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Evaluation of Biobased Solutions for Mycotoxin Mitigation on Stored Maize †

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Abstract: Maize (*Zea mays* L.) is highly susceptible to fungal post-harvest contamination. The main objective of this study was to evaluate the effectiveness of mustard powder and rice bran oil as post-harvest mitigation strategies towards maize quality control. The application of mustard powder (0.2%, *w*/*w*) showed an apparent inhibitory effect on aflatoxins biosynthesis, while the levels of fumonisins increased during the first six months of maize storage. Rice bran oil (1%, *v*/*w*) decreased the levels of fumonisins during the first six months when compared with the control. The application of mustard and rice bran oil for mycotoxin mitigation are promising, but further research is needed to confirm their effectiveness in stored maize.

Keywords: *Zea mays* L.; fumonisins; aflatoxins; mustard powder; rice bran oil; mitigation

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1. Introduction

Maize (*Zea mays* L.) is one of the most susceptible crops to contamination by mycotoxigenic fungi that produce mycotoxins [1]. Mycotoxins are fungal secondary metabolites mainly produced by the genera *Aspergillus*, *Penicillium*, *Claviceps*, *Alternaria*, and *Fusarium* [2]. The production of mycotoxins increases in reaction to stress induced by exogenous factors such as environmental extremes. The incidence of mycotoxins in maize grains is a huge concern for human and animal health due their probability of occurrence and toxicological properties. Aflatoxins, ochratoxin A, fumonisins, and zearalenone have been associated with hepatotoxicity, nephrotoxicity, and estrogenic effects [3].

Mycotoxins' contamination may occur at the field level, in farms after harvesting [4–8], and during the storage process [9–11]. Generally, its occurrence and prevalence is affected by agronomic practices, fungal activity, climatic conditions, and inadequate storage conditions, resulting in appreciable quality and quantity losses of around 10–20% [12].

The search for biobased solutions as natural alternatives to mitigate the occurrence of mycotoxins is a current challenge. The already available bioproducts envisage the ability to guarantee the absence of pathogenic organisms, as well as to reduce the use of chemical anti-fungal products with harmful health and environmental implications.

Mustard belongs to the *Brassicaceae* family and is rich in glucosinolates. The presence of isothiocyanate molecules plays an important role in plant defense due to their fungicidal, bactericidal, and insecticidal activity. Several studies have shown their beneficial effects against *Penicillium* species with the reduction in aflatoxins in nuts [13] and ochratoxin A, mainly in pita bread [14]. These natural preservative agents increase the shelf life of bread, reducing the level of fungal contamination by *Aspergillus*, *Fusarium*, and *Penicillium* [15].

Rice bran oil represents 18–22% of bran and has been associated with anti-inflammatory, anti-microbial, and anti-oxidant activity [16].

However, the application of natural compounds for the mitigation of mycotoxins in silos at the industrial scale is still limited. Owing to the higher toxicity of mycotoxins with regard to human and animals' health and the issue of regulating their occurrence, the main objective of this study was to evaluate the effect of the application of biobased solutions (mustard seeds and rice bran oil) in order to mitigate the occurrence of mycotoxins in maize grains stored for 10 months (one production campaign).

2. Material and Methods

2.1. Sampling

During the harvesting period in 2019, two samples of maize were collected in two experimental plots (M1 and M2), conducted in a farm located in the Tagus Valley region of Portugal. To the M1 plot, fertilization with macro- and micronutrients (N, P, and Zn) and a supplement with an anti-fungal treatment using F-BAC (EIBOL Ibérica, S. L. Valencia, Spain) were applied, while in the M2 plot, no reinforcement treatment was applied.

Each composite sample contained 10 kg of maize grains and was collected in October.

