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An Investigation on T-2 Toxin Contamination in Wheat Crops from 11 Cities, Iran

Omid Lakzaei-Azar*¹, Arash Chaichi-Nosrati¹, Leila Modiri¹, Seyyed Hamed Shirazi-Beheshtiha², Soheyl Shokri-Fashtali¹

¹ Department of Microbiology, Lahijan Branch, Islamic Azad University Lahijan, Iran

² Department of Clinical Sciences, Karaj branch, Islamic Azad University, Karaj, Iran

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ABSTRACT

Objective: The major *Fusarium* toxins found in cereals and cereal-based products that can be harmful to both human and animal health are some trichothecenes, such as deoxynivalenol (DON). **Methods:** A recent SCOOP project, Since toxinogenic fungi are cosmopolitan, mycotoxins are environmental pollutants present in virtually all parts of the world and causing diseases (mycotoxicoses). This cross-sectional study was carried out in Spring - Summer. T-toxin levels vary in different cities of Iran and are higher than national standard limits (20-21 µg/Kg per BW) and, therefore, according to the harvesting programs for the wheat crop. Thus the Ardebil city with a amount of 46.3ppb and Sari, Mazandaran province center, with the least amount of T-toxin by 27.2ppb have been defined respectively. **Results:** Because of the non-specific clinical signs of *Fusarium* mycotoxicoses, data about feed quality are an important part of the case history and an inevitable part of the preventive measures is regular foodstuffs monitoring with mycological and mycotoxicological examinations. The elimination of mycotoxins from feedstuff is an open problem because the scope for mycotoxin decontamination is very limited. In countries with adequate information about mycotoxin occurrence, regular tests to control foodstuffs and detect widespread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits.

INTRODUCTION

Mycotoxins are produced mainly by the mycelial structure of the filamentous fungi, commonly referred to as moulds. *Fusarium* species are plant pathogens commonly associated with cereals that, under favorable environmental conditions, can produce several secondary toxic metabolites. Mycotoxins can cause outbreaks in some diseases in humans and animals and therefore their presence in food and feed is a subject which is of national importance. The major *Fusarium* toxins found in cereals and cereal-based products that can be harmful to both human and animal health are some trichothecenes, such as deoxynivalenol (DON),

nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2), zearalenone (ZEA) and fumonisins (FB1, FB2 and FB3) (Visconti et al. 2005). Corn, wheat, barley, oats, rice, rye and other crops have been reported to contain the T-2 toxin. Natural occurrence has been reported in Asia, Africa, South America, Europe, and North America. Natural levels range from near zero to 10 ppm with a few exceptions showing levels of 15-40 ppm. The toxin production is greatest with increased humidity and temperatures of 6-24 °C (Riazipour et al. 2011). The Scientific Committee on Food (SCF) of the European Commission has recently established a full Tolerable Daily Intake (TDI) for a combined t-TDI for T-2 and HT-2 (0.06 µg/kg b.w. /day). A recent SCOOP project, aiming to evaluate the risk of dietary exposure to *Fusarium* toxins by the population of EU member states, showed that

*Corresponding Author: Omid Lakzaei-Azar, Department of Microbiology, Lahijan Branch, Islamic Azad University Lahijan, Iran, E-mail: mycotoximmune_achn@yahoo.com

Fusarium toxins are widely distributed in the food chain in the EU and the major sources of dietary intake of *Fusarium* toxins are cereal products, mainly based on wheat and maize. The Commission has also issued recommendations to prevent or reduce the contamination of *Fusarium* toxins in cereals and cereal products (European Commission, 2006). There is a need to develop and validate analytical methods for rapid, sensitive and accurate determination of these mycotoxins in cereals and cereal-based products in order to properly assess the relevant risk of exposure and to ensure that regulatory levels fixed by the EU or other international organizations are met (Goryacheva *et al.* 2007; Krska *et al.* 2005; Maragos, 2004; Pascale *et al.* 2007; Zheng *et al.* 2006). Epidemiological surveys have revealed that the predominant type-A (T-2 toxin, HT-2 toxin, DAS and neosolaniol) and type-B trichothecenes (DON, nivalenol and fusarenon-X) are widely distributed in cereals, whereas macrocyclic trichothecenes (types C and D) occur rarely in foods and feeds (Sabater Vilar *et al.* 2003). In the evaluation of *Fusarium* toxins the criteria for toxin selection have been: Of the toxins most commonly found in analytical surveys of cereals in the evaluation of *Fusarium* toxins the criteria for toxin selection have been the toxins for which there is a minimum of toxicological data (Bhatnagar *et al.* 2001).

