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ORIGINAL ARTICLE

A Comparative Regional and International Standard Limits prospects on T-2 Toxin pollutions in some States of Iran

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ABSTRACT

There is a great need to validate analytical methods for rapid, sensitive and accurate determination mycotoxins in order to properly assess the relevant risk of exposure and to ensure that regulatory levels fixed by the regional or international organizations are met. The aim of this study is to provide a measure of the significance of in cereals and cereal-based products impurities with T-2 toxin for human consumption in Iran. New harvested and cropped wheat samples obtained from 7 provinces .T-toxin levels, were exceeded the standard limits (20-21μg/Kg-bw) therefore, revealed are not safe for consumption. All found values were too much lower than toxin limits of acceptance by the FDA (100 ppb, are equal <50%), 60.0 ppb by the EC are equal <10% thus all examined cropped wheat got authorized for international consumption. An inevitable part of the preventive measures is regular foodstuffs monitoring with mycotoxicological examinations. Based on our collected results between wheat samples and wheat based-flour products, there are no significant differences in T-toxin pollution upon the national permitted limit as well as the CE and FDA advisory acceptance.

Keywords: *T-2 Toxin, Wheat, Iran, Regional and International Standard Limits.*

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INTRODUCTION

Mycotoxins are produced mainly by the mycelia structure of the filamentous fungi, commonly referred to as molds. *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) [1].

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 [2]. 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) [3].

A recent SCOOP project, aiming to evaluate the risk of dietary exposure to *Fusarium t*oxins by the population of EU member states, showed that *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 [3].

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 [4- 8].

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. 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 [9].

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 [10]. 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 [11]. A multitude of agricultural products are exposed to fungal contamination from the early stages of planting until their eventual consumption. 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 [2].

MATERIAL 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 grindin 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.

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 µl 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.

Test procedure

Inserting a sufficient number of wells into the microwell holder for all standards and samples to be run. Recorded standard and sample positions. Pipeted 50µl of standard or prepared sample to separate well Added 50µl of enzyme conjugate (red cap) to the bottom of each well then 50µl 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 microwell 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 (250µl per well) then emptied the wells again and remove all remaining liquid. Repeating the washing step two more times. Added 100µl of substrate/chromogenic to each well. Mixed gently by shaking the plate manually and incubate for 5 min at room temperature in the dark. Adding 100µl 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.

$$AbsorbanceStandard\% = \frac{\text{Absorbance of standard}}{Absorbance of NegCon} \times 100$$

RESULTS AND DISCUSSION

According to data's from the National Department of Agricultural supervisions and Plant Pathology Institute, the available Nobel provinces for cereal agriculture and related sites realized in three territories as the North, South and West mean while 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 Figures 1-2, the percentaged 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. According to the Figure 4-5, T-toxin levels in several different cities, were exuded the standard limits (20-21μg/Kg-bw) respecting to the measuring method therefore, according harvested wheat were not safe for consumption. Of examining how statistically obtained datas from analytical measurements conducted on wheat samples is shown in the graph Figure 1-6, 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 interverse 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 incamed the same alignment with the normal distribution of numerical datas and mentioned above findings were confirmed (Asymp. Sig (2-tailed) 0.555).

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 wide spread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits [12, 13]. Unfortunately in Iran, a limited number of mycotoxins including Aflatoxins, Fumonisins, 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 [14, 15], 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 [16] and different results have been reported from various studies on measuring *Fusarium* toxins including the T-2 toxin. Yazdanpanah et al. [16] 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 [17]. 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 [16]. Furthermore, by testing 23 samples of one wheat-based food, Yazdanpanah et al. [16] 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% (Table 1.1 , Figure 1-2) and the rate of Yazdanpanah et al. [16] and results of Riazipour et al [2] 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.

Muller (1998) proved in various years 27-61 % of corn [18] and 0-14 % of provender wheat (Hussein et al., 1989) harvested from south Germany were 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 [19]. Lepschy et al (1989). also showed that 38 % of wheat, barley, rye, oat and flour were contaminated with T-2 toxin [18]. 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 %) [13].

So the average found T-toxin of many studied samples concern it that the majority of samples were above the 40-30ppb degrees and are higher levels of acceptance limits as much as 20ppb, which almost makes up more than 70% and the residues have been at the range of 20-30ppb, which can be due to the cumulative effect of the toxin considered it as a serious threat.

