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EVALUATIONS OF FUMONISINS QUANTITIES IN WHEAT CROPS HARVESTED FROM NORTH, WEST AND SOUTH REGIONAL PROVINCES OF IRAN

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ABSTRACT

Wheat is one of the most important grains consumed in the world. Food contamination by toxigenic molds increased attention over the last three decades, which impact on food safety. Mycotoxin contamination in cereals is a potential risk to human and animal health. 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 was done by ELISA (Kits and RidaScreen fumonisins analysis R-Bio-Pharm GmbH) on the samples so that fumonisins to be analyzed. In examining wheat samples numbers/obtained measurements of toxin shown, the skewness 0.886, Kurtosis;-0.291 concerning the highest fumonisins measured zone were at interval ranges of 16-24ppb, and because the most number of samples have been accumulate tended the higher range of the curve to the maximum pollution of samples were in the range of 16-24 ppb have toxin threatening according to the standard value of 2µg/kg for feed could be serious attention to the cumulative effects of toxin, a serious risk and should not be overlooked about the cities and provinces where there.The maximum values of found respectively were more than standards up to 50%, so a serious risk are considered.The aim of this study was to determine the contamination of wheat grains in one of the chemical risk factors in superior territories in Iran.

KEY WORDS: *Fumonisin, Wheat, Territories of Iran*

Introduction

Mycotoxins are natural food and feed contaminants, mainly of grain are a serious problem in conventional and organic cereal production (Solarska*et al*., 2009).Currently, more mycotoxins are identified in the world, considering constitute a potential risk for human and animal health, not only dangerous for the Public Health, but they also deteriorate the marketable quality of the contaminated products, causing tremendous economic losses.(Rashedi *et al*., 2012).Wheat is one of the most important grains consumed in the world. Currently, 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 fumonisins (CVETNIC *et al*., 2004).The summary of the IARC evaluation of *Fusarium* mycotoxins is reported several Fusarium species. Ingestion of food contaminated with mycotoxins can cause mycotoxicosis which is an acute or chronic toxicity

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(Tanka et al., 2007).The European Commission regulates levels of fumonisinin as U.S.Food and Drug Administration specifies significantly higher limits of all three total fumonisins (FB1+FB2+FB3): a limit of 5000–100000µg/kg in human food and 2000–4000µg/kg in animal feed.Fumonisins, commonly occurs in cereal crops and their derivatives, However, low amounts are synthesized during crop growth whereas the highest amounts are produced by Fusarium during storage (Hazmi., 2010). Epidemiological evidence indicates a link between human esophageal cancer and ingestion of Fusarium-contaminated corn (WHO 2001). FB1, in cereals was 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 People's Republic of China (Rashedi*et al*., 2012, Pleadin et al., 2012, IARC. 2002). Major objectives on mycotoxin produced by genus *Fusarium* in cereals contribute to determine the distribution and level of fumonisins, DON and zearalenone in milled fractions and wheat milling performance study (TRIGO-STOCKLI *et al*., 1996),therefore, a rapid and sensitive technique for routine assays of mycotoxins in foods is necessary. There are several types of chromatography methods available for mycotoxins analysis. Traditionally the most popular methods are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) require extensive sample preparation and are expensive.The AOAC official method for FB1 FB2 and FB3 uses a strong anion based sample cleanup followed by LC based analysis and fluorescence detection. Immunoaffinity column use for mycotoxins has increased because of its simple cleanup procedure and lower detection level of mycotoxins. Additionally, simple extraction procedures, including Quick, Easy, Cheap, Effective, Rugged, and Safe QuEChERS or modified QuEChERS methods coupled to LC/MS or LC/MS/MS have been increasingly used as alternatives to traditional derivatization and extensive cleanup (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 compared with those obtained by other conventional methods such as TLC and HPLC (Feizy *et al* ., 2014). Several studies carried out in European/transcontinental countries, reported higher incidence of fumonisins in polluted cereals and in animal feed stuffs. Among several hundreds of mycotoxins, fumonisins are of Fusarium toxins and are among the most important mycotoxins regarding food safety (Aghili et al., 2010). The presence of different fumonisin analogs, are known, and the most abundant analog in nature is fumonisin B1 (FB1), followed by FB2 and FB3 (Rashedi*et al*., 2012, FAO.2006). FB1 has been implicated in animal disorders. Furthermore, an association between high rates of human esophageal cancer and high concentration of fumonisin in cereals has been reported in different countries (Trigo et al., 1996). In 2001, the Joint FAO /WHO Expert Committee on Food Additives and Contaminants (JECFA) established a Provisional Maximum Tolerable Daily Intake (PMTDI) for fumonisins (the sum of FB1, FB2 and FB3) of 2.0µg/kg of body weight per day (Ghazvinian et al., 2011). The International Agency for Research on Cancer (IARC) determined that the FB1 belongs to Group 2B, possible human carcinogen (Feizy *et al*., 2014). Fumonisins are mainly found as natural contaminants of corn and corn-based foods (Jeroen et al., 2013). However, there is evidence that they can occur in other crops and medicinal plants (Khosravi et al., 2013). Rice (Shaphard et al., 2000), bean (Tanaka et al., 2007), wheat, barley and soybean (Alizadeh et al., 2012) as the most important staple food for the human population Worldwide, in particular in the Middle East. Grains are the second highest

