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RESEARCH ARTICLE

Interactive effect of biological control (Aflasafe GH02) and different packaging types on aflatoxin levels in maize grain in two ecological zones

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Abstract

Aflatoxins are found in maize, groundnuts, and tree nuts. Most Ghanaians consume maize; therefore, aflatoxin exposure threatens their food security. Aflasafe GH02, a biological control agent, prevents contamination of crops with aflatoxins at preharvest and protects grains during storage. This study assessed the interactive effects of the agroecological zone, biological control (Aflasafe GH02), and three different storage bags on aflatoxin contamination. A 2 × 2 × 3 factorial laid out in a completely randomized design (CRD) was used for the experiment. After six months of storage, aflatoxin G1 (AFG1), aflatoxin B1 (AFB1), and aflatoxin B2 (AFB2) showed low contamination levels (< 3 μg/kg), except for AFG2, which had 12.97 μg/kg for control and 6.66 μg/kg for treated samples in both zones. Generally, lower contamination levels were observed in Purdue Improved Crop Storage (PICS) bags, followed by polypropylene bags (poly). Again, there were no significant changes in the levels of AFB1 of the maize stored in the forest zone in any packaging bags used for the experiment. The samples were not contaminated in all packaged bags in the savannah zone with AFG1. The study recommends that farmers use PICS to store their maize if the storage duration goes beyond five months to minimize the rise in aflatoxin contamination during storage. Farmers should also use biological controls during the preharvest stage to prevent contamination of maize during long-term storage.

Author summary

Aflatoxins, toxic compounds produced by Aspergillus species, pose a major threat to food security and public health, particularly in regions reliant on maize as a dietary staple. This study examines the effectiveness of Aflasafe GH02, a biological control agent, and different

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packaging materials in mitigating aflatoxin contamination during maize storage in two agroecological zones in Ghana. The results demonstrate that applying Aflasafe GH02 significantly reduces aflatoxin levels in stored maize, with Purdue Improved Crop Storage (PICS) bags showing superior performance in minimizing contamination compared to other packaging types. Notably, contamination levels of aflatoxins, including AFG1, AFB1, and AFB2, remained well below regulatory thresholds in treated samples after six months of storage, except for AFG2 in untreated maize. This study highlights the importance of combining biological controls with proper postharvest handling practices to prevent aflatoxin contamination during storage. The findings provide practical recommendations for farmers, emphasizing the use of PICS bags and biological controls to enhance the safety and longevity of stored maize. This work contributes to ongoing efforts to safeguard food security and reduce health risks associated with aflatoxin exposure in maize-dependent regions.

1. Introduction

Maize (*Zea mays L)* is the predominant grain cultivated in sub-Saharan Africa and is vital for sustaining people's livelihoods and ensuring food security [1]. Maize cultivation is practiced in Ghana's agroecological zones (AEZs), primarily by smallholder farmers under rainfed conditions [2]. The presence of aflatoxin in maize threatens food security in Ghana, as maize is a staple in the diet of most Ghanaians [3,4]. Aflatoxins, a group of chemically related toxins, are mainly synthesized by *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins can be found in several widely consumed foods, such as maize, groundnuts, and tree nuts [5]. Aflatoxins present a significant risk to individuals' and animals' immediate and prolonged well-being and commercial and international markets for products derived from maize [6]. AFB1, AFB2, AFG1, and AFG2 are the most potent mycotoxins out of the 18 possible aflatoxins generated by *Aspergillus spp* [7]. Aflatoxins are the most important mycotoxins, considering their prevalence, toxicity, and impact on trade and human health [8].

Prolonged consumption of low doses of aflatoxin has been associated with liver cancer, suppression of the immune system, heightened susceptibility to infectious diseases, impaired nutrient absorption, and hindered growth and development in children due to malnutrition [9,10]. Efforts have been made to limit the levels of aflatoxins in food and feed to protect consumers from the detrimental health consequences of aflatoxins [11–13]. The European Union (EU) suggested threshold for AFB1 is 1.0 μg/kg, while a 4.0 μg/kg limit is set for total aflatoxins [14]. In contrast, the United States federal recommendations for food and feed provide a maximum limit of 20 μg/kg of total aflatoxins [15,16]. A recent study found that mycotoxin contamination accounts for 77% of the total risk in sub-Saharan Africa, with aflatoxin itself representing 46% [17]. Multiple studies conducted in Ghana have detected significant levels of aflatoxin contamination in maize during the pre- and post-harvest stages of the maize value chain [18–23].

