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A STUDY ON FUSARIUM T TOXIN AND FUMONISIN CONTENTS RELATIVITIES IN WHEAT GRAIN CROPS OF IRAN

Mohammad Reza Poor Mohammadi, Arash Chaichi Nosrati*

Div. Microbiology, Dep. Molecular& Cell Biology, Fac. Basic sciences, Islamic Azad University, Lahijan branch, Lahijan , Gilan, Iran

Corresponding authors: (Email: achn@iau-lahijan.ac.ir)

ABSTRACT

Wheat is one of the most important grains consumed in the world. Food contamination with toxigenic moulds increased attention over the last three decades, which impact on food safety. Since Fusarium species are commonly associated with cereals can produce several secondary toxic metabolites the samples collected from provincial premier and preparation of cell extracts then toxin estimation were done by ELISA (Kits and Rida Screen T-toxin/Fumonisin analysis R-Bio-Pharm GmbH) on the samples so that T-toxin/Fumonisin to be analyzed. According to the amount of fusarium toxins measured and comparing to the amount of toxin observed in grains maintain compliance found that the amount of WT/WFum (NPar-Wilcoxon Signed Ranks Test for WT - WFum; Z ; -3.296a , Asymp-Sig ; 0.001, Pearson Correlation ; -0.240, Sig; 0.408) for T-toxin correlations in wheat incomparison to Fumonisin -toxin correlations in wheat have no significant correlation despite reverse relations for the both, but not statistically significant was supporting by the Pearson statistical determinations a significant correlation for the two variables incontrast were statisticaly meaningfull in numerical differences(P< 0/05). The determinative pollutions of wheat samples were in the (Mean; 38.436 ppb, Range;19.100 (Min; 27.200, Max; 46.300 ppb),Var;34.229, Std.Dev.;5.851, Skewness; -0.644, Kurtosis; -0.472) for T toxin, Mean; 19.151 ppb, Range ; 6.780 (Min; 16.560,Max; 23.340 ppb),Var ; 4.306, Std. Dev.; 2.075;Skewness; 0.886,Kurtosis; -0.291 for Fumonisin were showed a considerable disalignmented correlation specialy . Thus according to the standard values for feed and food could be serious attention to the cumulative effects of toxins, a serious risk and that should not be overlooked about the cities and provinces where conducted .The maximum values of found respectively were more than standards 50% up to 100%, so a serious risk are considered.

KEY WORDS: *T-toxin, Fumonisin, Wheat grain, provinces of Iran*

Introduction

Mycotoxins are natural food and feed contaminants, mainly of grain is a serious problem in cereal production, considering a potential risk for human and animal, are not only dangerous for the Public Health, but they also deteriorate the quality of food and feed, causing tremendous economic losses (Solarska et al., 2009, Rashedi et al., 2012). Food contamination with toxigenic moulds

