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DEOXYNIVALENOL AND DON – PRODUCING *Fusarium graminearum* ISOLATES IN WHEAT AND BARLEY CROPs IN NORTH AND NORTHWEST AREAS OF IRAN

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Abstract

Deoxynivalenol (DON) contamination of 227 wheat samples from Golestan, Ardabil, Azarbaijan, and 154 barley samples from Golestan province all located in the north and northwest of Iran produced in 2006 were determined using ELISA method. DON was detected in 44.97 % of the wheat samples collected from Golestan province at levels from 18.53 to 192.81 ng g^{-1}, with the average level of 40.99 ng g^{-1}. Also 78.36 percent of Golestan's barley samples were contaminated to DON with the average level of 57.60 ng g^{-1} and at levels from 15.19 to 280.6 ng g^{-1}. DON was not found in wheat samples of west Azarbaijan and only one sample from East Azarbaiejan was contaminated in this year, while it was detected in 92.30% of Ardabil's samples at levels from 32 to 316 ng g^{-1} with the average level of 130.23 ng g^{-1}. DON contamination of wheat and barley produced at north and northwest of Iran in 2006 was much lower than the world regulatory limit for cereals. Thirty five wheat samples collected from Parsabad Moqan area to determine frequency of *Fusarium graminearum* incidence and natural DON contamination levels in 2011 using HPLC - IAC. Mean of the incidence of *F. graminearum* was 70. 91% in irrigated system cultivars and DON was detected in 90% of samples ranged from 1.06 to 2.06 µg $g^{-1,}$ at harvest time and 3.08 to 7.49 µg g^{-1} after storing. DON potential production of 29 *F. graminearum* isolates obtained from wheat of north and northwest ranged from 17.82 to 2397.33 ng g^{-1} .

Keywords: Deoxynivalenol, Wheat, Barley, HPLC, ELISA, *Fusarium graminearum*, Iran.

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Introduction

There is an increasing worldwide awareness of the serious consequences that undesirable levels of mycotoxins may have on human and animal supplies; such as carcinogenic, mutagenic, teratogenic and estrogenic effects (Boutrif & Takeuchi 2010). Trichothecenes are the major Fusarium mycotoxins occurring on a worldwide basis in cereal grains. Deoxynivalenol is one of the trichothecene, which was produced by *Fusarium* species (CAST 2003). *Fusarium graminearum* (teleomorph: *Gibberella zeae*) isolates are concerned as major agents to produce deoxynivalenol (DON), nivalenol (NIV), and their derivatives including 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), 4-acetylnivalenol (4-ANIV), in wheat, barley and corn grains (Kimura *et al*. 2003). Many studies described the deleterious effects of DON on animal and human health. DON consumption causes acute and chronic toxicity and affects animal feeding behavior and immune function (Rotter *et al*. 1996; Lautraite *et al*. 1997; Schlatter 2004; Pestka *et al*. 2004), in domestic or laboratory animals, high doses of, DON caused feed refusal, decreased weight gain, vomiting, gastrointestinal and dermal irritation and immunological alterations (Rotter *et al*. 1996). DON reduces growth and feed consumption (anorexia) at low concentrations in the diet whereas it induces vomiting at higher acute doses (Rotter *et al*. 1996). DON is known to be clastogenic (Knasmuller *et al.* 1997). This mycotoxin is reported to bind to the ribosomal peptidyl-transferase site and inhibit protein and DNA synthesis, consequently exposure results in decreased cell proliferation (Shifrin and Anderson 1999). DON can significantly alter humoral immunity, cell-mediated immunity, and host resistance in a variety of experimental animal models (Pestka and Bondy 1990). Among the trichothecenes, DON is detected most frequently worldwide and in highest concentrations in cereal grains in Poland, Germany, Japan, New Zealand, and the Americas (Bottalico 1998; Placinta *et al*. 1999; Clear & Patrick 2000). DON is primarily produced during the vegetation period by *F. graminearum* and *F. culmorum*, the *Fusarium* species known to produce DON (Desjardins & Proctor 2001). *F. graminearum* Schw. is one of the most frequently found *Fusarium* species on cereals. Also the most common Fusarium mycotoxins in barley grain is deoxynivalenol which occurs more frequent with the concentration higher than other trichothecenes and therefore considered a major food safety issue (Bottalico 1998). *F. graminearum* has a broad host range and can cause Fusarium head blight of wheat and barley often called FHB which has been reported in wheat - growing areas worldwide but is especially prevalent in temperate climates when relatively cool temperatures and weather coincide during flowering stage (CAST 2003). Natural epidemics are often localized and sporadic and thus difficult to predict during early stages of symptom development, infected heads are not easily detected in farm fields because heads may show few symptoms. In severe infections; however, white head can progress throughout the entire head, colonizing kernels and sticks. If wet weather persists, blue- black perithecia and a pinkish mass of mycelium and macroconidia can form on wheat surface. Ascospores and macroconidia produced by *F. graminearum* growing on wheat stalks and other crop residues are the major inocula for wheat head scab. In addition, there was a positive correlation between FHB and rain fall in April. Epidemics also appear to be associated with wet weather late in the growing season (CAST 2003). In general, *Fusarium* species require a high water activity (a_w) to colonize grain usually above 0.90 a_w for growth (Lacey *et al*. 1991). DON levels in kernels of wheat and barley are highly correlated with disease severity ratings and with various measures of fungal load, including colony- forming units; however, even visibly healthy grain can contain significant DON (CAST 2003).

