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ZEARALENONE - PRODUCING ACTIVITY OF *FUSARIUM GRAMINEARUM* AND *FUSARIUM OXYSPORUM* ISOLATED FROM BULGARIAN WHEAT

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Abstract

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Several *Fusarium* species are important pathogens of cereals and corn, causing severe crop yield reduction. In addition, some isolates are able to produce mycotoxins. The most important *Fusarium* mycotoxins, which can frequently occur at biologically significant concentrations in cereals, are fumonisins, zearalenone (ZEA) and trichothecenes (deoxynivalenol, nivalenol and T-2 toxin). The aim of the current study was to establish ZEA-producing activity of some wheat *Fusarium* species (*F. graminearum* and *F. oxysporum*) originated from different geographical regions in Bulgaria. 40 wheat *Fusarium* isolates were screened for their ability to produce ZEA, by cultivating on the sterile wheat. To determine the presence of ZEA was used a monoclonal antibody-based affinity chromatography, *ZearalaTest*™ by using Fluorometer & HPLC, Series-4, VICAM®, USA. The results of the mycotoxicological analysis revealed that 22 (55%) of the wheat samples tested were contaminated with ZEA. Obtained data show also that the *F. graminearum* is among the main species producing ZEA in Bulgarian wheat. On the other hand, for the first time 7 (35%) in 20 isolates of *F. oxysporum* in Bulgarian wheat were found to synthesize ZEA.

Key words: *Fusarium graminearum*, *Fusarium oxysporum*, Zearalenone, wheat

Abbreviations: Zearalenone, ZEA

Introduction

Several *Fusarium* species are important pathogens of cereals and corn, causing severe crop yield reduction. In addition, some isolates are able to produce mycotoxins (Botallico and Perone, 2002). Mycotoxins are secondary metabolites produced by a wide variety of fungal species that cause nutritional losses and represent a significant hazard to the food chain (Denev, 2005; Scudamore, 2005; Magan and Aldred, 2007; Fink-Gremmels, 2005, 2012). Mycotoxins can cause acute intoxications under high contamination of feeds, but more important are facts of their cumulative, immunosuppressive and immunotoxic action (Kirov and Denev, 1990; Fink-Gremmels, 1999; Denev and Beev 2002; Denev, 1999, 2005; Oswald et al., 2005; Surai and Dvorska, 2005, Dospatliev and Palagacheva, 2009; Surai et al., 2010). The most important *Fusarium* mycotoxins, which can frequently occur at biologically significant concentrations in cereals, are fumonisins, zearalenone (ZEA) and trichothecenes (deoxynivalenol, nivalenol and T-2 toxin). They are common mycotoxins throughout the world, mainly associated with cereal crops, in particular corn, wheat, barley, rye, rice and oats (Goyarts et al., 2007; Valcheva and Valchev, 2007).

Zearalenone is a non-steroidal oestrogenic mycotoxin produced mainly by *F. graminearum, F. culmorum, F. cerealis, F. equiseti* and *F. semitectum* (Logrieco et al., 2003; Kumar et al., 2008). ZEA is among the most widely distributed *Fusarium* mycotoxins in agricultural commodities and is often found at relatively high concentrations, especially in corn. ZEA has both uterotrophic and estrogenic activity, and may cause reproductive disorders in farm animals, particularly swine. ZEA is responsible for recurring toxicoses in livestock, characterised by hyperestrogenism in swine, infertility and poor performance in cattle and poultry, and there is a possible impact on human health (Gromadzka et al., 2008; Metzler et al., 2010; Fink-Gremmels, 2012). The preliminary scanty evidence of the genotoxicity of ZEA, is limited to mice and cultured mammalian and human cells, and it is not classified as human carcinogen (IARC, 1993).

Three species: *F. verticillioides*, *F. graminearum* and *F. oxysporum* have been considered as the most important in Bulgarian cereals (Vrabcheva et al., 1996; Valcheva, 2003; Beev, 2004). However, in Bulgaria there is little information on the ability of above species to produce ZEA.

The aim of this study was to establish ZEA-producing activity of some wheat *Fusarium* species (*F. graminearum* and *F. oxysporum*) originated from different geographical regions of the country.

Materials and Methods

A total of 40 wheat isolates of the species *F. graminearum* (20) and *F. oxysporum* (20), originated from different geographical regions, were screened for their ability to produce ZEA by cultivating on the sterile wheat as recommended by Sherwood and Peberdy (1972), Berisford and Ayres (1976) and Popova (1984). All of used isolates were identified according to Gerlach and Nirenberg (1982).

The presence of ZEA was determined by using monoclonal antibody-based affinity chromatography, *ZearalaTest*™ with Fluorometer & HPLC, Series-4, VICAM®, USA, according to manufacturer instructions.

Results and Discussion

The results of the mycotoxicological analysis revealed that 22 (55 %) of the wheat samples tested were contaminated with zearalenone (Tables 1 and 2), at levels 84-1600 µg/kg.

Obtained data show that the *F. graminearum* is among the main species producing zearalenone in Bulgarian wheat (Table 1). Among 20 of tested isolates, 15 (75%) were toxigenic and the largest quantity of ZEA (600 µg/kg) was found to be produced by a species isolated from the region of Pleven.

