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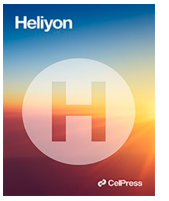

Controlling aflatoxin in maize: The effects of varieties, packaging materials, and agroecological zones

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ABSTRACT

Contamination of crops by aflatoxin is rampant in warm regions worldwide, including Sub-Sahara Africa. Contamination of maize and other foodstuffs with aflatoxin seriously threatens the health of humans and animals. The experimental design used was 2 × 2 × 3 factorial, laid out in a complete randomized design (CRD) consisting of two agroecological zones, two varieties, and three different packaging materials. At the end of the six months of storage, there was no contamination of the maize with aflatoxin G1. Again, there was no contamination of maize stored in the Forest zone with aflatoxin B1. High contamination levels of aflatoxin B1 (8.91 μg/kg), aflatoxin B2 (10.74 μg/kg), and aflatoxin G2 (14.49 μg/kg) occurred in the Wangdataa variety stored in jute. Purdue Improved Crop Storage (PICS) bags recorded lower contamination levels than jute and polypropylene (PP). Contamination was higher in the Savannah zone than in the Forest zone. The three packaging materials used gave maximum protection to all the maize stored in the Forest against aflatoxin B1 and aflatoxin G1. Farmers, traders, and all aggregators of maize in the Savannah zone should be discouraged from using jute bags to store maize in the Savannah zone for an extended period. Opeaburo should be planted and stored in the Savannah zone rather than Wangdataa. Farmers should be encouraged to use PICS bags to store maize in the Savannah zone to control aflatoxin B1, aflatoxin B2, and aflatoxin G2.

1. Introduction

Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus* [1]. Aflatoxins exist in many tropical and subtropical regions, and many crops and agricultural commodities are susceptible to aflatoxin contamination [2]. More than 20 aflatoxins have been identified, with aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), and aflatoxin M1(AFM1) being the most prevalent aflatoxins found in food [3,4]. AFs B1, B2, G1, G2, and M1 are the most significant aflatoxins because of their genotoxic carcinogenic features [5]. Aflatoxin is the most potent natural cause of cancer. It has been associated with a higher prevalence of hepatocellular cancer in Africa [6] and stunting [6–9]. Aflatoxin contamination has also been associated with animal micronutrient deficiencies [10]. Consequently, there has been global attention on aflatoxins due to their potential threat to human and animal health [11]. As part of global efforts, maximum limits have been set on the quantity of aflatoxin in products intended for direct human consumption. Maximum permissible limits for the European Union (EU), United States (US)/Ghana, and

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Kenya are 4 ng/g, 20 ng/g, 10 ng/g respectively [12].

Aflatoxin contamination of various crops also influences trade and economic growth [13–15]. Newspaper headlines on poisonous aflatoxins in kenkey, a maize-based Ghanaian staple, have repeatedly caused fear and panic among the populace [16].

Aflatoxins are pervasive and cannot be destroyed easily, but contamination can be reduced to safe and acceptable levels for human and animal consumption through strategic interventions [17]. Several pre- and postharvest interventions were developed to reduce the economic and health effects caused by aflatoxins in food and feed. These interventions, at the pre-harvest level, focus on the reduction of fungal infection in the field, such as good agricultural practices (GAP) [18,19], host plant resistance [20] and bio-control [21]. The interventions for postharvest are good manufacturing practices (GMP), which encompass harvesting at the right time and drying immediately after harvesting, as well as good transportation practices and storage improvements [9,22]. Despite the evolution of several aflatoxin control interventions, contamination remains a problem. Studies on aflatoxin contamination in Ghana continue to show significant volumes of contamination far above acceptable limits. A survey conducted by Ref. [23] showed that maize samples collected from silos and warehouses in Ejura contained aflatoxin levels in the range of 20–355 ng/g; while fermented maize dough from major processing sites contained aflatoxin levels of 0.7–313 ng/g [24]. also found high average aflatoxin levels in maize samples collected from North Kwahu (153 ng/g), Ejura Sekyerdumasi (121 ng/g), and Nkoranza (134 ng/g), considerably beyond the approved limits recommended by the USA and the European Union. Perrone et al. (2014) recently reported high aflatoxins in maize grains collected from open markets in Ghana and Nigeria [25]. also reported high levels of aflatoxins on maize samples collected from Ejura (30–70 ng/g) and Agboboloshie (102–484 ng/g) markets.

In Ghana, some studies have been conducted on occurrence, prevalence, interventions and health risk related to aflatoxins in maize and groundnuts [23–33]. However, limited studies have examined the effectiveness of the use of postharvest interventions in the various ecological zones in Ghana and its effects on aflatoxin contamination. Therefore, this research aimed to assess the effects of varieties of maize, agroecological zones, and three different storage bags on aflatoxin contamination of maize.

