ cfu/g for S1), and imported durum wheat (672.5×10^2 cfu/g in the range of $583.5 \times 10^2 \pm 805.39$ cfu/g for S60 to $7 \times 10^2 \pm 1.41 \pm$ cfu/g for S63), and finally by local durum wheat which contains the

Table 3

Fungal load of all genuses per sample collected from different type of wheat grains

Type of Wheat	Sample	Fungal load (UFC×10²/g)*					
		Total Flora	*Alternaria spp.*	*Aspergillus spp.*	*Cladosporium spp.*	*Fusarium spp.*	*Penicillium spp.*
Local soft wheat	S1	69.5± 95.46	/**	33.5 ± 44.55	5.5 ± 7.78	/	30.5 ± 43.13
	S2	106± 149.91	/	51 ± 72.12	/	/	55 ± 77.78
	S3	238± 159.81	/	217.5 ± 159.1	5 ± 7.07	/	15.5 ± 7.78
	S4	162± 214.96	/	36 ± 49.50	15 ± 21.21	/	111 ± 144.25
	S5	176± 248.90	/	45.5 ± 64.35	20 ± 28.28	/	110.5 ± 156.27
	S6	315± 190.92	/	140 ± 56.57	/	/	175 ± 134.35
Total		**1066.5**	**00**	**523.5**	**45.5**	**00**	**497.5**
Imported soft wheat	S7	**100% Rhizopus**	/	/	/	/	/
	S8	122.5± 43.13	/	56.5 ± 6.36	15 ± 7.07	5 ± 7.07	46 ± 35.36
	S9	121± 83.44	/	70 ± 42.43	/	/	51 ± 41.01
	S10	250± 226.27	/	70 ± 84.85	10 ± 0.	70 ± 56.57	100 ± 84.85
	S11	171.5± 19.09	/	18.5 ± 3.54	55 ± 7.07	88 ± 36.77	10 ± 14.14
	S12	36± 9.90	/	22.5 ± 27.58	11 ± 15.56	/	2.5 ± 2.12
	S13	1199.5± 89.80	/	50 ± 14.14	345 ± 63.64	624.5 ± 181.72	180 ± 42.43
	S14	890± 134.35	/	180 ± 98.99	105 ± 28.28	280 ± 169.71	325 ± 106.06
	S15	715± 7.07	/	160 ± 8.48	/	305 ± 8.48	250 ± 7.07
	S16	**100% Rhizopus**	/	/	/	/	/
	S17	**100% Rhizopus**	/	/	/	/	/
	S18	3± 4.24	/	2.5 ± 3.54	/	/	0.5 ± 0.71
	S19	221.5± 16.26	1.5 ± 2.12	/	220 ± 14.14	/	/
	S20	108.5± 21.92	30 ± 14.14	/	25 ± 7.07	53.5 ± 0.71	/
	S21	44.5± 7.78	20.5 ± 3.54	23.5 ± 3.54	0.5 ± 0.71	/	/
	S22	64± 33.94	/	5 ± 7.07	43.5 ± 19.09	5 ± 7.07	10.5 ± 14.85
	S23	229.5± 19.09	/	/	218 ± 33.94	10 ± 14.14	1.5 ± 0.71
	S24	37.5± 45.96	5 ± 7.07	2.5 ± 3.54	25 ± 35.36	/	5 ± 7.07

Table 3 continued

Type of Wheat	Sample	Fungal load (UFC×10²/g)*					
		Total Flora	*Alternaria spp.*	*Aspergillus spp.*	*Cladosporium spp.*	*Fusarium spp.*	*Penicillium spp.*
	S25	10 ± 14.14	/	5 ± 7.07	5 ± 7.07	/	/
	S26	13.5 ± 19.09	0.5 ± 0.71	/	/	/	13 ± 18.38
	S27	27.5 ± 34.65	1.5 ± 2.12	26 ± 36.77	/	/	/
	S28	5.5 ± 7.78	/	5.5 ± 7.78	/	/	/
	S29	2 ± 1.41	1 ± 1.41	/	/	/	1 ± 0
	S30	11.5 ± 16.26	0.5 ± 0.71	/	/	1 ± 1.41	10 ± 14.14
	S31	1 ± 1.41	1 ± 1.41	/	/	/	/
	S32	35 ± 7.07	5 ± 7.07	5 ± 7.07	5 ± 7.07		20 ± 0
	S33	22 ± 1.41	5 ± 7.07	6.5 ± 4.95	/	10.5 ± 13.43	/
Total		**4342**	**71.5**	**709**	**1083**	**1452.5**	**1026**

