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The Natural Occurrence of T-2 Toxin and Fumonisins in Maize Samples in Croatia

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Abstract Maize (*Zea mays*) considered to be one of the most frequent crops in Croatia, is often contaminated with *Fusarium* mycotoxins. The aim of this study was to investigate the possible contamination of maize with T-2 toxin and fumonisins on 46 samples from different regions of Croatia. The highest concentrations of T-2 toxin and fumonisins were 210 and 25,200 ng/g, with mean values of 110 and 4,509 ng/g, respectively, pointing to maize contamination with these mycotoxins after the period of the year with extremely high rainfall.

Keywords T-2 toxin · Fumonisins · Maize · Croatia

Cereal plants may be contaminated with mycotoxins, produced by fungi during its growth, handling and storage. Consumption of a mycotoxin-contaminated diet in humans and animals may induce acute and long-term chronic effects resulting in teratogenic, carcinogenic, oestrogenic or immune-suppressive effects (Binder et al. 2007). *Fusarium* species are widely distributed in nature and often cause deterioration of food and feeds. Mycotoxins produced by these species are divided into several classes: trichothecenes, fumonisins and zearalenone.

Trichothecenes are toxic to human beings and animals and can cause both acute and chronic diseases including vomiting, diarrhea, skin irritation, feed refusal, nausea, neural disturbances and abortion (Desjardings 2006; Eriksen et al. 2004). T-2 toxin is type A trichothecene mycotoxin mainly produced by *Fusarium (F.) poae, F. sporotrichioides, F. kyushuense* and *F. langsethiae* with the major producer *F. sporotrichioides* prevalent in North America, South America, Asia, Africa and Europe (Richard 2007). Due to the broad prevalence of this fungus, many different crops can be infected with T-2 toxin. T-2 toxin is a very potent cytotoxic and immunosuppressive toxin, which can cause acute intoxication and chronic diseases in both humans and animals (Peraica et al. 1999). The major effect of T-2 toxin is considered to be the inhibition of protein synthesis, which leads to a secondary disruption of DNA and RNA synthesis (Bennett and Klich 2003), and also affects the immune system (Creppy 2002).

Fumonisins have been found worldwide, primarily in maize, with more than 10 compounds that have been isolated and characterized. Fumonisins B_1, B_2 and B_3 are the major fumonisins produced. The most prevalent in contaminated maize is fumonisin B_1, which is believed to be the most toxic, i.e. nephrotoxic and hepatotoxic (Thiel et al. 1992; Musser and Plattner 1997; Voss et al. 2007). The concentrations of fumonisins in raw maize are influenced by environmental factors such as temperature, humidity, drought and rainfall during pre-harvest and harvest periods. High concentrations of fumonisins are associated with hot and dry weather, followed by periods of high humidity. High concentrations of fumonisins may also occur in raw maize that has been damaged by insects (Miller 2000).

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Maize (*Z. mays*), considered to be one of the most frequent crops in Croatia, is often contaminated with *Fusarium* (Jurjević et al. 1999). Earlier investigations conducted on different cereals revealed an occurrence of T-2 toxin (Sokolović and Šimpraga 2006; Vulić et al. 2011) and a high frequency of fumonisin B_1 cereals positive samples (Jurjević et al. 1999; Domijan et al. 2005). The aim of this study was to investigate the possible contamination of maize from different regions of Croatia with T-2 toxin and fumonisins after the period of the year with extremely high rainfall, using previously validated ELISA as a quantitative screening method for their determination.

Materials and Methods

A Ridascreen fumonisin and T-2 toxin kit for ELISA were provided by R-Biopharm (Darmstadt, Germany). Each kit contains a microtiter plate with 96 wells coated with antibodies; fumonisin (0, 0.025, 0.074, 0.222, 0.666 and 2 μg/mL) and T-2 toxin (0, 0.1, 0.2, 0.4, 0.8 and 1.6 ng/mL) standard solutions in water; peroxidase conjugated fumonisin/T-2 toxin; anti-fumonisin/T-2 toxin antibody; substrate and chromogen solutions (urea peroxide/tetramethylbenzidine) and a stop solution (1 N sulphuric acid). ELISA was performed using the calibrated instrument ChemWell (Awareness Technology 2910, Inc., USA).

A total of 46 maize representative samples were randomly sampled in different regions of the Republic of Croatia. Samples were collected during October of 2010 in maize fields during the harvest and then divided into 4 groups according to sampling regions (region 1—Bjelovarsko-bilogorska district; region 2—Koprivničko-križevačka district; region 3—Osječko-baranjska district; region 4—Zagrebačka district). Maize samples were ground into a fine powder in an analytical mill (Cylotec 1093, Tecator, Sweden) and stored at 4°C prior to analysis.

