

THE INCIDENCE OF THE STRAINS OF *FUSARIUM* SP. AND OF ZEARALENONE IN CEREALS ANALYZED FROM THE SOUTH WEST OF ROMANIA

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ABSTRACT

*In this paper, the authors presents the results of their research made in south west of Romania to evidence the level of foodstuffs and feedstuffs contamination with *Fusarium* sp. and zearalenone. The authors considers that this type of studies is very important because the foodstuffs and feedstuffs that were infected with different fungi could be contaminated with mycotoxins and these products could produce different affections to the people and to the animals. This is the reason because the contaminated food and feed must be eliminated of the people and animal consumption, if exceeds the maximum levels imposed by legislation. The results are synoptically presented in tables, comparatively with other author's results.*

KEY WORDS: *Fusarium, zearalenone, mycotoxins, oestrogenic*

INTRODUCTION

25% of the global crop is contaminated with mycotoxins (Kanora & Maes, 2009). Mycotoxins are toxic fungal metabolites can be classified into either field or storage fungi (FAO, 1997; Antonissen *et al*, 2014). The alteration of cereals with fungi and mycotoxins cause severe agro-economic damage all over the world and their consumption leads to impaired health of humans and animals. *Fusarium* is an ubiquitous fungi with several species involved as important pathogens of cereal crops. The most important *Fusarium* mycotoxins are fumonisins, zearalenone (ZEA or ZON) and trichothecenes (deoxynivalenol, nivalenol and T-2 toxin) (Filimon *et al*, 2012; Antonissen *et al*, 2014). The IARC has classified the toxins derived from *Fusarium* as possibly carcinogenic to humans (IARC, 1993). Zearalenone (a phenolic resorecyclic acid lactone) is a natural contaminant of grains infested with different species of *Fusarium* sp. The most common species of *Fusarium* incriminated in the development

of toxins are: *F.roseum* var. *graminearum*, *F. moniliforme*, *F. equiseti*, *F. sporotrichoides*, *F. tricinctum*, *F. oxysporum*, *F. culmorum* and *F. moniliforme*. The growth and mycotoxins production of such moulds are influenced very much by the eco-physiological factors, mainly the temperature and water activity of the attacked crops and products. *Fusarium* spp. are more adapted to the climate of North America and Europe (Mirocha *et al*, 1977; EFSA, 2011).

Zearalenone has been isolated for the first time in 1962 by Stob and collaborators, from a species of *Fusarium graminearum*, isolated from contaminated corn, having an humidity of 45% and a temperature of 12°C (EFSA, 2011). Urry *et al.* (1966) and Kuo *et al.* (1967) identified this compound as zearalenone. It appears that at 25°C, the fungus does not produce the toxin, but grows abundantly and at a humidity decreased under 14% it does not invade cereals. The presence of zearalenone in animal feed has been associated with oestrogen syndrome in mammals and birds (Driehuis *et al*, 2008). Zearalenone is classified as a mycotoxin with predominantly oestrogenic action. On sows before puberty, ZEA produces decreased fertility, an edema of the entire genital tract with the doubling the intestine weight and atrophy of the ovaries, uterine prolapse and rectal prolapse. Exposure to *Fusarium* can generally influence human and animal susceptibility to infectious diseases and allergy (Ianovici, 2008; Ianovici & Tudorică, 2009; Marin *et al*, 2010; EFSA, 2011).

Fusarium mycotoxins are capable of inducing both acute and chronic toxic effects on humans (Ehling *et al*, 1997; Ueno *et al*, 1983; Ianovici *et al*, 2011). The presence of these estrogenic substances may have caused the development of breasts of children (before age 8) in Italy (Fara *et al.*, 1979), and premature thelarke, pubarche, gynecomastia, and precocious pseudopuberty in Puerto Rico (Hayes, 1994).

It has been experimentally proved that zearalenone administered to animals is eliminated by renal diuresis in a proportion of 40-60% after 10-24 hours and the rest is metabolized. The liver produces the biotransformation of zearalenone in its derivatives (α and β zearalenol, α and β zearalanol), which can be eliminated through milk as well (Zinedine *et al*, 2007; Smith & Moss, 1985; EFSA, 2011). The elimination of zearalenone derivatives explains the estrogenic syndrome in very young gilts. Pigs are very susceptible to zearalenone (Kanora & Maes, 2009). Palynšik and collaborators, using feed contaminated with different species of *Fusarium* in goose feed, noticed a reduction of the amount of semen and azoospermia (Mirocha & Christensen, 1974). The use of α -zearalanol for growth promotion in food animals was banned in the European Union (EU) in 1985.

Roine and collaborators (1971) noticed that furages contaminated with *Fusarium* as well as with ZEA, in a concentration of 25 ppm, determined at cattle reproductive disorders, characterized clinically by vulvovaginitis and infertility.

