

Mycotoxin incidence in pre-harvest maize grains[†]

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† Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/

Submitted: 25 October 2020; Accepted: date; Published: date

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 (AFB₁, AFB₂, AFG₁ and AFG₂), fumonisins (FB₁ and FB₂), deoxynivalenol (DON), toxin T2 (T2), ocratoxin 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 its storage is still limited. The incidence of mycotoxins in maize grains is substantially influenced by the agricultural practices where the harvesting time is of utmost importance to minimize the risk of accumulation. The main objective of this work is to evaluate, for the first time, the incidence of different types of mycotoxins on maize grains harvested on three farmers located at the Tagus Valley region of Portugal. For this purpose, grains from three harvesting dates were analyzed by UHPLC-ToF/MS. It has been shown that fumonisins (FB₁ and FB₂) were the main mycotoxins in the samples analyzed. No other mycotoxins were detected. Among fumonisins, FB₁ was the most predominant. Additionally, our data also indicates that the risk of contamination by FB₁ and FB₂ increases with late harvestings. 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

1. Introduction

The occurrence of mycotoxins in maize grains is a big concern due 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* genus [2]. In Europe, mycotoxins originated from field to storage are predominantly 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 (FB₁ and FB₂), aflatoxins (AFB₁, AFB₂,

AFG₁ and AFG₂), deoxynivalenol, (DON), toxin T2 (T2), ochratoxin A (OTA) and zearalenone (ZEA) have been reported. All mycotoxins cause negative effects on human health and livestock production [4]. Thereby, regulatory maximum thresholds were established for mitigation of mycotoxins 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, insects 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 its storage is still limited. For this reason, the main objective of this work is to evaluate, for the first time, the incidence of different types of mycotoxins in maize grains harvested on three farms located at the Tagus Valley region of Portugal and at the same time to evaluate the impact of the harvesting time on the mycotoxins 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 at 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), C1 (without treatment). Sampling occurred in three harvesting dates 1st harv date, 2nd harv date (10 days late from 1st harv date) and 3rd harv date (10 days late from 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 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 homogenisation 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 hour 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-ToFMS 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) 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 mycotoxins determination on the maize samples from farms of Tagus Valley region of Portugal in 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 farmers of Tagus Valley region of Portugal

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

The mycotoxin content is express 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 (AFB₁, AFB₂, AFG₁, AFG₂), 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 analysed only fumonisins (FB₁ and FB₂) were detected. These results are coherent with a recent study from Spain which showed that only FB₁ and FB₂ had a consistent presence at pre-harvest with variable concentration never exceeding the legal limits in silo [11].

The concentrations of fumonisins type B (FB₁+FB₂) varied considerably but were always at levels below the limits established by the EU for unprocessed grains. Indeed, the level of FB₁ on the earlier

harvest date (1st harv date) was from 133.3 to 339.4 $\mu\text{g}/\text{kg}$ and for FB₂ between 114.5 and 180.7 $\mu\text{g}/\text{kg}$. On the second harvesting date, the concentration of FB₁ was found 136.3–844.1 $\mu\text{g}/\text{kg}$, for FB₂ were 117.8–326.2 $\mu\text{g}/\text{kg}$. On the later harvest date (3rd harv date), the concentrations of FB₁ and FB₂ ranged from 117.7–1182.4 $\mu\text{g}/\text{kg}$ and 108.9–495.7 $\mu\text{g}/\text{kg}$, respectively.

It is worth nothing that the interval of values for the measured concentrations increased from the first to the third harvesting date being the lower limit less variable than the upper limit. Among fumonisins, FB₁ was the most predominant in all the samples, as reported in other countries [14,15]. Samples collected in the plots B3 and C1 at the first harvesting date displayed a non-detectable level of fumonisins. Subsequent samples showed low levels of contamination what 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 harvested dates.

B2 showed the highest concentration of FB₁ and FB₂ on the later harvesting, 1182.4 $\mu\text{g}/\text{kg}$ and 495.7 $\mu\text{g}/\text{kg}$, respectively. On the other and, A3 exhibited the lowest concentrations of FB₁ and FB₂.

