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Research Article

STUDY ON THE AMOUNT OF *FUSARIUM* T-2 TOXIN IN THE SAMPLES OF BAKERY FLOUR PROVIDED BY TOP WHEAT PRODUCER PROVINCES

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ABSTRACT
Several different of *Fusarium* sp. produce many mycotoxins that called trichothecenes and Which are the most popular in health foods, it is important to make the primary and secondary maycotoxsicosis in human and animal, are known as T-2 toxin. Since, T-2 toxin produced by *Fusarium* genus in the grains has not been widely studied and toxin production and secretion methods remain unknown in many *Fusarium* sp., accordingly, this study was conducted. In this study examines the extent of T-2 toxin produced in the examples of the top state producers of wheat flour (Golestan, Mazandaran, Ardabil, Zanjan, Hamadan, Kermanshah, Khuzestan), as discussed by ELISA. After preparation of the samples of wheat, and 4 samples of 100 gr were randomly selected. In the next step, the wetting and conditioning sample process were done. After crushing and grinding, the samples have been ready to begin extraction operations toxin. Extraction of the soluble toxin was done by using solvent extraction. Then, until the extract volume has been below to 10 ml, the samples placed in water bath. The extraction filtration by using Whatman filter without charcoal was performed. Finally, to measurement the amount of toxin aimed at the competitive ELISA, estimating the samples was carried out by RIDA SCREEN T-2 kit (Art.Nr.:R5302). The toxin produced by 14 samples had different significant levels. The average rate of T-2 toxin production of the samples examined was from 11/1ppb to 41/2 ppb. Most samples were in the range of 25 ppb-30 ppb toxin production. The highest levels of toxin production of flour samples associated with Famenin city with the 41/2 ppb and the Gonbad city with the lowest rate of 11/1 ppb. This study showed that, the samples have the potential of contamination with T-2 toxin and the flour and cereal products companies, must the presence of T-2 toxin be investigated, like other mycotoxins to ensure food safety.

Keywords: *Fusarium, T-2 Toxin, ELISA, Mycotoxin*

INTRODUCTION
Trichothecenes are the most important group of mycotoxins in terms of diversity and being widespread so that more than 150 types of trichothecene have been identified. T-2 toxin is a trichothecene of very high toxicity which is 10 times more toxic than Deoxynivalenol. This toxin affects the skin 100 times more than mustard. It is also a heat-resistant toxin which will not be easily destroyed by autoclaving and is harmful to human and animal health due to its cytotoxicity effect and ability to suppress the immune system. This kind of toxin contaminates the crops, especially grain, is posing a serious problem for food hygiene, and causes severe acute or chronic poisoning in humans and domestic animals, even leading to death, as several epidemics of it have been reported in people and animals which ended up with death in thousand cases. It creates a range of clinical symptoms in the poisoned patient the most important of which is alimentary toxic aleukia (ATA).
Fusarium species are one of the most important fungal groups owing to their importance in medicine, hygiene, and agriculture. They produce a range of mycotoxins that cause contamination of food. When growing on food, different types of *Fusarium* produce different types of mycotoxins under the influence of factors such as ambient temperature, humidity, oxygen pressure etc. Considering the viability and stability of mycotoxins on food, even after being cooked, because of their resistance to heat, they bring about mycotoxicosis. The word mycotoxin is used for the compounds produced by fungi and toxic to other organisms. Mycotoxins along with other fungal metabolites such as antibiotics, alkaloids, and so on,

are compounds produced by fungal cells during the later stages of the growth of filamentous fungi and are known as secondary metabolites. Fungal toxins are produced following the growth of toxigenic molds on food. The toxicity resulting from the consumption of such foods is called primary mycotoxicosis. If mycotoxins find a way to livestock products such as meat and milk through food chain, the condition arising from using such products is termed secondary mycotoxicosis.

Mycotoxins cause four main types of toxicity including acute toxicity, chronic toxicity, mutagenicity property, and malformation. The main effect described for acute mycotoxicosis is to yield adverse effects on the liver and kidney which will lead to death if continues. Anyway, some mycotoxins are primarily involved in protein synthesis and thus cause skin allergy, necrosis, or weakened immune system. Another group of mycotoxins, which act as neurotoxins, may cause continuous vibrations in animals at low levels but permanent brain damage or death at high levels. Long-term impacts of ingesting small quantities of mycotoxins are different. Causing cancer especially in liver is the main chronic effect of many mycotoxins. Some of the toxins influence DNA replication, thus having mutagenic and teratogenic effects. In contrast to bacterial toxins, most mycotoxins do not have a structure made of protein and are relatively small molecules, which is why they are normally not recognized by the immune system of humans and animals. The symptoms of acute mycotoxicosis are usually quite different from that of the toxicity resulting from bacterial toxins. Mycotoxicosis symptoms are various due to the differences in their chemical structures.

Fusarium is one of the most common fungi that can cause respiratory allergic reactions in human being. Among this species, two important pathogenic strains *F. solani* and *F. oxysporum* have been reported. Under predisposed conditions such as AIDS, neutropenia, bone marrow transplantation, cancer, burns, trauma, and diabetes, this fungus can cause diseases such as endocarditis, endophthalmitis, disseminated infection, peritonitis, and keratitis. Fusariosis is an important fungal disease which is resistant to treatment and is often created by *F. solani*. The entries of *Fusarium* species are respiratory system and skin. In order, the main places of infection are skin, blood, and lung. After entering and residing, the fungus will be disseminated through blood and will involve organs such as lung, heart, liver, spleen, and kidneys. *Fusarium* is life-threatening for patients with leukemia or aplastic anemia.

By investigating the genotoxic effects of T-2 toxin on rabbits in 2012, Hanfer concluded that T-2 toxin by 2 mg/kg dosage in rabbit feed has genotoxic effects on rabbit lymphocytes. Mannan oligosaccharide supplement (MOS) in rabbit feed had a significant protective effect against T-2. These results show that MOS probably reduces the risk associated with the absorption of mycotoxins owing to its binding capacity and antioxidant properties.