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worldwide production after corn (Pleadin et al., 2012). Although contamination of wheat and rice with fumonisins has been reported in the United States and it has been studied extensively in the European Union unfortunately little information has come from Asia (Binder et al., 2007, Rameza et al., 2014). As 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 fumonisins in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO (2004).The risks of fumonisin B1 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 FB1, FB2, FB3, alone or in combination of 2µg/kg body weight (Riazipur 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 (fumonisins toxin) in superior territories in Iran.

Materials and Methods

Fresh wheat samples harvested from the early May to late September from 7superior wheat cultivating shores, including the southern provinces (khoozestan),Western (including Kermanshah, Hamedan) and Northern (including Zanjan, Ardebil, Mazandaran, Golestan), for every one hundred of samples provided .that, after preparation, wheat collected for use, drying/adjusting humidity, mixing and re-mixing for each sample were done, four samples of 100g were randomly selected in order to sample measurements per 10 tonnes of origin, sample control, sample stock and the sample was prepared for flour and Wheat samples were then taken and process were done by the Laboratory mills, after combination. Releasing toxins in solution using solvent extraction separation were done with the solvent containing 40ml methanol, 40mlethanol and 20ml of acetone up to 20ml for each 10g chopped/miled sample at first which transferred to a falcon tube container will previously 20ml NS and 20mL of solvent Extract to be shaken for 30 Minutes and heading and then transferred to a water bath to reduce values to 10ml, and then extracts separated using a filter paper WhatmanNo.1 flat that operating with simultaneous transfer of 10ml of deionized distilled water to wet the filter and also dilute the extract and speeding the movement take place. Finally 50 microliters were used for ELISA testing.To detect fumonisins 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 observance of 450nm (Rosi et al; 2007).

Results

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, where 14 out of 14 cities and shopping centers (Figure1). According to the amount of fumonisins measured in grain samples comparing to the amount of toxin observed in bread flour maintain compliance with the standards and practices conserving National average nutritional values approvals (autorising the

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mixing wheat flours) found that the amount of toxin in wheat and flour 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 sample numbers/obtained measurements of toxin shown, the skewness:0.886, Kurtosis:-0.291 concerning the highest fumonisins measured zone were at interval ranges of 16-24ppb, and because the most number of samples have been accumulated tended the higher range of the curve to the right, a normal curve is resulting in drawn (Figure 2).

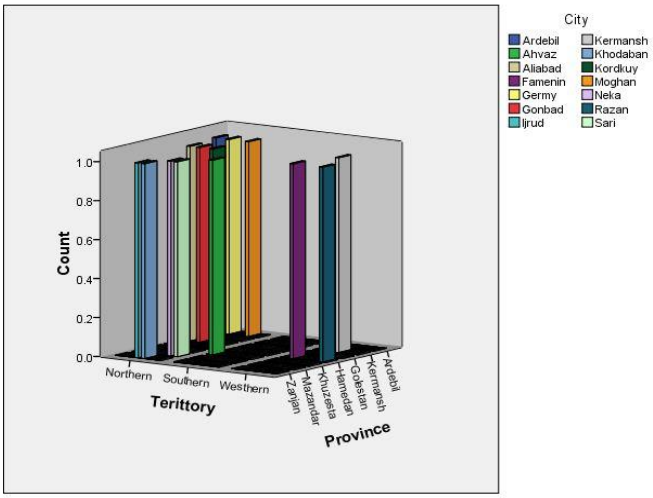

Figure1: histograms of the sampling cities, locations distribution in the North, South and West