2.2. Biobased Treatments

To the M1-T sample, 0.2 % (w/w) seed mustard was added, and M2-T was treated with 1% (v/w) rice bran oil. The maize grains were mixed for eight hours with mustard solution and rice oil in a pilot reactor (50 L) system (Juchheim Laborgeräte GmbH, Bernkastel-Kues, Germany) fermenter to ensure a homogeneous blending process. After the blending process, maize grains were stored in small barrels located inside the silos. Approximately 1 kg of maize was collected from each barrel after 2, 5, and 10 months of storage. The samples were ground in a Retsch rotor mill (SK 300) (Retsch GmbH, Haan, Germany) with a sieve with trapezoid holes of 1.00 mm and stored at −20 °C until the further analysis of mycotoxins.

2.3. Determination of Mycotoxins in Samples

2.3.1. Mycotoxin Extraction

The analytical procedure used to quantify the mycotoxin content in maize grains was previously described by Silva et al. [17].

2.3.2. Mycotoxin Ultra-High Performance Liquid Chromatography Combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) Analysis

Aflatoxins (AFB1, AFB2, AFG1, and AFG2), fumonisins (Fum B1 and Fum B2), toxin T2 (T2), and zearalenone (ZEA) were quantified using the method described by Silva et al. [17].

2.3.3. Deoxynivalenol (DON) Analysis

The detection and semi-quantitative screening of DON in maize were carried out using the method described by Freitas et al. [18].

2.4. Statistical Analysis

The statistical analyses applied to the analytical results were performed using SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL, USA). The mycotoxins were measured in triplicate.

3. Results and Discussion

Despite the screening of other mycotoxins, M1 and M2 samples of maize grains only revealed fumonisins and aflatoxins. The levels of mycotoxins quantified in the maize samples stored for 10 months, controls, and the one treated with the biobased solution of mustard seeds are described in Figure 1.

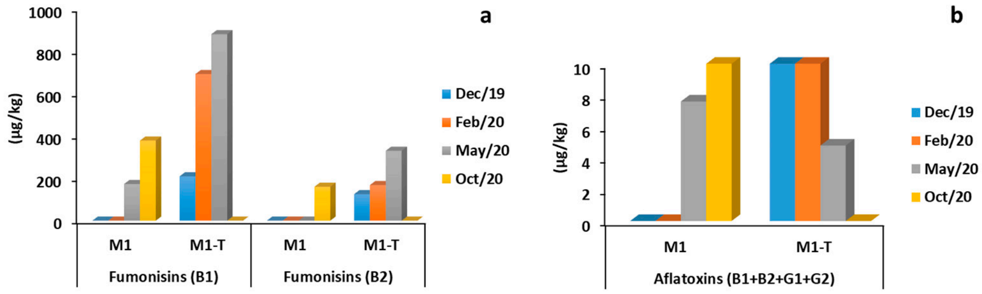

Figure 1. Levels of (**a**)—fumonisins (B1 and B2) and (**b**)—aflatoxins (B1 + B2 + G1 + G2) on control maize barrel (M1) and maize treated with mustard seeds (M1-T).

The levels of fumonisins and aflatoxins in M1 increased during the storage period. On the other hand, the contents of fumonisins were always below the limits established by the EU. After 10 months of storage, the levels of aflatoxins exceeded the authorized limits [19] of 10 μg/kg. Unexpectedly, fumonisins seemed to have a higher tendency of increasing in the M1-T barrel, where the treatment was applied. However, after 10 months, no B1 or B2 fumonisins were detected. The mustard treatment had also a positive effect in the reduction in the levels of aflatoxins after long periods of storage. It reduced the aflatoxin content by 50% between each measurement time: after 2 months of storage, aflatoxins reached 10 μg/kg, but after 5 months, this value was only 4.8 μg/kg, and after 10 months, no aflatoxins were found.

The levels of fumonisins found in the control maize sample (M2) and maize treated with rice bran oil (M2-T) stored in barrels for 10 months are described in Figure 2.

