Biotechnological
Communication

Biosci. Biotech. Res. Comm. 9(4): 804-808 (2016)

BBRC
Bioscience Biotechnology
Research Communications

Evaluation of fumonisin and zearalenone levels in wheat of silages in Golestan Province, Northeastern Iran

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ABSTRACT

Mycotoxins are secondary fungal metabolites that can contaminate stored foods and lead to various complications. Golestan province has a humid climate so on it is considered as a high risk area for fungal contamination of wheat products, therefore this study was aimed to evaluate the levels of fumonisin (FB) and zearalenone (ZEA) contamination in wheat, stored in the province's silages which has a humid climate. 35 samples of stored wheat were collected from silages of 14 cities in the province. FB and ZEA were extracted from samples and later their levels were measured using enzyme-linked immunosorbant assay method by the commercially available kits. Fungal mycoflora of the samples was identified too. 31 out of 35 samples were contaminated by ZEA, while only 3 samples had FB contamination. Temperature or relative humidity had no effect on the prevalence or concentration of these toxins. The mean ± SD for ZEA and FB were 3.77±2.46 ng/g and 0.034±0.11 ng/g, respectively which were lower than the maximum tolerated level for mycotoxins in food and feed. 29 out of 35 samples (82.84%) had one of four fungi types which went under detection. The detected levels of FB and ZEA in wheat samples of this province are lower compared with other similar studies in Iran and particularly northeastern of Iran. Despite the low concentrations in our study, it is recommended to monitor the occurrence of ZEA and FB in wheat and wheat products to ensure safety and consequently improved public health.

KEY WORDS: ZEARALENONE, FUMONISINS, WHEAT, ELISA

ARTICLE INFORMATION:

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Received 21st Nov, 2016
Accepted after revision 25th Dec, 2016
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007
Thomson Reuters ISI ESC and Crossref Indexed Journal
NAAS Journal Score 2015: 3.48 Cosmos IF : 4.006

Online Contents Available at: http//www.bbrc.in/

HIGHLIGHTS

- Fumonisin and Zearaleone are two of abundant mycotoxins in cereals
- Positive correlations were seen between mycotoxins and health problems such as cancers
- Mycotoxins monitoring in stored cereals may have potent financial and health benefits

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by various mould fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* which are naturally developed in food products. They generally grow on agricultural products due to pre/post-harvest states and transportation or storage conditions. The *Fusarium* fungus species are the most frequent pathogens in crops in humid climate. Fumonisins (FB) and zearalenone (ZEA) are two mycotoxins, produced by these species which can have serious effects on the safety of foods and feeds. ZEA binds to the mammalian estrogen receptors induces oestrogenic effects in mammals and interferes with conception, ovulation, implantation, fetal development, and viability of newborn animals. While B1, B2 and B3 are the most common types of fumonisins, fumonisin B1 (FB1) is the most toxic form which contaminates wheat and causes many diseases in both animals and humans (Shier, 2001, Šegvić Klarić, 2009, Pérez-Torrado, 2010, Shirima, et al., 2013, Arroyo-Manzanares, 2014, Feizy, 2014).

Fumonisins are absorbed through the digestive system and affect cell surface by interfering with the biosynthesis of sphingolipids, resulting in accumulation of sphingosine and membrane dysfunction (Stockmann-Juva, 2008). These compounds act as an accelerator for cancer and cause mutations through frequent accumulation of sphingoid free base (Bhandari, 2002). Epidemiologic studies have reported a direct correlation between FB intake and esophageal cancer (EC) in South Africa (Shirima, et al., 2013) and China (Yoshizawa, 1994). FB1 is also thought to be a risk factor for EC, especially in high risk areas (Yli-Mattila, 2010) such as Golestan province, located in northeastern Iran (Roshandel, et al., 2012).

It also has been categorized by the International Agency for Research on Cancer as a possible human carcinogen (Group 2B) (IARC, 2004). Wheat is one of the most important cereal crops for human consumption (Chehri, 2010) and mycotoxin contamination of crops, particularly wheat, maize, peanuts, and rice can have serious economic and environmental consequences. Moreover, the food and agriculture organization (FAO) estimated that 25% of the world's crops are affected by mycotoxins annually, with an average loss of 1 billion metric tons of food and food products each year (Schmaile, 2009). There are several methods available for mycotoxin analysis including ELISA (Sebaei, 2012 and Arroyo-Manzanares, 2014). Therefore, this study was aimed to use the ELISA technique to determine FB and ZEA levels in wheat grains collected from silages of Golestan province, Iran.