A recent study reported that maize samples from farmers in both the savannah and Forest zones exhibited AFB1 concentrations of 9.618 μg/kg. Additionally, they recorded higher concentrations of total aflatoxins, ranging from 11.6225 μg/kg to 23.53 μg/kg [23]. The effective implementation of appropriate agronomic and storage methods can prevent the contamination of maize and other foodstuffs by aflatoxins. Several studies have extensively documented the utilization of atoxigenic fungi to manage toxigenic fungal species [24–27]. Biocontrol frequently reduces preharvest crop aflatoxin contamination to safe levels and protects crops throughout storage [25–27]. This study evaluated the effect of AEZs, biological controls (Aflasafe GH02), and storage bags on aflatoxin contamination.

2. Materials and methods

2.1 Study area

The experiment was conducted in Wa, located in the Upper West Region, representing the savannah zone, and in Ejisu, in the Ashanti Region, representing the forest zone of Ghana, from November 2020 to July 2021. These two ecological zones are described in our previous study [28]. Fig 1 presents a map of the studied communities. The field experiment took place from May 2020 to January 2021. Soil analysis was conducted to determine nutrient composition and identify *Aspergillus spp.* presence [29].

2.2 Experimental design for planting

This study employed a 2 × 2 × 2 factorial design arranged in a randomized complete block design (RCBD). The plots were divided into two groups through randomization to distinguish treatment plots from control plots, with the entire procedure replicated three times. The RCBD was used alongside a split-plot design for enhanced experimental precision.

2.3 Microflora and soil nutrient analysis

Soil samples were collected from all four sites and transported to the KNUST Aflatoxin and Soil Science laboratories to analyze *Aspergillus spp.* and soil nutrient content. Soil analysis was conducted to determine pH and measure nitrogen, phosphorus, and potassium concentrations.

2.4 Planting and Aflasafe GH02 application

Seeds were sown directly with a planting distance of 70 cm between rows and 25 cm between individual plants. Maize seeds were uniformly planted at a depth of 5 cm in compact, moist soil to ensure optimal seed-to-soil contact, enhancing moisture absorption and promoting successful germination [28]. The planting followed a systematic arrangement in parallel rows. Aflasafe GH02 was applied using a hand broadcasting technique, where the product was manually distributed over the soil surface when maize plants reached 40 days of age at a rate of 10 kg per hectare [28]. Fig 2 illustrates the application of Aflasafe GH02 in a maize field.

2.5 Sample collection of grains at harvest

Maize was harvested at physiological maturity, after which the cobs were dried. Ten cobs were randomly selected, manually de-husked, and shelled from each field. The shelled maize was then dried until it reached a moisture content of 12%.

2.6 Experimental design used for the storage

The storage experiment utilized a 2 × 2 × 3 factorial design in a CRD, incorporating two AEZs, two levels of Aflasafe GH02 treatment, and three types of packing materials. The storage bags used were made of jute, polypropylene, and PICS. Maize samples were stored for six months in each AEZs. Temperature and humidity in the storage rooms were continuously monitored in each zone using Elitech data loggers, which were pre-configured to record measurements at hourly intervals throughout the storage period. Data was documented using ElitechLog software.

2.7 Laboratory analysis

Maize samples were sent to the Aflatoxins Laboratory at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, to analyze AFB1, AFB2, AFG1, and AFG2. Aflatoxins

