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Effectiveness of some aflatoxin interventions in reducing the aflatoxin contamination of maize in Ghana

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ABSTRACT

The presence of aflatoxin in food and feed represents a significant health hazard to both human and animal populations, hence exerting adverse effects on the environment and the global economy. The effects of maize variety, agroecological zones, and aflatoxin control measures on aflatoxin contamination were tested using a 2x2x3 factorial in a randomised complete block design (RCBD). The treatments consisted of two agroecological zones, two varieties of maize, and three aflatoxin control measures. The aflasafe was applied at a rate of 10 kg/ha and the nicoking at 200 ml per 15 liters of water. There was no contamination of maize samples grown in the Forest zone with aflatoxins during all the treatments. There was no contamination of maize samples with aflatoxins BI, B2, G1, and G2 for all the treatments in the Savannah zone except for the hoeing method. The highest contamination of Aflatoxin G2 (AFG2) (0.19 µg/kg) was recorded by the Wangdataa maize variety using hoeing as the means of the aflatoxin control method. The highest Aflatoxin G1 (AFG1) contamination (0.45 µg/kg) was produced by maize grown in the Savannah ecozone using the hoeing method of control. In conclusion, the study showed that all the control methods gave maximum protection to the maize while growing on the field except the hoeing method in the Savannah ecological zone. The study recommends that in the Savannah zone if a farmer chooses hoeing as a method to control aflatoxins, it is advisable to complement this approach with the use of biological or chemical methods for effective aflatoxin control.

Contribution/Originality: The study has brought to light the development of different types of aflatoxins in selected maize varieties grown under different aflatoxin management practices in diverse ecological zones. It points to the appropriate variety, aflatoxin management type, and ecological zone interaction for aflatoxin control in maize. Farmers and other value chain actors could be guided accordingly.

1. INTRODUCTION

Aspergillus flavus is a fungus that opportunistically affects oil-rich crops, including maize, peanuts, and tree nuts (Majumdar et al., 2018). It is important because it produces aflatoxin as a secondary metabolite in several crops' seeds before and after harvest. Aspergillus flavus and Aspergillus parasiticus, in particular, make naturally occurring poisons called Aflatoxins B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2

(AFG2) (Pascale et al., 2020). One of the factors that make aflatoxins one of the most problematic mycotoxins is that the causative fungi can manufacture aflatoxins not only during the pre-harvest period but also during the post-harvest stages, including storage (Guchi, 2015). Aflatoxin is the most poisonous mycotoxin and negatively affects human and animal health (Liu, Galani, Gong, & Orfila, 2020). According to a recent estimate by the World Bank, the number of liver cancer cases attributed to the ingestion of aflatoxin is approximately 90,000 (Jaffee, Henson, Unnevehr, Grace, & Cassou, 2018).

Aflatoxins are most common in dry stress situations when crops are getting close to maturity, when there is a lot of wetness after harvest, or when crop drying and storage conditions are poor. As a result, Southeast Asia, South Asia, and Sub-Saharan Africa are regions with a high risk of exposure to aflatoxins (Strosnider et al., 2006). The extent and prevalence of crop aflatoxin contamination are expected to increase worldwide with current climate change trends (Battilani et al., 2016). To safeguard consumers from the health effects of aflatoxins, limits on aflatoxins in food and feed have been set (JECFA, 2018). These limits vary from nation to nation. Due to non-compliance, importing countries have imposed strict standards, leading to numerous exporting countries, including Ghana, losing access to exclusive European markets and significant annual trade revenues (Dzirasah, 2015).

Maize (*Zea mays*) is the most important cultivated cereal crop in Sub-Saharan Africa (SSA), with over 70 million metric tons grown on more than 34 million hectares (FAOSTAT, 2016; Macauley, 2015). Maize is a staple food in Ghana and is consumed in various dishes, including banku, kenkey, Tuo Zaafi, and porridge. Its cultivation also provides the nation with money and foreign exchange (Aklaku, Sowley, & Ofosu, 2020). The cultivation and consumption of maize are faced with a major challenge: aflatoxin contamination. Good Agricultural Practices (GAP) can be defined as the use of the knowledge currently available to address environmental, economic, and social sustainability for on-farm production and post-production processes, resulting in wholesome and safe food and non-food agricultural products" (Dewi et al., 2022). Farmers can raise the quality and safety of food and other agricultural goods, boost output, and lower overall post-harvest losses by implementing key GAPs. Examples of GAP are weed control, control of pests, fertilizer application, use of resistant varieties, biocontrol, early harvesting, proper drying, sorting, etc. The appropriate adoption of GAP by farmers helps improve the safety and quality of food and other agricultural products, provides the added benefit of increased yield, and reduces overall post-harvest losses (Xu et al., 2022). Good pre- and post-harvest practices can help minimize aflatoxin contamination (Cleveland, Dowd, Desjardins, Bhatnagar, & Cotty, 2003; Kamala et al., 2018; Mahuku et al., 2019).