Micotoxic oestrogenic syndrome has been described at cattle, in our contry by Grigore and collaborators. They found that moldy feed produced serious breeding

disorders: abortion, placental retentions, sexual cycle disorders (false oestrus, repeated vulvovaginal swelling, vaginal prolapse). These changes were observed in females before puberty (cattle of 3-12 months). In the feed there were found *Fusarium*, *Aspergillus* and *Penicillium* (Coman & Popescu, 1985).

ZEN determinates intoxications in many other animal species as: bovines (Diekman & Green, 1992), lambs (Hufstedler *et al.*, 1996), meat poultry (Swamy *et al.*, 2002), horses (Minervini *et al.*, 2006), lab rodents (Yang *et al.*, 2006). Zearalenone has been detected as well in beer, beans, peanuts, bananas and soy (Gilbert, 1989; Kuiper-Goodman *et al.*, 1987).

MATERIALS AND METHOD

Mycological examination consists of two parts: the determination of the total mycological load and the determination of mycological spectrum – taxonomic classification of isolated fungus. The determination of the total number of molds (NTM/g) is made by seeding on special culture media of serial dilutions (decimals) from the sample and reading the plates after 2-10 days.

Highlighting the potential toxigenic of fungi was performed by a relatively simple test: irradiating cultures with ultraviolet (UV), with a wavelength of 254-366 nm, after 3-15 days of culture. The observation of typical fluorescent colonies or the environment around them, following UV irradiation, indicates a potentially toxigenic strain (Șchiop *et al.*, 1996).

The frequency of toxigenic strains vary from species to species. Various studies appreciate the frequency from 1/2 to 1/100; the simple determination of fungal species known in the literature as toxigenic does not constitute a argument to assert that feed or food analyzed from a mycological point of view does not correspond for consumption (Kuiper-Goodman *et al.*, 1987). It must be shown that the isolated strain is toxigenic. Also, to establish feed or food sanitation, it is necessary to consider that mycotoxins toxigenic strains develop only under certain environmental conditions. There are apparently paradoxical phenomenon, namely: products do not contain viable fungi but contain mycotoxins. This phenomena is due to the processing methods or food storage (Smith & Moss, 1985).

The determination of the total number of molds is made by seeding of serial dilutions (decimals) from the sample on specific culture media – agar Sabouraud. The samples analyzed were from batches of products suspected of being altered. The mycological spectrum was determined through microscopy, using determinants. Potential toxigenic strains and cultures were determined by UV irradiation. The toxigenic strains were determined by cultivating the isolated species on appropriate synthetic or natural medium (rye, maize) followed by the extraction of the micotoxins and their identification using enzyme-linked immunoassay ELISA. The determination

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of micotoxins from foodstuffs and feedstuffs harvested from the south west of Romania was made using the enzyme-linked immunosorbent assay ELISA.

RESULTS AND DISCUSSIONS

When the humidity of of the substrate is increased, between 22-30% and with temperatures between 24-27⁰C, *Fusarium* spp. develops in a time of 3 to 6 weeks and shall develop ZEA. The development of this mycotoxin is favored by lowered temperatures of 10-14⁰C. *Fusarium* develops on incompletely ripened grains or high humidity. The climatic conditions from our country, especially in the autumn, with warm days and chilly nights, ensure a good development of the *Fusarium* (Șchiop *et al.*, 1996). Fusariosis can affect in some regions, 5-10% from the wheat or corn crop. Maize varieties that do not reach maturity are affected (Resnik *et al.*, 1996; Smith & Moss, 1985). The following tables illustrates the degree of contamination by fungi belonging to the genus *Fusarium* and zearalenone cereals analyzed.

TABLE 1. The incidence of fungus of the genus *Fusarium* and ZEA in wheat samples from impaired quality batches.

County	Number of samples	Positive for <i>Fusarium</i>		Positive for ZEA		Readed value (μg/kg)		
		No.	%	No.	%	min	mean	max
Arad	72	22	30,5	4	5,5	25	45	75
Caraș-Severin	50	20	20,0	2	4,0	35	-	65
Mehedinți	50	8	16,0	1	2,0	-	-	60
Hunedoara	66	25	37,9	6	9,7	28	45	65
Timiș	98	20	20,4	4	4,0	17	35	80

TABLE 2. The incidence of fungus of the genus *Fusarium* and ZEA in corn samples from impaired quality batches.

County	Number of samples	Positive for <i>Fusarium</i>		Positive for ZEA		Readed value (μg/kg)		
		No.	%	No.	%	min	mean	max
Arad	86	31	36,0	6	6,9	45	75	90
Caraș-Severin	54	12	22,2	4	7,4	40	80	120
Mehedinți	48	15	31,2	2	4,1	75	-	155
Hunedoara	82	30	36,5	8	9,7	35	55	75
Timiș	120	28	23,3	7	5,8	25	60	135

TABLE 3. The incidence of fungus of the genus *Fusarium* and ZEA on combined feed samples from impaired quality batches.

