The influence of climate conditions on the occurrence of deoxynivalenol in maize harvested in Serbia during 2013-2015

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Abstract

The objective of this study was to investigate the influence of climate conditions on deoxynivalenol (DON) content in 1800 maize samples collected from main maize growing regions in Serbia during a period of three years.

DON concentration was determined by validated direct competitive Enzyme Linked Immunosorbent Assay (ELISA) method.

Presence of DON in maize samples from 2013, 2014 and 2015 years was detected in 2.5%, 96.0% and 15.5% of samples in the concentration range of 260.1-1388 μg/kg, 260.4-9050 μg/kg and 252.3-6280 μg/kg, respectively.

The obtained results indicate that different weather conditions recorded in examined years had a significant influence on DON occurrence in maize. Extremely rainy weather conditions in maize

growing season 2014 were favourable for DON production and even 292 (48.3%) samples were unsuitable for human consumption, since DON concentrations were greater than 1750 μg/kg. However, lack of rainfall and higher air temperatures during the years 2013 and 2015 contributed to lower contamination frequency of DON.

These findings confirmed that maize should be continuously monitored in order to protect human and animal population against the risk of DON contamination. Furthermore, monitoring of DON occurrence in maize from Serbia is required in order to collect data which is needed for establishing a Serbian as well as European database.

Keywords: maize, deoxynivalenol, Serbia, climate conditions

1. Introduction

Maize is very often contaminated with fungi of *Fusarium* species which can result in mycotoxins production during maize growth, harvest, storage, transport and processing. *Fusarium* species synthesize a wide range of mycotoxins of diverse structure and chemistry which are a potential health hazard for humans and animals (D´Mello, 1991; Placinta et al., 1999).

One of the major *Fusarium* mycotoxin that can occur in maize and maize-based products is deoxynivalenol (DON). DON (12,13-epoxy-3 α ,7 α ,15-trihydroxytrichothec-9-one-8-one) is a polar organic compound which belongs to the type B trichothecenes, and their occurrence is mainly associated with *Fusarium graminearum* and *Fusarium culmorum*, both of which are important plant pathogens (JECFA, 2001). Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world, and its occurrence is considered to be an indicator of the possible presence of other, more toxic trichothecenes (Lombaert, 2002; Desjardings, 2006; Eriksen & Pettersson, 2004; Wu, 2007).

Based on limited data and evidence in humans and experimental animals, DON was classified in group 3 by International Agency for research on Cancer (IARC, 1993). Although DON does not show a pronounced carcinogenic effect, its presence in food chain can cause a variety of toxic effects in both humans and animals. In humans, DON may influence inhibition of protein and DNA synthesis (CAST, 2003). Furthermore, DON can have a negative impact on hematologic cells, manifesting principally as thrombocytopenia and leucopoenia (Parent-Massin, 2004; Pestka, 2007). Consumption of feed contaminated with DON by livestock has been associated with a variety of adverse health effects including feed refusal, reduced weight gain, diarrhea and emesis (Kuiper-Goodman, 2002; Ma, & Guo, 2008).

Since presence of DON in maize may potentially affect human and animal health and cause great economical losses, maximum residue level (MRL) have been established in numerous countries over the world. The regulation of Serbia (Serbian Regulation, 2011) on the control of DON in food was harmonized with the Regulation (European Commission, 2006c) of European Union (EU) and adopted in 2011. Until then, maize intended for human consumption in Serbia had to be tested only for presence of aflatoxins and ochratoxin A. According to the new Serbian and EU Regulation, MRL for DON in unprocessed maize intended for human consumption is 1750 μg/kg. If maize is intended for animal feed, concentration of DON cannot be greater than 8000 μg/kg (Serbian Regulation, 2010; European Commission, 2006b).

Occurrence of DON in maize is considered a typical agricultural issue in regions with cooler climates where weather conditions are favourable for *Fusarium graminearum* and *Fusarium culmorum* growth and DON production (JECFA, 2001; Sudakin, 2003). Previous studies on the occurrence of DON in maize, have shown that presence of this toxin is dependent on many factors, in particular climate conditions throughout the cereal growth period (Domijan et al., 2005; Jajić et al., 2008a; Pleadin et al., 2012; Van der Fels-Klerx et al., 2013).