In Iran wheat and barley Fusarium head blight (FHB) were reported as an important wheat disease in Golestan and Mazandaran provinces (Golzar *et al*. 1993; Foroutan *et al* 1993; Zamnizadeh and Khorsandi 1995). Favorable climatic conditions in the north (Golestan) and northwest (Azarbyijan and Ardebil) of Iran causes growth of *Fusarium* species on wheat and barley resulted in epidemic of wheat scab disease (FHB), and DON production (Mirabolfathy 2010).

Since Gholestan and Azarbaiejan areas are located in the high risk areas for cereal DON contamination, this research conducted to determine the natural DON contamination of wheat and barley crop of above areas and evaluate the

DON production potential of *F. graminearum* isolates obtained from wheat product of these areas.

218 wheat samples included 183 from Golestan, 28 from Ardabil, 9 from East Azarbaijan, 6 from West Azarbaijan and 154 barley samples from Golestan provinces collected in 2006 and 35 samples from Parsabad Moqan in Ardabil province collected in 2011 from different fields or local storages. Each 10- 20 kg - sample was included 10 subsamples collected from 10 fields or storages throughout different stages including pre harvest, at harvest, before and after storing for 6 months. Samples were ground and sub sampled with analytical Romer mill series ΠTM, (MO, USA) and mixed well; a 100g-subsample was taken from each ground sample and stored at -20ºC.

Evaluation of occurrence of *F. graminearum* contamination

The frequency of *F. graminearum* infection estimated on winter wheat (*Triticum aestivum*) by investigating on thirty samples collected from Ardabil areas including two kind irrigated and dry land wheat system cultures at harvest time in 2011. Fifty wheat kernels of each sample were sterilized with 1% solution of sodium hypochlorite for 3 minutes. After surface disinfection, kernels were placed directly on potato dextrose agar (PDA) medium. The plates were incubated at 25° C for 7 days; the growing Fusarium isolates were identified morphologically based on macro and microscopic characteristics on CLA, SNA, PDA media (Burgess *et al*. 1994; Nelson *et al*. 1983; Leslie and Summerell 2006). Finally the percent of *F. graminearum* contaminated kernels was recorded.

Evaluation of DON contamination

DON contamination analysis of the samples collected in 2006 was conducted using competitive enzyme immunoassay method and confirmed with HPLC - IAC in 10 percent of the samples.

ELISA

Water suspension of each sample was prepared by adding 100 ml distilled water to 20 g of the ground samples and shaking at 150 rpm for 30 minutes. The suspension was filtrated through No.1 Whatman filter paper. 50µl of each filtrated sample and DON standard solutions including 0 , 3.7, 11.1, 33.3 and 100 ppb dropped in each micro titer wells which were coated with capture antibodies directed against anti – DON antibodies, then peroxidase conjugate DON, monoclonal anti- DON antibody and substrate (tetramethylbenzidine) were added according to manufacture recommendation (R-Bbiopharm kit). The measurement was made photometrically at 450 nm by ELISA-Reader (Bio-Tek ELx 800). The absorbance was inversely proportional to the DON concentration in the samples. For confirmation by HPLC each extracted sample cleaned up by DONPREP immunoaffinity column and DON was estimated by reversed-phase high performance liquid chromatography. The accuracy of the applied method (recovery test) was estimated using 2000 ± 400 ng g^{-1} wheat CRM powder and also two spiked samples were prepared at 500 ng g^{-1} and 1000 ng g^{-1} concentrations.