Daković *et al.,* (2009) study on the adsorption of T-2 by hectorite (formula: $NaO_3(Mg,Li)_3Si_4O_{10}(OH)_2$), it was found that the presence of certain active sites in hectorite is effective in T-2 adsorption. According to that research, the amounts of T-2 toxin adsorbed by hectorite in the pH of 3.0, 7.0, and 9.0 are 9.178 mg/g, 9.930 mg/g, and 19.341 mg/g, respectively. The obtained data show that T-2 toxin adsorption by hectorite is pH-dependent. In the research carried out to study T-2 toxin in the grains and cereals for human use in 2012, Riazipour *et al.,* concluded that experiment samples were all contaminated by T-2 at different levels within 7.9 - 65.9 µg/kg (average: 2.1 ± 17.9). The highest level was for wheat sample with contamination around 42.4 (±8.4)µg/kg. Also, in another study on T-2 in domestic or imported rice in 2009, it was found that all rice samples were more or less contaminated by T-2, but contamination did not exceed the allowable amount. Based on that study, the average contamination of domestic and imported rice was 11.2 and 13µg/kg, respectively.

The average contamination was 14.5 for Pakistani rice and 12.6 for Thai rice. Hofstetter in a study entitled Biotransformation on a successful way to deactivate T-2 in broiler chicks showed that among the four tested commercial products, only Mycofix was able to cope with malfunction effects resulting from dietary prescription of T-2 toxin. Preliminary results of exposure to chronic effects of T-2 toxin in rabbits in Tornyos *et al.,* (2011) showed that exposure to T-2 toxin at a rate of 0.1 mg/kg/day causes partial Leydig cell hyperplasia. Given the preliminary results, it seems that an adult rabbit may be able to bear a 0.05 mg/kg/day concentration of T-2.

Given the widespread contamination of food products to a variety of saprophytic fungi belonging to *Fusarium* genus, low level of food hygiene in the country, dietary patterns, undesirable storage conditions for food and grains and so forth, contamination of food to various mycotoxins in Iran is higher than the allowable amount, and the results of the present study, considering the contamination of foods to T-2, raised the necessity of paying serious attention to this subject and doing further complementary studies in this regard. The present research was aimed at investigating T-2 toxin production in flour samples of the top producers in the country and comparing the amount of the T-2 produced according to international standards.

MATERIALS AND METHODS

The present study is of prospective, cross-sectional, observational type. The sampling was done following the instruction of sampling from open and close sites (firm) CBS. The samples were taken from each fifty square hectare of the field, a sample group with placing in open plates in the site. 6 plates having malt extract agar, yeast extract agar, Czapek yeast extract agar, Czapek agar, Sabouraud dextrose agar, and potato dextrose agar all mixed with 100 ppm chloramphenicol and 50 ppm tetracycline were used to take a sample group. All the plates were aerobically incubated at 25±2 °C. In the range of 3, 7, and 15 days, all the plates were always (and also daily) checked, identified, marked, and samples were taken by a sterile glass needle and cultured in prepared plates. In plates and tubes containing agar butt slant from growth substrates of malt extract agar, yeast extract agar, potato dextrose agar, corn meal agar, Sabouraud dextrose agar, and Czapekdox agar, all mold samples were recaptured and incubated with preplans. At last, some samples were taken from *Aspergillus*colonies and cultured in plates containing Czapekdox agar, Czapek yeast extract agar (with and without 20% sucrose), malt extract agar, and Czapekdox agar (with and without 20% sucrose). The samples were grown at 25±2 °C, and after 3, 7, or 15 days checking, and simultaneously, culture slide from each sample was provided on 20%-sucrose Czapek yeast extract and Czapekdox agar substrates in order to grow with former pattern.

To provide extract from the obtained isolates of cultivation in liquid substrate to prepare and motivate the extract more and more, a full loop having 10^5 phialospore from the PBS mixture and conids of every grown isolate in Czapek extract agar plate were taken and re-cultured into a 50 mL Falcon tube containing the liquid substrate of Czapekdox broth with 1% malt extract agar. The re-cultured tubes were incubated in the darkness-light period at 25±3 °C and 200 rpm. After seven days, floating or deposited mass in the liquid, which was mold fungal, infant small filament (Germ tube), was deposited by centrifugation at 3000 rpm for 15 minutes and removed from culture medium of fungus using sterile filter paper. Every provided culture medium was observed in a PBS tube and sampled into every 5mL Falcon tube of buffer, 1mL cold acetone was added, and the separation was done by centrifugation at 15-3000 rpm. The supernatant was separated from the larger deposits and kept in another tube after marking at -20°C. For synchronization, the size of protein of each mixture obtained from each *Aspergillus* isolate was measured by Bradford method and thick samples were diluted up to 0.5 mg/ml. The thick samples were diluted and the dilute samples were again concentratedby this method until all extract samples had 0.5 mg/ml protein. Finally, the one-sample Kolmogorov-smirnov test was used for statistical analysis of the normal distribution of the measured mean values of toxin in biomass and medium culture. We used NORMAL Q-Q PLOT test for the scattering distribution of the measured values of toxin in biomass and culture medium of the studied species, and in order to determine the numerical difference of Citrinin production amount between the medium and biomass the Willcoxon Singned Rank Test was used. Also, we used Excel and Office 2010 and SPSS 16 to analyze the findings.

RESULTS AND DISCUSSION

Results

According to the information obtained from grain deputy in agricultural organizations of the country's provinces and the related website, provinces comprising top wheat producers in different years are in the north, south, and west of Iran.

Therefore, while geographical zoning of south, north, and west, considering the number of times a province takes the lead in annual wheat production, three provinces were selected from each zone as well as three cities from each province. Then, by lottery, the desired provinces and cities were determined for sampling. Among the provinces, Fars and Bushehr were first excluded due to heavy rains during the harvest period. Humidity can cause mold growth on the surface of samples and this means an error in tests. Hence, we disregarded these samples in order not to have an error in the test.

