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ORIGINAL ARTICLE

Measuring toxin Zearalenone in wheat harvested from three regions of North, West and South of Iran

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ABSTRACT
Zearalenone (Mycotoxin F2) is a mycotoxin produced by genus Fusarium in cereals. This mycotoxin is a non-steroidal metabolite with esterogenic-like effects that causes reproductive problems in animals specially swine (infertility, abortion) and hyper estrogenism in women.Fusarium species are commonly associated with cereals can produce several secondary toxic metabolites.In the samples collected from province a premier manufacturer and the collection and preparation of cellex tracts by ELISA Kits and Ryda screen Zearalenone analysis. of the samples produced Zearalenone analyzed.The maximum number of samples in the range of 12-14 ppb have toxin treated according to the standard value of 25 ppb is for animal feed can beserious attentionto the cumulative effects of toxin, serious risk and should not be over looked because of the country's cities an provinces where there. The maximum number of values found more than50% is standard, and a serious risk is considered. The aim of this study was to determine the contamination of wheat grain in one of the risk factors (toxin zearalenone) in Superior territory in Iran.
Keywords: Zearalenone, Wheat, Territories of Iran.

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INTRODUCTION

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus*, *Penicillium*and *Fusarium*. Mycotoxin contamination of grain is a serious problem in conventional and organic cereal production [1]. Currently; more than 400 mycotoxins are identified in the world. Considering their heat stability, these substances constitute a potential risk for human and animal health. The chemical and biological properties of mycotoxins and their toxic effects are extremely variable. These negative effects include carcinogecity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity and immunotoxicity and females being more sensitive than males. Mycotoxins are not only dangerous for the Public Health, but they also deteriorate the marketable quality of the contaminated products, causing tremendous economic losses [2].Wheat is one of the most important grains consumed in the world. Food contamination with toxigenic moulds increased attention over the last three decades. Most grain, such as wheat, maize, bean and rice, can be infested by filamentous and microscopic fungi. Some genera can produce toxic secondary metabolites, namely mycotoxins, which impact on food safety. Mycotoxins are secondary metabolites produced by fungi when they grow on agricultural products before or after harvest or during transportation or storage. Currently, more than mycotoxins have been identified in the world, but the most important groups of mycotoxins are the major health concern for humans and animals, and occur quite often in food including: AFs, OTA, trichothecenes (deoxynivalenol, nivalenol),ZEA and fumonisins. During growth in the fields, maize and other cereals are exposed to mycoflora. Substrate moisture (>20%), air temperature and relative humidity (< 90 %) provide "field fungi" excellent environmental conditions for development .The most frequent "field fungi" are *Fusarium* species, which can colonize the straw, grain and ear before the harvest [3]. Zearalenone, an estrogenic metabolite, commonly occurs with DON in cereal crops in the United States. Zearalenone and its derivatives (a-zearalenol, bzearalenol, zeranol, taleranol and zearalanone) can be produced by *Fusarium*.spp. In corn stems infected by *Fusarium*in the field and in rice culture. However, the highest amounts of Zearale ZEA none are produced by *Fusarium*during storage whereas low amounts are