2. Materials and methods

2.1. Study area

The experiment was conducted in Wa in the Upper West Region and Ejisu in the Ashanti region, representing Ghana's Savannah and Forest zone, respectively, from February 2021 to April 2021. Fig. 1 is a map showing the study areas. The Forest zone has a bimodal rainfall pattern, allowing for two cropping seasons in a year. They are the major and minor seasons. In the major season, there is heavy rainfall from April to July, followed by a moist period in August [34]. The minor cropping season starts from September through to

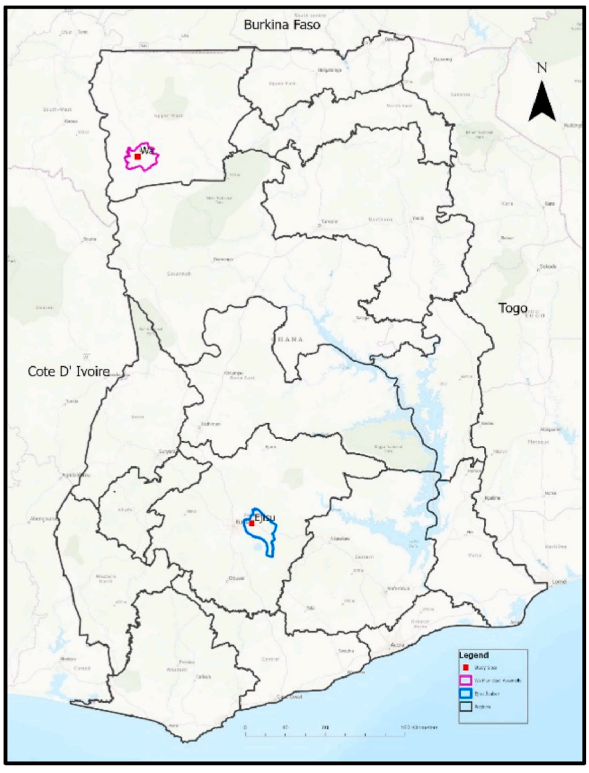

Fig. 1. Map of Ghana indicating the study areas.

November. Wa is located in Ghana's northwest, approximately between the longitudes of 9°32′W and 10°20′W and the latitudes of 1°40′N and 2°45′N. Around 579.86 Km^2 or 6.4 % of the entire landmass of the UWR make up its total land area. The elevation of Wa, which lies in the Savannah's high plains, is between 160 and 300 m above sea level [35]. The Savannah zone is characterized by a unimodal rainfall pattern, with only one cropping season (major season), from May to November [34]. December marks the beginning of the harmattan, and it continues till March. The harmattan season is characterized by a dry period and dust blown from the Sahara Desert to Ghana [36].

2.2. Experimental design for storage

The experimental design used was 2 × 2 × 3 factorial, laid out in a complete randomized design (CRD) consisting of two agro-ecological zones, two varieties, and three different packaging materials. The maize was harvested when it reached physiological maturity, and most of the cobs had dried. The cobs were handpicked, hand-shelled, and dried on tarpaulins on cemented platforms to a moisture level of 11–12 %. The samples were hygienically packed in three different storage bags. The storage bags used were in jute bags, polypropylene bags and PICS bags. Fig. 2 shows the bags used for the experiment. The maize samples were stored for six months in each agroecological zone. During the storage, the temperature and humidity of the storage room in each agroecological zone were recorded using Elitechlogger pre-programmed to record temperature and humidity every hour. The results were saved to the computer via ElitechLog software.

2.3. Laboratory analysis

2.3.1. Proximate analysis

The physicochemical analysis, such as moisture content, dry matter, crude protein, crude fat, crude fibre, ash, and carbohydrate of the maize varieties, was done using the Association of Official Analytical Chemists [37] method. All of the analyses were carried out in triplicate.

2.3.2. Aflatoxins analysis

The maize samples were sent to the Aflatoxins Laboratory of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, for aflatoxins analysis and the School of Agriculture Laboratory, University of Cape Coast, Ghana, for proximate analysis. The maize samples were analyzed for aflatoxins B1, B2, G1, G2, and total aflatoxins at the Laboratory using the method described by Ref. [38]. Extraction of aflatoxin from the maize sample was done using the protocol for analyzing aflatoxins described by Ref. [38]. The protocol was slightly modified to extract aflatoxin utilizing acetonitrile and acetic acid v/v (9:1) as the extraction solvent and additional agitation steps. Using a Preethi Mixer Grinder, samples were ground and homogenized. Transferring a 2 g sample weight into a 50 mL centrifuge tube, adding 5 mL of distilled water, and vortexing for 1 min 5 mL of the extraction solution was then added after the solution had been allowed to stand for 5 min. The resultant mixture was agitated at 250 rpm for 15 min and vortexed for 3 min with a Genie Vortex machine. The mixture was then added to a mass of 1.32 g of anhydrous $MgSO_4$ and 0.2 g of NaCl, vortexed for 1 min, and then agitated at 250 rpm for 5 min. Before injection, the upper organic layer of the tube was filtered through a 0.45 m nylon syringe after being centrifuged for 5 min at 4000 rpm. The filtered extract was injected into the high-performance liquid chromatography (HPLC) apparatus at a volume of 50 L.

2.3.3. HPLC analysis

HPLC analysis was performed using the Photochemical Reactor for Enhanced Detection (PHRED) in line with AOAC Official Method 2005.08 [39] for post-column derivatization. The determination of aflatoxins was done by using an Agilent 1200 Quaternary

Fig. 2. Picture of jute bags (a), polypropylene bags (b), and PICS bags (c).

