

Vesna S. Krnjaja¹, Jelena T. Lević²,
Slavica Ž. Stanković², Ana M. Stepanić²

¹ Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia

² Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia

FUSARIUM SPECIES AND THEIR MYCOTOXINS IN WHEAT GRAIN

ABSTRACT: Incidence of fungi and concentration of mycotoxin deoxynivalenol (DON), zearalenone (ZON) and fumonisin (FB₁) were studied in the grain of the winter wheat collected subsequently after harvesting in 2010. In the analyzed samples the highest incidence was determined for the species of *Fusarium* (84.7%) genus, significantly lower incidence was determined for the species of genus *Alternaria* (12.35%), and especially for species of genera *Acremoniella* (2.05%), *Acremonium* (0.65%) and *Penicillium* (0.25%). *F. graminearum* (99.05%) was the most present species of *Fusarium* genus, whereas the following species *F. sporotrichioides* (0.4%), *F. subglutinans* (0.4%), *F. poae* (0.05%), *F. proliferatum* (0.05%) and *F. verticillioides* (0.05%) were only sporadic. The presence of DON, ZON and FB₁ mycotoxins was established in all studied wheat samples. DON was detected in concentrations from 123 to 393 µg kg⁻¹ (average 214 µg kg⁻¹), ZON from 157.144 to 471.055 µg kg⁻¹ (average 299.934 µg kg⁻¹), and FB₁ from 2.715 to 16.488 µg kg⁻¹ (average 6.286 µg kg⁻¹).

KEY WORDS: *Fusarium* spp., wheat grain, incidence, mycotoxins

INTRODUCTION

Fusarium head blight (FHB) is one of the most serious diseases of cereals and causes significant losses in both yield and quality of cereals. When susceptible wheat genotypes are infected during flowering, the infected spikelets bleach prematurely. The disease progresses up and down the head and may infect all spikelets in a head when weather conditions are favorable (Parr y et al., 1995). Frequently, only a part of the head is affected by FHB. These partly white and partly green heads are diagnostic. Additional indications of FHB infection are pink to salmon-orange spore masses of the fungus, often seen on the infected spikelet and glumes during prolonged wet weather. This causes considerable yield and quality losses and accumulation of mycotoxins in the grain (Windle s, 2000).

Fusarium infected-grain often contains high concentrations of mycotoxins which are harmful to human beings and livestock. They are mainly from the group of trichothecenes, deoxynivalenol (DON) and nivalenol (NIV), and other mycotoxins, such as zearalenon (ZON) and fumonisins (FB₁) (B a i and S h a n e r, 1994; N a k a j i m a, 2007; S t a n k o v i ć et al., 2010).

Several *Fusarium* species cause this disease, and *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein) Petch) is one of the most important species. *F. graminearum* can be divided into two chemotaxonomic groups, DON chemotype and NIV chemotype, based on the production of different trichothecene mycotoxins. DON is the most prevalent mycotoxin in cereals, and DON chemotypes of *Fusarium* are found worldwide. On the other hand, NIV chemotype is found in more restricted regions – this chemotype is found in Asia, Africa and Europe, but not in North America. Several countries have established legislative limits for DON in cereals. Thus, generally, greater attention is focused on DON than NIV as a trichothecene mycotoxin (N a k a j i m a, 2007).

The acute symptoms of trichothecene poisoning are characterized by skin irritation, food refusal, vomiting, diarrhoea, hemorrhage, neural disturbance, miscarriage and death (J o f f e, 1986). Chronic ingestion of small amount of trichothecenes may result in an important secondary effect, the predisposition to infectious disease through suppression of the immune system (M i l l e r and A t k i n s o n, 1987).

Zearalenone is another toxic metabolite produced by many *Fusarium* species and often co-occurs in cereals with trichothecenes. Zearalenone causes estrogenic effects and reduces reproductive capability (B i a g i, 2009).

