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FUNGAL INFECTION AND OCCURENCE OF ZEARALENONE IN BARLEY HARVESTED IN 2003 IN SERBIA

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ABSTRACT

Mycological investigation in 11 winter barley samples was performed. All of barley samples were infected with moulds. The ratio of infected kernels varied from 11.7% (sample SSK8) to 13.0% (sample SSK9) after 14 days of incubation. The fungi were isolated from kernels and they were classified into 6 genera and 11 species. Genus *Fusarium* was presented with the highest number of species (5). *F.poae*, a toxigenic species of the genus *Fusarium*, was present in all samples tested. The highest ratio (about 66%) of all mycopopulations belonged to genus *Alternaria* spp. All barley samples contained zearalenone, but the concentration was low (from 5.2 to 52.0 μg kg^{-1}).

Key words: animal nutrition / feed mixtures / barley / fungal infection / zearalenone / Serbia and Montenegro

OKUŽBE Z GLIVAMI IN POJAV ZERALENONA V VZORCIH JEČMENA, POŽETIH V LETU 2003 V SRBIJI

IZVLEČEK

Preiskali smo 11 vzorcev ječmena na prisotnost plesni in vrsto plesni. Vsi vzorci ječmena so bili okuženi s plesnijo. Delež okuženih zrn se je po 14 dnevih inkubacije gibal od 11,7 % (vzorec SSK8) do 13,0 % (vzorec SSK9). Iz teh zrn smo glive izolirali in razvrstili v 6 rodov in 11 vrst. Rod *Fusarium* je bil zastopan v največjem številu različnih vrst (5). *F.poae*, toksično vrsto rodu *Fusarium*, smo našli v vseh testiranih vzorcih. Največji delež (okrog 66 %) vseh populacij plesni je pripadal rodu *Alternaria* spp. Vsi vzorci ječmena so vsebovali zearalenone, vendar so bile koncentracije majhne (od 5,2 do 52,0 μg kg^{-1}).

Ključne besede: prehrana živali / krmne mešanice / ječmen / glivice / okužba / zearalenon / Srbija in Črna gora

INTRODUCTION

Barley (*Hordeum sativum*) is a sort of grain (fam. *Gramineae*) which is used as a component of feed mixture.

Barley kernels can be attacked by microorganisms during vegetative period. But, more often microbial infection can occure during the storage of cereals.

A very important role in micropopulations of cereals, including barley, have fungi. As it is known, many of them are producers of varios toxic metabolites, such as aflatoxins, ochratoxins, trichothecenes, zearalenone and others (Marasas *et al*., 1984; Samson and van Reenen Hoekstra, 1988; Pittet, 1998; Stojanović *et al*., 2003; Kocić-Tanackov and Škrinjar, 2004).

In our country ochratoxin A and zearalenone are quite frequent contaminants of different types of agricultural commodities (Bočarov-Stančić *et al*., 1997; Škrinjar *et al*., 2002, 2003). The contaminations is influenced primarily by climatic conditions (relative humidity and

temperature), soil moisture, insect damage, mineral nutrition deficiencies and other factors. But, there is no doubt that the highest concentrations of toxins are associated with the postharvest fungal growth on poorly stored agricultural products.

Zearalenone, known as F-2 toxin is one of the main fungal toxin detected in our country. It is an estrogenic toxic metabolite produced by different species of genus *Fusarium* (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* etc.), which frequently colonize cereal crops, especially during the storage.

Despite its biological effects, the presence of zearalenone in feeds has long been a problem in animal husbandry, notably by causing infertility and related disorders particularly in swine and sheep (Pittet, 1998).

The natural occurrence of zearalenone in a variety of agricultural commodities (wheat, barley, corn) has been reviewed in extensive detail by many investigators (Marasas *et al*., 1984; Kuiper-Goodman *et al*., 1987; Pittet, 1998; Škrinjar *et al*., 2002, 2003).

The aim of this study was to investigate a fungal infection of barley kernels during the storage and the presence of zearalenone in them, as well.

MATERIAL AND METHODS

Mycological investigation (total viable count of moulds, identification of mould species) in several winter barley samples (11) was performed. All samples were analyzed on the presence of zearalenone, too.