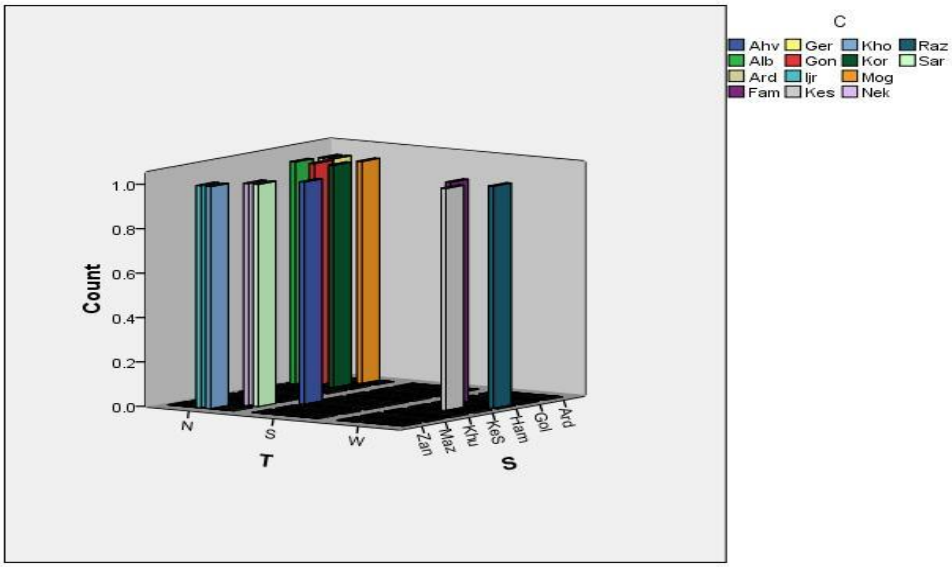

Figure 1-1: Frequency distribution of sampling sites in the north, south, and west by dividing the sampled provinces and cities. N, S, and W symbolize the north, south, and west of the country including: Zan (Zanjan), Maz (Mazandaran), Khu (Khuzestan), KeS (Kermanshah), Ham (Hamedan), Gol (Golestan) and Ard (Ardebil)

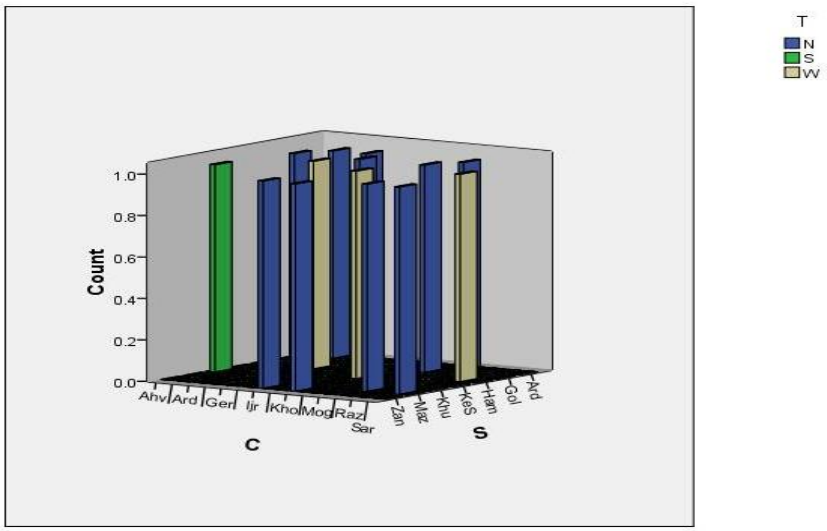

Figure 1-2: Distribution of the sampled cities in the studied provinces: Ahv (Ahvaz city in Khuzestan Province), Ard (Ardebil city), Alb (Aliabad in Golestan), Fam (Famnin in Hamedan), Ger (Germi in Ardebil), Gon (Gonbad in Golestan), Ijr (Ijrud in Zanjan), Kho (Khodabande in Zanjan), Kal (Kalale in Golestan), KeS (Kermanshah), Mog (Dashte Moghan in Ardebil), Nek (Neka in Mazandaran), Raz (Razan in Hamedan), and Sar (Sari in Mazandaran)

According to figure 1-3, frequency percentage of the number of samples obtained from the geographical zones shows that maximum percentage appertains to the north of the country (N) with 10 samples from 10 cities and frequency percentage of 71.4%, and minimum percentages are for the west (W) with 3 samples and 3 cities and frequency distribution of 21.4% and for the south (S) with 1 sample from 1 city and frequency distribution of 7.1%.

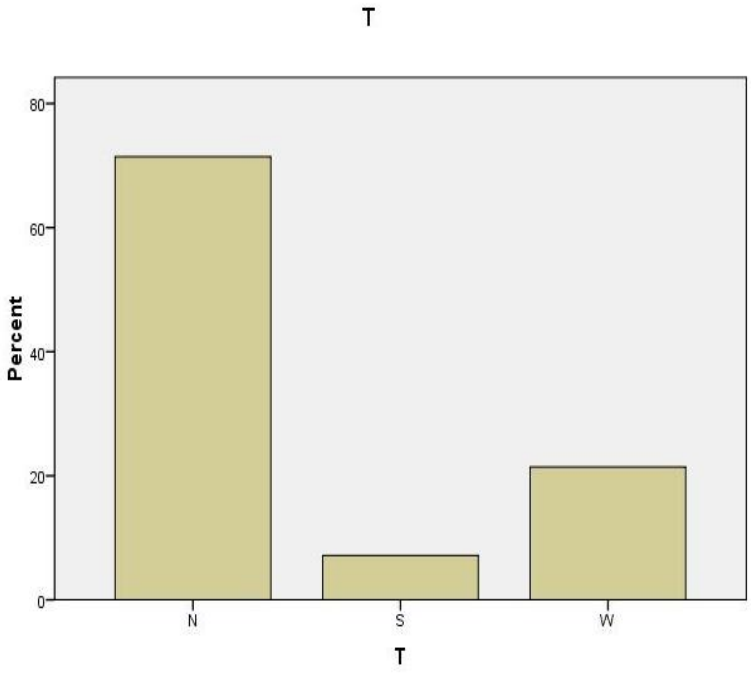

Figure 1-3: Frequency distribution of the samples provided in three geographical zones of the country

As can be seen in figure 1-4, frequency percentage of the number of obtained samples in terms of provincial distribution indicates that Ardebil (Ard) and Golestan (Gol) provinces each with 3 samples and 21.4% have the maximum number, and Hamedan (Ham), Mazandaran (Maz) and Zanjan each with 2 samples and 14.3%, and kermanshah (KeS) and Khuzestan (Khu) each with 1 sample and 7.1% include the minimum number. All the cities include 1 sample and the frequency of 7.1% appertains to each.