Since in the recent years Serbia represents a leader in terms of maize production and exports in Europe and among the top ten exporters in the world, control of mycotoxins as well as DON occurrence is necessary (Maslac, 2015). Although Serbian regulations for MRL of DON in maize were harmonized with EU regulations it should be noted that monitoring program is still not prescribed. Data on the natural occurrence of DON gathered in Serbia are still insufficient since they are based on the analysis of a smaller number of maize samples. Therefore, the aim of this study was to collect more data on the matter by considering greater number of analyzed maize samples and to investigate the influence of climate conditions during maize growing season in three different years on the occurrence of DON in maize.

2. Materials and method

2.1. Samples, kits and chemicals

A total of one thousand eight hundred (n=1800) maize samples were collected in period 2013 - 2015 year. Samples were collected from Northern (Autonomous Province of Vojvodina) and Central Serbia which represent the most important maize growing areas in Serbia (Figure 1). Every year after harvest 600 maize samples were taken from Srem, Bačka and Banat regions in Vojvodina and from seven different districts (Mačvanski, Kolubarski, Podunavski, Šumadijski, Braničevski, Pomoravski and district of Belgrade city) in Central Serbia.

Sampling was performed according to EU requirements (European Commission, 2006a) in order to overcome irregular mycotoxins distribution. Particular numbers of incremental samples were combined in order to obtain aggregate samples of approximately 5-10 kg. Aggregate samples were homogenized and quartered to obtain a 500 g of laboratory samples which were refrigerated at 4 °C until the analysis.

Determination of DON was done by Enzyme Linked Immunosorbent Assay (ELISA) method using Quantitative DON Test kit (Neogen Veratox® 5/5, Lansing, USA). Range of quantitation for this test kit is between 250 and 2000 µg/kg. Except for the contents of the test kits, distilled water (Millipore, BedFord, MA, USA) was used for ELISA analysis.

2.2. Sample extraction

500 g of each representative sample was ground to a 1 mm particle size using laboratory mill (Knifetec™ 1095 mill, Foss, Hoganas, Sweden). Sub-samples of 10 g were extracted with 50 ml of distilled water and shaken vigorously for ten minutes on laboratory Griffin flask shaker (Griffin and George, Wembley, England). Extracts were filtered through a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The obtained filtrates were collected, vortexed (Vortex mixer, Velp Scientifica) and used for analysis.

Samples with DON concentration greater than 2000 µg/kg were additionally analyzed after dilution.

2.3. DON analysis by ELISA

The instructions given by the manufacturer for DON determination were strictly followed. ELISA method is based on the competition between free DON in the samples and standards with enzyme-labeled DON (conjugates) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color. The intensity of the color is inversely proportional to the concentration of mycotoxin in the sample or standard. Intensity of the color in each well was measured at 650 nm in a microwell reader (Thermolabsystem, Thermo, Finland). The optical densities of the standards form the standard curve and the samples optical densities are plotted against the curve to calculate the exact concentration of mycotoxins. Analysis of all 1800 maize samples were carried out in accredited laboratory of the Institute of Food Technology, University of Novi Sad. Laboratory is accredited in agreement with standard SCS ISO/IEC 17025 (2006). Furthermore, the validation process of applied ELISA method was conducted in accordance with European Official Decision procedure (European Commission, 2002b). The limit of detection (LOD) and limit of quantification (LOQ) of Neogen Quantitative DON Test kit were 100 µg/kg and 250 µg/kg, respectively. Those values were determined by ELISA test kit producer (Neogen Veratox® 5/5, Lansing, USA), while the precision of the method at LOQ was checked by analyzing test standard solution at the LOQ level during every analysis. The repeatability, reproducibility and trueness of the applied ELISA method were determined after analysis of two certified reference materials (CRMs) as well as spiked maize samples at three different levels (500, 1000 and 3000 µg/kg). CRMs with certified DON content of 900±100 µg/kg (CRM 1) and 700±100 µg/kg (CRM 2) were supplied by Trilogy Analytical Laboratory (Trilogy® Reference Material, D-W-164, Washington, USA). The results of methodology validation are shown in Table 1. As can be seen, the obtained validation parameters were in compliance with recommendations given in Regulation 2006/401/EC (European Commission, 2006a). Furthermore, performances of the ELISA method were checked in international interlaboratory studies (Progetto Trieste, Test Veritas, Padova, Italy; Neogen Corporation, Technical Services Division, Natural Toxins, Lansing, USA; The Grain and Feed Trade Association, GAFTA, London, United Kingdom). Obtained Z values from 0.06 to 0.23 confirmed analytical quality of the applied ELISA method.