Fumonisins are a group of toxic metabolites produced by *Fusarium* strains which contaminate cereals worldwide. Among fumonisins, fumonisin B₁ is always the most abundant. Porcine pulmonary edema is caused by fumonisins (B i a g i, 2009). Precaution should be taken to avoid inhalation of mycotoxin-containing spores and dust and direct skin contact with infected grains (T r e n h o l m et al., 1989). The control of fusarium head blight has relied on the resistant varieties and use of fungicides. Resistant cultivars are very rare and application of fungicides may be used for control of fusarium head blight (N o u r o z i a n et al., 2006).

Taking into consideration the importance of the harmful effect of *Fusarium* species and their mycotoxins in grains, especially wheat, the presence of pathogenic fungal species was studied, especially the presence of species of *Fusarium* genus, and presence of fusariotoxins, deoxynivalenol (DON), zearalenone (ZON) and fumonisin (FB₁) in wheat grains, immediately after harvesting in 2010.

MATERIALS AND METHODS

Wheat samples were collected immediately after harvesting in July, 2010. Twenty samples were selected for microbiological and 10 for mycotoxicological analysis. Collected samples were stored in refrigerator at 4°C before analysis.

Moisture content was determined immediately after sampling. Average moisture content in the studied samples ranged from 10.75 to 15.73% (average 12.3%).

Samples of wheat grain for microbiological analysis were rinsed in tap water for one hour, disinfected in sodium hypochlorite solution (NaOCl) in the ratio of 3:1 for 5 minutes and subsequently rinsed several times in distilled water. After the grains were dried on filter paper, 100 grains of each sample were distributed in 20 Petri dishes containing 2% water agar (WA) and incubated at room temperature of $20 \pm 2^\circ\text{C}$ during 7 days. Based on morphological appearance of fungi colonies, species of various genera were identified, paying particular attention to the species of *Fusarium* genus (Ellis, 1971; Burgess et al., 1994; Watanabe et al., 1994).

Based on the results of microbiological analysis of the samples, 10 samples were selected for mycotoxicological analysis. Samples were tested for presence of DON, ZON and FB₁ mycotoxins by using ELISA (Enzyme linked immunosorbent assay) methods. Samples were ground to fine powder. For the analysis of presence of ZON and FB₁ toxins, 5 g of each ground sample was mixed with 25 ml of methanol and 1 g of NaCl, whereas for the analysis of DON toxins, samples were mixed with water and 1 g NaCl. Samples prepared in this way were put in blender and mixed for 3 minutes. They were subsequently filtered through Whatman filter paper 1 and filtrate was collected. Dilution filtrate was carried out according to manufacturer instructions and differed depending on the concentration of toxin in the sample. ELISA procedure was performed following the manufacturer's recommendations (Tecna S. r. l., Trieste, Italy). Absorbance was determined using the spectrophotometer Elisa reader BioTek EL x 800™ (Absorbance Microplate Reader) at 450 nm.

RESULTS

Based on microbiological analysis of wheat grains, the presence of five fungi genera was established: *Acremoniella*, *Acremonium*, *Alternaria*, *Fusarium* and *Penicillium*. Species of *Fusarium* genus were the most frequent (84.7%), followed by species from genera *Alternaria* (12.35%), *Acremoniella* (2.05%), *Acremonium* (0.65%), and *Penicillium* (0.25%) (Table 1).

Tab. 1 – Frequency of pathogenic fungi in wheat grain samples

Fungal genera	Frequency (%)
<i>Acremoniella</i>	2.05
<i>Acremonium</i>	0.65
<i>Alternaria</i>	12.35
<i>Fusarium</i>	84.7
<i>Penicillium</i>	0.25

F. graminearum, *F. poae* (Peck) Wollen., *F. proliferatum* (Matsushima) Nirenberg, *F. sporotrichioides* Sherb., *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas, and *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* (Sacc.)) Nirenberg (Table 2) were identified species of *Fusarium* genus. The most frequent species was *Fusarium graminearum* (99.05%), whereas other species were isolated only in 0.40% (*F. sporotrichioides* and *F. proliferatum*) and 0.05% (*F. poae*, *F. proliferatum* and *F. verticillioides*).

