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Research Article

AN INVESTIGATION ON THE PATTERN OF DEOXYNIVALENOL (DON) MEAN VALUES IN *ASPERGILLUS* ISOLATES, BASED ON SUBGENUS AND SPECIES CORRELATIONS

***Parvaneh Rahsseparpoor**
Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
**Author for Correspondence*

ABSTRACTS
Mycotoxins are secondary metabolites which produced under optimum conditions on the food stocks of human and animals. Trichotoxins are the greatest important mycotoxins produced through the movalenat pathway. Trichothecene mycotoxins are a group of structurally similar fungal metabolites that are capable of producing a wide range of toxic effects. Although Deoxynivalenol is one of the least acutely toxic trichothecenes, should be treated as an important food safety issue because it is a very common contaminant of grains. Gilan and Mazandaran areas, the North of Iran, are with favorite conditions for *Aspergillus* growth. In present study we will study the production of Deoxynivalenol toxin in cell extract of *Aspergillus* species isolated indigenous in North of Iran. Firstly, sampling, culture and isolation was performed in Gilan and Mazandaran provinces. After using settle plates, recognition of the species, using ELISA, we quantitatively analysed Deoxynivalenol produced by available species. In subgenus, the greatest DON concentration mean values was belonged to Unclassifiable (6.997 ppb) and least concentration was Ornati (2.727 ppb). In species of genus, the greatest DON concentration mean values was belonged to *A. sp*VI (34.935 ppb) and least concentration was *A. carbonarius* (1.036 ppb). Thus it could believed that species of the genus *Aspergillus* have to be more considered as well as the most well known potent DON producers genera such as *Fusarium* spp.

Keyword: *Aspergillus, DON, Subgenus, Species, Iran*

INTRODUCTION

Deoxynivalenol is one of trichothecenes known to be produced mainly by Fusarium species. Vomitoxin (IUPAC name: (3α,7α)-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one), also known as Deoxynivalenol (DON), is a type B trichothecene, an epoxy-sesquiterpenoid, occurs predominantly in grains such as wheat, barley, oats, rye, maize and less often in rice, sorghum and triticales (Gautam and Macky, 2011).
At the cellular level, the main toxic effect is inhibition of protein synthesis via binding to the ribosome. In animals, moderate to low ingestion of toxin can cause a number of as yet poorly defined effects associated with reduced performance and immune function (Rotter *et al.,* 1996.
The main overt effect at low dietary concentrations appears to be a reduction in food consumption (anorexia), while higher doses induce vomiting (emesis) (Rotter *et al.,* 1996).
At low dosages of deoxynivalenol, hematological, clinical, and immunological changes are also transitory and decrease as compensatory/adaptation mechanisms are established, in part because of differences in deoxynivalenol metabolisms, with males being more sensitive than females. The capacity of deoxynivalenol to alter normal immune function know a days has been of particular interests (Rotter *et al.,* 1996).
While in vivo, deoxynivalenol suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects which are similar to human IgA nephropathy (Rotter *et al.,* 1996).
Further toxicology studies and an assessment of the potential of deoxynivalenol to be an etiologic agent in human disease are warranted (Rotter *et al.,* 1996).
FDA's (U.S. Food and Drug Administration) Advisory Levels for Deoxynivalenol (vomitoxin). For grain and grain byproducts destined for beef cattle and feedlot cattle older than four months, as well as for chickens (FDA recommends that commodities containing this level of vomitoxin not exceed 50 percent of the ration for these species) 10 p.p.m. For grain and grain byproducts destined for all other animal species (FDA recommends that commodities containing this level of vomitoxin not exceed 40 percent of the ration) 5 p.p.m.

EU DON Regulatory Levels Based on Tolerable Daily Intake X Uncertainty

Product	Greatestleve(µg/kg)
Unprocessed cereals other than durum wheat, oats and maize	1250
Unprocessed durum wheat and oats	1750
Cereal flour, including maize flour, maize grits and maize meal	750
Breads, pastries, biscuits, cereal snacks and breakfast cereals	500
Pasta (dry)	750
Processed cereal-based food for infants and young children and baby food	200

MATERIALS AND METHODS

Sampling, Culture and Isolation

From the first May to late October (2011) in the provinces of Gilan and Mazandaran, (Northern states of Iran), following the agenda, the sampling process on indoor and outdoor sites by (CBS firms) was performed (Klich, 2002a; Kozakiewicz, 1989; Samson *et al.,* 2001).