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increased attention over the last three decades, whith impact on food safety. Fusarium species, colonise before the harvest (CVETNIc et al., 2004). The most important Fusarium mycotoxins are Fumonisins, TCs such as T-2, HT-2, DON, DAS, FUS-X, NIV, diacetylnivalenol, neosolaniol and ZEA. They are common mycotoxins throughout the world, mainly associated with cereal crops, in particular corn, wheat, barley, rye, rice and oats (Ghazvinianet al., 2011, Shephardet al., 2000, Mankevicieneet al .,2011). Infection of cereal grains with Fusarium species can trigger serious human and animal diseases (Jereonet al., 2013, Mankevicieneet al ., 2011,Hazmi., 2010) In addition to corn or corn-based foods and feeds (Trigo et al.,1996, Sadeghi et al., 2012, Rashedi et al., 2012), Fusarium species are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia might occur more frequently in the warmer and subtropical part of the world (Alizadehet al., 2012, Feizyet al., 2014,Trigoet al ., 1996). Fumonisins, the TCs and ZEA are hazardous for human and animal health as they are stable to heating and are not degraded during normal food processing and consequently, commonly found on cereals grown in the temperate regions of Europe, America and Asia. The toxin is probably the most important of the northern temperate or modcrate regions (Ghazvinian et al., 2011). The toxin is commonly found world-wide on cereals such as wheat, rye, barley, oats and corn as Wheat is one of the most important grains consumed in the world epidemiological evidence indicates a link between human esophageal cancer and ingestion of Fusarium contaminated corn (WHO 2001). These are in cereals associated with the incidence of a high rate of human esophageal cancer in Africa , in northern Italy , in Iran , the Southeastern of the United States and with promotion of primary liver cancer in certain endemic areas of the China (Feizyet al., 2014, Pleadin et al., 2012,). There are no confirmed biomarkers for human exposure (WHO 2001), whereas higher concentrations interfere with the health status, major objectives on mycotoxin produced by genus Fusarium in cereals contribute to determine the distribution and level of Tcs, Fum,Don and Zea in milled fractions and wheat milling performance study (TRIGO-STOCKLI et al., 1996)although the allegation that TCs were responsible for the reported symptoms is controversial (Ghazvinian et al ., 2011). Following By now, causing most concern are T-2, which is the most acute toxic TC, HT-2, and NIV (Feizy et al., 2014, Feizy et al ., 2014) . Cytotoxic effects were observed at slightly higher doses of TCs (Ghazvinian et al., 2011). Generally, occurs together cereals products isolated from grains and beans were found to and its derivatives are generally found in various cereal crops such as wheat, corn, barley, oats and rye and processed grains (malt, beer and bread) (Jeroen et al., 2013). Since T-2 tetraol is the most stability metabolite, it is the most appropriate metabolite for diagnostic testing (Marasas et al., 1996, Trigo et al., 1996). The ATA Committee noted that IARC (1993) concluded that no data were available on the carcinogenicity to humans (Feinberg et al.1989, KARAMI et al.,2008 , Solarskaet al.,2009,Mallmannt et al.,2001 , Remza et al., 2014 ,Feizy et al., 2014). , Ghiasian et al., 2006). DON is the most often occurring TC and is prevalent in crops used for food and feed production (Solarska et al., 2009 , Riazipour et al., 2012). DON is less toxic than other TCs such as T-2, however, there have been reports that in Asia of illness in humans, associated with the consumption of cereals contaminate with DON and possibly much lower doses of other TCs (Feizy et al., 2014). In 1993, IARC placed DON in Group 3, not classifiable as to its carcinogenicity to humans (Trigo et al., 1996, Tanaka et al., 2007). A provisional maximum tolerable intake (PMTDI) of 1 μg/kg body weight (BW) was set by (Mallmannt et al., 2001). NIV occurs more often in years with dry and warm growing seasons. NIV is more frequently reported in Europe, Australia and Asia than in

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America. Both mean levels and incidence of positive samples of NIV are lower than for DON even in the Nordic countries and Europe (Egmond et al., 2004,Tanaka et al., 2010, Marasas et al., 1996, Khosravi et al., 2013) is abundant in various cereal crops such as corn, barley, mixed feed samples and other grains from various regions in the world. Co-existence of DAS and T-2 in animal feeds and human foods represent a health threat to humans and animals in some parts of the world (Alizadeh et al., 2012). Toxic effects of DAS in humans and animals seemed similar, Also, it is not mentioned very frequently in the research, as the main interest for TCs concerns DON, T-2 and HT-2 (Riazipour et al., 2012). Therefore, a rapid and sensitive technique for routine assay of mycotoxins in foods is necessary. There are several types of chromatographic methods available for mycotoxins analysis. Methods for the detection of mycotoxins are mainly based on chromatography and immunochemistry. 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)(Jeroen et al ., 2013). 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 (Feizy et al ., 2014). Several studies carried out in Erupian/transcontinental countries, reported the high incidence of Fusarium toxins in cereals and in animal feeding stuffs. Mycotoxin contamination in cereals is a potential risk to human and animal health. Among several hundreds of mycotoxins, T, Fum and Zea toxins are among the most important mycotoxins regarding food safety (Anne et al., 1996). Further more, an association between high rates of human esophageal cancer and high concentration in cereals has been reported in different countries (Trigoet al ., 1996). Wheat and rice are as the most important staple food for the human population Worldwide, in particular in the Middle East These grains areof the highest worldwide production as well as corn (Pleadin et al., 2012). Although contamination by the legal limits vary significantly both from country to country and by mycotoxin type and matrix; the determination methods need to provide accurate and reproducible results both within and between laboratories. Current regulations of fusarium toxins in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO (2004). The risks of fusarium toxins have been evaluated by The World Health Organization's International Programme on Chemical Safety (IPCS) and the Scientific Committee on Food (SCF) of the European Commission. They determined a tolerable daily intake (TDI) for Fumonisins, TCs such as T-2, HT-2, DON, NIVand ZEA, alone or in combination of µg/kg/body weight. (Riazipour et al., 2012)Cereal products are important in our food chain and economy. Therefore, foodstuffs need to be controlled/analyzed during food processing and all mycotoxin analyses for the entire food chain has importance for human health. It is important to continue to monitor the occurrence of these mycotoxins in cereals and cereal products. The aim of this study was to determine the contamination of wheat grains as one of the important risk factors in Superior terittories in Iran.

