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Full Length Research Paper

Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins

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Four storage systems of maize commonly used by farmers in Benin, West Africa, were tested to determine their impact on infection of maize by *Fusarium* and subsequent contamination with fumonisins. The study showed that *Fusarium* incidence was significantly higher when maize was stored on a cemented floor in a house, a non ventilated facility (40.3 ± 17.4%), than in the other tested systems ($p < 0.05$). The lowest *Fusarium* incidence was recorded when maize was stored in a bamboo granary that is a ventilated facility (25.5 ± 13.5%) ($p < 0.05$). All maize samples from the tested storage systems were found to be fumonisin-positive, with levels ranging from 0.6 to 2.4 mg/kg. Fumonisin level, overall, was found to decrease over the storage period, but not significantly in all the tested storage systems. Damage by lepidopterous pests was significantly and positively correlated with both infection of maize with *Fusarium* and contamination by fumonisin. In contrary, damage by coleopterous insects was significantly and negatively correlated with infection of maize with *Fusarium* and contamination by fumonisin. Avoiding the use of non-ventilated systems to store maize and reducing insect infestation in field and during storage are very important recommendations for farmers.

Key words: Maize, storage systems, *Fusarium*, fumonisins, insect infestation.

INTRODUCTION

In Benin like in most Sub-Sahara African countries, maize is generally harvested late and is stored in cob form either in wooden granaries, under the roofs of the farmers' houses, or on floor in houses (Fiagan, 1994). Maize is also stored in grain form in clay containers, mud silos, or in bags. Most of these systems create inadequate storage conditions unfavourable for good drying of maize, particularly in humid and semi-humid zones. They consequently promote fungal infection and subsequent production of mycotoxins. There are two important mycotoxigenic fungi mostly found associated with stored maize. These are *Aspergillus flavus* that produces aflatoxins (Hell et al., 1995), and *Fusarium verticillioides* (previously known as *F. moniliforme* Sheldon), which produces fumonisins (Marasas et al., 1979).

Fumonisins are recently discovered mycotoxins (Gelderblom et al., 1988). They cause fatal diseases in horses and swine, possess cancer-promoting activity in

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rats, and are associated with porcine pulmonary oedema (Nelson et al., 1994). Oesophageal cancer in humans has been related to consumption of maize with high concentrations of fumonisins (Marasas, 1995; Wang et al., 2000). The International Agency for Research on Cancer (IARC), consequently, has evaluated fumonisin FB_1 as possibly carcinogenic to humans, belonging to the group 2B carcinogens (IARC, 2002).

It is likely that storage systems of maize with components such as time of harvest, type of storage structure, hygiene and insect infestation, interact and influence fungal infection and mycotoxin contamination. Hell et al. (2000) found higher aflatoxin levels when maize was stored under or on top of the roof of farmers' houses, than in ventilated granaries. In Nigeria, Udoh et al. (2000) showed that insect infestation in maize stores was correlated with aflatoxins.

With respect to *Fusarium* infection and fumonisin contamination in maize, recent countrywide surveys conducted in Benin showed high levels of fumonisin in many villages whereas maize cobs were stored in various storage systems (Fandohan et al., 2005). These latters were suspected, among others, to be linked to the elevated fumonisin levels (Gnonlonfin, 2000). The present study was undertaken to further consider the influence of different storage systems commonly used in Benin, West Africa, on the natural occurrence of both *Fusarium* and fumonisin contamination in maize. The effect of insect damage on *Fusarium* infection and subsequent fumonisin contamination in maize was also evaluated.

MATERIALS AND METHODS

Research site

The experiment was conducted in a village situated in the Southern Guinean Savannah of Benin. The annual rainfall pattern of this zone is bimodal with precipitation averaging 1100 – 1500 mm, allowing for two maize growing seasons. Maximum temperatures range from 26 to 35°C and annual relative humidity averages 85 – 90%. In this village, maize is commonly harvested late (about one month after maturity), and cobs with husks are usually stored on floor in house.

Maize cultivar

The maize cultivar used was the 90-day cultivar DMR-ESR-W, an improved IITA variety most commonly recommended to farmers in Benin. This variety has been found to be resistant to downy mildew (*Peronosclerospora sorghi*) and to maize streak virus (Schulthess et al., 2002).