Type of Wheat	Sample	Fungal load (UFC × 10²/g)*						
		Total Flora	*Alternaria spp.*	*Aspergillus spp.*	*Cladosporium spp.*	*Fusarium spp.*	*Penicillium spp.*	*Ulocladium spp.*
Local durum wheat	S34	57.5 ± 34.65	5 ± 7.07	5 ± 7.07	36.5 ± 6.36	/	11 ± 14.14	/
	S35	72 ± 15.56	10 ± 0	10 ± 0	25 ± 7.07	5.5 ± 7.78	21.5 ± 0.71	/
	S36	62.5 ± 44.55	/	11 ± 0	35.5 ± 36.06	/	16 ± 8.49	/
	S37	26 ± 7.07	1 ± 0	15 ± 7.07	/	/	10 ± 14.14	/
	S38	30 ± 14.14	5 ± 7.07	/	/	/	25 ± 7.07	/
	S39	41.5 ± 57.98	0.5 ± 0.07	16 ± 23.34	/	/	25 ± 35.36	/
	S40	5 ± 7.07	/	5 ± 7.07	/	/	/	/
	S41	25 ± 21.21	/	25 ± 21.21	/	/	/	/
	S42	25 ± 21.21	/	/	/	/	25 ± 21.21	/
	S43	5 ± 7.07	/	/	/	/	5 ± 7.07	/
	S44	/	/	/	/	/	/	/
	S45	10 ± 14.14	/	/	/	/	10 ± 14.14	/
	S46	0.5 ± 0.71	/	/	/	/	0.5 ± 0.71	/
	S47	25 ± 35.36	/	15 ± 21.21	/	/	10 ± 14.14	/

Table 3 continued

Type of Wheat	Sample	Fungal load (UFC × 10^2/g)*						
		Total Flora	***Alternaria spp.***	***Aspergillus spp.***	***Cladosporium spp.***	***Fusarium spp.***	***Penicillium spp.***	***Ulocladium spp.***
	S48	3 ± 2.12	/	1.5 ± 2.12	0.5 ± 0.71	/	0.5 ± 0.71	0.5 ± 0.71
	S49	2 ± 1.41	/	1.5 ± 0.71	/	/	0.5 ± 0.71	/
	S50	2 ± 2.83	/	/	/	/	2 ± 2.83	/
	S51	**100% Rhizopus**	/	/	/	/	/	/
	S52	1 ± 1.41	1 ± 1.41	/	/	/	/	/
	S53	95 ± 134.35	50 ± 70.71	25 ± 35.36	20 ± 28.28	/	/	/
	S54	100 ± 14.14	20 ± 28.28	25 ± 35.36	25 ± 35.36	/	30 ± 42.43	/
	S55	**100% Rhizopus**	/	/	/	/	/	/
Total		**588**	**92.5**	**155**	**142.5**	**5.5**	**192**	**0.5**
Imported durum wheat	S56	**100% Rhizopus**	/	/	/	/	/	/
	S57	8.5 ± 7.78	/	2.5 ± 0.71	/	/	6 ± 7.07	/
	S58	11.5 ± 3.54	/	/	/	0.5 ± 0.71	11 ± 4.24	/
	S59	**100% Rhizopus**	/	/	/	/	/	/
	S60	583.5 ± 805.39	/	/	/	583.5 ± 805.39	/	/
	S61	**100% Rhizopus**	/	/	/	/	/	/
	S62	55 ± 21.21	/	/	/	5 ± 7.07	50 ± 28.28	/
	S63	7 ± 1.41	/	0.5 ± 0.71	/	6.5 ± 2.12	/	/
	S64	7 ± 7.07	/	/	/	/	7 ± 1.41	/
Total		**672.5**	**00**	**3**	**00**	**595.5**	**74**	**00**

*Result is given as mean value ± SD; **No contaminated sample.

least total fungal load (588×10^2 cfu/g in the range of $72 \times 10^2 \pm 15.56$ cfu/g for S35 to $0.5 \times 10^2 \pm 0.71 \pm$ cfu/g for S46).

The different genus isolated from wheat grains samples and identified were *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Ulocladium* and *Rhizopus*. Moreover, mycological analysis of different samples using plating technique revealed the diversity and differences in frequency and relative density of each genus identified from each type of wheat and each sample.

According to the results summarized in (Table 4), the higher percent frequency in the imported soft wheat grains was 62.96% for *Aspergillus spp.* and 59.26% for *Penicillium spp.* and the least was 40.74% for *Fusarium spp* and *Alternaria spp.* In comparison, local soft wheat samples were 100% contaminated by *Aspergillus* and *Penicillium* species and 66.67% were contaminated by *Cladosporium spp.* Higher frequency for imported durum wheat was observed by *Fusarium* and *Penicillium* genus with 44.44% and the lowest frequency was 2.22% for *Aspergillus spp.* the frequency percent for local durum wheat samples revealed that *Penicillium* genus exhibited the higher rate (68.18%), followed by *Aspergillus* genus (54.55%), *Alternaria* genus (36.36%) and *Cladosporium* genus (27.27%) and the least amounts were shown by *Fusarium* and *Ulocladium* genus (4.55%).

