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PRESENCE OF T-2 AND HT-2 TOXINS IN MAIZE

ABSTRACT: Data on the occurrence of T-2 and HT-2 toxins in maize from Serbia and Europe are very limited. In this study a total of 50 maize samples harvested during September and October 2012 in Serbia were analyzed. Presence of T-2/HT-2 toxins was determined by enzyme-linked immunosorbent assay (ELISA) method. Among the 50 analyzed maize samples even 26 (52.0%) samples were contaminated with T-2/HT-2 toxins. Concentration interval between 25-60 µg/kg and 60-200 µg/kg were found in 46.0% and 6.0% of analyzed maize samples, respectively.

KEY WORDS: T-2/HT-2 toxins, maize, ELISA

INTRODUCTION

T-2 and HT-2 toxins are members of a large group of fungal sesquiterpenes, commonly marked as type of A trichothecenes. They are produced by various Fusarium species, including F. sporotrichoides, F. poae, F equiseti, F. acumninatum, as well as species from the genera Myrothecium, Cephalosporum, Verticimonosporum, Trichoderma, Trichothecium and Stachybotrys (EFSA, 2011). One of the major producers of T-2 and HT-2 toxins is F. sporotrichoides, which can grow in temperature interval ranging from −2 to 35 °C and with water activities above 0.88 (Richard, 2007; Creppy, 2002). Previous studies have shown that the presence of T-2 and HT-2 toxins is quite often related to grains such as wheat, maize, oats, barley, rice, beans, soya beans and their derived products (SCF, 2001). HT-2 toxin is a metabolite of T-2 toxin and it is formed in microbial transformation via deacetylation reaction (Zhou et al., 2008). In general, trichothecenes are very stable compounds during storage, milling, processing, and cooking at high temperatures. T-2 toxin is more rapidly metabolized than HT-2 toxin which is also the main metabolite in vivo (Erikson and Alexander, 1998); also, they can be metabolized by various animals but can also be metabolized by plants and fungi (Dohnal et al., 2008). T-2 toxin is one of the most toxic trichothecenes with potent inhibitory influence on DNA, RNA and protein synthesis, and it
also shows immunosuppressive and cytotoxic effects both in vivo and in vitro. T-2 toxin is a very potent cytotoxic and immunosuppressive toxin, which can cause acute intoxication and chronic diseases in both humans and animals (Ueno et al., 1983; Peric et al., 1999; Zhou et al., 2008). The symptoms of acute intoxication are nausea, vomiting, abdominal pain, diarrhea, bloody stools and weight loss. The major effect of T-2 toxin is inhibition of protein synthesis which leads to secondary disruption of DNA and RNA synthesis (Richard, 2007; Creppy, 2002; Bennett and Klich, 2003). The immune system is also a target of T-2 toxin and the effect includes changes in leukocyte count, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection and blastogenic response to lectins (Creppy, 2002).

Recent data, collected by the EU member states in order to evaluate the risk of dietary exposure to Fusarium toxins, have shown that T-2 and HT-2 toxins are quite common contaminants in cereals in the EU (SCF, 2001). The joint FAO/WHO expert committee on food and additives (JECFA, 2001) established a permissible limit of tolerable daily intake (TDI) value of 0.06 µg/kg body weight for T-2 toxin and HT-2 toxin, alone or in combination.

Worldwide, 13 countries have reported legal maximum levels (MLs) or recommendations for T-2 and HT-2 toxins in food and feed products. The following European countries have prescribed regulations for T-2 and HT-2 toxins: in Armenia the maximum allowed level is 100 µg/kg for T-2 toxin in all types of food; in Hungary 300 µg/kg for T-2 toxin in milled products, and 300 µg/kg for the sum of T-2/HT-2 toxins cereal constituents of muesli; in Moldova 100 µg/kg for T-2 toxin in cereals and cereal flour; in Norway 100 µg/kg for T-2 and HT-2 toxins in cereals and cereal products, and 50 µg/kg for T-2 and HT-2 toxins in cereals and cereal products for infants and young children; in Russia 100 µg/kg for T-2 toxin in barley, and in Ukraine 100 µg/kg for T-2 toxin in grains, flour, wheat middlings, bread products, and all seeds used for immediate human consumption and for processing in the products for human consumption. As it can be seen, MLs for T-2 and HT-2 toxins in food are mainly regulated in Eastern Europe. Further, other countries (Canada, China, Croatia, Iran, Israel, Norway, Serbia, and Ukraine) have set maximum levels for T-2 toxin in feed products, mostly ranging from 25 to 1000 µg/kg (EFSA, 2011).