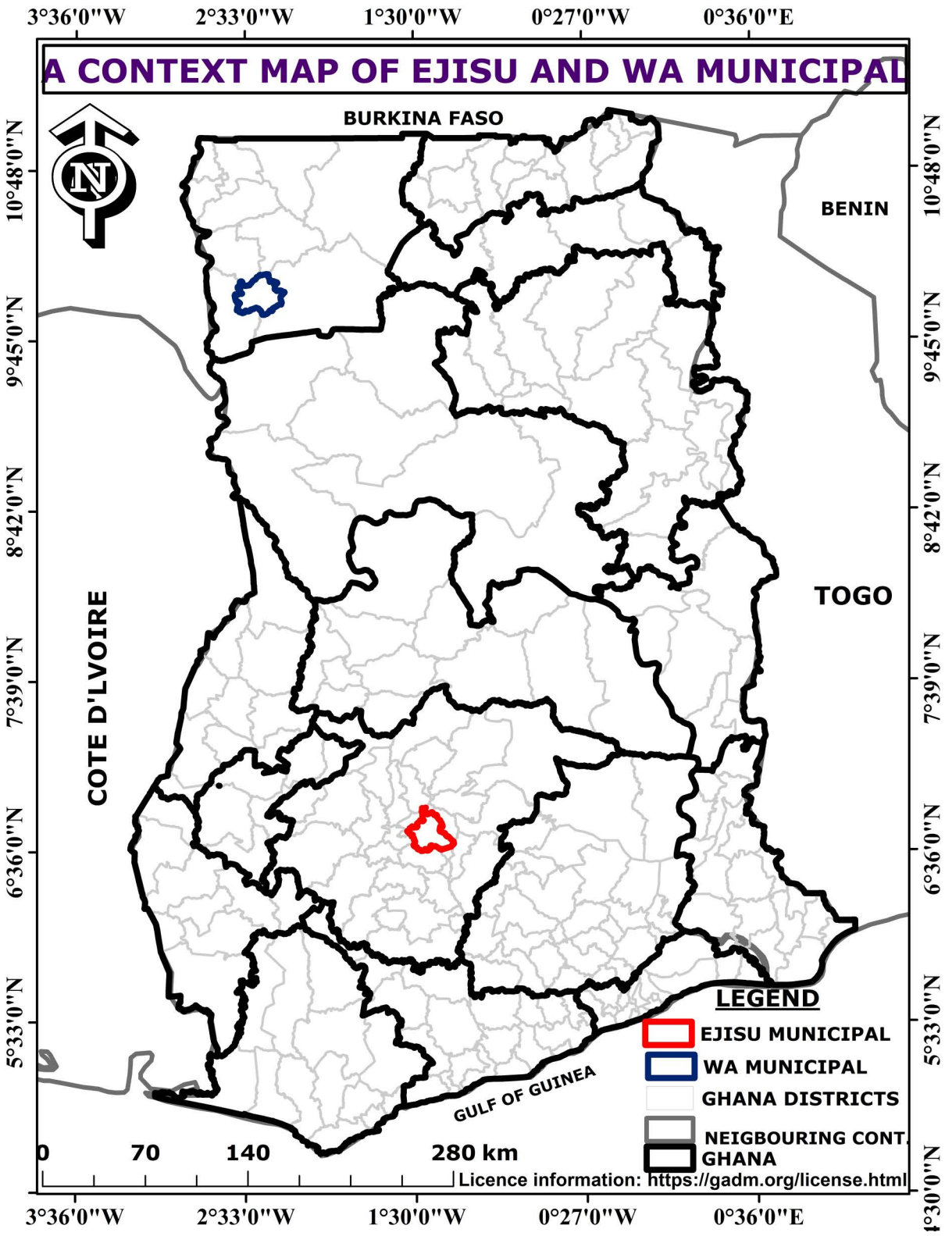

Fig 1. Map of Ghana indicating the study areas for the farm experiment.

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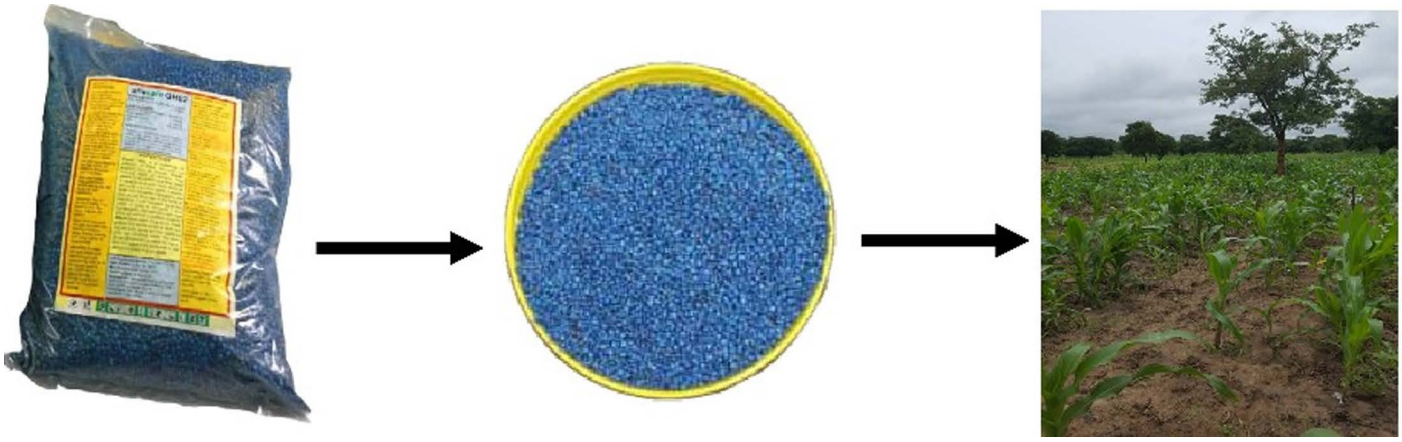

Fig 2. Aflasafe GH02 applied on a maize field.

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were extracted from the samples using a solvent mixture of acetonitrile and acetic acid at a 9:1 ratio by volume, with additional agitation steps to optimize extraction.