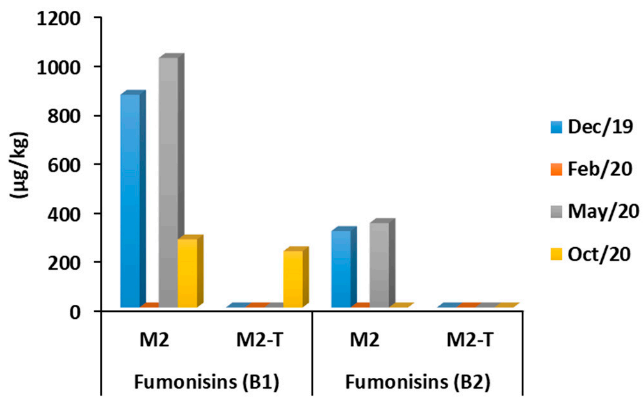

Figure 2. Levels of fumonisins (B1 and B2) on control maize barrel (M2) and maize treated with rice bran oil (M2-T).

The control maize sample (M2), which revealed high levels of fumonisins (B1) from 869 μg/kg to 1019 μg/kg during the first 5 months of storage (May/2020), showed a reduction to 278 μg/kg after 10 months of storage (Oct/2020). The values of fumonisin (B2) ranged from 312 μg/kg at harvest time on Dec/2019 to 345 μg/kg after 5 months of storage; later, B2 fumonisin was not detected. The different levels of fumonisins in the two control samples (M1 and M2) at harvest time could be correlated with the application of F-BAC treatment in the M1 plot, which reduced the incidence of mycotoxigenic fungi. The fertilization with macro- and micronutrients (N, P, and Zn) and the anti-fungal treatment using F-BAC could mitigate the occurrence of mycotoxins in maize grains during the first 5 months of storage. The levels of fumonisins detected during storage were lower than the values found in the same variety of maize harvested in the same location in 2018 [9]. Previous results showed that levels of B1 fumonisin and B2 fumonisin also decreased in stored maize, from 1666 µg/kg to 1527 µg/kg for B1 fumonisin and 473 µg/kg to 353 µg/kg for B2 fumonisin after 4 months of storage in barrels [9]. In Spain, the accumulation of B1 fumonisin decreased from 509.56 to 188.42 µg/kg, and B2 fumonisin decreased from 131.08 µg/kg to undetected leveks in grain maize after three months of storage [11].

The application of rice bran oil exhibited a positive effect in the mitigation of the accumulation of mycotoxins during storage in barrels. In the first 5 months of storage, mycotoxins were not detected neither from *Fusarium* (toxin T2, zearalenone, and deoxynivalenol) nor from *Penicillium* (ochratoxin A) and *Aspergillus* (aflatoxin) accumulation. However, after 10 months of storage, 230 µg/kg of B1 fumonisin was found. Our results indicate that rice bran oil loses activity after 5 months. Further experiments must be carried out with other concentrations of rice bran oil and/or additional applications. A second application after 6 months of storage is expected to maintain the effect of rice bran oil as an inhibitor of mycotoxin accumulation.

4. Conclusions

The present study assessed the use of biobased solutions (mustard seeds and rice bran oil) to mitigate mycotoxin accumulation during 10 months of storage in barrels, simulating real "in silo" conditions.

The results obtained with mustard and rice bran oil applications for mycotoxin mitigation in stored maize are promising; mustard seeds revealed a good effect in reducing the levels of aflatoxins below the stablished limits, while in the use of rice bran oil, no mycotoxin accumulation was verified over 5 months of storage.

Further research is needed to establish the ideal concentration of mustard seeds and rice bran oil used and/or the specific moment to apply it in storage maize, with the objective to deliver useful recommendations to different maize chain stakeholders.

Supplementary Materials: The following are available online at 1st International Online Conference on Agriculture—dvances in Agricultural Science and Technology at https://www.mdpi.com/article/10.3390/IOCAG2022-12306/s1. Session: From Field to Consumers: Challenges and Approaches to High-Quality Agricultural Products. https://doi.org/10.3390/IOCAG2022-12306. Presentation: Evaluation of Biobased Solutions for Mycotoxin Mitigation on Stored Maize.

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