Table 4

Frequency and Relative density of all genuses per type of wheat grains

Fungal Genus	Type of Wheat							
	Imported Soft Wheat		Local Soft Wheat		Imported Durum Wheat		Local Durum Wheat	
	Fr %	RD %**	Fr %	RD %	Fr %	RD %	Fr %	RD %
Alternaria spp.	40.47	1.65	00	00	00	00	36.36	15.73
Aspergillus spp.	62.96	16.33	100	49.09	2.22	0.45	54.55	26.36
Cladosporium spp.	51.85	24.94	66.67	4.27	00	00	27.27	24.23
Fusarium spp.	40.70	33.45	00	00	44.44	88.55	4.55	0.94
Penicillium spp.	59.26	23.63	100	46.65	44.44	11	68.18	32.65
Ulocladium spp.	00	00	00	00	00	00	4.55	0.09

* Fr %: Percent frequency; ** RD%: percent of relative density.

On the other hand, the percent relative density was also calculated for different genus. The results obtained show that the higher relative density observed in imported soft wheat was for *Fusarium spp.* (33.45%), followed by *Penicillium spp.* (23.63%), *Aspergillus spp.* (16.33%) and *Cladosporium spp.* (10.33%) and the lowest was for *Alternaria spp.* (1.65%). On the contrary, in the case of local soft wheat, the highest relative density recorded was for *Aspergillus* and *Penicillium* species with 49.09 % and 46.65% respectively and the least value was for *Cladosporium spp.* (4.27%). In imported durum wheat, the rate of relative density observed was 88.55% for *Fusarium spp.* 11% for *Penicillium spp.* and 0.45% for *Aspergillus spp.* The higher relative density in local durum wheat was revealed for *Penicillium spp.* (32.65%), *Aspergillus spp.* (26.36%), *Cladosporium spp.* (24.23%), *Alternaria spp.* (15.73%) and the least was for *Fusarium spp.* (0.94%) and *Ulocladium spp.* (0.09%).

235 purified isolates were represented by 42 species including: *Alternaria alternata, A. infectoria, Aspergillus clavatus, A. fischerianus, A. flavus, A. fumigatus, A. japonicus,*