Materials and Methods

Fresh wheat samples harvested from the early May to late September 2014 from 7 superior wheat cultivating shores, including the southern provinces (khoozestan), Western (including Kermanshah, Hamedan) and Northern (including Zanjan, Ardebil, Mazandaran, Golestan), for every one

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hundreds of samples provided that, after preparation, drying/adjusting humidity, mixing and re-mixing for each four particular samples of 100g per 10 tone of origin were randomly selected in order to sample measurements, sample control , sample stock . The sample was prepared for flour, and Wheat samples were then were taken and processed were done by the Laboratory mills, Releasing toxins in solution using solvent extraction separation were done with the solvent containing 40 ml methanol, 40 ml ethanol and 20 ml of acetone up to 20 ml For each 10 g chopped/miled sample at first which transferred to a falcon tube container will praviously 20 ml NS and 20 mL of solvent Extract to be shaken for 30 Minutes and heading and then transferred to a water bath to reduce values to 10 ml, and then extracts separated using a filter paper Whatman No.1 flat that operating with simultaneous transfer of 10 ml of deionized distilled water to wet the filter and also dilute the extract and speeding the movement take place. Finally 100 micro liters were used for ELISA testing. To detect toxin levels in the fungal biomasses and the culture medium samples using the Competitive ELISA Procedure as described by R-Bio-Pharm GmbH was used and measured at the absorbance of 450nm (Rosi et al., 2007).

RESULTS

Of wheat samples collected from the North, West and South of Iran, in seven provinces, including 14 cities and shopping centers (Figure 1), according to sampling distribution criterias that is indicateable the number of samples obtained from regions shows, the Northerns belonging a frequency of 71.4 percent, the highest, Westherns by a frequency of 21.4 % finally the lowest are in Southern bring its frequency of 7.1 percent(fig-1). According to the amount of T toxin measured in grain samples (Mean; 38.436, Range; 19.100 (Min; 27.200, Max; 46.300),Var; 34.229, Std. Dev ; 5.851, Skewness; -0.644, Kurtosis ; -0.472) comparing to the amount of Fum. toxin observed in (Mean; 19.151, Range; 6.780 (Min; 16.560, Max; 23.340),Var; 4.306, Std.Dev.; 2.075; Skewness; 0.886, Kurtosis; -0.291) maintain compliance with the standards and practices conserving National average nutritional values approvals found that the amount of toxins in wheat have no significant correlation despite reverse relation, but not statistically significant Supporting statistical determination and significant correlations . In examining wheat samples numbers/obtained measurements of toxin shown, normalized distribution frequency of obtained wheat samples for T/Fum. toxin,in different ranges concerning the highest T toxin measured zone were at interval ranges of 25-50ppb, Fum(16-24ppb) because the most number of samples have been accomulate tended the higher range of the curve to the right, a normal curve were resulted.

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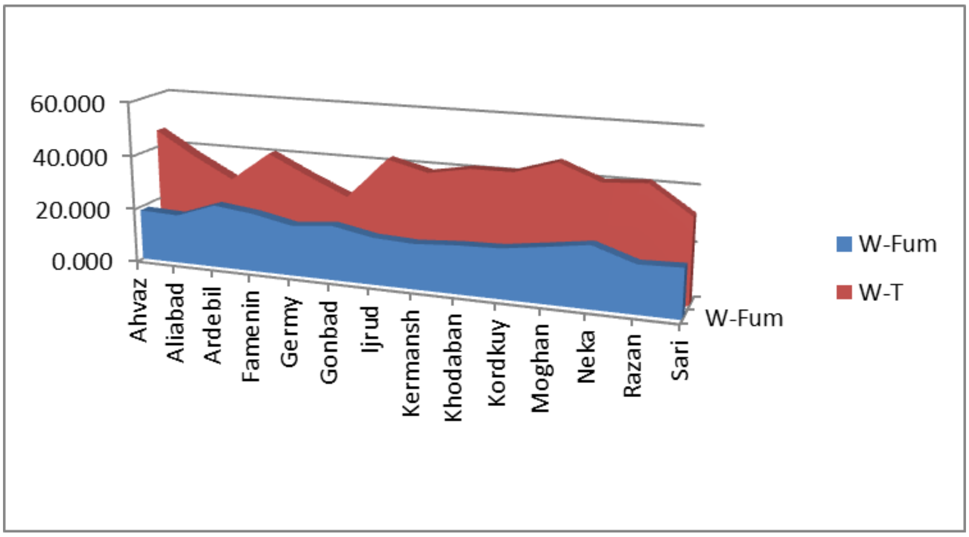