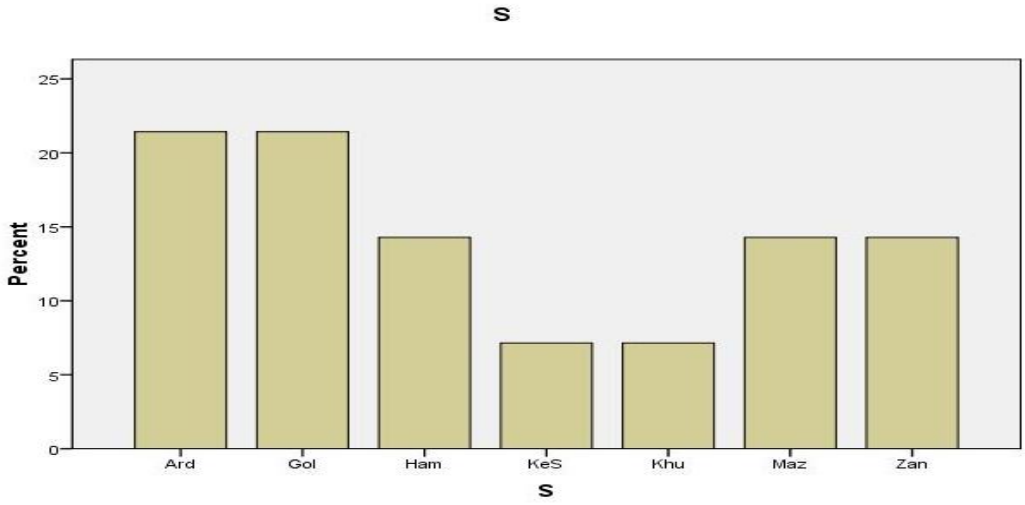

Figure 1-4: Frequency percentage of the obtained samples in the studied provinces

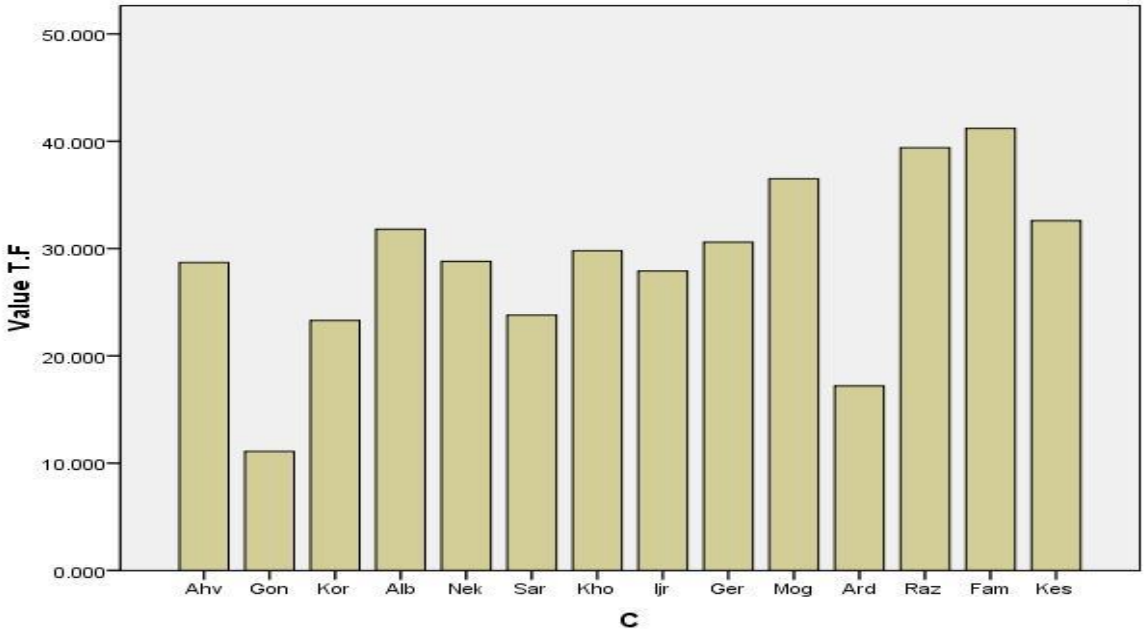

Figure 1-5: Amounts of T-2 toxin measured in flour samples of the studied cities (index line is an indication of Iran standard)

According to the figure, amount of T-2 is different in different cities and exceeds the standard of Iran (20 µg/kg body weight) in most cases. In the measurement method for the amount of toxin in this research, toxin assessment unit was ppb. So, according to the figure and in order of wheat harvest season from left to right, the city of Ahvaz in Khuzestan province (Ahv), Gonbadekavus in Golestan (Gon), Kordkuy in Golestan (Kor), Aliabad in Golestan (Alb), Neka in Mazandaran (Nek), Sari in Mazandaran (Sar), Khodabande in Zanjan (Kho), Ijrud in Zanjan (Ijr), Germi in Ardebil (Ger), Dashte Moghan in Ardebil (Mog), Ardebil city (Ard), Razan in Hamedan (Raz), Famnin in Hamedan (Fam), and Kermanshah province (KeS) had T-2 amounts of 28.7, 11.1, 23.3, 31.8, 28.8, 23.8, 29.8, 27.9, 30.6, 36.5, 17.2, 39.4, 41.2, and 32.6 ppb, respectively. Therefore, maximum and minimum amounts of T-2, i.e. 41.2 and 11.1 ppb, belonged to the city of Famnin in Hamedan province and the city of Gonbadekavus in Golestan province, respectively.

Based on and paired t-test correlation test, the relationship between the amount of T-2 in wheat powder and baking flour made from it is divergent and not statistically significant (sig: 0.1, sig: 0.161).

The divergent relationship we observed is because of the fact that the amount of toxin is changed by mixing and formulating the flour provided from usable wheat of factories before packaging in order to adjust food content and to comply with different standards for foods in the production of which wheat flour is used.