Pump with Fluorescence Detector (Ex: 360 nm, Em: 440 nm) and Sunfire® C18 Column (150 × 4.60 mm, 5 μm). Methanol and water were used as the mobile phase at a rate of 1 ml/min and a stable 40 °C for the column. Using LCTech UVE, post-column derivatization was achieved. Romer Labs® aflatoxin standard of 5.02 ng/L in acetonitrile served as a basis for preparing Aflatoxin Mix (G1, G2, B1, B2) standards (ng/g). Aflatoxin concentrations in the samples were determined using the retention standard solution runs and calibration curves for each toxin. Figs. 3–5 represent the standard chromatograph, contaminated and aflatoxin-free chromatographs.

2.3.4. Method performance

Linearities for Aflatoxin G2, G1, B2, and B1 were 0.998, 0.999, 0.999, and 0.998, respectively. The limit of Quantification was Aflatoxin G2 (0.2 ppb), Aflatoxin G1 (0.1 ppb), Aflatoxin B2 (0.2 ppb) and Aflatoxin B1 (0.1). Recovery tests were performed to evaluate precision and accuracy. Blank samples were spiked at 5 (five) replicated maize samples at 13 ng/g, 26 ng/g, and 104ug/g with recoveries of 97 ± 1.07 %, 98 ± 1.35 %, and 99 ± 0.93 %, respectively. Periodically ran blanks had no detectable level of the desired analyte. Trueness was further validated using certified reference material (TR-A1000) from Triology Laboratory, USA. The value obtained from ten replicates was 20.65 ± 0.71 μg/kg and was within the acceptable range of the certified value of 21.0 ± 2.9ug/kg. g/kg. For replicates, the coefficient of variance was less than 15 %. By spiking blank samples with an aflatoxin standard, quality assurance was established by testing for accuracy and truthfulness. Run-off blank samples that were confined to the absence of aflatoxins. Less than 15 % of the variation was found in the coefficient of variation for replicates. Aflatoxin concentration was estimated as,

Aflatoxin, ng/g = A x (T/I) x (1/W)

Where, A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (ul),

I = volume eluate injected into LC (ul), W = mass (g) of a commodity represented by the final extract.

2.3.5. Data analysis

The data sets were analyzed as a complete randomized design (CRD). Differences between treatment means were separated by Fischer's Least Significant Difference at 1 % probability level. Shapiro – Wilk normality test was conducted, Analysis of Variance (ANOVA) was used to determine significant differences among samples using the statistics 9.1 statistical package.

3. Results and discussion

3.1. Temperature and humidity

Tables 1 and 2 present data on temperature and humidity during the storage period for the Forest and Savannah zones, respectively, while Table 3 presents results on the proximate analysis of the maize varieties.

From Tables 1 and 2, except for November (27.9 °C), the Savannah zone recorded higher temperatures than the Forest zone during storage. The average temperature during the storage time for the Savannah zone (30.9 °C) was higher than the Forest zone (28.8 °C). Generally, the Forest zone recorded higher average humidity (76.7 %) than the Savannah zone (44.8 %).

From Table 3, no significant difference was recorded among the following parameters: moisture, dry matter, crude fibre, ash, and carbohydrate in the two varieties. Although there was no significant difference, Wangdataa had higher dry matter (91.30 %), ash (1.26 %), and carbohydrate (78.55 %) than Opeaburo. There was a significant difference between crude fat and crude protein.

3.2. Results of maize samples before storage

There was no contamination of the maize samples with aflatoxins AFB1 and AFB2 for all the treatments in the two ecozones. The mean concentration of AFG1 before storage at ecozones was 0.15 μg/kg and 0.00 μg/kg for the Savannah and Forest zones, respectively, while Opeaburo and Wangdataa recorded means of 0.08 μg/kg and 0.07 μg/kg respectively before storage (Table 4). The concentration of AFG2 before storage based on ecozones was 0.06 μg/kg

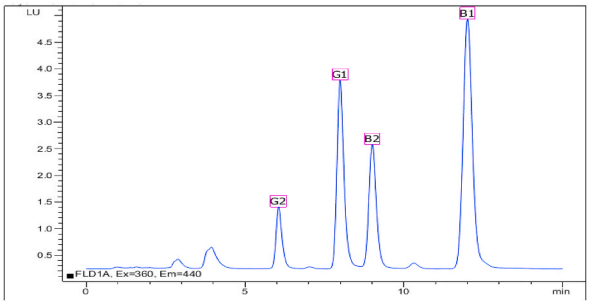

Fig. 3. Standard Chromatograph for Aflatoxins (G2 = 0.5 ppb, G1 = 2 ppb, B2 = 0.5 ppb, B1 = 2 μg/kg).

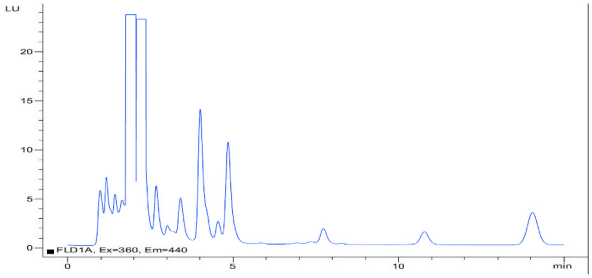

Fig. 4. Chromatograph for aflatoxin-contaminated maize sample.

