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RESEARCH PAPER

Complementarity of biochar on aflasafe biocontrol agent to aflatoxin contamination in groundnuts (*Arachis hypogea* L.) under screen house conditions

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Abstract

Biochar amendment is acknowledged to suppress the effect of pathogenic fungi and favour plant resistance against soil-borne pathogen effect. The study tested the sole role of Biochar's efficacy in managing Aflatoxin in groundnuts and when integrated with Aflasafe (aflatoxin biocontrol). This study was done at NM-AIST screen house where groundnut seeds were planted in pots filled with the mixture of soil and Biochar at the rates of 2.5%, 5% and 7.5% in April 2022. At a blooming stage, toxigenic and atoxigenic *A. flavus* strains were applied. After 120 days, groundnut seeds were harvested and sent to the ILRI laboratory in Kenya for aflatoxin quantification. Biochar showed a suppressive effect on toxigenic *A. flavus* by significantly reducing aflatoxin contamination in groundnuts. A negative correlation between Biochar and aflatoxin content was observed when the biochar rate was increased to 5%; above this rate, there was a slight increase in aflatoxin content. Aflatoxin contamination was observed to be comparatively less in the integration of Biochar and Aflasafe biocontrol. For example, the application of 5% Biochar and 2×10^6 aflasafe showed a 99.97% reduction compared to the control, while sole Biochar and aflasafe reduced aflatoxin by 85.6% and 90%, respectively. Biochar-amended soils indicated a dramatic increase in pH, CEC, Mn, P, K, Ca, B, Zn and Si. Over space and time, the long-term positive effects of Biochar potentially offer options for scaling up Aflatoxin control at pre-harvest crop growth and development stages.

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Introduction

Groundnut (*Arachis hypogaea* L.) is one of the leguminous crops (Daudi *et al.*, 2018). This crop is a native New World crop where early pioneers found it cultivated broadly in both Mesoamerica and South America (Abady *et al.*, 2019; Daudi *et al.*, 2018; Singh Tomar *et al.*, 2022). It is reported that groundnut remnant pericarp tissue recovered from archaeological sites in Peru dates its purposeful agricultural use to around 3900-3750 years ago (Daudi *et al.*, 2018). The domestication of this crop is supported by archaeological records between 300 and 2500 BC in Peruvian desert oases and likely first occurred in the valleys of the Paraguay and Parana rivers in the Chaco region of South America (ICRISAT, 2016). In Africa, groundnuts were presented from Brazil by the Portuguese in the 16th century (Daudi *et al.*, 2018). Frank Samuel, head of the United Africa Company, came up with the idea in 1946 of cultivating groundnuts in Tanganyika for the production of vegetable oil (Katundu *et al.*, 2014). Groundnut is the most significant crop for smallholder farmers in Tanzania, providing food, feed, and income for families (Mfaume *et al.*, 2019). The crop is grown in different types of soils, but more preferably those with more than fifty per cent sand with pH ranges between 4.8 to 7 and rainfall range of 600 mm to 1500 mm (Daudi *et al.*, 2018). Nutritionally, groundnut is rich in fat, protein, carbohydrates, vitamins, and minerals (Abady *et al.*, 2019). Groundnuts mostly succumb to Aflatoxin contamination at the pre-harvest stage due to their anatomical structure (root crop) (Kuhumba *et al.*, 2018). Aflatoxin contamination in groundnuts is increasing tremendously regardless of the interventions made due to the inherent nature of the crop and soil as the sole media for both crop and aflatoxin-causing inoculum. According to the European Rapid Alert System for Food and Feed (2020) database, among other mycotoxins, Aflatoxin was the most common mycotoxin in groundnuts (Pickova *et al.*, 2021). FAO (2003) asserts that 25% of the world's food products (maize and groundnuts) were significantly affected by Aflatoxins. In Africa, the annual monetary loss due to Aflatoxin-contaminated groundnut in 2019 was reported to be over $250 million (Mfaume *et al.*, 2019). It is also reported that the annual economic impact caused by the Aflatoxin effect on humans in Tanzania was approximated to be $1,100 (Mfaume *et al.*, 2019). In 2016, Tanzania reported 65 hospitalized patients and 19 deaths in high groundnut-producing districts (Chemba and Kondoa) due to Aflatoxin (Massomo, 2020).

