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FUNGAL CONTAMINATION OF MAIZE GRAIN SAMPLES WITH A SPECIAL FOCUS ON TOXIGENIC GENERA

Vesna Krnjaja[1], Aleksandar Stanojković[1], Slavica Stanković[2], Miloš Lukić[1], Zorica Bijelić[1], Violeta Mandić[1], Nenad Mićić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
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Abstract: In this study, the total fungal count and contamination with toxigenic fungi from *Aspergillus, Fusarium* and *Penicillium* genera of 127 maize grain samples collected from animal farms in subrbs of Belgrade area during 2012-2015, were determined. The total fungal count was determined using a dilution method, and standard mycological procedures were used to identify potential toxigenic fungi genera.
In the tested samples of maize grains, the total fungal count was from 1 x 10^1 to 3 x 10^6 cfu g^{-1}. No statistically significant differences between investigated years in regard to the mean total fungal count were determined. According to the Regulation on the quality of animal feed of the Republic of Serbia, the total fungal count above permitted limit (2 x 10^5 cfu g^{-1}) was established in 9.52, 7.89, 20.69 and 55.56% tested samples in 2012, 2013, 2014 and 2015, respectively.

Potentially toxigenic fungi from *Aspergillus, Fusarium* and *Penicillim* genera have been identified as the most common in all the samples. In most of the samples, based on the average value for the four-year period (2012-2015), *Fusarium* species (92.22%) have been identified, followed by the species of the genera *Aspergillus* (80.83%) and *Penicillium* (48.68%). A weak positive correlation was established between the moisture content of the samples and the total fungal count in 2012 (r=0.41), in 2013 (r=0.27) and in 2014 (r=0.36) and the medium positive correlation (r=0.61) in 2015.

Based on the results of mycological analysis of grain maize it can be concluded that the test samples in a relatively large number did not meet the criteria of hygienic quality. Therefore, regular and continuous control of the mycological quality of maize grain as the most important nutrient in animal nutrition is necessary as a preventive measure to reduce and control contamination of grain with mycotoxigenic fungi.

Key words: maize, total fungal count, toxigenic fungi

Introduction

Maize is a major food crop for people and also uses as feed for livestock. It makes about 50-70% of poultry feeds *(Jokić et al., 2004)* and it is the most important source of energy for pig feeding *(Edwards, 2002)*. However, maize grains are suitable substrate for fungal infection. There are a many potential toxigenic fungi species that contaminate grain, from which species from *Aspergillus, Fusarium* and *Penicillium* genera are dominant species, as contaminants of maize and producents mycotoxins. The contamination of maize by toxigenic fungi and their mycotoxins is the process that it can occur yet in the field during harvest and later during the storage until the consumption *(Zorzete et al., 2008)*.

Environmental conditions and climatic factors are the most important to the contamination of maize grains before and after harvest. The moisture content of grain and temperature are the most important factors for the growth of potentially mycotoxigenic fungi and spread of infection to the maize grain before and after harvest (*Kana et al., 2013*). Species of the genera *Fusarium, Aspergillus* and *Penicillium* are the most important pathogens isolated from the animal feed in Serbia, from the mycotoxicological aspect. In the environmental conditions of Serbia, *Fusarium* species are usually isolated from the maize grain which, in any given year, may cause a significant reduction in yield, increased mycotoxins contamination and the mass occurrence of mycotoxicosis in animals, particularly pigs *(Lević, 2008)*.

Given the prevalence of the most common types of toxin-producing fungi of the genera *Fusarium, Aspergillus* and *Penicillium* in Serbia, of particular importance to the health of animals is to consider the presence of mycotoxins as secondary metabolites of fungi. In the production of poultry, in particular, the presence of aflatoxin and ochratoxin, produced by *Aspergillus* and *Penicillium* species, is examined, as the most important carcinogenic contaminants of poultry feed (*Leggieri et al., 2015*). *Fusarium* mycotoxins (T-2 toxin and zearalenone) cause disorders in the reproduction of pigs and estrogenism (*Zain, 2011*). Fumonisins are *Fusarium* mycotoxins which are known as causes of equine and porcine leukoencephalomalacia pulmonary edema (*Placinta et al., 1999*). The mycotoxins commonly co-occur in the maize grains, so the presence of a range of mycotoxins is not an unusual occurrence in prepared animal mixtures (*Streit et al., 2012).*

The aim of this paper was to determine the total fungal count and to identify potentially mycotoxigenic fungi genera in maize grain samples during the fourth-year period (2012-2015) and also to assess the potential danger of the presence of these contaminants in the food chain.