Figure 1: Normalized distribution frequency of obtained wheat samples Fum-T toxin, of different ranges, of the different cities

NPar-Wilcoxon Signed Ranks Test for WT - WFum;Z; -3.296a, Asymp-Sig.; 0.001, Pearson Correlation ; -0.240, Sig; 0.408

Counter currently a significant degrees of correlation between the numerical differences between the processed wheat T/Fum (NPar-Wilcoxon Signed Ranks Test; Z; -3.296a, Asymp-Sig ; 0.001, Pearson Correlation ; -0.240, Sig; 0.408) toxin values and their disalignment quite reasonable, entirely due to the presence of toxin-producing agents in the process of harvesting, handling, storage, meal preparation and suspectively to take place in the packages 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,that not be neglegable (Figure 1).

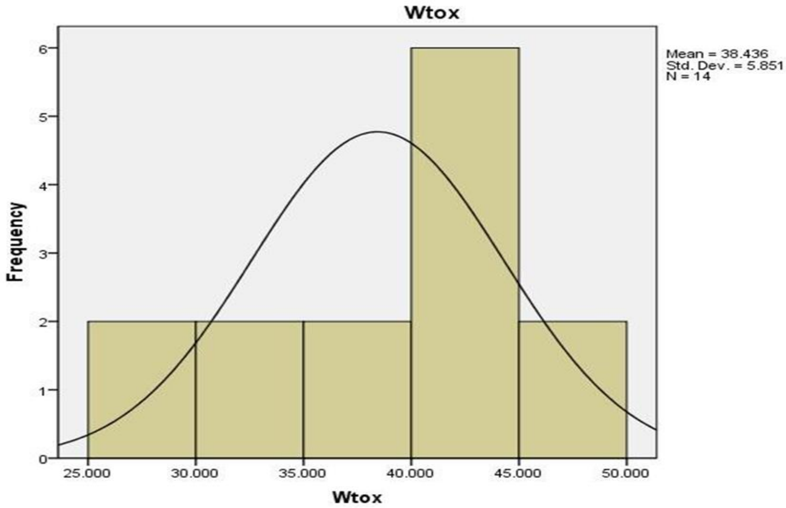

Figure 2: Normalized distribution frequency of obtained wheat samples T toxin

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Mean; 38.436, Range; 19.100 (Min; 27.200, Max; 46.300),Var; 34.229, Std.Dev.; 5.851, Skewness; -0.644, Kurtosis;-0.472