In Ghana, some studies have been conducted on the occurrence, prevalence, interventions, and health risks related to aflatoxins in maize and groundnuts (Agbetiameh et al., 2020; Akowuah, Mensah, Chan, & Roskilly, 2015; Florkowski & Kolavalli, 2013; Hell, Cardwell, Setamou, & Poehling, 2000; James et al., 2007; Jolly et al., 2007; Kpodo, 1995; Perrone et al., 2014; Sugri et al., 2015; Udoh, Cardwell, & Ikotun, 2000). However, limited studies have examined the effectiveness of good agricultural practices in the various ecological zones of Ghana and their effects on aflatoxin contamination.

Therefore, this research aimed to assess the effects of varieties of maize, agroecological zones, and aflatoxin control methods on aflatoxin contamination in maize.

2. MATERIALS AND METHODS

2.1. Farm Experiment

The agricultural experiment was conducted in two distinct agroecological zones spanning the country. The two agroecological zones under consideration were the Forest zone, located in the Ashanti Region, and the Savannah zone, situated in the Upper West Region. The specific locations were Wa in the Upper West Region and Ejisu in the Ashanti Region. Wa is situated in the northwest of Ghana, roughly between the latitudes of 1°40′N and 2°45′N and the longitudes of 9°32′W and 10°20′W. Its area is roughly 579.86 Km², or 6.4% of the UWR's total landmass. Wa is located in the Savannah high plains, typically between 160 and 300 meters above sea level (Ahmed

et al., 2020). The Ejisu-Juaben Municipality, which is situated in the center of the Ashanti Region, has Ejisu as its capital city. It is located between latitudes 1°15′ and 1°45′ north and 6°15′ and 7°00′ west (Acheampong & Dinye, 2015).

Figure 1 illustrates the map of Ghana, indicating the two study areas. The Forest zone has a bimodal rainfall pattern, allowing for two cropping seasons per 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 (Nkrumah et al., 2014). The minor cropping season starts in September and runs through November. The Savannah zone is characterized by an unimodal rainfall pattern, with only one cropping season (major season) from May to November (Nkrumah et al., 2014). December marks the beginning of the Harmattan, and it continues till March. The season of Harmattan is characterized by a dry period and dust blown from the Sahara Desert to Ghana (Oppong-Anane, 2006). The experiment was conducted in the field from May 2020 to January 2021. The soil was analyzed to ascertain the nutrient composition and the presence of aflatoxin-causing fungi. The land was ploughed before planting. The treatments were two agroecological zones (Forest and Savannah), two maize varieties (Opeaburo and Wangdataa), and three methods of aflatoxins control (biological (aflasafe), chemical, and hoeing).