2.4. *Statistical analysis*

Statistical analysis of variance was carried out by Duncan´s multiple comparison tests using STATISTICA software version 12.5 (StatSoft Inc. 2015, USA). P values <0.05 were regarded as significant.

3. Results and Discussion

The results of DON contamination in 1800 examined maize samples are presented in Table 2. As can be seen, the obtained data indicate significant differences in the occurrence of DON in samples collected over period of three years (2013-2015). Only 15 (2.5%) among 600 analyzed maize samples from 2013 year were contaminated with DON, with mean concentration of 642.3±364.7 µg/kg. However, in maize samples from 2014 frequency of DON occurrence was very high, since DON was detected in 576 (96.0%) of analyzed samples. The mean value of these results was 3063.3±1264.4 µg/kg and they were distributed in the following way: 284 (47.3%) samples had concentration between 250 and 1750 µg/kg, 290 (48.3%) samples between 1750 and 8000 µg/kg and 2 (0.3%) samples had DON concentration greater than 8000 µg/kg. Furthermore, 507 (84.5%) maize samples from 2015 year did not contain DON in concentration greater than 250 µg/kg (LOQ), while 83 (13.8%) and 10 (1.7%) samples contained DON in concentration range from 250-1750 and 1750-8000 µg/kg, respectively.

According to both EU and Serbian regulations MRL of DON for maize intended for human consumption is 1750 µg/kg. As can be seen, none of the contaminated maize samples from 2013 year exceeded the MRL. Contrary to this, even 292 (48.3%) samples collected from 2014 year were unsuitable for human consumption, since DON concentrations were greater than 1750 μg/kg. In two samples from 2014 year DON concentration exceeded concentration of 8000 μg/kg which is MRL for maize intended for animal feed. Furthermore, analysis of 600 maize samples from 2015 year showed that in 10 (1.7%) samples DON concentrations were higher than MRL.

The findings obtained in this study indicate significant differences in the occurrence of DON in maize samples harvested during different maize growing seasons. Presence or absence of DON in samples from different maize growing seasons could be explained by weather conditions during the investigated periods. According to literature data, occurrence of DON is most prevalent in the northern temperate regions where weather conditions are favourable for *Fusarium graminearum* and *Fusarium culmorum* growth and DON production (JECFA, 2001; Sudakin, 2003). These fungi are especially prevalent, and with them DON, in years with abundant precipitation and lower air temperatures at the end of summer and the beginning of autumn (Lević et al., 2004). The optimal temperature for *F. graminearum* growth is 25 °C, at a water activity above 0.88, while for growth of *F. culmorum* it is 21 °C, at a water activity above 0.87 (Reid & Hamilton, 1996; JECFA, 2001).

