



Effect of Aflasafe® Application on the Productivity, Grain Quality, and Aflatoxin Reduction in Maize (*Zea mays* L.)

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ABSTRACT

Aflatoxin contamination remains a major threat to food safety, crop quality, and public health in sub-Saharan Africa. Aflasafe, a biocontrol product containing atoxigenic *Aspergillus flavus* strains, has proven effective in reducing aflatoxin levels, yet its broader agronomic impacts are less understood. This study evaluated the effects of Aflasafe applied at vegetative and flowering stages on maize growth, yield, nutritional composition, and postharvest quality. Application at the flowering stage led to marked improvements in vegetative growth, including plant height, leaf area, and stem girth. Yield components such as ear number, grain count, and overall yield were also significantly enhanced. Proximate composition analysis indicated elevated levels of crude fat, protein, and carbohydrate contents. In addition, grains treated with Aflasafe showed higher germination rates and seed vigor, together with reduced populations of toxigenic *A. flavus* and aflatoxin concentrations that remained within internationally accepted safety limits. These results demonstrate Aflasafe's dual potential as a biocontrol and growth-promoting agent, advancing sustainable maize production, food security, and public health resilience.

Keywords: Aflasafe, Biocontrol, Crop yield, Fungal contamination, Proximate composition.

INTRODUCTION

Maize (*Zea mays* L.) is a globally significant cereal crop, serving as a fundamental source of food, feed, and industrial raw materials. In sub-Saharan Africa, and particularly in Nigeria, maize is not only a dietary staple but also an essential driver of rural livelihoods and national food security (Ekpa et al., 2019; Wossen et al., 2023). Its adaptability to diverse agro-ecological zones and its role as a primary

source of calories have made it indispensable in both subsistence and commercial farming systems (Asfaw et al., 2024). Despite its critical importance, maize production faces persistent biotic challenges that undermine both yield and grain safety, among which aflatoxin contamination represents a major concern (Erenstein et al., 2022; Nji et al., 2022).



Aflatoxins are toxic and carcinogenic secondary metabolites produced primarily by *Aspergillus flavus* and *A. parasiticus* (Okechukwu et al., 2024). Aflatoxin B1, the most potent variant, is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (Kumar et al., 2017; Marchese et al., 2018). Chronic exposure in humans has been associated with hepatocellular carcinoma, immune suppression, and impaired growth in children, while in animals it contributes to reduced productivity and economic losses (Rushing & Selim, 2019; Benkerroum, 2020). These toxins are particularly prevalent in tropical regions, where warm and humid conditions favor the proliferation of toxigenic fungi. Aflatoxin contamination can occur both in the field and during storage, posing a multi-stage threat to maize safety across the value chain (Kumar et al., 2017; Massomo, 2020).

Conventional management strategies, such as fungicides and post-harvest decontamination, have shown limited success in mitigating aflatoxin risks, particularly at the smallholder level (Kumar et al., 2017; Shabeer et al., 2022; Mdindikasi et al., 2024). These approaches are often constrained by high costs, regulatory limitations, and concerns over environmental and human health. As a result, attention has shifted towards sustainable, biologically based solutions that are safe, affordable, and compatible with existing farming practices (Muhie, 2022). Biological control using atoxigenic strains of *A. flavus* has emerged as a promising alternative. These non-aflatoxigenic strains act through competitive exclusion, displacing their toxigenic counterparts by occupying the same ecological niche (Khan et al., 2019; Chofamba, 2021). Aflasafe®, a biocontrol product developed by the International Institute of Tropical Agriculture (IITA) and partners, leverages this mechanism using native, atoxigenic strains

specifically adapted to local agro-ecological zones.