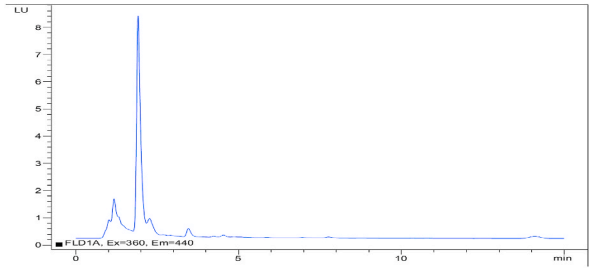

Fig. 5. Chromatograph for aflatoxin-free (blank) maize sample.

Table 1
Temperature and humidity data for Forest Zone during storage.

Month	Temperature(°C)	Humidity (%)
February	29.8	63.0
March	28.2	74.6
April	28.6	74.7
May	28.6	79.6
June	27.4	82.1
July	27.3	86.5
Average	28.3	76.7

Source: Field data 2021

Table 2
Temperature and humidity data for Savannah zone during storage.

Month	Temperature(°C)	Humidity (%)
November	27.9	83.5
December	30.3	34.5
January	30.2	21.7
February	32.5	21.7
March	33.4	44.8
April	31.3	62.8
Average	**30.9**	**44.8**

Source: Field data 2021

for the Savannah and 0.00 μg/kg for the Forest zone. Opeaburo recorded 0.00 μg/kg, and Wangdataa recorded 0.06 μg/kg contamination levels (Table 4). The concentration level before storage for all the types of aflatoxin was <0.1 μg/kg.

3.3. Effect of ecozones and varieties on AFB1 contamination after storage

The interaction of ecozones and maize varieties is statistically significant (Table 5). Higher AFB1 contamination (6.22 μg/kg) was produced by the Wangdataa variety stored in the Savannah ecozone, followed by Opeaburo (1.79 μg/kg) also stored in the Savannah zone. There was no contamination of the maize samples with AFB1 (0.00 μg/kg) for both varieties stored in the Forest zone. For the varieties only, Wangdataa gave a higher AFG1 (3.11 μg/kg) contamination and recorded lower contamination by Opeaburo (0.89 μg/kg). For the ecozones only, maize stored in the Savannah had a higher AFB1 contamination (4.00 μg/kg).

Table 3
Proximate composition of Wangdataa and Opeaburo maize varieties.

	Wangdataa (%)	Opeaburo (%)	LSD
Moisture	8.69a	8.76a	0.13
Dry matter	91.30a	91.24a	0,22
Crude protein	13.01a	12.41b	0.37
Crude fat	3.94b	4.15a	0.13
Crude fibre	3.41a	3.75a	0.38
Ash	1.26a	1.15a	0.37
Carbohydrate	78.55a	78.35a	0.63

Source: Field data 2021

Table 4
Level of contamination of maize before and after storage.

AEZ	VAR	PA	CAH B1 (μg/kg)	CAS B1 (μg/kg)	% inc B1 (%)	CAH B2 (μg/kg)	CAS B2 (μg/kg)	% inc B2 (%)	CAH G1 (μg/kg)	CAS G1 (μg/kg)	% inc/red G1 (%)	CAH G2 (μg/kg)	CAS G2 (μg/kg)	% inc G2 (%)
Savannah	O	Jute	0.00	6.04^{ab}	100	0.00	5.15^{ab}	100	0.16	0.00	**100**	0.00	15.72^{a}	100
		Poly	0.00	4.71^{ab}	100	0.00	0.00^{b}	0	0.16	0.00	**100**	0.00	14.15^{a}	100
		PICS	0.00	0.00^{b}	0	0.00	0.00^{b}	0	0.16	0.00	**100**	0.00	7.42^{ab}	100
	W	Jute	0.00	13.99^{a}	100	0.00	13.97^{b}	100	0.14	0.00	**100**	0.00	15.72a	99
		Poly	0.00	0.00^{b}	0	0.00	6.37^{ab}	100	0.14	0.00	**100**	0.00	11.42^{ab}	99
		PICS	0.00	0.85^{b}	100	0.00	0.00^{b}	0	0.14	0.00	**100**	0.00	8.41^{ab}	99
Forest	O	Jute	0.00	0.00^{b}	0	0.00	0.26^{b}	100	0.00	0.00	0	0.00	11.89^{ab}	100
		Poly	0.00	0.00^{b}	0	0.00	0.32^{b}	100	0.00	0.00	0	0.00	11.97^{ab}	100
		PICS	0.00	0.00^{b}	0	0.00	0.07^{b}	100	0.00	0.00	0	0.00	9.88^{ab}	100
	W	Jute	0.00	0.00^{b}	0	0.00	2.68^{ab}	100	0.00	1.03^{a}	100	0.00	11.17^{ab}	100
		Poly	0.00	2.49^{b}	100	0.00	2.54^{ab}	100	0.00	2.49^{a}	100	0.00	8.81^{ab}	100
		PICS	0.00	0.00^{b}	0	0.00	0.17^{b}	100	0.00	0.00	0	0.00	3.49^{b}	100

Significance levels, *($P < 0.05$) and ** ($P < 0.01$) for testing the differences between treatments.
AEZ, Agroecological zones; VAR, Variety; PA, Packaging Materials; CAH, Concentration at harvest; CAS, Concentration at storage; O, Opeaburo; W, Wangdataa.
% inc, % increase = {(CAS - CAH)/CAS} *100.
% red (*bold), % reduction = {(CAH - CAS)/CAH} *100.

