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THE CONTENT OF DEOXYNIVALENOL AND ZEARALENONE IN CERTAIN PARTS OF *FUSARIUM* INFECTED WHEAT HEADS

ABSTRACT: During the year 2006, climatic conditions were favourable for the appearance of head blight in the majority of localities in which wheat was grown in our country. In the locality of Apatin, in certain plots, the amount of detected infection was up to 25 infested heads per m². During the harvest, heads with distinct disease symptoms and sporulation of *Fusarium graminearum* fungi were gathered. Grains from the parts of heads with manifested disease symptoms were separated into separate samples, together with the grains above and below the infested head part. Apart from ocular evaluation, the percentage of grain infestation by *Fusarium* genus fungi was determined in all three sample categories, using wet chamber method. Deoxynivalenol (DON) was determined in the samples after extraction, using acetonitrile-water (84:16, v/v) solution. Quantitative amount of DON was determined using liquid chromatography with DAD detector at 220 nm. The content of DON in the samples was as follows: grains with manifested disease symptoms 353,4 ppm (µg/g), grains above the infested head part 0,225 ppm (µg/g), grains below the infested part 0,125 ppm (µg/g). The content of zearalenone in the samples was determined using thin layer chromatography method. This toxic agent was determined only in the samples taken from the head part in which disease symptoms were clearly manifested in the amount of 2,1 ppm (µg/g).

KEY WORDS: deoxynivalenol, *Fusarium* head blight, *Fusarium graminearum*, zearalenone

INTRODUCTION

Wheat heads, in our agroecological conditions, are often exposed to the *Fusarium* genus fungi infection which causes *Fusarium* head blight. Apart from inevitable decrease in yield, mycotoxins, that are almost always produced by these fungi in the infested grains, represent the greatest danger for humans and animals. Apart from *Fusarium* genus, also developed on the grain, there

are numerous saprophytic fungi from the genera of *Alternaria*, *Mucor*, *Bipolaris*, *Epicoccum*, *Cladosporium*, *Penicillium*, *Stemphylium* etc., which, by their enzymatic activity, destroy proteins and in great degree decrease the technological quality of the product (Clear and Patrick, 1993; Šarić et al., 1997; Korona et al., 1995; Csosz, 2002; Bagi et al., 2004; Bagi et al., 2005; Balaž et al., 2006).

Among fungi which are regularly isolated from wheat seeds, it is well known that toxins are produced by species belonging to genera of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Mucor*, *Rhizopus*, *Streptomyces* etc. Among toxins produced by *Fusarium* genus species, the most frequent and the most important ones, in a great number of agricultural products, are deoxynivalenol (DON) and zearalenone (ZEA) toxins, which are known to cause serious health problems to people and animals (Peraica et al., 1999; Schnerr et al., 2002; Sundstol Eriksen, 2003).

The aim of this work was to determine the presence and concentration of toxins in the infested grains taken from the head part with clearly manifested *Fusarium* head blight symptoms, as well as in grains from the head parts above and below the manifested symptoms. In the part of an intensively infested head, to a greater or lesser extent, shrunk grains were formed which are, together with chaff, partially removed during the harvest. However, from the point of view of food safety, it is extremely important whether DON or ZEA are accumulated in other parts of the head, where grains are formed, and which are then combine harvested and transferred into food.

MATERIAL AND METHODS

The intensity of *Fusarium* head blight infestation, during the vegetative period of 2005/06, was monitored in the locality of Apatin on Renesansa variety. Meteorological factors are represented in Table 1, according to decades, in the period from March to July, when they could affect the intensity of the occurrence of this disease. Crops preceding wheat on the plot were: sugar beet in 2002, wheat in 2003, corn in 2004, sunflower in 2005.

Tab. 1 — Meteorological factors in the locality of Sombor (from April to June 2006)

Month	Decade	Temperature (°C)	The amount of precipitation (mm)
March	I	2.0	6.2
	II	2.5	22.4
	III	10.6	22.5
Average		5.2	51.1
April	I	10.4	32.5
	II	12.0	74.5
	III	15.9	0.0
Average		12.8	107
May	I	13.2	10.9
	II	17.5	7.8
	III	18.0	35.4

Average		16.2	54.1
June	I	13.1	73.2
	II	21.7	13.4
	III	25.3	14.0
Average		20.0	100.6
July	I	22.0	22.8
	II	23.2	2.8
	III	25.7	24.9
Average		23.7	50.5

The evaluation of the intensity of head infestation was done in June 20, 2006, in the pheno-phase of early waxy ripeness (scale BBCH 7.77), when the average number of infested heads was determined on the sample of 600 heads/m², at four randomly chosen places on the plot.