Previous studies have demonstrated Aflasafe®'s efficacy in reducing aflatoxin levels in maize across various field settings (Agbetiamah et al., 2020; Atehnkeng et al., 2022; Bonkougou et al., 2024; Ola et al., 2022). However, the broader agronomic implications of its application, including effects on crop productivity and grain quality, remain underexplored. This study was therefore undertaken to evaluate the impact of Aflasafe® application on maize cultivated under aflatoxin-prone conditions in Ilorin, Kwara State, Nigeria. Specifically, it aimed to assess its effectiveness in enhancing grain yield, improving nutritional grain quality, and reducing aflatoxin contamination.

MATERIALS AND METHODS

Experimental Site, Soil Sampling, and Experimental Design

The study was carried out during one cropping season at an experimental field located in Kilanko, Offa Garage Road, Ilorin West Local Government Area, Kwara State, Nigeria. The location is within the Southern Guinea Savanna agroecological zone, characterized by high relative humidity and moderate annual rainfall suitable for maize production.

Before planting, soil samples were collected to determine the initial physicochemical properties of the soil. Sampling was done at a depth of 0–15 cm using a hand trowel. Ten subsamples were randomly collected across the field, thoroughly mixed to form a composite sample, air-dried for 24 hours, and sieved through a 2 mm mesh. The prepared sample was analyzed at the Nigerian Stored Products Research Institute (NSPRI), Ilorin, to determine soil texture, organic carbon, organic matter, total nitrogen, soil pH, available phosphorus, and exchangeable cations



including calcium, magnesium, sodium, and potassium using standard laboratory procedures (Ganiyu et al., 2020; Tiruneh et al., 2021; Takamoto et al., 2023; Oliveira et al., 2024).

The experiment was arranged in a completely randomized design with three treatments. Maize was planted at a spacing of 30 cm within rows. To minimize border effects, the first and last rows of each plot were used as guard rows, and data were collected only from the inner rows. Treatments consisted of Aflasafe® application at different maize growth stages: T1, no Aflasafe® application (control); T2, Aflasafe® application at the vegetative stage; and T3, Aflasafe® application at the flowering stage.

Maize Variety and Aflasafe® Application

The maize variety used was Sammaz 29, a high-yielding and widely adapted variety. Seeds were obtained from the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. Aflasafe®, sourced from the International Institute of Tropical Agriculture, Ibadan, Nigeria, was applied at a uniform rate of 10 kg/ha by manual broadcasting in accordance with the manufacturer's recommendations. Application at the vegetative stage was carried out at 5–6 weeks after planting, approximately three weeks before flowering, while application at the flowering stage was conducted at 9 weeks after planting.

Data Collection

Morphological Parameters

Morphological characteristics were assessed on tagged plants, starting two weeks after sowing and continuing at two-week intervals. The parameters measured included plant height, leaf number, and stem girth. Leaf area estimation followed the method of Schrader et

al. (2021) using the formula: Leaf area = $0.5 \times \text{leaf length} \times \text{leaf breadth}$.

Physiological Parameters

Physiological assessment focused on total chlorophyll content. Fresh leaf samples underwent extraction in 80% acetone, and chlorophyll concentration was quantified spectrophotometrically using Arnon's equations, as described by Wagay et al. (2019).

Yield-Related Parameters

Yield components were evaluated at physiological maturity. Measurements included cob length, cob circumference, number of cobs per plant, number of seeds per cob, cob weight per plant, seed weight per plant, and total plot yield. Cob yield determination used ten representative plants per plot after air-drying to 12% moisture content (Akinuoye-Adelabu & Modi, 2017). Seed yield per hectare calculation followed the procedure described by Beyene et al. (2022).

Biochemical Parameters

Biochemical analyses included proximate composition and antioxidant-related compounds. Proximate analysis of maize grains for moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate contents followed the method of Qamar et al. (2016). Vitamin C determination used the 2,6-dichlorophenol indophenol titration method (Nielsen, 2010). Total carotenoid extraction employed an acetone–hexane solvent system, and spectrophotometric quantification followed Arvayo-Enríquez et al. (2013).

