Original Research Paper

Ochratoxin A and Deoxynivalenol Mycotoxin Profile in Triticale Seedlings with Different Susceptibility to the Root Rot

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Article history
Received: 02-10-2022
Revised: 10-12-2022
Accepted: 19-12-2022

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Abstract: Cereals in the southeastern part of Kazakhstan can be affected by the crown and common root rot and spot blotches mostly caused by *Bipolaris sorokiniana* and *Fusarium* spp. Triticale is a man-made crop obtained from the crossing of wheat (*Triticum* sp.) × rye (*Secale cereale* L.) and it is mainly cultivated as a feed grain for livestock in Kazakhstan. Mycotoxins are secondary metabolites of fungi secreted by the toxigenic species *Alternaria, Aspergillus, Fusarium*, and *Penicillium*. The study aimed to determine the effect of the varietal factor and the type of mycotoxin on their content in 15-day triticale seedlings. The spread and development indices of root rot were calculated and the Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) was performed to determine the concentration of mycotoxins. The content of micro-toxins depended both on their type and the triticale variety (P-value <0.001). The maximum content of mycotoxins Ochratoxin A and Deoxynivalenol in some seedlings reached 100-120 mcg/mL, on average 12.4 and 15.1 mcg/mL, respectively. In Rondo, Fidelio 5, and Valentin varieties, the mycotoxin content was 11.2; 16.9, and 8.6 mcg/mL, with a spread of 79.0-93.0%, the development of common root rot reached 30.5-34.2%, while in Idea, TI 17, Nevo and Dokuckaevsky 9, the average mycotoxin content was lower, within 1.4-3.9 mcg/mL, with a spread of 50.0-65.0% and the development of common root rot reaching 20.5-25.0%. In the triticale Alnaiskii 5 variety, these indices equaled 0.7 mcg/mL; 55.0 and 19.2%, respectively. A high positive correlation (0.8) was found between the content of mycotoxins and the infestation of triticale seedlings with root rot.

Keywords: Mycotoxin Profile, Triticale Seedling, Genotype, Ochratoxin A, Deoxynivalenol

Introduction

Cereals in the southeastern part of Kazakhstan can be affected by crown and spot blotches and common root rot mostly caused by *Bipolaris sorokiniana* and *Fusarium* spp., (Sultanova *et al*., 2021; Bozoğlu *et al*., 2022; Rysbekova and Sultanova, 2022). Among 400 species of mycotoxins, Ochratoxin A (OTA), aflatoxins Deoxynivalenol (DON), and T-2 toxins are dangerous for animals and humans (Pascari *et al*., 2019; Sun *et al*., 2019).

Ochratoxin A (OTA) is a toxic secondary metabolite and is produced by several species of *Aspergillus* and *Penicillium* fungi (Sun *et al*., 2018; 2019). People are chronically and constantly exposed to OTA due to its presence in oats, wheat, and barley (Ye *et al*., 2019). Currently, studies of this mycotoxin are aimed at developing a biotin-streptavidin-amplified enzyme-linked immunosorbent assay using a biotinylated nanobody in cereals (Sun *et al*., 2019) a sensitive and selective biotin-streptavidin-amplified enzyme-linked immunosorbent assay to detect OTA (Sun *et al*., 2018) a Polyvinylidene Fluoride (PVDF) membrane-based dot immunoassay (Tang *et al*., 2018), Fluorescence Detection (FLD)-based method for simultaneous determination (Dhanshetty and Banerjee, 2019), using silver Nanoparticles (AgNPs) in combination with reduced graphene oxide (Kunene *et al*., 2021), occurrence and safety evaluation of ochratoxin a in cereal-based baby foods (Khoshnamvand *et al*., 2019) and change of amino acid residues for mycotoxin OTA in cereals (Zhang *et al*., 2020).

DON is a mycotoxin produced in pathogens by the fungi *Fusarium graminearum* and *F. culmorum* (Femenias *et al*., 2020). Recent studies on this mycotoxin are devoted to its detection in baby food products prepared based on cereals (Herrera *et al*., 2019; Pascari *et al*., 2019; Mruczyk *et al*., 2021), the development of new methods for the identification of DON in food samples and animal feed (Kong *et al*., 2019; Liu *et al*., 2022), its presence in grain crops (Femenias *et al*., 2020; Wang *et al*., 2022), assessment of the effects of this mycotoxin on human health (Wang *et al*., 2019; Narváez *et al*., 2022).

The study aimed to determine the effect of the varietal factor and the type of mycotoxin on their content in 15-day triticale seedlings. The study consists of five sections, namely the introduction, materials and methods, results, discussion, and conclusion.