Experimental design

Three improved indigenous storage systems were tested along with the traditional system, all arranged in a randomised block design, and replicated three times. These were:

- Maize cobs with husks, harvested late, and stored for 8 months on cemented floor in a house (traditional system);
- Maize cobs with husks stored for 8 months in an aerated woven bamboo granary (improved system);
- Maize cobs with husks stored for 8 months on a platform (improved system);
- Maize cobs with husks stored for 4 months on a platform, shelled, and grains stored in a mud silo for 4 months (improved system).

The bamboo granary, platform and mud silo were installed outside, each covered with a thatched roof. They were not accessible to rodents. Each wooden pole supporting the bamboo granary and platform had a simple but effective device to exclude rodents. There was no insect control.

Sampling method

During storage, 50 cobs were randomly sampled from each system at 0, 1, 4, 5, 7 and 8 months after stocking. The cobs were collected in each granary at top (10 cobs), in centre and on sides of the granary (30 cobs), and at bottom (10 cobs). Thereafter, the cobs were dehusked and manually shelled. A sub-sample of 1 kg of grains was taken from each sample. This sub-sample was divided into two lots. The first lot (700 g), unground, was intended for grain moisture, insect and fungal evaluations, whereas the second (300 g), ground, was intended for fumonisin analyses.

Laboratory analyses

Grain moisture content was determined on-farm just after sampling using an electronic moisture meter (model HOH-EXPRESS HE 50, PFEUFFER, Germany). Insect evaluation was performed by sieving each maize sample; all insects found were collected, counted and identified using keys from Weidner and Rack (1984) and NRI (1991). Damage caused to grains by lepidopterous and coleopterous insects was separately assessed on the basis of a 1000-grain sample (Pantenius, 1988).

Mycological analyses were performed using the plating method. Four replicates of 25 grains (100 grains) from each sample were surface disinfested in a 10% sodium hypochlorite solution for 2 min and rinsed twice in distilled water. The grains were plated in Petri dishes containing Potato Dextrose Agar (PDA) with five grains per Petri dish. The Petri dishes with grains were incubated for five days at 25°C exposed to a 12:12-hour light and dark regime, after which fungal genera were identified (Singh et al., 1991). Thereafter, *Fusarium* species were isolated, transferred onto carnation leaf agar (CLA) in Petri dishes and incubated at 25°C for seven days exposed to a 12:12-hour light/dark regime. *Fusarium* species were identified using keys from Nelson et al. (1983) and Pitt and Hocking (1999).

Total fumonisin content was determined in samples collected at 0, 4 and 8 months after stocking using the VICAM method (VICAM Science Technology, 1998), as described by Fandohan et al. (2005).

Statistical analyses

Statistical analyses were performed using SPSS for Window version 10.0 (SPSS Inc., Chicago, Illinois). Analysis of variance (ANOVA) and Tukey's HSD test were used to compare the means of fungal incidence and total fumonisin level detected in each storage system and throughout the storage period. Pearson correlation test was used to assess relationships among *Fusarium* incidence, fumonisin level and damage by lepidopterous and

Table 1. Mean fungal incidence in maize in different storage systems over all storage period.

Storage systems	No. of samples	Infected maize grains (%)		
		Fusarium	*Aspergillus*	*Penicillium*
Storage in bamboo granary	18	25.5 ± 13.5 a	24.4 ± 11.0 a	19.9 ± 8.0 a
Storage on platform	18	37.1 ± 9.2 ab	33.8 ± 16.9 ab	24.5 ± 10.1 a
Prestorage on platform + storage in mud silo	18	30.2 ± 15.8 ab	33.5 ± 16.3 ab	23.3 ± 9.7 a
Storage on cemented floor in house	18	40.3 ± 17.4 b	46.8 ± 23.6 b	40.5 ± 18.2 b

No. of samples: Number of maize samples collected from each storage system during the storage period
Values shown are the mean (± Standard Deviation) percentage of maize grains infected by fungi in the storage systems
Means in a column followed by the same letter are not significantly different from each other (Tukey HSD, P = 0.05)