Microbiological Parameters

Microbiological evaluation involved isolation and identification of fungal species from rhizospheric soil and maize leaf tissues collected at different growth stages. Soil fungi isolation used the serial dilution plate method on Potato Dextrose Agar supplemented with

streptomycin, while endophytic fungi isolation followed surface sterilization of leaf segments before plating on the same medium (Khiralla et al., 2016; Shitole et al., 2019). Culture incubation occurred at $28 \pm 2^\circ\text{C}$ for 5–7 days, after which distinct colonies underwent sub-culturing to obtain pure isolates. Fungal identification relied on macroscopic characteristics such as colony morphology, pigmentation, and texture, as well as microscopic features including hyphal and spore structures, with standard mycological keys (Kidd et al., 2023). Aflatoxin B1, B2, G1, and G2 concentrations were quantified through thin-layer chromatography according to Castro and Vargas (2001).

Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) using SPSS software. Treatment mean separation

employed the Least Significant Difference test at a 5% significance level.

RESULTS AND DISCUSSION

Physicochemical Properties of Experimental Soil

The physicochemical properties of the experimental soil are presented in Table 1. The soil was classified as sandy loam, with low organic matter (0.67%) and organic carbon (0.58%), and a slightly alkaline pH of 6.9. These characteristics are typical of cultivated tropical soils and suitable for field-based evaluation of biocontrol agents such as Aflasafe. The soil recorded low calcium (3.99 cmol/kg) and sodium (0.83 cmol/kg) contents, moderate potassium (0.53 cmol/kg) and magnesium (0.566 cmol/kg) levels, and low available phosphorus (3.607 mg/kg). These findings agree with Bandyopadhyay et al. (2016), who reported that Aflasafe performs effectively across diverse soil types and agroecological conditions.

Table 1: Physicochemical results of the experimental surface soil.

Soil characteristics		
Physical properties	Particle size	Percentage distribution (%)
	Sand	50
	Silt	24
	Clay	26
	Textural class	Sand loamy
Chemical properties		
	pH	6.90
	OC	0.58
	TKN	0.585
	OM	0.676
	A.P (MGKG)	3.607
	Ca (cmol)	3.997
	Mg (cmol)	0.566
	K(cmol)	0.53
	Na (%)	0.83
	Conductivity	149.3

OC= Organic Carbon, OM= Organic Matter, TKN= Total Kjeldah Nitrogen, AP = Available Phosphorus, Ca= Calcium, Mg = magnesium, K= Potassium, Na= sodium, and Conductivity.

Effect of Aflasafe on Morphological Growth Parameters

Aflasafe application significantly influenced maize vegetative growth. Plant height increased progressively throughout the eight-week sampling period, with the highest values recorded under flowering-stage application (T3), followed by vegetative-stage application (T2), and the control (T1). Leaf number followed an irregular pattern, with significant increases at 2, 4, and 6 weeks under vegetative-stage application, while flowering-stage application recorded the highest leaf number at 8 weeks (Figure 1).

Stem diameter and leaf area increased with crop age across all treatments. Significant differences in stem diameter occurred under flowering-stage application. Leaf area development under flowering-stage application closely resembled the control, while vegetative-stage application resulted in comparatively smaller leaf area at 6 and 8 weeks (Figure 2). These observations align with Agbetiameh et al. (2020) and Atehnkeng et al. (2022), who reported enhanced plant height, leaf number, stem diameter, and leaf area in Aflasafe-treated maize. Robust leaf development under flowering-stage application suggests improved photosynthetic capacity and biomass accumulation.