A group of sample was applied using settle plates technique by six plates with Malt extract agar, Yest extract agar, Czapek- Yest extract agar, Czapek- agar, Sabouraud dextrose agar and Potato dextrose agar while all impregnated with 100ppm Chloramphenicol and 50ppm tetracycline , a sample group plates were withdrawn after 30, 60, 90 minutes and 15, 30, 60 minutes. All plates were incubated aerobicaly in 25±2°C (Klich 2002a; Kozakiewicz 1989; Odds *et al.,* 1983; Samson *et al.,* 2001).

Till 15 days all plates were investigated for all the young colony to be identified, marked, newly growth colonies are harvested and planted in prepared Malt extract agar, Yeast extract agar, Potato dextrose agar, Corn male agar, Saboraud's dextrose agar, Czapek- Yeast agar and Czapek- Dox agar plates, all the new found mould samples were restored and were followed by prestove program like macro and microscopic properties in the 5, 10, 15 days span and then were recorded (Klich 2002a; Kozakiewicz 1989; Pittet 1998; Rodger 2001).

At the end, of 300 Aspergillus colonies the 150 ones randomly selected colonies transfer to in plates with Malt extract agar, czapek- Doux agar, Czapek- yest extract (with and without sucrose 20%), Czapek- Dox Agar (with and without sucrose 20%) which has been examined for morphological Macro and Microscopic incubation at 37°C and after 3, 7, 14 and sometimes 25 or 30 days examination and simultaneous slide culture from each sample on the Czapek- Dox Agar, Czapek- Yest extract 20% sucrose for growth normaly by perverse model was provided (Klich 2002a, 2002b; Kozakiewicz 1989; Samson *et al.,* 2001).

Morphological Studies

For morphological studies and macro and microscopic photobiometery the front and back of one week or two weeks aged colonies (two to four weeks for black Aspergillusy colonies) were selected. Measureing the width, check out the colors, pigments, and extrolits, taking photographes, cells, and umbrellas, hyphae, stypes, the conidies crown and micrometerics on conidiophores, vesicles and conidies and also the emergence and micrometery of Sclertia or Ascs were done (Klich 2002a; Kozakiewicz 1989; Samson *et al.,* 2001).

Providing Cellular Extracts

A loop full of the mixture of PBS and each isolate in each agar plate been harvested and transferred in to 50ml Falcon tube with a fluid bed Czapek-Dox broth containing one per cent Malt extract agar and then subcultured. With 200 rpm, 25±2°C in and photo periodic conditions incubated and inspected daily (Green *et al.,* 2003; Oda *et al.,* 2006; Odds *et al.,* 1983). After seven days of float or sink in the tubes of fluid and small Germ tube were purified by centrifuging at around 3000 rpm to 15 minutes and cellular biomasses were harvested. Masses washed for three consecutive times with 25ml of PBS with centrifugation (3000 rpm for 15 min), and stoked in a - 20°C were stored (Ausubel *et al.,* 2002; Shadzi *et al.,* 1993). Defrosting the samples soaked in ice fields, 48 hours each in a desiccator and then 2g of it was harvested. Mass of every dry mould filament was mixed 3 times in a 15ml Falcan tube, each time with 3 subsequent replication (each 7 minutes) with 5 ml sampling buffer using a tube mixer and glass globes (pearl) and each time 25 minutes grinding was performed. Mouldy mixture to each tube filtered samples

and one ml of cold acetone added and of around 3,000 Rpm centrifuged (15 minutes) remaining a larger separation deposited (Moallaei *et al.,* 2006; Shadzi *et al.,* 1993). Supernatant samples treated by 1 to 5 ratio with cold acetone and then meintand in a cold 20°C for one to three days and finally were centrifuged at around 20 000 RPM to 20 minutes in the cold- 20°C fridged centrifuged. Deposits and with drawals made from the concentrated samples were diluted in dilution of the concentrated extracts of the same method was applied to all samples (Ausubel *et al.,* 2002; Medina *et al.,* 2005; Puente *et al.,* 1991). Then detection of DON were done by direct competitive ELISA in Aspergillus species using RIDASCREEN® DON (Art. No.: R5906) which is a competitive enzyme immunoassay for the quantitative analysis of DON in feed and foods.