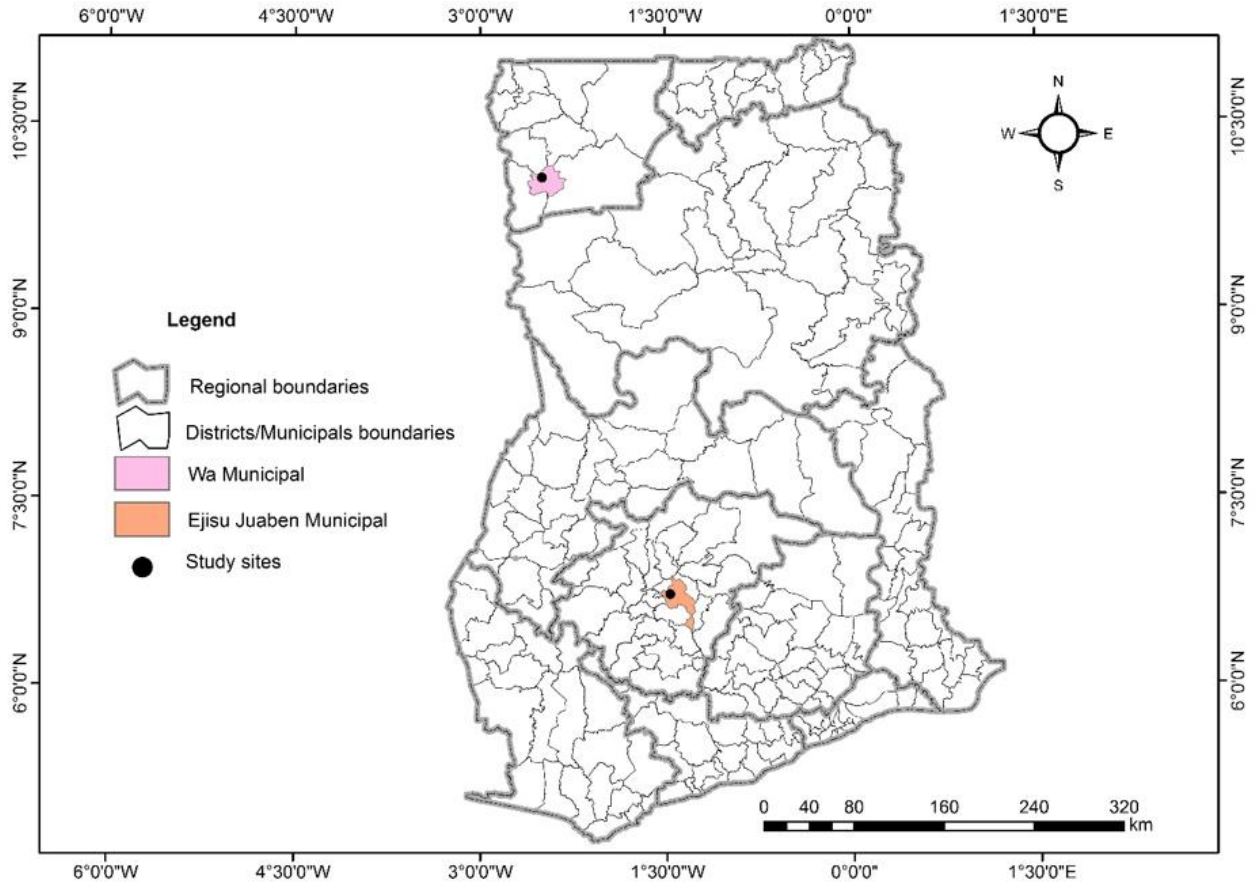

Figure 1. Map of Ghana indicating the study areas.

2.2. Experimental Design for Planting

The experiment was carried out in a 2x2x3 factorial in a Randomized Complete Block Design (RCBD). After the randomization, the plots were divided into two to separate the treatment from the control. The treatment was replicated three times. A split-plot design was used in conjunction with RCBD.

2.3. Plot Layout

Two main fields were created: fields A and B in each zone. Field A was the control field, while field B was the treatment field (application of aflasafe). In each zone, the treatment field was 200 m away from the control field. The control fields must be sufficiently separated from the treatment fields to avoid underestimating efficacy (Agbetiameh et al., 2020).

2.4. Microflora and Soil Nutrient Analysis

Samples of soil were taken from all four sites and taken to Kwame Nkrumah University Science and Technology Aflatoxin Laboratory and Soil Science Laboratory for aflatoxin-causing organisms and soil nutrient analyses, respectively. The soil was analysed for pH, Nitrogen, Phosphorus, and Potassium.

2.5. Land Preparation

The land was ploughed, and the debris was left on the soil surface without burning for 7 days before planting. Pegging and lining were done after the debris was dried. This was done to divide the land into blocks and plots.

2.6. Planting

Seeds were planted directly with a spacing of 70cm between rows and 25cm between plants. A planting stick was used to make a hole at a depth of 5cm, and two seeds of maize were placed in the hole and covered with soil. All the maize seeds were planted at an even depth of 5cm into firm, moist soil to ensure good seed-to-soil contact for moisture uptake and subsequent germination. Planting was done in rows.

2.7. Maize Cultivar

The maize cultivars used were Opeaburo and Wangdataa. Opeaburo is a hybrid variety that matures in 110 days (Afranaa Kwapong & Ankrah, 2023). Wangdataa is a local variety that matures in 90 days (Ayipio, Abu, Agyare, Azewongik, & Bonsu, 2018). The seeds were bought from the Antika shop in Wa, in the Upper West Region. Table 1 describes the maize varieties planted.

2.8. Fertilizer Application

Nitrogen, Phosphorus, and Potassium (NPK) compound fertilizer (60-60-60 kg/ha) was applied when the maize plants were 14 days after planting and top-dressed with nitrogen (30 kg/ha) at 5 weeks after planting. The method used for the application was the side placement method.

2.9. Chemical Control

Chemical weed control was used as one of the methods of aflatoxin control. The name of the herbicide used for weed control was Nicoking, which contains 40-oil dispersion (OD) Nicosulfuron. Nicoking is a selective and systemic post-emergence herbicide that is used to control annual and perennial grasses and broadleaf weeds in maize. The weeds were sprayed with a knapsack sprayer fitted with a low-volume nozzle at 200 ml per 15 litres of water. The chemicals were applied twice during the growing season. The first was done when the maize was 14 days old. The second was done when the maize was 38 days old.

2.10. Hoeing

Hoeing was used as one of the methods of aflatoxin contamination control. This treatment served as the control. Hoeing was done twice during the cropping season. The first hoeing was done when the maize was 14 days old. The second was done when the maize was 38 days old.

2.11. Aflasafe Application

The GH (02) Aflasafe was applied by the hand broadcast method. The product was broadcasted by hand on the soil surface when the maize was 40 days old after planting at a rate of 10 kg/ha, as described by Agbetiameh et al. (2019). This time corresponded with 2–3 weeks before crop flowering. All agronomic operations, such as weeding and fertilizer application, were finalised before the aflasafe application to reduce movement in the field for about 7 to 10 days after treatment so the product remained on the soil surface. The soil was moist when the aflasafe was applied.

2.12. Sample Collection of Grains at Harvest and Weather Data

The maize was harvested when it reached physiological maturity, and most of the cobs had dried down. Ten ears of maize were randomly collected at harvest from all the fields. The cobs were handpicked, hand-shelled, and immediately taken to the KNUST aflatoxin laboratory for the preharvest aflatoxin analyses.

2.13. Weather Data

Secondary data consisted of meteorological data (rainfall, relative humidity, and temperature) from the Ghana Meteorological Department, Wa, and Kumasi, which covered January to December 2020.