To protect the health of consumers, the commission of the European Communities established the maximum level for most studied mycotoxins in cereals (EC, 2006; EC, 2007), while regulations regarding HT-2 and T-2 toxins in cereals have not yet been established.

Maximum levels of T-2 and HT-2 toxins in food are not regulated even in Serbia, but it is regulated for compounds and complementary feed mixtures for chickens, piglets and calves, and compound and complementary feed mixtures for pigs and poultry with maximum level of T-2 toxin of 500 µg/kg, 1000 µg/kg, respectively. Also, the maximum allowed level for total trichothecenes is 300 µg/kg for feed mixtures for chickens, piglets and calves, and 600 µg/kg for feed mixtures for cows and poultry (Službeni Glasnik RS, 2010).
Due to the fact that there is no available data on the occurrence of T-2 and HT-2 toxins in maize from Serbia, the aim of this study was to investigate the presence of sum of T-2/HT2 toxins in maize for human consumption and animal feed products.

MATERIALS AND METHOD

Samples

A total of 50 maize samples were collected in Serbia. Samples were collected after harvest, during September and October, 2012. All samples were stored at 4 °C in a refrigerator before analysis.

Sample preparation

After grinding, 5 g of maize sample was extracted with 25 ml of methanol/deionized water (70/30; v/v) solution and it was shaked vigorously for 3 minutes, followed by extract filtering through a filter paper (Whatman, Black Ribbon). The filtrate obtained was diluted 1:1 (e.g. 1 ml in 1 ml) with deionized water and it was mixed by Vortex.

Determination of sum of T-2/HT-2 toxins

Content of T-2/HT-2 toxins was determined by the enzyme immunosorbent assay method (ELISA). All samples were analyzed in duplicate with Quantitative T-2/HT-2 Toxins test kit (Neogen Veratox®, Lansing, USA). Range of quantification for T-2/HT-2 toxins test kit was between 25 – 250 µg/kg and analysis were done according to the manufacturer’s description.

Free T-2 or HT-2 toxins in the samples and controls are allowed to compete with enzyme- labeled HT-2 toxin (conjugate) for the antibody binding sites. After a wash step, substrate was added and it reacted with the bound conjugate to produce blue color. More blue color meant less T-2/HT-2 toxins. The test was read in a microwell reader (Thermolabsystem, Thermo, Finland) to yield optical densities. The optical densities of the controls formed a standard curve, and the sample optical densities were plotted against the curve to calculate the exact concentration of T-2/HT-2 toxins. According to the manufacturer’s description the detection limit for T-2/HT-2 toxins was 25 µg/kg.

RESULTS AND DISCUSSION

In this study, 50 maize samples were analyzed to evaluate the sum of T-2/HT-2 toxins contamination. The obtained results (Table 1) showed that
T-2/TH-2 toxins were found in 26 (52.0%) samples with concentrations over 25 µg/kg. Concentrations of T-2/TH-2 toxins in the positive samples ranged from 25.3 to 185.2 µg/kg. Positive results were classified into two groups. Results for the sum of T-2/HT-2 showed that 24 (48.0%) of the analyzed maize samples contained less than 25 µg/kg, while concentrations that ranged from 25-60 µg/kg and 60-200 µg/kg were found in 23 (46.0%) and 3 (6.0%) samples, respectively. Mean level of all positive samples was 25.3 µg/kg.