Since toxinogenic fungi are cosmopolitan, mycotoxins are environmental pollutants present in virtually all parts of the world and causing diseases (mycotoxicoses) to living organisms are described (Steyn, 1998). As yet only a few mycotoxins have been related to important food- and feed- borne diseases, the potential impact on human and animal health of many of them remains to be elucidated (Fink *et al.* 1999). A multitude of agricultural products are exposed to fungal contamination from the early stages of planting until their eventual consumption (Boermans *et al.* 2007; Bullerman *et al.* 2007). If the contaminating fungi belong to a toxin producing species, it may produce mycotoxin as a secondary metabolite at some stage of its growth. The aim of this study is to provide a measure of the significance of cereal impurities with T-2 toxin being the most poisonous member of the trichothecenes family. This was achieved by a scrutiny of cereals for human consumption in some of the wheat centers that serve a multiple number of meals in Iran (Riazipour *et al.* 2012).

2. MATERIALS AND METHODS

This cross-sectional study was carried out both in the field and in laboratory situations in Spring- Summer (March-August) 2013. New harvested and cropped wheat samples obtained from early March-late August from 7 provinces with a high level productions in the state including the southern provinces (Khoozestan), West (including Kermanshah, Hamedan) and North (Zanjan, Ardebil, Mazandaran, Golestan) a series per one hundred

tone of each batch storages provided. After preparation, the wheat specimens collected for use dried, and adjusted its humidity on 14%, mixing and re-mixing were done for each grouped samples, taken four sample groups of 100 Grams randomly selected as a sample in order to measure, control, Stoke and samples for grinding then mealing or flour wheat samples taken longer phase of the laboratory mill followed by the wheat samples were been ready for extraction (Iran Standard and Industrial Research Institute, Work Guideline No. 2087). In this method, the number of specimens was taken from three parts different of each batch (both sides and middle of the batches). Next, by mixing these three parts, approximately 200 g was taken as a final sample and kept in a closed container in the refrigerator.

2.1. Preparation of samples

The samples have been stored in a cool place, protected from light. A representative sample (according to accepted sampling techniques) have been ground and thoroughly mixed prior to proceeding with the extraction procedure. weighing 5 g of ground sample and added it to a suitable container with 25 ml of methanol (70%), shaking vigorously for 3 min (manually with shaker), filtered the extract through Whatman No.1 filter, diluted 1 ml of the obtained filtrate with 1 ml of distilled or deionized water finally used 50 μ l of the filtrate per well in the test sample size may be increased if required, but the volume of methanol/water must be adapted Accordingly.

2.2. Test procedure

Inserting a sufficient number of wells into the micro well holder for all standards and samples to be run. Recorded standard and sample positions. Pipette 50 μ l of standard or prepared sample to separate well Added 50 μ l of enzyme conjugate (red cap) to the bottom of each well then 50 μ l of the anti-T-2 toxin antibody solution to each well. Mix gently by shaking the plate manually and incubation for 10 min, at room temperature, dumping the liquid out of the wells into a sink. Taped the micro well holder upside down onto a clean filter towel to remove all remaining liquid from the wells. Using a multichannel pipette, filled the wells with deionized water (250 μ l per well) then Emptied the wells again and remove all remaining liquid. Repeating the washing step two more times. Added 100 μ l of substrate/chromogen to each well. Mixed gently by shaking the plate manually and incubate for 5 min at room temperature in the dark. Adding 100 μ l of stop solution to each well. Mixing gently by shaking the plate manually and measured the absorbance at 450 nm to be Read within 10 minutes after addition of stop solution. For single determinations we recommend logit/log evaluation and for double or multiple determinations cubic spline should be used the course of

the standard curve is shown in the quality Assurance Certificate enclosed in the test kit.