Table 1-1: Paired Samples Test to show the significance of the relationship

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	Df	Sig. (2-tailed)
Pair 1	T.W - T.F	6.785714E0	17.081923	4.565336	-3.077094	16.648523	1.486	13	**.161**

Table 1-2: Displays paired samples correlations which are not statistically significant considering the numerical difference between average amounts of T-2 in wheat (T.W) and in flour (T.F)

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	T.W & T.F	14	-.457	**.100**

Table 1-3: Paired samples correlations to show divergence or convergence of the samples

One-Sample Kolmogorov-Smirnov Test

		T.F
N		14
Normal Parameters[a]	Mean	28.76429
	Std. Deviation	8.119266
Most Extreme Differences	Absolute	.172
	Positive	.104
	Negative	-.172
Kolmogorov-Smirnov Z		.643
Asymp. Sig. (2-tailed)		.802

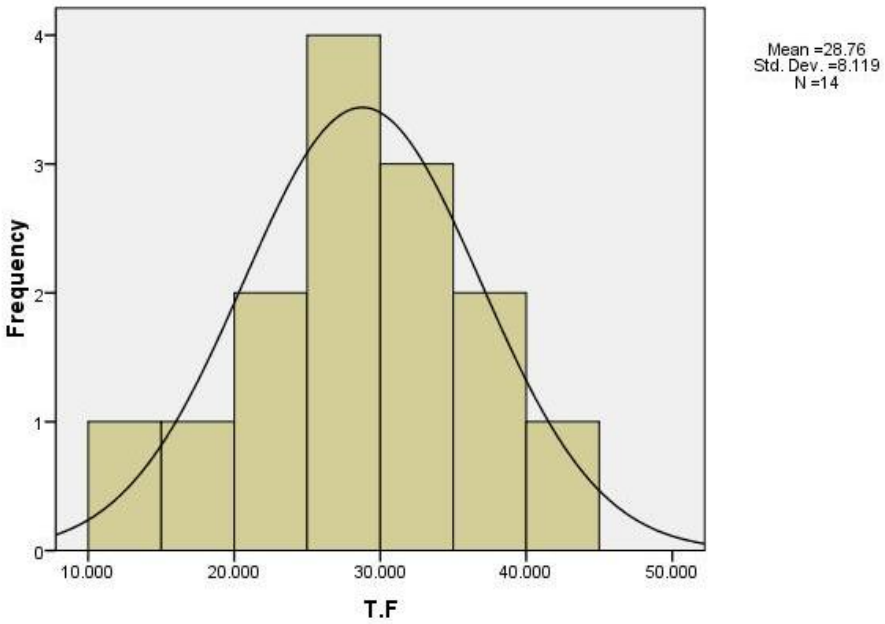

Figure 1-6: Mean frequency percentage of T-2 obtained from the studied wheat samples, and distribution in different ranges (0.0-55 ppb)

According to the statistical study of the distribution of the mean toxin values which are measured and their compliance with the terms of the normal curve, we find out that, during the preparation of baking flour and as a result of mixing different flours, measurable toxin amounts will at most level out at 25-30 ppb, and this is confirmed by various and repeated tests considering Table 1-3 and Figures 1-7 and 1-8.
Table 1-3 shows the above nonparametric study using One-Sample Kolmogorov-Smirnov Test in which toxin distribution values follow the near normal resultant in all the flour (T.F) samples. It also demonstrates that we had the least possible error in the process of randomly selecting a sampling location, sample preparation, toxin measurement, and digital analysis.

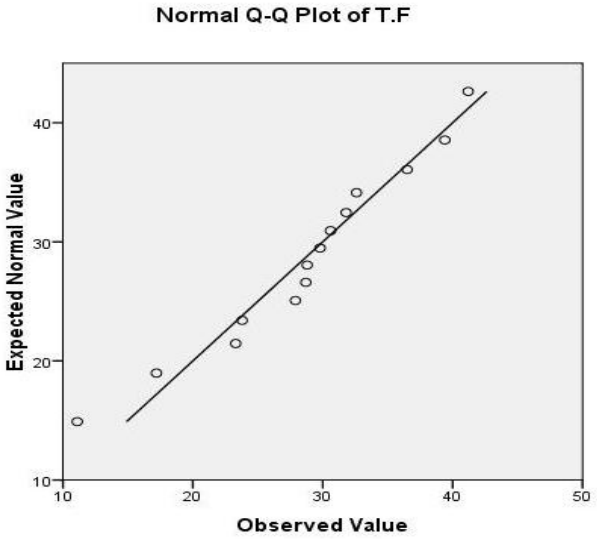

Figure 1-7: Scattering of the data obtained from toxin amounts measurement based on Normal Q-Q Plot test regarding T-2 toxin in flour

According to the figure, the closer the dots are to the line, the higher the normality of the figure is. This figure confirms the normal distribution curve.

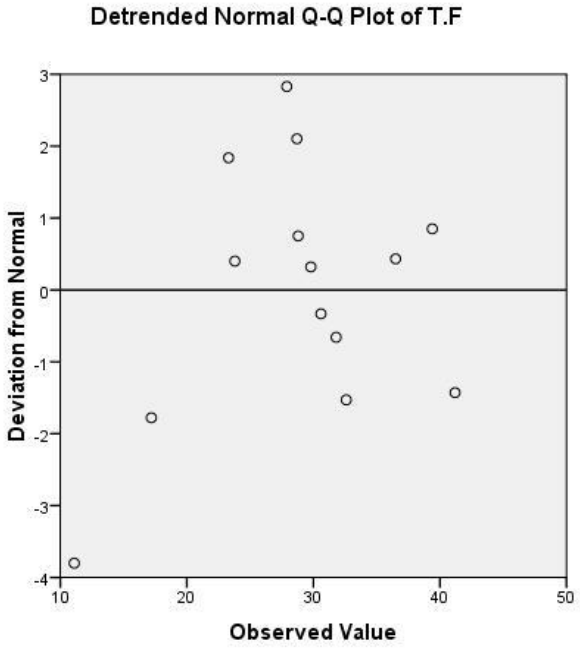

Figure 1-8: Looking into normal distribution of the data from two sides confirms Figure 1-7

Discussion

Having toxic potential and carcinogenic effects, mycotoxins are regarded as important regulatory issues all over the world. In countries provided with adequate information on mycotoxin prevalence, regular tests are being performed in order to control foodstuffs in terms of important and prevalent toxins and this leads to the exclusion of products with higher than allowable limits from the consumption circle. In our country, a limited number of mycotoxins including aflatoxins, zearalenone, fumonisins, and ochratoxins

are measured solely for exports and 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. Pondering the effects of these toxins on consumers, it is crucial that adequate information about how often people are exposed to these kinds of toxins is provided. It has been proved that fungi, which produce trichothecenes, exist in a number of different foodstuffs and different results have been reported from scant studies on measuring Fusarium toxins including the T-2 toxin.

