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AN ASSESSMENT OF AFLATOXIN LEVELS IN WHEAT GRAINS AND WHEAT FLOURS SAMPLES OF 5 PREMIER PROVINCES OF IRAN

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ABSTRACT

Food contamination by toxigenic molds increased attention over the last three decades, which impact on food safety, therefore, a rapid and sensitive technique for routine assays of mycotoxins in foods is necessary to continue to monitor the occurrence of these mycotoxins in cereals and cereal products. In the present research, wheat samples were provided from some provinces in different places and their aflatoxin production levels were studied by ELISA method (Kits and Rida Screen Aflatoxin analysis R-Bio-Pharm GmbH). The average and relative deviation of samples from the 14 cities, were 8.32ppb and 3.78ppb, respectively, indicating the presence of aflatoxin in newly harvested domestic wheat crops. After statistical studies and the determination of correlation and significance in $p< 0.05$, it was found that toxin level in wheat and processed flour cannot be inversely proportional to each other (PC:-0.135) and this correlation was not statistically significant (sig:0.65). The determinative pollutions of wheat samples were in the Mean:8.314,Range:12.300(Min:2.500,Max:14.800ppb),Var;14.263,Std.Dev:3.777,Skewness: 0.056, Kurtosis: -0.856 for aflatoxin. The determinative pollutions of wheat flour samples were in the Mean: 9.529, Range: 24.500 (Min: 3.300, Max: 27.800ppb),Var; 40.070, Std.Dev: 6.330, Skewness:1.895, Kurtosis:5.049 for aflatoxin. Statistic definitions for Wafla–Fafla (Z: -0.157a, Asymp-Sig:0.875, Pearson Correlation -0.135, Sig: 0.645) and were not shown a considerable disalignmented correlation specially at a Pearson Correlation Significance value. 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 were conducted.The maximum values of found respectively were more than standards 30% up to 100%, so a serious risk are considered.

KEY WORDS: *Aflatoxin, Wheat grains and flours, Provinces of Iran*

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Introduction

Wheat is the most important cereal crop in terms of cultivated area, production, and consumption in Iran. Owing to the abundant production and the main role of wheat and its flour products in the diet of humans and animals, they can play a very important role in endangering human health in case of contamination with health-threatening factors. Among fungal toxins, which have been noticed in several studies, are aflatoxins. Aflatoxins are a big group of mycotoxins which are produced by some species of *Aspergillus* named *Aspergillus flavus* and *Aspergillus nomius* on foods including legume, cereals, and feed. These species have a worldwide distribution and can cause aflatoxicosis disease in domestic animals and human. Both the Europe Commission and the United Nations Commission on Human Rights have recommended preventing or reducing the contamination with fungal toxins in cereals and cereal crops, aflatoxin is produced by different fungi the most important of which is *Aspergillus flavus* or related group that has a very wide distribution in nature and cereal crops are counted as one of its suitable growth substrates (ICMSF, 1996). Wheat contamination with fungi and aflatoxins has been noted in several studies. The highest contamination with aflatoxin was detected for B_1 with the average of 16.3µg/kg (Halt M., 1994). In Iran, the majority of the studies done with food in terms of aflatoxin and the factors producing it about pistachio were because of its export value, in other studies, the aflatoxin present in the diet has been examined as one of the possible risk factors for esophagus cancer in the Caspian coastal area Mazandaran and Golestan provinces, but not for Gilan province (Hormozdiari H et al., 1975). Currently, the basic principles of multimycotoxin, implementation, advantages and limitations of these methods are being investigated. Recent research projects with the purpose of assessing the risk of food exposure to fungal toxins in the populations of Europe Union (EU) countries have shown that fungal toxins have been widely distributed in the food chain in the EU. Food consumption for the entire population of adults was generally under TDIs of the related toxins whereas it was close to TDIs or, in some cases, more than normal level of high-risk groups such as infants and young children (Cotty and Jaime-Garcia, 2007). There is a need to develop and validate analytical methods for rapid, sensitive, and accurate determination of the mycotoxins present in cereals and their related products based on proper examination and evaluation of the risks associated with exposure to these mycotoxins in order to make sure completely. For this reason, there is constant supervision of the Europen Union and other international organizations have examined the high toxicity of aflatoxin and its effect on public health, aflatoxin level was determined in wheat flour samples of Golestan province in the north of Iran too. In order to study using standard sampling methods, thus, considering the negative effects of aflatoxin on health, aflatoxin contamination should be taken into consideration in future programs. Reducing aflatoxin contamination is possible by reducing wheat storage time and controller humidity (Taheri et al., 2012). In the present research, wheat samples, 1 kg for each 10 tons, from newly harvested wheat in the provinces of majority in wheat cultivation and production area were provided and studied using ELISA techniques.