Absorbance of standard

$$\text{Absorbance Standard \%} = \frac{\text{Absorbance of standard}}{\text{Absorbance Neg Com}} \times 100$$

According to dates from the National Department of Agricultural supervisions and Plant Pathology Institute, the available noble provinces for cereal agriculture and related sites realized in three territories as the North, South and West meanwhile for many years, the top wheat-producing provinces of the country were located taking into consideration the number of provinces per annual wheat production of each provinces in each region, three cities and three provinces were randomly chosen from each territories then a random sampling of

local provinces and cities sample size were determined(Figure 1.1). According to the contents of Table 1 and Figure 3, the percentage frequency of obtained samples from triple regions showed that the Northern Iran (N) with 10 samples-cities, by the frequency of 71.4% located at the highest, Western Iran (W) with 3 samples-cities of frequencies about 21.4 % and Southern Iran (S) with only 1 sample-city, at a frequency rate of 7.1% came in respectively.

Table1:

Frequency of the taken samples based on geographical distribution

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	N	10	71.4	71.4	71.4
	S	1	7.1	7.1	78.6
	W	3	21.4	21.4	100.0
	Total	14	100.0	100.0	

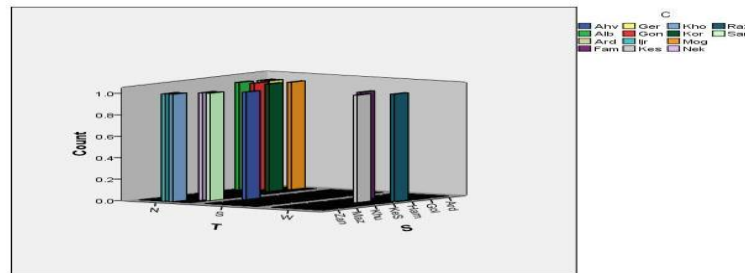


Figure 1:

Distribution of sampling sites in the North, South and West territories simplified cities Which includes the divided provinces (N-north (Ard (Ardebil), Maz (mazandaran) Gol (Golestan)), S-south(Khu (Khuzestan)) and (W-West (Zan (Zanjan) , Kes (Kermanshah)and Ham (Hamedan) of the country

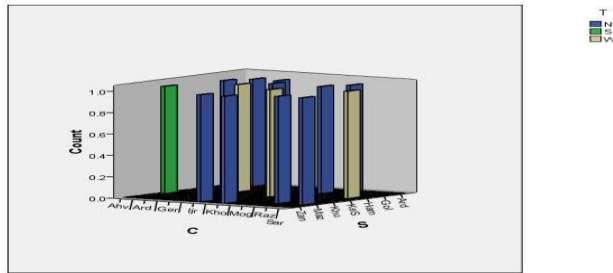


Figure 2:

Diagram of sampled cities distribution in the regional provinces of Iran

Ahv (Ahvaz Khuzestan), Ard (Ardabil city) Alb (Ali Abad province), Fam (fameninHamedan province), Ger (Germi Ardebil province), Gon (gonbad of Golestan province), Ijr (Ayjrudof zanzan), Kho (Khodabandeh, Zanzan), Kor (kord koy Golestan), KeS (Kermanshah Province), Mog (plain Mog Ardabil province) Nek (Neka, Mazandaran Province), Raz (Razan, Hamedan province), Sar (Sary State Mazandaran).

