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Mycotoxin Incidence in Pre-Harvest Maize Grains †

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Abstract: The occurrence of mycotoxins causes substantial reductions in maize (*Zea mays* L.) grain quality worldwide. The predominant mycotoxins found in maize grains are aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), fumonisins (FB_1 and FB_2), deoxynivalenol (DON), toxin T2 (T2), ochratoxin A (OTA) and zearalenone (ZEA). In Europe, the predominant mycotoxins originated by field contaminations are produced by mycotoxigenic fungi mainly belonging to the *Fusarium* genus. Accurate fungal identifications, mycotoxin detection and occurrence estimations are important, however, in Portugal, the knowledge about the incidence of mycotoxin types in pre-harvested maize grains and during their storage is still limited. The incidence of mycotoxins in maize grains is substantially influenced by the agricultural practices where the harvesting time is of upmost importance to minimize the risk of accumulation. The main objective of this work was to evaluate, for the first time, the incidence of different types of mycotoxins on maize grains harvested on three farms located in the Tagus Valley region of Portugal. For this purpose, grains from three harvesting dates were analyzed by UHPLC-ToF-MS. It was shown that fumonisins (FB_1 and FB_2) were the main mycotoxins in the samples analyzed. No other mycotoxins were detected. Among fumonisins, FB_1 was the most predominant. Additionally, our data also indicated that the risk of contamination by FB_1 and FB_2 increases with late harvesting. Therefore, a good knowledge of climatic conditions may lead to the establishment of adequate field practices, particularly, the forecast of an early harvesting time.

Keywords: *Zea mays* L.; harvesting time; *Fusarium*; fumonisins; mycotoxins; UHPLC-ToF-MS

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1. Introduction

The occurrence of mycotoxins in maize grains is a big concern due to their potential risk for animal and human health, emphasized by the worldwide importance of maize as a commodity in feed and food uses [1].

Mycotoxins are secondary metabolites produced by fungal toxigenic species belonging to different genera including the *Aspergillus*, *Penicillium* and *Fusarium* genera [2]. In Europe, mycotoxins originated from field to storage are predominantly a consequence of fungal infections by *Fusarium* species reacting to the stress caused by environmental extremes [3]. In maize grains and maize-derived food and feed products, the presence of fumonisins (FB_1 and FB_2), aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), deoxynivalenol, (DON), toxin T2 (T2), ochratoxin A (OTA) and zearalenone (ZEA) has been reported. All mycotoxins cause negative effects on human health and livestock

production [4]. Thereby, regulatory maximum thresholds were established for mitigation of mycotoxin occurrence in food and feed products [5,6].

The incidence of such metabolites in maize grains depends on many factors such as agricultural practices, genetic background, insect damage, storage conditions and environmental conditions [7]. Among the agricultural practices, the harvesting time has been shown to be significant on the levels of different mycotoxins, mainly fumonisins, deoxynivalenol and zearalenone [5]. Few studies reported that the risk of fumonisins contamination in forage maize for whole-crop silage increases with later harvesting times [8]. In Portugal, the knowledge about the incidence of mycotoxin types in pre-harvested maize grains and during their storage is still limited. For this reason, the main objective of this work was to evaluate, for the first time, the incidence of different types of mycotoxins in maize grains harvested on three farms located in the Tagus Valley region of Portugal and at the same time to evaluate the impact of the harvesting time on the mycotoxin contamination level of maize.

2. Methods

2.1. Sampling

During the 2019 campaign, 24 samples of maize of Pioneer varieties P0933 (B, C) and P1049 (A) were collected from eight plots (A1, A2, A3, A4, B1, B2, B3 and C1) in three farms, located in the Tagus Valley region of Portugal. The eight different plots correspond to the application of different field treatments which are A1 (Coragen-200 mL/ha), A2 (without treatment), A3 (Nergetic 30–200 kg/ha), A4 (Patentkali-200 kg/ha), B1 (F-BAC), B2 (without treatment), B3 (Nefusoil) and C1 (without treatment).

Sampling occurred on three harvesting dates: 1st harv date, 2nd harv date (10 days after 1st harv date) and 3rd harv date (10 days after 2nd harv date). From each plot, ears were taken at random. Each primary sample was composed of 25 ears (approx. 5 kg) which were ground (Retsch rotor mill SK 300) with a sieve of trapezoid holes of 1.00 mm to obtained flour according to the European Union requirements [9]. The flours of each sample were mixed for homogenization, and three subsamples of 50 g samples were stored at −20 °C in sterile plastic tubes until analysis.