HPLC analysis

Extraction and clean-up

DON quantitative analysis of all wheat samples collected in 2011 and 10 percent of wheat and barley samples collected in 2006 evaluated using reversed-phase high performance liquid chromatography (HPLC) - IAC method, 200 ml de-ionized water was added to 25 g of each ground sample accompanied with 2 g NaCl and shook for 30 min at 150 rpm by Gallenkamp shaker. Samples were filtered through Whatman No.1 filter paper, 10 ml of the filtrate was filtered through GF/A glass microfiber filter, and then 2 ml of the extract (equivalent to 0.25 g sample) was passed through the DONPREP immunoaffinity column at a flow-rate of about 1 drop/ second. For high level contaminated samples collected in 2011, 2ml of ten time diluted of each extract passed through the immunoaffinity column. The column was rinsed with 5 ml de-ionized water at the same flow rate; DON was eluted with 1.5 ml methanol and collected in a 4 ml-cleaned dark glass vial. The eluted DON was evaporated under nitrogen stream at 40ºC and dissolved in 1 ml of the HPLC mobile phase solution (acetonitrile: methanol: water, 6: 6: 88 V/V).

A high performance liquid chromatography system equipped with auto sampler (Waters 717), binary HPLC pump (Waters 1525) and a dual λ absorbance UV detector (Waters 2487), was used for the analysis. The reverse phase column was a Waters Nova-pak® C-18, 3. 9 mm×250 mm, 4 µm particle size (Waters Milford, MA, USA) at 40°C. A mixture of water: acetonitrile: methanol (88:6:6;

V/V) at a flow rate of 1.0 mL min^{-1} was used as mobile phase in isocratic elution. The detection was performed at the wavelength of 218 nm (Karami-Osboo *et al*. 2010).

The quantification of DON was estimated by measuring the area under the chromatogram at the DON retention time compare with the relevant calibration curve obtained from the same experiment.

Reagents and standards

Standard of DON was purchased from Sigma (St. Louis, MO, USA). Stock solution was prepared by dissolving the solid standard in methanol(1mg ml^{-1}). Acetonitrile, methanol and water were analytical or HPLC grade were purchased from MERCK Company (Germany), DONPREP immunoaffinity columns and Certified Reference Material (CRM) were obtained from R-BIOPHARM RHONE LTD (Glasgow, Scotland). Filter papers (Whatman No.1) and glass microfiber (GF/A) were from Whatman (Maidstone, UK.).

DON- producing isolates

To determine DON-producing potential of *F. graminearum* isolates, wheat grain samples which collected from infected wheat fields of North and North West of Iran in the spring of 2006 were sterilized with 1% solution of sodium hypochlorite for 3 minutes. After surface disinfection, kernels were placed directly on potato dextrose agar (PDA) medium. The plates were incubated at 25° C for 7 days; the growing Fusarium isolates were identified morphologically based on macro and microscopic morphological characteristics on CLA, SNA, PDA media (Burgess *et al*. 1994; Nelson *et al*. 1983; Leslie and Summerell 2006). The isolates were kept on PDA in 5ºC. Thirty representative *F. graminearum* isolates selected randomly and sub cultured on potato dextrose agar and incubated at 25° C for 7 days. To check DON production, the isolates were grown on the rice powder media (rice powder was blank of DON based on HPLC-IAC tests which was done before). To prepare the sterilized rice medium, 5 ml distilled water added to 5 g DON - blank rice powder in each plate and autoclaved for two consecutive days in 121 ºC for 15 minutes at 15 psi. Five agar plugs (5×5mm) of each isolate plated on each plate. After 10 days incubation at 25ºC, the plate content crushed with 15 ml sterilized distilled water using a mortar and pestle and shook for 30 min at 100 rpm, Extracts were filtered using Whatman No 1 filters and analyzed for DON using R-Bbiopharm ELISA kit as described before. For checking the accuracy of the used method, DON amounts of culture - extracts of three isolates (G17= 27.9, G6 = 2357, G19= 2397) were determined using HPLC-IAC method as described before.

Recovery test for HPLC method

To prepare 500 and 1000 ng g^{-1} spiked samples, appropriate amount of standard solution (10 µg ml^{-1}) of DON was added to 25 g of a blank wheat sample and left for one hour to evaporate solvent prior to extraction. Extraction and clean up were carried out as the same for the other samples and CRM.

Result

Accuracy of the methods

Average recovery levels in ELISA were 83.8% for 500 ng g^{-1} and 88.9% for 1000 ng g^{-1}(Table 1) and in HPLC method mean recovery tests for 500 and 1000 ng g^{-1} spiked sample were 93.5 and 73.5 percent (n=5) respectively (Figure I). Detection limit was 10 ng g^{-1}. A positive correlation was found between all points of standard curve in range 50 to 10000 ng ml^{-1}, it also has a good linearity with R^2=0.9999

DON-producing potential of *F. graminearum* isolates

To clarify differences among *F. graminearum* isolates from different geographical zones, the DON- producing potential of 30 isolates collected from North and North West wheat fields were investigated. All of *F. graminearum* isolates which were the dominant species were obtained from infected wheat kernels produced DON. The isolates possessed great variation in quantity of DON production ranged from 17.82 to 2397.33 ng g^{-1}. Ardabil's isolates produced less DON than Golestan's isolates. The mean of DON amount production of Golestan isolates was 421.85 ng g^{-1} and for Ardabil was 112.22 ng g^{-1} (Table 2).