Table 5
Effect of ecozones and variety on AFB1contamination after storage.

Varieties	Ecozones		
	Savannah	Forest	Means
Opeaburo	1.79^{b}	0.00^{c}	0.89^{b}
Wangdataa	6.22^{a}	0.00^{c}	3.11^{a}
Means	4.00^{a}	0.00^{b}	

HSD (0.01)	DF: 1
Varieties = 0.31rowhead	F: 400.03
Ecozones = 0.31rowhead	P: 0.0000
Varieties* Ecozones = 0.54rowhead	SE: 0.1563

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

3.4. Effect of ecozones and packaging materials on AFB1 contamination after storage

The interaction of ecozones and packaging materials is statistically significant (Table 6). Higher AFB1 contamination (9.26 μg/kg) is produced by maize samples in jute packaging material stored in the Savannah ecozones. There was no contamination (0.00 μg/kg) in all the packaging bags in the Forest zone. For the packaging materials, jute gave the highest AFB1 contamination (4.62 μg/kg), and the lowest was PICS (0.21 μg/kg). For the ecozones only, maize stored in the Savannah ecozone had a higher AFB1(4.00 μg/kg) contamination, and maize stored in the Forest zone had a lower contamination AFB1(0.00 μg/kg).

Table 6
Effect of ecozones and packaging materials on AFB1 contamination at storage.

Packaging	Ecozones		
	Savannah	Forest	Means
Jute	9.26[a]	0.00[c]	4.62[a]
Poly	2.36[b]	0.00[c]	1.18[b]
PICS	0.42[c]	0.00[c]	0.21[c]
Means	4.00[a]	0.00[b]	

HSD (0.01)	DF: 2
Packaging = 0.43rowhead	F: 589.21
Ecozones = 0.31rowhead	P: 0.0000
Packaging* Ecozones = 0.72rowhead	SE: 0.1914

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

3.5. Effect of varieties and packaging materials on AFB1 contamination after storage

The interaction of varieties and packaging materials is statistically significant (Table 7). The highest AFB1 contamination (8.91 μg/kg) was recorded by the Wangdataa maize variety stored in jute. The lowest (0.00 μg/kg) of AFB1 contamination was observed in Opeaburo stored in PICS and Wangdataa stored in poly. For the packaging materials only, maize stored in jute obtained the highest AFB1 contamination (4.62 μg/kg), and the lowest was PICS (0.21 μg/kg). For the varieties only, Wangdataa recorded a higher AFB1 (3.11 μg/kg), and Opeaburo recorded a lower contamination of AFB1 (0.89 μg/kg).

3.6. Effect of ecozones and variety on AFB2 contamination after storage

The interaction of ecozones and varieties is statistically significant (Table 8). Higher contamination of AFB2 (9.17 μg/kg) was recorded by the Wangdataa variety stored in the Savannah zone, and Opeaburo recorded a lower contamination (0.04 μg/kg) in the same zone. For varieties only, Wangdataa obtained a higher contamination of AFB2 (4.68 μg/kg). For the ecozones only, maize stored in the Savannah zone had a higher contamination of AFB2 (4.60 μg/kg), and a lower contamination of AFB2 (0.20 μg/kg) was recorded in the Forest ecozone.

3.7. Effect of ecozones and packaging materials on AFB2 contamination after storage

The interaction of ecozones and packaging materials is statistically significant (Table 9). The highest contamination of AFB2 (10.63 μg/kg) was produced by maize samples in jute packaging material stored in the Savannah zone. The lowest contamination (0.00 μg/kg) of AFB2 was recorded by maize stored in PICS in the Savannah zone but was not significantly different from maize stored in PICS in the Forest zone. For the packaging materials only, jute gave the highest AFB2 (5.47 μg/kg) and PICS (0.06 μg/kg) lowest. For the ecozones only, maize stored in the Savannah had higher contamination of AFB2 (4.60 μg/kg).

3.8. Effect of varieties and packaging materials on AFB2 contamination after storage

The interaction of varieties and packaging materials was significant (Table 10). The highest contamination of AFB2 (10.74 μg/kg) was produced by Wangdataa stored in jute packaging material. The lowest contamination of AFB2 (0.03 μg/kg) was recorded by Opeaburo stored in PICS but was not significantly different from the Wangdataa variety stored in PICS. For the packaging materials only, jute recorded the highest contamination of AFB2 (5.47 μg/kg). For the varieties only, Wangdataa had higher contamination of

Table 7
Effect of varieties of maize and packaging materials on AFB1 contamination after storage.

Packaging	Varieties		
	Opeaburo	Wangdataa	Means
Jute	0.34[c]	8.91[a]	4.62[a]
Poly	2.36[b]	0.00[c]	1.18[b]
PICS	0.00[c]	0.42[c]	0.21[c]
Means	0.89[b]	3.11[a]	

HSD (0.01)	DF: 2
Varieties = 0.31rowhead	F: 880.97
Packaging = 0.43rowhead	P: 0.0000
Packaging*Varieties = 0.72rowhead	SE: 0.1914

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

Table 8
Effect of ecozones and variety on AFB2 contamination after storage.