Tab. 1. – Contamination frequency (CF), interval (CI) and mean level (CM±SD) of T-2/HT-2 toxins

<table>
<thead>
<tr>
<th>CF (%)</th>
<th>CI (µg/kg)</th>
<th>CM (µg/kg) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 µg/kg</td>
<td>48.0</td>
<td>34.7 ± 7.95</td>
</tr>
<tr>
<td>25–60 µg/kg</td>
<td>46.0</td>
<td>154.1 ± 31.2</td>
</tr>
<tr>
<td>60–200 µg/kg</td>
<td>6.0</td>
<td>122.9–185.2</td>
</tr>
</tbody>
</table>

Given the fact that high mycotoxin concentrations are usually associated with climate changes, in particular humidity and temperature as the factors most critical for mould formation and mycotoxin production, the obtained medium average contamination levels observed in this study could be explained by the fact that in 2012 the investigated parts of our country were extremely warm and dry throughout the vegetation period of maize (Republic Hydrometeorological Service of Serbia, 2012).

Most of the previously mentioned countries that prescribed regulations for T-2 and sum of T-2/HT-2 toxins set 100 µg/kg as the maximum limit for cereals. According to the obtained results, 3 (6%) of the 50 analyzed maize samples contained concentration of T-2/HT-2 toxins that was greater than 100 µg/kg.

Due to the lack of available data on the occurrence of T-2/HT-2 toxins in maize from Serbia, we analyzed the results from the region. Jakošević et al. (2010) reported that analyzed grains from Slovenia were not contaminated with HT-2 and T-2 toxins. Vulić et al. (2011) investigated the presence of T-2/HT-2 toxin in 25 feed samples and 25 commodities from Croatia. They reported that 76% of analyzed feed samples were contaminated with T-2/HT-2 in the concentrations that ranged from 6.09 to 67.7 µg/kg. Out of all analyzed commodities oat, barley, wheat, corn and soya beans contained the highest concentrations which were 32.9, 23.1, 6.91, 5.02 and 3.17 µg/kg, respectively.

Furthermore, Binder et al. (2007) reported that 1 out of 18 maize samples collected in different European farms between October 2003 and December 2005 was positive for T-2 toxin. Greater number of cereal samples (63 maize, 51 wheat, 34 barley and 33 oat samples) was analyzed by Pleđin et al. (2013). The examined samples were collected from different fields situated in one of the six Croatian regions, and analyzed by using ELISA method. T-2 toxin was detected in 36 (57%) analyzed maize samples with average concentrations of 24.0 µg/kg. The obtained concentration of T-2 toxin ranged from 5 to 42 µg/kg.
Results obtained in this study are similar with the results obtained from the region regarding T-2/HT-2 occurrence in maize. Based on the results, it can be concluded that it will be necessary to continue the control of the presence of T-2/HT-2 in maize and other types of food and feed.

CONCLUSION

Considering the high toxicity of T-2 and HT-2 toxins, there is a need for collecting more data on their occurrence in food and feed and setting the maximum limits for these mycotoxins in food from Serbia.

REFERENCES

ПРИСУСТВО T-2 И HT-2 ТОКСИНА У КУКУРУЗУ

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Резиме

Подаци о појави T-2 и HT-2 токсина у кукурузу су веома ограничени како у Србији тако и у Европи. У току истраживања анализирани су узорци кукуруза који су сакупљени на територији Србије непосредно након бербе 2012. године. Имуноафинитетном методом (ELISA) анализирано је присуство суме T-2 и HT-2 токсина у 50 узорака кукуруза. Од укупног броја анализираних узорака кукуруза у 24 (48,0%) није детектовано присуство T-2 и HT-2 токсина, док је 26 (52,0%) узорака било контаминирано концентрацијом T-2 и HT-2 токсина у опсегу од 25 до 60 µg/kg, док је 6,0% испитаних узорака кукуруза садржало концентрацију у опсегу од 60 до 200 µg/kg.

КЉУЧНЕ РЕЧИ: T-2/HT-2 токсини, кукуруз, ELISA

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