Materials and Methods

Sampling newly harvested wheat in 7 wheat-producing provinces, the provinces of the south (Khuzestan), the west (Kermanshah, Hamedan) and the north (Golestan, Mazandaran, Zanjan and Ardebil), 1 kg for each 100 storage, tons of wheat collected for each sample, four 100g were

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randomly chosen which were prepared in order to flour making procedures (Lakzaiazar et al., 2014). Then, wheat samples were taken a grinding stage, then, to start toxin extraction.Toxin was released into the separating solution by using extraction solvent (contains 40ml methanol, 40ml ethanol and 20ml acetone) followed by being vigorously shaken and mixed for 30 minutes, next step, they are transferred to the bain-marie (water bath) for a period during which the volume of the extract reaches less than 10ml, then, the extract is filtered using Whatman filter number 1 which was active charcoal fire. This operation is in parallel with the transfer of 10ml deionized distilled water in order to wet the filter and also to dilute the extract and increase the flow speed.To detect aflatoxin levels in the wheats biomasses and the processed flour medium samples using the Competitive ELISA Procedure as described by R-Bio-Pharm GmbH Rida screen competitive immunoassay enzyme for the quantitative analysis of aflatoxin was used and measured at the observance of 450nm (Rosi et al; 2007).

Results

The amount of the samples harvested and sampling site distribution was evaluated, samples were prepared, after that, measurement and numerical value analysis were obtained According to our results, for wheat aflatoxin were fluctuated from 2.00 to 14.00 and 0.00-30.00ppb for wheat flour respectively. Given, aflatoxin levels follow a normal distribution (statistic definitions, Mean: 8.314, Range:12.300 (Min: 2.500, Max: 14.800),Var: 14.263, Std.Dev: 3.777, Skewness: 0.056, Kurtosis: -0.856) wheat flour aflatoxin were fluctuated from 0.06 to 0.86, respectively. Given, aflatoxin level in follows a normal distribution (Statistic definitions; Mean: 9.529, Range: 24.500 (Min: 3.300, Max: 27.800), Var: 40.070, Std.Dev: 6.330, Skewness:1.895, Kurtosis: 5.049 (Fig 1 and 2).

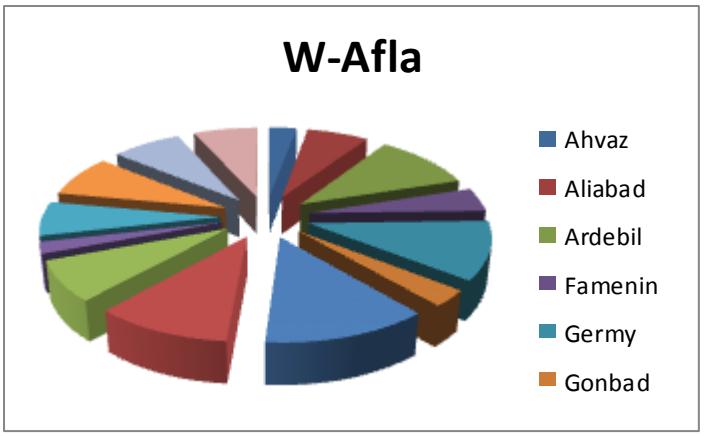

Fig 1- The concentration of the wheat toxin distributed in each city

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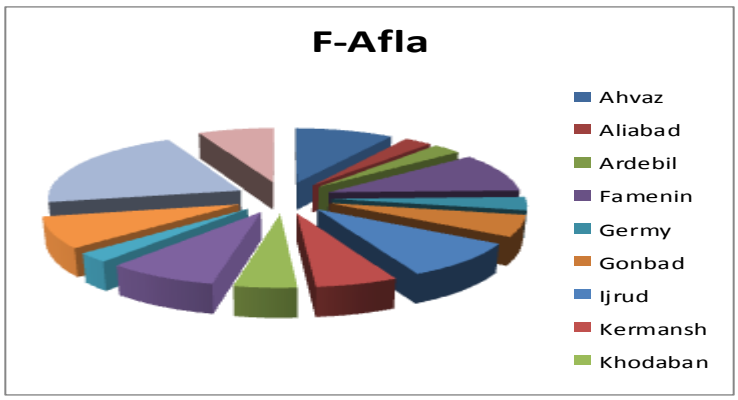