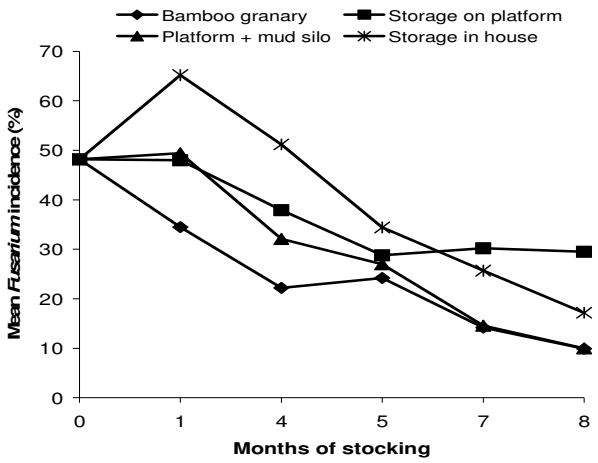

Figure 1. Changes in *Fusarium* incidences in the different storage systems throughout the storage period.

coleopterous insects. Before analyses, insect numbers were transformed to log (x+ 1), but the data are presented untransformed.

RESULTS

The major fungal genera encountered on maize in the tested storage systems were *Fusarium* sp., *Aspergillus* sp. and *Penicillium* sp. Their incidence was higher on the maize stored on the cemented floor in a house compared to the other systems (Table 1). *F. verticillioides* was the *Fusarium* species mostly found in all the storage systems. Overall, its incidence was significantly higher when maize was stored on cemented floor in a house (40.3 ± 17.4%) and lower in the maize stored in the bamboo granary (25.5 ± 13.5%) ($p < 0.05$) (Table 1). There was a significant decrease in *Fusarium* incidence throughout the storage period in all the storage systems ($p < 0.01$). This decreasing trend was much lower in the maize stored on the cemented floor in a house, than in the other systems (Figure 1). Unexpectively, *Fusarium* incidence increased in the maize stored on platform from the fifth month. In contrast to *Fusarium*, *Aspergillus* incidence significantly increased in all the systems throughout the storage period ($p < 0.01$), and this trend was markedly greater in the maize stored on floor in a house (Figure 2).

All maize samples from the different storage systems were found to be fumonisin-positive, with levels ranging from 0.6 to 2.4 mg/kg (Table 2). Fumonisin contamination did not significantly differ from one storage system to another ($p > 0.05$). Higher fumonisin levels were detected in freshly harvested maize (2.4 mg/kg) i.e at 0 month of storage. A decrease trend of the toxin level was generally observed in all the tested systems throughout the storage period, but this decrease was significant only in the bamboo granary ($p < 0.05$) (Figure 3). The toxin level in the maize stored on platform firstly decreased from 0 to 4 months of storage as in all the storage systems, but slightly increased later (Figure 3).

With respect to the presence of insects, coleopterous species were predominant throughout the storage period. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), *Cathartus quadricollis* Guerin (Coleoptera: Cucujidae), *Tribolium* sp. and *Carpophilus* sp. were the species and genera mostly found in all the storage systems. These insects became numerous from the fourth month of storage (Table 3). *P. truncatus* was most common in both the bamboo granary (318.1) and the platform (54.5) (data not shown). The lepidopterous insects were rather lesser in the systems, most frequently found at harvest and during the first month of storage (Table 3). The borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) and to a lesser extent the cob feeder *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) were mostly encountered.

Table 2. Mean total fumonisin level in maize samples in different storage systems over all storage period.

Storage systems	No. of samples	Total fumonisin level (mg/kg)	
		Range	Mean
Storage in bamboo granary	9	0.9 – 2.4	1.8 ± 0.5
Storage on platform	9	1.5 – 2.4	2.0 ± 0.4
Prestorage on platform + storage in mud silo	9	0.6 – 2.4	2.0 ± 0.5
Storage on cemented floor in a house	9	1.7 – 2.4	2.2 ± 0.5

No. of samples: Number of samples collected from each storage system during the storage period
Values shown are the range and the mean (± Standard Deviation) total fumonisin level detected in the maize collected from each storage system

