



## ORIGINAL ARTICLE

# An Assessment of Aflatoxin Levels in Wheat Samples of 5 top Provinces of Iran

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### ABSTRACT

Wheat is the most important cereal crop in terms of cultivated area, production, and consumption in the world as well in Iran. Fungi are among microorganisms with highly powerful metabolic activity, such during the growth on wheat are accompanied by secretion of compounds termed toxins, which, on the one hand, they cause different diseases in wheat plant or the decay of wheat grains in the farms and warehouses. In the present research, wheat samples were provided from 5 provinces of the country (Mazandaran, Guilan, Zanjan, Kermanshah, and Khuzestan) in different places and their aflatoxin production levels were studied by using ELISA method. The average and relatively deviation of samples from the 14 cities, were 8.32ppb and 3.78ppb, respectively, indicate the presence of aflatoxin in newly harvested domestic wheat crops. After Pearson's statistical studies and the determination of correlation and significance in  $p < 0.05$ , it was found that toxin level in wheat and processed flour can be inversely proportional to each other (PC: -0.135), and this correlation was not statistically significant (sig: 0.65). Finally, by examining the results obtained in this study and similar studies, which used different methods to measure aflatoxin, and the numbers obtained, we found out that ELISA has appropriate renewability, simplicity, selectivity, sensitivity, speed, and cost and can be considered a highly suitable method for the determination of mycotoxins in food products.

**Keywords:** Aflatoxin, Mycotoxins, Iran, Wheat

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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. In the farm and the warehouse, wheat can be invaded by different microorganisms, especially fungi. (Saari). Among fungal toxins, which have been noticed in several studies, are aflatoxins. This toxin can cause acute liver damage, cirrhosis, tumor induction, and teratogenic effects in humans or animals. 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. Wheat contamination with fungi and aflatoxins has been noted in several studies. In the studies done on flour products in Croatia, *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. The highest contamination with aflatoxin was detected for B<sub>1</sub> with the average of 16.3µg/kg. In Iran, the majority of the studies done on 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 Caspian coastal area (Mazandaran and Golestan provinces). 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 [7]. 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. Four major aflatoxins, B1, B2, G1, and G2, plus metabolic products of B1 named M1 and M2 are important as direct contaminants of foods and feed, accumulate in different tissues such as liver, lung, kidney, and immune, genital, neural, and digestive systems. This toxin causes liver cancer by affecting p53 gene. Also, the toxin causes mutation in animal and plant cells and chronic toxicity in animals. 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 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 for high-risk groups such as infants and young children. Firstly, it should be noted that fungal mycotoxin levels are low in general. However, their levels vary. Therefore, the danger is more in some years which this difference depends on climatic conditions and the way products are processed. According to the surveys carried out, mycotoxin level and mycotoxin changes depend on climate of the area, which cereals are more susceptible to mycotoxins in rainy seasons. During seasons with lighter rain, especially summer, and the season in which the crop is ripe, the infection is lower. And this level of variation is because of changes in climate which are uncontrollable [2]. 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 [6]. For this reason, there is constant supervision by the Europe Union and other international organizations. They are based on new technology including lateral flow device (LFD), enzyme flow-based membrane process through immunoassay, fluorescence polarization (FP) by immunoassay and near infrared spectroscopy (NIR), molecular imprinted polymer (MIP), plasmon resonance (SPR), and Biosensors [1] have examined the aflatoxin contamination in wheat flour samples of Golestan province in the north of Iran. Due to 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. In order to study the seasonal changes, summer and winter sampling has been done by using standard sampling methods [1]. Thus, considering 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 (1). In the present research, wheat samples, 1 kg for each 10 tons, from newly harvested wheat in the provinces of Kermanshah, Hamedan, Ardebil, Golestan, Zanjan, and Mazandaran 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), from first April to late August, 1 kg sample was provided for each 100 storage tons. After preparation of wheat collected samples, for each sample, four 100 g were randomly chosen which were prepared for measurement, control, stock, and flour making procedures. Then, wheat samples were taken a grinding stage. then, wheat samples were prepared to start toxin extraction. Toxin was released into the separating solution by using extraction solvent which contains 40 mL methanol, 40 mL ethanol, and 20 mL acetone. For each grinded wheat sample, first, 10 g is removed and transferred to a suitable Falcon tube container, 20 mL physiologic serum and 20 mL extraction solvent were then added 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 10 mL, Then, the extract is filtered using Whatman filter number 1 which was active charcoal free. This operation is in parallel with the transfer of 10 mL deionized distilled water in order to wet the filter and, also, to dilute the extract and increase the flow speed.