ELISA Assay

As the basis of the test was the antigen-antibody reaction, microtiter wells were coated with capture antibodies directed against anti –deoxynivalenol antibodies used for Deoxynivalenol standards and sample solutions, then deoxynivalenol enzyme conjugate and anti – deoxynivalenol antibodies were added thus Free deoxynivalenol and deoxynivalenol enzyme conjugate to be competed for the deoxynivalenol antibody binding sites (competitive enzyme immunoassay). Anable the same time, the deoxynivalenol antibodies to be also bound by the immobilized capture antibodies. Any unbound enzyme conjugate were then removed in a washing step. Then substrate/chromogen were added to the wells, bounded enzyme conjugate converted the chromogen into a blue product. Addition the stop solution leaded to a color change from blue to yellow. The measurement was made photometrically at 450 nm. The absorbance was inversely proportional to the deoxynivalenol concentration in the samples.

RESULTS

Table 1 and Figure 1 showing statistically analysis and the frequency of identified species. According to table the greatest frequency species was *A. flavus* in contrast *A. af flavus* and *A. sp*V were the lowest frequency species.

Table 1: The frequency of identified species on samples

Species	Count isolates	Percent	Cumulative Percent
A. af flavus	1	.9	.9
A. af nidulans	2	1.9	2.8
A. alliaceus	2	1.9	4.7
A. awamori	3	2.8	7.5
A. candidus	4	3.7	11.2
A. carbonari	6	5.6	16.8
A. flavus	18	16.8	33.6
A. foetidus	4	3.7	37.4
A. fumigatus	5	4.7	42.1
A. melleus	3	2.8	44.9
A. niger	4	3.7	48.6
A. niveus	3	2.8	51.4
A. ochraceus	4	3.7	55.1
A. ostianus	3	2.8	57.9
A. parasiticus	5	4.7	62.6
A. sojae	9	8.4	71.0
A. spIII	7	6.5	77.6
A. spIV	2	1.9	79.4
A. spV	1	.9	80.4
A. spVI	2	1.9	82.2
A. terreus	6	5.6	87.9
A. unguis	4	3.7	91.6
A. wentii	3	2.8	94.4
S. ornata	6	5.6	100.0
Total	107	100.0	

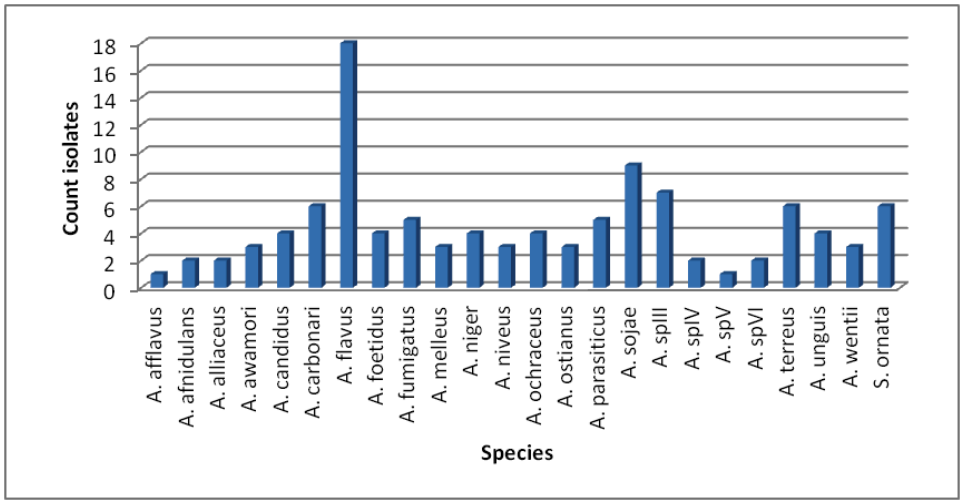

Figure 1: The frequency of identified species on samples

Table 2 and figure 2 showing of totally 107 *Aspergillus* isolates, in the obtained results, the greatest frequency was belonged to subgenus *Circumdati* with 66 isolates (61.7%) and the least frequency of subgenus *Fumigati* with 5 isolates (4.7%).