Materials and Methods

During the four-year period (2012-2015), the total fungal count was determined and toxin-producing fungal species identified in a total of 127 samples of maize grains which were collected successively (multiple times) every year (during harvest and storage) from different farms in the vicinity of Belgrade. In 2012, 2013, 2014 and 2015 analyzed a total 42, 38, 29 and 18 samples respectively. The size of laboratory sample was 1 kg. After laboratory admission, the samples were analysed for fungal contamination immediately, or were stored 2-3 days at controlled temperature prior the analysis. The moisture content of the tested maize grain samples was determined using a laboratory moisture meter (OHAUS MB35, USA), and mycological analysis was performed according to the method ISO 21527-2 (2008).

Identification of toxigenic genera of fungi was performed according to Watanabe (2002). The frequency of positive, i.e. samples contaminated by toxigenic fungi, was calculated according to the formula: Fr (%) = the number of samples were a fungal genus occurred/the total number of samples x 100.

Statistical analysis was performed with nonparametric test, using the SPSS software (IBM, Statistic 20). To determine the normality, the Shapiro-Wilk (SW) test was used, and to determine homogeneity of variance, the Levene's test. Because the Shapiro-Wilk test showed significant difference compared to the normal distribution, the significance of differences was tested using the Mann-Whitney U - test.

The correlation among individual values for moisture content and total fungal count was determined using the Pearson correlation coefficient.

Results and Discussions

The total fungal count and identification of toxigenic fungi are important indicators of hygienic quality of maize grain as feed of plant origin that is used as an important component in animal feed.

The average moisture content in tested samples of maize grain was 12.19% (2012), 12.37% (2013), 13.81% (2014) and 12.42% (2015). Mycological analysis of tested maize samples established the total fungal count in the range from 1×10^1 to 3×10^6 cfu g^{-1}. The tested samples of maize showed 9.52% (2012), 7.89% (2013), 20.69% (2014) and 55.56% (2015) of the samples with total fungal count above the limit (2×10^5 cfu g^{-1}) according to the Regulation on the quality of animal feed for the feed of vegetable origin of Republic of Serbia (*Official Gazette, 4/2010, and 27/2014 113/2012*) (Table 1). A large number of samples with total fungal count above allowed limit in 2015 (55.56%) can be explained by the extremely favorable climatic conditions during the maize growing season in 2014 (April- October), when, according to the data of Republic Hydro-meteorological Service of Serbia for the Belgrade area, the total precipitation of 675.3 mm was recorded and the mean daily temperatures were >20°C. Those conditions had a favorable impact on the increase of the infective potential of toxigenic fungi in maize in the field, and later during the storage in 2015. In tested maize samples, an average total fungal count was high and was not statistically significant ($P \leq 0.05$) between the studied years (2012 - 2015) (Table 2). This is probably due to the high moisture content (> 15%) in samples of maize grains which were analyzed during the harvest and due to the poor storage conditions (uncontrolled conditions of temperature and humidity).

Similar to our results, in Turkey, analyzing 30 samples of maize grains originating from different locations, *Alptekin et al. (2009)* have determined the total fungal count of 5×10^5 cfu g^{-1} to 5.2×10^7 cfu g^{-1}. In Argentina, *González Pereyra et al. (2012)*, during a two-year study (2006-2007), in the analysis of samples of feed for cattle which contained 60 to 70% of maize grain, have found that the total fungal count ranged from 0 to 2.10×10^8 cfu g^{-1}, and statistically significant differences were determined in regard to the average total fungal count in samples of tested mixtures for cattle between the investigation years. These statistical differences are explained by suitable climatic factors and environmental variations during the sampling period (May to November).