2.14. Laboratory Analysis

The maize samples were sent to the Aflatoxins Laboratory, Kwame Nkrumah University of Science and Technology, for analysis. The maize samples were analyzed for aflatoxins B1, B2, G1, and G2 using the method described by Sirhan, Tan, Al-Shunnaq, Abdulra'uf, and Wong (2014) with slight modifications. Acetonitrile-acetic acid v/v (9:1) was used as the extraction solvent. 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 minute. Then, 5 mL of the extraction solution was added after the solution had been allowed to stand for 5 minutes. The resultant mixture was agitated at 250 rpm for 15 minutes and vortexed for 3 minutes with a Genie Vortex machine. The mixture was then added to a mass of 1.32 g of anhydrous MgSO4 and 0.2 g of NaCl, vortexed for 1 minute, and then agitated at 250 rpm for 5 minutes. Before injection, the upper organic layer of the tube was filtered through a 0.45-m nylon syringe after being centrifuged for 5 minutes at 4000 rpm. The filtered extract was injected into the high-performance liquid chromatography apparatus at a volume of 50 L (HPLC).

2.15. HPLC Analysis

For post-column derivatization, an HPLC analysis was done with the Photochemical Reactor for Enhanced Detection (PHRED), which was in line with the Association of Official Analytical Chemists, 2005.08 (AOAC, 2006) for post-column derivatization. For post-column derivatization, use the photochemical reactor for enhanced detection (PHRED). The determination of aflatoxin was done by using an Agilent 1200 Quaternary Pump with Fluorescence Detector (Ex: 360 nm, Em: 440 nm) and a Sunfire® C18 Column (150 x 4.60 mm, 5 um). Methanol and water were used as the mobile phases at a rate of 1ml/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 the preparation of Aflatoxin Mix (G1, G2, B1, B2) standards (ng/g). Aflatoxin concentrations in the samples were determined using retention standard solution runs and calibration curves for each toxin.

2.16. 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 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 run blanks had no detectable level of the desired analyte. Trueness was 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:

$$\text{Aflatoxin, ng/g} = A \times (T/I) \times (1/W)$$

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

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

2.17. Data Analysis

The data sets were analyzed using a randomized complete block design. Differences between treatment means were separated by Fischer's Least Significant Difference at the 5% probability level. An 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. Soil, Temperature, Humidity, and Rainfall Analysis

Table 2 presents the results of the soil fertility analysis. The Forest zone had higher soil nutrient content in N (0.217%), K (0.395%), Ca (5.02%), Mg (1.20%), H (0.210%), organic carbon 2.075% and organic matter (3.577%) than the Savannah zone. Nutrient levels in the Savannah zone were N (0.140%), K (0.262%), Ca (3.60%), Mg (0.80%), P (37.59%), Na (0.0591%), and Al (0.307%). Table 3 presents the results of the soil analysis for aflatoxin-causing organisms. The results showed that soil samples from the Savannah zone had higher levels ($2.30 \times 10^4 \pm 0.00$ - $2.45 \times 10^5 \pm 0.04$) of the aflatoxins (moulds) causing organisms than those from the Forest zone. Table 4 presents the data on temperature, humidity, and rainfall for 2021, the period in which the experiment was conducted. From the results, the Savannah zone surprisingly had a higher total rainfall (1192.6mm) than the Forest zone (1189.6mm). The Ashanti region recorded the highest humidity (75.7%) compared to the Savannah zone (53.5%).

Table 1. Maize varieties planted.

				Average yield	
Variety	**Type of variety**	**Grain colour/ Texture**	**Maturity days**	**Bags/Acre**	**Tonnes/Hectare**
Wangdataa	Drought and Striga tolerant	White/Flint-dent	90	4.7	19
Opeaburo	Normal/Hybrid	White/Flint-dent	110	7.5	30

Source: Recommended Production Practices for Maize in Ghana (Adu et al., 2014).

Table 2. Results of soil analysis.

Sample name (Soil)	pH	AVAIL P mg/kg	% Total N	Exch. bases (cmol/kg)				Exch. acidity		% Org. carbon	% Org. matter
				K	Ca	Mg	Na	Al	H		
SZA	6.36	37.59	0.140	0.262	3.60	0.80	0.0591	0.307	0.169	0.479	0.825
SZB	6.01	19.74	0.105	0.262	3.80	0.60	0.0172	0.281	0.167	0.599	1.032
FZA	5.59	11.1	0.140	0.395	5.20	1.80	0.0133	0.281	0.162	2.075	3.577
FZBS	5.42	22.78	0.217	0.228	4.20	1.20	0.0133	0.264	0.210	1.157	1.995

Note: SZA (Savannah Zone A): Soil sample used for the control, and SZB (Savanah Zone B): Soil sample used for the treatment. FZA (Forest Zone A): Soil sample used for the control, and FZB (Forest Zone B): Soil sample used for the treatment.

Table 3. Results of soil analysis for the presence of Aspergillus spp in the soil.

