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PRESENCE OF DEOXYNIVALENOL IN WINTER WHEAT TREATED WITH FUNGICIDES

V. Krnjaja[1], J. Lević[2], S. Stanković[2], T. Petrović[3], V. Mandić[1], Z. Tomić[1], A. Obradović[2]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
[3]Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
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Abstract: Natural occurrence of *Fusarium* spp. and concentrations of mycotoxin deoxynivalenol (DON) in the grain of the winter wheat moderately susceptible to *Fusarium* head blight (FHB) has been studied. Grain samples were collected from wheat crops intended mainly for human consumption. All wheat crops were treated with fungicides before (a.i. flutriafol – formulated as Fluoco, applied in dose of 0.5 l ha^{-1}) and during the flowering phase of growing (a.i. thiophanate-methyl + epoxiconazole formulated as Eskorta plus and a.i. thiophanate-methyl formulated as Funomil, applied in doses of 0.75 and 0.5 l ha^{-1}, respectivily). Among of *Fusarium* species only *F. graminearum,* as a well known producer of DON, was identified. This fungus was identified in 15 of 19 samples (78.9%) with incidence in positive samples of 2 to 28% (average, 14.0%). Presence of DON was established in 13 of a total 19 investigated wheat grain samples (68.4%). In positive samples DON was detected in concentrations from 69 to 918 μg kg^{-1} (average, 478 μg kg^{-1}). DON showed a significant and positive correlation at P≥0.05 with grain moisture content (r = 0.52*). Between the frequency of *F. graminearum* and concentration of DON and between the frequency of *F. graminearum* and grain moisture content, positive correlation was determined, but without statistical significance (r = 0.44 and r = 0.29, respectively).

Key words: Fusarium head blight, *Fusarium graminearum*, deoxynivalenol, winter wheat

Introduction

Wheat is one of the most important crop cultures grown in Serbia, on approximately 500,000 ha, with average yield of 3,700 kg/ha *(Statistical Yearbook of Serbia, 2012)*. It has been used for thousands of years to provide food for humans. For livestock feeding wheat grain can be used as concentrated livestock feed, whereas whole plant can be used as fodder.

There are several toxigenic species of *Fusarium* that are also a major pathogens of cereal plants, causing *Fusarium* head blight (FHB) in wheat. It is the major wheat disease occurring worldwide, especially in temperate climate regions, causing reductions in yield and quality of wheat *(Parry et al., 1995)*. In our agro-climatic condition, *F. graminearum* has been isolated as the most present species from *Fusarium*-infected grains *(Lević et al., 2008; Krnjaja et al., 2011)*. This fungus produces different mycotoxins, mainly deoxynivalenol, which contaminate grain *(Nakajima, 2007a; Stanković et al., 2012)*.

Fusarium species produce a wide range of mycotoxins of diverse structure and chemistry. Deoxynivalenol (DON) is a trichothecene mycotoxin with toxic effects on animals and human health. Testing of grains and animal feed on the occurrence of *Fusarium* mycotoxins attracts considerable attention and has been the subject of extensive investigations over the recent years. DON concentration in *Fusarium*-damaged grain generally increases with the percentage of damaged grain in a given sample. It was reported that the amount of DON produced by *F. graminearum* was positively correlated with fungal biomass *(Wegulo, 2012)*.

Semaškiené et al. (2006) have established a slight reduction in *Fusarium* infection in the plants treated with fungicides compared to untreated plants of spring cereals. *Wegulo (2013)* has been demonstrated the importance of applying of integrated management in cultivation of wheat, with an emphasis on application of fungicides and host resistance.

In this paper the results of the occurrence of *F. graminearum* and DON concentrations in samples of winter wheat grains collected from crops treated by fungicides have been presented.

Materials and Methods

The total of 19 samples of wheat grains of variety Takovčanka collected in 2013 from crops cultivated in Institute for Animal Husbandry, Belgrade, were used for mycological and mycotoxicological analysis. Wheat crops were treated twice with fungicides during the period of wheat growing. In the first half of April, the samples were treated with fungicide based on flutriafol (formulated as Fluoco) at a dose of 0.5 l ha^{-1} wheras the combination of fungicides based on epoxiconazole + thiophanate-methyl (formulated as Excorta Plus) and thiophanate-methyl (formulated as Funomil) at doses 0.75 and 0.5 l ha^{-1}, respectively, has been applied in the second half of May, in the flowering phase. Average weight of 1 kg per sample of wheat grains was taken immediately after the harvest in July 2013 using standard methods *(European Commission, 2006)*. The moisture content of wheat grains was determined using a moisture analyzer (OHAUS MB35, USA).