Natural DON contamination of wheat and barley in 2006

The results of DON analysis in wheat and barley samples produced in 2006 revealed that the mean of

Table 1. The result of recovery test obtained from spiked and CRM (Certified Reference Material) samples

	Number of test	Mean of contamination(µg/g)	Mean of recovery(µg/g)	Standard devision	Related standard division%	Recovery %
CRM	4	2.000	1.947	0.15	7.6	97.3
SPIKE(500ppb)	5	0.500	0.445	0.07	14	88.9
SPIKE(1000ppb)	2	1.000	0.838	1.4	0.003	83.8

Table 2. DON production (ng/g) of wheat *F. graminearum* isolates collected from different Golestan and Ardabil provinces

Code	Location	Area	DON/(ng/g)
G-1	Golestan	Ali abad	44.69
G-2	Golestan	Kord Kooy	116.10
G-3	Golestan	Kord Kooy	90.01
G-4	Golestan	Ali abad	9.96
G-6	Golestan	Fazel Abbad	2357.89
G-7	Golestan	Gorgan	44.96
G-8	Golestan	Kord Kooy	17.82
G-9	Golestan	Khanbebin	89.38
G-10	Golestan	Ali abad	58.09
G-11	Golestan	Azad Shahr	48.76
G-12	Golestan	Gonbad	44.77
G-13	Golestan	Ramian	29.74
G-14	Golestan	Ramian	182.47
G-15	Golestan	Gonbad	41.33
G-16	Golestan	Ali abad	56.29
G-17	Golestan	Gorgan	27.93
G-18	Golestan	Ali abad	1489.80
G-19	Golestan	Ali abad	2397.33
G-20	Golestan	Gonbad	466.35
G-21	Golestan	Azad shahr	33.29
G-22	Golestan	Kord Kooy	1930.62
G-23	Golestan	Gonbad	49.98
G-24	Golestan	Gorgan	41.70
G-25	Golestan	Kordkoy	1236.81
G-26	Golestan	Ali abad	107.65
A-1	Ardabil	Moghan	38.86
A-2	Ardabil	Moghan	116.88
A-3	Ardabil	Moghan	50.3
A-4	Ardabil	Moghan	242.85

incidence of DON contamination for wheat and barley in Golestan province were 44.97 % and 78.36% respectively. The contamination levels were 18.53 to 192.81 ng g^{-1} for wheat and 15.19 to 280.6 ng g^{-1} for barley (tables 3 and 4).

The mean of DON contamination (ng/g) and the proportion of contaminant samples (%), throughout different stages for wheat and barley crop of Golestan province in 2006 were showed in table 5.

None of sample collected from West Azarbaiejan was contaminated and only one sample from East Azarbaiejan was contaminated in this year (tables 6 and 7), while DON was detected in 92.30% of Ardebil's samples at levels from 32 to 316 ng g^{-1} (tables 8). Fusarium head blight (FHB) or scab is a destructive disease of barley in many countries. A better understanding of the interrelationships between plant traits and FHB