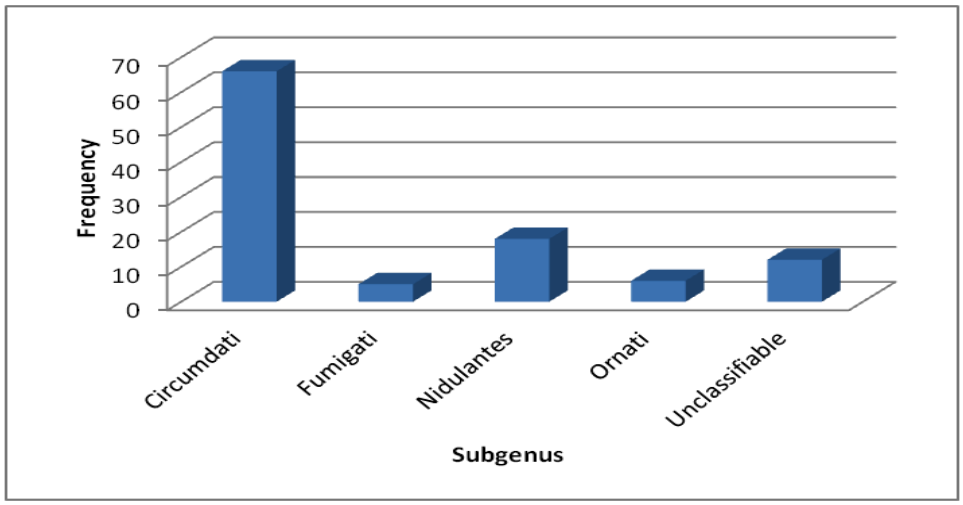

Figure 2: The frequency of *Aspergillus* isolates at the subgenus states

Table 2: The frequency of *Aspergillus* isolates at the subgenus states

Subgenus	Count isolates	Percent	Cumulative Percent
Circumdati	66	61.7	61.7
Fumigati	5	4.7	66.4
Nidulantes	18	16.8	83.2
Ornati	6	5.6	88.8
Unclassifiable	12	11.2	100.0
Total	107	100.0	

Table 3 and figure 3 showing DON concentration mean values in the biomass based on the subgenus. The greatest concentration values was belonged to Unclassifiable (6.997 ppb) and least concentration was Ornati (2.727 ppb).

Table 3: DON concentration mean values in the biomass based on subgenus

Subgenus	Count isolates	DON Mean concentration (ppb)
Circumdati	66	6.58223
Fumigati	5	4.53680
Nidulantes	18	2.90244
Ornati	6	2.72683
Unclassifiable	12	6.97733
Total	107	5.69574