### **ELISA determination**

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 (kit Art. Nr.: R5302) was used and measured at the absorbance of 450nm [3].

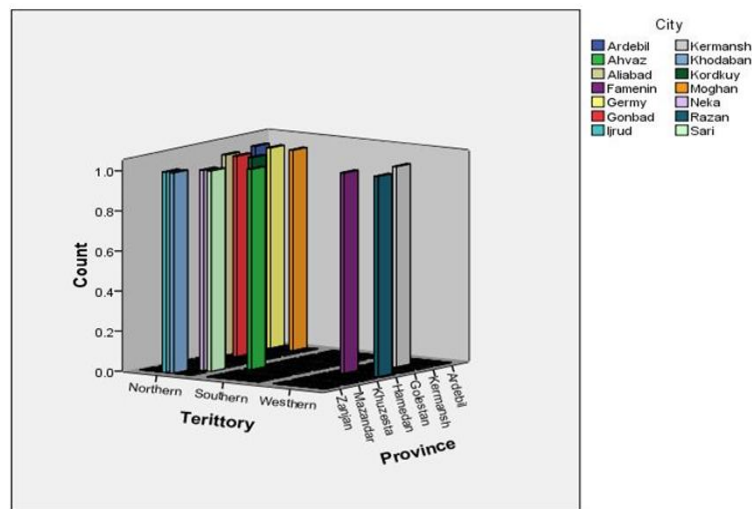
## **RESULTS AND DISCUSSION**

The wheat collected samples were provided from 7 provinces, Mazandaran, Hamedan, Zanjan, Kermanshah, Khuzestan, Golestan, and Ardebil. The regions selected for sampling were chosen based on their recent 5-year production volumes. These regions are located in the south (Khuzestan province), the west (Kermanshah province), and the north (Ardebil, Zanjan, Golestan, and Mazandaran provinces) of

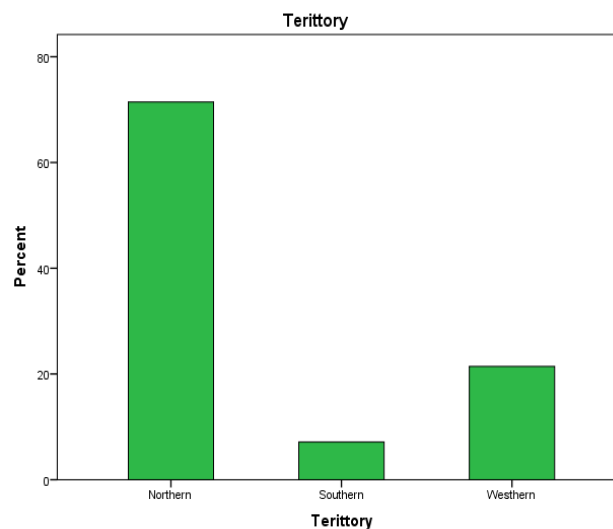
Iran. Unfortunately, the samples obtained from Bushehr and Fars provinces were excluded from extraction and measurement process due to water damage and microbial health disorder, especially fungal biomass [Figure 1], [10].

