

Pomegranate Peel Extract, Used as a Natural Supplement in the Ensiling Process, May Help to Reduce the Contamination of Silage with Fungi and Mycotoxins

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Abstract

In Israel, fungal and mycotoxin levels in wheat and maize silage were examined in a study at six animal feed facilities. The fumonisin mycotoxins FB1 and FB2 were found in each and every sample of maize silage examined. Intriguingly, there was no correlation found between the presence of the fungal species that might produce certain mycotoxins and the silages of wheat and maize. In laboratory-prepared maize silage, we conducted additional research into the effects of pomegranate peel extract (PPE) on *Fusarium* infection and fumonisin biosynthesis. PPE inhibited *Fusarium proliferatum*'s biosynthesis of FB1 and FB2, resulting in a 90 percent decrease in fumonisin production in silage samples compared to untreated controls. Under PPE treatment, qRT-PCR analysis revealed that key genes involved in the fumonisin-biosynthesis pathway were down regulated, corroborating this finding. Our findings suggest promising new applications for natural compounds that have the potential to replace conventionally used synthetic chemicals and reduce fungal and mycotoxin contamination in agricultural foodstuffs.

Keywords: Mycotoxins • *Fusarium* • *Penicillium*

Introduction

Mycotoxins that are produced as secondary metabolites by filamentous fungi primarily belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* can contaminate silage, which is one of the primary sources of animal feed for dairy cattle. Mycotoxins can cause severe acute and chronic toxic effects when consumed, posing a serious threat to human and animal health. The ensiled forages are acidified by lactic acid-producing bacteria while silage is stored in anaerobic conditions. Under low oxygen conditions, most mycotoxigenic fungi cannot grow in the acidic silage environment. However, due to their high stability, mycotoxins produced by mycotoxigenic fungi in the field may not change during the silage process. For instance, the concentration of zearalenone (ZEN) in maize silage remained virtually unchanged for a total of 12 weeks. On the other hand, the main field producer of ZEN, *Fusarium culmorum*, vanished from the silage after 11 days, indicating that ZEN was produced prior to ensiling [1,2].

Description

It is anticipated that the application of various antifungal compounds will limit the amount of mycotoxin contamination produced during ensiling and prevent the growth of mycotoxigenic fungi. Biocontrol agents have been shown in a number of studies to reduce aerobic spoilage and reduce or prevent fungal contamination in silage. Compared to untreated silage, the yeast and mold counts in maize silage that had been inoculated with *Lactobacillus buchneri* and *Pediococcus pentosaceus* were lower. Additionally, that study

demonstrated that treatment with potassium sorbate reduces aerobic spoilage and fungal contamination in maize silage. However, the concentrations of *Fusarium*-producing toxins in maize silage were unaffected by the application of these additives. Alternative, eco-friendly methods of controlling these toxic substances during installation are required because the current methods are insufficient to eliminate or reduce mycotoxin contamination to safe threshold levels [3,4].

Plants produce a huge assortment of mixtures that are liable for many natural and pharmacological properties, including antimicrobial exercises. In treated plant hosts, it has been reported that some plant-derived compounds have direct antifungal activity. Polyphenols like gallic acid, ellagic acid and its glycosylated derivatives, anthocyanins, proteins, bioactive peptides and polysaccharides are found in abundance in various parts of the pomegranate fruit, particularly the peel. Pomegranate peel extracts (PPEs) have been found to have strong antioxidant, antitumor, antibacterial and antifungal properties, according to a number of in-vitro and in vivo studies. Besides, our new work exhibited that past its antifungal action, PPE can repress aflatoxin creation by *Aspergillus flavus*. PPE inhibited the production of aflatoxin B1 in that study without affecting the growth of the fungus, indicating that the extract may have an effect on specific genes that encode enzymes for the aflatoxin-biosynthesis pathway. We looked into PPE as a potential way to stop agricultural products from producing mycotoxin because of these findings [5,6].