The provision standards for T-2 in food according to the type of product and the sensitivity of wheat in some countries, strategic, and values differ, there is below than 25ppb for animal-feed Plants or for human consumption has been announced 20 ppb that differ in countries with too much differ amounts, such as, 20 µg/kg (Slovakia) to maximum levels up to 300 µg/kg (Hungary) are designated [20].

Although the distribution of T-2 concentration in the range of intended counter are not correlated (Figure 6) But it should be noted that the highest frequency of T-2 toxin concentrations in the range of 30-

40ppb could be seen in Northern; Ardebil, Southern; Khuzestan and Northern; Golestan, were seen. Reasonably could showed that *Fusarium* in all provinces have to be examined in temporary holding wheat fields or barns, silo, or the transportation solution exists (Figure 3).Thus, the highest possibility for T-toxin production out of the majority of samples collected , the Northern region of Iran , then the Southern of Iran and the Western are concomitantly included. (Figure 1, 2 Table 1). Based on our collected results between wheat samples and wheat based-flour products manufacturing process, there are no significant differences in the level of the T-toxin pollution above the national permitted limit as well as the CE and FDA advisory acceptance levels. Considering all the results of this study we can be said to explain that *Fusarium* could be the main T-2 producer, and at intervals after planting ,cultivation and harvesting process eventually transportation or accommodation activities remains, with the impact grains long-term. Remaining viable on grains for a long term periods, of farm and food products shall cause unsuspected or desirable pollution problems, this level of contamination varies according to geographical regions, but during the process of turning wheat into flour such as milling contamination levels of toxins, including T-2 may be a somewhat rises or falls that have not been neglected too.

Table 1. 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**	

N:north, S: south,W: west

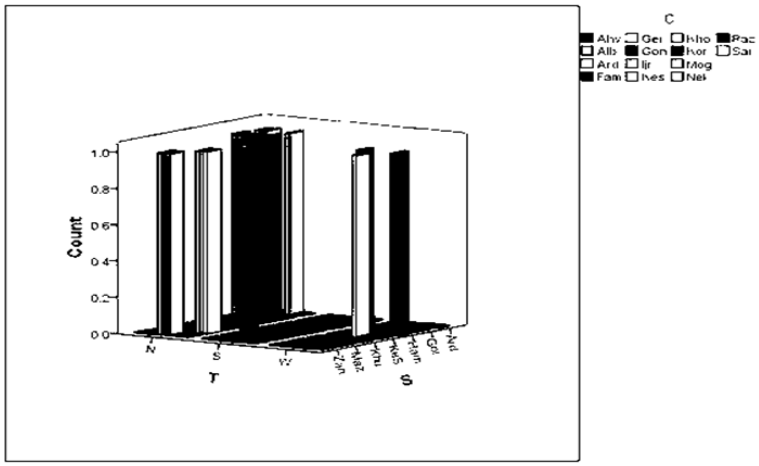

Figure 1: Distribution of sampling sites in the North, South and West territories simplified cities Which includes the divided provinces (N-north(Ard (Ardebil),Maz(mazandaran) Gol (Golestan)), S-south(Khu (Khuzestan)) and(W-West(Zan (Zanjan) , Kes (Kermanshah)and Ham (Hamedan)) of the country.

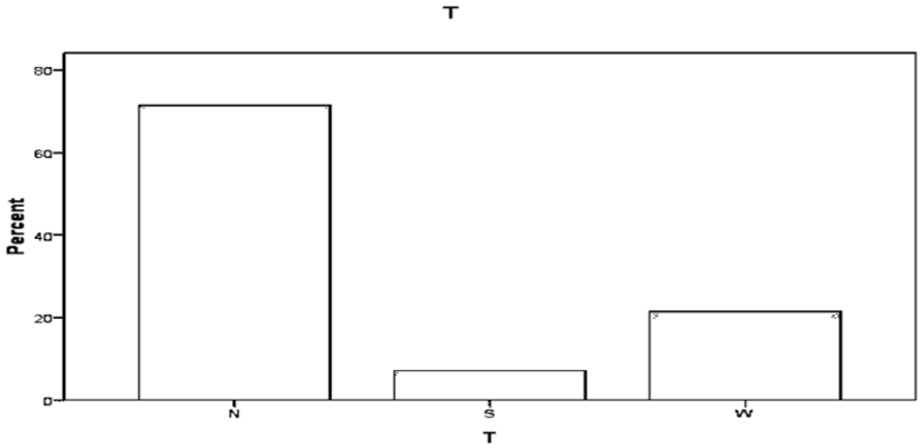

Figure 1-2: provides examples of the sample propagation percentages in three geographic regions.