For the mycological analysis the wheat grains, were first disinfected in 1% sodium hypochlorite solution (NaOCl) 3-5 minutes and rinsed twice in distilled water. After drying of grains on filter paper, 50 grains of each sample was distributed in 5 Petri plates (9 cm in diameter) containing water agar (WA) (10 grains per one plate) and kept in incubator (Memmert) at 25ºC during 7 days. Identification of colonies of fungi that overgrowth the wheat grains was done by microscopic examination of mycelium and spores, according to *Burgess et al. (1994)* and *Watanabe (1994)*. The frequency and incidence of individual species was calculated per sample according to *Lević et al. (2012).*

The presence of DON was detected by enzyme-linked immunosorbent assay (ELISA). Five grams of sample was mixed with 1 g of NaCl and homogenized in 25 ml of 70% (v/v) methanol in a 250 ml Erlenmeyer flask on the orbital shaker (GFL 3015, Germany) for 30 minutes. Homogenate was filtered through a Whatman filter paper 1. The filtrate was further analysed according to the manufacturer's instructions Celery Techna ® ELISA kits. Absorbance was measured at a wavelength of 450 nm on an ELISA reader spectrophotometer (Biotek EL x 800TM, USA).

Correlation between individual values obtained for grain moisture content, frequency of *F. graminearum* and concentration of DON was determined using Pearson's correlation coefficient.

Results

Moisture content of the samples of wheat grains ranged from 12.1 to 15.0%, with an average of 13.7%, for all tested samples (data not presented).

Based on mycological analysis *F. graminearum,* as well known producer of DON, was identified. This fungus was found in 15 out of 19 samples with incidence of 2 to 28% (average, 14.0%) in positive samples. In all investigated samples, species of genus *Alternaria* were identified, with an incidence of 84.8% (range 70-100%). *Epicoccum* spp. and *Penicillium* spp. were determined in 15.8% of samples, and *A. flavus* and *Rhizopus* in 5.3% of the samples, while in 10.5% of samples the isolated species have not been sporulated (sterile mycelia) (Table 1).

Table 1. Frequency and incidence of fungal species in samples of wheat grain

Fungal species	Frequency		Incidence (%)	
	Positive/total sample	Percentage	Range	Average
Alternaria spp.	19/19	100.0	70-100	84.8
Aspergillus flavus	1/19	5.3	2	2
Epiccocum spp.	3/19	15.8	6	6
Fusarium graminearum	15/19	78.9	2-28	14.0
Penicillium spp.	3/19	15.8	2-8	6
Rhizopus spp.	1/19	5.3	10	10
Sterile mycelia	2/19	10.5	14-16	15

Mycotoxicological analysis of wheat samples revealed the presence of DON in 68.4% of the tested samples. The concentration of DON in positive samples of wheat ranged from 69 to 918 μg kg^{-1} with an average concentration of 478 μg kg^{-1} (Table 2). The concentration of DON was exceeded the permissible limit (750 μg kg^{-1}) prescribed by the Serbian Regulation *(Official Gazzete, 2011)* in only two samples (data not shown).

Table 2. Concentrations of DON mycotoxin in wheat grain samples

Item	DON
Sample size[a]	13/19
Incidence %	68.4
Range (μg kg^{-1})	69-918
Mean[b] (μg kg^{-1})	478

[a] Number of positive samples/Number of total samples
[b] Mean concentration in positive samples

A positive correlation was established between concentration of DON and the grain moisture content ($r = 0.52^*$, $P \geq 0.05$), between concentration of DON and the frequency of *F. graminearum* ($r = 0.44$), as well as between the grain moisture content and the frequency of *F. graminearum* ($r = 0.29$).