S/No	Code	0.01	0.001	Counts	LOG CFU	Average	St. dev.	Results (CFU/g)
1	SZA	23	3	23000	4.361728	2.30E+04	0.00	$2.30 \times 10^4 \pm 0.00$
		23	3	23000	4.361728			
2	SZB	TNTC	26	260000	5.414973	2.45E+05	0.04	$2.45 \times 10^5 \pm 0.04$
		TNTC	23	230000	5.361728			
3	FZB	16	0	16000	4.20412	1.50E+04	0.04	$1.50 \times 10^4 \pm 0.04$
		14	0	14000	4.146128			
4	FZA	4	0	4000	3.60206	4.00E+03	0.00	$4.00 \times 10^3 \pm 0.00$
		4	0	4000	3.60206			

Note: SZA (Savannah Zone A): Soil sample used for the control, and SZB (Savanah Zone B): Soil sample used for the treatment. FZA (Forest Zone A): Soil sample used for the control, FZB (Forest Zone B): Soil sample used for the treatment, and LOG CFU (Logarithm of Colony Forming Units).

Table 4. Monthly weather data for Savannah and Forest Zones.

Month	Relative humidity		Maximum temperature		Minimum temperature		Rainfall	
	Savanah	Forest	Savanah	Forest	Savanah	Forest	Savanah	Forest
Jan	22	61	35	34.6	20.9	21.3	0	0
Feb	19	67	37.3	36.1	23.1	22.3	0	0
Mar	41	71	39.2	34.8	26.7	23.3	3.9	124.1
Apr	54	78	36.9	33.5	26	23	150	96.6
May	65	79	34.4	33.1	24.8	22.9	188	165.3
Jun	73	81	31.7	31.1	23	22.5	163	255
Jul	77	80	30	30.6	22	22.3	139.7	59
Aug	72	80	29.9	30.3	21.6	21.9	85.2	6.8
Sep	79	81	30.3	30.5	21.3	22.3	373.7	182.5
Oct	66	80	32.4	31.1	21.4	22.7	89.1	216
Nov	36	77	35.7	32.7	20.3	22.7	0	57.2
Dec	38	74	36.6	32.3	20.6	22.4	0	27.1
Total	642	909	409.4	390.7	271.7	269.6	1192.6	1189.6
Average	53.5	75.7	34.1	32.5	22.6	22.4	99.3	99.1

3.2. Results on the Effect of Variety of Maize, Agroecological Zone, and Aflatoxin Control Methods

3.2.1. Effect of Treatment on AFB1 and AFB2

There was no contamination of the harvested maize with the aflatoxins AFB1 and AFB2 for all the treatments in the two zones.

3.2.2. Effect of Ecozones and Aflatoxin Control Methods on AFG1 Contamination at the Farm Level

The interaction between Ecozones and aflatoxins control methods was statistically significant (Table 5). The highest AFG1 contamination (0.45 µg/kg) was produced by maize grown in the Savannah ecozone using the hoeing method of weed control. For the methods only, hoeing gave the highest AFG1 contamination (0.23 µg/kg), and the least was chemical and biological (0.00). For the ecozones, maize grown in the Savannah had the highest G1 (0.15 µg/kg), and the least were those grown in the Forest ecozone (0.00).

Table 5. Effect of ecozones and aflatoxin control methods on AFG1 contamination at the farm level.

Methods	Ecozones		
	Savannah	Forest	Means
Hoeing	0.45^a	0.00^b	0.23^a
Biological	0.00^b	0.00^b	0.00^b
Chemical	0.00^b	0.00^b	0.00^b
Means	0.15^a	0.00^b	
Highest significant difference, HSD (0.05): Methods= 0.02, Ecozones= 0.01, Methods* Ecozones= 0.03			

Note: Means with same letters (a,b) are not significantly different ($P < 0.05$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg.

3.2.3. Effect of Ecozones and Varieties on AFG1 Contamination at the Farm Level

The interaction of ecozones and maize varieties is statistically significant between the two zones (Table 6). The highest AFG1 contamination was produced by both Opeaburo (0.16 µg/kg) and Wangdataa (0.14 µg/kg) maize varieties grown in the Savannah ecozone. The least was recorded by both varieties in the Forest ecozone. For the varieties only, Opeaburo gave the highest G1 contamination (0.08 µg/kg), and the least was Wangdataa (0.07 µg/kg). For the ecozones only, maize grown in the Savannah had the highest AFG1 contamination (0.15 µg/kg), and the least were those grown in the Forest ecozone (0.00).

Table 6. Effect of ecozones and varieties on AFG1 contamination at the farm level.