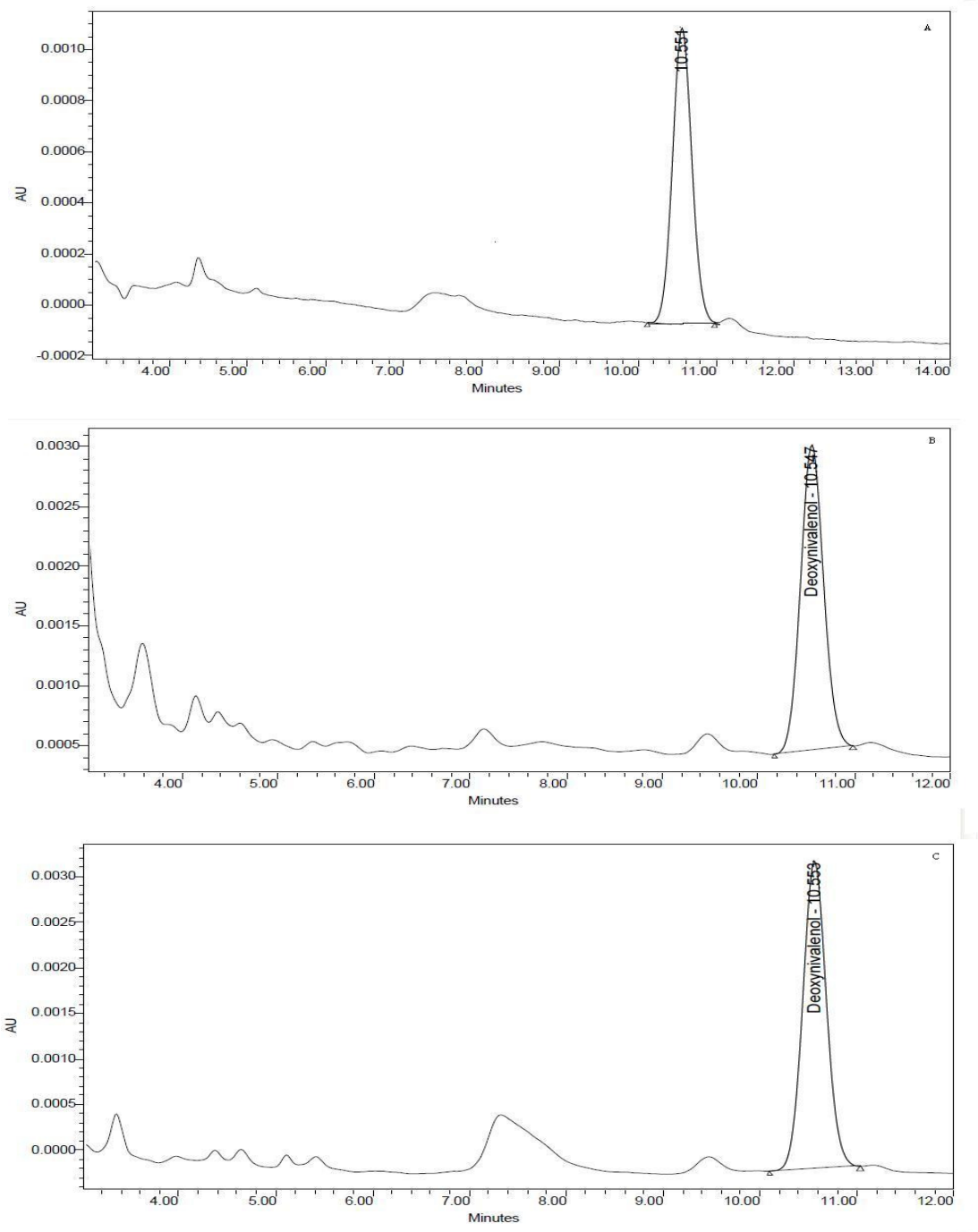

Fig. 1. HPLC chromatograms of (A) Deoxynivalenol standard 250 ng ml^{-1}, (B) wheat CRM 2000± 400 ng g^{-1} (C) wheat sample. HPLC condition, column: 250× 3.9 mm, C18 reverse phase ODS, 4 µm; mobile phase; acetonitrile: methanol: water (6: 6: 88); flow rate: 1.0 ml min^{-1}; UV detector: 218 nm.

Table 3. DON contamination of wheat crop produced in different areas during different stages in Golestan province in 2006

Locations	Number of samples	Number of non contaminated samples	Mean and range of contamination (ng/g)	
		Preharvest		
Gonbad	5	5	ND*	
Kord-koy	5	1	131.3	
Gorgan	3	0	129.27	
Kalaleh	5	4	51.65	
Daland	2	1	26.2	
Ramian	3	2	28.17	
Aq-Qala	3	3	ND	
Total for prehawet	26	16	52.37	ND*-193
		At harvest		
Gonbad	14	7	49.62	
Torkaman	1	0	18.59	
Kord-koy	8	4	71.9	
Gorgan	6	2	70.74	
Kalaleh	14	11	22.5	
Ramian	7	1	34.1	
Aq-Qala	8	5	36.32	
Total for harvort	58	30	43.39	18-140
		Before storage		
Ali-abbad	5	0	50.5	
Gonbad	12	10	37.9	
Gorgan	6	1	76.76	
Aq-Qala	1	0	38.5	
Ramian	1	0	ND	
Total for prestorage	20	11	38.29	ND-89
		After storage		
Gonbad	14	8	35.68	
Gorgan	16	5	40.31	
Ali abaad	22	6	42.1	
Golestan	12	10	23.81	
Aq-Qala	5	4	24.3	
Minoodasht	5	5	ND	
Kord-koy	5	3	43.1	
Total for post-storage	79	41	29.9	ND-82
Total wheat	183	98	40.99	

*ND= Non detected

Table 4. DON contamination of barley crop produced in different areas during different stages of Golestan province in 2006