Discussion

In the samples of wheat collected in 2013, the *F. graminearum* species was identified as a FHB disease-causing species, with an average incidence of 14.0%. In the study of *Krnjaja et al. (2011b)*, it has been reported the incidence of *F. graminearum* of 82.50% in wheat grains collected in 2009, not treated with fungicides, whereas in untreated samples collected in 2010, the incidence of *F. graminearum* was 99.05% (*Krnjaja et al., 2011a*). Since in this study the grains were treated with fungicide, the lower incidence of *F. graminearum* was obtained, compared to the previous results of *Krnjaja et al. (2011a,b)*. This finding confirmed the assumption that the application of fungicides could be a suitable preventive measure for the control of the occurrence of *Fusarium* spp. during the growing season of wheat.

In this study, 68.4% DON positive samples of wheat were determined with an average concentration of 478 μg kg^{-1}, whereas *Krnjaja et al. (2011a,b)* have established 100% DON positive samples with an average concentrations of 490 μg kg^{-1} in 2009 and 214 μg kg^{-1} in 2010. It could be assumed that these differences in an average concentration of DON has been the results of differences among the tested varieties of wheat, the differences in grain moisture content at harvest as well as climatic conditions, particularly in terms of temperature and rainfall during flowering and early stages of grain development.

By applying the fungicide tebuconazole and thiophanate-methyl, *Wachowska et al. (2012),* have effectively controlled FHB and isolated the lower level of *Fusarium* spp. in treated wheat grains in comparison with the control plants. By examining the efficiency of seven fungicides in order to reduce mycotoxins *Nakajima (2007b)* has found that preparations based on thiophanate-methyl have significantly reduced the content of DON and nivalenol (NIV) mycotoxin in wheat grain. *Metcalfe et al. (2000)* have found that the use of the sterol 14-α-demethylase inhibitors (DMI) fungicides (fluquinconazole, flutriafol and prochloraz) in full recommended doses had not caused resistance of pathogenic fungi of wheat to these fungicides.

Nakajima (2007b) found that the efficacy of the control of DON and NIV was consistently lower than that of FHB severity and assumed that critical control point of DON and NIV might be different from that of FHB severity. The high levels of DON and NIV could be produced beyond 20 days after anthesis even by early infection and infection at a late stage. For this reason, the frequency of fungicide application has been important in the prevention of infection by *Fusarium* spp. and likewise for mycotoxin reduction. The general recommendation for timing of fungicide application is the beginning of flowering phase of growth, in which the plants are most susceptible to *Fusarium* infection. Likewise, developing control strategies that cover the late stage as well as the early stage would be desirable to reduce the risk of mycotoxin contamination in wheat *(Nakajima, 2007b)*.

Based on the regulated maximum permissible concentration of mycotoxins in food and feed in Serbia *(Official Gazette of RS, 2011)*, it could be concluded that the examined batches/lots of unprocessed wheat grains can be used as food and feed because its contained a concentration of DON under the safe limit (1250 $\mu g\ kg^{-1}$). However, for direct human consumption only wheat containing DON less than 750 $\mu g\ kg^{-1}$ can be used.

Conclusion

In conclusion, the use of fungicides in wheat crops significantly reduces the presence of the disease-causing species of FHB and thus prevents considerable DON production. These results may be of great importance especially in years when outbreaks of causal agents of wheat head fusariosis are expected. The use of fungicides in conjunction with other preventive measures to protect the wheat from the appearance of FHB is important to promote the development of integrated pest management strategy.

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Prisustvo deoksinivalenola u ozimoj pšenici tretiranoj s fungicidima