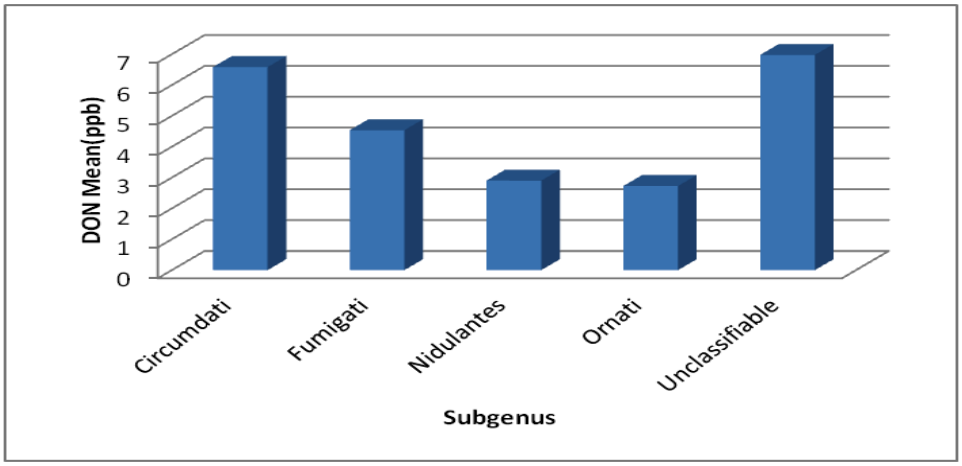

Figure 3: DON concentration mean values in the biomass based on subgenus

Table 4 and figure 4 showing DON concentration mean values in the biomass based on species. The greatest concentration was *A. sp*VI (34.935 ppb) and least concentration was *A. carbonarius* (1.036 ppb). Table 5 showing DON concentration frequency based on the subgenus in the range of 0-100 ppb in the biomass. In subgenus *Circumdati* the greatest frequency of DON concentration was in the range of 0-10ppb (54 isolates) and the least DON concentration frequency was in the range of 60-70ppb (1), in subgenus *Funmagati* the greatest frequency was in the range of 0-10ppb (3) and the least frequency was in the range of 10-20ppb (2), in subgenus *Nidulantes* the greatest frequency was in the range of 0-10ppb (16) and the least frequency was in the range of 20-30ppb (2), in subgenus *Ornati* the greatest and lowest frequencies, were 0-10ppb (5) and 10-20(1) respectively, in subgenus Unclassifiable the greatest and least frequencies were 0-10ppb (11) and 80-90 ppb (1) respectively.

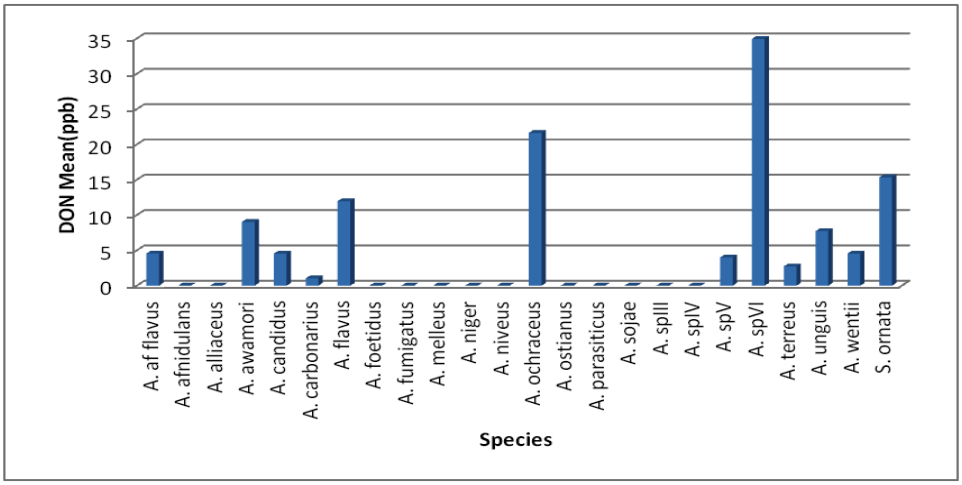

Figure 4: DON concentration mean values in the biomass based on species

Table 4: DON concentration mean values in the biomass based on species

Species	Count isolates	DON Mean concentration (ppb)
A. af flavus	1	4.55511
A. af nidulans	2	.00000
A. alliaceus	2	.00000
A. awamori	3	9.03389
A. candidus	4	4.53680
A. carbonarius	6	1.03633
A. flavus	18	11.96114
A. foetidus	4	.00000
A. fumigatus	5	.00000
A. melleus	3	.00000
A. niger	4	.00000
A. niveus	3	.00000
A. ochraceus	4	21.62800
A. ostianus	3	.00000
A. parasiticus	5	.00000
A. sojae	9	.00000
A. spIII	7	.00000
A. spIV	2	.00000
A. spV	1	3.99925
A. spVI	2	34.93450
A. terreus	6	2.72683
A. unguis	4	7.72633
A. wentii	3	4.54533
S. ornata	6	15.34200
Total	107	5.69574

Table 5: DON concentration frequency based on the subgenus in the range of 0-100 ppb in the biomass

			Biomass/ELISA-DON										
			0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
Subgenus	Circumdati	Count	54	3	2	4	0	0	1	0	2	0	66
		%within Subgenus	81.8%	4.5%	3.0%	6.1%	0.0%	0.0%	1.5%	0.0%	3.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	60.7%	50.0%	100.0%	66.7%	0.0%	0.0%	100.0%	0.0%	66.7%	0.0%	61.7%
		%of Total	50.5%	2.8%	1.9%	3.7%	0.0%	0.0%	.9%	0.0%	1.9%	0.0%	61.7%
	Fumigati	Count	3	2	0	0	0	0	0	0	0	0	5
		%within Subgenus	60.0%	40.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	3.4%	33.3%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
		%of Total	2.8%	1.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
	Nidulantes	Count	16	0	0	2	0	0	0	0	0	0	18
		%within Subgenus	88.9%	.0%	.0%	11.1%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	18.0%	.0%	.0%	33.3%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	16.8%
		%of Total	15.0%	.0%	.0%	1.9%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	16.8%
	Ornati	Count	5	1	0	0	0	0	0	0	0	0	6
		%within Subgenus	83.3%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	5.6%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
		% of Total	4.7%	.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
	Unclassifiable	Count	11	0	0	0	0	0	0	0	1	0	12
		%within Subgenus	91.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	8.3%	0.0%	100.0%
		%within Biomass/ELISA-DON	12.4%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	33.3%	0.0%	11.2%
		%of Total	10.3%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.9%	0.0%	11.2%
Total		Count	89	6	2	6	0	0	1	0	3	0	107
		%within Subgenus	83.2%	5.6%	1.9%	5.6%	0.0%	0.0%	.9%	0.0%	2.8%	0.0%	100.0%
		%within Biomass/ELISA-DON	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%
		%of Total	83.2%	5.6%	1.9%	5.6%	0.0%	0.0%	.9%	0.0%	2.8%	0.0%	100.0%