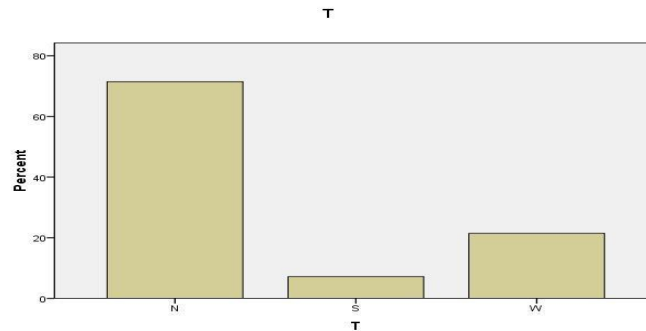


Figure 3:

Providing sample propagation percentages in three geographic regions

According to the contents of Table 1 and Figure 3, the percentage frequency of samples obtained from regions showed that between northern Iran (N) with 10 samples-

cities, and the frequency of 71.4% in the highest, West of Iran (W) with 3 samples- cities and the frequencies 21.4 % and south (S) with number 1 sample- cities, at a frequency rate of 7.1%.

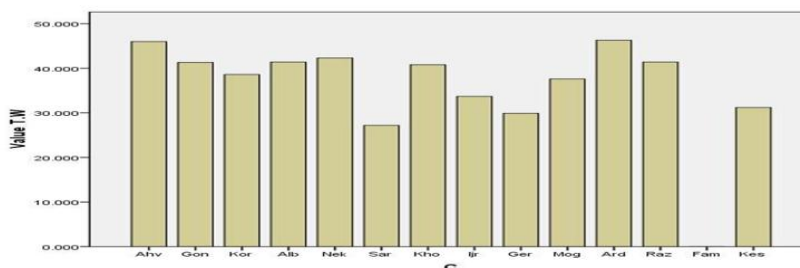


Figure 4:

Measured T-toxin levels in wheat samples of the studied cities

According to the Figure 4, T-toxin levels vary in different cities of Iran and are higher than national standard limits (20-21 µg/Kg the human body weight) of measuring the amount of toxins in the research evaluation unit ppb and , therefore, according to the charts in order to harvesting programs for the wheat crop from left to right ,Khuzestan province State city ;Ahvaz (Ahv), 46ppb, Gonbad city of Golestan province (Gon), 41.3ppb, the Kalaleh city of Golestan province (kal), 38 ppb, Ali Abad province 41.3ppb, Neka , Mazandaran (Nek), 42.3ppb and the state CitySari, Mazandaran Province (Sar), 27.2ppb, Khodabandeh city, Zanzan Province (Kho), 40.8ppb,

Ayjrud county city agricultural State Branch (Ijr), 33.7ppb, Germmy city of Ardabil province (Ger), 29.9ppb and Meghann county city agricultural State Branch (Mog) 37.6 and Ardabil province State city (Ard), 46.3, for the city of Razan (Raz) and famenin (Fam) for the Hamedan province were respectively 41.4ppb, 0.00ppb and finally the amount of quantified toxins for Kermanshah Province State city (KeS) was 31.2 ppb have the highest T-toxin poison into account . Thus, according to (Figure 4) the Ardabil city with an amount of 46.3 ppb and Sari, Mazandaran province with the least amount of T-toxin 27.2 ppb have been defined respectively.

According to, 2way paired t-tests , statistically showed no statistically significant relationships or significant differences between the numerical experimental dates between the targeted couples and between the wheat and flour Samples measured toxin quantities and even found tend to observed relationships indicating that the mixing and formulation process made by wheat flour

producers regarding to pre -packed food rules, intakes to regulate nourishment contents and full compliance with food standards, making different amounts of toxin by which may change to be reduced , according to 2-tailed Paired Samples test (Sig. 0.161) of the numerical differences of average T-toxin in wheat and flour samples were not significant.