V. Krnjaja, J. Lević, S. Stanković, T. Petrović, V. Mandić, Z. Tomić, A. Obradović

Rezime

U radu je proučavana prirodna pojava *Fusarium* spp. i koncentracija mikotoksina deoksinivalenola (DON) u zrnu ozime pšenice srednje osetljive prema fuzariozi klasa (FHB). Uzorci zrna su prikupljeni sa proizvodnih useva pšenice namenjene uglavnom za ljudsku upotrebu. Svi usevi pšenice bili su tretirani sa fungicidima pre (a.m. flutriafol – formulisana kao preparat Fluoco, primenjen u dozi 0,5 l ha^{-1}) i tokom cvetanja biljaka (a.m. tiofanat-metil + epoksikonazol formulisana kao preparat Eskorta plus i a.m. tiofanat-metil formulisana kao Funomil, primenjeni u dozi 0.75 i 0.5 l ha^{-1}, respektivno). Među *Fusarium* vrstama jedino je identifikovana *F. graminearum*, koja je poznati producent DON. Ova gljiva je bila identifikovana u 15 od 19 uzoraka (78.9%) sa incidencom od 2 do 28% (prosek 14.0%) u pozitivnim uzorcima. Prisustvo DON je utvrđeno u 13 od ukupno 19 proučavanih uzoraka pšenice (68.4%). U pozitivnim uzorcima DON je detektovan u koncentracijama od 69 do 918 μg kg^{-1} (prosek 478 μg kg^{-1}). DON je pokazao značajnu i pozitivnu korelaciju pri $P \geq 0.05$ sa sadržajem vlage zrna ($r = 0.52^*$). Između učestalosti *F. graminearum* i koncentracije DON i učestalosti *F. graminearum* i sadržaja vlage zrna utvrđena je, takođe, pozitivna korelacija ali statistički nije značajna ($r = 0.44$ i $r = 0.29$, respektivno).

References

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory Manual for *Fusarium* Research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.

EUROPEAN COMMISSION (2006): Commision regulation (EC) No. 401/2006. Official Journal of the European Union, L70, 12.

KRNJAJA V.S., LEVIĆ J.T., STANKOVIĆ S.Ž., STEPANIĆ A.M. (2011a): *Fusarium* species and their mycotoxins in wheat grain. Proc. Nat. Sci, Matica Srpska Novi Sad, 120, 41-48.

KRNJAJA V.S., STANKOVIĆ S.Ž., LEVIĆ J.T., (2011b): The presence of toxigenic *Fusarium* species and fusariotoxins deoxynivalenol and zearalenone in winter wheat. Biotechnology in Animal Husbandry, 27, 1, 63-73.

LEVIĆ J., STANKOVIĆ S., KRNJAJA V., BOČAROV-STANČIĆ A., IVANOVIĆ D. (2012): Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia. Pesticide and Phytomedicine 27, 1, 33-40.
LEVIĆ J., STANKOVIĆ S., KRNJAJA V., KOVAČEVIĆ T., TANČIĆ S. (2008): Fusarium head blight and grain yield losses of Serbian wheat. Cereal Research Communications 26, Suppl. B, 513-514.
METCALFE R.J., SHAW M.W., RUSSEL P.E. (2000): The effect of dose and mobility on the strenght of selection for DMI fungicide resistance in inoculated field experiments. Plant Pathology, 49, 546-557.
NAKAJIMA T. (2007a): Making evidence-based good agricultural practice for the reduction of mycotoxin contamination in cereals. Food and Fertilizer Technology Center, Annual Report, 05-17, 111-120.
NAKAJIMA T. (2007b): Progress and outlook for the control of nivalenol and deoxynivalenol contamination due to Fusarium head blight in wheat. Mycotoxins, 57, 2, 129-134.
PARRY D.W., JENKINSON P., McLEOD L. (1995): Fusarium ear blight (scab) in small grain cereals - a review. Plant Pathology, 44, 207-238.
OFFICIAL GAZETTE OF RS (2011): Regulation on amendment of Regulation on the maximum allowed residue levels of pesticides in food and animal feed for which maximum allowed residue quantities of plant protection products. No. 28/11.
STANKOVIĆ S., LEVIĆ J., IVANOVIĆ D., KRNJAJA V., STANKOVIĆ G., TANČIĆ S. (2012): Fumonisin B1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. Food Control 23, 384-388.
STATISTICAL YEARBOOK OF SERBIA (2012): Statistical Office of the Republic of Serbia, Belgrade, pp. 410.
WACHOWSKA U., MIKOłAJCZYK W., KUCHARSKA K. (2012): The effectiveness of tebuconazole and thiophanate-methyl in reducing the *Fusarium* pathogens development on winter wheat heads. Progress in Plant Protection, 52, 3, 671-675.
WATANABE T. (1994): Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C. pp. 410.
WEGULO S.N. (2012): Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins, 4, 1157-1180.
WEGULO S.N., BOCKUS W.W., HERNANDEZ NOPSA J.F., PEIRIS K.H.S., DOWELL F.E. (2013): Integration of fungicide application and cultivar resistance to manage Fusarium head blight in wheat. Chapter 2. DOI: 10.5772/53096

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