2.8 High-performance liquid chromatography (HPLC) analysis

HPLC analysis used a Photochemical Reactor for Enhanced Detection (PHRED) according to AOAC Official Method 2005.08 [30] for post-column derivatization. Aflatoxin detection was performed with an Agilent 1200 Quaternary Pump equipped with a Fluorescence Detector (Ex: 360 nm, Em: 440 nm) and a Sunfire C18 Column (150 x 4.60 mm, 5 μm). Methanol and water were used as the mobile phases at a 1 mL/min flow rate, with a constant column temperature of 40 °C. Post-column derivatization was achieved using an LC Tech UVE device. Standards for aflatoxin mix (AFG1, AFG2, AFB1, AFB2) were prepared using a Romer Labs aflatoxin standard (5.02 ng/L in acetonitrile), and aflatoxin concentrations in the samples were quantified through calibration curves and retention time standards for each toxin.

2.9 Method performance

Recovery tests were performed to evaluate the accuracy and correctness. The empty samples were supplemented with five duplicate maize samples at concentrations of 13, 26, and 104 μg/g, yielding recoveries of 97 ± 1.07%, 98 ± 1.35%, and 99 ± 0.93%, respectively. Periodic testing was performed, but no detectable quantity of the desired chemical was detected. The accuracy was further verified using a certified reference material (TR-A1000) acquired from a Trilogy laboratory in the United States [17]. The average outcome derived from 10 repeated experiments was 20.65 ± 0.71 μg/kg, falling within the acceptable range of the certified value of 21.0 ± 2.9 μg/kg. The coefficient of variance for each replicate was less than 15%. Quality assurance was ensured by validating the precision and accuracy of the samples by using an aflatoxin standard. A thorough examination of the blank samples was conducted, specifically focusing on detecting the absence of aflatoxins. The coefficient of variance for duplicates showed a variation of less than 15%. The aflatoxin concentration was determined by applying the formula: Aflatoxin concentration (μg/kg) = A x (T/I) x (1/W)

Within the provided context, the variables are explicitly specified as follows: In this context, A denotes the quantity of aflatoxin measured in nanograms after being injected as eluate. T refers to the final test solution eluate volume, measured in microliters. I represent the

volume of eluate injected into the liquid chromatography (LC) system, measured in microliters. Finally, W represents the commodity's mass measured in grams of the final extract.

2.10 Statistical analysis

Analysis of Variance (ANOVA) was conducted to examine the datasets that measured aflatoxin levels after storage. This study aimed to detect significant changes in the samples. The analysis was conducted using Statistix 9.1 (9th Edition) statistical software. The datasets were examined using a CRD. Disparities among the treatment means were assessed using the Fischer Least Significant Difference test with a significance level of 5%.

3. Results and discussion

3.1 Temperature and humidity data

Except for November (27.9 °C), the savannah zone consistently experienced higher temperatures than the forest zone during storage, as shown in Tables 1 and 2 [26]. The mean temperature during the storage period for the savannah (30.9 °C) exceeded that of the forest zone (28.8 °C). The forest zone exhibited a higher average humidity of 76.7%, in contrast to the savannah zone, with an average humidity of 44.8%.

3.2 Effect of biological control of aflatoxin and ecozones on AFG1 levels in maize

No substantial variation was observed in the levels of AFG1 in either the treated or the control samples in either zone (Table 3). Nevertheless, AFG1 (0.11 μg/kg) was low in treated samples stored in the forest zone.

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: Kaburi et al. [29].

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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: Kaburi et al. [29].

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Table 3. Effects of biological control of aflatoxin and ecozones on AFG1 levels in maize.

	Ecozones		
Biocontrol	Savannah	Forest	Means
Aflasafe	0.00[a]	0.11[a]	0.06[a]
No Aflasafe	0.00[a]	0.00[a]	0.00[a]
Means	0.00[a]	0.06[a]	
HSD (0.01):	DF: 1		
Biocontrol = 0.15,	F: 1.00		
Ecozones=0.15,	P: 0.3273		
Biocontrol* Ecozones= 0.27	Standard error: 0.079		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets differ significantly ($p < 0.01$). HSD, highest significant difference. Unit: μg/kg.

https://doi.org/10.1371/journal.pstr.0000160.t003

3.3 Effect of packaging materials and biocontrol on AFG1 levels in maize

Regardless of the biological control or packaging bags used, there were no notable disparities in the levels of AFG1. The amounts varied from 0.00 μg/kg to 0.17 μg/kg, as indicated in Table 4.

3.4 Effect of ecozones and biological control on levels of AFG2 in maize

There was a substantial correlation between ecozones and biological controls. Maize in the forest zone that did not receive the Aflasafe GH02 treatment had higher levels of AFG2 (12.01 μg/kg) (Table 5). Maize treated with Aflasafe GH02 in the savannah zone showed a reduced level of AFG2 (7.38 μg/kg). In the case of the biocontrol, maize that was not treated with Aflasafe GH02 exhibited a higher concentration of AFG2 (11.73 μg/kg). Concerning the ecozones, the maize stored in the savannah zone exhibited a contamination level of AFG2 at 10.58 μg/kg.