The fumonisins (FB₁+FB₂) of maize grains on three harvesting dates are showed in Figure 1.

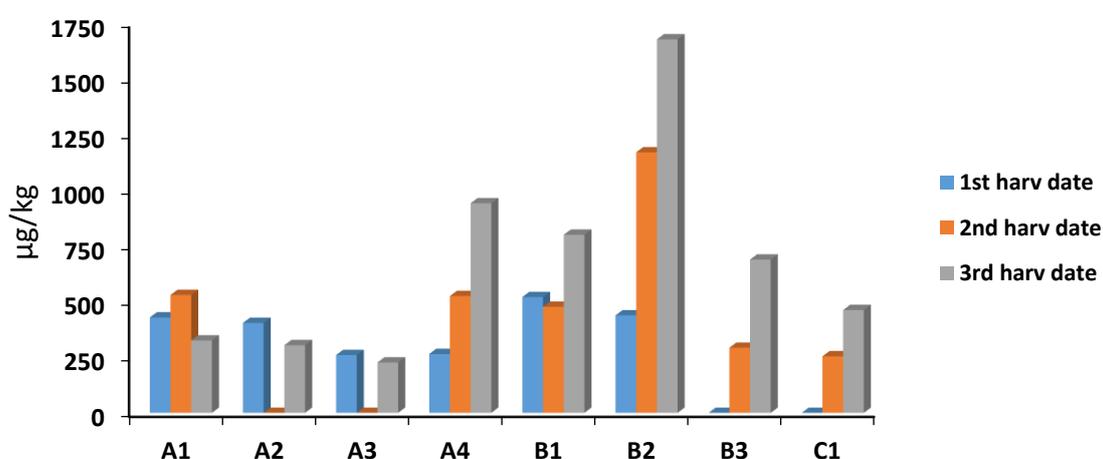


Figure 1. Total of fumonisins (FB₁+FB₂) of maize from farms of Tagus Valley region of Portugal (A, B, C) in three harvesting dates.

The results of fumonisins (FB₁+FB₂) were decreased on the maize samples from the farmer A, not detected at 2nd harv date, but without significant differences between de first and the third harvesting dates. Regarding to the samples from B and C farmers, the concentrations of fumonisins clearly increased in the later harvested dates. In general, the highest levels of fumonisins were observed at the third harvesting date which is suggestive of higher risk of contamination by FB₁ and FB₂ with late harvestings, but the variability of weather conditions should be accounted. Some studies [2,16] reported that fumonisins contamination of maize and its by-products is a concerning issue in countries located in Southern Europe. The reported studies also state that accumulation of fumonisins in maize grains is progressive and influenced by the changes in weather conditions, so that regular monitoring and measures to diminish their levels are needed.

4. Conclusion

The main mycotoxins present on the maize grain from farmers of Tagus Valley region of Portugal were the fumonisins B₁ and B₂. This preliminary study of the effect of harvesting time on the mycotoxin contamination highlights the recommendation for earlier harvested, taking in 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 harvested years.

Author Contributions: Conceptualization, B.C. and C.B.; methodology, A.F. and A.S.; formal analysis, A.S., A.F. and A.S.; investigation, B.C., A.F., A.S., E.A., T.P. and C.B.; data writing—original draft preparation, B.C.;

writing—review and editing, B.C., A.S., E.A. and C.B.; project administration, E.A., T.P. and C.B.; funding acquisition, T.P., C.B.

Funding: This research was funded by National Funds by Rural Development Program through the Operational Group QUALIMILHO- New sustainable integration strategies that guarantee quality and safety in the national maize, PDR2020 n^o 101-031295 (2017-2020). This work was supported by FCT-Portuguese Foundation for Science and Technology through the R&D Unit, UIDB/04551/2020 (GREEN-IT - Bioresources for Sustainability). The work was supported by UIDB/00211/2020 with funding from FCT/MCTES through national funds.

Conflicts of Interest: authors declare no conflict of interest.

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