County	Number of samples	Positive for <i>Fusarium</i>		Positive for ZEA		Readed value (μg/kg)		
		No.	%	No.	%	min	mean	max
Arad	53	24	45,2	11	20,7	35	60	85
Caraș-Severin	44	18	40,9	5	11,3	50	75	125
Mehedinți	27	17	63	9	33,3	60	95	175
Hunedoara	57	19	33,3	12	21,8	25	55	95
Timiș	90	27	30	5	8,8	45	60	110

In the samples analyzed by us, the incidence of fungus belonging to the *Fusarium* genus, the potential producers of zearalenone was comprised between 16 and 37,9% in wheat samples and 22,2 up to 36.5% in corn samples, while the incidence of contamination with zearalenone was from 2 to 9,7% in wheat samples and between 4,1 and 9,7% in corn samples. In combined forages for swine, the incidence of fungus from the *Fusarium* genus was between 30 and 63%, the incidence of zearalenone was from 8,8 to 33%.

The incidence of zearalenone in products of vegetable origin has been investigated by many scientists, from different countries, because the presence of mycotoxins in cereals and othe products which underlie human alimentation constitutes a public health issue. The results of these investigations is presented in Table 4.

TABLE 4. Data regardin the incidence and degree of contamination of food with zearalenone

Sample	Country	No. of samples	Incidence (%)	Dose (µg/kg)	Autors
Imported maize	United Kingdom	139	42	100 - 584	Scudamore and Patel, 2000
Cereals	Germany	84	38	2 - 67	Schollenberger et al., 2005
Maize	Italy	46	30	8 - 969	Cavaliere et al., 2005
Wheat	Germany	407	41	35 - 451	Meister, 2009
Rye	Germany	514	16	22 - 37	Meister, 2009
Wheat	Bulgaria	54	2	Mean 29,0	Manova & Mladenova, 2009
Barley	Bulgaria	18	11	Mean 10,0	Manova & Mladenova, 2009
Maize	Bulgaria	19	21	Mean 80,6	Manova & Mladenova, 2009
Maize	Romania	54	33	> 100	Tabuc et al., 2009
Wheat	Romania	35	40	> 100	Tabuc et al., 2009
Barley	Romania	21	71	> 100	Tabuc et al., 2009

Whereas the species of *Fusarium* produce a large number of toxins, zearalenone can be found in forages among other mycotoxins. After the metabolism, mycotoxins and their degradation products infest into the milk, meat and eggs (Tanaka et al, 1990). In 1988, Tanaka et al. published a paper about the occurrence of ZEA from 19 countries including Germany, Italy, Poland and UK. The paper reported the contamination of wheat, barley, maize, oat, sorghum, rye and rice by ZEA. Placinta *et al.* (1999) reported the contamination of samples of wheat, barley, oat, rye and feeds from Bulgaria, Germany, Finland, Netherlands, Norway and Poland by ZEA at levels from few lg/kg to 8 mg/kg. In Yugoslavia, ZEA was found at high levels (up to 10 mg/kg) in corn (Balzer *et al.*, 1977) and in dairy cattle feeds (Skrinjar *et al.*, 1995). Zakharova *et al.* (1995) reported a low contamination of cereal crop from Russia in 1993 and 1994 by ZEA. In Hungary, Fazekas *et al.* (1996) reported the contamination of mouldy and stored corn with ZEA that ranged between 0.01 and 11.8 mg/kg. In the Netherlands, the occurrence of ZEA was reported in wheat (Tanaka *et al.*, 1990) and in feed ingredients (Veldman *et al.*, 1992). We can observe that the incidence of zearalenone in cereals analyzed is contained in between very wide limits from 2 to 71%, the maximum value being identified in Romania.

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The our results obtained are similar with the ones obtained in the studies done on the cereal samples coming from the south-east of Romania (Tabuc *et al*, 2004, Tabuc *et al*, 2009; Tabuc *et al*, 2011) and western Romania (Stroia *et al*, 2010).

The adverse effects of mycotoxins on human and animal health are well known and that is why explicit remarks are made and the maximum permissible limits are set for these kind of contaminants in food and feed (Șchiop *et al*, 1996; Scudamore *et al*, 1997).

CONCLUSIONS

The values determined confirmed the presence of ZEA, but the values did not exceed the maxim permitted levels for each type of cereals analyzed. Comparing the results obtained by us, with similar studies performed in Romania, we obtained similar values. The incidence of zearalenone in cereals analyzed in Romania is close to the values of incidence of the mycotoxin from analyzed cereals in Germany, Italy and England.

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