A toxin production between 92-330 µg/kg was seen in three isolates from Troyan area while the other three isolates from the same area did not produce ZEA (Table 1). Isolates originated from the region of Silistra and Elhovo also were found to produce ZEA in significant quantities, respectively, 250 and 130 µg/kg. Some isolates from Stara Zagora region were referring to highly productive in terms of ZEA production (120-480 µg/kg). Cumulative amounts of ZEA at 100 µg/ kg were also found to be produced by isolates from areas of Montana, Cherven Bryag and Smyadovo, while only one of two isolates from Sofia region (Elin Pelin, Bojurishte) was toxigenic - 84 µg/kg.

Some articles revealed that *F. oxysporum* is one of the main producers of ZEA (Mirocha et al., 1977; El-Kady and El-Maraghy, 1982; Chelkowski and Manaka, 1983b) However, Bulgarian isolates (from cereals) of the same species were not capable to produce ZEA as reported by several authors (Stankushev et al., 1977, Dimitrov et al., 1982, Popova 1984). Unlike the studies cited above, for the first time 7 (35%) in total of 20 isolates of *F. oxysporum* were found to synthesize ZEA in quantities exceeding 50 µg / kg, and in 6 of them (30%) ZEA production was above 100 µg / kg (Table 2).

Results of mycotoxicological studies with isolates of *F. oxysporum* have shown that they can be considered as potentially dangerous to animal and human health (JECFA, 2001). Two isolates of *F. oxysporum* (K3, F7) possessing high toxigenic activity were isolated from the wheat originated from the Sofia region (Elin Pelin, Bojurishte), whereas the rest were from the region of Botevgrad, Varna and Silistra (Table 2). For one isolate originating from wheat produced in the region of Sliven (F93) the ZEA-producing activity was estimated at 90 µg/kg. The capacities of the other isolates to produce zearalenone were below the limit of detection.

Table 1
Toxigenic isolates of *F. graminearum* originated from different geographical areas.

Species	Isolates	Zearalenon, µg/kg	Region
F. graminearum	F 52	0	Troyan
	F 51	0	Troyan
	F 54	110	Troyan
	F 30	0	Troyan
	F 41	210	Troyan
	F 43	330	Troyan
	F 40	92	Troyan
	F 53	130	Elhovo
	F 70	250	Silistra
	T 41	131	Silistra
	S 5	480	Stara Zagora
	S 6	120	Stara Zagora
	S 7	180	Stara Zagora
	S 8	0	Stara Zagora
	T 5	280	Montana
	T 31	182	Cherven Bryag
	F 69	1600	Pleven
	T 52	84	Elin Pelin
	T 55	0	Bojurishte
	T 66	130	Smyadovo

Table 2
Toxigenic isolates of *F. oxysporum* originated from different geographical areas

Species	Isolates	Zearalenon, µg/kg	Region
F. oxysporum	K 3	150	Sofia
	F 7	120	Sofia
	F 107	0	Chirpan
	T 36	100	Pleven
	F 32	0	Pleven
	F 71	200	Botevgrad
	F 57	0	Troyan
	F 29	0	Troyan
	F 56	0	Troyan
	T 10	0	Popovo
	T 11	0	Popovo
	WA 3	0	Stara Zagora
	WB 1	0	Stara Zagora
	F 44	0	Stara Zagora
	T 42	130	Silistra
	T 75	140	Varna
	T 61	0	Shumen
	F 93	90	Sliven
	F 48	0	Kardjali
	F 108	0	Dobrich

Data analysis showed that wheat is a suitable substrate for ZEA-producing species of the genus *Fusarium*. Our studies on ZEA-producing capability of some isolates from the regions of Troyan and Stara Zagora clearly showed that their mycotoxigenic ability varied widely, between isolates belonging even to the same species. This means that contamination of wheat with these species itself is not a sufficient evidence for the presence of ZEA in it. It was found that the formation of mycotoxins in wheat depends on the stability of varieties, toxigenic potential of *Fusarium* species a biotic and biotic environmental factors etc. (Glenn, 2007). However, it should be noted that contamination of wheat and other cereals with toxigenic species of microscopic fungi is one of the important prerequisites for the formation of ZEA (Richard, 2007). This is confirmed by the results of our research in which it was found that 75 % of isolates of the species *F. graminearum* and *F. oxysporum* (35 %) cultivated on wheat are toxigenic.

Conclusions

It was found that the main producer of ZEA in the tested samples is *F. graminearum*. However, ZEA productive activity has been demonstrated in 35% of isolates of *F. oxysporum*.

More than half of the isolates were found to be toxigenic, making them extremely hazardous to health and animal productivity, the quality of the produced animal products and their safety in humans. It was confirmed that the presence of toxigenic species in cereals, is not always associated with the presence of mycotoxins in samples. On the other hand, the absence of strains producers in the samples does not guarantee their purity in terms of mycotoxins. It is therefore necessary mycotoxicological continuous monitoring of food and feed production, in view of reliable risk assessment for animal and human health.

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