Fig 2- The concentration of the wheat toxin distributed in each city

The average and standard deviation of 14 samples from the same number sampled cities, which were 8.32 and 3.78 (mean: 8.314 and 9.529) respectively, indicate the presence of aflatoxin in newly harvested domestic wheat was crucial. The aflatoxin level measured in fresh wheat samples is compared to the aflatoxin level observed in bakery flour, processed samples which are provided complying with permitted toxin average, standard food value average, and by approved approaches such as mixing the wheat from country's different areas. Wheat samples were in the Mean: 8.314, Range:12.300 (Min: 2.500, Max: 14.800ppb), Var:14.263, Std.Dev:3.777, Skewness: 0.056, Kurtosis: -0.856 for aflatoxin. The determinative pollutions of wheat flour samples were in the Mean: 9.529, Range: 24.500 (Min: 3.300, Max: 27.800ppb), Var: 40.070, Std.Dev.; 6.330, Skewness: 1.895, Kurtosis: 5.049 for Aflatoxin. Statistic definitions for Wafla – Fafla (Z:-0.157a, Asymp-Sig:0.875) and were not shown a considerable disalignmented correlation specially at a Pearson Correlation Significance value (NPar-Wilcoxon Signed Ranks Test for FAfla-WAfla;Z; -0.157a, Asymp-Sig: 0.875, Pearson Correlation -0.135, Sig:0.645). After statistical studies and the determination of correlation and significance, it was found that toxin level in wheat and its level in mixed flour can be inversely proportional to each other (PC:0.135) and this correlation was not statistically significant (sig:0.65).The numerical difference between values was obtained through determining the correlation and significance degree. Given the toxin level in processed wheat, it was found that the aflatoxin level present in wheat is divergent and this divergence is not significant, and the numerical difference obtained in the degree of - 0.16 is accidental.

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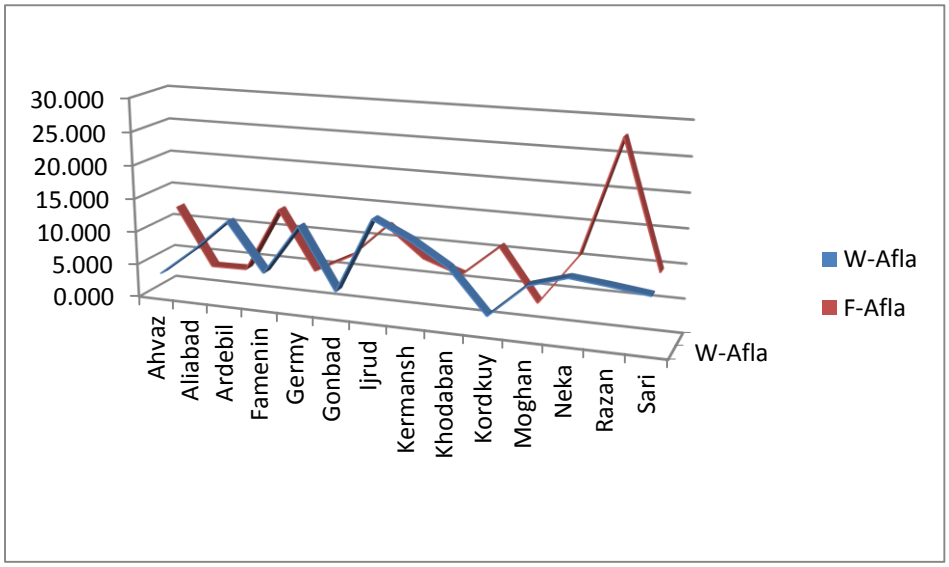

Fig 3- Toxin level in wheat and its level in mixed flour can be inversely proportional to each other NPar-Wilcoxon Signed Ranks Test for FAfla - WAfla:Z: -0.157a, Asymp-Sig: 0.875, Pearson Correlation -0.135, Sig:0.645