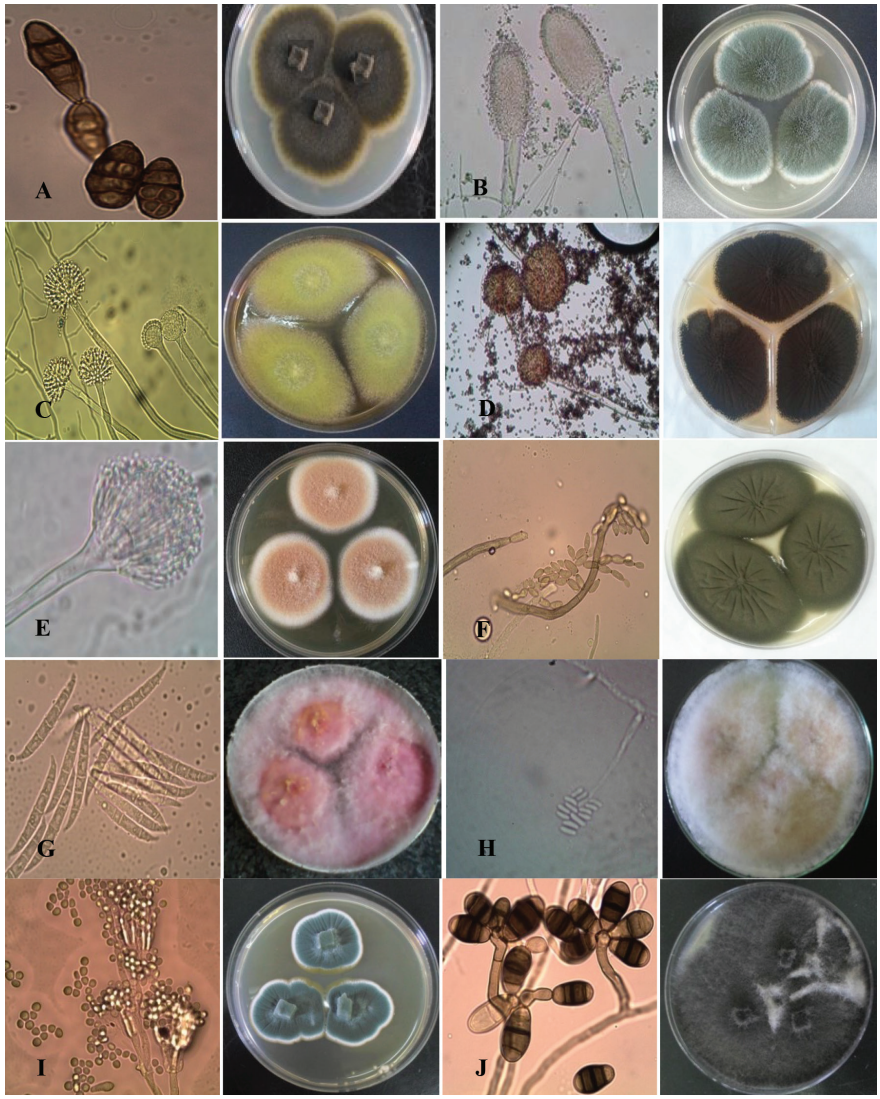

Fig 1. Microscopic (G×100) and macroscopic aspect on MEA plate of some fungi. A. *Alternaria alternata*; B. *Aspergillus clavatus*; C. *Aspergillus flavus*; D. *Aspergillus niger*; E. *Aspergillus terreus*; F. *Cladosporium cladosporioides*; G. *Fusarium graminearum*; H. *Fusarium verticillioides*; H. *Penicillium digitatum* and J. *Ulocladium botrytis*

A. nidulans, A. niger, A. niveus, A. oryzea, A. parasiticus, A. sydowi, A. terreus, A. ustus, Cladosporium cladosporioides, C. herbarum, Fusarium equiseti, F. oxysporum, F. poae, F. solani, F. graminearum, F. subglutinans, F. verticillioides, Penicillium chrysogenum, P. citreonigrum, P. digitatum, P. expansum, P. fellutanum, P. funiculosum, P. glabrum, P. glandicola, P. italicum, P. janthinellum, P. oxalicum, P. paxilli, P. pinophilum, P. simplicissimum, P. thomii, P. variabile and *Ulocladium botrytis*. (Fig. 1) presents some identified fungal species.

Determination of F. graminearum *chemotype DON*

In our comparative study, we state that the imported wheat samples analyzed were highly contaminated by *Fusarium* species, especially, by *F. graminearum* with 65%. Consequently, eight of them were screened for their capacity to produce DON using TLC. Accordingly, for the TLC plate given in (Fig. 2), five on eight isolates were positive for

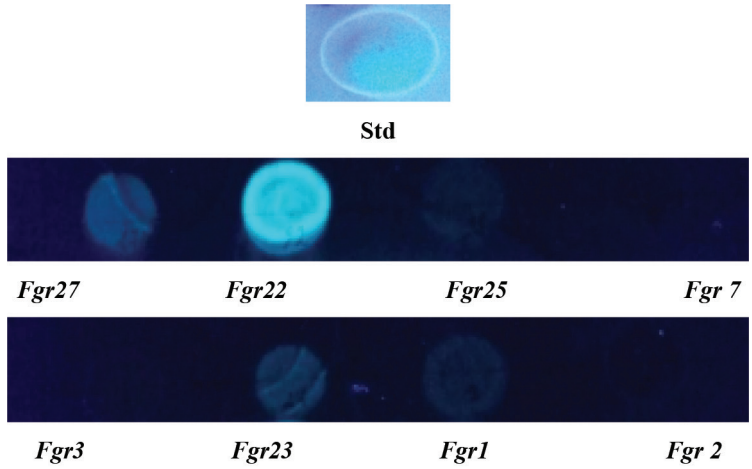

Fig. 2.

Fig 2. TLC analysis of *F. graminearum* chemotype DON. Std. standard (100 mg/ml); Fgr. Different isolates of *F. graminearum*

the deoxynivalenol synthesis. Indeed, it clearly appears that Fgr 22 isolate produce the higher quantity of DON which exceeds 100 μg/ml (in comparison with the control TLC plate), followed by Fgr 27, Fgr23, Fgr 25 and then Fgr1. However, the isolates Fgr2, Fgr3 and Fgr7 showed negative chemotype DON. Therefore, the screening of imported wheat grains contamination with DON was further conducted. It should be noted that local wheat grains were contaminated by one species of *Fusarium* which was *F. oxysporum.*

DON contamination

The 64 wheat samples analyzed by quantitative ELISA kit expose the difference between the quantities of DON. The results revealed that the minimum of DON quantity was inferior to 0.04 ppm (= 40μg/Kg) and the maximum was superior to 5 ppm (=5000 μg/Kg) on soft wheat grains. Furthermore, results on durum wheat grain, the quantities of DON oscillate between 0.12 ppm and 15 ppm (Table 5). Therefore, 62.96% of soft wheat grains and 55.56% of durum wheat imported from France, far exceed the limits required. In comparison, soft and durum wheat samples inspected respect the limits of DON required by the regulation, except for samples n° 36, 37, 38 and 50 which exceed 15 ppm (18.18%).