The amount of the samples harvested and sampling site distribution were evaluated, samples were prepared, after that, measurement and numerical values analysis were obtained according to Graph 4, for wheat aflatoxin were fluctuated from 0.06 to 0.86, respectively. Given, aflatoxin level in follows a normal distribution [fig 4]. The average and standard deviation of 14 samples from the same number sampled cities, which were 8.32 and 3.78, 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. After Pearson's statistical studies and the determination of correlation and significance in  $p < 0.05$ , 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). Wilcoxon curve was also studied in the present research. 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 to the degree of -0.16 is accidental [fig 5]. 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<sub>1</sub> with the average of 16.3 µg/kg. In Iran, the majority of the studies done on food in terms of aflatoxin and the factors producing it about crops were of export values. In other studies, the aflatoxin present in the diet has been examined as one of the possible risk factors for esophagus cancer in Caspian coastal area (including Mazandaran and Golestan provinces. According to the results obtained in the present 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 were the presence of aflatoxin itself which should be taken into greatest consideration. Average amount of aflatoxin production in the samples collected from 14 cities was 8.3, being far less than the global allowable limit, 30 ppb even national permitted averages. Aflatoxin contamination range was between 1.3-7.1 ppb, 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 [5]. Aflatoxin levels in the research conducted by Halt et al., Abdullah et al., and Escobar were 16.3 ppb, 11.25-252.5 ppb, and 1-20 ppb, respectively in comparison another's 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. In the study, the status of the cereals waiting to be consumed in warehouses of few cooking centers has been examined in terms of contamination with one important mycotoxin from Tr chothecenes family. Findings showed that all samples provided from the cereals present in the warehouses were more or less contaminated with mycotoxin T-2 [9]. Average aflatoxin levels of the whole samples were 0.82 and 1.99 ng/g in summer and winter, respectively. B<sub>1</sub> 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. Aflatoxin in winter was more than that in summer. Highest aflatoxin B<sub>2</sub> contamination frequencies were in winter (98%) and in summer (51%). In winter, the relationship between humidity and aflatoxin B<sub>1</sub> level and total aflatoxin was significant. Despite the flour samples contamination, were more than the national rules of standard based on Iranian Standard Institute was not observed, but it was a lot higher than similar studies and the present study (8.3 ppb). Hence, considering 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. Results have shown that, in all the 35 samples analyzed, there have been lots of mycotoxins such as aflatoxins, ochratoxins A, zearalenone, deoxynivalenol, and fumonisin FB<sub>2</sub>, and fumonisin FB<sub>1</sub> has been observed in 11 samples (31.4%) with a level from 36.3 to 2.891 mg/g. The presence of the subspecies *Aspergillus*, the subspecies *Fusarium*, and the subspecies. The effect of fumigation in storage periods on microorganism growth, through the reduction of fungi percentage in sink, has also been studied. This fact occurs due to detrimental effect of phosphine on fungi growth. Since mycotoxins cannot be removed from the grain during the milling process, preventing their growth by decreasing the temperature, humidity, and contamination with pest in the sink is of great