Heads with symptoms of *Fusarium* head blight were gathered during the wheat harvest (July 10, 2006). Determination of *Fusarium* head blight causal agent was conducted using method by Burgess et al. (1988). In the greatest number of cases, infection by the fungus was determined in the mid-plant parts of the head. By separating the infested part from the non-infested upper and lower head parts, three grain categories were formed in order to investigate the presence and content of mycotoxins. Apart from ocular separation, seed infestation was also determined using the method of incubation in moist chamber (Pitt and Hocking, 1985).

The content of deoxynivalenol (DON) in grains from different parts of ear, was determined by method of liquid chromatography (HPLC). The samples were ground, homogenized, and 25.0 g of the sample were extracted with 100 cm³ of CAN-water (84:16, v/v). six cm³ of crude extract was cleaned up on CACC column (activated charcoal — alumina — Celite — cation exchange resin). The cleaned up extract was evaporated to dryness, dissolved in 3 cm³ of ethyl acetate, and quantitatively transferred to an evaporation vessel by triple washing with 1.5 cm³ the ethyl acetate (Jajić, 2004). The eluate was evaporated to dryness only. The purified, evaporated residue was redissolved in 300 µl of methanol, and a 15 µl of aliquot solution was injected into the LC system under the following chromatographic conditions: mobile phase, a mixture of solvents ACN-water (16:84, v/v), λ = 220 nm, flowrate 0.6 cm³/min. Calibration curves used for quantitative determination were constructed on the basis of the area under DON chromatographic peaks, using standard working solutions.

The content of zearalenone was determined by thin layer chromatography (TLC) method. Extraction and purification were performed according to Balzer et al. (1978) method. Evaporated residue was redissolved in 100 µl of chloroform. 10, 25 and 50 µl of extract were applied to the plate using a micropipette, along with 10, 15, 25 and 50 µl of standard zearalenone solution (C = 5 µg/cm³). Quantitative determination was based on the comparison of fluorescence intensity of sample spots and standard solution spots.

RESULTS

The average intensity of *Fusarium* head blight infestation in the locality of Apatin was 24,25% (Table 2), which is considerably more than the intensity of infection in average years (B a g i, 1999).

Tab. 2 — The intensity of *Fusarium* head blight infestation in the locality of Apatin in 2006

	Number of infested heads/m ² Replication				
	I	II	III	IV	X
Infestation intensity	25	26	27	19	24,25

Based on morphological and breeding characteristics of isolates from the infested heads on PDA and CLA substrates, *Fusarium graminearum* fungus was determined. Grain infestation, according to the investigated categories, was determined using the wet chamber method (Table 3).

Tab. 3 — The infestation of grains taken from different head parts

Sample	The percentage of <i>F. graminearum</i> infestation (%)
Grains from the head part with manifested disease symptoms	100
Grains from the head part above the infested spot	5
Grains from the head part below the infested spot	2

The content of deoxynivalenol in the samples was as follows: grains with manifested disease symptoms 353,4 ppm ($\mu\text{g/g}$), grains above the infested head part 0,225 ppm ($\mu\text{g/g}$), grains below the infested head part 0,125 ppm ($\mu\text{g/g}$). The presence of zearalenone was determined in the grain samples from the head part in which symptoms were clearly manifested in the amount of 2,1 ppm ($\mu\text{g/g}$).

DISCUSSION

Mycotoxins in groceries represent serious threat to human and animal health (H a r i s et al., 1999; Š k r i n j a r et al., 2005). The types and quantities of toxins formed in the cereal grains infested by *Fusarium* genus fungi depend on the fungus type and isolate, the time when the infestation appeared, grain crops genotype, as well as on the environmental conditions, above all temperature and humidity (P e r k o w s k i et al., 1995; B o č a r o v - S t a n č i ć, A. 1996; B a g i et al., 2000; P a u l et al., 2005). Among numerous toxins, extremely important are the ones belonging to the group of trichothecene, which include DON as well as zearalenone. Correlation was determined between the formation of DON toxin and the degree of pathogenicity of *F. graminearum* isolate, which indicates the role of DON in the isolate virulence (H a r r i s et al., 1999; G o s w a m i and K i s t l e r, 2005). It is considered

that zearalenone is an estrogenic toxin which affects the formation of fruiting stadium of *F. graminearum* (H o m d o r k et al., 2000).