synthesized during crop growth [4]. Zearalenone induces feminization at dietary concentrations of less than 1 ppm, whereas higher concentrations interfere with conception, ovulation, implantation, fetal development, and the viability of newborn animals. Previous studies conducted on the effect of milling on DON in wheat were mostly on eastern Canadian wheats: hard white wheat, hard spring wheat, Ontario soft white winter and Quebec red hard spring wheat, and durum and red spring wheat. Milling studies conducted so far on U.S. wheats were on hard red winter wheat and soft wheat. The effect of milling on zearalenone in Korean wheat was reported. The crop year from July 1992 through June 1993 for all districts in Kansas was characterized by above-normal total precipitation. For most of the spring and summer of 1993, heavy and frequent rains saturated fields in central and eastern Kansas. Such a condition favors growth and toxin production by *E graminearum,* the fungus that produces DON and zearalenone. Our major objective was to determine the distribution and level of DON and zearalenone in milled fractions from the 1993 Kansas hard red winter wheat milling performance study [5]. The presence of ZEA in food stuffs may cause hyperestrogenism in women, as they are highly sensitive to the action of estrogenous hormones. Their excessive levels may result in numerous manifested systemic disorders, such as decreased libido, ovarian and uterine dysfunctions, an ovulation, infertility and neoplasmic lesions in the entire reproductive system [6].Therefore, a rapid and sensitive technique for routine assay of mycotoxins in foods is necessary. Over the last years, the importance and application of immunoassays, especially enzyme-linked immunosorbent assay (ELISA), has grown significantly. ELISA test kits became very popular recently due to their relatively low cost and easy application and their results could be comparable with those obtained by other conventional methods such as TLC and HPLC (7). There are several types of chromatographic methods available for mycotoxins analysis. Traditionally the most popular methods used for mycotoxins analysis are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). These methods require extensive sample preparation and are expensive. Methods for the detection of mycotoxins are mainly based on chromatography and immunochemistry. A number of liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods detect a large range of mycotoxins and their metabolites in a variety of food and feed commodities In 2007, Sulyok et al. reported an LC-MS/MS method capable of detecting 87 analytes with just a single extraction step after which the diluted crude extract was measured directly(8). Several studies carried out in Europe and in transcontinental countries, reported the high incidence of ZEA in cereals and in animal feeding stuffs. Bottalico (1998) reported the occurrence of ZEA and its derivatives at levels up to 2758 g.kg-1 and up to 175 g.kg-1 in cereal grains worldwide and in European countries (respectively). In the United Kingdom, maize for human consumption and maize products used in animal feed have been found to have high concentrations of ZEA and its derivatives at ranges of 4 to 584 g.kg-1 and 55 to 1400 g.kg-1 (respectively) (4). ZEA was regulated in 1996 by 6 countries, by the year 2003 ZEA was regulated by 16 countries. Limits for ZEA in cereals currently vary from 50 to 1000 μg/kg. Current regulations of ZEA in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO (2004).Toxicity of Zearalenone and its metabolites, described as estrogenic properties, is related to the chemical structure of the mycotoxins, a structure similar to naturally occurring estrogens (estradiol, estrone and estriol). Interaction of such compounds with human estrogen receptors in competition with 17-bestradiol was also reported. The estrogenic potency of ZEA has been shown to be several times higher than that of other environmental estrogens in various test assays (4).The aim of this study was to determine the contamination of wheat grain in one of the risk factors (toxin zearalenone) in Superior territory in Iran.

MATERIALS AND METHODS

Samples Preparation

Fresh wheat samples harvested from early May to early September from 7 provinces producing wheat countries, including thesouthern provinces (khozestan),West (including Kermanshah, hamedan) and North (including Zanjan, Ardebil, Mazandaran, Golestan) forever one hundred store kilogram sample provided was noticed that, after preparation, wheat samples collected for use (Removal of debris and odor filter with source of current 2 in 2 special Flour Factory), drying and adjust humidity, mixing and re-mixing for each of the samples was done , then four samples of 100g were randomly selected in order to sample measurements, sample control , samples tock and the sample was prepared for flour, and Wheat samples were then were taken and the continuation processed one by the Laboratory mills, and after communication, the wheat will be ready to start the extraction of toxins. Releasing toxins in solution using solvent extraction separation is done with the solvent containing 40 ml methanol, 40 ml ethanol and 20 ml of acetone for each sample at first were taken 10g chopped and transferred to a suitable container will then 20 mL serum physiology and 20 mL of solvent Extract was added and shaken for 30Minutes and heading. And then transferred to a water bath for lower10 mL the volume of extract Falcon tube, and then

extract using a filter Whatman No.1 that has no active charcoal flat that this operation with simultaneous transfer of 10 ml of deionized is tilled water to wet the filter and also dilute the extract and speeding the movement takes place 50 microliters are used for testing.

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. Pipetted 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-Zearalenone 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 to well 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 substrate/chromogen 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 log it/log evaluation and for double or multiple determinations cubic spine should be used. The course of the standard curve is shown in the quality Assurance Certificate enclosed in the test kit.