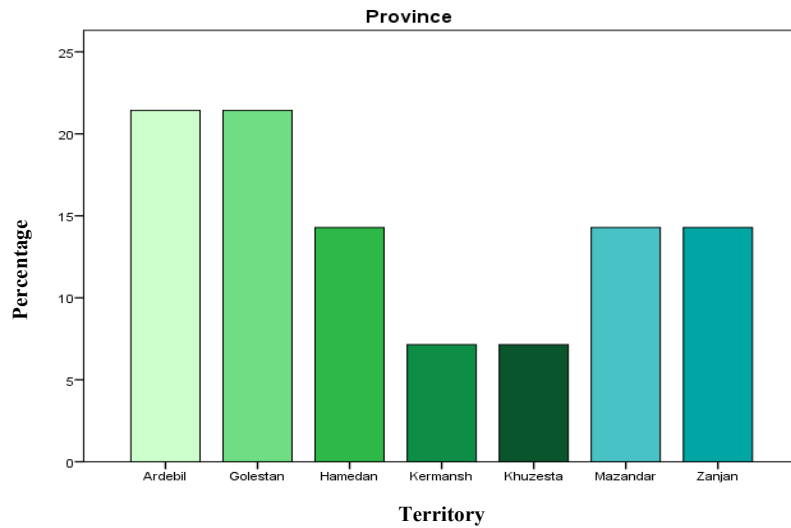
importance. The aflatoxin level measured in this research was so close to that in ours, measurement method, however, was not mentioned in this research (4). dealt with detecting aflatoxin and ochratoxin of wheat and flour in the north of Iran. In their study, in order to determine the presence of toxins 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 by Italian Technalab Company commercial kit. The experiment was done in Mazandaran province in which, among 14 flour producers, 7 cases were randomly chosen and sampled. Based on production rate and number of flour packs (40 kg) according to the random table, Regarding mean contamination of the samples, lowest and highest contamination belongs to number 6 in unit 1 and unit 2. Minimum contamination was observed in the sample of unit 3, No. 1, and its maximum was in the samples of units 5 to 6 (6.71 ppb). The least contamination existed in sample 2, unit 2 (5 ppb) and maximum of the contamination observed was in sample 4 unit 6 (21.42 ppb). The values obtained for aflatoxin in this research were nearly the same as our findings except unit 6 that had maximum amount. However, none of these amounts exceed the permissible limit, i.e. 30 ppb (4).



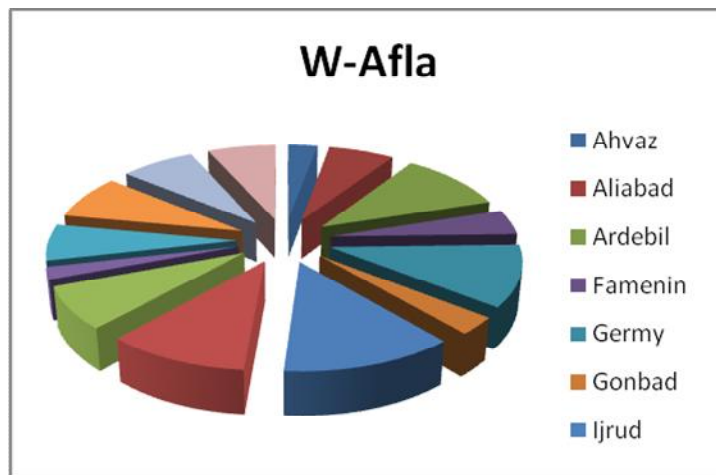
**Figure 1.** Histograms of the sampling cities locations distribution in the North, South and West



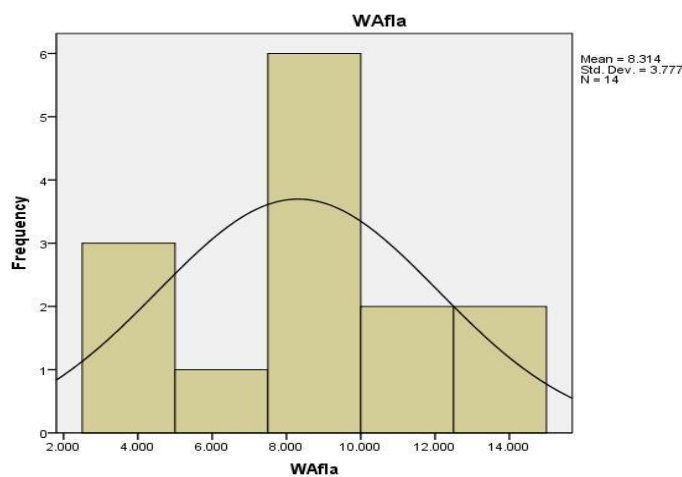
**Figure 2.** Frequency distribution percentage obtained from three regions, the north, the west, and the south, of the country



**Figure 3.** Frequency distribution percentage of the samples obtained from the 7 provinces.



**Figure 4.** The concentration of the wheat toxin distributed in each city



**Figure 5.** Normalized distribution frequency of obtained wheat samples Aflatoxin,of the different ranges

**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 secure 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 hum an health and Animals are raised. This results 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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