MATERIAL AND METHODS

Samples were collected from 35 wheat silages in 14 cities of Golestan province, according to the guideline provided by the standard and industrial research institute of Iran (ISIR) number 2087, in a way that for every 1500 tons of wheat, 1 Kg should be taken as sample. The samples were filled in well-packed sterile plastic bags and then stored in 2-8 °C until the time of analysis. Questionnaires were also designed for each silo which contained the following items: silo location, structural type of silo (metal, concrete, etc.), storage temperature, relative humidity (%), total capacity of the silo and the planting and harvesting area of the stored products.

The fungal mycoflora of current wheat samples were determined as following: Each sample (20 g) was surface disinfected for 2 minutes with 0.2% sodium hypochlorite solution and rinsed three times with sterile distilled water. From each sample, 40 grains were randomly selected and then put in Petri plates (90 mm diameter, 10 grains/dish) containing Sabouraud's dextrose agar (Merck, Darmstadt, Germany) with 5% chloramphenicol in duplicate. Petri plates were incubated at 25°C for 6 to 10 days. Each pure culture was characterized and identified based on their morphological and microscopic characteristics using the keys of Pitt and Hockings (1997) and Raper and Fennel (1965).

First, 50 – 100 jars of wheat were ground into a fine powder and then 3 jars of this powder were added to 3 ml of 80% methanol and mixed for 15 minutes at room temperature. It was later centrifuged for 10 minutes at 2000*g and the supernatant liquid was used as the sample for further FB detection test.1 gram of well-pulverized wheat powder was added to 4 ml of 60% methanol and then mixed for 15 minutes at room temperature. After centrifugation for 10 minutes at 2000*g, the supernatant liquid was used as the sample for ZEA detection test.

In order to measure FB, 50µl of the obtained supernatant from the FB extraction step were added to 150µl of dilution buffer provided by the FB measurement ELISA kit (Euro Proxima, Cat number: 5121FUM), according to the manufacturer's instruction. For ZEA measurement, 25µl of the obtained supernatant from the ZEA extraction step was added to 475µl of dilution buffer provided

Table1: The descriptive characteristics of the investigated wheat silages in the Golestan province, Iran				
Ave. Temperature and (Range) (C°)	Ave. relative humidity and (Range) (%)	Ave. capacity (tons) and (Range)	Ave. period of wheat storage (year)	Type of silo
31.37±2.85 26-37	35.51±7.77 21-58	18700 2500-100000	1	Metal: 34.5% Concrete: 8.5% Traditional: 37% Mechanized: 20%

by the ZEA measurement ELISA kit (EuroProxima, Cat number: 5121ZON), according to the manufacturer's instruction. The tests were performed in duplicate and the results were reported as ng/g.The data were analyzed by SPSS (version 21) statistical software. The normality of data distribution was evaluated using Kolomogrov-Smirnov Shapiro-Wilk and Speraman correlation coefficient test was used to investigate the relationships between mentioned toxin levels and other parameters due to the non-parametricity of the data.

Overall, 35 wheat silages were investigated in this study and Table 1 shows the basic descriptive characteristics of all the tested silages from the Golestan province, Iran. It also has to be mentioned that the average period of wheat reservation for all the silages was one year (table 1).

RESULTS

FB contamination was found in only three samples with concentrations equal to 0.4 ng/g, while 31 samples (88%) were contaminated by the ZEA mycotoxin. The ZEA-free samples were related to four silages which were also free of FB contamination. The found FB levels in the samples were ranging from 0 to 0.4 ng/g with a mean ± SD of 0.034±0.11 ng/g. The levels of ZEA in the tested samples were ranging from 0 to 10.40 ng/g with a mean ± SD of 3.77±2.46 ng/g.Overall, there were 4 silages without any toxic samples and it was also found that all the FB contaminated samples were accompanied by ZEA contamination.Further analysis was performed to explore the probable difference of contamination between the silages located in the East or West of the province and no significant variation was detected (table 2).

The Pearson correlation coefficient test showed no relationship between the presence and level of mycotoxin contamination with temperature or relative humidity percentage in the tested silages. Also, the type or capacity of the silages had no significant effect on the level of these toxins. While, all three FB contaminations were found in traditional silages (the silages without any developed structure or building), contamination-free silages were of metal and mechanized types. However, the analysis showed no significant statistical difference between these types of silages.

Table 3 shows the frequency and percentage of the samples owning various types of fungi (table 3).