Tab. 2 – Frequency of *Fusarium* species in wheat grain samples

<i>Fusarium</i> species	Frequency (%)
<i>F. graminearum</i>	99.05
<i>F. poae</i>	0.05
<i>F. proliferatum</i>	0.05
<i>F. sporotrichioides</i>	0.40
<i>F. subglutinans</i>	0.40
<i>F. verticillioides</i>	0.05

The analysis of fusariotoxins showed presence of DON, ZON and FB₁ in all studied samples. ZON was detected in the highest average concentration (299.934 µg kg⁻¹) and in the widest range (157.144-471.055 µg kg⁻¹), followed by FB₁ with 6.286 µg kg⁻¹ (ranging from 2.715-16.488 µg kg⁻¹) and DON with 214 µg kg⁻¹ (ranging from 123-393 µg kg⁻¹) (Table 3).

Tab. 3 – Concentrations of *Fusarium* mycotoxins in wheat grain samples

Mycotoxins	Mycotoxin concentration (µg kg ⁻¹)	
	Average	Range
DON	214	123-393
ZON	299.934	157.144-471.055
FB ₁	6.286	2.715-16.488

DISCUSSION

In these studies of mycobiota of wheat grain, the most frequent were *Fusarium* (84.7%), followed by *Alternaria* species (12.35%). Out of *Fusarium* species, the most frequent was *F. graminearum* (99.05%), whereas other species were present in very low percentage, from 0.05% (*F. poae*, *F. proliferatum* and *F. verticillioides*) to 0.4% (*F. sporotrichioides* and *F. subglutinans*). In similar studies, L e v i ć et al. (2008) established the presence of *F. graminearum* up to 55.5%. According to S t a n k o v i ć et al. (2007) two year results showed that the greatest number of wheat grain samples was infected with species of the genera *Fusarium* (81.8 and 65.0%), and *Alternaria* (36.3 and 17.5%) with the intensity ranging from 9.4 to 84.0% in 2005, and from 23.4 to 80.6% in 2006. Out of 13 identified species belonging to the genus *Fusarium*,

F. graminearum had the highest frequency (35.2 and 12.5%) and the intensity of up to 67.2 and 21.9% in 2005 and 2006, respectively, followed by *F. poae*, but only in 2005 (20.4%), and *F. proliferatum* in 2006 (19.7%) (Stanković et al., 2007).

During a two year investigation (2000-2001) of *Fusarium* spp. on wheat grains, Walwijk et al. (2003) established that the species of *F. graminearum* was the most dominant in presence that was, on average, 58.4 and 58.6% in 2000 and 2001, respectively.

According to data of the Republic hydrometeorological service of the Republic of Serbia, May, 2010 was the month with tropical days, high temperatures and abundant rainfall, especially in the second and third ten days, when wheat was in the pheno stage of blooming. Such weather conditions were favorable for the development of *F. graminearum* and Fusarium head blight.

Presence of DON, ZON and FB₁ in all investigated samples of wheat grain in our research indicated that the weather conditions during the vegetation period of winter wheat in 2010 were very favorable for development of the studied fusariotoxins. ZON was detected in the highest concentration of 299.934 µg kg⁻¹ (ranging from 157.144-471.055 µg kg⁻¹), followed by FB₁ with 6.286 µg kg⁻¹ (ranging from 2.715-16.488 µg kg⁻¹) and DON with 214 µg kg⁻¹ (ranging from 123-393 µg kg⁻¹). According to the data of Jajić et al. (2008), concentration of DON in samples of wheat grain ranged from 124 to 1235 µg kg⁻¹, depending on the conditions in the years of study. According to Pačladi et al. (2004), grain samples, from three studied cultivars of durum wheat inoculated in the field with *F. graminearum* and *F. culmorum*, and non-treated by fungicides, had DON concentration ranging from 500 to 1.040 µg kg⁻¹.

According to Kamoun et al. (2009), infection levels in freshly harvested wheat grain were very low and the maximum DON level of the positive samples was 53 µg kg⁻¹. The investigation of the incidence of fusariotoxins in winter wheat in Belgium, conducted from 2002 to 2005, showed that *Fusarium* infection and DON contamination were mainly influenced by location and environmental parameters. Mean DON levels ranged from 0 to 15.000 µg kg⁻¹. Seasonal and local weather conditions, before and during the flowering, along with local crop husbandry measures (crop rotation, soil preparation), seemed to be of great importance for the explanation of the variations in the obtained results. *F. graminearum* and *F. culmorum* were in general the most frequently occurring *Fusarium* spp. in Flanders over the 4 years, but the composition of the *Fusarium* population varied strongly depending on the location and year (Isebaert et al., 2009).