3.5 Effect of biological control of aflatoxin and packaging material on levels AFG2 in maize

As shown in Table 6, a substantial interaction was observed between the biological control and the packaging materials. Maize that was not treated with Aflasafe GH02 and was stored in a jute sack had a higher AFG2 level of 12.97 μg/kg. In the biological control, maize samples not subjected to the Aflasafe GH02 treatment had a contamination level of AFG2 at 11.73 μg/kg. In contrast, those who underwent Aflasafe GH02 treatment had a contamination level of 8.55 μg/kg. Regarding the packing material exclusively, maize samples stored in jute sacks exhibited a higher AFG2 level of 12.47 μg/kg, whereas maize samples stored in PICS had a lower level of 6.76 μg/kg.

3.6 Effect of ecozones and biological control of aflatoxin on AFB1 levels in maize

The impact of ecozones and biological controls on AFB1 contamination levels is presented in Table 7. There was a substantial correlation between ecozones and biological controls. Maize held in the savannah zone without the Aflasafe GH02 treatment had a higher concentration of AFB1 (2.03 μg/kg). No traces of AFB1 (0.00 μg/kg) were found in the maize samples from the forest zone, indicating no contamination. In terms of biocontrol, maize that was not treated with Aflasafe GH02 exhibited a higher level of AFB1 contamination (1.02 μg/kg) compared to maize that was treated with Aflasafe GH02 (0.16 μg/kg). Contamination of AFB1 in maize was higher in the savannah zone, with a concentration of 1.18 μg/kg.

Table 4. Effect of packaging materials and biocontrol on AFG1 levels in maize.

	Biocontrol		
Packaging	Aflasafe	No Aflasafe	Means
Jute	0.17[a]	0.00[a]	0.08[a]
Poly (PP)	0.00[a]	0.00[a]	0.00[a]
PICS	0.00[a]	0.00[a]	0.00[a]
Means	0.06[a]	0.00[a]	
HSD (0.01):	DF:1		
Biocontrol = 0.15,	F: 1.00		
Packaging= 0.22,	P: 0.3827		
Packaging*Varieties=0.36	Standard error: 0.096		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD, highest significant difference. Unit: μg/kg.

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Table 5. Effects of ecozones and biological control on AFG2 levels in maize.

	Ecozones		
Biocontrol	Savannah	Forest	Means
Aflasafe	9.72[c]	7.38[d]	8.55[b]
No Aflasafe	11.44[b]	12.01[a]	11.73[a]
Means	10.58[a]	9.69[b]	
HSD (0.01):	DF: 1		
Biocontrol =0.29,	F:199.19		
Ecozones=0.29,	P:0.0000		
Biocontrol* Ecozones= 0.50	Standard error: 0.15		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD, highest significant difference. Unit: μg/kg

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Table 6. Effect of biological control and packaging material on AFG2 levels in maize.

	Biocontrol		
Packaging	Aflasafe	No Aflasafe	Means
Jute	11.97[b]	12.97[a]	12.47[a]
Poly (PP)	9.47[c]	12.91[a]	11.19[b]
PICS	4.22[d]	9.295[c]	6.76[c]
Means	8.55[b]	11.73[a]	
HSD (0.01):	DF:2		
Biocontrol =0.29,	F: 131.77		
Packaging=0.41,	P: 0.0000		
Packaging*biological=0.67	Standard error: 0.17		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD: Highest Significant Difference. Unit: μg/kg

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3.7 Effect of biological control of aflatoxin and packaging material on the level of AFB1 in maize

According to the data in Table 8, there was a substantial interaction between biological control and packaging. Maize samples not treated with Aflasafe GH02 and stored in poly had the

Table 7. Effects of ecozones and biological control of aflatoxin on AFB1 levels in maize.

	Ecozones		
Biocontrol	Savannah	Forest	Means
Aflasafe	0.32[b]	0.00[b]	0.16[b]
No Aflasafe	2.03[a]	0.00[b]	1.02[a]
Means	1.18[a]	0.00[b]	
HSD (0.01):	DF:1		
Biocontrol= 0.32,	F:199.19		
Ecozones=0.32,	P:0.000		
Biocontrol* Ecozones=0.57	Standard error: 0.16		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD: Highest Significant Difference. Unit: μg/kg

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Table 8. Effects of biological control and packaging materials on AFB1 levels in maize.

	Biocontrol		
Packaging	Aflasafe	No Aflasafe	Means
Jute	0.00[b]	0.68[b]	0.34[b]
Poly	0.00[b]	2.36[a]	1.18[a]
PICS	0.48[b]	0.00[b]	0.24[b]
Means	0.16[b]	1.02[a]	
HSD (0.01):	DF:1		
Biocontrol=0.32,	F: 54.55		
Packaging=0.46,	P: 0.0000		
Packaging*Varieties=0.76	Standard error: 0.20		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD: Highest Significant Difference. Unit: μg/kg

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highest amount of AFB1 (2.36 μg/kg). Only maize samples not treated with Aflasafe GH02 exhibited a greater level of AFB1 (1.02 μg/kg) than the biological control. Maize samples contained in the polyethylene packing material showed the highest level of AFB1 contamination, measuring at 1.18 μg/kg.