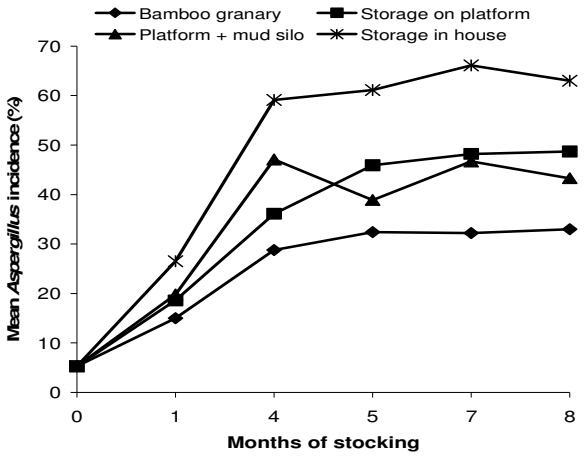

Figure 2. Changes in *Aspergillus* incidences in the different storage systems throughout the storage period.

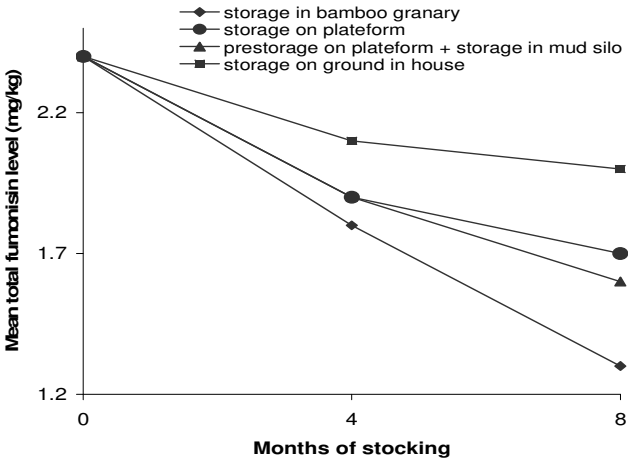

Figure 3. Changes in total fumonisin level in maize in the different storage systems throughout the storage period.

Significant positive correlations were found between the number of lepidopterous insects and *Fusarium* infection. Both *M. nigrivenella* and *C. leucotreta* were significantly related to *Fusarium* infection ($r = 0.653$, $p < 0.01$) and ($r = 0.438$, $p < 0.01$), respectively, and to fumonisin contamination ($r = 0.834$, $p < 0.01$) and ($r = 0.631$, $p < 0.01$), respectively (Table 4). A significant and positive relationship was also found between the lepidopterous insect damage and both *Fusarium* infection ($r = 0.802$, $p < 0.01$) and fumonisin contamination ($r = 0.852$, $p < 0.01$). On the other hand in contrary, both the number and damage of the coleopterous insects were significantly and negatively correlated with *Fusarium* infection and fumonisin contamination (Table 4).

DISCUSSION

These results showed that storing maize on a cemented floor in a house is not an appropriate practice. This system that is commonly used in several parts of Benin and in many other West African countries appears to be more favourable for fungal infection and fumonisin contamination, compared to the other tested storage systems. It is often observed that storage conditions in farmers' houses are confined. Maize cobs are usually stored in a corner of the house, which is not always well ventilated. In such conditions, grain moisture content diminishes slowly, mainly during the first months of the storage period, and remains high enough to promote fungal infection and mycotoxin development. In a recent study in Benin, Hell et al. (2000) found that storing maize in non-ventilated conditions such as under the roof of the farmer's house has a higher risk of aflatoxin development. These authors related their findings to the fact that this storage system is used in the humid zones of Benin, where the rainfall pattern is bimodal with averages ranging from 1200 mm to 1500 mm, the mean relative humidity of air mostly around 90%, and part of the harvest occurring during a rainy period. High humidities, therefore, are likely to create suitable

Table 3. Mean number of insects in maize (per 1 000 grains sample) during the storage period.

Months after stocking	*Mussidia*	*Cryptophlebia*	*Sitophilus*	*Prostephanus*	*Tribolium*	*Carpophilus*	*Cathartus*
0	12.7	2.0	4.0	0	0.7	5.3	8.7
1	2.5	0.4	18.6	0	0.3	5.7	46.8
4	0	0	295.8	32.5	33.1	21.3	285.7
5	0	0	671.8	143.3	107.6	18.3	473.0
7	0	0	641.1	253.8	110.7	16.4	710.4
8	0	0	570.7	134.2	100.7	23.9	633.4
Overall	2.5	0.4	367.0	94.0	58.8	15.2	359.7

Table 4. Correlation among insect infestation, *Fusarium* incidence and fumonisin contamination in maize.