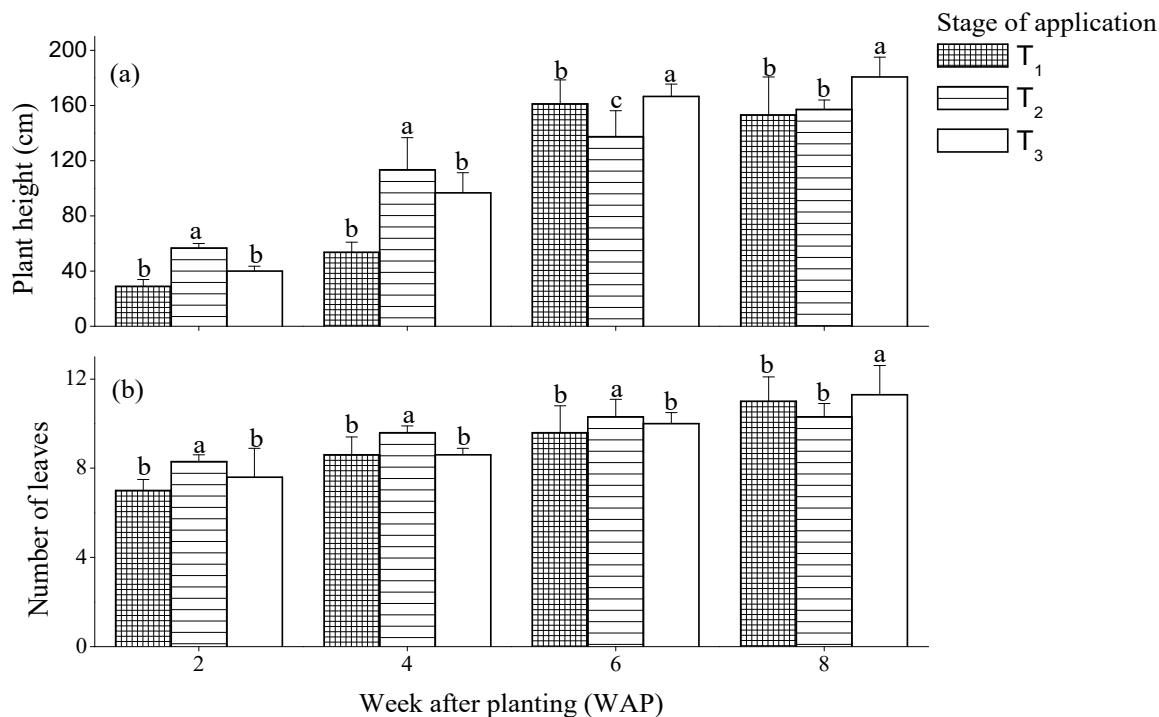


Figure 1 (a–b): Plant height and number of leaves at different crop growth periods as influenced by Aflasafe application. Values represent means of three measurements. T1 = control (no Aflasafe application); T2 = Aflasafe application at the vegetative stage; T3 = Aflasafe application at the flowering stage..