Materials and Methods

In 2021, seeds for the study were selected from triticale plants (Fig. 1), during the period of full ripeness of grain on stationary experiments of the Department of the Gene Pool of Field Crops and Plant Protection of the Kazakh Research Institute of Agriculture and Crop Production in the Almaty region (coordinates 43.237589, 76.692629). Previously, triticale grain varieties had been evaluated for glume mold infestation. For this purpose, triticale grains (9 varieties and lines) were evaluated for glume mold infestation (100 grains in 4-fold repetition). Next, triticale varieties and lines were analyzed for laboratory germination and germination energy was evaluated on days 3, 5, and 7 in a thermostat. The repetition was 4-fold, 10 grains were germinated in one petri dish.

Laboratory Experiment

The spread of common root rot (*P*) was calculated by the formula:

$$P = n \times 100 \div N \quad (1)$$

where:
n = The total number of plants in the samples
N = The number of diseased plants

Mycotoxins from 15-day-old triticale seedlings were extracted using the Quick Easy Cheap Effective Rugged Safe (QuEChERS) method (Kuldybayev *et al*., 2021) and analyzed via LC-MS/MS according to (Lozowicka *et al*., 2022), followed by the validation with Document No. SANTE/11813/2017 (SANTE 2017).

Statistical Analysis

Data statistical processing was performed using R software version 4.1.1. Analysis of Variance (ANOVA) was conducted under the Kruskal-Wallis test. The statistical significance was established as $P \leq 0.05$ (Kuldybayev *et al*., 2021).

Fig. 1: Infestation of triticale seedlings with common root rot (Idea variety); Note: 0: Healthy seedlings; 1: Slightly affected by root rot; 2: Moderately affected by root rot; 3: Severely affected by root rot

Results

It was found that the blue mold infestation in triticale grains reached 12.5-16.5%. There were no significant differences in this indicator in the various samples (*P*-value-0.5127).

The indicators of mycotoxin content were measured in 15-day-old seedlings of 9 triticale cultivars in 8-fold repetition. Mycotoxins were not detected in 60 out of 72 measurements (Fig. 2 and 3). The maximum mycotoxin content reached 127.6 mcg/mL. Therefore, we applied statistical processing by the nonparametric Kruskal-Wallis test for the bound values.

According to the content of the mycotoxin type factor, the maximum content of OTA and DON was found. In some seedlings, the indicators reached 100-120 mcg/mL, on average 12.4 and 15.1 mcg/mL, respectively (Fig. 4, Table 1). A small content of mycotoxins Aflatoxin B1 and T2 toxin was found, on average equaling 0.7 and 0.4, respectively (Fig. 4 and 5, Table 1).

A high positive correlation was found between the content of mycotoxins and the infestation of seedlings with the common root rot, equaling 0.8. Thus, the average content of mycotoxins in Rondo, Fidelio 5, and Valentin varietals was 11.2; 16.9, and 8.6 mcg/mL, with a spread of 79.0-93.0%, and the development of root rot was 30.5-34.2. In the varieties Idea, TI 17, Nevo, and Dokuckaevskii 9, the average mycotoxin content was lower, in the range of 1.4-3.9 mcg/mL, with a spread of 50.0-65.0% and with the development of the disease equaling 20.5-25.0%. In the triticale Alnaiskii 5 variety, these indices were 0.7 mcg/mL; 55.0 and 19.2%, respectively (Table 2).

Table 1: Boxplot of the impact of triticale genotype factor on the content of mycotoxins in 15-day seedlings of triticale genotypes (KazNARU, 2022)

Mycotoxin	Content conc, (mcg/mL)
OTA	12.4
DON	15.1
Aflatoxin B1	0.7
Zearalenon	0.0
T2 toxin	0.4

P value <0.001***

Table 2: The impact of genotype factor on mycotoxins and common root rot incidence in triticale (KazNARU, 2022)

			Common root rot incidence, %	
No.	Variety	Mycotoxin content conc, (mcg/m)	Spread	Incidence
1	Idea	3.900	65.0	24.0
2	TI 17	2.100	50.0	20.5
3	Nevo	1.700	65.0	24.0
4	Dokuckaevskii 9	1.400	65.0	25.0
5	Altayskii 5	0.700	55.0	19.2
6	Rondo	11.200	93.0	34.0
7	Fidelio 5	16.900	86.0	34.2
8	Valentin	8.600	79.0	30.5
9	Run	3.800	46.0	23.0

