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Original article

Assessment of different drying methods and application of some chemical compounds in prevention of corn contamination by Fumonisin

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Summary. The present study aimed to assess prevention of fungal growth and *fumonisin* production in corn by chemical methods at different levels. The anti-fungal properties of a series of chemical compounds at different levels were tested on yeast chloramphenicol glucose agar. The positive samples to *fusarium* were separated to measure *fumonoisin* content by ELISA. Among the chemical compounds, sorbic acid at 0.5-1.5% concentrations, sodium metabisulphite at 0.5-1.5% concentrations, benzoic acid at 0.5-1.5% concentrations, and propionic acid at 1.5% concentration completely inhibited *Fusarium verticillioides* growth and *fumonisin* production. Not only did sulfur dioxide gas (SO_2) and sodium metabisulphite at 1.5% concentration completely inhibited *Fusarium* growth, but they also prevented the growth of all fungi and no mold was observed on the plates. Thus, propionic acid had tolerable (50-75%) anti-fungal properties, but was ineffective in complete prevention of *Fusarium*.

Key words: anti-fungal, corn, chemical compounds, Fumonisin, Fusarium

Introduction

Corn *(Zea mays L. spp)* is a major crop in human and livestock food rations and corn kernels are subject to infection by a variety of toxigenic fungi that may occur in the field or store (1). The most common types of these fungi are *Aspergillus flavus*, *Fusarium verticillioides*, and *Fusarium proliferatum* (2, 3). Mycotoxins are the secondary metabolites made by fungi that grow naturally in food crops, especially in cereals and grains, which can be produced in the field pre-harvest or during harvest, transportation, and storage stages (4). Growth of fungi that leads to production of toxins in corn depends on several factors, including low moisture of the soil, too low temperature during the night or too high temperature in the day, and soil nutrient deficiencies (5, 6). Application of antifungal agents, such as sorbates, propionates, and benzoates, is a post-harvest strategy to prevent the growth of fungi and their toxin production (7). Fumonisins are one type of water-soluble mycotoxins that were first defined and characterized in 1988. Fumonisins are produced by the common corn fungus *Fusarium verticillioides* (formerly *Fusarium moniliforme*). *Fusarium verticillioides* is the most important species, which grows as a corn pathogen (8) and Fumonisin B_1 (FB_1) is the most abundant and the most toxic of all the known fumonisins (9). According to the International Agency for Research on Cancer (IARC), the cancer risk of FB_1 to humans has been currently categorized as Group 2B (potentially carcinogenic substance) (10). There are reports from all over the world regarding fumonisins contamination in corn and corn-based food and feed (11). Moreover, epidemiological evidence has shown a relationship between

the consumption of fumonisin-contaminated corn and occurrence of human esophageal cancer in several regions of the world (12). Thus, due to the risk of human and animal health, contamination of corn by *F. Verticillioides*, *F. Proliferatum*, and fumonisins is a growing concern throughout the world. Furthermore, the Codex Committee of Food and Agriculture Organization (FAO) created a single general code of practice for prevention of mycotoxin contamination in cereals, including a specific provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg of body weight per day for fumonisins B_1, B_2, and B_3 on the basis of a safety factor of 100 (13). Therefore, it is necessary to obtain data on concentration of this mycotoxin in human foodstuffs and use suitable detoxification methods. Using chemical compounds is one of the procedures to prevent fungal growth and, consequently, mycotoxin production. Hence, the present study aims to assess several chemical compounds for their efficacy to inhibit mold growth and fumonisin production.

Materials and Methods

Sampling

In this study, many corn clusters were collected from one of the big farms located in the west of Tabriz in October 2013. Sampling was carried out randomly in different parts of the farm. Then, 51 samples were divided into seventeen trio groups; fourteen groups were treated by different compounds and three control groups were considered without any treatments. The experimental groups were dried in the 50°C oven, while two of the control groups were dried outdoor (under shade and sun) and one was dried in the 50°C oven. The samples with final moisture (15%) were stored in sterile plastic envelopes for the rest of the tests to be done.