Locations	Number of samples	Number of non contaminated samples	Mean and range of contamination (ng/g)	
		Pre harvest		
Aq-Qala	4	0	64.31	
Total for the stage	4	0	64.31	22 - 114
		At harvest		
Gonbad	13	4	59.93	
Torkaman	13	0	74.09	
Kalaleh	8	5	33.22	
Aq-Qala	14	0	81.88	
Total for the stage	48	9	62.28	19-166
		Before storage		
Torkaman	12	2	138.21	
Gonbad	11	2	39.15	
Gorgan	1	0	159.96	
Aq-Qala	8	2	65.17	
Kalaleh	5	3	19.76	
Chenaran	2	2	ND	
Maraveh	1	0	16.83	
Total for the stage	40	11	62.72	ND - 280
		After storage		
Gonbad	21	10	56.72	
Kalaleh	7	6	22.12	
Gorgan	6	6	ND	
Aq-Qala	14	3	61.22	
Torkaman	14	0	65.46	
Total for the stage	62	25	41.10	ND - 124
Total barley	154	45	57.60	

*ND= Non detected

resistance should help in the development of effective and efficient breeding strategies for FHB-resistant cultivars. Choo *et al*. (2004) studies indicated that many of the quantitative trait loci (QTL) for FHB resistance coincide with the QTL for plant height, heading date, and spike characteristics. Clear *et al*. (1997) showed that a major portion of mycotoxins accumulated in the hulls of barley. The contamination levels of wheat and barley produced at North and North West areas of Iran in 2006 were lower than the advisory level for DON in the world (ISIRI, 2002).

Natural DON contamination of wheat produced in 2011 at Ardabil province

In 2011 DON was detected in 90% of samples collected from Ardabil area at harvest time ranged from 1.06 to 2.06 µg g^{-1}, with the average amount of 1.7 µg g^{-1} (table 9). In the spring of this year the exceptional environmental conditions including heavy rain, high humidity for more than 3 days, and 20- 22˚C temperature occurred at wheat flowering time, which was favorable to outbreak Fusarium head blight on sensitive cultivars resulted in high levels of DON production in wheat at fields.

DON levels of samples collected after two

Table 5. The mean of DON contamination (ng/g) and the proportion of contaminant samples (%), throughout different stages for wheat and barley crop of Golestan province in 2006

Crops	Pre harvest			At harvest			Before storage			After storage		
	NS	CS	MC	NS	CS	MC	NS	CS	MC	NS	CS	MC
Wheat	26	38.5	52.37	58	48.3	43.4	20	45	38.3	79	48.1	29.9
Barley	4	100	64.31	48	81.25	62.28	40	72.5	62.72	62	59.7	41.10

NS= Number of samples, CS= contaminated samples (%), MC= Mean of contamination (ng/g)

Table 6. DON contamination of wheat crop (ng/g) produced in different areas of West Azarbaijan province in 2006

Location	Amount of contamination	Number of samples
Mahabad	ND*	1
Makoo	ND	1
Salmas	ND	1
Orumieh	ND	1
Miandoab	ND	1
Bokan	ND	1

*ND= Non detected

Table 7. DON contamination of wheat crop (ng/g) produced in different areas of East Azarbaijan province in 2006

Location	Amount of contamination	Number of samples
Varzaghan	ND*	1
Azar shahr	ND	1
Till	ND	1
Bandar sharafkhaneh	ND	1
Ahar	ND	1
Sarab	67.6	1
Sarab	ND	1
Sarab	ND	1
Ajab-Shir	ND	1

*ND= Non detected

Table 8. DON contamination of wheat crop (ng/g) produced in different areas of Ardabil province in 2006

Location	Mean of contamination (ppb)	Numvber of samples
Aslandouz	51.25	6
Pars abad	224.7	5
Germi	26.4	2
Bilesavar	32	4
Namin	316.8	7
Ardabil	ND	1
Jafar abad	ND	1
Meshkin shahr	ND	1
Kivi Kousar	ND	1