Varieties	Ecozones		
	Savannah	Forest	Means
Opeaburo	0.04[b]	0.22[b]	0.13[b]
Wangdataa	9.17[a]	0.18[b]	4.68[a]
Means	4.60[a]	0.20[b]	
HSD (0.01)		DF: 1	
Varieties = 0.19rowhead		F: 4428.90	
Ecozones = 0.19rowhead		P: 0.0000	
Varieties* Ecozones = 0.34rowhead		SE: 0.0974	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/k.

Table 9
Effect of ecozones and packaging materials on AFB2 contamination after storage.

Packaging	Ecozones		
	Savannah	Forest	Means
Jute	10.63[a]	0.30[c]	5.47[a]
Poly	3.18[b]	0.19[c]	1.69[b]
PICS	0.00[c]	0.11[c]	0.06[c]
Means	4.60[a]	0.20[b]	
HSD (0.01)		DF: 2	
Packaging = 0.27rowhead		F: 2019.60	
Ecozones = 0.19rowhead		P: 0.0000	
Packaging* Ecozones = 0.45rowhead		SE: 0.1193	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

Table 10
Effect of variety and packaging materials on AFB2 contamination after storage.

Packaging	Varieties		
	Opeaburo	Wangdataa	Means
Jute	0.19[c]	10.74[a]	5.47[a]
Poly	0.16[c]	3.21[b]	1.69[b]
PICS	0.03[c]	0.08[c]	0.06[b]
Means	0.13[b]	4.68[a]	
HSD (0.01)		DF: 2	
Varieties = 0.19rowhead		F: 2057.79	
Packaging = 0.27rowhead		P: 0.0000	
Packaging*Varieties = 0.45rowhead		SE: 0.1193	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

Table 11
Effect of ecozones and varieties on AFG2 contamination at storage.

	Ecozones		
Varieties	Savannah	Forest	Means
Opeaburo	11.36[a]	11.73[a]	11.54[a]
Wangdataa	12.07[a]	6.97[b]	9.52[b]
Means	11.71[a]	9.35[b]	
HSD (0.01)		DF: 1	
Varieties = 0.93rowhead		F: 66.80	
Ecozones = 0.93rowhead		P: 0.0000	
Varieties* Ecozones = 1.64rowhead		SE: 0.4730	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

AFB2 (0.13 μg/kg).

3.9. Effect of storage on AFG1

All the maize samples were not contaminated in both zones with AFG1 after storage.

3.10. Effect of ecozones and varieties on AFG2 contamination after storage

The interaction of ecozones and varieties was statistically significant (Table 11). Higher contamination of AFG2 (12.07 μg/kg) was produced by Wangdataa stored in the Savannah zone. A lower contamination of AFG2 (6.97 μg/kg) was recorded by Wangdataa stored in the Forest zone. For ecozones only, samples stored in the Forest zone recorded a lower contamination of AFG2 (9.35 μg/kg). For varieties only, Opeaburo had higher contamination of AFG2 (11.54 μg/kg).

3.11. Effect of ecozones and packaging material on AFG2 contamination after storage

The interaction of packaging materials and ecozones was statistically significant for AFG2 (Table 12). The highest contamination of AFG2 (14.95 μg/kg) was produced by maize stored in jute packaging material in the Savannah zone. Contamination of AFG2 (5.45 μg/kg) was recorded by maize stored in PICS in the Forest zone but was not statistically significantly different from maize stored in PICS (7.62 μg/kg) in the Savannah zone. For the packaging materials only, jute recorded the highest contamination of AFG2 (13.66 μg/kg. For ecozones only, maize stored in the Savannah zone had higher contamination of AFG2 (11.71 μg/kg).

3.12. Effect of varieties and packaging materials on AFG2 contamination after storage

The interaction of packaging materials and variety is statistically significant (Table 13). The highest contamination of AFG2 (14.49 μg/kg) was produced by Wangdataa stored in jute packaging material. The lowest contamination of AFG2 (4.55 μg/kg) was recorded by Wangdataa stored in PICS. For the packaging materials only, jute gave the highest AFG2 (13.66 μg/kg). For the varieties only, Opeaburo had a higher contamination of AFG2 (11.54 μg/kg).

4. Discussion

4.1. Storage environment

The production of aflatoxins can occur at a wide range of temperatures. However, the optimal temperature for aflatoxin production is 25–35 °C [40]. From Tables 1 and 2, the Savannah zone recorded higher temperatures than the Forest zone during storage. The average temperature during the storage time for the Savannah zone (30.9 °C) was higher than that of the Forest zone (28.8 °C). The average temperature during the storage time was within the optimal temperature for producing aflatoxins. According to Ref. [41], 95 % relative humidity increases the production of aflatoxins considerably. The Forest zone recorded higher humidity than the Savannah zone during storage. About 85 % of relative humidity is optimal for producing aflatoxins [41]. With the exemption of the relative humidity for June in the Forest (86.5 %), all the rest were less than (85 %). Generally, the Forest zone (76.7 %) recorded higher average humidity than the Savannah zone (44.8 %).