Varieties	Ecozones		
	Savannah	Forest	Means
Opeaburo	0.16[a]	0.00[c]	0.08[a]
Wangdataa	0.14[a]	0.00[c]	0.07[b]
Means	0.15[a]	0.00[b]	
HSD (0.05): Varieties= 0.01, Eco zones= 0.013, Varieties* Eco zones= 0.03			

Note: Means with same letters (a, b,c) are not significantly different (P < 0.05). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg,

3.2.4. Effect of Varieties and Aflatoxins Control Methods on AFG1 Contamination at the Farm Level

The interaction between aflatoxin control methods and maize varieties was statistically significant (Table 7). The highest AFG1 contamination (0.25 µg/kg) was produced by the Opeaburo maize variety grown using hoeing as the aflatoxin control method. The least AFGI contamination (0.00) was recorded by both varieties grown using biological and chemical aflatoxin control methods. For the varieties only, Opeaburo gave the highest AFG1 contamination (0.08 µg/kg), and the least was Wangdataa (0.07 µg/kg). For methods only, hoeing recorded the highest contamination (0.23 µg/kg).

Table 7. Effect of varieties and aflatoxin control methods on AFG1 contamination at the farm level.

Methods	Varieties		
	Opeaburo	Wangdataa	Means
Hoeing	0.25[a]	0.20[b]	0.23[a]
Biological	0.00[c]	0.00[c]	0.00[b]
Chemical	0.00[c]	0.00[c]	0.00[b]
Means	0.08[a]	0.07[b]	
HSD (0.05): Varieties= 0.01, Methods= 0.02, Methods* Varieties= 0.03			

Note: Means with same letters (a, b,c) are not significantly different (P < 0.05). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg.

3.2.5. Effect of Ecozones and Methods of Aflatoxins Control on AFG2 Contamination at the Farm Level

The interaction of ecozones and methods of aflatoxin control was significant (Table 8). The highest contamination of AFG2 (0.19 µg/kg) was produced by maize grown in the Savannah ecozone using hoeing. For the methods only, hoeing gave the highest AFG2 contamination (0.09 µg/kg), and the least was by chemical and biological methods (0.00). For the ecozones only, maize grown in the Savannah had the highest AFG2 contamination (0.60 µg/kg), and the least were those grown in the Forest ecozone (0.00).

Table 8. Effect of ecozones and methods of aflatoxins control on AFG2 contamination at the farm level.

Methods	Ecozones		
	Savannah	Forest	Means
Hoeing	0.19[a]	0.00[b]	0.09[a]
Biological	0.00[b]	0.00[b]	0.00[b]
Chemical	0.00[b]	0.00[b]	0.00[b]
Means	0.60[a]	0.00[b]	
HSD (0.05): Methods= 0.01, Eco zones= 0.007 Methods* Eco zones= 0.02			

Note: Means with same letters (a, b,) are not significantly different ($P < 0.05$). ($P < 0.05$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg.

3.2.6. Effect of Ecozones and Varieties on AFG2 Contamination at the Farm Level

The interaction between ecozones and variety was statistically significant (Table 9). The highest contamination of AFG2 (0.12 µg/kg) was produced by the Wangdataa maize variety in the Savannah zone. For the variety only, Opeaburo had the least contamination in AFG2 (0.00). For the ecozones only, the Savannah had the highest AFG2 (0.06 µg/kg).

Table 9. Effect of ecozones and methods of aflatoxins control on AFG2 contamination at the farm level.

Varieties	Ecozones		
	Savannah	Forest	Means
Opeaburo	0.00[b]	0.00[b]	0.00[b]
Wangdataa	0.12[a]	0.00[b]	0.06[a]
Means	0.06[a]	0.00[b]	
HSD (0.05): Varieties=0.007, Eco zones=0.007 Varieties* Eco zones= 0.01			

Note: Means with same letters (a, b) are not significantly different ($P < 0.05$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg.

3.2.7. Effect of Varieties and Methods of Aflatoxin Control on AFG2 Contamination at the Farm Level

The interaction between varieties of maize and methods of aflatoxin control is statistically significant (Table 10). The highest contamination of AFG2 (0.19 µg/kg) was recorded by the Wangdataa maize variety using hoeing as an aflatoxin control method. The least amount of AFG2 contamination was recorded using biological and chemical aflatoxins control methods. For the varieties only, Wangdataa gave the highest AFG2 contamination (0.06 µg/kg), and the least was Opeaburo (0.00). For the control methods only, hoeing gave the highest AFG2 contamination (0.09 µg/kg), and the least were biological and chemical methods (0.00).

Table 10. Effect of variety and methods of aflatoxins control on AFG2 contamination at the farm level.

Methods	Varieties		
	Opeaburo	Wangdataa	Means
Hoeing	0.00[b]	0.19[a]	0.09[a]
Biological	0.00[b]	0.00[b]	0.00[b]
Chemical	0.00[b]	0.00[b]	0.00[b]
Means	0.00[b]	0.06[a]	
HSD (0.05): Varieties= 0.01, Methods= 0.01, Methods* Varieties= 0.02			

Note: Means with same letters(a, b,) are not significantly different ($P < 0.05$). Treatment means were calculated from three replicated values. HSD – Highest Significant Difference. Unit for aflatoxin: µg/kg.