2.2. Sample Preparation

Two grams of maize of each subsample were placed into a 50 mL polypropylene tube, added 100 μL internal standard zearalanone (10 μg/mL) and extracted with 10 mL of acetonitrile 80% (*v*/*v*) for 1 h in an orbital shaker (Kotterman 4010, Uetze/Hanigsen, Germany). Extracts were centrifugated at 3000 rpm for 10 min, the supernatant was recovered, and the previous procedure was repeated. For analysis of fumonisins, 1 mL of the extract was diluted with 1 mL of ultra-pure water. For the analysis of the other mycotoxins, 8 mL of the extract was evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was redissolved with 1 mL of acetonitrile 40% (*v*/*v*) and vortexed for 30 s. All the extracts were filtered through a PVDF mini-uniprep™ and injected into the UHPLC-ToF-MS system.

2.3. Determination of Mycotoxins in Samples

Detection and quantification were performed with a Nexera X2 Shimadzu UHPLC coupled with a 5600 ToF-MS detector (SCIEX, Foster City, CA, USA) equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI), with the chromatographic conditions as described by Silva et al. [10].

2.4. Statistical Analyses

The mycotoxins were measured in triplicate. One-way analysis of variance (ANOVA) and Tukey's test were used to assess significant differences between samples. Differences

were considered significant at $p < 0.05$. The statistical analyses applied to the analytical results were performed using SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

The results of mycotoxin determination in the maize samples from farms of the Tagus Valley region of Portugal for the three harvesting times are shown in Table 1.

Table 1. Influence of harvesting time on the occurrence of mycotoxin contamination in maize grains from farms of the Tagus Valley region of Portugal.

Plots	Harvesting Data	FB_1	FB_2
A1	1st	261.5 ± 78.2ab	167.1 ± 34.8bc
	2nd	347 ± 25.9ab	182.1 ± 20.3bc
	3rd	216.1 ± 47.7ab	109.3 ± 2.5a
A2	1st	240.4 ± 46.8ab	163.5 ± 21.8b
	2nd	nd	nd
	3rd	189.4 ± 23.8ab	114.7 ± 2.0a
A3	1st	133.3 ± 11.1a	126.4 ± 26.3a
	2nd	nd	nd
	3rd	117.7 ± 0.0a	108.9 ± 0.0a
A4	1st	149.9 ± 26.6a	114.5 ± 5.7a
	2nd	350.4 ± 36.7abc	174.7 ± 8.5bc
	3rd	727.3 ± 76.8abc	214.3 ± 6.2bc
B1	1st	339.4 ± 19.1abc	180.7 ± 8.5bc
	2nd	335.2 ± 99.9ab	141.2 ± 17.9a
	3rd	568.8 ± 216.9abc	231.5 ± 58.1bc
B2	1st	273.3 ± 97.6ab	164.7 ± 42.7bc
	2nd	844.1 ± 67.9c	326.2 ± 75.7c
	3rd	1182.4 ± 233.4abc	495.7 ± 47.4d
B3	1st	nd	nd
	2nd	169.0 ± 44.2ab	123.5 ± 12.7a
	3rd	480.8 ± 127.3abc	207.6 ± 29.7bc
C1	1st	nd	nd
	2nd	136.3 ± 4.6a	117.8 ± 5.8a
	3rd	303.2 ± 36.0ab	158.7 ± 8.9a

The mycotoxin content is expressed in μg/kg; nd: not detected; the absence of common letters (a–d) indicates significant differences at $p < 0.05$.

In our study, there was no detection of ochratoxin A (OTA) in any maize samples at any harvesting time, indicating a low incidence of this type of mycotoxin in maize, as already described in other studies [2,7,11]. Aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), deoxynivalenol (DON) and zearalenone (ZEA) were not detected either, despite the common detection of these mycotoxins in maize-based food products in other countries [12,13]. In all the maize samples analyzed, only fumonisins (FB_1 and FB_2) were detected. These results are coherent with a recent study from Spain which showed that only FB_1 and FB_2 had a consistent presence at pre-harvest with variable concentration never exceeding the legal limits in a silo [11].