Discussion

The purpose of this study was, on the one hand, to evaluate the safety of consumed wheat grains in Bechar, and on the other hand, to bring about the novel fungal species isolated from imported wheat and their toxin. The present study revealed that most of local and imported wheat grains samples were contaminated with the common genus of fungi: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Cladosporium*. Indeed, several sci-

Table 5

Quantity of deoxynivalenol (DON) in wheat grain by ELISA test

Type of Wheat	Sample		DON (ppm*)
	Nb	%	
Imported soft wheat	8	12.5	< 0.04
	1	1.56	0.852
	1	1.56	0.970
	1	1.56	1.358
	1	1.56	1.456
	1	1.56	1.498
	1	1.56	1.584
	1	1.56	1.952
	1	1.56	1.963
	1	1.56	2.102
	1	1.56	2.265
	1	1.56	2.365
	1	1.56	2.658
	1	1.56	3.596
	1	1.56	4.267
	5	7.81	> 5
Local soft wheat	3	4.69	< 0.04
	1	1.56	0.154
	1	1.56	0.698
	1	1.56	0.949
Imported durum wheat	5	7.81	< 0.12
	1	1.56	3.657
	3	4.69	> 15
Local durum wheat	11	17.19	< 0.12
	1	1.56	0.178
	1	1.56	0.356
	1	1.56	0.532
	1	1.56	0.548
	1	1.56	0.835
	1	1.56	1.320
	1	1.56	1.528
	4	6.25	> 15

* 1ppm DON = 1μg DON/g = 1000μg/Kg.

entific investigations in cereal fungal contamination were revealed around the world and demonstrated that this contamination was caused especially by the cited genus and the results obtained were in agreement with the previous findings (Ferreira-Geraldo et al., 2006; Pitt and Hocking, 2009; Chehri et al., 2010; Franco et al., 2011; Battilani et al., 2016; Nagaraja et al., 2016; Al-Haik et al., 2017; Djaaboub et al., 2018). According to Klich (2002) fungus propagules get on grain most often with dust from soil, from the surface of plant remnants. Although molds take an important role of the balance of the ecosystem due to the decomposition of plant residues and contribute to the soil fertility (Tančić Živanov et al., 2017), they play a consequential part in the spoilage of strategic food. Thus, fungal contamination is the cause of the loss of 5 to 10% of the world's food production (Pitt and Hocking, 2009; Guimaraes et al., 2018a and b). Additionally, several fungal species can synthesize the toxic metabolites which have a harmful effect on animal and human health. Deoxynivalenol is one of five agriculturally important mycotoxins ubiquitous on grains around the world which is produced by *Fusarium graminearum* strains (Miller, 2016). In this context, the European commission declared in 2003 that this species is one of the most frequently found *Fusaria* on European cereals. Moreover, in recent years, *F. graminearum* chemotype DON was dominant in European cooler wheat-growing areas (Xu et al., 2005; Van der Fels-Klerx et al., 2012). In the present study, the results of fungal investigation demonstrate the dominance and the frequency of *Fusarium* species especially of *F. graminearum* chemotype DON on imported wheat grains. It should be noted that the threshold for DON required by regulation 856/2005 JOCE June, 6, 2005 in cereals intended for human consumption is 1250 μg/Kg and is 1750 μg/Kg for durum wheat (Boutigny et al., 2009). Our data demonstrate that 62.5% of *F. graminearum* isolates tested produce an average of 25 to 200 mg DON/ml. Thus, all of imported samples were contaminated by DON, and 62.96% of soft wheat grains and 55.56% of durum wheat exceeded the maximum allowable limit. Obviously, this confirms results which reported high levels of DON produced in European cereals (Langseth and Rundberget, 1999; Yazar and Omurtag, 2008; Foroud and Eudes, 2009; Boutigny et al., 2009; McCormick et al., 2011). Mastanjević et al. (2018) reported that mycotoxins, such as DON, can resist high temperatures and lower pH values and they are also capable of surviving the drying temperatures during cooking. Otherwise, local wheat grains were predominated by *Aspergillus* and *Penicillium* species with 49.09% and 46.65% respectively on soft wheat and 26.36% and 32.65% respectively on durum wheat. Several studies had reported that these genera were the main contaminants of Algerian grains (Tahani et al., 2008; Riba et al., 2010; Aoues et al., 2017) particularly in subtropical and warm temperate regions such as Bechar district where it is very hot, there is low rainfall and dry conditions. The results obtained were in accordance with those reported by several researchers for cereal grains and seeds (Weidenbörner et al., 2000; Berghofer et al., 2003). Moreover, different studies had revealed the contamination of local grains with aflatoxin B1 and ochratoxin A, and hence, there concentration in the samples is of high significance (Riba et al., 2008). Other genera, such as *Alternaria*, *Cladosporium* and *Rhizopus*, were also reported as prevalent fungi in cereal and wheat (Pitt and Hocking, 2009; Riba et al., 2008 and 2010). The relative density and frequency of different genera of molds and their associated toxins depend to the geographical location, environmental factors (pH, Aw, temperature, substrate), interaction with other microorganisms (bacteria, molds, yeast), cultural and storage prac-