3.3. Discussions

The soil used for the experiment in the Forest zone was observed to be richer in nutrients than that in the Savannah zone. The Forest zone, by its nature, had thicker vegetation than that in the Savannah zone and was hence richer in nutrients because of the constant falling and decomposition of leaves, leading to higher organic matter. The Forest zone had higher nutrient content in the following soil nutrients: N (0.217%), K (0.395%), Ca (5.02%), Mg (1.20%), H (0.210%), organic carbon (2.075%), and organic matter (3.577%) than the Savannah zone, while the Savannah zone had higher nutrient content in the following nutrients: P (37.59%), Na (0.0591%), and Al (0.307%) than the Forest zone. The high level of P (37.59%) is because of the constant bush burning every season in the Savannah zone. The soil used for the experiment was observed to be less rich in nutrients than that reported by Ama et al. (2022), (5.94%) Ca (0.75%), K (0.085%), and N (16.46%), but higher than (0.026%), K (0.35%) Ca, and 0.02% N of soil as reported by Mhlontlo, Muchaonyerwa, and Mnkeni (2007).

From the soil analysis results on aflatoxins causing moulds, it was observed that soil samples from the Savannah had higher levels of the aflatoxins causing moulds than those from the Forest zone. The aflatoxins-causing mounds are always present in soil (Yu, Cleveland, Nierman, & Bennett, 2005). One of the factors that determines the severity of the contamination is the condition of the soil (Wagacha & Muthomi, 2008). When conditions are unfavourable for the fungi, they turn into sclerotia and can lay dormant for an incredibly long time (Yu et al., 2005). This implies that maize grown in the Savannah zone will be prone to infestation by fungi, which has an impact on food safety and the health of consumers. Farmers in the Savannah zone should make sure that they practice the preharvest interventions for aflatoxins control to prevent contamination of the maize while it is growing on the field.

From the results (Table 4), the Wa (Savannah zone) had a higher total annual rainfall (1192.6mm) than the Forest zone (1189.6mm). This is an uncommon occurrence. In 1982, Wa recorded a higher total rainfall of (891.87) than Kumasi, and it happened again in 1996, when Wa still recorded a higher rainfall of (1199.87) than Kumasi (1040.47). These occurrences are attributable to climate variability and, therefore, indicate that efforts at combatting climate variability and climate change should be intensified. Climate variability and change occur because various human activities degrade the environment and the ozone layer. As a result of the unpredictable nature of the weather, the timing of planting and other operations is always affected. Contamination of crops by aflatoxins is expected to increase globally due to climate change (Battilani et al., 2016). It has been established that climate change can increase the prevalence of aflatoxins in crops such as maize. The Ashanti region recorded higher humidity (75.7%) compared to the Savannah zone (53.5%). The most harmful aflatoxin to humans and animals is aflatoxin B1 due to its close association with hepatocellular carcinoma, which can cause liver cancer (Qureshi et al., 2015). However, it was observed that all the samples were not contaminated with aflatoxin B1 and B2. A. flavus is capable of producing aflatoxins B1 and B2 (Kumar, Pathak, Bhadauria, & Sudan, 2021). This implies that the control methods used prevented the occurrence of *A. flavus* during the growing period of the maize.

Farm hygiene was practiced during the growing periods of the maize on the field. Control of weeds was done twice in the growing season of the maize. There was also the application of fertilizer to the growing maize. Applying soil nutrients can reduce plant stress, especially during the formation and development of seed, by maintaining enough soil pH and plant nutrition (Mutunga, Ndungu, & Muendo, 2017). When crops are stressed because of a lack of nutrients, it facilitates the proliferation of Aspergillus flavus, which produces secondary metabolites. Good agronomic practices are crucial for reducing aflatoxin contamination since fungal infestation begins at the preharvest stage (Cleveland et al., 2003; Mahuku et al., 2019). The study confirmed that all the varieties used in this research were resistant to B1 and B2 infections while growing on the field.

A. parasiticus is responsible for producing the Aflatoxins G1 and G2 (Kumar et al., 2021). At the end of the preharvest experiment, 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) and within the permissible limits of

Ghana, Codex Alimentarius, and the EU. This can be attributed to the number of moulds that were found in the soil before the experiment. The soil in the Savannah zone used for the control experiment had higher moulds (2.30 x 10^4 ± 0.00 CFU/g) than the Forest zone. The contamination in the Savannah zone only occurred in the samples where hoeing was used for aflatoxin control. When hoeing is used as the only aflatoxin control measure during cropping, much care should be taken to avoid contamination, particularly in the Savannah zone.

There was no contamination of any of the two varieties of maize used in the Forest ecozone with aflatoxin AFG1. AFG1 slightly contaminated the two varieties in the Savannah zone but was not significantly different ($P < 0.01$) from each other. The level of contamination was less than (<1.00) for the two varieties. The amount of AFG1 recorded was far below the EU standard of 4ug. The differences can be attributed to the soil condition before the maize was grown on it. The soil used for the control in Savannah had very low levels of N (0.105%), M (0.6%), and organic matter (1.03%) compared to the rest of the soil. There were more counts (260000, Table 3) of the fungal species in the soil found in the control zone of the Savannah zone than in the Forest zone. The variation between the two ecozones can also be attributed to the variation in rainfall and temperature during the growing season. Grains in the Savannah zone were harvested towards the end of August. There was uninterrupted rainfall in August in the Savannah zone (Table 4). This might have contributed to the slight infection recorded in the Savannah zone.