The frequencies of the samples examined in this study in three zones of the north, 71.4%, the west, 21.4%, and the south, 7.1%, comply with Yazdanpanah *et al.,* and Rezapour *et al.,* (2012). In the examinations, maximum amount of T-2 production, i.e. 41.2 ppb, belonged to flour sample for the city of Famnin in Hamedan province, and given that the second place for toxin production also belongs to the city of Razan in the same province, the situation of flour production in this province is worrying. The minimum amount of T-2 toxin production was for flour sample of Gonbad city in Golestan province with the amount of 11.1 ppb. Riazipour *et al.,* in a study on 46 samples from grains in 9 food cooking centers in Tehran showed that over 14% of the samples were contaminated in a level higher than the allowable level for human consumption. Also, in another research on T-2 in domestic or imported rice in 2009, it was found that all rice samples were more or less contaminated by T-2, but contamination did not exceed the allowable amount. Based on his study, the average contamination of domestic and imported rice was 11.2 and 13 ppb, respectively. The average contamination was 14.5 ppb for Pakistani rice and 12.6 ppb for Thai rice. Yazdanpanah *et al.,* (2012) by testing 35 immediately harvested wheat samples from northern areas of Iran (the cities of Gonbad and Gorgan) showed that although some evidence of the prevalence of contamination to other Fusarium toxins such as newelnon, newsolanyol and zearalenon was found, not a single sample was contaminated with T-2 toxin. In another study, he showed that Fusarium poisons including T-2 toxin often contaminate 24 different corn-based human foods, the contamination of majority of which was low, however. Furthermore, by testing 23 samples of wheat-based food products, Daraei *et al.,* reported a high prevalence of Fusarium toxins, but apparently none of them was contaminated by a high dosage of these toxins. In the studies conducted in other parts of the world, Muller proved that 27-61% of corn and 0-14% of provender wheat harvested from the south of Germany were contaminated with T-2 toxin in various years. Lepschy showed that 38 % of wheat, barley, rye, oat and flour samples were contaminated with T-2 toxin. In contrast, Verabcheva showed that only one sample of 140 wheat samples, which aimed for human consumption, was contaminated with T-2 toxin. In Halger's study, not a single sample from the central and western parts of the U.S was contaminated. Schollenberger indicated that, except for two non-contaminated samples, all of the other 125 wheat, barley, corn and German corn foodstuff samples were contaminated with one or more mycotoxins. Considering all the results obtained from these studies, it can be said that since Fusarium genus is the major producer of T-2 toxin, this fungus can contaminate farms and food products for years by affecting the stability of grains in the long term during cultivation and after cultivation intervals. This level of contamination is different according to the geographical areas, but in most cases, during the process of turning wheat into flour, the amount of contamination to toxins, especially T-2 toxin, will decrease.

Conclusion and Suggestions

The extent of grains contamination to T-2 toxin in our research indicates the provision of conditions for contamination of the grains for human consumption with fungi producing trichothecenes and it seems like if favorable conditions for mycotoxins growth and production are provided even for a short time, there will be the risk of high production levels of this toxin and other mycotoxins. As indicated by Park, storing the corn samples contaminated to trichothecenes including T-2 in 5°C for 8 days in the laboratory will cause an increase in their poison amount from 14-15 to 110-538 ng/kg. For countries which adopted T-2 toxin standards for human foodstuffs, the permitted amount of T-2 varies from 20µg/kg (Slovakia) to 300µg/kg (Hungary). However, most countries (such as Russia, Bulgaria, Armenia, and Estonia) have accepted 100µg/kg as their standard. Totally, average amount of toxin found in the examined samples was 28.76ppb and the majority of samples have an amount higher than 20ppb that can be regarded as a serious danger given the amount and cumulative effect of the toxin. In our country, the allowable limit has been

identified for some mycotoxins (Iran Standard and Industrial Research Institution, Regulation No. 5925). Accordingly, the allowable limit for T-2 toxins in animal food has been recommended to be 25µg/kg. Due to the fact that the allowable limit of other mycotoxins for human consumption is usually lower than those for animal food, it is expected that the T-2 toxin permissible level in human food will be lower than 25µg/kg. None of the samples was contaminated at higher than allowable limits based on most countries' standards, but according to Iranian National Standards, 81.7% of the tested grains had higher than permissible levels of contamination for consumption and they are not allowed to be consumed even by animals.

Based on the results of this study, which shows the extent of grains contamination with T-2 toxin, and since flour is a strategic product in many countries including Iran, it is suggested that the level of mycotoxins including T-2 should be measured before buying the grains for human use and that they should be discarded from the human consumption cycle in the case of contamination higher than the allowable limits. Taking into account the fact that marked differences have been reported in the amounts of contamination of agricultural crops to mycotoxins, we highly recommend doing a study based on the effect of geographical location on levels of T-2 toxin contamination for crops using grains with identified cultivation sources. In order to prevent the growth of fungi in storage and the production of mycotoxins, it is important that grains are not stored for a long time, and continuing this strategy is recommended.

REFERENCES

Aude Naert K, Van Broeck R, Bekaert B, De Witte F, Heremans B, Messens K, Höfte M and Haesaert G (2009). Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. *European Journal of Plant Pathology* **125** 445-458.

Bennett EJ and Chung KJ (1992). Medical Mycology. *Philadelphia*, Chapter 27 745-747.