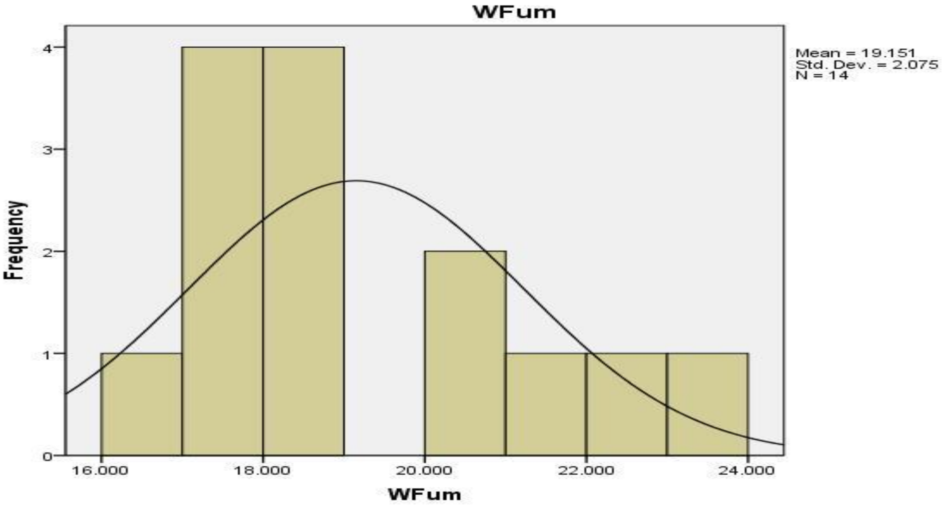

Figure 3: Normalized distribution frequency of obtained wheat samples Fumonisin
Mean; 19.151, Range; 6.780 (Min; 16.560, Max; 23.340), Var; 4.306,Std. Dev.; 2.075; Skewness; 0.886, Kurtosis; -0.291

According to the amount of T/Fum toxins measured in grain samples comparing to the amount of observed maintain compliance with the standards and practices conserving National average nutritional values approvals , found that the amount of toxin in wheat grains toxins have no significant correlation despite reverse relation, (PC;-0.077) but not statistically significant (Sig; 0.793) Supporting by the Pearson statistical determinations a significant correlation (P< 0/05). In examining wheat samples numbers/obtained measurements of T-toxin shown, Mean; 38.436, Range; 19.100(Min; 27.200, Max; 46.300),Var; 34.229, Std.Dev.; 5.851, Skewness; -0.644, Kurtosis; -0.472 concerning the highest fumonisins (Mean; 19.151, Range; 6.780 (Min; 16.560, Max; 23.340),Var; 4.306, Std.Dev.; 2.075; Skewness; 0.886,Kurtosis; -0.291) measured zone were at interval ranges of 16-24ppb, and for T-toxin 25-50ppb,because the most number of samples have been accomulate tended the higher range of the curve to the right, a normal curve is resulting in drawn(Figure 2 and 3). Counter currently a significant degrees of correlation between the numerical differences between the wheat grain T-toxin values and fumonisin and its disalignment quite reasonable, (NPar-Wilcoxon Signed Ranks Test for WT - WFum; Z; -3.296a, Asymp-Sig.; 0.001, Pearson Correlation ; -0.240, Sig;0.408) entirely due to the presence of toxin-producing agents in the process of harvesting, handling, storage, meal preparation and suspectively to take place in the packages 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, that not be neglegable.

Discussion

In moderate and 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. Contamination of feed with a Fusarium toxin can lead

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to disorders. An inevitable part of the preventive measures is regular food stuffs monitoring with mycological and mycotoxicological examinations. In 1999, the worldwide contamination of Fusarium mycotoxins (DON, NIV, ZEA, DAS, T-2, HT-2) in cereal grains have been reported by Placinta et al. , Schollenberger et al., 2006. In 2000, mycotoxin contamination (DON, NIV, ZEA) in rice have been suggested by Tanaka et al. (Pleadin et al., 2012) In 2001 the SCOOP (Scientific Co-operation on Questions relating to Food) have been reported data of Fusarium toxins (DON, NIV, FUS-X, T-2, HT-2, DAS, ZEA) in collected cereals (The Netherlands, Norway, Portugal, Sweden, UK, Italy, Germany, France, Finland , Denmark, Belgium, Austria) . Between 2003 and 2005, the studies of DON, T-2 toxin, ZEA and FUS (FB1+FB2+FB3) in cereal samples collected from European and Mediterranean markets and Asian-Pacific region have been reported by Binder et al (Karami et al., 2008). The limit values of Fusarium mycotoxins in cereal and cereal products (in the USA, EU) are given by (Hazmi et al., 2010, Leslie et al., 2006 Solarska et al.,2009). The International Agency for Research on Cancer (IARC) has evaluated the cancer risk of Fusarium mycotoxins to humans and grouped them as group 2B (probably carcinogenic). Having carcinogenic potential and poisonous effects, mycotoxins are considered to be one of the most important regulatory issues. However, based on available information on the occurrence of Fusarium mycotoxins, FDA accepted that typical Fusarium mycotoxins levels found in products intended for human consumption are much lower than the recommended levels . A provisional maximum is fixed for tolerable daily intake (PMTDI) for FB1, B2 and B3 single or in combination of Fusarium mycotoxins werer 2 μg/kg of body weight per day on the basis of the NOEL of 0.2 mg/kg of body weight per day and safety factor of 100. In countries with adequate information about mycotoxin occurrence, regular tests to control foodstuffs and detect widespread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits (Shephard et al., 2000; Tanaka et al., 2007). In 2001 the SCOOP (Scientific Co-operation on Questions relating to Food) have been reported data of Fusarium toxins (DON, NIV, FUS-X, T-2, HT-2, DAS, ZEA) in wheat, corn, barley, oat, rye [4]. Unfortunately, a limited number of mycotoxins including Aflatoxins, Fumonisins, Zearalenone and, Ochratoxins are only being measured only in export products, but they are not usually checked in foodstuffs for domestic consumption in Iran. (Egmond et al., 2003, Ghiasian et al., 2006) , Contamination of feed with mycotoxins is often a worldwide problem since there is no universal procedure that removes most of the mycotoxins without any affect on the nutritional value or not make it more expensive to produce. With the aim of minor losses in the industry, considerable attention is paid to the prevention of Fusarium mycotoxins contamination, and studies on different types of raw materials and compound feed, depending on various factors, are of great importance. In general, there is a lack of investigations on the presence of mycotoxins in food and feed. (Pleadin et al ., 2012). In relation to the results of previous researchs and also with the published data worldwide, it can be concluded that a certain number of wheat grain samples in this research had significantly high Fusarium mycotoxins concentrations, also, comparing the obtained concentrations of T tox, Fum with the maximum recommended concentrations for these mycotoxins in feed the results indicated an increased contamination of who feed with Fum ,T and ZEA or even Don, with mean concentrations of more higher than recommended for food and feed, respectively (Pleadin et al ., 2012). A higher Fusarium mycotoxins concentration than the maximum recommended was determined in about 60% of the total number of samples, with a maximum concentration of T(50ppb), Fum (24ppb) determined in the northern then the other part of the country.