After grinding, for analyses of both analytical parameters, to 5 g of samples 25 mL of methanol/distilled water (70/30) was added. The extraction was performed by vigorously shaking on a shaker for 10 min, followed by extract filtering through a filter paper (Whatman, Black Ribbon). The filtrate obtained was further diluted with distilled water (fumonisin) or dilution buffer (T-2 toxin) according to the kit's manufacturer's instruction and applied into the ELISA kit wells. When calculating the concentration of fumonisin and T-2 toxin in the samples, the results obtained from the calibration curve were multiplied by the corresponding dilution factor and taking into account mean recovery value.

For both analytical methods, competitive ELISA were performed according to the kit's manufacturer's instructions. All standards and samples were analyzed in duplicate, respectively. Fifty μL of standard and prepared samples and then 50 μL of diluted enzyme conjugate were added to the microwells. Diluted anti-fumonisins/T-2 toxin antibody was added and the microwell holder was mixed gently and incubated for 30 min (fumonisins) or 1 h (T-2 toxin) at room temperature in the dark. After incubation, the wells were washed 3 times with 250 μL of distilled water. Then, 100 μL of substrate/chromogen solutions were added to each well and incubated for 15 min (fumonisins) or 30 min (T-2 toxin) at room temperature in the dark. The reactions were stopped by adding 100 μL of stop solution and absorbance was measured at 450 nm. Statistical data analysis was performed by using Statistica Ver. 6.1. Software (StatSoft, Inc. 1984–2003, USA).

Results and Discussion

Surveillance studies of *Fusarium* mycotoxins occurrence in maize showed great variations from 1 year to another, influenced by many factors including environmental conditions such as climate, temperature and humidity, insect infestation and pre- and post harvest handling. Earlier investigations in Croatia pointed to increased concentrations of these mycotoxins during the wet years, protracted winter and temperature fluctuations. Published data was explained with very rainy and wet periods, which caused an increased contamination of cereals with moulds and the subsequent formation of mycotoxins (Pepeljnjak and Šegvić 2004).

Maximum allowed levels of T-2 toxin in cereals (also maize), as a food and feed component, have not been set in Europe nor in Croatia yet. For fumonisins (B_1 and B_2) the maximum allowed level in unprocessed maize intended for human consumption is 4,000 ng/g (Official Gazette of the Republic of Croatia 2011) whereas for maize and maize products intended for animals consumption the European Union recommendations with a maximum fumonisins value of 60,000 ng/g was accepted also in Croatia (Commission Recommendation 2006/576/EC).

In this investigation we analyzed 46 maize samples for T-2 toxin and fumonisins, sampled from four different regions in Croatia during harvesting. The intention was to examine, i.e. collect the data of concentrations of these mycotoxins after an extremely wet period of the year 2010, and determine whether the concentration exceeded the maximal allowed values set up in legislation for maize as a food and feed ingredient (fumonisins).

The estimated methods limit of detection (LOD), calculated from the mean value of ten determinations of blank maize samples plus three standard deviations, were 7 ng/g for T-2 toxin and 25 ng/g for fumonisins. Methods mean recoveries, determined at the fortification levels of 50 and

300 ng/g for T-2 toxin and 50 and 500 ng/g for fumonisins, were 88% and 79%, respectively.

The determined concentrations of T-2 toxin and fumonisins are shown in Figs. 1, 2, respectively.

The number of positive samples of T-2 toxin and fumonisins in all the investigated regions generally, was 24.4% and 67.4%, respectively. Minimum and maximum values of the determined concentrations and number (percentage) of positive samples are shown in Table 1.

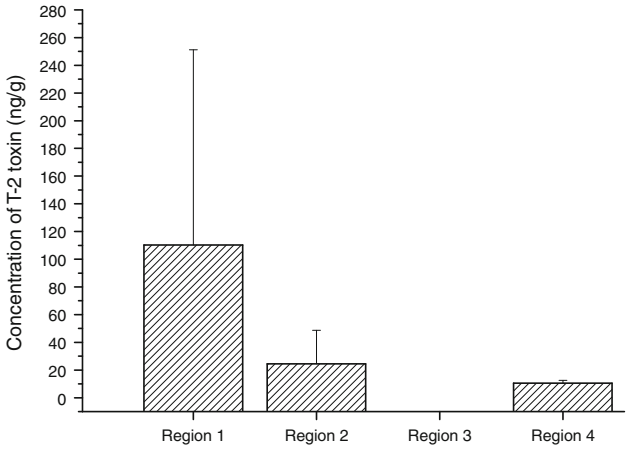

Fig. 1 Determined concentrations (mean ± SD) of T-2 toxin in maize samples from different regions in Croatia

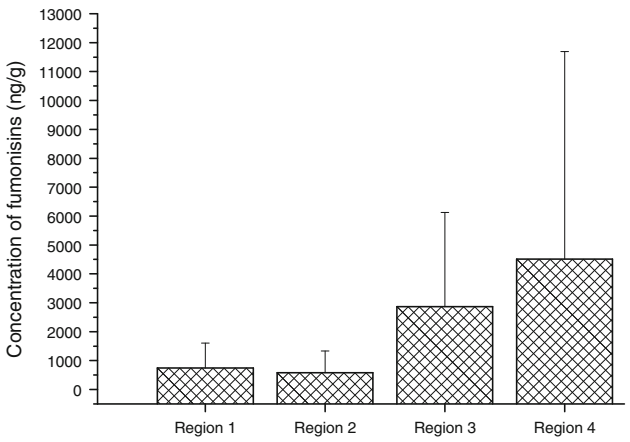

Fig. 2 Determined concentrations (mean ± SD) of fumonisins in maize samples from different regions in Croatia