Table 3: The occurrence of fungi in the samples		
Fungi type	No	%
Aspergillus flavus	16	45.71%
Aspergillus niger	2	5.71%
Penicillium spp	7	20%
Yeast	4	11.42%
Total	29	82.84%

According to the guidelines of Iranian institute of standard and industrial research (ISIR) number 2087 for food and feed's maximum tolerated level of mycotoxins, the maximum permitted levels of FB1 and ZEA should not exceed 5 ng/g and 200 ng/g, respectively. Although in this study ZEA contamination was present in 88% of the collected samples, all of them were at levels lower than the cutoff point reported by the ISIR guidelines. Moreover, the amount of the three FB contaminated samples was notably lower than the maximum permitted level.

Table 2: The comparison of FB and ZEA contamination in wheat silages located in the east and west of the province			
	East (n=17)	West (n=18)	*p-value*
ZEA (mean ± SD)	3.1059 ± 1.8084	4.4111 ± 2.8569	0.118
FB (mean ± SD)	0 ± 0	0.67 ± 0.1534	0.083

DISCUSSION

In our study, the presence or rate of mycotoxin contamination was not associated with temperature or relative humidity percentage of silages. Also, the type or capacity of the silages had no significant effect on these toxins. However, all three FB contaminations were related to traditional silages and also the silages without any toxins were metal and mechanized types, but the statistical analysis showed no significant difference which can suggest the effect of other non-mentioned factors in the establishment of mycotoxin contamination. Joshaghani (2013) conducted a study on the mycoflora of fungal contamination among the same wheat silages in this province and found that 10 out of 34 samples were contaminated with aflatoxin B_1 with hazardous levels only in one case. They reported *Aspergillus niger* having the highest frequency in their samples while in our study, *Aspergillus flavus* was the most prevalent fungi in the samples and also 82.84% of wheat samples showed the presence of fungi. According to the toxicogenity potency of some of these moulds, the risk of mycotoxin increase exist when long-lasting reservation of wheat in unfit circumstances.

There was no significant difference between the mean levels of ZEA and FB from silages located in the West and East of the province which is against their potential role in cancer pathogenesis, since there is a big difference in the frequency of cancer between the East and West of Golestan province (Pourshams, et al., 2005, Islami, et al., 2009). In a study by Bettencourt (2005), the contamination level of 60 maize meal and flour samples was assessed in Sao Paolo, Brazil and the average concentration of these toxins in maize meal and flour was found to be 6170 ng/g and 2740 ng/g, respectively which are remarkably higher than our findings. Šegvić-Klarić (2009) studied on the prevalence of ZEA contamination in household cereals and foods reported that 92% of foods had ZEA toxins and a high prevalence for ZEA compared to FB, which were similar to our findings. Curtui (1998) analyzed 55 wheat and corn samples destined for animal consumption and demonstrated that all wheat samples and 13% of corn samples were contaminated by ZEA which are in agreement with our findings.

Feizy (2014) reported 12.6% FB_1 contamination and no ZEA contamination in wheat samples of Mashhad, Iran, which are different from our findings. In the study of Chehri (2010), FB_1 was found as the dominant type of FB toxin with 68.2% prevalence rate, ranging 22-455 ng/g which are extremely higher compared to the total FB level in this study. Hedayati (2006) analyzed the samples obtained from 12 flour producing factories and showed that all the granaries had ZEA contamination with minimum concentration of 29 ng/g, which is in agreement with our results. In another study in Iran by Roohi (2012), 42 flour samples from bakeries and confectioneries were assessed for FB contamination and 18 samples from bakeries and 17 samples from the studied confectioneries had FB contamination. Alizadeh and others (2012) analyzed 132 grain silo samples (66 rice and 66 corn) and reported 50% and 40.9% FB_1 contamination in the corn and rice samples, respectively. Despite the heterogeneity of our samples with the former study, there was still a very high concentration of FB_1 (223.64 ng/g for corn and 21.59 ng/g for rice). In two parallel studies by Yazdanpanah and others (2006) and Shephard and others (2000) on corn samples of Mazandaran and Isfahan province in Iran, Mazandaran province had a higher level of FB in comparison to Isfahan which had results similar to our findings.

Wheat and other starchy grains are the staple food of many countries all over the world, especially Asian and African countries. Also, the use of grains in animal feeding further highlights the issue of their safety. Furthermore, development of advanced technology and possibility to store foods and particularly grains, propounds the importance of considering food storage safety. Therefore, more extensive studies are required to evaluate the levels of mycotoxin contamination in these foods for improved public and environmental health.

CONCLUSION

Low levels of mycotoxins are detected in the silages of Golestan province. ZEA contamination is more prevalent compared to FB, while none of the silages exceeds maximum tolerated levels of mycotoxins according to the safety of food and feed guidelines. Furthermore, there is an important necessity to monitor food products before consumption.

ACKNOWLEDGEMENTS

The authors would like to thank the Deputy of Research and Technology of Golestan University of Medical Sciences, Iran for financial support.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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