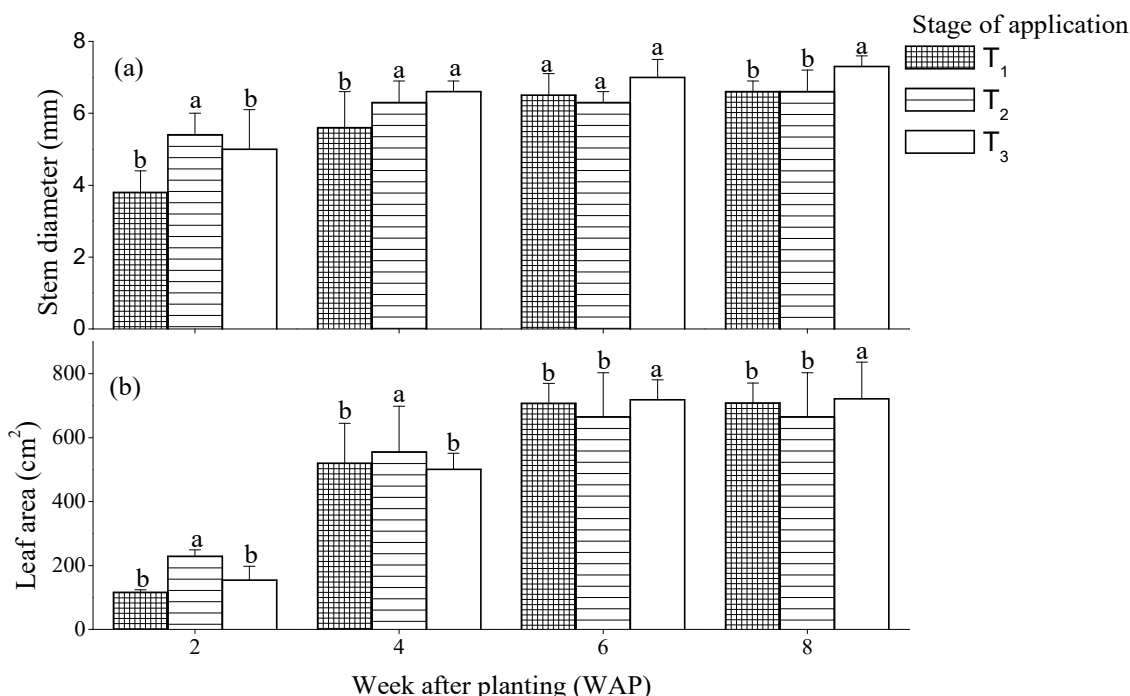


Figure 2 (a–b): Stem diameter and leaf area at different crop growth periods as influenced by Aflasafe application. Values represent means of three measurements. T1 = control (no Aflasafe application); T2 = Aflasafe application at the vegetative stage; T3 = Aflasafe application at the flowering stage

Effect of Aflasafe on Yield and Yield Components

Yield responses to Aflasafe application are shown in Tables 2 and 3. Cob yield and seed yield per hectare differed significantly among treatments, with the highest values recorded under flowering-stage application (T3). Other yield components such as number of cobs per plant, number of seeds per plant, cob length, cob circumference, and 1000-seed weight showed numerical increases under flowering-stage application, although not all differences were statistically significant.

Significantly higher cob seed weight and seed number occurred under flowering-stage application, with mean values of 227.60 g and 354.33 g, respectively. Lower values were

recorded in the vegetative-stage application and control treatments. These results corroborate findings by Kaburi et al. (2025) and Mustapha Dadzie et al. (2021), who reported superior yield performance in Aflasafe-treated maize. The results confirm that Aflasafe enhances not only food safety but also crop productivity and economic returns.

Table 2: Effect of Aflasafe on Yield.

Treatment	Cob yield (Kg/ha)	Seed yield (Kg/ha)
T1	1630.74±705.5 ^c	584.29±25.95 ^b
T2	2877.77±346.83 ^b	631.85±73.50 ^b
T3	3288.88±135.78 ^a	975.89±102.59 ^a
p-value	0.01	0.02

T₁ = Control; T₂= vegetative stage and T₃= flowering stage

Table 3: Effect of Aflasafe on Yield components.

Treatment	Number of seeds per plant(g)	Cob length per plant (g)	Cob Seed weight per plant (g)	Cob circumference Per plant	1000-seed weight(g)
T1	302.22±7.25 ^b	13.44±1.094 ^b	204.20±1.94 ^b	12.55±1.78 ^b	204.20±11.90 ^c
T2	303.44±29.98 ^b	12.22±1.72 ^b	225.9±5.512 ^a	11.33±1.45 ^b	225.90±1.33 ^a
T3	354.33±5.67 ^a	16.88±1.49 ^a	227.60±7.694 ^a	14.77±1.02 ^a	227.60±5.88 ^a
P-value	0.02	0.07	0.02	0.03	0.02

T₁ = Control; T₂= vegetative stage and T₃= flowering stage

Effect of Aflasafe on Physiological Parameters

Chlorophyll a, chlorophyll b, and total chlorophyll contents are presented in Table 4. No significant differences occurred among treatments for chlorophyll a or chlorophyll b. Chlorophyll a values ranged from 1.0985 to 1.0990, while chlorophyll b ranged from 1.1595 to 1.1695. A slight increase in chlorophyll b occurred in Aflasafe-treated plants, consistent with reports by Senghor et al. (2021).