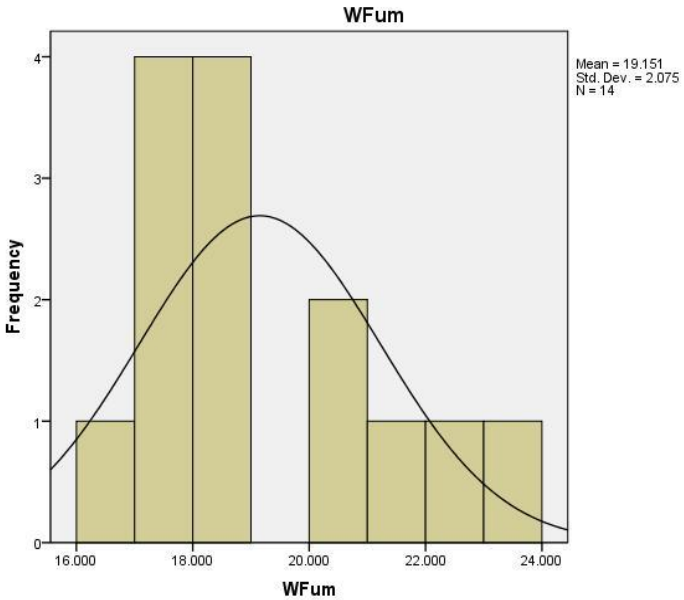

Figure 2: Normalized distribution frequency of obtaining wheat samples Fum-toxin, of the different ranges

Range: 6.780, Minimum: 16.560, Maximum: 23.340, Skewness:0.886, Kurtosis:-0.291

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Discussion

In moderate climates, the occurrence of *Fusarium* and their toxins in cereals is predisposed primarily by wet and cold vegetation periods requisite preventive measures against the multiplication of fungi and toxin production include tearing of well-dried grains at optimal conditions. An inevitable part of the preventive measures are regular foodstuffs monitoring with Mycological and mycotoxicological examinations. However, based on available information on the occurrence of fumonisins, the FDA accepted that typical fumonisin levels found in corn and corn 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 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. Some national estimates of intake of FB1 in Europe and in the world have displayed (Trigo et al., 1996) with adequate information about mycotoxin occurrence,regular tests to control foodstuffs and detect widespread and serious toxins 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 cereals (wheat, corn, barley, oat, rye) collected from 12 countries (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 fumonisins (FB1+FB2+FB3) in cereal samples collected from European and Mediterranean markets and Asian-Pacific region have been reported by Binder et al .The limit values of Fusarium mycotoxins in cereal and cereal products (in the USA, EU and Turkey) are given by (Hazmi et al.,2010)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 effect on the nutritional value or not make it more expensive to produce. 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 feed samples had significantly high Fumonisins concentrations,also, comparing the obtained concentrations mycotoxins in feed an increased contamination of who feed with Fumonisins, DON and ZEA with mean concentrations of more higher than recommended for food and feed, respectively (Pleadin et al ., 2012). A higher fumonisin concentration than the maximum recommended was determined in about 60% of the total number of samples, with a maximum concentration of 24.960ppb determined in the northern then the other part of the country shores. In some study it was also observed that the samples in which the low concentrations of ZEA were determined have predominantly more concentrations of fumonisin, or both mycotoxins always could be detected, or mostly the results indicate on both higher concentrations as in our study performed on processed wheat flour obtained by mixing imported wheat crops.Fumonisin side-effects on health are undeniable,due to the chronic and acute effects for consumers is qualified to provide sufficient information about its exposure to the general population. In the past studies, investigated in by ELISA method indicated that all samples were contaminated with fumonisin. Also results showed that most samples had contamination higher than of Europe standards but had