Currently, the main Aflatoxin management strategies in use include good agronomic practices (GAP), biological control, timely planting and harvesting, good post-harvest handling, good storage techniques, and chemical control (Beltran and Bandyopadhyay, 2021). The performance of all of these techniques in combination was reported to be not effective in reducing aflatoxin contaminations (Abdelaziz *et al.*, 2022; Hell and Mutegi, 2011; Johnson *et al.*, 2018; Linz *et al.*, 2014). There is a need to conduct studies on Aflatoxin effective management methods that would be integrated and offer good results. The use of Biochar for soil amendment and pathogen management is widely reported in the literature explaining its effect in some pathosystems. It is reported that Biochar is more effective in controlling soil-borne pathogens and has suppression efficacies of 86% for fungi, 100% for oomycetes, 100% for viruses, 96% for bacteria, and 50% for nematodes (Iacomino *et al.*, 2022). As fungal soil-borne pathogens are concerned, their effect was reported in *F. oxysporum* f. spp., *Verticillium dahlia*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium cepivorum* and *Sclerotium rolfsii* (Hou *et al.*, 2022; Iacomino *et al.*, 2022; Jaiswal *et al.*, 2014; Medeiros *et al.*, 2021; Singh and Kumar, n.d.). Biochar effectiveness is the function of raw materials used, soil type, soil quality and pyrolysis temperature (Frenkel *et al.*, 2017b; Rahman, *et al.*, 2022; Sobczak *et al.*, 2020). Mechanisms reported so far include initiation of systemic resistance, augmentation of rhizosphere aptitude of the microbial community, raising soil pH, and adsorption of phytotoxic compounds of plant and/or microbial origin (Bonanomi and Scala, 2015; Lu *et al.*, 2016; Medeiros *et al.*, 2021).

It has also been documented that Biochar can be used as a carrier material to deliver both nutrients and microbial inoculants to agricultural soils (Bolan *et al.*, 2021; Bonanomi and Scala, 2015; Kamali *et al.*, 2022). The unique physical and chemical properties of Biochar support beneficial microbial growth and activities in a diverse manner, whereby preventing them from desiccation during the dry period is the main mechanism reported so far (Egamberdieva *et al.*, 2016; Frenkel *et al.*, 2017c; Quilliam *et al.*, 2012; Wang *et al.*, 2020; Xiang *et al.*, 2022). These unique properties can be capitalized in integrating Biochar with beneficial atoxigenic Aspergillus flavus to increase their effectiveness as biocontrol agents.

Though atoxigenic A. flavus biocontrol was highly recommended for controlling aflatoxin in the recent years (Bandyopadhyay *et al.*, 2016; Maxwell *et al.*, 2021; Plateaux *et al.*, 2014), its efficacy is site-specific and subject to change due to farming and environmental factors. Much as biochar has complementary and enhancing multiple micro crop environments is desirable.

Therefore, it is here hypothesized that the integration of Biochar and atoxigenic Aspergillus flavus fungi strains (Aflasafe) would increase the efficacy of Aflasafe in smallholder farmers of Tanzania. Proven scientific information on this integration hypothesis is still scanty (Duan *et al.*, 2019; Hossain *et al.*, 2020; Kalus *et al.*, 2020). The study aims to assess the effectiveness of Biochar on Aflasafe in managing aflatoxin contamination in groundnut farming systems. The research will evaluate the efficacy of Biochar in-screen-house. It is expected that when Aflasafe is applied to the soil amended with Biochar in a groundnut planted field, Aflasafe effectiveness will be improved.

Materials and Methods

Study location

A study was conducted on March of the year 2022 at the NM-AIST screen house in Arusha, Tanzania. The area is located at latitude 3.40°14′20″ N, longitude 36.79°58′20″ E and altitude of 1199 m.a.s.l. The area has a temperature that ranges between 10 and 30 °C (50 and 86 °F) and an average annual rainfall of 1,180 millimetres (46.46 in). The humidity varies between 65 dries weather to 90% during the cool weather and main rain seasons (Fig. 1).