3.8 Effect of ecozones and biological control on AFB2 levels in maize

The analysis in Table 9 reveals a substantial interaction between the ecozones and biological controls. Maize samples not treated with Aflasafe GH02 and stored in the savannah zone exhibited a higher amount of AFB2, precisely 0.97 μg/kg. Maize samples treated with Aflasafe GH02 and stored in the forest zone showed reduced levels of AFB2 at 0.36 μg/kg. Maize that was not treated with Aflasafe GH02 as a biocontrol had a higher contamination level of AFB2 (0.68 μg/kg). In ecozones, maize in the savannah zone had a greater concentration of AFB2, measuring at 0.80 μg/kg.

3.9 Effect of biological control of aflatoxin and packaging material on levels of AFB2 in maize

Statistical analysis revealed a substantial correlation between the biological control of aflatoxin and the packing material used for AFB2 (Table 10). Maize samples treated with

Table 9. Effects of ecozones and biological control on levels AFB2 in maize.

	Ecozones		
Biocontrol	Savannah	Forest	Means
Aflasafe	0.63[b]	0.36[c]	0.49[b]
No Aflasafe	0.97[a]	0.39[c]	0.68[a]
Means	0.80[a]	0.38[b]	
HSD (0.01):	DF:1		
Biocontrol= 0.13,	F:10.80		
Ecozones=0.13,	P: 0.0031		
Biocontrol* Ecozones= 0.23	Standard error: 0.07		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD: Highest Significant Difference. Unit: μg/kg

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Table 10. Effect of biological control of aflatoxin and ecozones on level AFB2 of maize.

	Biocontrol		
Packaging	Aflasafe	No Aflasafe	Means
Jute	1.06[a]	0.89[a]	0.98[a]
Poly (PP)	0.57[b]	0.156[c]	0.36[b]
PICS	0.42[bc]	0.44b[c]	0.43[b]
Means	0.68[a]	0.49[b]	
HSD (0.01):	DF:2		
Biocontrol 0.13,	F: 7.20		
Packaging=0.18,	P:0.0036		
Packaging*Biocontrol=0.31	Standard error: 0.08		

Values are expressed as the mean of three replicates of the aflatoxin levels in maize. All mean scores with different column alphabets are significantly different ($p < 0.01$). HSD: Highest Significant Difference. Unit: μg/kg

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Aflasafe GH02 and stored in a jute bag had the highest contamination level of AFB2, measuring 1.06 μg/kg. However, the samples maintained in the jute without the Aflasafe GH02 treatment exhibited no statistically significant changes. As a biological control, maize samples treated with Aflasafe GH02 exhibited higher AFB1 contamination, measuring at 0.68 μg/kg. Maize samples in the jute packing material showed the highest level of AFB1 contamination, measuring 0.98 μg/kg.

4. Discussion

The optimal temperature range for aflatoxin production is 25 °C – 35 °C [31]. Tables 1 and 2 show that, except in November (27.9 °C), the savannah zone experienced higher temperatures than the forest zone during storage. The mean temperature during the storage period for the savannah (30.9 °C) exceeded that of the forest zone (28.8 °C). The average temperature during storage was within the ideal range for aflatoxin production. A recent study found that a relative humidity of 95% significantly increases the development of aflatoxins [32]. Except for November (83.5%), the forest zone had greater humidity than the savannah zone during storage. The ideal humidity level for producing aflatoxins is approximately 85% [32]. Except for the relative humidity in June in the forest, which was 86.5%, all other values were below 85%. The forest zone exhibited a higher average humidity of 76.7% than the savannah zone, with an average humidity of 44.8% [29].

4.1 Effect of biological control of aflatoxin and ecozones on AFG1 levels in maize

There was no statistically significant relationship between the ecozones and the biological control of AFG1. Nevertheless, a minimal concentration of AFG1 (0.11 μg/kg) was identified in the forest area in the treated samples during a storage period of six months. Before storage, the samples were free from any contamination of AFG1. Several investigations conducted in Ghana have identified fungal growth and aflatoxins in treated samples using a biological control (Aflasafe GH02) [24,33]. Contamination was also observed during the storage period. Spores are inherently present and can contaminate the grains at any stage of the manufacturing process.