RESULT AND DISCUSSION

The total number of wheat samples collected from the North, West and South of Iran, in seven provinces, including Khuzestan, Golestan, Ardebil, Zanjan, Kermanshah, Hamedan, Iranconducteda14 out of 14 citiesand shopping center was wheat (Figure 1.1). According to (table 1 and figure 2), indicate that the number of samples obtained from regions shows,that from the North Country (Northern) with10 samples from the same city and a frequency of 71.4 percent, the highest, West Country (Western) with three samples of the same number and frequency of 21.4% south of the country (Southern) and the number one example of the city and its frequency is 7.1 percent. Measured according to the amount of zearalenone in grain samples and fresh compared to the amount of toxin zearalenone observed in samples of bread flour to maintain compliance with the standards and practices Average nutritional value approved by mixing wheat flour was provided by the country. After reviewing the statistical Pearson determine the significant correlation in the P<0/05 was found that the amount of toxinin wheat and flour prepared inversely proportional. (PC = 0/19) but not statistically significant. (Sig = 0/74)

In examining how numbers obtained from measurements some of toxin in wheat samples in the following diagram shown, the skewness to the right of the highest some toxin zea measured at intervals of 12 and 14, and because the number of samples who have occupied the high range of the curve to the right, resulting in a normal curveisdrawn.With a significant degree of correlation between the numerical difference between the values obtained from the venom of the Wheat flour is processed from the specified amount of toxin zearalenone in wheat Counter current and its imports from the disalignment quite reasonable, and the numerical difference Obtained with a Z: -2/54 and Sig: 0/01entirely due to the presence of toxin-producing agent in the process of Harvesting, handling, storage, meal preparation and is expected to take the package and also the Increased further. However, the per capita consumption of wheat flour in bread and bread made especially for the cumulative effect of the toxin (Figure1.3).

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 to ring of well-dried grains at optimal conditions. An inevitable part of the preventive measures is regular foodstuffs monitoring with mycological and mycotoxicological examinations. Contamination of feed with a *Fusarium* toxin can lead to impaired immune functions, metabolism disorders, decreased performance, and increased susceptibility to adverse environmental influences. 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 [9,10].Unfortunately in Iran, a limited number of mycotoxins including aflatoxins, fumonisins, zearalenone and, ochratoxins are only being measured in export products, but they are not usually checked in foodstuffs for domestic consumption [17,18]. Contamination of feed with mycotoxins is often a worldwide problem since there is no universal procedure that removes most of the mycotoxins without any effect on the nutritional value or not makes it more expensive to produce. With the aim of minor losses in the livestock industry, considerable attention is paid to the prevention of mycotoxins