Table 9. *F. graminearum* (%) and DON contamination (µg/g) of wheat crop produced in 2011 in Pars abad and Bilesavar of Ardabil Province

samples	Location	Variety	*F. graminearum* (%)	DON (µg/g)
1	Bilesavar Jafar abad	N8019	46	1.72
2	Pars abad Azar Moqan	N8019	54	1.80
3	Pars abad Oultan	N8019	90	1.72
4	Pars abad Haji Avaz	N8019	20	1.89
5	Pars abad Azar Moqan	N8019	60	1.96
6	Pars abad Takle	N8019	54	1.90
7	Bilesavar Rouh kandey	N8019	90	1.31
8	Bilesavar Rouh kandey	N8019	50	1.96
9	Bilesavar Rouh kandey	N8019	64	1.94
10	Bilesavar Rouh kandey	N8019	64	1.74
11	Parsabad Azar Moqan	N8019	66	1.57
12	Azar Moqan	N8019	64	1.92
13	Bilesavar Rouh kandey	Atila 50	3	ND
14	Pars abad Karim nejad	N8019	86	1.79
15	Pars abad Haji Avaz	N8019	74	1.63
16	Pars abad Oultan	N8019	90	1.90
17	Pars abad Nasir pour	N8019	100	1.84
18	Pars abad Haji Avaz	N8019	76.7	1.14
19	Pars abad Azar Moqan	Shiroudi	60	0.84
20	Pars abad Azar Moqan	Shiroudi	60	1.72
21	Pars abad Azar Moqan	N8019	50	1.06
22	Pars abad Oultan	N8019	84	2.06
23	Pars abad Oultan	Chamran	94	1.20
24	Karim nejad	N8019	90	1.08
25	Bilesavar Jafar abad	Kouhdasht	13	ND
26	Bilesavar Jafar abad	N8019	70	1.16
27	Bilesavar Jafar abad	Zagros	16	ND
28	Bilesavar Jafar abad	N8019	93	1.81
29	Pars abad Oultan	Kouhdasht	9	0.14
30	Parsabad Molla kandi	N8019	94	1.24

Table 10. .DON contamination of wheat stored at Parsabad storages for 3 months in 2011

Location	Oultan	Tazekandi	Taavoni	Goushlou	Oultan
DON (ng/g)	4491	7486	3085	3524	5751

months storing ranged from 3.08 to 7.49 µg g^{-1}, with the average amount of 4.87 µg g^{-1} which were 3 to seven time of the world regulatory limit for cereals (1000ng $g^{-1)}$ (table 10). High humidity (more than 13%) and a_w (0.97- 0.99) of kernels in the local traditional storages of Ardabil area increased DON contamination during storage period.

Frequency of *F. graminearum* incidence

High FHB disease severity occurred in 2011 throughout Ardabil province. The incidence of *F. graminearum* contamination in wheat samples collected from dry–land areas were 3, 11 and 16 % for cultivars Atila, Kouhdasht, and Zagros respectively which were the lowest incidence. On

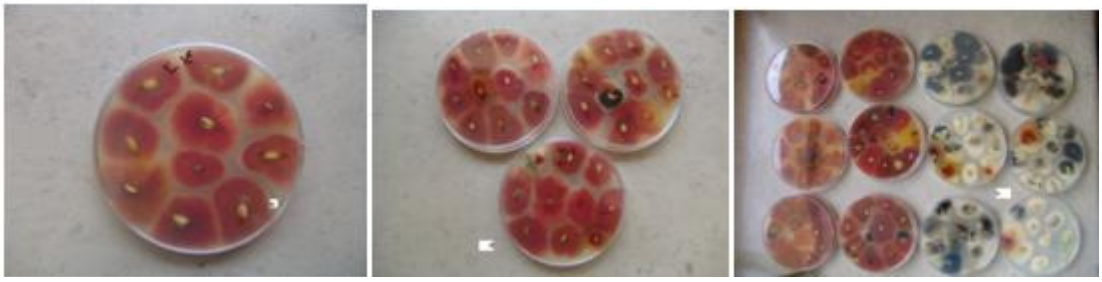

Fig. 2. comparing the *F. graminearum* incidence frequency of two kinds of irrigated (a,b) and dry- land wheat (c) cultivars produced in Parsabad and Bilesavar of Ardabil Province in 2011

the other hand under the same environmental conditions the incidence of *F. graminearum* was high in irrigated system cultivars including N8019, Chamran and Shiroudy with the range from 20 to 100 percent and the mean of 70. 91% (table 9 and fig. 2).

Three to 100% of grains were infested in 2011 at climatologically differing localities Parsabad. Disease progress was accelerated by rainfall during the flowering season. The species most frequently isolated was *F. graminearum*. The mean deoxynivalenol (DON) content varied from 224.7 ng kg^{-1}in 2006 to 1.9 µg kg^{-1}in 2011 which was correlated with disease severity. The wheat varieties cultured in dry land wheat farming systems showed lower rates of infection to Fusarium head blight and lower DON contamination (Table 9) than irrigated farming system's varieties. In Parsabad wheat fields the FHB infections were sever and white head was progressed throughout the entire head, colonizing kernels and sticks and a pinkish mass of mycelium and macroconidia were formed on wheat surface.