The contamination level was sufficiently low to be within the permitted limit of parts per billion (ppb) set by the EU. The toxicity level of aflatoxin varies depending on the specific form of the aflatoxin present. The order of toxicity, from most to least harmful, was AFB1, AFG1, AFB2, and AFG2 [34]. AFG1 is the second most poisonous aflatoxin after AFB1. The results demonstrated that maize stored for six months exhibited reduced levels of contamination, showing that Aflasafe GH02 application and effective agricultural methods can effectively mitigate the issue of aflatoxin contamination. It has been argued that biological controls exhibit carryover effects. To optimize the effectiveness of biological control, it is essential to ensure that maize is treated appropriately during postharvest activities, including shelling, drying, packaging, and storage.

4.2 Effect of packaging material and biological control of aflatoxin on AFG1 levels in maize

Following the storage period, minimal contamination was observed in jute storage bags. Sudini et al. [35] found that PICS bags produced less toxin than cotton bags under similar conditions. No significant differences were observed between treatment and control groups. The samples treated with the biological control (Aflasafe GH02) in the forest zone exhibited low levels of AFG1. The primary factors contributing to aflatoxin contamination in crops and agricultural products is *Aspergillus spp.*, regional climate, conditions during growth and storage, genotype of the planted crop, soil conditions, and insect infestation [36,37]. Various factors, including fungal populations, insect infestation, pre- and post-harvest handling, and environmental conditions such as climate, temperature, humidity, O_2, and CO_2, influence the contamination of stored foodstuffs by toxigenic fungi [38]. Minor infections in maize samples may have occurred for the aforementioned reasons. *Aspergillus spp*. can proliferate quickly during storage because of abundant rainfall during and after harvest, as well as inadequate drying of the crop before storage [39].

4.3 Effect of ecozones and biological control on AFG2 levels of maize

AFG2 contamination of the samples occurred during the field trial, and the toxins increased during storage. Upon careful examination of the data, it was evident that the contamination level of AFG2 surpassed those of the other toxins being investigated. AFG2 is the least harmful of the four aflatoxins studied [34]. The correlation between ecozones and biological management significantly affected the presence of AFG2. Maize cultivated in the forest zone not treated with Aflasafe GH02 had the highest contamination with AFG2, with a 12.01 μg/kg concentration. Recent studies state that AFB production is equivalent to the production of AFG at low temperatures [40]. In this study, contamination exceeded the level of AFB produced during storage. Biological control was implemented during the field growth phase of the maize. The maize samples remained uncontaminated following the harvest. After a storage

period of six months, both the treated and untreated samples exhibited contamination, with the untreated samples showing the highest level of contamination. biocontrol agents enhanced the protection of treated samples compared to untreated samples.
Consequently, maize remains susceptible to contamination during the postharvest handling phases. Among the ecozones, the savannah zone had the most significant level of AFG2 contamination in stored maize, at 10.58 μg/kg. This can be ascribed to the elevated temperatures documented in the savannah region during storage. Maize samples for the experiment were collected in August during the intense rainfall period in the savannah zone. Throughout the drying phase, precipitation persisted in the region, potentially leading to elevated contamination levels compared to the forested area.

4.4 Effect of biological control of aflatoxin and packaging material on AFG2 levels in maize

Statistical analysis revealed a substantial correlation between biological control of aflatoxin and the type of packaging material used. Maize samples not treated with Aflasafe GH02 and stored in jute had the highest AFG2 contamination, measuring 12.97 μg/kg. However, this contamination level did not significantly differ from the polybag samples. Biocontrol, which has a carryover effect that provides storage protection, is frequently effective in reducing preharvest crop aflatoxin contamination to safe levels [17,25–27]. Unlike the jute bag, the PICS bag can shield grains from absorbing moisture from the surrounding surroundings. Humidity facilitates AF proliferation. PICS bags can decrease the entry of oxygen and regulate the release of carbon dioxide, thereby inhibiting the growth of aflatoxins and insects in stored grains [41]. This could explain the small degree of contamination observed in the samples housed in the PICS.

4.5 Effect of ecozones and biological control of aflatoxin on AFB1 levels of maize

After a storage period of six months, neither the treated nor untreated samples exhibited AFB1 above (2.50 μg/kg). This indicates that all samples were effectively shielded from AFB1 in both ecozones. AFB1 is considered the most hazardous variety of aflatoxin for animals and humans due to its strong association with hepatocellular carcinoma, a form of liver cancer [42]. Maize samples treated with Aflasafe GH02 and stored in the forest zone for six months showed no contamination (0.00 μg/kg) AFB1. The contamination levels of AFB1 in the savannah zone were extremely low, measuring less than 0.50 μg/kg. This is well below the permissible limit of 1.0 μg/kg set by the EU for AFB1. The amounts observed in the untreated samples were relatively small, suggesting farmers could achieve long-term maize storage without contamination by implementing effective postharvest handling methods. The contamination levels in the savannah zone were significantly lower after six months of storage than samples taken from farmers within two to four weeks of harvesting [43].