P value <0.001*** <0.001***

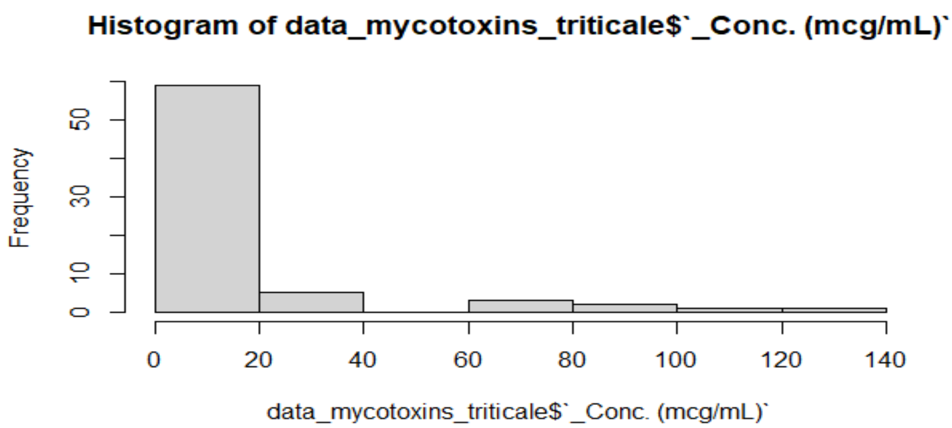
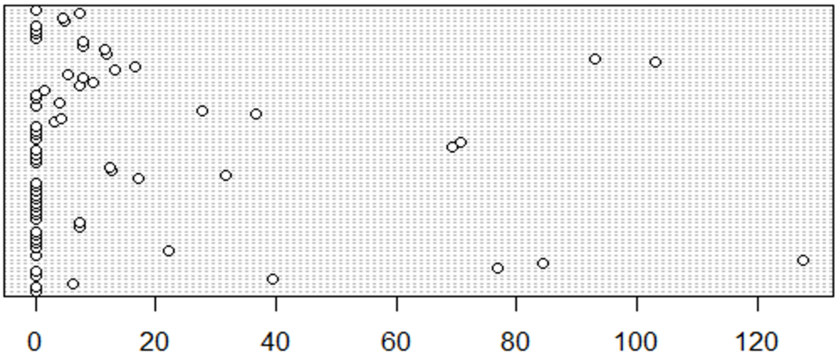


Fig. 2: General distribution of mycotoxin content in 15-day seedlings of triticale genotypes (KazNARU, 2022)



Min.	1st Qu	Median	Mean	3rd Qu.	Max.
0.0	0.0	0.0	5.6	0.6	127.6

Fig. 3: Mycotoxin content in 15-day seedlings of triticale genotypes (KazNARU, 2022)

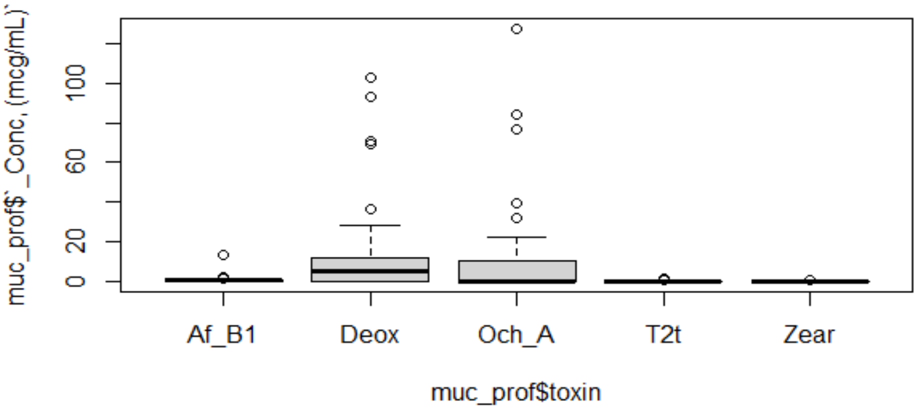


Fig. 4: Boxplot of mycotoxin factor to the content of mycotoxins in 15-day seedlings of triticale genotypes (KazNARU, 2022); Note: Af_B1: Aflatoxin B1; Deox: DON; Och_A: OTA; T2t: T2 toxin; Zear: Zearalenon