Climate conditions in Serbia can be described as moderate-continental. However during recent years significant weather condition changes were recorded. Based on everything stated above, weather conditions (air temperatures and amount of precipitation) for period of maize planting, growing and harvesting (April-September) in 2013-2015 years were examined in order to investigate their influence on absence or presence of DON in maize samples. Weather conditions parameters for studied maize growing season 2013-2015 are shown in Table 3 (Republic Hydrometeorological Service of Serbia, 2013, 2014, 2015). According to weather conditions parameters year 2014 was significantly different in comparison to 2013 and 2015 years. The statistical analysis of variance showed significant differences for 2014 in terms of air temperature and amount of precipitation. Lower values of temperature deviation as well as number of days with temperatures higher than 35 °C were noted during maize growing season in 2014 in comparison with years 2013 and 2015. Furthermore, 2014 was characterized by significantly higher number of rainy days as well as amount of precipitation. It could be assumed that different weather conditions during maize growing season 2014 had a great influence on DON presence in even 96.0% of examined maize samples. Therefore, weather conditions parameters in 2014 were additionally analyzed and showed in Figure 2. The data in Figure 2 shows monthly average values of air temperatures (a) and amount of rainfall (b) in Serbia for the period from April to September in comparison to average values of these parameters from long-term period (1981-2010). As can be seen, monthly average values of air temperatures in year 2014 were similar or slightly higher than values for the same months in the long-term period (1981-2010). However, the amount of rainfall was significantly higher during the maize growing season 2014 in comparison to the amount of rainfall in long-term period (1981-2010). Spring 2014 in Serbia was characterized by changeable weather conditions with lower temperature an extremely high amount of precipitation. For example, average amount (172 mm) for sum of precipitation recorded in May 2014 represents maximum recorded values since meteorological observations exist in Serbia. Furthermore, average amount of precipitation during maize growing season 2014 (April-September) was around 700 mm, and based on this fact it was the rainiest period in the recent years (Figure 3). Such high amount of precipitation was associated with an increase in the amount of moisture in the grain which resulted in favourable conditions for the growth of certain *Fusarium* species and synthesis of DON. Moisture level of the maize in the fields at the beginning of the harvest was above 20% and farmers delayed harvest, keeping the maize longer in the fields to reduce the moisture levels. Otherwise, farmers would have to pay the additional cost for artificial drying of the maize (Maslac, 2015). In addition, it should be noted that even rainy years promote growth and development of certain *Fusarium* species which are capable of producing different mycotoxins, causing root, stem ear rot, as well as severe reductions in crop yield (between 10% and 30%) (Logrieco et al., 2002), a large amount of rainfall in 2014 year influenced increases of maize yield to record maximum of 7.7 MT/ha (Maslac, 2015). In some parts of Serbia (Northern Province of Vojvodina) maize yields reached a record high of 10-15 MT/ha. Also Serbia exported a record quantity of 3 million MT of maize. Contrary to this, higher air temperatures and lack of rainfall during maize growing seasons 2013 and 2015 influenced lower maize yield than expected. In 2015 almost 25% of maize production was seriously damaged because of the extreme summer drought. Due to drought conditions and fact that in Serbia less than 5% of the maize growing land is irrigated, which makes it particularly vulnerable, the moisture content in maize at the beginning of the harvest in 2013 and 2015 years was around 13% (Maslac, 2016). Such moisture content in maize represents unfavorable dry conditions for some *Fusarium* species and DON synthesis, which is evidenced by lower DON contamination frequency of 2.5% and 15.5% in examined maize samples from years 2013 and 2015, respectively.

The findings obtained in this study regarding the influence of climatic conditions (mainly air temperature and amount of rainfall) on the occurrence of DON in maize are in compliance with findings from several previous studies (Sutton, 1982; Vigier et al., 1997; Logrieco et al., 2002; Van der Fels-Klerx et al., 2013). Those studies also confirmed that higher contamination frequency of DON in maize is characteristic for a year with abundant precipitation and lower air temperature, which are favorable for *F. graminearum* and *F. culmorum* growth and DON synthesis. Even findings from numerous studies indicate that DON is one of the most frequent mycotoxins in temperate and cooler regions, climate changes in the recent years influence presence of DON in many other regions over the world (Streit et al., 2013). Therefore, occurrence and prevalence of DON is variable from region to region, and year to year, and also depend on geographic location, annual variations in rainfall patterns, national wealth, related agricultural and regulatory infrastructure (Placinta et al., 1999; Turner, 2010).

With the aim to introduce comprehensive insight into the occurrence of DON in maize from Serbia during the previous years (2004-2012) results of published studies are investigated and presented in Table 4. Even thought previous studies from Serbia were conducted on a smaller number of samples, the obtained results also indicate the influence of weather conditions, especially amount of precipitation, on DON occurrence in maize. Therefore, the obtained results from those studies were commented in dependence of recorded precipitation (Figure 3). Jajić et el. (2008a, 2008b) reported that 50.0%, 43.9% and 38.1% of analyzed maize samples from 2004, 2005 and 2006 years were contaminated with DON, respectively. As can be seen from the Figure 3, the maize growing season (April-September) during the period from 2004 to 2006 was characterized by higher amount of precipitation in comparison to long-term period (1981-2010). Furthermore, lack of rainfall during the year 2007 influenced lower contamination frequency (25.2%) of DON (Jajić et al., 2008b), while none of the analyzed maize samples from the year 2008 was contaminated with DON (Matić et al., 2009). Contamination of maize with DON in Serbia was also investigated in year 2010. In that study Jakšić et al. (2012) found that rainy maize growing season (Figure 3) influenced the presence of DON in 22 (91.7%) among 24 analyzed maize samples with a wide range of DON concentrations (154-16528 µg/kg). Moreover, our previous investigation (Kos et al., 2014) showed that recorded drought conditions during maize growing season 2012 caused an absence of some *Fusarium* fungi which resulted in DON appearance in only 2 (2.2%) among 90 analyzed maize samples. On the other hand, drought conditions during the same year influenced presence of aflatoxins in 68.5% of the maize samples even though occurrence of these toxins in maize from Serbia is not noticed previously (Kos et al., 2013).