The length of the period over which *Fusarium* genus fungi could infect heads, depends predominantly on climatic conditions during sensitive wheat phenophase, which lasts for about ten days, starting from the beginning of blooming to the grain formation (B a l a ž, 1987). In spikelets infected at the moment of blooming, grains are not formed, and in spikelets in which at the moment of infestation grain was developed to a certain degree, shrunk, poorly filled grains appear. Shrunk grains also appear in case that fungus infests head spindle, in the head part above the manifested spot. Most of the shrunk grains are removed, together with chaff and grain clippings during combine harvest, which also depends on the combine type and adjustment i.e. on the strength of air current that separates lighter head parts from the wheat grains.

To sum up, toxins transferred into groceries on the one hand, originate from those head parts in which poorly filled grains, are formed and which are infected by *Fusarium* genus fungi, not removed during combine harvest, and on the other, the quantity of toxins depends on the conditions of wheat storage, since in inadequate conditions toxic fungi are spread to noninfested grains, i.e. fungi activity in already infested grains produces mycotoxins. According to the obtained results, the quantity of DON and ZEA toxins is the greatest in grains from the head part, with manifested symptoms of *Fusarium* head blight, and in which 100% grain infestation was determined, which matches the results of other authors according to whom great positive correlation was determined between fungus biomass in the infested grains and the quantity of DON (S n i j d e r s and P e r k o w s k i, 1990). DON toxin in non-infested parts (above and below the infested head part) is not created, i.e. it does not move with the movement of plant assimilates. Minimal quantities of DON determined in these grains are the result of the presence of a few percents of infested grains, which could be determined only by incubation on moist blotter. The obtained data lead to the conclusion that mycotoxins are not transferrable from the head parts containing high quantity of fungi and toxin biomass into other head parts, i.e. into grains. They also point to the liability of conclusions made by Z h o u et al. (2002), who recommend the choice of favourable type of resistance against the spread of parasite within plant tissue, with the aim of preventing DON accumulation in grain crops. By preventing the growth of hyphae within the plant, the creation of toxins in the infested tissue is localised.

Based on these results, it can be concluded that in the measures with the aim of preventing toxins from appearing in groceries, apart from the measures for preventing the head infestation to develop (soil cultivation, fertilisation, crop rotation, chemical protection, resistant genotypes), and optimal storage conditions (humidity, temperature, the presence of insects etc.), measures of combine adjustments could also be included, which enable the removal of substantial part of infested grains, together with chaff which could drastically reduce the danger of mycotoxins to become part of nutrition chain. More precise role of the appropriate combine adjustment in removing infested grains should be investigated more thoroughly in the future, and included as an important measure in avoiding mycotoxicosis in humans and animals.

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САДРЖАЈ ДЕОКСИНИВАЛЕНОЛА И ЗЕАРАЛЕНОНА У ПОЈЕДИНИМ ДЕЛОВИМА ФУЗАРИОЗНИХ КЛАСОВА ПШЕНИЦЕ

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Резиме

Током 2006. године климатски услови су били повољни за појаву фузариозе класа пшенице у већини локалитета гајења ове културе у нашој земљи. У локалитету Апатин на појединим парцелама констатована је зараза и до 25 заражених класова по m². За време жетве прикупљени су класови са јасним симптомима бо-

лести и спорулације гљиве *Fusarium graminearum*. Из класова су у посебне узорке одвојена зрна из дела са симптомима обољења, као и зрна изнад и испод захваћеног дела класа. Поред окуларне оцене, проценат захваћености зрна гљивама из рода *Fusarium* је одређен у све три категорије узорака, методом на филтер папир-у. У узорцима одређен је деоксиниваленол (ДОН) након екстракције са смешом ацетонитрил-вода (84:16, v/v). Квантитативни садржај ДОН-а је одређен течном хроматографијом са ДАД детектором на 220 nm. Садржај ДОН-а је у узорцима био следећи: зрна са испољеним симптомима обољења 353,4 ppm ($\mu\text{g/g}$), зрна изнад захваћеног дела класа 0,225 ppm ($\mu\text{g/g}$), зрна испод захваћеног дела 0,125 ppm ($\mu\text{g/g}$). Садржај зеараленона у узорцима одређен је методом танкослојне хроматографије, при чему је овај токсин утврђен само у узорцима из дела класа на којем су се јасно испољавали симптоми и то у количини од 2,1 ppm ($\mu\text{g/g}$).