tices (Van der Fels-Klerx et al., 2012; Trabelsi et al., 2017; Chilaka et al., 2018; Vismera et al., 2019). Besides, mycotoxins production depended of a combination of phenotypic plasticity and genetic variation of different isolates of the same species.

In conclusion, we revealed in this study a high frequency and the dominance of *Fusarium* on imported wheat grains. In comparison, with local wheat we note the occurrence of the genus *Aspergillus* and *Penicillium.* Although the percentage of *F. graminearum* chemotype DON was not important, we detected high levels of DON content in French wheat grains. Thus, there is a serious and persistent problem in the processing or in the storage of wheat and its derivates. Consequently, additional investigations on the evaluation of DON levels in imported cereal are necessary to provide more data and to evaluate the exposure of the Algerian population to DON and health risks of this toxin.

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Literature

Aiko, V. and Mehta, A. (2016): Prevalence of toxigenic fungi in common medicinal herbs and spices in India. 3 Biotech. 6, 1–10.

Al-Haik, W. M., Abdullah Bawazir, A. M., Aly, M. M., Al-Haddad, A. M. and Shantaram, M. (2017): Antimicrobial activity of lactic acid bacteria against toxigenic fungi. Int. J. Curr. Res. Aca. Rev. 5, 1–8.

Aoues, K., Boutoumi, H. and Benrima, A. (2017): Phytosanitary status of local durum wheat stored in Algeria. Revue Agrobiologia. 7, 286–296.

Balzer, A., Tardieu, D., Bailly, J. D. and Guerre, P. (2004): The trichothecenes: toxins nature, natural occurrence in food and feeds, and ways of struggle. Revue Méd. Vét. 155, 299–314.

Battilani, P., Toscano, P., Van der Fels-Klerx, H. J., Moretti, A., Camardo Leggieri, M., Brera, C., Rortais, A., Goumperis, T. and Robinson, T. (2016): Afltoxin B1 contamination in maize in Europe increases due to climate change. Scientific Reports. Nature 6, 24328.

Bennett, J. W. and Klich, M. (2003): Mycotoxins. Clin. Microbiol. Rev. 16, 497–516.

Berghofer, L. K., Hocking, A. D., Miskelly, D. and Jansson, E. (2003): Microbiology of wheat and flour milling in Australia. Int. J. Food Microbiol. 85,137–149.

Boutigny, A. L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M. N., Pinson-Gadais, L. and Richard-Forget, F. (2009): Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and Tri gene expression in *Fusarium* liquid cultures. Mycological Reserearch 13, 746–753.

Burgess, L. W., Klein, T. A., Bryden, W. L. and Tobin, N. F. (1987): Head blight of wheat caused by *Fusarium graminearum* Group I in New South Wales in 1983. Australasian Plant Pathology 16, 72–78.

Chehri, K., Salleh, B., Yli-Mattila, T., Soleimani, M. J. and Yousefi, A. R. (2010): Occurrence, pathogenicity and distribution of *Fusarium spp.* in stored wheat seeds Kermanshah Province, Iran. Pakistan J. Biological Sciences 13, 1178–1186.

Chehri, K., Maghsoudlou, E., Asemani M., and Mirzaei, M. R. (2011): Identification and pathogenicity of *Fusarium* species associated with head blight of wheat in Iran. Pak. J. Bot., 43, 2607–2611.

Chen, H. K., Wang, G. and Jiang, X. (1982): Studies on *Fusarium* species infecting spikes of wheat and barley in Zhejiang Province. Acta Phytopathologica Sinica 12, 1–12.

Chilaka, C. A., De Boevre, M., Atanda, O. O. and De Saeger, S. (2018): Quantification of *Fusarium* mycotoxins in Nigerian traditional beers and spices using a multi-mycotoxin LC-MS/MS method. Food Control 87, 203–210.

Djaaboub, S., Moussaoui, A., Meddah, B., Makhloufi, S., Gouri, S. and El Khatib, R. (2018): Antifungal activity of some indigenous lactic acid bacteria isolated from soft wheat. J. Pure and Applied Microbiology. 12, 111–118.

Ferreira-Geraldo, M. R., Tessmann, D. J. and Kemmelmeier, C. (2006): Production of mycotoxins by *Fusarium graminearum* isolated from small cereals (wheat, triticale and barley) affected with scab disease in southern Brazil. Brazilian J. Microbiology 37, 58–63.