Anti-fungal compounds

In this study, propionic acid, benzoic acid, sorbic acid, sodium metabisulphite (Merck, Germany) and SO_2 gas (Kavian gas, Iran) were utilized.

Inoculation and assessment of growth

At first, 12 trio groups of the samples were individually washed with different concentrations (0.5%, 1%, and 1.5%) of the relevant chemical substance (propionic acid, benzoic acid, sorbic acid, and sodium metabisulphite) at the room temperature for 15 minutes. To achieve uniform distribution of the chemical compound, the clusters of corn were placed in sealed containers and were shaken. Besides, 2 trio groups of the samples were exposed to SO_2 gas (5 ppm) for 6 and 12 hours. Afterward, all the treated samples were placed in the oven (50°C) for 24-48 hours for completely drying. On the other hand, the three control groups were dried individually in the shade, sun (air-drying), and oven (50°C) without any treatments. Then, 5 maize kernels in each trio group were separated from the clusters, rinsed with sterile distilled water to remove surface contaminants, and were plated on Yeast Chloramphenicol Glucose Agar (YGC Agar) (Merck, Germany) aseptically. Medium culture was prepared at appropriate concentration (4× 104 ppm), sterilized in autoclave at 121°C for 15 minutes, mixed well, and dispensed into plates. The plates were located at room temperature to incubate for 5 days. In the cases with no fungal growth, a minimum incubation period of 5 days was allowed to ensure that growth did not really occur. During this time, the grown colonies were examined macroscopically and counted by experts. The experiment was repeated for four times. After all, the plates that were found to be positive for *Fusarium verticillioides,* were separated for further assessments.

Determination of fumonisins

The research by Abbas et al. (2006) (5) revealed that the more convenient Enzyme Linked Immunosorbent Assay (ELISA) method yielded reliable results such HPLC for measurement of fumonisin, because the values obtained through the two methods were not significantly different. Therefore, Direct Competitive ELIZA (DC-ELISA) method (Ridascreen® R3401, R-Biopharm AG, Darmstadt, Germany) was employed in this study for fumonisins content. In doing so, 5 gr of milled corn were mixed with 25 mL of methanol/distilled water (70/30) in a shaker for 3 min-

Table 1. Fumonisin B_1 (FB_1) content in the control groups without any treatments

	Total mold (%)[a]	*Fusarium* (%)[a]	FB1 content (ppm±SD) [b]
Shade (Air-dried)	100	65	4±0.59
Sun (Air-dried)	90	25	4±0.28
Oven (50 °C)	40	15	1.8±0.19

[a]*Number of corn kernels in each group was 20 and then, the percentage of growth was calculated.*
[b]*The limit of determination of the method used in the present trial was 2 µg/kg.*

utes, filtered, and diluted with distilled water. Then, the assessment was done with 50 µg/lit of the sample. After that, fumonisin concentration was calculated in a dry weight basis. Moisture content of the samples was also determined by drying a 10g sample in an oven at 105°C for 17 h. The determination limit of the method used in the present trial was 2 µg/kg.

Statistical analysis

The relationship between different concentrations of the chemical compounds and mold growth was assessed using Spearman's correlation test ($p<0.05$). Additionally, the fumonisin levels observed in the control groups were compared using one-way Analysis of Variance (ANOVA) and Tukey's test with a probability value of 0.05 to determine the statistical significance. The association between the concentrations of chemical acids and mold count was also determined by ANOVA and Duncan's test ($p<0.05$). All the analyses were performed using the SPSS statistical software, version 21.

Results

All the corn samples in the control groups were positive for FB1. Table 1 presents the results of fumonisin and FB1 was detected in the control groups. Accordingly, the percentages of fusarium and total mold contamination in the control groups were averagely 35% and 77%, respectively. Besides, a significant difference was observed between the oven-dried and the two air-drying methods in the control groups regarding the percentage of *Fusarium* and total mold contamination ($p<0.05$) (Table 1). The study results indicated a significant difference between the air-dried and oven-dried methods concerning the level of FB_1 content ($p<0.05$), but no significant difference was found between the two air-drying methods in this regard ($p>0.05$).