To the best of authors knowledge, the number of recently published data in the literature related to DON occurence in maize from other countries in the same region as Serbia is rather low, especially data that could be comparable to our results since neighboring countries have similar climatic conditions as Serbia. Only published data are from neighboring country Croatia, which also confirmed the influence of weather conditions on DON presence in maize. Pleadin et al. (2012) reported that among 40 analyzed maize samples collected after rainy maize growing season 2010, even 34 (85.0%) samples were contaminated with DON. Furthermore, in 20 (50.0%) samples contamination level of DON was above MRL level of 1750 μg/kg. However, even though 2011 maize growing season in Croatia was characterized by lack of rainfall and higher air temperatures, the same group of authors (Pleadin et al., 2013) detected DON in 45 (71.1%) among 63 analyzed maize samples. Common presence of DON in maize from Croatia was also confirmed in period from 1998 to 2004 years (Sokolović & Šimpraga, 2006). Therefore, Pleadin et al. (2013) claimed that DON is the most common mycotoxin in Croatia.

Furthermore, there is only sporadic literature data investigating the occurrence and levels of DON in maize from European countries during the last decade. Therefore, authors decided to revise recently available published data from over the world (Table 5). As can be seen, the greatest numbers (1655) of maize samples were analyzed in Argentina in the period 2000-2005. Even though in some samples DON concentration was very high (3600 μg/kg), only 28 (1.7%)

of examined maize samples were contaminated with DON. Although numbers of analyzed maize samples from other countries, showed in Table 5, are not very high, finding from those studies confirmed that climate conditions had a great influence on absence or presence of DON in maize. Increasing trend of DON occurrence in maize during the recent years influenced by climatic changes was also noticed in an intensive study reported by Streit et al. (2013). This study included analyses of regulated mycotoxins (aflatoxins, ochratoxin A, zearalenone, deoxynivalenol and fumonisins) in raw materials collected during 2004-2011 years from all over the world. With contamination frequency of 55% DON was the most prevalent mycotoxin detected in the samples. Furthermore, analysis of 4675 maize samples showed that North Asian maize was very often contaminated with DON (90%), while samples from Europe were contaminated with average contamination frequency of around 60%. Less favorable climatic conditions in South-East Asia influenced presence of DON in 32% of analyzed maize samples. Moreover, one more intensive study obtained in Report SCOOP task 3.2.10 regarding occurrence data of *Fusarium* toxins indicate that DON was the most common trichothecene in cereals from twelve European countries. Among 520 analyzed maize samples, DON was found in 463 (89%) with maximum concentration of 8850 µg/kg (Schothorst & van Egmond, 2004; Döll & Dänicke, 2011).

Based on the findings in this and other studies during the last years, it can be concluded that *Fusarium* fungi and DON are frequent contaminants of maize. Furthermore, after analysis of 1800 maize samples from three maize growing seasons, it could be noticed that amount of precipitation represents a climatic factor with the strongest influence on the occurrence of DON in maize. The obtained results indicate the necessity for constant monitoring of DON occurrence in maize from Serbia in order to protect human and animal health. This monitoring is especially important during years in which weather conditions may be suitable for the growth of *Fusarium* fungi on crops. Based on high number of analyzed samples this study represents one of the most intensive studies in the recent years for this region and obtained results could be useful for developing a database together with risk assessment models for Serbia as well as for Europe.