4.6 Effect of biological control and packaging material on AFB1 levels in maize

Statistical analysis revealed a substantial correlation between biological control of aflatoxin and the type of packaging material used. Maize samples not treated with Aflasafe GH02 and stored in poly had the highest AFB1 contamination, measuring 2.36 μg/kg. Polypropylene (PP) bags are insufficient to protect grains during storage. The bag facilitates the absorption of moisture from the atmosphere and the exchange of gases. Polypropylene bags lack hermetic properties, and there is evidence suggesting that using these bags to package grains for storage

can promote fungal infection and aflatoxin generation [18,20]. Only the maize samples not treated with Aflasafe GH02 showed the highest AFB1 contamination (1.02 μg/kg), specifically for the biological control. Samples treated with Aflasafe GH02 exhibited better protection during storage than those not treated with Aflasafe GH02. Aflasafe GH02 is ecologically sustainable and operates by introducing indigenous toxigenic strains into the soil to outperform the toxigenic strains [21] effectively. This measure mitigates the risk of contamination in the field and provides additional protection during storage.

4.7 Effect of ecozones and biological control on AFB2 contamination at storage

After storage for six months, none of the samples, whether treated or untreated, showed any contamination with AFB2 up to a concentration of 2.00 μg/kg. Both the treated and untreated samples had highly satisfactory outcomes. The contamination levels were minimal. The correlation between ecozones and biological management had a notable effect on the presence of AFB2. Throughout the investigation, the contamination levels in the savannah zone were consistently higher than in the forest zone. The fungus flourishes in regions characterized by elevated temperatures. Typically, the temperatures in the savannah zone were consistently high during March and April. The Upper West Region, situated between latitudes 8 and 11, experiences an arid environment with a relative humidity rarely exceeding 70% from July to September. During this period, the region also experienced maximum temperatures ranging from 28 °C to 35 °C [44]. Harvesting occurred in August, and during the entire drying process of the samples, there were ongoing rainfall events. Samples in the forest zone were collected in December, following the minor season, which facilitated the drying of maize, as there was no rainfall during this period. Aflasafe GH02 has undergone rigorous testing and has been scientifically validated as highly effective in mitigating aflatoxins during both the pre and postharvest phases. Research conducted in Africa demonstrated that the use of Aflasafe GH02 can significantly reduce aflatoxin contamination in maize and groundnut crops by 80-99% at several stages, including cultivation, storage, and throughout the value chain [45].

4.8 Effect of biological control of aflatoxin and packaging material on AFB2 levels in maize

The correlation between the biological control of aflatoxin and the choice of packing material has a notable effect on AFB2. Maize samples treated with Aflasafe GH02 and stored in jute had the highest AFB2 contamination, measuring 1.06 μg/kg. Nevertheless, no statistically significant difference was observed between the samples maintained in the jute without Aflasafe GH02 treatment. The treated samples exhibited significantly higher contamination levels than the untreated samples. The level of contamination in the treated samples could not be attributed to the effectiveness of the Aflasafe GH02. Following the completion of the harvest, neither the treated nor the untreated samples showed any contamination with aflatoxins. Contamination can occur at any handling stage. A study found that crops treated with Aflasafe GH02 had 80–100% fewer aflatoxins than untreated crops in all three AEZs (P 0.05 μg/kg) [46]. The crops treated with Aflasafe GH02 had a maximum total aflatoxin concentration of 2.5 ppb at any location during the research. This suggests that there were minor differences. The measured levels were very low as none of the contaminated samples exceeded 2.00 μg/kg.

5. Conclusion

Aflatoxin in maize poses a significant risk to food security in tropical regions such as Ghana, where maize is a fundamental dietary component. Biocontrol is an effective method for

reducing preharvest crop aflatoxin contamination to safe levels. It also provides storage protection owing to its carryover impact. This study evaluated the effects of the AEZs, biological control (Aflasafe) GH02, and three distinct storage bags on aflatoxin contamination. Upon completion of the storage period, the contamination levels of AFG1, AFB1, and AFB2 were very low (< 3 μg/kg), except for AFG2. AFG2 exhibited 12.97 μg/kg contamination levels for the control samples and 6.66 μg/kg for the treatment samples in both zones. The PICS bags generally exhibited lower contamination levels than the poly bags. Again, there was no AFB1 contamination in maize stored in the forest zone, regardless of the packaging bag used for the experiment. None of the samples in the sealed bags in the savannah zone were contaminated with AFG1. The current study was conducted in only two out of the six AEZs in Ghana, and only two varieties were used.

This study suggests that farmers should exercise meticulous postharvest handling practices to avoid contamination during storage when employing biological control methods to manage aflatoxins. This study also suggests that farmers should utilize PICS for the long-term storage of maize, particularly when implementing biological control methods to manage aflatoxins. To mitigate the risk of maize contamination during extended storage, farmers should employ biological control methods throughout the preharvest phase.

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