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consonant with Iran national standard, such amount of higher than standard was not observed surprisingly confirm our results about the original wheat crops and processed wheat flour for bread making.According to the JECFA average of absorption of all the samples is less than the tolerable daily uptake. Daily intake of fumonisin reasonably showed that such are recognized dangerous of view and have stringent security to eliminate or reduce this toxin is thought by the authorities since 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 fumonisin in cereals in Iran. Khosravi et al.,2013 published data 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. In HPLC analysis, most of the rice samples (96.7%) collected were found to be positive for FB1 with mean levels ranging from not detected to 56.2 mg/kg for fresh samples and from 4.3 to 42.8 mg/kg for storing ones. FB1 levels varied from one zone to another and throughout the storage time, showing a decreasing trend in most zones.Rice samples with a high prevalence of diverse species of toxigenic fungi, in particular Fusarium species, and high levels of FB1 in many samples indicate the need for proper surveillance and monitoring exclusively for the prevention of fungi and FB1 in rice produced in Mazandaran province. Survey of contamination of fumonisin mycotoxin in cereals and other crops in other countries has led to different results. Schollenberger showed that only two cases were free -fusarium pollution toxins and other samples were contaminated with one or more mycotoxins of samples of wheat, barley, corn and corn products in Germany, shows many similarities to have been done in such a way so that the high prevalence of mycotoxins in samples had evidence. Alizadeh et al.2012 reported that Fumonisin B1 (FB1 a toxic and carcinogenic mycotoxin produced in cereals)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 were measured by thin layer and high pressure liquid chromatographies. The mean level of FB1 and the proportions of FB1 contaminated samples were compared between low and high EC-risk areas of the province. The mean of FB1 levels in corn and rice samples were 223.64 and 21.59 µg/g,respectively. FB1 contamination was found in 50% and 40.9% of corn and rice samples, respectively, even found high levels of correlations between FB1 contamination in rice and the risk of EC. Therefore, fumonisin contamination in commonly used staple foods, especially rice, corn or wheat, may be considered as a potential risk factor for EC in this high risk region.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 a lesser extent, in humans(Chehri*et al* ., 2010).In studies conducted in this investigation, the data relating to the north of the country, with 71.4%, the West21.4% and south of the country with 7.1% and in terms of results and the toxin fumonisin with conduction studyis consistent 50% of the frequent samples studied in this investigation have shown the Northern region location of samples collection have contributed to the production of wheat, is consistent with the toxin conduction study.Although the distribution of fumonisin concentration in the ranges considered, are not significantly correlated are countercurrent (Figure 1andTable1,Figure 3). But it should be noted that most of fumonisin concentrations were in the range of 16-25ppb, which may indicate endemic fungal causative agents of fumonisinin the conducted geographical areas.Given that the largest amount of toxin production observed in the range of 16-25ppb ppb,

therefore, this suggests the possibility of Fusarium infection in all studied wheat fields or ware houses for temporary maintenance or transportation process Storepit there(Figure 3andTable1).The highest possible average toxin production due to the plurality of samples collected from the area north and south and then to the West of country (Figure 1 and 2).

Conclusion

In recent years increasingly are aware of the release of mycotoxins in the world tropical regions. Therefore,the possibility of contamination of crops in a field of fungi and their toxins depending microbial flora ofcerealssuch as wheat, barley, rye, millet, etc and products resulting from direct contact with initial or environmental sources, are of tenmainly during the growing, harvesting and storing molds even when grains kept under the humid conditions. *Fusarium* spp. are maybe also cause spoilage. Studies have shown that after harvesting, the use of appropriate methods of processing, drying and storage it is necessary to prevent and spread of contamination. Packaging is an important step in cereal crops good conditioning. According to the results of this research can be said that of all the major steel-producing Fusarium toxin, fumonisin, 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. 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 lined Production or the food to be less time consuming, less chance of infestation .Due to the relatively large proportion of human and animal feed grains and oil seeds and their products are formed animal feed contaminated with mycotoxins can have undesirable consequences in terms of public health and food secure and safety.The results of this study represent contamination of wheat samples from different parts of Iran is important, but the ability of a significant considered as a potential threat to human health and animals are raised. This result reveals 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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