Foroud, N. A. and Eudes, F. (2009): Trichothecenes in Cereal Grains. Int. J. Mol. Sci. 10, 147–173.

Franco, T. S., Garcia, S., Hirooka, E. Y., Ono, Y. S. and Dos Santos, J. S. (2011): Lactic acid bacteria in the inhibition of *Fusarium graminearum* and deoxynivalenol detoxification. J. Appl. Microbiology 111, 739–748.

Gilbert, J. and Tekauz, A. (2000): Review: recent developments in research on Fusarium head blight of wheat in Canada. Canadian J. Plant Pathology 22, 1–8.

Guimarães, A., Venancio, A. and Abrunhosa, L. (2018a): Antifungal effect of organic acids from lactic acid bacteria on *Penicillium nordicum.* Food Additives and Contaminants: PART A, 1–16.

Guimarães, A., Santiago, A., Teixeira, J. A., Venâncio, A. and Abrunhosa, L. (2018b): Anti-aflatoxigenic effect of organic acids produced by *Lactobacillus plantarum.* International J. Food Microbiology. 264, 31–38.

Hornok, L. (2007): Sexual and vegetative compatibility/incompatibility in *Fusarium* Species. Acta Phytopathol. et Entomol. Hung. 42, 291–296.

James, A. and Zikankuba, V. L. (2018): Mycotoxins contamination in maize alarms food safety in Sub-Sahara. Africa Food Control 90, 72–81.

Kaktcham, P. M., Zambou Ngoufack, F., Foko Kouam, E. M., Oana, C., Matei, F., Cornea, C. P. and Israel-Roming, F. (2018): Antifungal activity of lactic acid bacteria isolated from peanuts, gari, and orange fruit juice against food aflatoxigenic molds. Food Biotechnology 32, 237–256.

Khalef, A., Zidane, C., Charef, A., Gharbi, A., Tadjerouna, M., Betbeder, A. M. and Creppy, E. E. (1993): Human ochratoxicosis in Algeria. In: E. E. Creppy, M. Castegnaro, G. Dirheimer (eds.): Human Ochratoxicosis and its Pathologies. INSERM/John Libbey Eurotext, Montrouge and France, pp. 123–127.

Kim, H. S., Lee, T., Dawlatana, M., Yun, S. H., and Lee, Y. W. (2003): Polymorphism of trichothecene biosynthesis genes in deoxynivalenol- and nivalenol-producing *Fusarium graminearum* isolates. Mycol. Res. 107, 190–197.

Klich, M. A. (2002): Biogeography of *Aspergillus* species in soil and litter. Mycologia. 94, 21–27.

Korn, U., Müller, T., Ulrich, A. and Herta-Müller, M. E. (2011): Impact of aggressiveness of *Fusarium graminearum* and *F. culmorum* isolates on yield parameters and mycotoxins production in wheat. Mycotox. Res. 27, 195–206.

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

Leslie J. F. and Summerell, B. A. (2006): The *Fusarium* Laboratory Manual. Blackwell Professional, Ames, Iowa and USA. 368 p.

Luz, C., Saladino, F., Luciano, F. B., Manes, J. and Meca, G. (2017): In vitro antifungal activity of bioactive peptides produced by *Lactobacillus plantarum* against *Aspergillus parasiticus* and *Penicillium expansum*. LWT – Food Science and Technology. 81, 128–135.

Malachova, A., Stockova, L., Wakker, A., Varga, E., Krska, R. and Michlmayr, H. (2015): Critical evaluation of indirect methods for the determination of deoxynivalenol and its conjugated forms in cereals. Analytical and Bioanalytical Chemistry 407, 6009–6020.

Mastanjević, K., Šarkanj, B., Mastanjević, K., Šantek, B. and Krstanović, V. (2018): *Fusarium culmorum* mycotoxin transfer from wheat to malting and brewing products and by-products. World Mycotoxin J. 12, 55–66.

McCormick, S. P., Stanley, A. M., Stover, N. A. and Alexander, N. J. (2011): Trichothecenes: from simple to complex mycotoxins. Toxins 3, 802–814.

Miller, J. D. (2016): Mycotoxins in food and feed: A challenge for the twenty-first century. In: D.-W. Li (ed.): Biology of Microfungi, Fungal Biology. Springer International Publishing, Switzerland, pp. 469–493.

Nagaraja, H., Chennappa, G., Poorna Chandra Rao, K., Mahadev Prasad, G. and Sreenivasa, M. Y. (2016): Diversity of toxic and phytopathogenic *Fusarium* species occurring on cereals grown in Karnataka State, India, 3 Biotech. 6, 57.