Determination of total viable count of moulds per one kernel was done as follows. Sample (100 kernels) were immersed in a 100% of 0.4% of sodium chypochlorite and shaked on shaker for 2 min. After that, chlorine solution was decanted and the sample rinsed with sterile distilled water (2×100 ml). Under septic conditions kernels were placed on Petri dishes (ϕ13 cm) containing sterile filter paper (25 kernels/Petri dish) and 10 ml of sterile distilled water. Incubation was performed 14 days at 25 °C.

Identification of isolated fungi was carried out according to Nelson *et al*. (1983) and Samson and van Reenen-Hoekstra (1988).

Qualitative and quantitative determination of zearalenone was examined by using the fluorometric method (Fluorometric "Vicam" series 4), Zearala Test^{TM}.

RESULTS AND DISCUSSION

Mycological investigation

As can be seen from Table 1, between 71 (sample SSK9) and 89 barley kernels (sample SSK1) was infected with moulds already after 7 days of incubation. It was found that infection degree increased about 20% during further incubation time in some cases. After 14 days total viable count of moulds per sample (100 kernels) arranged from 115 (sample SSK2) to 130 (sample SSK9).

Numerous fungi were isolated from barley samples. They were classified into 6 genera (*Alternaria, Aspergillus, Fusarium, Rhizopus, Scopulariopsis* and *Ulocladium*) and 11 species (Table 2).

Genus *Fusarium* was present with the higest number (5) of different species (*F. acuminatum, F. avenaceum, F. culmorum, F. poae, F. sporotrichioides*). All of these species are known as producers of various toxic metabolites (Nelson *et al*., 1983). Except *F. acuminatum*, all other *Fusarium* species, isolated in these experiments are producers of zearalenone.

Table 1. Number of infected kernels and total viable count of moulds (per sample/ 100 kernels)

Sample-Sign	No. of infected kernels after		Total count of moulds after	
	7 days	14 days	7 days	14 days
SSK1	89	94	117	123
SSK2	82	91	101	115
SSK3	80	90	107	125
SSK4	87	92	112	120
SSK5	78	89	104	118
SSK6	73	87	102	121
SSK7	84	92	113	121
SSK8	79	95	88	117
SSK9	71	92	79	130
SSK10	83	97	94	123
SSK12	76	96	84	125

Table 2. Mould species isolated from barley

Genus	Species
Alternaria	*alternata* (Fr.) Keissler
	brassicicola (Schw.) Wiltshire
Aspergillus	*niger* van Tieghem
Fusarium	*acuminatum* Ellis & Everhart
	avenaceum (Fr.) Sacc.
	culmorum (W. G. Smith) Sacc.
	poae (Peck) Wollenweber
	sporotrichioides Sherb.
Rhizopus	*stolonifer* (Ehrenb.) Lind.
Scopulariopsis	*fusca* Zach
Ulocladium	*chartarum* (Preuss) Simmons

It was determined that a share of *Fusarium* spp. in mycopopulation varried from 11% (sample SSK10) to 30% (sample SSK6) (Fig. 1). *F. poae* was the most frequent species of genus *Fusarium*. It was isolated from all samples tested (Fig. 2). About 27% of barley samples were infected with *F. acuminatum* and *F. avenaceum* and about 9% with *F. culmorum* and *F. sporotrichioides*.

It is necessary to point out, having in mind total mycopopulations isolated from barley (1100 kernels), that the highest incidence of fungal infection was observed with *Alternaria* species (Fig. 3). Genus *Alternaria* was presented by two species, *A. alternata* and *A. brassicicola*. As it was established in our earlier investigations (Škrinjar *et al*., 1997). *A. alternata* was extremly frequent. Naimely, all samples were infected by it (Table 3).