Table 6: DON concentration frequency in the fungi cell biomasses of the conducted species in the range of 0 to 100 ppb intervals

Species		Biomass/ELISA-DON										Total
		0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	
A. afflavus	Count	1	0	0	0	0	0	0	0	0	0	1
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	1.1%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	.9%
	%of Total	.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	.9%
A. afnidula	Count	2	0	0	0	0	0	0	0	0	0	2
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	2.2%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
	%of Total	1.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
A. alliaceus	Count	1	0	0	0	0	0	1	0	0	0	2
	%within Species	50.0%	.0%	.0%	.0%	0.0%	0.0%	50.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	1.1%	.0%	.0%	.0%	0.0%	0.0%	100.0%	0.0%	.0%	0.0%	1.9%
	%of Total	.9%	.0%	.0%	.0%	0.0%	0.0%	.9%	0.0%	.0%	0.0%	1.9%
A. awamori	Count	3	0	0	0	0	0	0	0	0	0	3
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	3.4%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
	%of Total	2.8%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
A. candidus	Count	3	1	0	0	0	0	0	0	0	0	4
	%within Species	75.0%	25.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	3.4%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
	%of Total	2.8%	.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
A. carbonarius	Count	6	0	0	0	0	0	0	0	0	0	6
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	6.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
	%of Total	5.6%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
A. flavus	Count	16	0	0	0	0	0	0	0	2	0	18
	%within Species	88.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	11.1%	0.0%	100.0%
	%within Biomass/ELISA-DON	18.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	66.7%	0.0%	16.8%
	%of Total	15.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	1.9%	0.0%	16.8%
A. foetidus	Count	4	0	0	0	0	0	0	0	0	0	4
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	4.5%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
	%of Total	3.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%

A. fumigatus	Count	3	2	0	0	0	0	0	0	0	0	5
	%within Species	60.0%	40.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	3.4%	33.3%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
	%of Total	2.8%	1.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
A. melleus	Count	2	1	0	0	0	0	0	0	0	0	3
	%within Species	66.7%	33.3%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	2.2%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
	%of Total	1.9%	.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
A. niger	Count	4	0	0	0	0	0	0	0	0	0	4
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	4.5%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
	%of Total	3.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
A. niveus	Count	1	0	2	0	0	0	0	0	0	0	3
	%within Species	33.3%	.0%	66.7%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	1.1%	.0%	33.3%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
	%of Total	.9%	.0%	1.9%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
A. ochraceus	Count	4	0	0	0	0	0	0	0	0	0	4
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	4.5%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
	%of Total	3.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
A. ostianus	Count	3	0	0	0	0	0	0	0	0	0	3
	%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	3.4%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
	%of Total	2.8%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
A. parasiticus	Count	1	0	2	2	0	0	0	0	0	0	5
	%within Species	20.0%	.0%	40.0%	40.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	1.1%	.0%	33.3%	100.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
	%of Total	.9%	.0%	1.9%	1.9%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	4.7%
A. sojae	Count	7	0	2	0	0	0	0	0	0	0	9
	%within Species	77.8%	.0%	22.2%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
	%within Biomass/ELISA-DON	7.9%	.0%	33.3%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	8.4%
	%of Total	6.5%	.0%	1.9%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	8.4%
A. spIII	Count	6	0	0	0	0	0	0	0	1	0	7
	%within Species	85.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	14.3%	0.0%	100.0%
	%within Biomass/ELISA-DON	6.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	33.3%	0.0%	6.5%
	%of Total	5.6%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.9%	0.0%	6.5%