contamination, and studies on different types of raw materials and compound feed, depending on various factors, are of great importance. The problem of mycotoxins is particularly expressed during rainy years, when the percentage of mould contamination which leads to subsequent formation of mycotoxins significantly increase. Among farm animals pigs are specially sensitive to mycotoxins, while ruminants are able to degrade the toxins in the rumen. In general, there is a lack of investigations on the presence of mycotoxins in animal feed [13]. In relation to the results of previous research for DON and ZEA in Croatia and also with the published data worldwide, it can be concluded that a certain number of feed samples in this research had significantly high concentrations. Also, comparing the obtained concentrations of DON and ZEA with the maximum recommended concentrations for these mycotoxins in feed the results indicated an increased contamination of pig feed with DON and ZEA, with mean concentrations of 1454±1444 μg/kg and 153±161 μg/kg, higher than recommended in 8 (20%) and 2 (5%) of the 40 samples of pig feed, respectively. Higher DON concentrations than recommended were also observed in 2 of 29 (7%) samples of feed for calves with a mean concentration of 1140±1288 μg/kg. DON and ZEA were determined with great variations of concentrations between the samples. The concentration of DON was generally [103 samples analyzed) higher than the recommended in about 10% of samples with a maximum concentration of 4107 μg/kg. A higher ZEA concentration than the maximum recommended was determined in about 2% of the total number of feed samples, with a maximum concentration of 558 μg/kg determined in feed for calves in the eastern part of the country. In this study it was also observed that the samples in which the low concentrations of DON were determined have also low concentrations of ZEA, or both mycotoxins were not detected, or mostly the results indicate on both higher concentrations as in our earlier study performed on feed for fattening pigs [13]. Zearalenone side-effects on health are undeniable. Due to the chronic and acute effects of zearalenone for consumers is qualified to provide sufficient information about its exposure to the general population. In the present study, zearalenone mycotoxin in Tafton, Sangak, Barbari and Lavash bread distributed in Kermanshah was investigated in by ELISA method. Our results indicate that all samples contaminated with zearalenone. Also results showed that most bread samples had contamination higher than of Europe standards but had consonant with Iran national standard, such as amount of higher than standard was not observed. Daily intake of zearalenone in the samples showed that Kermanshah breads are recognized dangerous of view this toxin and have stringent security to eliminate or reduce this toxin is thought by authorities. Not aggregation in occasion the effects of mycotoxins on human health, economic status and sensitivity to the toxin has caused the standard employed for each country is different. Few studies have examined the contamination of zearalenone in cereals. Kazemi et al in 2009 studied storage wheat in Azarbijan state perceived that 90% from sampels were polluted by mycotoxins. In a study conducted by Hadiani on corn in Mazandaran, was found 7.5% of cultivated maize containing zearalenone that are less than the maximum of authorized limit of the toxin in Iran But in the Hedayati study on the wheat samples 80.5% of samples were contaminated and the contamination of the 64.4 percent was higher than the authorized standard (200 mg per kg). The results of the present study were in contrast with the Hadyany and Hedayati results so that uses standard of Iran and to compare results of this study none of the samples were diagnosis contaminated. In the Yazdanpanah study on the assessment encounter of Tehran's population of the zearalenonemycotoxin of 72 samples of rice, bread, popcorn and wheat flour were collected. According to the Iran standard, all samples were below the maximum tolerable ZEA contamination in the food that is similar to results obtained from this study. According to the JECFA average of absorption this toxin of all the samples is less than the tolerable daily uptake. In the study Karami et al 8.6% of the 175 samples wheat of Golestan state were ZEA-contaminated, unlike our study; the extent of the contamination was not wide. Mean and range of pollution was determined, respectively, 72 and 39 to 104ng per g that was to be less than the recommended amount of zearalenone in wheat .Survey of contamination of zearalenone mycotoxin in cereals and other crops in other countries have led to different results. Schollenberger showed that of 125 samples of wheat, barley, corn and corn products in Germany, only two cases were free *Fusarium* pollution toxins and other samples were contaminated with one or more mycotoxins. The results of this study shows many similarities with have been done in such a way so that the high prevalence of mycotoxins in samples had evidence. In the Chełkowski study for determine zearalenone in wheat before harvest 106 wheat field in central, northern and southern Netherlands were studied. *Fusarium* species were analyzed in 1311 wheat samples. *Fusarium* was found in 48% of surveyed samples. Dominant species were detected in the center *Fusarium*, with prevalence of 1%and in the north and south of Germany respectively 53% and 55%. Manova and Associates study in Bulgaria, 91 samples were collected from wheat and corn crops of grain centers. Tests indicate that zearalenonecontamination of maize and wheat, respectively, compared to148mgkg and6/36 g/kg. The results of the study on the amount of toxin in wheat somewhat similar to Barbari but incompatible with other breads were examined. In our study wide scale the contamination of bread in our study to

zearalenone is indicator a lack of proper storage conditions of grain and opportunistic fungi. Park et al received during their investigation if mycotoxin-contaminated corn samples of to be stored for 8 days at 5°C contamination levels those increase of 35-14 ng per g to 110-538. Daily intake of zearalenone in the studied sample indicates Kermanshah city breads of view presence of the zearalenone mycotoxin are considered hazardous and should be stringent security measures to be considered to eliminate or reduce these toxins by the authorities. Daily intake of zearalenone in the sample indicates the presence of the zearalenone mycotoxin in Kermanshah city breads are considered hazardous and should be stringent security measures to eliminate or reduce these toxins by the authorities to be considered the Our results suggest that the type of bread and flour. Terms of contamination, there is no significant difference Taftoon and Barbari and was accounted the lowest and highest contamination levels of the toxin. Unfortunately, the origin of the wheat samples contaminated with zearalenone that were examined in this study was not unclear exactly, that is the product harvested from Kermanshah state or other states and other countries imported. Attention to this subject that breadis one of the most widely used food substances in cereal series, over prevalence contamination to zearalenone in wheat samples of various aspects can be considerable seriously. Thus, according to the results of this study indicate that the extent of contamination flour to the zearalenone toxin. In case of contamination with levels above the limit of the cycle is eating out. Attention to growth opportunist fungi toxin productive to be advice that long maintain of cereals and flour in silos and bread provision cent [14].The occurrence of mycotoxins produced by *Fusarium*.spp. in small cereal grains, particularly in wheat, is of great concern worldwide, because their presence in processed feeds and foods seems unavoidable. Consequently, they have been associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, in humans. In this survey, the members of *Gibberellafujikuroi* complex, especially *F. verticillioides*and *F. proliferatum,* were detected at the highest frequencies after *F. graminearum*. Our results are in agreement with other studies in the USA, Canada, Argentina and Europe [16].