The results showed no contamination of the two maize varieties with AFG1, where biological and chemical aflatoxin control methods were used. The use of biological control to control aflatoxin helps to reduce the contamination of crops by aflatoxins and reduces contamination to a safe level, and this also has a carry-over effect that offers protection in storage (Bandyopadhyay et al., 2019; Ezekiel et al., 2019; Senghor et al., 2020). The two varieties recorded slight infections from the hoeing method, with Opeaburo recording the highest contamination (0.25 µg/kg). This variation can be attributed to the differences in the maturity period of the maize. Opeaburo takes 120 days to mature, while Wangdataa takes 90 days to mature. The longer the maize stays on the field, the higher the chances of it being contaminated (Xu et al., 2022) through excessive rain, the maize plant falling on the ground, and attack by insects and pests.

The interaction of ecozones and methods of aflatoxin control was significant (Table 8). The highest contamination of AFG2 (0.19 µg/kg) was produced by maize plants grown in the Savannah ecozone using the hoeing method. There was no contamination of the maize samples by chemical or biological control methods. Aflatoxins can be transmitted to crops through the contact of the produce with soil. During hoeing, the soil is loosened, and when it rains, the soil can easily be splashed on the maize fruit, and the soil particles can help in the easy dispersal of the spores when the wind blows. This might have accounted for the small contamination of maize samples with AFG2 (0.19 µg/kg) in the Savannah ecozone using the hoeing method. The soil surface was not disturbed by using the chemical control method. The differences in the temperature and humidity between the two zones might have accounted for the contamination of the maize grown in the Forest zone using the hoeing method (Table 4).

There was no contamination of Opeaburo with AFG2 in both ecozones. The Wangdataa variety grown in the Forest zone did not record any contamination. There was slight contamination of the Wangdataa variety in the Savannah zone with AFG2 (0.12 µg/kg). The growth of the fungi that cause aflatoxin contamination is influenced by the amount of pH present in the soil. Low pH ($3 > pH > 1$) will reduce the growth of the fungus. Soil that is slightly higher in pH ($6 > pH > 3$) will encourage fungal growth and production of aflatoxins (Eshelli, Harvey, Edrada-Ebel, & McNeil, 2015). The soil used for the experiment in the Savannah zone had a higher pH (6> pH) than the Forest zone. The variation that occurred at the level of contamination can be attributed to the differences in the soil pH. Contamination can be caused by prolonged drought and high temperatures during silking of the maize plant. Temperatures in the Savannah zone are usually higher compared to the Forest zone. The Savannah zone had an average temperature of (Min 22.6^{0}C, Max 34.1^{0}C) C, which was slightly higher than the average

temperature (Min 22.4^{0}C, Max 32.5^{0}C) of the Forest zone. The slight contamination recorded may be because of the high temperature. High temperatures promote the growth of the fungi.

There was no contamination of Opeaburo with AFG2, irrespective of the method used for the on-farm aflatoxin control methods. However, the Wangdataa variety recorded slight contamination of AFG1 (0.19 µg/kg) using the hoeing method to control aflatoxin. Contamination of crops by aflatoxins can occur on the field during harvesting, storage, and transport by the fungi (Kumar et al., 2021). The level of infection recorded is far below the permissible limits of the EU, which has the highest standard in terms of permissible limits. Wangdataa is a local variety that can withstand drought and Striga attacks (Adu et al., 2014). From the results, Opeaburo can withstand aflatoxins better than the Wangdataa variety. Opeaburo is a hybrid variety.

4. CONCLUSIONS

There was an absence of aflatoxin contamination seen in all maize samples cultivated within the Forest zone, irrespective of the treatments applied. In the Savannah zone, with the exception of the hoeing method, all treatments yielded maize samples that were free from contamination by aflatoxins BI, B2, G1, and G2. The Wangdataa maize variety demonstrated the highest reported contamination of AFG2 at a concentration of 0.19 µg/kg while employing hoeing as the strategy for controlling aflatoxin. Maize cultivated in the Savannah ecozone utilising the hoeing technique of aflatoxin management had the most substantial AFG1 contamination, measuring 0.45 µg/kg. Nevertheless, the degree of contamination observed was far lower than the acceptable threshold. The study suggests that farmers should consider using the Wangdataa and Opeaburo cultivars because they demonstrate resistance against aflatoxins. It is recommended that farmers incorporate the chemical and biological strategies outlined in this study to mitigate the presence of aflatoxins. Whenever a farmer opts for hoeing as a means of aflatoxin control in the Savannah zone, it is advisable to supplement this approach with the simultaneous utilisation of biological or chemical approaches for aflatoxin management. The effective management of aflatoxins during maize cultivation was achieved through the utilisation of resistant cultivars, biological control methods such as aflasafe, and chemical control measures like nicoking in the two distinct agroecological zones. Farmers in both regions, particularly those in the savannah zone, are advised to adopt effective preharvest aflatoxin management strategies in order to prevent the contamination of their maize crops with aflatoxins. This is crucial due to the favourable environmental circumstances that facilitate the proliferation of these harmful poisons.

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