	A. spIV	Count	2	0	0	0	0	0	0	0	0	0	2
		%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	2.2%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
		%of Total	1.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
	A. spV	Count	1	0	0	0	0	0	0	0	0	0	1
		%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	1.1%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	.9%
		%of Total	.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	.9%
	A. spVI	Count	2	0	0	0	0	0	0	0	0	0	2
		%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	2.2%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
		%of Total	1.9%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	1.9%
	A. terreus	Count	6	0	0	0	0	0	0	0	0	0	6
		%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	6.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
		%of Total	5.6%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
	A. unguis	Count	4	0	0	0	0	0	0	0	0	0	4
		%within Species	100.0%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	4.5%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
		%of Total	3.7%	.0%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	3.7%
	A. wentii	Count	2	1	0	0	0	0	0	0	0	0	3
		%within Species	66.7%	33.3%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	2.2%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
		%of Total	1.9%	.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	2.8%
	S. ornata	Count	5	1	0	0	0	0	0	0	0	0	6
		%within Species	83.3%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	100.0%
		%within Biomass/ELISA-DON	5.6%	16.7%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
		%of Total	4.7%	.9%	.0%	.0%	0.0%	0.0%	.0%	0.0%	.0%	0.0%	5.6%
Total		Count	89	6	6	2	0	0	1	0	3	0	107
		%within Species	83.2%	5.6%	5.6%	1.9%	0.0%	0.0%	.9%	0.0%	2.8%	0.0%	100.0%
		%within Biomass/ELISA-DON	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0 %	0.0%	100.0%
		%of Total	83.2%	5.6%	5.6%	1.9%	0.0%	0.0%	.9%	0.0%	2.8%	0.0%	100.0%

Table 6 the definitive concentration frequency of DON in biomass of the species in the range 0 to 100 ppb indicating that species *A. af flavus* has the only one isolate observed in the range of 0 to 10 ppb, *A. af nidulans* 2 isolates *A. alliaceus* in the range of 0 to 10 ppb and even 60 to 70 ppb has one isolate in each ranged intervals, *A. awamori* showed 3 isolates only at o to 10 ppb the same as *A. candidus* 3 isolates for the range of 0 to 10 ppb and 10 to 20. of *A. carbonarius* 6 isolates observed only in the range of 0 to 10 ppb, *A. flavus* isolates were 16 for 0 to 10 ppb and 2 for 80 to 90 ppb. Isolates of *A. foetidus* were only 4 in the range of 0 to 10 ppb like *A. fumigatus* 3 isolates for the range 0 to 10 ppb and 2 for the range 10 to 20. Reflecting to *A. melleus* 2 isolates were in the range of 0 to 10 ppb and one in the range of 10 to 20 ppb. *A. niger* 4 isolates observed only in the range of 0 to 10 ppb like *A. nievus* one isolate for the 0 to 10 ppb in contrast 2 isolates in the range of 20 to 30 ppb. In the *A. ochraceus* observed cases only 4 in the range 0 to 10 ppb, and *A. ostianus* 3 isolates concomitantly the only one case for *A. parasiticus* in the range of 0 to 10 ppb and 2 isolates for the each range of 20 to 30 ppb and 30 to 40 ppb. About cases *A. sojea* 7 cases in the range of 0 to 10 ppb and 2 isolates in the range of 20 to 30 ppb were observed. In the species of *A. sp* III we observed 6 isolated in the range of 0 to 10 ppb and one in the range of 80 to 90 ppb. *A. terreus* observed isolates were only 4 in the range of 0 to 10 ppb, likely 2 cases of *A. wenti* this range 0 to 10 ppb and one for the range of 10 to 20 ppb. In the species *S. ornata* 5 cases were obtained toxigenic at the range of 0 to 10 ppb and only one isolates in the range of 10 to 20 ppb (Table-2).

DISCUSSION

Along this line, in the study on the relationship of DON mean concentration measured in the subgenera it must be considered that Unclassifiable isolates and subgenera *Circumdati* and *Fumigati* had the greatest number of toxicogenic isolates and provided the highest toxicogenic mean values of DON but significant difference was not observed in the correlation of above mentioned variables (table 3, figure 3).