In studies conducted in this study, the data relating to the north of the country, with 71.4%, the West21.4% and south of the country with 7.1% and interms of results and the toxin zearalenone with conduction studyis consistent (Figure 1 and Table1) .The frequency of the samples studied in this investigation have shown the location of collection Northern region have contributed to the production of wheat, whichis consistent with conduction study. Although the distribution of zearalenone concentration in the ranges considered, are not significantly correlated are Countercurrent (Table 3 and Figure 3). But it should be noted that most of the toxin zearalenone concentrations in the range of 12-14ppb, which may indicate endemic fungal causative agents of Zearalenone is a geographical area .Given that the largest amount of toxin production observed in the range of 12-14 ppb, therefore, this suggests The possibility of Fusarium infection in all studied in wheat fields or ware houses for temporary maintenance or transportation process Store it there (Figure 3 and Table1).The highest possible average toxin production due to the plurality of samples collected from the area North and south and to the West country (Figure 1 and 2).Based on the results of samples collected from the milling process and there are no significant differences, although pollution levels above the limit.

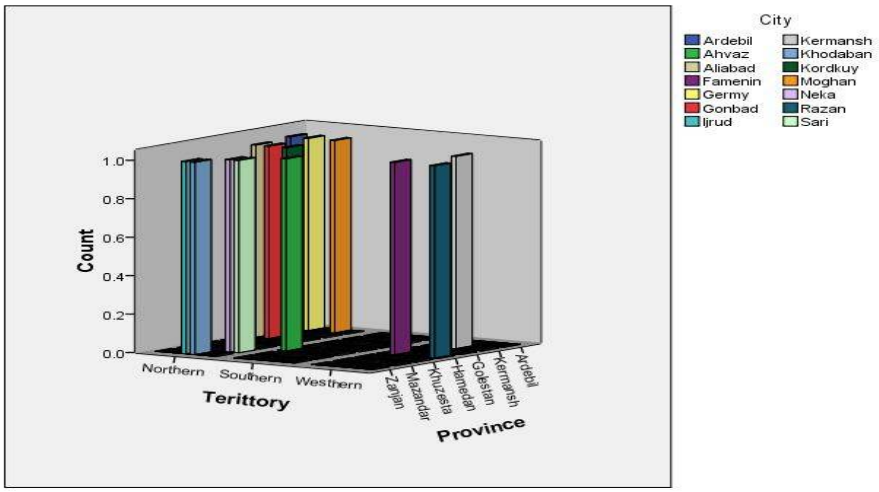

Figure 1.Distribution histograms of the sampling locations in the North, South and West Divided cities sampled

According to the results of this research can be said of all the major steel-producing *Fusarium* toxin zearalenone is at intervals after planting and cultivation remains, and in the longer termer mains and can

cause contamination of farm and food products the reform years. This level of contamination varies according to geographical regions, but in the process of turning wheat into flour contamination by toxin such as zearalenone toxin may be somewhat reduced and sometimes increased. Comparing the results of studies in other countries, it can be concluded that the major items to potential contamination of addition, fungi and toxins exist and should be harvested at all items human nutrition ingredients, apply to the use of international standards and conditions for shipping they keep creating. Another interesting point is that the harvest at end of the line Production or the food to be less time consuming, less of infection.

		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

Table1.1: Frequency of samples obtained based on the geographical distribution

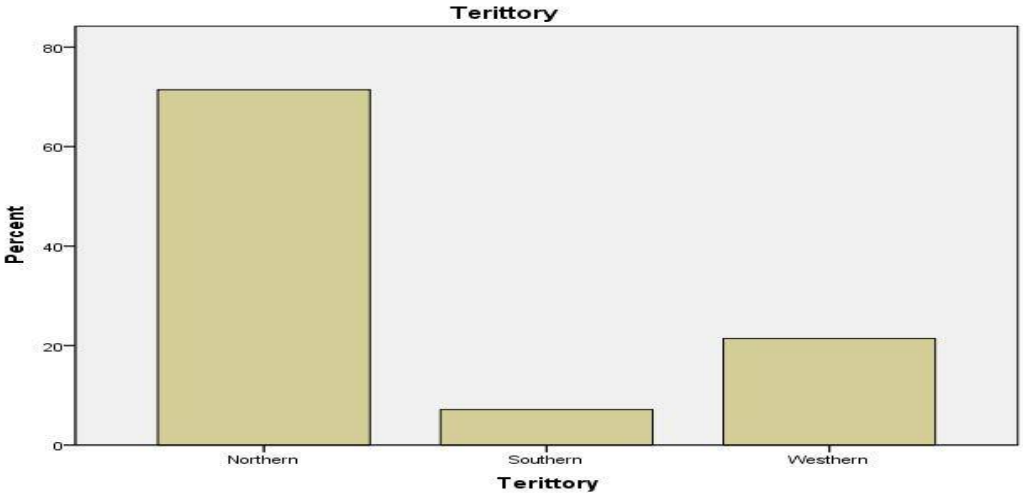

Figure2: Indicate that the samples obtained from the three regions of North,West and South.