Table 4: Aflasafe effect on chlorophyll determination of maize leave

Factors	Chlorophyll A	Chlorophyll B	Total chlorophyll
Treatment			
T1	1.099±0.00 ^a	1.167±0.00 ^b	1.266±0.00 ^b
T2	1.0985±0.00 ^b	1.1695±0.00 ^b	1.268±0.00 ^b
T3	1.099±0.00 ^a	1.1595±0.00 ^a	1.259±0.00 ^a
p-value	0.2	0.6	0.7

T₁ = Control; T₂= vegetative stage and T₃= flowering stage

Effect of Aflasafe on Proximate Composition and Energy Value

The proximate composition of maize grains is presented in Table 5. Aflasafe application did not significantly affect moisture, ash, crude fiber, crude fat, or crude protein, except for carbohydrate and energy values. Moisture content ranged from 26.04% to 30.1%, with the highest value recorded under flowering-stage application. While increased moisture supports physiological activity and yield, it necessitates careful postharvest drying and storage management (Ziegler et al., 2021).

Crude fiber content peaked under vegetative-stage application, while crude fat was highest under flowering-stage application. Protein content was highest under vegetative-stage application. These results align with Kaburi et al. (2025) and Atehnkeng et al. (2022), who reported enhanced nutritional quality in Aflasafe-treated maize. Higher carbohydrate content and energy value under flowering-stage application indicate improved energy density and food value.

Table 5: Effect of Aflasafe on Proximate composition and energy value on maize grain at harvest

Treatment (T)	Moisture (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Energy value (kcal)
T1	26.04 ± 0.26 ^b	1.14 ± 0.26 ^a	1.12 ± 0.63 ^b	2.56 ± 0.60 ^b	4.01 ± 0.40 ^b	61.05 ± 15.10 ^b	588.52 ± 0.90 ^b
T2	28.90 ± 0.09 ^b	1.07 ± 0.19 ^b	1.76 ± 0.47 ^a	2.50 ± 0.10 ^b	4.77 ± 0.52 ^a	60.98 ± 16.60 ^b	590.45 ± 0.73 ^b
T3	30.10 ± 0.06 ^a	1.07 ± 0.24 ^b	1.52 ± 0.58 ^b	3.46 ± 0.32 ^a	3.14 ± 0.31 ^b	64.76 ± 12.50 ^a	626.55 ± 1.27 ^a
p-value		0.01	0.02	0.01	0.01	0.01	0.01

T₁ = Control; T₂= vegetative stage and T₃= flowering stage

Effect of Aflasafe on Phytonutrient Composition

Vitamin C, lycopene, and beta-carotene contents are presented in Table 6. Vitamin C ranged from 6.11% to 12.68%, with the highest value recorded in the control treatment. Lycopene and beta-carotene contents remained constant across treatments, with no significant differences observed.

Table 6: Effect of Aflasafe on Phytonutrient composition of maize at harvest

Treatment (T)	Vitamin c	Lycopene	Beta carotene
T ₁	12.68±0.08 ^a	1.01±0.00 ^a	1.00±0.00 ^a
T ₂	6.11±0.02 ^b	1.01±0.00 ^a	1.00±0.00 ^a
T ₃	6.73±0.12 ^b	1.01±0.00 ^a	1.00±0.00 ^a
pvalue	0.02	0.01	0.01

Effect of Aflasafe on Fungal Load and Species Composition

Fungal load and species distribution in soil and maize leaves are presented in Table 7. Thirteen fungal species were identified, with *Aspergillus* species predominating. The highest fungal load occurred in the control soil and leaves, while the lowest fungal load occurred under vegetative-stage application. Soil fungal counts declined from 9.0×10^2 CFU/g in the control to 5.0×10^2 CFU/g under vegetative-stage application.

Leaf fungal loads followed a similar pattern, with vegetative-stage application recording the lowest counts. These results agree with Kaburi et al. (2025) and Senghor et al. (2021) and indicate effective suppression of fungal populations through Aflasafe application.