Discussion

Among fungal toxins, which have been highly noticed in several studies, are aflatoxins. This toxin can cause acute liver damage, cirrhosis, tumor induction, and teratogenic effects in humans or animals .Wheat contamination with fungi and aflatoxins has been noted in several studies, whereas *Aspergillus* with 34.87% has been defined as the dominant contaminating fungus, *Aspergillus flavus* with 9.94% had the greatest contamination level among *Aspergillus* species and the highest contamination with aflatoxin was detected for B_1 with the average of 16.3µg/kg (Halt M., 1994). In Iran, the majority of the studies done with food in terms of aflatoxin and the factors producing it about crops were of export values results obtained in the recent research, no clear and direct relationship between the climates of different areas of the country and no drastic presence of aflatoxin in domestic wheat samples was observed, but the important point made in the results was the presence of aflatoxin itself which should be taken into greater consideration. Average amount of aflatoxin was 8.3ppb, being far less than the global allowable limit, 30ppb even national permitted averages. The aflatoxin contamination range was between 1.3-7.1ppb, which is noticeably different from the results obtained in this study as Several reasons can justify this fact, including sample type (whether wheat is imported or is from that very area), sampling method, conditions and preservation place of wheat, which could possibly influence the level of contamination (Hedayati et al. ;2005). Aflatoxin levels in the research conducted by Halt et al., Abdullah et al., and Escobar were 16.3ppb, 11.25-252.5ppb, and 1-20ppb, respectively in comparison another investigated the existence of mycotoxins in different grain warehouses which is similar to the present research but with the difference that it has covered a wider range. Riazipour et al. (2006), findings showed that all samples provided from the cereals present in the warehouses were more or less contaminated with mycotoxin in an average aflatoxin levels of the whole samples 0.82 and 1.99 ng/g in summer and winter, respectively. B_1 aflatoxin levels, 3.1% and 7.4%, have identified more than the

permissible level of global law in the samples related to summer and winter. Despite the flour samples, contamination, were more than the national rules of the standard based on Iranian Standard Institute was not observed, but it was a lot higher than similar studies and the present study (8.3ppb). Hence, considering the negative effects of aflatoxin on health, aflatoxin contamination should be taken into consideration in future programs. Reducing aflatoxin contamination is possible by reducing wheat storage time and controller humidity. Brick et al. (2011), studied the fungus and mycotoxins present in wheat grain after the harvest such as aflatoxins (AFB1,AFB2,AFG1,AFG2),ochratoxinsA (OTA),zearalenone (ZON), deoxynivalenol (DON) and fumonisin (FB1, FB2) were assessed. Results have shown that, there have been lots of myxotoxins with a level from 36.3 to 2.891mg/g. This fact occurs due to the detrimental effect of the milling process, preventing their growth by decreasing the temperature, humidity, and contamination with pest in the sink is of great importance. The aflatoxin level measured in this research was so close to that in ours, measurement method, however, was not mentioned in this research. Mahmoudi et al. (2012), dealt with detecting aflatoxin and ochratoxin of wheat and flour in the north of Iran. In their study, in order to determine the presence of toxins and their levels, biological ELISA methods, similar to the method used in our study, were utilized. Toxins were compared to Iranian International Standard, Ochratoxin A has been measured commercial kit. Regarding mean contamination of the samples, lowest and highest contamination belongs to (6.71ppb). The least contamination existed in (5ppb) and a maximum of the contamination observed was in (21.42ppb). The values obtained for aflatoxin in this research were nearly the same as our findings except maximum amounts However, none of these amounts exceed the permissible limit 30ppb (Mahmoudi et al., 2012). Ramesh et al. (2013), aimed to study market samples of food grains and grain flours in terms of aflatoxin B1 contamination. The research has been conducted in order to study aflatoxin contamination in the food grains and grain flour locally, India. Food grains (Bengal chickpeas, Bajra/cumbu, corn, barley, sorghum) and green grains have been analyzed in terms of aflatoxin B1 in a way that every AOAC method has been implemented by thin layer chromatography with high performance under standard references.

Conclusion

In recent years increasingly are aware of the release of mycotoxins in the world tropical regions. Therefore, the possibility of contamination of crops of cereals such as wheat, barley, rye, millet, etc. and products resulting from direct contact with initial or environmental sources, even when grains kept under the humid conditions 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. Because of lack of ventilation, humidity and lack of proper treatment a variety of fungal causative agents of the samples have shown in some samples was low despite the appearance of fungal infection were not so much, but have 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 consequences in terms of public health and food security and safety. The results of this study represent contamination of wheat samples from different parts of Is important, but the ability of a significant considered as a potential threat to human health and animals is 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.

Acknowledgments
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