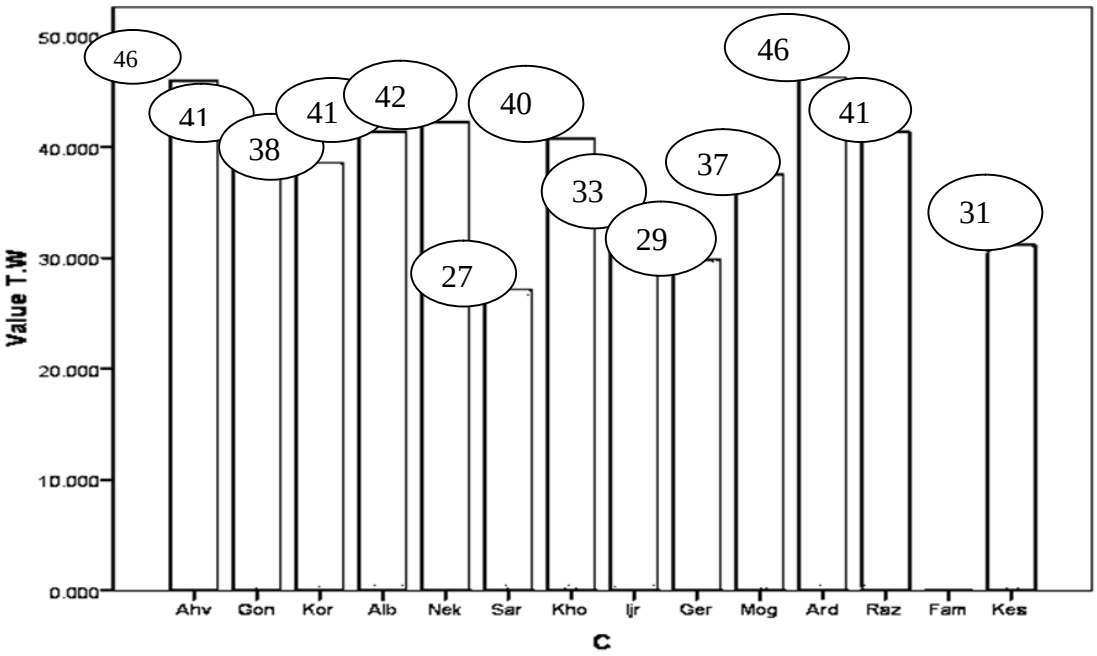

Figure 1-3: Measured T-toxin levels in wheat samples of the studied cities (line; indicating Iran national standard limit, 20 ppb).

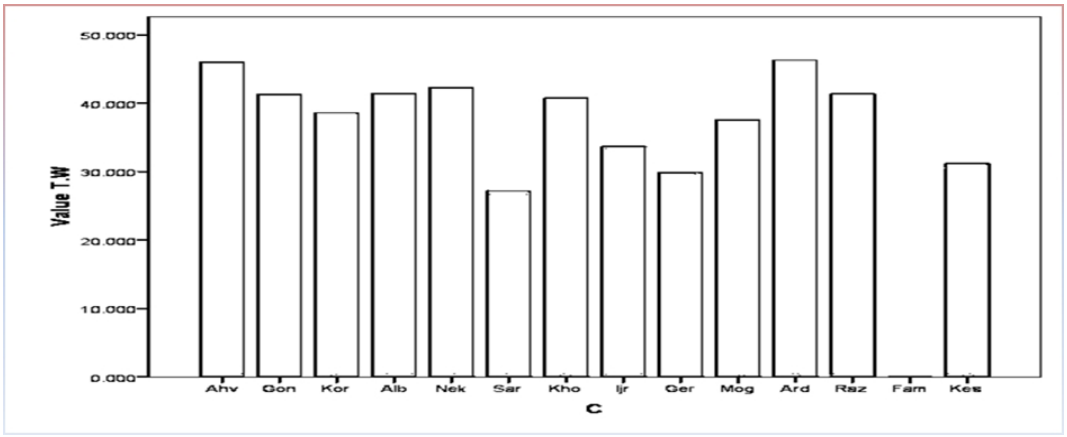

Figure 1-4: The mean toxin measured quantities in compare with FDA standard limits (FDA = 100ppb). All found values were too much lower than the amount of toxin limits of acceptance by the FDA for T-2 which is 100 ppb are equal <50%,Thus, all examined cropped wheat outraised for consumption.