In addition, in the study on the DON concentration mean in the biomass, based on the species from Unclassifiable isolates, isolate 6 (*A. sp*VI) produced the DON toxin more than its peers *Aspergillus* isolated of *A. ochraceus* and follow *S. ornata* had the greatest amount of toxin production among the studied isolates and more interestingly, toxin production in the members of subgenus *Circumdati* was lower than mentioned and conducted species which are more reliable for toxin production and have the most isolates (table 4, figure 4).

Amongst identified species the Maximum produced DON were drived by species graups; *A. sp* VI with 2 isolates (34.93 ppb), *A. ochraceus* with 5 isolates (21.62 ppb) and *S. ornata* with 3 isolates (15.34 ppb). Minimum DON production observed in the *A. carbonarius* with 6 isolates (1.03 ppb), *A. terreus* with 6 isolates (2.72 ppb), *A. sp* V with 3 isolates (3.99 ppb) concomitantly and DON intermediate producers were only the *A. flavus* with 7 isolates (11.96 ppb), *A. awamori* with 18 isolates (9.03 ppb), and *A. unguis* with 3 isolates (7.72 ppb). In identified species *A. af nidulans*(with 2 isolates), *A. alliaceus*(6 isolates), *A. foetidus* (3 isolates) *A. fumigatus*(4 isolates), *A. melleus*(2 isolates), *A. niger*(4 isolates)*A. niveus*(3 isolates), *A. ostianus*(1 isolates), *A. parasiticus*(4 isolates), *A. sojae*(2 isolates), *A. sp* III*(*1 isolates), *A. sp* IV (4 isolates) never produced DON (0 ppb) or never detected by theused kit (- ppb). Although the amount of toxin produced in a large number of isolates was trace but the cumulative effect of toxin in the body weight should not be overlooked (Table 6).

Conclusion

Amounts of DON concentration obtained of *Aspregillus* species in our study was not more than FDA's Advisory Levels for DON or level the safe limit for baby foods and young children and level in unprocessed wheat according to the European Commission.

According to amount of DON measured in samples of corn in the presence of toxin-producing *Fusarium* in Golestan and Ardabil (Moqan) Provinces, Iran, Karami-Osboo *et al.*, (2004-2005), 76.7% of samples in were a range of 54.4-518.4ng/g) while and the amount of toxin measured in samples of wheat in Jeddah, Saudi, were in a range of 15 to 800µg/kg in the collected samples in the absence of *Fusarium* specie, Shows that when *Fusaria* are toxin-producing flora toxin then cases of *Aspergilluses* are toxin-producing amounts are more. So our guess that some *Aspergillus* species parallel and play role a Simultaneously the

same as toxigenic Fusarium isolates produce DON is or like toxicants (Karimi-Osboo *et al.,* 2010). So our advantages about DON producing *Aspergilli* could be beleaved simply.

According to the Al-Hazmi's (2010) findings working on wheat samples from Jeddah, Saudi Arabia, could be accepted that without the presence of *Fusarium* species, by which DON and its related compounds might to be produced, have more popularity, even *Aspergillus* species isolates have seriously considered of view to produce DON and same compounds too. So imagine us the fact that proves at the beginning of *Aspergillus* species the ability to produce DON and also determines the amount of toxin produced by them in their biomasses and toxin leakage size or the in growth medium (Al-Hazmi, 2011).

According to the growing time limits of 14 days, has been performed in the lab, the authority of the *Aspergillus* species in compared with same time for *Fusarium* species DON production time that in study Akinsanmi *et al.,* (2003, Queensland and northern New South Wales) and after the days then carefully we can review or compare our research data with the data obtained in their researches.

According to genes the promote or regulate toxin production in *Aspergillus* and *Fusarium* species, special those that are pathogenic effects on plants, and their known decisive role in toxin production could be suggested that Same genes and regulatory process similarly to what exists in *Fusarium* species and provides the possibility production of DON and family molecules in *Aspergillus* considered, might prove by which DON or their similarities and differences, until be To exploit the inhibition of toxin production in food products (McDonald *et al*., 2005).

Thus it could believed that species of the genus *Aspergillus* have to be more considered as well as the most well known potent DON producers genera such as *Fusarium* spp, can be related to some of gene mutation and gene diversity in the *A. spergillus* spp needing to genomicrobiochemical investigations by related techniques.

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