The effect of chemical compounds on fungal growth and FB_1 production by Fusarium

All the chemical compounds screened in the present trial were almost effective compared to the controls. The results revealed a correlation between treatment with chemical compounds and reduction in mold growth.

Propionic acid

At the 1.5% concentration, propionic acid exerted inhibitory effects on *Fusarium* growth and FB_1 production. However, *Fusarium* was detected in the samples treated with propionic acid at 0.5% and 1% levels. Hence, it could be concluded that these two concentrations had a tolerable effect on prevention of fusarium growth and FB_1 production. On the other hand, no remarkable impact was observed on prevention of other molds growth. Also, no significant differences were found between different concentrations with respect to reduction of colony counts in this group ($p>0.05$) (Table 2).

Benzoic acid

Benzoic acid reduced the colony counts of total molds. Additionally, increasing the concentration to 1% and 1.5% led to more severe reduction of colony counts ($p<0.05$). It should also be mentioned that complete suppression of *Fusarium* growth and FB_1 production occurred at all levels (0.5-1.5%) (Table 2).

Table 2. The efficacy of chemical compounds in preventing *Fusarium verticillioides* growth and FB_1 production

	Concentration (%)	Total mold growth[a] (%)	*Fusarium* growth[a] (%)	FB1 content[b] (ppm±SD)
Propionic acid (v/v)	0.5	90	5	0.3 ± 0.08
	1	30	10	0.5 ± 0.05
	1.5	85	0	ND[c]
Benzoic acid (w/v9)	0.5	70	0	ND
	1	35	0	ND
	1.5	25	0	ND
Sorbic acid (w/v)	0.5	15	0	ND
	1	15	0	ND
	1.5	10	0	ND
Sodium metabisulphite (w/v)	0.5	20	0	ND
	1	20	0	ND
	1.5	0	0	ND

[a] *Number of corn kernels in each group was 20 and then, the percentage of growth was calculated.*
[b] *The limit of determination of the method used in the present trial was 2 µg/kg.*
[c] *ND, not detected*

Sorbic acid and sodium metabisulphite

These two groups of chemical compounds showed similar results at 0.5%, 1%, and 1.5% concentrations; both completely inhibited *Fusarium* growth and FB_1 production. Also, no significant difference was observed among the three levels with regard to reduction in colony counts ($p>0.05$). Moreover, sodium metabisulphite at the highest level (1.5%) perfectly prevented all the molds and the samples treated with this compound remained sterile (Table 2).

Sulfur dioxide gas

Sulfur dioxide gas (SO_2) (5ppm) suppressed all the molds, *Fusarium* growth, and FB_1 production at both 6- and 12-hour exposure times (Table 3).

Discussion

Maize is a plant, which is most susceptible to contamination by *Fusarium* species. FB_1 production by *F. verticillioides* can occur in maize kernels before harvest, after harvest prior to drying, pre-storage and storage periods, and during processing into food and feed products (14). Prevention of mycotoxins contamination is essential since there are few ways to completely overcome health problems in human and animals (15). The United States Food and Drug Administration (FDA) has provided a guideline to allow a maximum content of 2 ppm fumonisin in corn and corn products for human consumption (16). Our results showed that the chemical compounds at all concentrations (0.5-1.5%) reduced FB1 content lesser than 2 ppm in corn. Nevertheless, the air-dried methods presented ineffectiveness to inhibit FB1 content above than 2 ppm. Ghosh et al. (1996)(17) reported complete inhibition of mold

Table 3. The efficacy of sulfur dioxide gas (SO_2) in preventing *Fusarium verticillioides* growth and FB_1 production

	Exposure time (hr)	Total mold growth[a] (%)	Fusarium growth[a] (%)	FB1 content[b] (ppm±SD)
So_2 gas	6	0	0	ND[c]
So_2 gas	12	0	0	ND