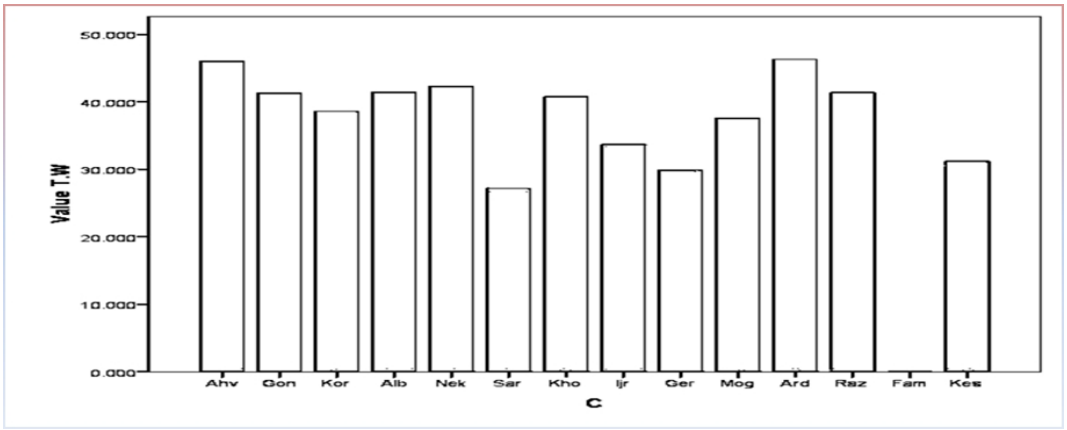

Figure 1-5: Comparing produced T-2 toxin in relation to CE standard (CE = 0/06µg or 60ppb)

All found values were estimate about <10%lower than the amount of toxin limits of acceptance determined by the EC for T-2 which is equal to 60.0 ppb,thus, all examined cropped wheats outraised for consumption.

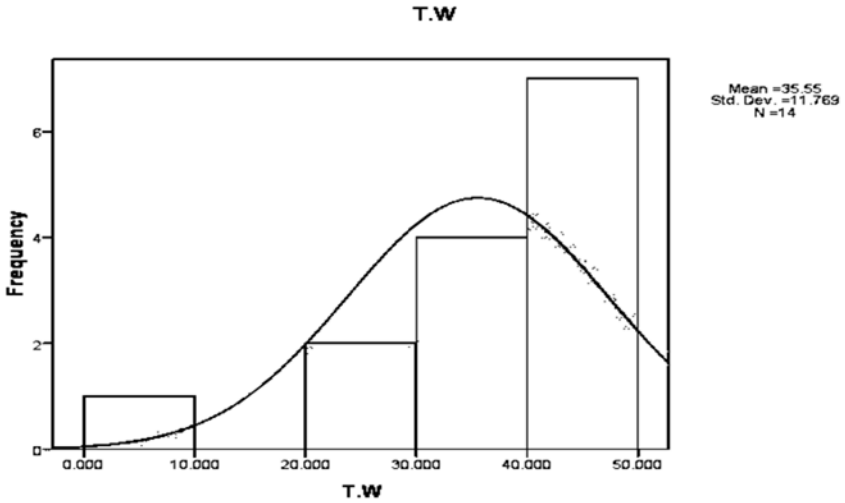

Figure 1-6: Diagram of obtained wheat samples toxin quantities normal distribution in different intervals (0.0 - 55ppb) using Paird Samples Correlations.

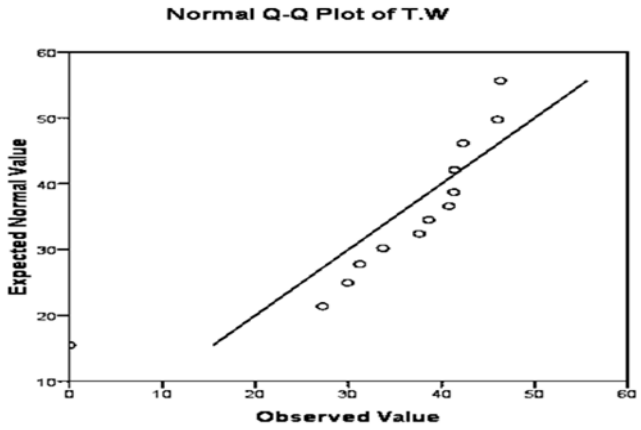

Figure 1-7: Normal QQ Plot Diagram of the T-toxin scattering data.

