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Evaluation of biobased solutions for mycotoxin mitigation on stored maize †

Bruna Carbas [1,2], Andreia Soares [1], Sílvia Barros [1], Ana Carqueijo [1], Andreia Freitas [1,3], Ana Sanches Silva [1,4,5], Daniela Simões [1], Tiago Pinto [6], Eugénia de Andrade [1,7], Carla Brites[1,7] *

[1] National Institute for Agricultural and Veterinary Research (INIAV), I.P., Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal. andreia.soares@iniav.pt; silvia.barros@iniav.pt; ana.carqueijo@iniav.pt; daniela.simoes@iniav.pt
[2] Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro (CITAB-UTAD), 5000-801 Vila Real, Portugal. bruna.carbas@iniav.pt
[3] REQUIMTE/LAQV - R. D. Manuel II, Apartado, 55142 Oporto, Portugal. andreia.freitas@iniav.pt
[4] Centre for Animal Science Studies (CECA), ICETA, University of Porto, 4051-401 Porto, Portugal. andreia.freitas@iniav.pt ana.silva@iniav.pt
[5] University of Coimbra, Faculty of Pharmacy, 3000-548 Coimbra, Portugal.
[6] ANPROMIS—Associação Nacional dos Produtores de Milho e do Sorgo, 1549-012 Lisboa Portugal; anpromis@anpromis.pt
[7] GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal. eugenia.andrade@iniav.pt
* Correspondence: carla.brites@iniav.pt*
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Abstract: Maize (*Zea mays* L.) is highly susceptible to fungal contamination at post-harvest. 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 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.

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 crop for contamination by mycotoxigenic fungi and 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 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, 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 to guarantee the absence of pathogenic organisms, as well as to reduce of use of chemical antifungal products with harmful health and environmental implications.

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

Rice bran oil represents 18 – 22% of bran and have been associated to anti-inflammatory, antimicrobial and antioxidant activity [16].

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

2. Material and methods

2.1. Sampling

At the harvested 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 plot M1 a fertilization with macro- and micronutrients (N, P, and Zn) and a supplement with an antifungal treatment using F-BAC (EIBOL Ibérica, S. L. Valencia, Spain) was applied, while in plot M2, no reinforcement treatment was applied.

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

2.2 Biobased treatments

M1-T sample was added 0.2 % (w/w) of seed mustard, and M2-T was treated with 1% (v/w) of rice bran oil. The maize grains were mixed during eight hours with mustard solution and rice oil in a pilot reactor (50L) system (Juchheim Laborgeräte GmbH) 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) with a sieve of trapezoid holes of 1.00 mm and stored at −20 °C until further analysis of mycotoxin.

2.3. Determination of mycotoxins in samples

2.3.1. Mycotoxin extraction

The analytical procedure used to quantify the mycotoxins content of maize grains is 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 by method described by Silva et al. [17].

2.2.3. Deoxynivalenol (DON) Analysis

Detection and semi-quantitative screening of DON in maize were performed 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 reveal 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.

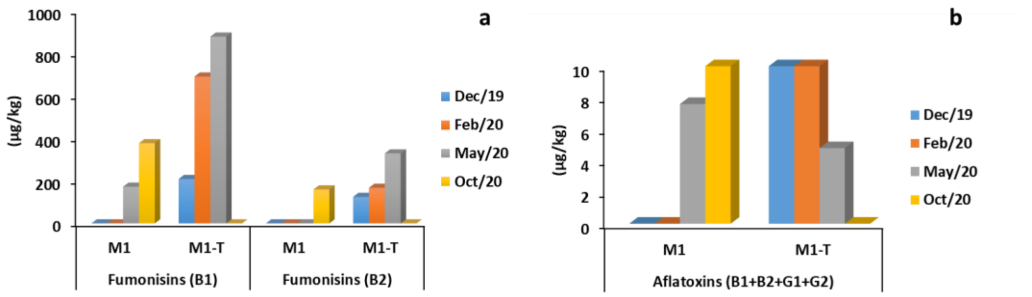

Figure 1. Levels of **a)-** fumonisins (B1 and B2) and **b)-** aflatoxins (B1+B2+G1+G2) on control maize barrel (M1) and maize treated with mustard seeds (M1-T)

The levels of fumonisins and aflatoxins in M1 increased during the storage period. On the other hand, 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 M1-T barrel, where the treatment was applied. However, after 10 months, no fumonisins B1 and B2 were detected. The mustard treatment had also a positive effect in the reduction of the levels of aflatoxins after long periods of storage. It reduced the aflatoxins content in 50% between each measurement time: after 2 months of storage, aflatoxins reached 10 µg/kg, but after 5 months, it was only 4.8 µg/kg and after 10 months no aflatoxins were found.

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

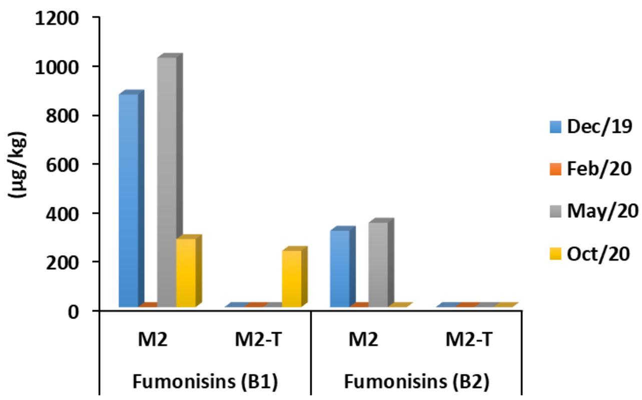

Figure 2. Levels of fumonisins (B1 and B2) on control maize barrel (M2) and maize treated with rice bran oil (M2-T)

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/20) showed a reduction to 278 μg/kg after 10 months of storage (Oct/20). The values of fumonisin (B2) ranged from 312 μg/kg, at harvest time in Dec/19, to 345 μg/kg after 5 months of storage, later fumonisin B2 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 antifungal treatment using F-BAC, could mitigate the occurrence of mycotoxins on maize grains during the first 5 months of storage. The levels of fumonisins detected during storage were lower than the values found in same variety of maize harvested in the same location in 2018 [9]. Previous results showed that levels of fumonisin B1 and fumonisin B2 also decreased in stored maize, from 1666 μg/kg to 1527 μg/kg of fumonisin B1 and 473 μg/kg to 353 μg/kg of fumonisin B2 after 4 months of storage in barrels [9]. In Spain, the accumulation of fumonisin B1 decreased from 509.56 to 188.42 μg/kg, and fumonisin B2 from 131.08 μg/kg to not detected in grain maize after three months of storage [11].

The application of rice bran oil exhibited a positive effect to mitigate 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 fumonisin B1 were found. Our results indicate that the rice bran oil loses activity after 5 months. Further experiments must be done, with other concentrations of rice bran oil and/or additional applications. A second application after 6 months of storage is expected to keep the effect of rice bran oil as an inhibitor of mycotoxins accumulation.

4. Conclusion

The present study accesses the use of biobased solutions (mustard seeds and rice bran oil) to mitigate the mycotoxin accumulation during 10 months of storage in barrels simulating the 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 to reduce the levels of aflatoxins below the stablished limits, while using rice bran oil no mycotoxin accumulation was verified for 5 months of storage.

Further research is needed to stablish 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 the different maize chain stakeholders.

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