In our study, high concentration of ZON in wheat grain samples was established (average 299.934 µg kg⁻¹). According to Stanković et al. (2007), ZON was determined in the range from 37 to 331 µg kg⁻¹, where 64.52% of positive samples had, on average, 133.4 µg kg⁻¹. The presence of ZON was determined in three most cultivated varieties in Serbia (Evropa-90, Pobeda and Renesansa) with an average for positive samples being 171.67, 110.50 and 114.83 ppb, respectively (Stanković et al., 2007). Contamina-

tion of high number of wheat grain samples (78.0%) with ZON (160-500 $\mu\text{g kg}^{-1}$) was also recorded by S t o j a n o v i ć et al. (2002).

Concentration of FB_1 in wheat grain in our investigation ranged from 2.715 to 16.488 $\mu\text{g kg}^{-1}$, which was not in concordance with lower percentage of isolated producers (0.05-0.40%). Similar results were obtained by S t a n k o v i ć et al. (2008). According to these authors, concentration of FB_1 in the studied samples of wheat grain ranged from 2.000 to 20.000 $\mu\text{g kg}^{-1}$, whereas the producers of this mycotoxin (*F. verticillioides*, *F. proliferatum* and *F. subglutinans*) were isolated in low percentage (0.8-8.0%).

CONCLUSION

Global climatic changes and increased warming of the atmosphere in recent years have had huge impact on an increase in the development of pathogenic and toxigenic fungi species in grains. Due to high temperatures and high air humidity during vegetation period of wheat, conditions favorable for spreading of *Fusarium* species are constantly present and recorded in Serbia. Accumulation of secondary fungal metabolites, mycotoxins, in crops, and later during the storage of grains, represents risk to human and animal health. Therefore, permanent and regular control of the quality of grains is a prerequisite for healthy nutrition of both humans and animals. Necessary measures of quality control also provide data on quantitative parameters/indicators of harmful and toxic substances in the food chain. These data generally create possibility for the development of public awareness of preventive measures against possible danger. Awareness of the danger caused by the presence of contaminants in food chain can significantly contribute to the application of measures which would ensure the required quality of food, thus preserving the quality of human and animal health.

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FUSARIUM VRSTE I ЊИХОВИ МИКОТОКСИНИ У ЗРНУ ПШЕНИЦЕ

Весна С. Крњаја¹, Јелена Т. Левић², Славица Ж. Станковић², Ана М. Степанић²

¹ Институт за сточарство, Аутопут 16, 11080, Београд-Земун, Србија

² Институт за кукуруз „Земун Поље“, Слободана Бајића 1, 11185, Београд-Земун, Србија

Резиме

Учесталост гљива и концентрација микотоксина деоксиниваленола (DON), зеараленона (ZON) и фумонизина (FB₁) проучавана је у зрну озиме пшенице прикупљене непосредно након жетве у 2010. години. У испитиваним узорцима највећу заступљеност имају врсте из рода *Fusarium* (84,7%), а знатно мање врсте из рода *Alternaria* (12,35%), а посебно из родова *Acremoniella* (2,05%), *Acremonium* (0,65%) и *Penicillium* (0,25%). Од врста рода *Fusarium* најзаступљенија је врста *F. graminearum* (99,05%), док су спорадичне врсте *F. sporotrichioides* (0,4%), *F. subglutinans* (0,4%), *F. poae* (0,05%), *F. proliferatum* (0,05%) и *F. verticillioides* (0,05%). Присуство микотоксина DON, ZON и FB₁ установљено је у свим испитиваним узорцима зрна пшенице. DON је био детектован у концентрацијама од 123 до 393 µg kg⁻¹ (просек 214 µg kg⁻¹), ZON од 157,144 до 471,055 µg kg⁻¹ (просек 299,934 µg kg⁻¹) и FB₁ од 2,715 до 16,488 µg kg⁻¹ (просек 6,286 µg kg⁻¹).