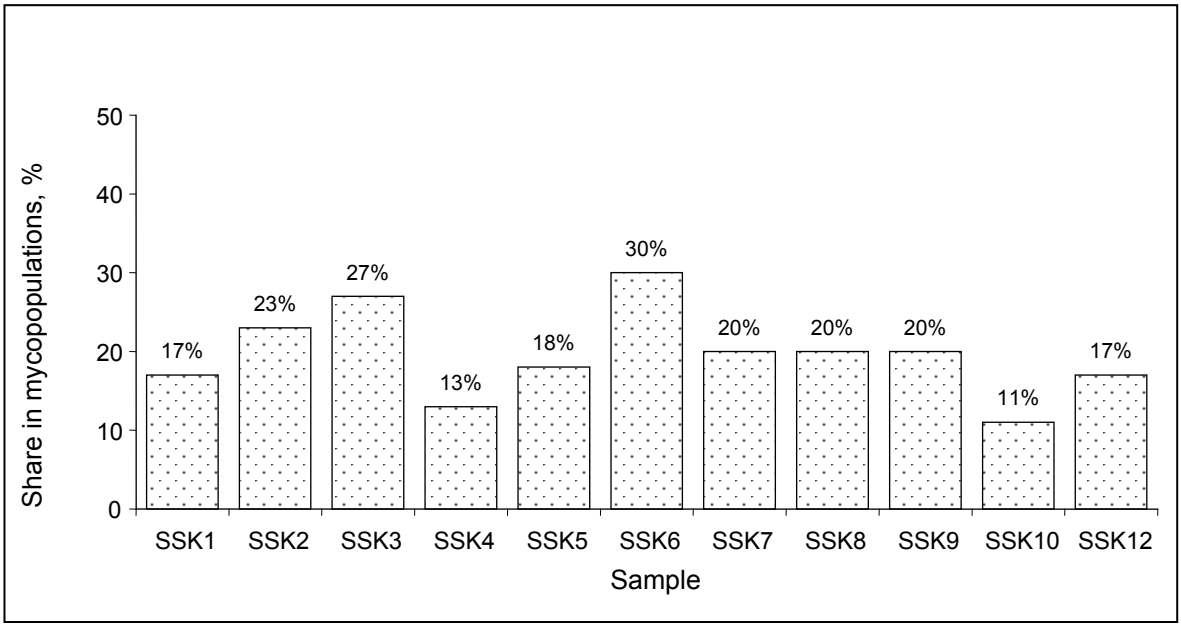

Figure 1. Share of *Fusarium* spp. in mycopopulations isolated from barley samples.

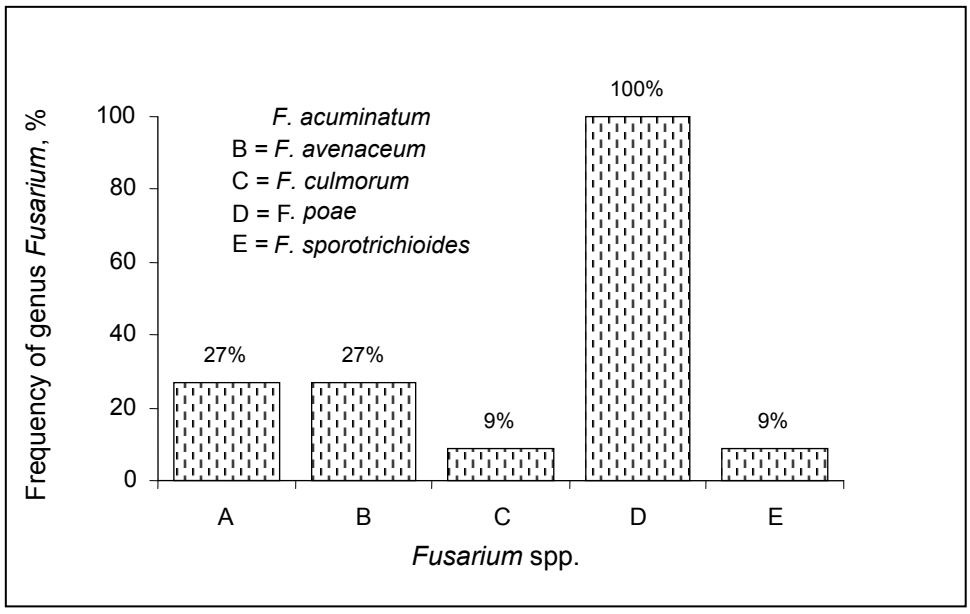

Figure 2. Frequency of *Fusarium* spp. in total mycopopulations of genus *Fusarium*.