From Table 3, no significant difference was recorded among the following parameters: moisture, dry matter, crude fibre, ash, and carbohydrate. Although there was no significant difference, Wangdataa had higher dry matter (91.30 %), ash (1.26 %), and carbohydrate (78.55 %) than Opeaburo. Carbohydrate-rich substrate supports the production of aflatoxins more than oils as carbohydrate easily provides carbon which is needed for the growth of the fungal [14]. The two varieties had a statistically significant difference in crude fat and protein. The production and accumulation of aflatoxins rise in full-fat substrates compared to low-fat substrates [42].

4.2. Condition of maize samples before storage

Aflatoxin B1 is the most harmful to humans and animals due to its close association with hepatocellular carcinoma, which can cause liver cancer [43]. However, it was observed that all the samples were not contaminated with aflatoxin B1 and B2 before storage. Aflatoxins B1 and B2 are synthesized by A. flavus [44].

Aflatoxins G1 and G2 are produced by *A. parasiticus* [44]. Before storage, there was no contamination of the maize samples with AFG1 in the Forest zone. There was very slight contamination at the Savannah zone, which was less than (<1.00 μg/kg) and within the permissible limits of Ghana, WHO, and EU. The soil samples in both zones were analyzed for the presence of moulds before the planting was done. The soil in the Savannah zone used for the experiment was observed to have higher moulds ($2.30 \times 10^4 \pm 0.00$ CFU/g) than the Forest zone. The slight contamination of the maize samples before storage can be attributed to the number of moulds that were found in the soil before the experiment.

4.3. Effects of storage treatment on AFB1 and AFB2 levels of maize

The Shapiro – Wilk normality test revealed that the data were normally distributed. The Forest zone had lower AFB1 and AFB2

Table 12
Effect of ecozones and packaging materials on AFG2 contamination after storage.

Packaging	Ecozones		
	Savannah	Forest	Means
Jute	14.95[a]	12.37[bc]	13.66[a]
Poly	12.57[b]	10.24[c]	11.40[b]
PICS	7.62[d]	5.45[d]	6.54[c]
Means	11.71[a]	9.35[b]	
HSD (0.01)		DF: 2	
Packaging = 1.32rowhead		F: 0.13	
Ecozones = 0.93rowhead		P: 0.8767	
Packaging* Ecozones = 2.19rowhead		SE: 0.5793	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

Table 13
Effect of varieties and packaging materials on AFG2 contamination at storage.

Packaging	Varieties		
	Opeaburo	Wangdataa	Means
Jute	12.83[a]	14.49[a]	13.66a
Poly	13.28[a]	9.53[b]	11.40[b]
PICS	8.52[b]	4.55[c]	6.54[c]
Means	11.54[a]	9.52[b]	
HSD (0.01)		DF: 2	
Varieties = 0.93rowhead		F: 30.18	
Packaging = 1.32rowhead		P:0.0000	
Packaging*Varieties = 2.19rowhead		SE: 0.5793	

Means within the same row with no superscript in common are significantly different ($P < 0.01$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. DF: Degrees of freedom. SE: Standard Error. Unit for aflatoxin: μg/kg.

contamination levels than the Savannah zone during storage. Toxin generation during storage is influenced by the relationship between kernel moisture and temperature and the type and length of storage [31]. The samples had the same moisture content in both zones and were all stored for six months. The production of aflatoxins can occur at various temperatures; however, the ideal temperature for aflatoxin production is 25–35 °C [40]. Temperatures in the Savannah zone are very high compared to the Forest zone, especially between March and April. This might be one of the reasons why the Savannah zone recorded higher levels of contamination than the Forest zone. The average temperature for the Savannah zone during the storage was 30.9 °C, which was higher than the average temperature of 28.3 °C in the Forest zone. Comparing the level of the AFB1 accumulated in the maize samples stored in the Savannah zone after the storage period to the European Union's recommended limit of 1.0 ng/g for AFB1 (Hamed et al., 2006), The level of contamination can be described as high because the level of contamination exceeded the recommended level by the EU standards. The level of contamination was higher in the jute sack than in poly and PICS bags. According to Ref. [45], PICS bags can limit oxygen flow and regulate carbon dioxide escape, stopping the growth of fungi and insects in the stored grains. PICS bags are hematic.

The presence of oxygen and carbon dioxide affects the production of aflatoxins. Fungal growth and the subsequent production of aflatoxins are hindered when the level of carbon dioxide is higher and a lower level of oxygen [46] Due to the many air spaces on the jute bag, oxygen will be higher than carbon dioxide compared to the PICS. This explains why the jute sack recorded the highest level of infestation compared to the PICS and poly.

All samples were not contaminated in the three different packaging bags with AFB1 in the Forest zone (Table 6). The Forest zone had lower temperatures compared to the Savannah zone. The variation in the performance of the packaging bags in the Forest and Savannah zones can, therefore, be attributed to the differences in temperature and humidity. This implies that if maize is well handled from preharvest to postharvest, any of the three packaging materials can be used for storage, especially in the Forest zone. Although polypropylene (PP) bags are presently used for storing grains, the grains can be contaminated by fungal and aflatoxins, particularly when those bags contain *A. flavus* spores due to the reusing of bags [26]. The bags used for the experiment were new.