CONCLUSION

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.

REFERENCES

1. Visconti A. [2001].Lattanzio V.M.T.,Pascale M.,Haidukovaski M. Analysis Of T-2 and HT-2 toxins in cereal grains by Immunoaffinity clean-up and liquid chromatography with fluorescence detection journals of chromatography 2005; A.1075: 151-158.

2. Riazipour M, ImaniFooladi AA, Bagherpour G. [2012]. Survey of T-2 Toxin Present in Cereals Destined for Human Consumption. Jundishapur J Microbiol. 5(3): 497-501.
3. European Commission. Recommendation of 17 August 2006 on the prevention and reduction of Fusarium toxins in cereals and cereal products. Official Journal of the European Union2006. L234, p. 35-40.
4. Maragos C.M. [2004]. Emerging technologies for mycotoxin detection. Journal of Toxicology. 23: 317-344.
5. Krska R., Welzig E., Berthiller F., Molinelli A., Mizaikoff B. [2005]. Advances in the analysis of mycotoxins and its quality assurance. Food Additives and Contaminants. 22: 345-353.
6. Zheng M.Z., Richard J.L., Binder J. [2006]. A review of rapid methods for the analysis of mycotoxins.Mycopathologia. 161: 261-27.
7. Goryacheva I.Y., de Saeger S., Eremin S.A., van Peteghem C. [2007]. Immunochemical methods for rapid mycotoxin detection: Evolution from single to multiple analyte screening – a review. Food Additives and Contaminants. 24**:** 1169-1183.
8. Pascale M., Visconti A. [2007]. Overview of detection methods for mycotoxins, in: J.F. Leslie, R. Bandyopadhyay and A. Visconti (eds.), Mycotoxins: detection methods, management, public health and agricultural trade. CABI Publishing, Wallingford, U.K., Chapter**;** 15, p. 173-185
9. Bhatnagar D, Yu J, Ehrlich KC. [2002]. Toxins of filamentous fungi.ChemImmunol. 81: 167-206
10. Steyn PS. [1998]. The biosynthesis of mycotoxins.Revuede medicine Veterinaire. 149: 469–478
11. Fink-Gremmels J . [1999]. Mycotoxins: their implications for human and animal health; Vet Q. 21 (4): 115-20**.**
12. Shephard GS, Marasas WF, Leggott NL, Yazdanpanah H, Rahimian H, Safavi N. [2000]. Natural occurrence of fumonisins in corn from Iran. J Agric Food Chem. 48(5):1860-4.
13. Tanaka K, Sago Y, Zheng Y, Nakagawa H, Kushiro M. [2007]. Mycotoxins in rice. Int J Food Microbiol. 119 (1-2): 59-66.
14. Egmond HP, Jonker M, [2003]. Food, Nations AOotU, Milieu RvVe. Worldwide regulations for mycotoxins in food and feed in 2003. Food and Agriculture Organization of the United Nations; 2004.
15. Ghiasian SA, Maghsood AH, Yazdanpanah H, Shephard GS, Van Der Westhuizen L, Vismer HF, et al. [2006]. Incidence of Fusarium verticillioides and levels of fumonisins in corn from main production areas in Iran. J Agric FoodChem**.** 54(16) :6118-6122.
16. Yazdanpanah H, Daraei B, Saifi Z, Rasekh HR, Shafaati A, Mansour-Khani MJK1. [1998]. Natural occurrence of Fusarium mycotoxins in a corn-based product from the Iranian market. Toxicology Letters. 95(1001):155.
17. Vrabcheva T, Gessler R, Usleber E, Martlbauer E. [1996]. First survey on the natural occurrence of Fusarium mycotoxins in Bulgarian wheat. Mycopathologia. 136 (1): 47-52.
18. Lepschy-v Gleissenthal J, Dietrich R, Martlbauer E, Schuster M, Suss A, Terplan G. [1989]. A survey on the occurrence of Fusarium mycotoxins in Bavarian cereals from the 1987 harvest. Z Lebensm Unters Forsch. 188(6): 521-6.
19. Hussein HM, Franich RA, Baxter M, Andrew IG. [1989]. Naturally occurring Fusarium toxins in New Zealand maize. Food Addit Contam. 6(1): 49-57.
20. Reddy KRN, Salleh B, Saad B, Abbas HK, Abel CA, Shier WT. [2010]. An overview of mycotoxin contamination in foods and its implications for human health.Toxin Reviews. 29 (1): 3-26.