Wide studies have been conducted on *F. graminearum* as the causal agent of Fusarium head blight diseases of wheat and barley throughout wide areas in Iran. Residues of infected wheat, barley and corn play important role as the inoculums sources of infection, especially whereby wheat and barley are planted in rotation with corn in the majority of fields in these areas of Iran. Monitoring of *F. graminearum* spores at the time of wheat flowering would identify the best time for spraying fungicides (CAST 2003) result in inhibition of DON production in wheat and barley.

During 1990-92 because of the favorite environmental conditions for Fusarium head blight disease in Iran, wheat scab became epidemic and caused considerable losses. *F. graminearum, F. culmorum*، *F. semitectume* and *F. proliferatum* were isolated from infected plants (Golzar *et al*. 1998; Zamani-Zadeh & Forutan 1992).

The severity of Fusarium head blight and DON levels differed considerably between years, reflecting climatic effects. In a given year, the severity of Fusarium head blight also differed between cropping systems. The level of grain contamination by mycotoxins therefore depends on both climate and cropping system.

Discussion

In this research natural contamination of DON was determined in wheat and barley crops of North and North West of Iran where the climatic conditions are favorable for Fusarium head blight spreading rapidly and extensively in spring resulted in DON production in wheat at high levels. Evaluation of DON in wheat and barley samples collected in 2006 indicated that the mean of frequency of DON contamination incidence in Golestan and Ardabil areas where located at high-risk areas to incident for Fusarium head blight was 59.7% (218 out of 365 samples).

It seems the controversy of the results of 2011 with the results obtain from 2006 is related to environmental conditions. Climate is thought to be a key determinant of Fusarium head blight (Langseth *et al*. 1995; Lacey *et al*. 1999; Kriss *et al*. 2012).

The sensitive wheat variety, N8019, which was cultured at the majority of irrigated system fields in Ardabil seems to be the main reason because under the same favorable environmental conditions for wheat scab disease outbreak at this high-risk area in this year, DON was not detected in the wheat yielded from dry land varieties (Kouhdasht, Zagros and Atila). In 2011 the symptoms of FHB was sever at field in Ardabil which seems it was

correlated with DON accumulation, but there is a need to develop a better understanding of resistance to toxin accumulation and rapid screening methods for this trait (Sneller *et al*. 2012). On the other hand *F. graminearum* wheat isolates collected from wheat fields throughout North and North West provinces of Iran possessed great variation in quantity of DON production (Table 1). It's supposed the outbreak of high DON - producing or high virulent *F. graminearum* populations were endemic in 2011 year which could be the main reason for high DON contamination wheat crop. Within-field variation of F. *graminearum* isolates for aggressiveness and deoxynivalenol productions causes different effect on wheat head blight incident (Talas *et al*. 2012). Based on the Malihipour *et al*. (2012) study who compared the phylogenetic, chemotypic, and pathogenic abilities of 58 isolates from Canada, Mexico, and Iran, Canadian and Iranian isolates clustered in one group and were identified as *F. graminearum* lineage 7 (= *F. graminearum* sensu stricto) within the *F. graminearum* (Fg) clade which were the most aggressive and based on Tri12 gene 25.6% of the Iranian isolates were determined to be NIV chemotype.

In general, *Fusarium* species require a high water activity (a_w) to colonize grain, usually above 0.90 aw for growth (Lacey *et al*. 1991). DON concentrations appear to be low in grain contaminated in the field but can increase in storage if the moisture is greater than 34% (Wilson and Abramson 1992). The amount of DON in wheat increased significantly (from 3ppm to 7 ppm) during storage period at unfavorable local storing condition in Ardabil which indicated that the storage condition plays a vital role to increase the contamination. In 2006 the mean of contamination levels before storing were approximately more than after storage including 38.3 and 29.9ng g-1 for wheat, and 62.72 and 41.10 ng g-1 for barley respectively (Table 4), separating unqualified wheat lots for feed consumption before storage or mixing with wheat products of other provinces could be the reasons caused decreasing the DON contamination levels. An advisory level was not set for raw wheat intended for milling because normal manufacturing practices and additional technology available to millers can substantially decrease DON levels in the finished wheat product. US Food and Drug Administration advisory level for deoxynivalenol in wheat-derived products like flour, bran and germ that may be consumed by human is 1µg ng-1. So the contamination level of Golestan wheat and barley product in 2006 was lower than advisory level for deoxynivalenol. The results from the study of Wegulo *et al*. (2011) indicated that fungicide efficacy in reducing FHB and DON has been greater in moderately resistant cultivars than in susceptible ones. This showed that integrating cultivar resistance with fungicide application can be an effective strategy for management of FHB and DON in winter wheat.

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