Berry RD (1975). *The Filamentous Fungi*, **1**, Glasgow, Chapter 15 123-135.

Bottalico A and Perrone G (2002). Toxigenic Fusarium species and mycotoxinsassociated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology* **108** 611-624.

Brown DW, McCormick SP, Alexander NJ, Proctor RH and Desjardins AE (2002). Inactivation of a cytochrome p-450 is a determinant of trichothecene diversity in Fusarium species. *Fungal Genetics and Biology* **36** 224-233.

Brown DW, McCormick SP, Alexander NJ, Proctor RH and Desjardins AE (2001). Genetic and biochemical approach to study trichothecene diversity in Fusarium sporotrichioides and Fusarium graminearum. *Fungal Genetics and Biology* **32** 121-133.

Burgess LW, Summerell BA, Bullock S, Gott KP and Backhouse D (1994). *Laboratory Manual for Fusarium Research*, 3rd edition, Sydney (University of Sydney Press).

Bushelman SJ, Callen JP, Roth DN and Cohen LM (1995). Disseminated Fusarium solani infection. *Journal of the American Academy of Dermatology* **32**(2Pt2) 346-351.

Carter JP, Rezanoor HN, Desjardins AE and Nicholson P (2000). Variation in Fusarium graminearum isolates from Nepal associated with their host of origin. *Plant Pathology* **49** 452-460.

Carter JP, Rezanoor HN, Holden D, Desjardins AE, Plattner RD and Nicholson P (2002). Variation in pathogenicity associated with the genetic diversity of Fusarium graminearum. *European Journal of Plant Pathology* **108** 573-583.

Chandler EA, Simpson DR, Thomsett MA and Nicholson P (2003). Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes and characterisation of chemotypes of Fusarium graminearum, Fusarium culmorum and Fusarium cerealis. *Physiological and Molecular Plant Pathology* **62** 355-367.

Charmley LL, Trenholm HL, Prelusky DA and Rosenberg A (1995). Economic losses and decontamination. *Natural Toxicology* **3** 199-203.

Collins MS and Rinald MG (1977). Cutaneous infection in man caused by Fusarium moniliforme. *Sabouraudia* **15** 151-160.

Copeland AR (1994). *Methods protein analysis. The Dupont Merck Pharmaceutical Company Experimental Station* (International Thomson Publishing) New york 50-68.

Desjardins AE (2000). Occurrence of Fusarium species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *Journal of Agricultural and Food Chemistry* **48** 1377-1383.

Desjardins AE (2006). *Fusarium Mycotoxins: Chemistry, Genetics and Biology* (APS Press) St. Paul, M.N.

Desjardins AE, Manandhar G, Plattner RD, Maragos CM, Shrestha K and McCormick SP (2000). Occurrence of Fusarium species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *Journal of Agricultural and Food Chemistry* **48** 1377-1383.

Eudes F, Comeau A, Rioux S and Collin J (2000). Phytotoxicité de huit mycotoxines associées à la fusariose de l'épi chez le blé. *Canadian Journal of Plant Pathology* **22** 286–292.

Gale LR, Chen LF, Hernick CA, Takamura K and Kistler HC (2002). Population analysis of Fusarium graminearum from wheat fields in eastern China. *Phytopathology* **92** 1315-1322.

Gang G, Miedaner T, Schuhmacher U, Schollenberger M and Geiger HH (1998). Deoxynivalenol and nivalenol production by Fusarium culmorum isolates differing in aggressiveness toward winter rye. *Phytopathology* **88** 879-884.

Haratian M, Sharifnabi B, Alizadeh A and Safaie N (2008). PCR analysis of the Tri13 gene to determine the genetic potential of Fusarium graminearum isolates from Iran to produce Nivalenol and Deoxynivalenol. *Mycopathology* **166** 109-116.

Hearn VM and Wilson EV (1990). Immunochemical studies of Aspergillus fumigatus mycelial antigens by polyacrylamide gel electrophoresis and western blotting. *Journal of General Microbiology* **136** 1525-1535.

Horner WE, Helbling A, Salvaggio JE and Lehrer SB (1995). Fungal Allergens. *Clinical Microbiology Reviews* **8**(2) 161-179.

Jaradat ZW (2005). T-2 mycotoxin in the diet and its effects on tissues. In: *Reviews in Food and Nutrition Toxicity*, edited by Watson RR and Preedy VR, Boca Raton (CRC Press) **4** 173–212.

Ji L, Cao KL, Hu T and Wang S (2007). Determination of Deoxynivalenol and Nivalenol Chemotypes of Fusarium graminearum Isolates from China by PCR Assay. *Journal of Phytopathology* **155** 505-512.

Kimura M, Tokai T and Takahashi-Ando N (2007). Molecular genetic studies of Fusarium trichothecene biosynthesis; pathways, genes and evolution. *Bioscience, Biotechnology and Biochemistry* **71** 2102- 2123.

Križková L, Ďuračková Z, Šandula J, Sasinková V and Krajčovič J (2001). Antioxidative and antimutagenic activity of yeast cell wall mannans in vitro. *Mutation Research* **497** 213–222.

Križková L, Žitňanova I, Mislovičová D, Masárová J, Sasinková V, Ďuračková Z and Krajčovič J (2006). Antioxidant and antimutagenic activity of mannan neoglycoconjugates: Mannan-human serum albumine and mannan-penicillin G acylase. *Mutation Research* **606** 72–79.

Lee T, Han YK, Kim KH, Yun SH and Lee YW (2002). Tri13 and Tri7 determine deoxynivalenoland nivalenol-producing chemotypes of Gibberella zeae. *Applied and Environmental Microbiology* **68** 2148-2154.

Lemmens M, Burstmatyr H and Ruckenbauer P (1993). Variation in Fusarium head blight susceptibility of international and Austrian wheat breeding material. *Die Bodenkultur* **44** 65-78.

Maertens L (2011). Strategies to Reduce Antibiotic Use in Rabbit Production. *Journal of Agricultural Science and Technology A* **1** 783-792.

Mandeel A, Gamal Y and Mohammad A (1996). Analysis of SDS-PAGE dissociated proteins of pathogenic and nonpathogenic fusarium species. *Mycopathologia* **127** 159-166.