Table 1 The presence of T-2 toxin and fumonisins in maize samples

Parameter	LOD (ng/g)	Min (ng/g)	Max (ng/g)	Positive/total no. of samples	Positive samples (%)
T-2 toxin	7	10.5	210	10/46	24.4
Fumonisins	25	31	25,200	31/46	67.4

The highest concentration of T-2 toxin was determined in Region 1 with a mean value of 110 ng/g, and maximal value of 210 ng/g. All the determined values of T-2 toxin were below 500 ng/g as was earlier set as the maximum allowed level in the Republic of Croatia (until 2010). The results obtained on the same samples show that the highest fumonisins concentrations were determined in Region 4, with mean values of 4,509 ng/g and a maximal value of 25,200 ng/g. This value is lower than the maximum allowed level (60,000 ng/g) which was set for fumonisins in maize and maize products intended for animal feeding, but higher (approximately 6 times) than the maximum allowed level (4,000 ng/g) which was set for fumonisins in unprocessed maize intended for human consumption. There was no correlation between the determined T-2 toxin and fumonisins concentrations according to the regions sampled.

Available data of T-2 toxin occurrence in Europe reported that oat is the cereal most frequently contaminated with T-2 toxin (Langseth and Rundberget 1999; Mankevičiene et al. 2007). Considering the explicit toxicity of this mycotoxin, there is a need for further data collection and a definition of the values of the maximum allowed level in different cereals (Vulić et al. 2011), also in maize as a food and feed component. Binder et al. 2007 summarized data from October 2003 to December 2005 of the mycotoxins occurrence in Europe and the Mediterranean Region in cereals. T-2 toxin was analyzed in 18 maize samples of which one sample was found to be positive with a concentration of 188 ng/g (Binder et al. 2007). Schollenberger et al. (2006) reported a survey of *Fusarium* toxins in different grains and feedstuffs from Germany. The incidence of T-2 toxin in maize samples from their survey was 51% with a maximum toxin level of 108 ng/g. Meister (2008) reported a T-2/HT-2 toxins incidence of 80% in maize samples from Norway with concentrations ranging from 2 to 106 ng/g. The published data on the representation of T-2 toxin in Croatia, in particular the various types of feed during the period since 1998–2004, showed the value of 100–500 ng/g with 16.8% of T-2 toxin feed positive samples (Sokolović and Šimpraga 2006). In an investigation on the occurrence of T-2/HT-2 toxins by Vulić et al. (2011), conducted on 17 maize samples, the maximum determined level was 5.02 ng/g with an incidence of 76%. All the mentioned studies have indicated a frequent occurrence of T-2 toxin in the European Union and also in Croatia.

Trucksess et al. (1995) indicate the presence of low concentrations (4–82 ng/g) of fumonisins in sweet maize. Broken kernels of maize which have been screened from bulk lots of maize before any milling process contain higher concentrations of fumonisins than whole kernels, and are often used in animal feeds. Higher fumonisin concentrations were found in maize screenings and in concentrations of 160 to 330 mg/kg caused porcine diseases (Ross et al. 1991).

In the investigation of Binder et al. 2007 data for maize indicated that fumonisins were detected in 9 out of 16 samples tested, with a mean value of 836 ng/g and the highest level of 2,174 ng/g. In Croatia Domijan et al. (2005) analyzed samples of maize from different parts of Croatia, collected after the autumn harvest. Analyses showed concentrations of fumonisin B_1 ranging from 196.8 to 1,377.6 ng/g, and fumonisin B_2 from 684 to 3,084 ng/g. In this study fumonisin B_1 was the most common mycotoxin that has been detected in all the analyzed samples, as well as in the study of Jurjević et al. (1999) where 97% and 93% of maize samples contained fumonisin B_1 in two consecutive years. These results indicate a high frequency of maize contamination with fumonisins in previous years in Croatia, although this study pointed out more significant levels than those earlier determined.

According to data from the Meteorological and hydrological institute of Croatia the period from August to October of 2010 (before harvesting maize) in all the investigated regions was cold with extremely high rainfall. Higher concentrations of T-2 toxin and fumonisins determined in this investigation in some maize samples, in comparison to results of earlier years in Croatia and results in other European countries, probably might be explained with high humidity and low temperatures, i.e. frequent rain and temperatures significantly lower than the average in the period of growing maize, which subsequently increased its contamination with moulds and the formation of these *Fusarium* mycotoxins. Such contamination of maize with fumonisins in concentrations higher than the maximum allowed limit, may present a risk for human health if it is used as a food component.

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