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In this study it was also observed that the samples in which the low concentrations of ZEA were determined have less fumonisins and predominantly more concentrations of T toxin, or both mycotoxins always could be detected, or mostly the results indicate on both higher concentrations as in our study performed on processed wheat obtained by mixing imported wheat crops. In the past studies, investigated in by ELISA method indicated that all samples were contaminated ,also results showed that most 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 surprisingly confirm our results about the original wheat crops. Khosravi et al, 2013, published datas on Mycoflora profiles of fresh and stored rice grains showed that Aspergillus species (37.3%, 40.7%) were the predominant fungal agents, followed by Fusarium (21.6%, 16.2%) as the second agents respectively. Survey of contamination of mycotoxin in cereals and other crops in other countries have led to different results. Alizadeh et al.2012 reported that Fumonisin B1 (FB1 a toxic and carcinogenic mycotoxin produced in cereals due to fungal infection) contamination of rice and corn samples and its relationship with the rate of esophageal cancer (EC) in a high risk area in northeastern Iran geographical subdivisions of Golestan province . Therefore, fumonisin contamination in commonly used staple foods, especially rice, may be considered as a potential risk factor for EC in this high risk region. Daily intake of Fusarium toxins are considered hazardous and should be stringent those results suggesting that the type of bread and flour in terms of contamination, showed no significant differences and were accounted the lowest and the highest contamination levels of the toxin. Attention to this subject that wheat is one of the most widely used food substances in cereal series, over prevalence contamination in wheat samples of various aspects can be considerable seriously (Sadeghi et al ., 2014). 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 foods seems unavoidable. Our results are in agreement with other studies in the USA, Canada, Argentina and Europe (Chehri et al ., 2010). Although the distribution of Fusarium toxins concentration in the ranges considered, are not significantly correlated are countercurrent (Figure 1). But it should be noted that most concentrations were in the range of which may indicate endemic fungal causative agents of fusarium toxins in the conducted geographical areas (Figure 2 and3).Given that the largest amount of toxin production observed in the range of ppb, therefore, this suggests the possibility of Fusarium infection in all studied wheat fields or ware houses for temporary maintenance or transportation process .Based on the results of samples collected there are no significant differences, although pollution levels above the limit.According to the results of this research can be said that of all the major steel-producing Fusarium toxin, is at intervals after planting and cultivation remains, and in the longer term remains and can cause contamination of farm and food products there for years. This level of contamination varies according to geographical regions,but contamination by toxins toxin may be some what reduced and sometimes increased. Comparing the results of studies in other countries, it can be concluded that the major items of potential contamination of food due to 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 the end of the line Production or the food to be less time consuming, less chance of infection .

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