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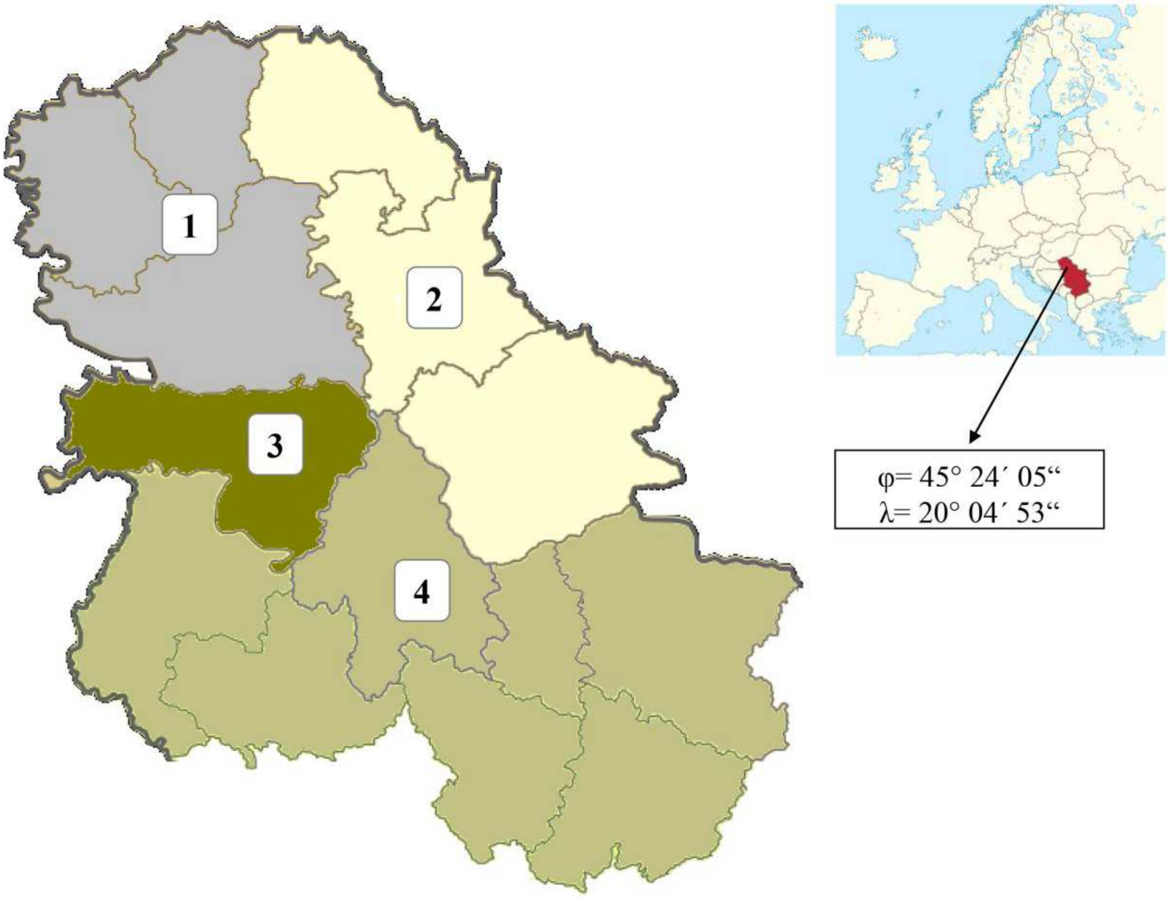
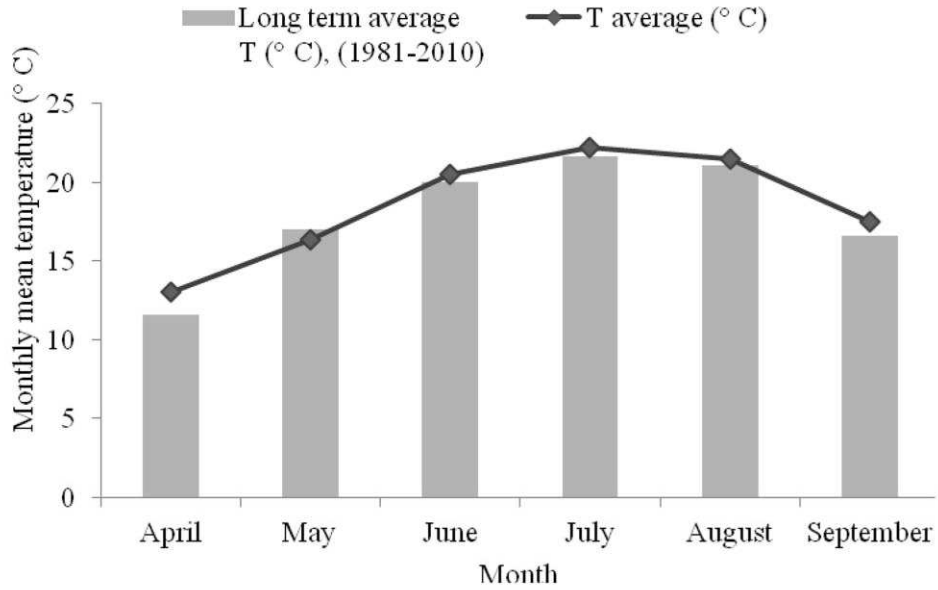
Figure 1. Regions of maize sampling locations