Table 7: Fungi load as influenced by application of aflasafe at different stages of growth

Parameter	T1	T2	T3
Soil			
Total fungal count	9.0×10^2 6.0×10^3	5.0×10^2 2.0×10^3	7.0×10^2 5.0×10^3
Organisms identified	<i>Aspergillus flavus</i> <i>Penicillium chrysogenum</i> <i>Mucor</i> spp, <i>Aspergillus parasiticus</i> , <i>Aspergillus niger</i> <i>Cladosporium</i> spp <i>Aspergillus terreus</i> <i>Aspergillus nomius</i>	<i>Fusarium</i> <i>oxysporum</i> , <i>Rhizopus</i> <i>stolonifer</i> , <i>Mucor</i> spp., <i>Culvularia lunata</i> , <i>Penicillium</i> <i>chrysogenum</i>	* <i>Aspergillus niger</i> <i>Aspergillus terreus</i> <i>Mucor</i> spp <i>Rhizopus stolonifer</i> <i>Penicillium chrysogenum</i>
Leaves			
Total fungal count	8.0×10^2 5.0×10^3	4.0×10^2 3.0×10^3	6.0×10^2 4.0×10^3
Organisms identified	<i>Aspergillus paracitus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus nomius</i> , <i>Penicillium chrysogenum</i> , <i>Fusarium oxysporum</i> , <i>Aspergillus niger</i> , <i>Cladosporium</i> spp., <i>Aspergillus parasiticus</i> , <i>Rhizopus stolonifer</i> , <i>Culvularia lunata</i> , <i>Aspergillus terreus</i>	<i>penicillium</i> <i>chrysogenum</i> , <i>Fusarium</i> <i>oxysporum</i> , <i>Rhizopus</i> <i>stolonifer</i>	<i>Mucor</i> spp., <i>Fusarium oxysporum</i> , <i>Rhizopus stolonifer</i> , <i>Penicillium chrysogenum</i> , <i>Cladosporium</i> spp

Effect of Aflasafe on Aflatoxin Contamination

Aflatoxin concentrations in maize grains are presented in Table 8. The control treatment recorded the highest aflatoxin B1 concentration (35 ppb), while flowering-stage

application recorded 12 ppb. Vegetative-stage application resulted in the lowest aflatoxin levels. These results confirm previous findings that Aflasafe reduces aflatoxin contamination to compliant levels (Mustapha Dadzie et al., 2021; Bonkougou et al., 2024).

Table 8: Aflatoxin concentration in maize sampled in the field

Treatment	B1	% Reduction of Aflasafe	B2 Ppb	G1	G2
T ₁	35.00±1.73 ^b	100	0.00	0.00	0.00
T ₂	10.00±2.51 ^a	28.75	0.00	0.00	0.00
T ₃	12.00±.57 ^a	34.28	0.00	0.00	0.00
Pvalue	0.05	0.05	0.00	0.00	0.00

Effect of Aflasafe on Post-harvest Germination and Seed Vigor

Post-harvest germination results are presented in Table 9. Vegetative-stage application recorded the highest germination percentage (88.83%) and fastest germination rate. These findings indicate that Aflasafe application preserved seed viability and vigor, consistent with Muñoz-Zavala et al. (2025).

Table 9: Post-harvest germination of maize grain test as influenced by application of Aflasafe

Treatment	% Germination	Speed of germination
T ₁	86.533±3.752 ^c	6.376±1.409 ^c
T ₂	88.833±2.233 ^a	11.39±1.994 ^a
T ₃	86.633±6.666 ^b	8.222±1.444 ^b
Pvalue	0.005	0.9

CONCLUSION

The application of Aflasafe not only suppresses aflatoxin contamination but also positively impacts plant growth, yield, grain nutritional composition, and postharvest quality. Application at flowering appears particularly beneficial for yield and proximate composition, whereas application at the vegetative stage improves protein content and seed vigor. These findings highlight the multiple benefits of Aflasafe and support its

practical use in maize production systems to improve food safety, yield, and economic returns.

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