All randomly selected maize silage samples from six animal feed centers across Israel contained the mycotoxins fumonisin B1 and B2 (FB1 and FB2, respectively), which are produced by several *Fusarium* species. This suggested that even in well-preserved silage, mycotoxins could remain. In addition, PPE prevented the production of FB1 and FB2 by *Fusarium proliferatum* during the ensiling of maize on a laboratory scale, indicating that this extract may be able to prevent the contamination of animal feed with mycotoxin.

Over the course of two years (2018–2019), six animal feed centers in Israel's northern, central and southern districts collected 320 samples of silage for dairy cattle, 160 of wheat and 160 of maize. The silages were approximately six months old when the samples were taken. The example aliquots were taken from silage stacks, in a space something like 1 m far off from the sides, top and base, utilizing a silage drill roughly 1 m behind the cutting essence of the silage stack. The samples were transported to the laboratory in cool

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Received: 01 November 2022, Manuscript No. jfim-23-85456; Editor assigned: 03 November 2022, Pre QC No. P-85456; Reviewed: 15 November 2022, QC No. Q-85456; Revised: 21 November 2022, Manuscript No. R-85456; Published: 28 November 2022, DOI: 10.37421/2572-4134.2022.8.260

plastic bags after being collected. Each example was separated into two subsamples: a first subsample of 100 g was examined for parasitic settlement counts following landing in the research facility and the leftover example was put away at -20°C until additional mycotoxin examination. A pH electrode was used to take a reading from the aqueous extract of the silage sample. The parameters for the method's performance and validation were chosen in accordance with regulation no. 401/2006. Multi-mycotoxin standard solutions were spiked at three concentrations into three wheat and three maize silage samples that had not been contaminated with the major mycotoxins or were only slightly contaminated with them. As described, extraction and analysis were carried out. At three distinct points in time, the spiking experiments were carried out in triplets. The precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and specificity of the validation were all established [7,8].

A variety of pomegranates was purchased from local markets. Using a laboratory grinder, the fruit peels were freeze-dried and ground into a fine powder. 500 milliliters of 80% methanol were used to extract the 100 g of dried powder after 72 hours in the dark at room temperature. Whatman no was used to filter the suspension. 1 filter paper and concentrated at 45 degrees Celsius using a rotary evaporator. The extract was then freeze-dried and a concentrated stock solution of PPE (100 mg/ml) was made in sterile water and stored at 4 degrees Celsius until needed. Red onion cv.-derived fumonisin-producing *Fusarium proliferatum* strain YO3 During the field study, Mata Hari was used. Before each experiment, the strain was brought up from below 80°C by subculturing it on PDA plates and being kept at 28°C . Spores (macroconidia) were collected in sterile saline from cultures that had been grown for four days. A hemocytometer count was used to adjust the macroconidial suspension to the required concentration. Placing the inoculum on PDA plates to measure CFU counts confirmed the concentration of the inoculum [9,10].

Conclusion

The maize hybrid "Overland" was used in the 2019 field trial in Israel's Kibbutz Yotvata. By injecting 5-ml of a suspension of macroconidia through the silk canals (inside the husk cavity and above the cob), the ears were inoculated with *F. proliferatum* at the beginning of silk formation, which occurred between 4 and 7 days after silk emergence. There were three replicates in the experiment, each with two rows. A control group that was not treated consisted of ears of maize inoculated with clean saline water. Up until ear maturity, the field was maintained for an additional forty days. At this point, all of the plants from the treated plots, including the ears, were harvested, bagged and brought to the laboratory for analysis.

Acknowledgement

None.

Conflict of Interest

None.

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How to cite this article: Chen, Meifeng. "Pomegranate Peel Extract, Used as a Natural Supplement in the Ensiling Process, May Help to Reduce the Contamination of Silage with Fungi and Mycotoxins." *J Food Ind Microbiol* 8 (2022): 260.