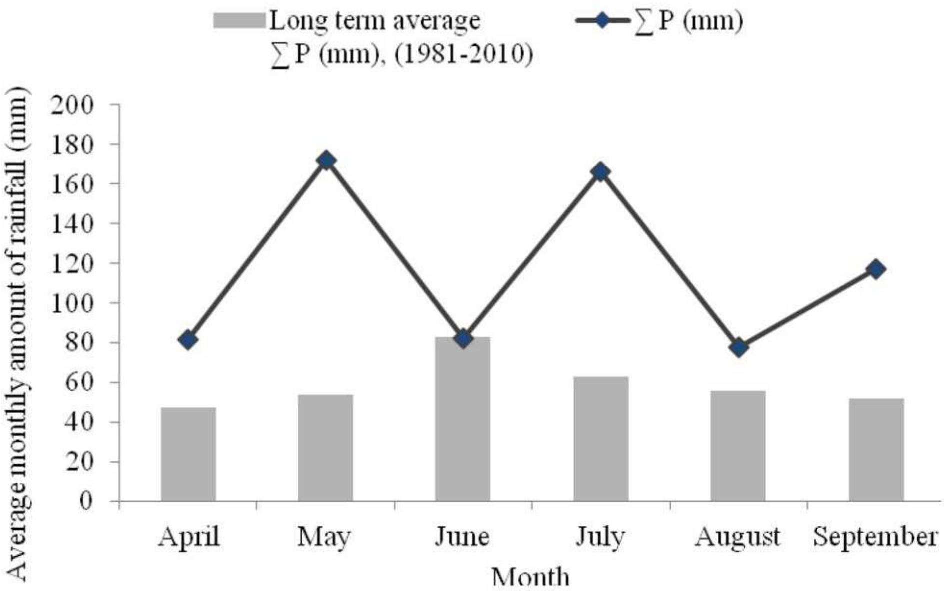


1. Bačka, 2. Banat, 3. Srem, 4. Central Serbia

Figure 2. Monthly average temperatures (a) and amount of rainfall (b) in 2014 year (April-September) in comparison with average values for the period 1981-2010.



a)



b)

Figure 3. Comparison of average amount of rainfall in Serbia from April to September in period 2000-2015 with long-term period (1981-2010).