Variety Wangdataa recorded a higher level of AFB2 (9.17 μg/kg) contamination than variety Opeaburo. The fungi need a substrate that is rich in carbohydrates in order to grow. The carbohydrate-rich substrate supports the production of more toxins than substrate-rich in oil because carbohydrate easily provides the carbon needed for the growth of the fungal [14]. Wangdataa has a higher carbohydrate (78.55 μg/kg) content than Opeaburo (78.35 μg/kg). The variation between the two varieties stored in the same zone indicates that Wangdataa is more prone to infestation by aflatoxins than Opeaburo.

Among the three packaging materials used for the storage, PICS prevented the growth of AFB2 than the remaining bags. PICS is a triple-layer hermetic storage bag. Aflatoxin-producing fungi and other moulds are deprived of humidity and oxygen when PICS is used

to store maize. Consequently, there is a reduction in growth rate, leading to a decrease in aflatoxin levels [47]. The variation among the two varieties clearly indicates that Opeaburo is a very resistant variety against aflatoxin infestation. For Opeaburo, all the storage bags used maintained the quality of the maize for six months with minimal infestation. Although Wangdataa has proven susceptible to AFB2 infestation, the PICS bag maintained its quality. Wangdataa has less fat (3.94 %) as compared to Opeaburo (4.15 %). However, the production and accumulation of aflatoxins rise in substrates with high-fat content compared to those with low-fat content [44]. However, the opposite was observed in this experiment.

4.4. Effects of storage treatment on AFG1 and AFG2 levels of maize

After the storage period, there was no contamination for all the maize samples stored in both zones with AFG1. Comparing the values obtained from the infestation of AFB1 and AFB2 with AFG2, it was observed that the contamination of maize with AFG2 was higher than AFB1 and AFB2. According to Ref. [48], when temperatures are high, the production of AFB1 and AFB2 is normally higher than AFG1 and AFG2 but equal at low temperatures. This study does not conform to the above statement, but rather, higher levels of AFG2 (12.07 μg/kg) were produced by the Wangdataa maize variety stored in the Savannah zone (Table 11). This implies that various varieties of maize would behave differently regarding the level of infestation by the various types of aflatoxins. Contamination levels for the Wangdataa variety have always been lower in the Forest zone than in the Savannah zone. This implies that Wangdataa can withstand fungal infection in the Forest zone than in the Savannah zone.

In terms of the level of infection of the stored samples with AFG2 to variety and ecozones, the same trend of infection was observed as in AFB1 and AFB2. Jute packaging material recorded the highest level of AFG2 contamination. Moist conditions favour the growth of fungi and the production of aflatoxins. When used for storage, Jute bags allow for moisture reabsorption by the grains when the humidity level is high [49]. The PICS bags have a triple layer with one outer layer made of PP and two inner layers made of HDPE, thus making it difficult for grains stored in it to reabsorb moisture, hence, the less infestation by aflatoxins [45].

Research done by Ref. [17] showed that PICS bags were more effective in suppressing the growth of Aspergillus flavus and the successive contamination of maize with aflatoxins compared to non-hermetic containers among the different moisture contents. Although Wangdataa has not shown resistance to contamination of aflatoxins, it is managed better if stored in the PICS bag. A critical look at the study shows that each toxin behaves differently with the different varieties. From this study, in terms of the maize samples with aflatoxins AFB1, AFB2, AFG1, and AFG2 infections. Infection levels by AFG2 were the highest, followed by AFB2 and AFB1, respectively. There was no infection by AFG1 after storage. The degree of toxicity associated with aflatoxins varies depending on the kinds present, with AFTs-B1 > AFTs-G1 > AFTs-B2 > AFTs-G2 being the most dangerous [50]. Aflatoxin B1 is the most toxic and poses a severe health risk, especially in Africa, where most people consume a relatively large amount of maize or groundnut, which is highly susceptible to aflatoxin contamination [17]. The storage treatment controlled the most toxic aflatoxins, B1 and G1.

5. Conclusion

At the end of the six months of storage of the two varieties of maize in both zones in the three different storage bags, there was no contamination of the two maize varieties with AFG1. Again, there was no contamination of maize stored in the Forest zone in all the packaging bags used for the experiment with AFB1. Higher contamination levels of AFB1, AFB2, and AFG2 occurred in the Wangdataa variety stored in jute. PICS bags recorded the lowest contamination levels than jute and poly. Contamination was higher in the Savannah zone than in the Forest zone. The study recommends that Wangdataa and Opeaburo varieties be stored in all three packaging bags: PICS, poly, and jute in the Forest zone to prevent contamination of maize with AFB1 and AFG1. Farmers, traders, and all aggregators of maize in the Savannah zone should be discouraged from using jute bags to store maize in the Savannah zone. PICS should be used to store Opeaburo and poly to store Wangdataa in the Savannah zone to control AFB1. Farmers should avoid using jute bags to store maize for extended periods to prevent contamination by AFB2, AFB1, and AFG2. In order to control all the AFB1, AFB2, and AFG2, farmers should be encouraged to use PICS bags to store maize.

Data availability statement

Data associated with this study has been deposited at OSF project, https://osf.io/7xtwv/.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Sandra Ama Kaburi: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Francis Appiah:** Resources, Supervision, Validation, Writing – review & editing. **Francis Padi Lamptey:** Conceptualization, Methodology, Writing – review & editing. **Gifty Serwaa Otoo:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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