[a] *Number of corn kernels in each group was 20 and then, the percentage of growth was calculated.*
[b] *The limit of determination of the method used in the present trial was 2 µg/kg.*
[c] *ND, not detected*

growth and aflatoxin biosynthesis by *A. flavus* by 0.5% propionic acid. Propionic acid at 0.1% concentration was also the most effective anti-fungal compound with 100% inhibition of aflatoxin biosynthesis in the study by Gowda et al. (2004)(18). Nonetheless, the present study showed that a higher concentration (1.5%) of propionic acid was needed for complete inhibition of *Fusarium* growth and fumonisin production. Biro et al. (2009) (19) demonstrated that application of sodium benzoate was effective in inhibition of fumonisin formation. Dixit and Singh (2012) (20) also indicated that benzoic acid was the most potent inhibitor of fumonisin elaboration and complete inhibition of FB_1 production was recorded at 0.2% concentration. Another study also disclosed that benzoic acid at 0.2% concentration completely inhibited aflatoxin production (18). On the contrary, the current study revealed complete suppression of *Fusarium* growth and FB_1 production at 0.5%, 1%, and 1.5% concentrations of benzoic acid. Of course, 1% and 1.5% concentrations resulted in more severe reduction of colony counts of total mold. Young et al. (1987) (21) reported transformation of 85% of Deoxynivalenol (DON) into DON-sulfonate in a contaminated maize (4.4 ppm) after treatment by sodium bisulfite solutions at 80˚C for 18 h. Dixit and Singh (2012) (20) also applied potassium metabisulphite and sodium metabisulphite and observed complete inhibition of FB1 production at 0.4% concentration. In our study, sodium metabisulphite at 1.5% concentration perfectly prevented all the molds, fusarium growth, and FB_1 production. It should also be noted that sodium metabisulphite at 0.5%, 1%, and 1.5% concentrations completely inhibited *Fusarium* growth and FB_1 production. Moreover, sorbic acid at 0.5%, 1%, and 1.5% concentrations completely inhibited *Fusarium* growth and FB_1 production. Similarly, Dixit and Singh (2012) (20) reported that sorbic acid at low concentration levels (0.2%) resulted in 86.66% inhibition. Pujol et al. (1999) (22) stated that steeping at 0.2% SO_2 aqueous solution at 60˚C for 6 h might reduce FB_1 content of the naturally contaminated corn kernels. Our results also demonstrated that SO_2 gas (5ppm) suppressed all the molds, *Fusarium* growth, and FB_1 production at both 6- and 12-hour exposure times. According to the EMAN (2000), methods for decontaminating mycotoxins, such as the fumonisin content of food, should be easy to use and economical and should not lead to formation of compounds that are still toxic, may reverse to reform the parent mycotoxin, or alter the nutritional and palatability properties of the grain or grain products (13). On the other hand, performance of each procedure for decontaminating mycotoxins may alter by dissimilar conditions. For example, the inhibitory effect of sorbic acid increased at low pH levels (23) or there were significant different tolerances among strains from different parts of the world due to the infection agents and concentrations (24). Thus, further studies are required to be conducted on the issue.

Conclusion

In conclusion, among the chemical compounds applied in this study for evaluation of their anti-fungal properties, SO_2 gas (5 ppm) and sodium metabisulphite at 1.5% concentration were the most effective with 100% inhibition of fumonisin biosynthesis by *F. verticillioides*. In addition, not only did SO_2 gas (5 ppm) and sodium metabisulphite at 1.5% concentration completely inhibited *Fusarium* growth, but they also prevented the growth of all fungi and did not show any molds on the relevant plates. Other compounds could not suppress all fungal growth generally, but limited them. Considering the importance of *Fusarium* in fumonisin production, the following compounds could inhibit its growth: propionic acid at 1.5% concentration, benzoic acid, sorbic acid, and sodium metabisulphite at all levels. Furthermore, when there is no possibility of using chemical compounds for drying corns, applying oven (50˚C) is the best treatment method.

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