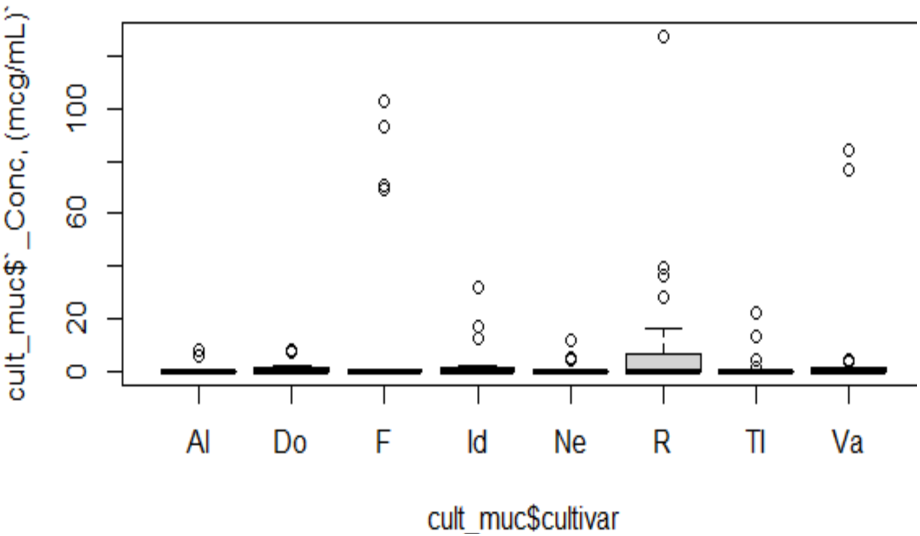


Fig. 5: Boxplot of mycotoxin. factor to the content of mycotoxins in 15-day seedlings of triticale genotypes (KazNARU, 2022); Note: Al: Altayskii 5; Do: Dokuckaevskii 9; F: Fidelio 5; Id: Idea; Ne: Nevo; R: Rondo; TI: TI 17; Va: Valentin

Discussion

OTA has carcinogenic and immunotoxic effects on humans, therefore, selective and sensitive monitoring of the molecules of this substance in food samples is of great importance in its determination (Bostan *et al*., 2017). The maximum permissible level of OTA in food products established by the European Commission is 2-10 mcg/k (Bostan *et al*., 2017).

The most common contaminant of grain crops is DON. DON is produced by fungi from the genus *Fusarium*, which can cause the FHB of cereals (Tanaka *et al*., 1988). Studies of DON on various animal species have proven its teratogenicity, cytotoxicity, genotoxicity, and immunotoxicity as a result of chronic exposure, as well as nausea, vomiting, and refusal of food in case of acute exposure (Kong *et al*., 2019).

Common methods for detecting OTA and DON include liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, and liquid chromatography (Lozowicka *et al*., 2022). We performed the determination of micro-toxins in triticale samples using liquid chromatography. Our study indicated a positive correlation between triticale infestation with root rot and mycotoxin content. Spanic *et al*. (2019) determined a greater level of trichotecens in wheat with Fusarium Head Blight (FHB) symptoms. Moreover, the level of mycotoxins in cereals can be related to the susceptibility of varieties to diseases. High levels of mycotoxins in triticale varieties Fidelio 5 and Rondo and greater indices of root rot spread imply that these varieties are susceptible to root rot. Similar results were obtained in wheat varieties inoculated with *F. graminearum* (Xian *et al*., 2022). However, an increased level of mycotoxins was not determined in susceptible to root rot soybean cultivars as compared with resistant ones (Kuldybayev *et al*., 2021). It indicates plant-specific molecular defense against fungal pathogens and in consequence diversified effect on mycotoxin secretion. Bryła *et al*. (2016) noticed the highest concentration of mycotoxins in triticale cultivars compared to wheat, barley, and oat.

Conclusion

Triticale is an important cereal intended for animal feed. Therefore, studies that examined resistance to root rot cultivars with low mycotoxin contamination are particularly needed. The study aimed to determine the effect of the varietal factor and the type of mycotoxin on their content in 15-day triticale seedlings using the method of liquid chromatography coupled with tandem mass spectrometry. The content of micro-toxins depended on the type of mycotoxin, the triticale variety, and the infestation of triticale seedlings with root rot. Altayskii 5 and Dokuckaevskii 9 are resistant to root rot triticale cultivars with low mycotoxin contamination confirmed in laboratory conditions. The promising results of this study are a premise to expand the study in field conditions and the selection of triticale varieties guaranteeing safe animal feed and suitable for cultivation in Kazakhstan.

Acknowledgment

The authors thank the reviewers for their contribution to the peer evaluation of this study.

Funding Information

The paper has been written based on the results obtained from the project funded by the Ministry of Science and Education of the Republic of Kazakhstan BR10765017: Development of approaches of integrated systems for the protection of fruit, vegetable, grain, forage, legumes and plant quarantine in southeastern Kazakhstan. The study was partially supported by the Ministry of Science and Education in Poland (SIB-03).

Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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