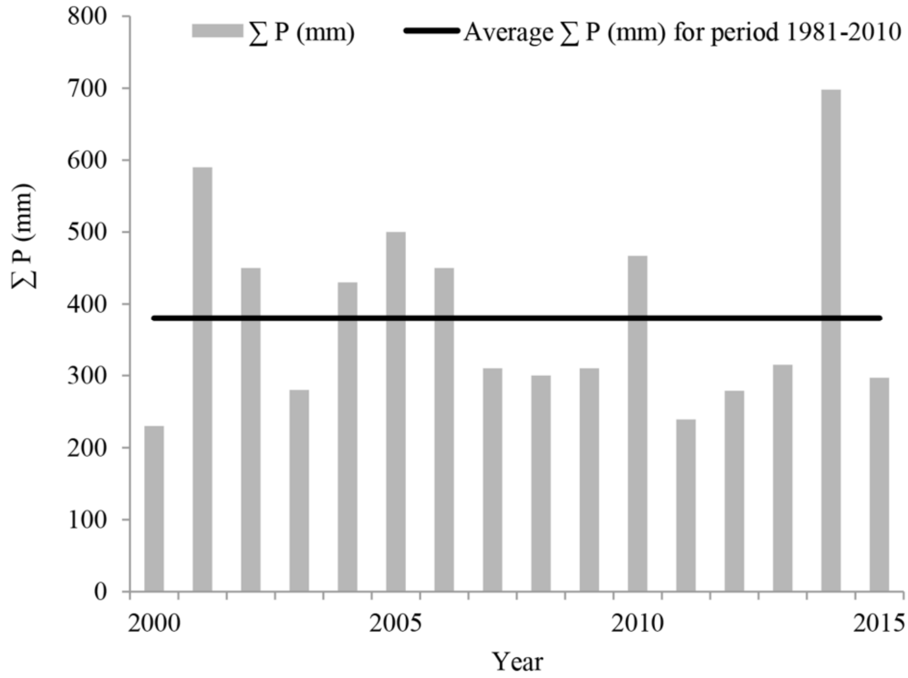


Table 1. Validation parameters of ELISA method for determination of deoxynivalenol.

Validation parameters	Quantitative DON Test				
	CRM 1	CRM 2	Spiking concentration (μg/kg)		
			100	350	3
RSD_r	6.09	3.9	4.6	2.3	4.7
RSD_R	9.54	5.4	6.2	3.0	5.2
R	105.9	103.5	93.4	92.8	87.9

RSD_r: relative standard deviation calculated under repeatability conditions (%).
RSD_R: relative standard deviation calculated under reproducibility conditions (%).
R: recovery (%).

Table 2. Occurrence of deoxynivalenol in maize samples harvested in period 2013-2015.

Year	Incidence rate N (%)				Range	Mean ±SD	Me
	<250	250-1750	1750-8000	>8000			
2013	585 (97.5)	15 (2.5)	-	-	260.1-1388	642.3±364.7	54
2014	24 (4.0)	284 (47.3)	290 (48.3)	2 (0.3)	260.4-9050	3063.3±1264.4	176
2015	507 (84.5)	83 (13.8)	10 (1.7)	-	252.3-6280	921.1±952.7	57

N: number of samples.
Range: range of deoxynivalenol concentration (µg/kg).
Mean ± SD: mean concentration (µg/kg) ± standard deviation (µg/kg).
Median: median of deoxynivalenol concentration (µg/kg).

Table 3. Weather conditions parameters in the period April-September (2012-2016).

Year	Deviation T average (° C)	N Tmax> 30 ° C	N Tmax> 35 ° C	N precipitation	∑ P (mm)	∑ P (%)
2012	2.3^{d}	63^{d}	18^{c}	40^{a}	270^{a}	67^{a}
2013	1.2^{b}	37^{b}	8^{b}	53^{ab}	326^{b}	87^{b}
2014	0.7^{a}	14^{a}	0^{a}	75^{c}	780^{d}	192^{d}
2015	1.9^{c}	53^{c}	14^{bc}	45^{a}	313^{b}	75^{a}
2016	1.4^{b}	25^{ab}	1^{a}	50^{ab}	485^{c}	116^{c}

Different letters in the same column indicate significant differences (P<0.05) between values according to the Duncan´s multiple range test.
T: temperature; N:numbers of days; P: precipitation; ∑: sum.

Table 4. Deoxynivalenol occurrence and levels in maize in recent surveys in Serbia.

Year	N	Incidence rate N (%)	Range of concentration (µg/kg)	Reference
2004	10	5 (50)	40-2460	Jajić et al., 2008a.
2005	66	29 (43.9)	40-2210	
2006	21	8 (38.1)	140-1340	Jajić et al., 2008b.
2007	119	30 (25.2)	270-1720	
2008	17	n.d.	n.d.	Matić et al., 2009.
2010	24	22 (91.7)	154-16528	Jakšić et al., 2012.
2012	90	2 (2.2)	600-700	Kos et al., 2014.

N: number of samples.
n.d. - not detected.

Table 5. Deoxynivalenol occurrence and levels in maize in recent surveys worldwide.

Country	Year	N	Incidence rate N (%)	Range of concentration (μg/kg)	Reference
Argentina	2000-2005	165 5	1.7	n.d.-3600	Garrido et al., 2012.
China	2009-2012	132	77	3.3-834.4	Han et al., 2014.
Italy	2009-2011	140	21.4	3-428	Leggieri et al., 2015.
Poland	2011-2012	30	66.6	n.d.-90	Czembor et al., 2015.
India	n.a.	25	6	10-1070	Mishra et al., 2015.
Tanzania	n.a.	60	63	68-2196	Kamala et al., 2015.

N: number of samples.
n.d. - not detected; n.a. - not available.