The concentrations of fumonisins type B (FB_1 + FB_2) varied considerably but were always at levels below the limits established by the EU for unprocessed grains. Indeed, the level of FB_1 on the earlier harvest date (1st harv date) was from 133.3 to 339.4 μg/kg and for FB_2 between 114.5 and 180.7 μg/kg. On the second harvesting date, the concentration of FB_1 was found to be 136.3–844.1 μg/kg; for FB_2, it was 117.8–326.2 μg/kg. On the later harvest date (3rd harv date), the concentrations of FB_1 and FB_2 ranged from 117.7 to 1182.4 μg/kg and from 108.9 to 495.7 μg/kg, respectively.

It is worth noting that the interval of values for the measured concentrations increased from the first to the third harvesting date, the lower limit being less variable

than the upper limit. Among fumonisins, FB_1 was the most predominant in all the samples, as reported in other countries [14,15].

Samples collected in plots B3 and C1 at the first harvesting date displayed a non-detectable level of fumonisins. Subsequent samples showed low levels of contamination which may indicate the occurrence of agricultural conditions disfavoring the incidence of fungal presence at late maturity stages. However, the concentrations of fumonisins clearly increased in the later harvest dates.

B2 showed the highest concentration of FB_1 and FB_2 in the later harvesting: 1182.4 µg/kg and 495.7 µg/kg, respectively. On the other hand, A3 exhibited the lowest concentrations of FB_1 and FB_2.

The fumonisins (FB_1 + FB_2) of maize grains on three harvesting dates are shown in Figure 1.

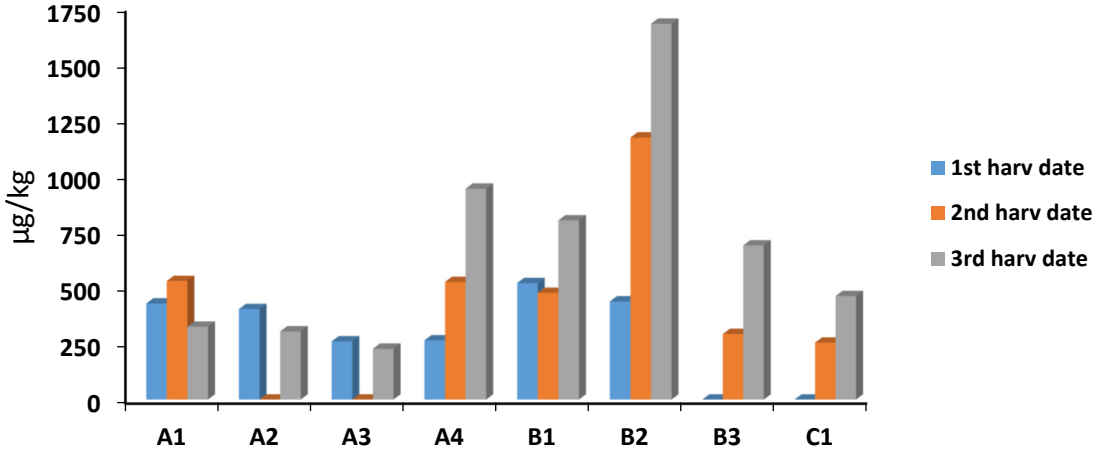

Figure 1. Total of fumonisins (FB_1+FB_2) of maize from farms of the Tagus Valley region of Portugal (A,B,C) on three harvesting dates.

The results of fumonisins (FB_1 + FB_2) were decreased in the maize samples from farm A, not detected at the 2nd harv date, but without significant differences between the first and the third harvesting dates. Regarding the samples from B and C farms, the concentrations of fumonisins clearly increased on the later harvest dates. In general, the highest levels of fumonisins were observed at the third harvesting date which is suggestive of a higher risk of contamination by FB_1 and FB_2 with late harvesting, but the variability of weather conditions should be accounted for. Some studies [2,16] reported that fumonisin contamination of maize and its by-products is a concerning issue in countries located in Southern Europe. The reported studies also stated that the accumulation of fumonisins in maize grains is progressive and influenced by the changes in weather conditions, and thus regular monitoring and measures to diminish their levels are needed.

4. Conclusions

The main mycotoxins present in the maize grain from farms of the Tagus Valley region of Portugal were the fumonisins B_1 and B_2. This preliminary study of the effect of harvesting time on the mycotoxin contamination highlights the recommendation for earlier harvest, taking into account the full maturation and dryness of maize grain. However, to corroborate our results, further research is recommended including more data from other regions of Portugal, as well as from other harvest years.

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