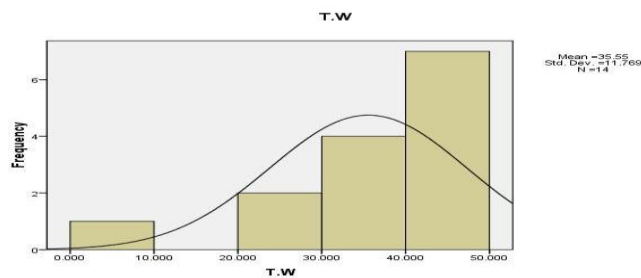
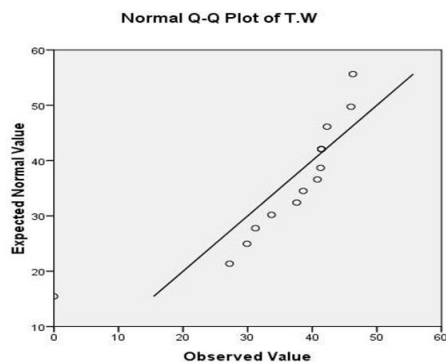
**Figure 5:**

Diagram of obtained wheat samples toxin quantities normal distribution in different intervals (0.0 - 55ppb) using Paired Samples Correlations.



Of examining how statistically obtained data's from analytical measurements conducted on wheat samples is shown in the graph Figure 3, figures tends to the right due to the presence of T-toxin highest values measured in the toxic ranges 30-40 and 40-50 ppb are interferes the lowest in the non toxic range of 20-30 . To ensure about the normal distribution test results, repeated statistic measurements Re-tested by One-Sample Kolmogorov-Smirnov Test income the same alignment with the normal distribution of numerical data's and mentioned above findings were confirmed (Asymp. Sig (2-tailed)0.555).

Figure 6:

Normal QQ Plot Diagram of the T-toxin scattering data

Discussion

In moderate climates, the occurrence of *Fusarium* and their toxins in cereals is predisposed primarily by wet and cold vegetation periods. Requisite preventive measures against the multiplication of *fungi* and toxin production include storing of well-dried grains at optimal conditions. An inevitable part of the preventive measures is regular foodstuffs monitoring with mycological and mycotoxicological examinations. Because of the non-specific clinical signs of *Fusarium* mycotoxicoses, data about feed quality are an important part of the case history. Contamination of feed with a *Fusarium* toxin can lead to impaired immune functions, metabolism disorders, decreased performance, and increased susceptibility to adverse environmental influences. The elimination of mycotoxins from feedstuff is an open problem because the scope for mycotoxin decontamination is very limited. As a preventive measure, it is possible to use agents that can inactivate mycotoxins, particularly substances with a high absorption capacity and specific enzymes, also, various microorganisms can be used to inhibit growth of the fungi, or to degrade mycotoxins. In summary, providing for quality edible and safe products is vital and the research priority is to eliminate toxic residues produced by fungi. Having carcinogenic potential and poisonous effects, mycotoxins are considered to be one of the most important regulatory issues. In countries with adequate information about mycotoxin occurrence, regular tests to control foodstuffs and detect widespread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits (Shephard et al. 2000; Tanaka et al. 2007). Unfortunately in Iran, a limited number of mycotoxins including *Aflatoxins*, *Fumonisin*s, *Zearalenon* and, *Ochratoxins* are only being measured in export products, but they are not usually checked in foodstuffs for domestic consumption. Trichothecenes are a major family of mycotoxins and T-2 toxin is the most poisonous. Rendering the effects of these toxins for consumers (Egmond et al. 2003; G hiasian et al. 2006), it is crucial that adequate information