Nelson, P. E., Plattner, R. D., Shackelford, D. D. and Desjardins, A. E. (1991): Production of fumonisins by strains of *Fusarium moniliforme* from various substrates and geographic areas. Appl. Environ. Microbiol. 57, 2410–2412.

Orlando, B., Grignon, G., Vitry, C., Kashefifard, K. and Valade, R. (2019): *Fusarium* species and enniatin mycotoxins in wheat, durum wheat, triticale and barley harvested in France. Mycotoxin Research 35, 369–380.

Pawlowska, A. M., Zannini, E., Coffey, A. and Arendt, E. K. (2012): Green preservatives: Combating fungi in the food and feed industry by applying antifungal lactic acid bacteria. Advances in Food and Nutrition Research. 5, 215–236.

Pitt, J. I. and Hocking, A. D. (2009): Fungi and Food Spoilage. 3rd ed. Spinger: (Springer Science + Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), 519 p.

Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A. and Sabaou, N. (2008): Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. International J. Food Microbiology. 122, 85–92.

Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrihi, A. and Sabaou, N. (2010): *Aspergillus* section *Flavi* and aflatoxins in Algerian wheat and derived products. Food and Chemical Toxicology. 48, 2772–2777.

Saladino, F., Luz, C., Manyes, L., Fernandez-Franzon, M. and Meca, G. (2016): *In vitro* antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf bread shelf life improvement. Food Control 67, 273–277.

Shephard, G. S., Westhuizen, L. V. D., Gatyeni, P. M., Katerere, D. R., and Marasas, W. F. O. (2005): Do fumonisin mycotoxins occur in wheat? J. Agric. Food. Chem. 53, 9293–9296.

Tahani, N., Serghini-Caid, H., Ouzouline, M. and Elamrani, A. (2008): Mycologia of soft wheat: technological quality of grain and consequences on final products. Reviews in Biology and Biotechnology. BioAlliance Canada-Morocco. 7, 27–32.

Tan, M. K. and Niessen, L. M. (2003): Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of, *Fusarium* species causing crown rot and head blight of cereals Mycol. Res. 107, 811–821.

Tančić Živanov, S., Nešić, L., Jevtić, R., Belić, M., Ćirić, V., Lalošević, M. and Veselić, J. (2017): Fungal diversity as infuenced by soil characteristics. Zemdirbyste-Agriculture. 104, 305–310.

Thiel, P. G., Marasas, W. F. O., Sydenham, E. W., Sheperd, G. S., Gelderblom, W. C. A., and Niewenhuis, J. J. (1991): Survey of fumonisin production by *Fusarium* species. Appl Environ. Microbiol. 57, 1089–1093.

Trabelsi, R., Sellami, H., Gharbi, Y., Krid, S., Cheffi, M., Kammoun, S., Dammak, M., Mseddi, A., Gdoura, R. and Triki, M. A (2017): Morphological and molecular characterization of *Fusarium spp*. associated with olive trees dieback in Tunisia. 3 Biotech. 7, 28.

Van der Fels-Klerx, H. J., De Rijk, T. C., Booij, C. J. H., Goedhart, P. W., Boers, E. A. M., Zhao, C. and Van der Lee, T. A. J. (2012): Occurrence of Fusarium Head Blight species and Fusarium mycotoxins in winter wheat in the Netherlands in 2009. Food Addit. Contam. A. 29, 1716–1726.

Vismera, H. F., Shepharda, G. S., Van der Westhuizenb, L., Mngqawaa, P., Bushula-Njahc, V. and Leslie, J. F. (2019): Mycotoxins produced by *Fusarium proliferatum* and *F. pseudonygamai* on maize, sorghum and pearl millet grains in vitro. International J. Food Microbiology 296, 31–36.

Weidenbörner, M., Wieczorek, C., Appel, S. and Kunz, B. (2000): Whole wheat and white wheat flour-the mycobiota and potential mycotoxins. Food Microbiology.17, 103–107.

Xu, X. M., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G., Cooke, B. M., Doohan, F. M., Brennan, J. M., Moretti, A., Tocco, G., Mule, G., Hornok, L., Giczey, G. and Tatnell, J. (2005): Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. Eur. J. Plant Pathol. 112,143–154.

Yazar, S. and Omurtag, G. Z. (2008): Fumonisins, Trichothecenes and Zearalenone in Cereals. Int. J. Mol. Sci. 9, 2062–2090.

Yli-Mattila, T., Gagkaeva, T., Ward, T. J., Aoki, T., Kistler, H. C. and O'Donnell, K. A. (2009): Novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia 101, 841–852.

Zhang, J. B., Li, H. P., Dang, F. J., Qu, B., Xu, Y. B., Zhao, C S. and Liao, Y. C. (2007): Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycological Research 111, 967–975.