Table 1. Level of fungal contamination of investigated maize grain samples during 2012-2015

Fungal counts		Frequency (%)			
cfu g^{-1} *	log_{10}cfu	Year 2012	Year 2013	Year 2014	Year 2015
2.1×10^5 - 3×10^6	5.32-6.48	9.52	7.89	20.69	55.56
1.1×10^4 – 2×10^5	4.04-5.30	66.67	71.05	68.97	16.67
1×10^1 - 1×10^4	2 - 4	23.81	21.06	10.34	27.77

*Colony forming units per g of sample

Table 2. Mean of total fungal counts (log_{10}cfu g^{-1}) in investigated maize grain samples during 2012-2015

Maize grain samples	cfu g^{-1} (log10)	SD
Year 2012	4.41	0.74
Year 2013	4.57	0.58
Year 2014	4.82	0.72
Year 2015	4.52	0.94
Level of significance	0.087 (ns)	

cfu g^{-1} - colony forming units per g of sample; ns - not significant - $P>0.05$

The mycological analysis of maize grain showed toxigenic species of the genera *Aspergillus, Fusarium* and *Penicillium*. On average for all investigation years (2012-2015), the most of samples (92.22%) was contaminated with toxigenic species of the genus *Fusarium*, followed by 80.83% of samples contaminated with *Aspergillus* and 48.68% of the samples contaminated with *Penicillium* species. In 2012 and 2013, the most of samples were contaminated with *Aspergillus* species, 98.48 and 94.74%, respectively; while in 2014 and 2015 the majority of samples were contaminated with *Fusarium* species, 96.55 and 100%, respectively. A larger number of samples was contaminated with *Penicillium* species in 2012 (76.19%) and 2013 (63.16%) compared to 2014 (27.59%) and 2015 (27.78%) (Table 3). Similar to our results, in Italy, *Covarelli et al. (2011)*, in the analysis of the maize grains originating from different locations of Umbria region, within a two year period (2006-2007), have isolated *Fusarium* species in the highest percentage (up to 76.8%), *Aspergillus* (up to 14.5%) and *Penicillim* species (up to 9.2%). Likewise, in Saudi Arabia, from 20 samples of grain of yellow maize originating from different markets, *Mahmoud et al. (2013)* have isolated most commonly the species from the *Fusarium* (31.74%) and *Aspergillus* (30.83%) genera, and the *Penicillium* (13.75%) and *Alternaria* species (1.66%) have also been isolated but to a lesser extent. Also, *Mudili et al. (2014),* in the analysis of 150 freshly harvested maize samples during 2010-2012, have determined that the *Fusarium* species have been the most present in the investigated sites in India, followed by some species of the genera *Aspergillus* and *Penicillium*. Contrary to the above results, *Alptekin et al. (2009)* has found that the occurrence of species of the genus *Penicillium* was significantly higher than the species of the genera *Aspergillus* and *Fusarium,* in 30 tested samples of maize originating from different localities in Turkey. Furthermore, in Republic Srpska, *Trkulja et al. (2014),* from 83 samples of maize grain intended for human and animal consumption sampled before harvest in 2013, have isolated in almost all samples the *Aspergillus* species, and species of the genera *Fusarium, Penicillium* and *Alternaria* isolated in up to 33, 23 and 18% of the samples, respectively.

Table 3. Frequency of contaminated maize grain samples with potentially toxigenic fungi from *Aspergillus*, *Fusarium* and *Penicillium* genera

Fungal genus	Frequency of fungal contaminated samples (%)				
	Year 2012	Year 2013	Year 2014	Year 2015	Average (2012-2015)
Aspergillus	98.48	94.74	68.97	61.11	80.83
Fusarium	88.10	84.21	96.55	100	92.22
Penicillium	76.19	63.16	27.59	27.78	48.68

Using Pearson's correlation coefficient, in the tested samples of maize, a weak positive correlation was determined between the moisture content and the total fungal count, r=0.27 (2013), r=0.36 (2014) i r=0.41 (2012) and medium positive correlation of r=0.61 (2015). Similarly, in the studies of *Alptekin et al. (2009),* a positive correlation (r = 0.378) was determined between the relative humidity (RH) and fungal count which was not statistically significant (P> 0.05).