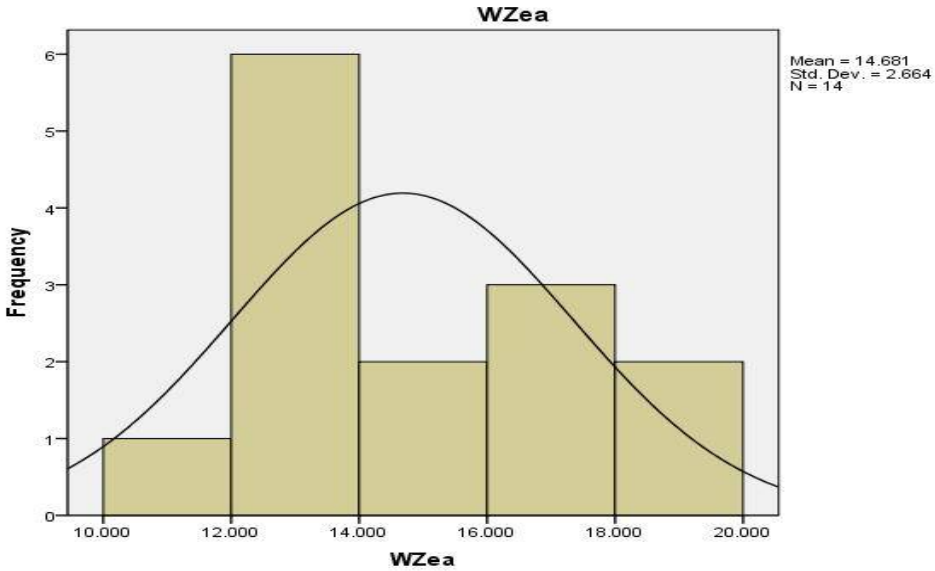

Figure3: Normalized frequency of Zea-toxin,obtained from samples of the wheat and the distribution between different

CONCLUSION

In recent years increasingly are aware of the release of mycotoxins in tropical regions of the world. Fungi capable of causing infection in the organs of plants and animals are alive or dead. Therefore, the possibility of contamination of crops in a field of mushrooms and their toxins there. Microbial flora of cereal such as wheat, barley, rye, millet, etc. The products resulting from direct contact with dirt, insects and other sources. Microbial flora of cereals, are often mainly during the growing, harvesting and storing molds.When grains Keptunder the wet conditions *Fusarium* are may be also causes spoilage. Products such as corn and wheat, and barley are usually kept at the farm level are infected. Studies have shown that after harvesting, the use of appropriate methods of processing, drying and storage it is necessary to prevent and the spread of contamination. Packaging is an important cereal crop in good condition Because of lack of ventilation, humidity and lack of proper treatment can cause a variety of fungal is infection. Comparison of fungal contamination of the samples have shown some samples with high levels of pesticide contamination was low despite the appearance of fungal infection in others not so much, but the amount of mushrooms has high levels of toxin production. Due to the relatively large proportion of human and animal feed grains and oilseeds and their products are formed animal feed contaminated with mycotoxins can have undesirable on sequences in terms of safety. For producers of crops and livestock and poultry growers, food merchants, Food and animal feed producers, consumers and the economy of countries around the world have followed. The results of this study represent contamination of wheat samples from different parts of the *Fusarium* is the species. This pollution not only from such economic damage caused by fungal decay products Is important, but the ability of a significant number of strains isolate din production levels High pathogenic fungal toxin zearalenone can be considered as a potential threat to human health hand Animals are raised. This results in the need for extensive epidemiological studies on the incidence, distribution and Genetic and biological diversity of the fungi with the aim of developing and implementing appropriate strategies and effective fungal contamination and mycotoxin control of human and animal foods and agricultural products reveals.

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