Parameter	Lepidopterous insects			Coleopterous insects					
	Number of *Mussidia*	Number of *Cryptophlebia*	Grain damage (%)	Number of *Sitophilus*	Number of *Prostephanus*	Number of *Tribolium*	Number of *Cathartus*	Number of *Carpophilus*	Grain damage (%)
Fusarium incidence	+0.653**	+0.438**	+0.802**	-0.611**	-0.642**	-0.573**	-0.339**	-0.463**	-0.645**
Fumonisin level in maize	+0.834**	+0.631**	+0.852**	-0.792**	-0.671**	-0.696**	-0.505**	-0.641**	-0.581**

** Correlation is significant at the 0.01 level (2-tailed).

conditions for fungal growth and mycotoxin production, mainly with respect to *Fusarium* in such a non-ventilated storage system.

In contrast, the woven bamboo granary and platform are typically ventilated storage structures. They are potentially less favourable for fungal growth and results obtained in this study showed them to be less conducive to *Fusarium* infection and fumonisin development during maize storage. Hell et al. (2000) also found ventilated bamboo granaries to be associated with lower aflatoxin levels in the humid regions. It is thought that this structure allows maize grains to dry more rapidly, provided that its diameter is not more than 2 m (FAO, 1992). The increase of *Fusarium* incidence observed in the platform system after 5 months of storage is not encouraging. Humidity build-up was noted later in parts of the maize stored on two of the platforms used during the study, likely due to rain leakage in the thatched roof found to be defective in places, which probably promoted fungal growth and subsequent mycotoxin production.

Regarding the mud silos, they are durable granaries, better adapted to dry regions, but non-ventilated storage facilities. Mud silos are considered as favourable for fungal infection and mycotoxin development, mainly if grain moisture content is above 15% or if storage occurs during the rainy season, where the relative humidity of air is higher than 90% (Fandohan, 2000). Prasad et al. (1987) found mud silos in India associated with high aflatoxin contamination. Hell et al. (2000) postulated that humidity build-up might occur through convection, permitting spores of fungi to persist for a long time in this granary, leading to a high risk of aflatoxin contamination. To overcome this risk, maize should be sufficiently dried (moisture content less than 15%) to ensure unfavourable conditions for fungal growth. The system used in this study, consisting in prestoring maize on a platform until drying before its final storage in a mud silo, appears suitable. This system significantly reduced grain moisture content from 22.5% at harvest to less than 11% after four months, creating unfavourable conditions for *Fusarium* growth.

Although the storage systems tested in this study significantly influenced *Fusarium* infection in stored maize, their effect on fumonisin contamination was not quite meaningful as, overall, there was no significant difference from one system to another in this respect. Previous studies on aflatoxins, however, found that storage systems significantly influenced aflatoxin contamination in maize (Hell et al., 2000; Udoh et al., 2000). Whereas aflatoxins are potentially serious problems during storage, fumonisin production primarily occurs in the field before harvest unless storage conditions become favourable (high relative humidity, high moisture content of stored maize) (Munkvold and Desjardins, 1997; Bolger et al., 2001).

Though not significant in most of the tested systems, the decrease trend in fumonisin level observed during the storage period is reassuring with respect to the stability of

the toxin in contaminated stored grains. Instability of fumonisins was also found in previous studies in naturally or artificially contaminated food products over time. Scott et al. (1999) found fumonisins to be unstable in naturally contaminated ground rough rice, maize starch and maize meal over storage time. Orsi et al. (2000) observed an overall decrease of fumonisin content in stored maize after 140 days of a one year-storage period in Brazil. More recently, Kim et al. (2002) found FB_1 and FB_2 to disappear completely in artificially contaminated Thai white rice flour after ten hours. These authors also observed up to 75% and 90% of decrease, respectively, in maize meal and in the flour of white rice, after two months of storage. About 30% decrease of total fumonisin B was also observed in maize cultures of *F. verticillioides* kept at 4°C over 13 - 20 years (Gelderblom et al., PROMEC, Medical Research Council, Tygerberg, South Africa, 2002, unpublished data). In contrast, Ngoko et al. (2001) found FB_1 to increase with storage time in maize collected in different zones of Cameroon.