about how often people are exposed to these kinds of toxins is provided. It has been reported that *fungi*, which produce trichothecenes, exist in a number of different foodstuffs and different results have been reported from various studies on measuring *Fusarium* toxins including the T-2 toxin. Yazdanpanah et al. (2011) by testing 35 immediately harvested *wheat* samples showed that although some evidence of impurity with other *Fusarium* toxins such as; *Nevalenol*, *Deoxynevalenol* and *Zearalenon* were found, not a single sample was contaminated with T-2 toxin by *ELISA* (Reddy et al. 2010) In another study, he showed that *Fusarium* poisons such as T-2 toxin, often contaminate 24 different corn-based human foods, however, the amounts in the majority of cases were low (Yazdanpanah et al. 2001). Furthermore, by testing 23 samples of one wheat-based food, Yazdanpanah et al. (2011) reported a high prevalence of *Fusarium* toxins; however, none of them were contaminated with a high dosage level of these toxins. The statistical analysis performed in this study showed that the frequency of wheat samples from north of Iran were 71.4%, of South 7.1% finally West 21.4% and the rate of Yazdanpanah et al. (2011) and results of Rezapour et al. (2012) has not been closed. In spite of that, different results have been reported from studies in other countries about the occurrence of contamination with T-2 toxin in grains and other crops. Schollenberger (2006) indicated that except for two pure samples, all of the other 125 wheat, barley, corn and German corn foodstuff samples, were contaminated with one or more kind of mycotoxin (Vrabcheva et al. 1996). Muller (1998) proved in various years 27-61 % of corn (12) and 0-14 % of provender wheat harvested from south Germany was contaminated with T-2 toxin. Moreover, in the study by Hussein, et al. 85 % of New Zealand corn samples had one or several *Fusarium* mycotoxins and T-2 toxin was found in 65 % of them (Hussein et al. 1989; Lepschy et al. 1989). Also showed that 38 % of wheat, barley, rye, oat and flour were contaminated with T-2 toxin (Tanaka et al. 2007; Verabcheva et al. 1996), on the contrary, showed that only one specimen of 140 wheat samples which were destined for human consumption, was contaminated with T-2 toxin (0.7 %). And in the Halger (1984) study, not a single sample from the central and western parts of the U.S was contaminated with T-2 toxin. So the average found in the toxin of many studied samples concern it that the majority of samples were above the 40-

30ppb degree and the majority of the samples with higher levels of 20ppb, which almost makes up more than 70% and The rest of the samples have been 20-30ppb range, which can be due to the cumulative effect of the toxin considered it as a serious threat. The Vision standard T-2 in food according to the type of product and the sensitivity of wheat in some countries, strategic, and values differ, there is much in 25ppb to animal-feed Plants or for human consumption has been announced 20 ppb that differ in countries with much lower amounts, such as, 20 µg/kg (Slovakia) to maximum levels up to 300 µg/kg (Hungary) are designated (Reddy et al. 2010). Although the distribution of T-2 concentration in the range of intended counter are not correlated (Figure 4) But it should be noted that the highest frequency of T-2 toxin concentrations in the range of 30-40ppb in Ardebil, Khuzestan, Golestan, were seen could showed that *Fusarium* in all provinces have to be examined in temporary holding wheat fields or barns, silo, or the transportation solution exists. Thus, the highest possible toxins out of the plurality of samples collected from the northern region of Iran and then to the south of Iran and the West are included. (Figure 1, 2, 3 Table 1). Based on the results collected and flour products manufacturing process, there are no significant differences in pollution levels above the permitted limit. Considering all the results of this study can be said to explain the main *Fusarium T-2* is the manufacturer, and at intervals after planting and cultivation remains, with the impact grain long-term remaining viability remaining grains can years of farm and food products that cause pollution., this level of contamination varies according to geographical regions, but during the process of turning wheat into flour milling contamination levels of toxins, including T-2 may be a somewhat rises or falls too.

Conclusions

In moderate climates, the occurrence of *Fusarium* and their toxins in cereals is predisposed primarily by wet and cold vegetation periods. Requisite preventive measures against the multiplication of fungi and toxin production include storing of well-dried grains at optimal conditions. An inevitable part of the preventive measures is regular foodstuffs monitoring with mycological and mycotoxicological examinations. Because of the non-specific clinical signs of *Fusarium* mycotoxicoses, data about feed quality are an important part of the case history.

Contamination of feed with a *Fusarium* toxin can lead to impaired immune functions, metabolism disorders, decreased performance, and increased susceptibility to adverse environmental influences. The elimination of mycotoxins from feedstuff is an open problem because the scope for mycotoxin decontamination is very limited. As a preventive measure, it is possible agents that can inactivate mycotoxins, particularly substances with a high absorption capacity and specific enzymes, Also, various microorganisms can be used to inhibit growth of the fungi, or to degrade mycotoxins. In summary, providing for quality edible and safe products is vital and the research priority is to eliminate toxic residues produced by *fungi*.

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Conflict of Interest

The author declares that there are no conflicts of interest.

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