Zearalenone

It was found that all barley samples were contamited with zearalenone at concentrations from 5.2 to 52.0 μg kg^{-1} (Table 4). In spite of the fact that concentrations were low, so frequent occurrence of zearalenone is underisable because of its biological effects.

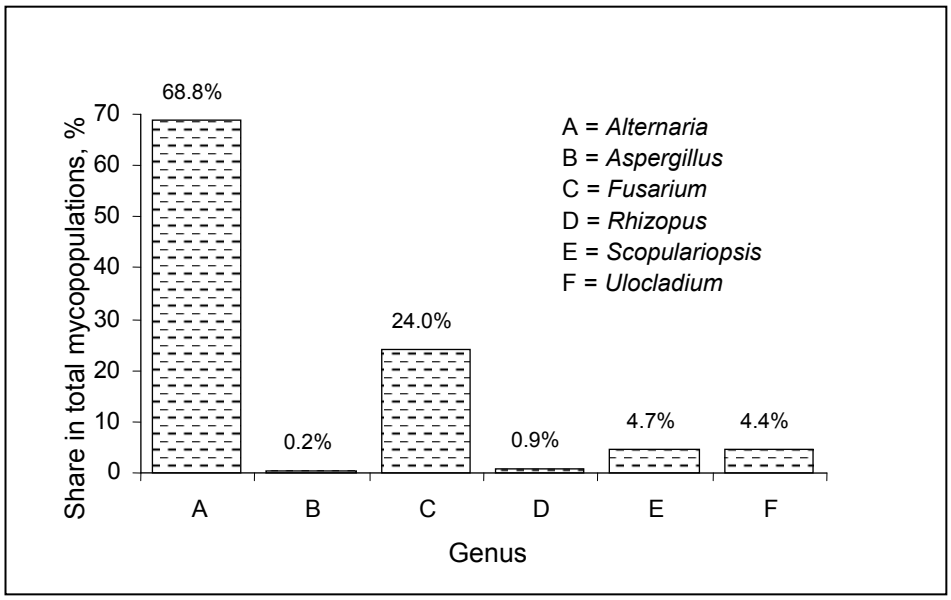

Figure 3. Share of fungal genera in total mycopopulations.

Table 3. Mycopopulations isolated from barley samples

Sample- Sign	Mould species
SSK1	*Alternaria alternata* *Fusarium avenaceum* *F. culmorum* *F. poae* *Mycelia sterilia* *Rhizopus stolonifer*
SSK2	*Alternaria alternata* *Fusarium poae* *Mycelia sterilia*
SSK3	*Alternaria alternata* *Fusarium poae* *Mycelia sterilia*
SSK4	*Alternaria alternata* *Fusarium acuminatum* *F. culmorum* *F. poae* *Mycelia sterilia* *Ulocladium chartarum*
SSK5	*Alternaria alternata* *A. brassicicola* *Fusarium acuminatum* *F. poae* *Mycelia sterilia*
SSK6	*Alternaria alternata* *Fusarium acuminatum* *F. poae* *Mycelia sterilia*
SSK7	*Alternaria alternata* *Fusarium poae* *Mycelia sterilia* *Scopulariopsis fusca*
SSK8	*Alternaria alternata* *A. brassicicola* *Fusarium poae* *Mycelia sterilia* *Rhizopus stolonifer* *Ulocladium*
SSK9	*Alternaria alternata* *Fusarium poae* *Mycelia sterilia* *Scopulariopsis fusca* *Ulocladium*
SSK10	*Alternaria alternata* *Aspergillus niger* *Fusarium poae* *Mycelia sterilia*
SSK12	*Alternaria alternata* *A. brassicicola* *Fusarium avenaceum* *F. poae* *F. sporotrichioides*

Table 4. Presence of zearalenone in barley samples

Sample – Sign	Zearalenone $\mu g \cdot kg^{-1}$
SSK1	22.0
SSK2	5.2
SSK3	8.6
SSK4	5.8
SSK5	7.1
SSK6	8.2
SSK7	52.0
SSK8	24.0
SSK9	37.0
SSK10	19.0
SSK12	16.0

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