Further studies are necessary to thoroughly explain this situation. Some factors including environmental conditions, intrinsic characteristics of stored products and chemical reactions are suspected. Munkvold and Desjardins (1997) argued against the view that fumonisin concentration increases in maize stores during storage, as long as conditions of grain moisture content and temperature are maintained at recommended levels. It is also suggested that fumonisin molecules might bind with the starch of the product during storage to form a complex, which is not detectable (Kim et al., 2002). Scott et al. (1999) reported that reaction of FB_1 with reducing sugars such as D-glucose is likely to explain the rapid fumonisin loss observed in maize starch. Kim et al. (2002) suspected the moisture content of the product, its texture and metal ions present in the product to influence fumonisin loss. In the case of the current study, environmental conditions during storage are likely to have affected fumonisin content in maize. Dry season and rainy season alternated during the eight months of storage.

Insects, mainly lepidoptera, played an important role in *Fusarium* infection in this study. A strong relationship existed between insect damage and *Fusarium* infection. Schulthess et al. (2002) reported similar results considering the effect of *F. verticillioides* on the infestation of maize by various insects. Infact, depending on the feeding habits or preferences of the larvae of these insects, they can attack maize stems, cobs, silks or grains, cause injuries at these parts of the plant, spreading fungal inoculum within the plant during their movement and feeding (Dowd, 1998). *M. nigrivenella* usually attacks maize cobs and damages grain from the tip of the cob (Setamou et al., 1998). By boring a channel, the insect breaks the testa of grains, which constitutes a natural barrier for fungal growth, promoting easy spread of fungi. *M. nigrivenella* damage also predisposes maize to pre- and postharvest infestation with storage coleopterous insects. The latter preferentially enter the holes produced by the *M. nigrivenella* larvae, thus further enhancing in the dissemination of fungal inoculum (Setamou et al., 1998).

Coleopterous insects are found to be less implicated in the infection process of maize with *Fusarium*, although these insects were more numerous than lepidopterous insects throughout the storage time. There is, however, ample evidence of their involvement in fungal infection in field, and the sap beetles, *Carpophilus* sp. are the insects best known to spread *Fusarium* in maize (Dowd, 1998). Fennell et al. (1975) found that amongst the sap beetles collected in maize fields in Missouri, 60% were contaminated with *Fusarium* sp. and only 7% with *A. flavus.* The implication of the sap beetles in vectoring *Fusarium* may vary from one area to another within a country, and depend also on many agronomic factors including husk coverage of maize cobs, silk channel, grain pericarp thickness, the abundance of beetles, maize variety and weather conditions (Dowd, 1998). Because of their association with the damage caused by the lepidopterous pests, the presence of the sap beetles may not be recognised when evaluated at harvest, unless great care is taken to look for characteristic feeding marks and the presence of frass (Dowd, 1998). All this may explain the negative correlation found between *Carpophilus* spp. and *Fusarium* infection in this study.

Sitophilus spp. and *Cathartus* spp. were negatively and significantly correlated with the incidence of *F. verticillioides* during the storage time in this study. Cardwell et al. (2000), however, found significant and positive correlations between the number of these insects and the fungus in a previous work. These authors artificially infected maize cobs in the field whereas in the current work, maize cobs were left to be naturally infected and evaluated during an eight-month storage period.

Maize storage systems used by farmers may, therefore, influence *Fusarium* infection and subsequent fumonisin contamination. Any storage system creating conditions favourable for fungal growth and fumonisin production is not, therefore, to be recommended. Damages caused by insects are found to promote *Fusarium* infection in the tested storage systems. Consequently, any action undertaken to reduce insect infestation before harvest and during storage could help to reduce *Fusarium* infection and subsequent fumonisin contamination. Recommendations in this respect include using maize cultivars less susceptible to lepidopterous insects, harvesting without delay to avoid insect infestation in the field, sorting out damaged cobs or grains at harvest, where possible insect control, and choice of storage structure to ensure good drying. Further studies are needed to clarify instability of fumonisins in naturally contaminated food products during storage as it has been observed in the current study.

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