Meca G, Meneghelli G, Ritieni A, Manes J and Font G (2012). Influence of different soluble dietary fibers on the bioaccessibility of the minor Fusarium mycotoxin beauvericin. *Food and Chemical Toxicology* **50** 1362–1368.

Meissonnier GM, Raymond I, Laffitte J, Cossalter AM, Pinton P, Benoit E, Bertin G, Galtier P and Oswald IP (2009). Dietary glucomannan improves the vaccinal response in pigs exposed to aflatoxin B1 or T-2 toxin. *World Mycotoxin Journal* **2** 161–172.

Miedaner T, Reinbrecht C and Schilling AG (2000). Association among aggressiveness, fungal colonization and mycotoxin production of 26 isolates of Fusarium graminearum in winter rye head blight. *Journal of Plant Diseases and Protection* **107** 124-134.

Mirocha CJ, Abbas HK, Windels CE and Xie W (1989). Variation in deoxynivalenol, 15acetyldeoxynivalenol, 3-acetyldeoxynivalenol and zearalenone production by Fusarium graminearum isolates. *Applied and Environmental Microbiology* **55** 1315-1316.

Mudge AM, Macky D, Dong R, Dardiner DM, White RG and Manners JM (2006). A role for isolation of tichothecene mycotoxin by Fusarium graminearum and Fusarium culmorum or barley and wheat. *Mycopathology* **128** 19-23.

Murphy JW (1996). Cell-mediated immunity. In *The Mycota,* edited by Miller JD and Howard DH (Springer) New York **7** 67–97.

Nelson PE, Toussoun TA and Marasa WFO (1983). *Fusarium Species: An Illustrated Manual for Identification*. University Park, P.A. (Pennsylvania State University Press).

Nicholson P, Rezanoor HN, Simpson DR and Joyce D (1997). Differentiation and quantification of the cereal eyespot fungi Tapesia yallundae and Tapesia acuformis using a PCR assay. *Plant Pathology* **46** 842-856.

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW and Joyce D (1998). Detection and quantification of Fusarium culmorum and Fusarium graminearum in cereals using PCR assays. *Physiological and Molecular of Plant Pathology* **53** 17-37.

Nirenberg H (1976). Unterstructure uber die morphologische und biologische differnzierung in der Fusarium-Sektion Liseola. *Mitteilungen aus der Biologischen Bundesansatlt fur Landund Forstwirtschaft*, Berlin-Dahlem **169** 11-17.

O'Donnell K, Kistler HC, Tacke BK and Casper HH (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineage of Fusarium graminearum, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences of the United States of America* **97** 7905-7910.

Ohno N (1991). Purification and properties of amylases extracellulary produced by an imperfect fungus. *Bioscience, Biotechnology, and Biochemistry (BBB)* **56**(3) 456-471.

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

Pennington ES (1985). A study of clinical sensitivity to airborne molds. *Allergy* **12** 388.

Rabassa VR, Schwegler E, Goulart MA, Lopes MS, Hoffmann DA, Lisboa FP, Vendramin L, Roll VFB, Diaz GJ, del Pino FAB and Corrêa MN (2010). Metabolic parameters of ewes receiving diets containing a flatoxin and zearalenone with addition of modified glucomannan. *Brazilian Journal of Veterinary Research and Animal Science* **47** 67–73.

Reynoso M, Ramirez ML, Torres AM and Chulze SN (2011). Trichothecene genotypes and chemotypes in Fusarium graminearum strains isolated from wheat in Argentina. *International Journal of Food Microbiology* **145** 444-448.

Rezar V, Frankic T, Narat M, Levart A and Salobir J (2007). Dosedependent effects of T-2 toxin on performance, lipid peroxidation, and genotoxicity in broiler chickens. *Poultry Science* **86** 1155–1160.

Rocha O, Ansari K and Doohan FM (2005). Effects of trichothecene mycotoxins on eukaryotic cells: a review. *Food Additives and Contaminants* **22** 369–378.

Ryaziupour Majid *et al.*, (1998). Measurment of mycotoxine in a military area **10**(winter) 35-43.

Ryu J, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H and Ueno Y (1988). The acute and chronic toxicities of nivalenol in mice. *Fundamental and Applied Toxicology* **11** 38-47.

Schmale DG, Leslie JF, Zeller KA, Saleh AA, Shields EJ and Bergstrom GC (2006). Genetic structure of atmospheric populations of Gibberella zeae. *Phytopathology* **96** 1021-1026.

Scoz LB, Astolfi P, Reartes DS, Schmale DG, Moraes MG and Del Ponte EM (2007). Trichothecene mycotoxin genotypes of Gibberella zeae in Brazilian wheat. *Plant Pathology* **58** 344-351.
Svidzinsky TIE (1995). Isoenzyme profile of paracoccidioides brasiliensis. *Journal of Medical and Veterinary Mycology* **33** 280-285.
Tóth B, Mesterházy A, Horváth Z, Bartók T, Varga M and Varga J (2005). Genetic variability of central European isolates of the Fusarium graminearum species complex. *European Journal of Plant Pathology* **113** 35-45.
Verma J (1994). Fusarium solani: immunochemical characterization of allergens. *International Archives of Allergy and Immunology* **104** 175-183.
Verma J, Sridhara S, Singh BP and Gangal SV (1995). Studies in shared antigenic/ allergenic components among fungi. *Allergy* **50** 811-816.
Vidal DR (1989). Invivotoxicity of T.2 toxin a fusarium mycotoxin to mouse peritindal macrophages infect. *Immunology* **57**(7) 2260- 2264.
Ward TJ, Bielawski JP, Kistler HC, Sullivan E and O'Donnell K (2002). Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. *Proceedings of the National Academy of Sciences of the United States of America* **99** 9278-9283.
Wiebe MG (2002). Mycoprotein from Fusarium venenatum: a well-stablished product for human consumption. *Applied Microbiology and Biotechnology* **58**(4) 421-427.
Wiebe MG (2004). Quorn Mycoprotein- overview of a successful fungal product. *Mycologist* **18**(1) 17-20.