Conclusion

Based on the results obtained in this four-year study, it can be concluded that most of the samples of maize grain were contaminated with mycotoxigenic fungi of the genera *Aspergillus, Fusarium* and *Penicillium,* and total fungal count above permitted limit was established in 9.52% (2012), 7.89% (2013) 20.69% (2014) and 55.56% (2015) of the samples. On average for all years of investigation, *Fusarium* species were identified in most maize samples (92.22%), followed by 80.83% of the samples contaminated with *Aspergillus* and 48.68% of the samples contaminated with *Penicillium* species. These results are due to agro-ecological and climatic conditions, especially the total precipitation and temperature, suitable for fungal infection of maize grains still in the field in the investigated area. Similarly, poor environmental conditions of storage after maize harvest contribute to the development of unwanted biological contaminants into the grain. Consequently, the permanent control of the mycological condition of maize during harvest and during storage is the main preventive measure against undesirable consequences to the health of humans and animals arising from the consumption of food with a high content of harmful contaminants such as mycotoxigenic fungi.

Kontaminacija uzoraka zrna kukuruza gljivama s posebnim osvrtom na toksigene rodove

Vesna Krnjaja, Aleksandar Stanojković, Slavica Stanković, Miloš Lukić, Zorica Bijelić, Violeta Mandić, Nenad Mićić

Rezime

Ukupan broj gljiva i kontaminacija s potencijalno toksigenim vrstama iz rodova *Aspergillus, Fusarium* i *Penicillium* određivani su u 127 uzoraka zrna kukuruza koji su sakupljeni na farmama u okolini Beograda tokom četvorogodišnjeg perioda (2012-2015). Primenom metode razređenja određen je ukupan broj gljiva, dok su standardne mikološke metode korišćene za identifikaciju potencijalno toksigenih rodova gljiva.

U ispitivanim uzorcima zrna kukuruza ukupan broj gljiva je bio od 1 x 10^1 do 3 x 10^6 cfu g^{-1}. Između ispitivanih godina nisu ustanovljene statističke značajne

razlike u prosečnim vrednostima ukupnog broja gljiva. Prema Pravilniku Republike Srbije o kvalitetu hrane za životinje, u hranivima biljnog porekla, ukupan broj gljiva iznad dozvoljenog limita (2 x 10^5 cfu g^{-1}) ustanovljen je u 9,52, 7,89, 20,69 i 55,56% ispitivanih uzoraka u 2012., 2013., 2014. i 2015. godini, respektivno.

Od potencijalno toksigenih gljiva identifikovane su *Aspergillus, Fusarium* i *Penicillim* vrste kao najučestalije u svim ispitivanim uzorcima. U najvećem broju uzoraka, na osnovu prosečnih vrednosti u četvorogodišnjem periodu (2012-2015), identifikovane su *Fusarium* vrste (92.22%), zatim vrste iz rodova *Aspergillus* (80.83%) i *Penicillium* (48.68%). Između sadržaja vlage ispitivanih uzoraka i ukupnog broja gljiva ustanovljena je slaba pozitivna korelacija u 2012. (r=0.41), 2013. (r=0.27) i 2014. godini (r=0.36) i srednje pozitivna korelacija (r=0.61) u 2015. godini.

Na osnovu dobijenih rezultata mikološke analize zrna kukuruza može se zaključiti da ispitivani uzorci u relativno velikom broju ne zadovoljavaju kriterijume higijenskog kvaliteta. Zbog toga, redovna i stalna kontrola mikološkog kvaliteta zrna kukuruza kao najvažnijeg hraniva u ishrani životinja je neophodna preventivna mera za smanjenje i kontrolu kontaminacije zrna s mikotoksigenim gljivama.

Ključne reči: kukuruz, ukupan broj gljiva, mikotoksigene gljive

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