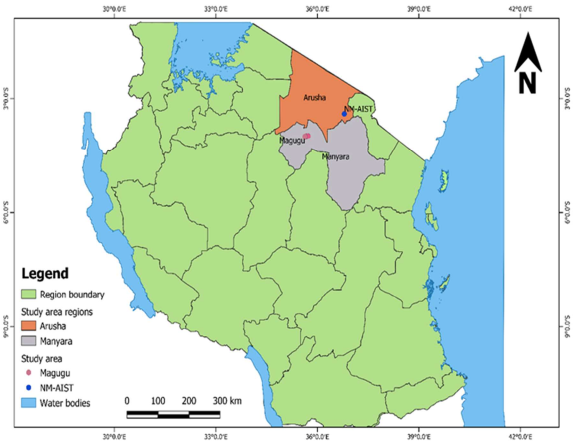


Fig. 1. Map showing the study area

Materials

Soil: A composite soil sample was collected from five sites in Magugu ward groundnut growing areas, then through halving, coning, and quartering, a representative 300 kg soil sample that was used for the pot experiment was obtained. This soil was the best soil for growing groundnuts on the Tanzania Northern zone (sand soil above 50%). Magugu is in Babati district-Manyara region, located at Latitude -3.9954° or 3° 59' 43" S; Longitude. 35.78172° or 35° 46' 54" E. Magugu has an average temperature of 27°C and relative humidity of 80%. Toxigenic *A. flavus*: The toxigenic fungi were collected from Mikocheni Mycology Laboratory. Aflasafe: Aflasafe was bought from agro shops in Arusha Tanzania. Maize cobs: Maize cobs were collected from farmers around NM-AIST for preparing biochar due to the necessity of using the locally available materials for easy accesabilty. Groundnut seeds: A highly susceptible Aflatoxin-free groundnut seed (Red Mwitunde variety) was taken from TARI Naliendele.

Methods

Biochar production and characterization

Biochar production

The collected maize cob samples were carried to the NM-AIST laboratory for pyrolysis.

Pyrolysis was done using a macro furnace at the standard effective temperature of 500°C for one hour, cooled by natural conversion, pulverized using a grinder, and sieved using a 2mm sieve.

Biochar characterization

The microscopic analysis of biochar was carried out in Motlatsi Phari Institution-South Africa on 2 December 2023 at the magnification of 200^{xx} and Electrical heating temperature of 500 kV. A research microscope, Nikon Eclipse E-200, with fluorescence attachment, was used to know Biochar morphological characteristics using a Scanning Electron Microscope (SEM) (Fig. 2). The porosity and pore size of Biochar were scrutinized using Brunauer-Emmett-Teller (BET). Characterization was done to understand the physical morphology for the determination of the ability of materials to absorb solvents. Chemical characterization of Biochar was done at the TARI-Uyole laboratory in Tanzania.

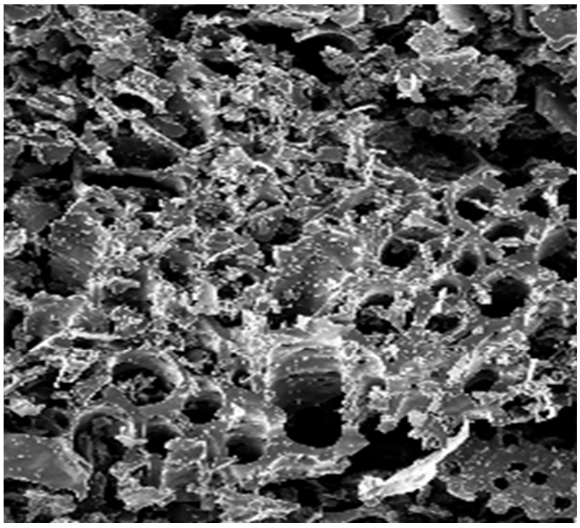


Fig. 2. SEM image of maize cob Biochar taken by Motlatsi Phari institution (South Africa) showing micro and macro pores in maize cob Biochar

Inoculum preparation and experimental layout

Inoculum preparation

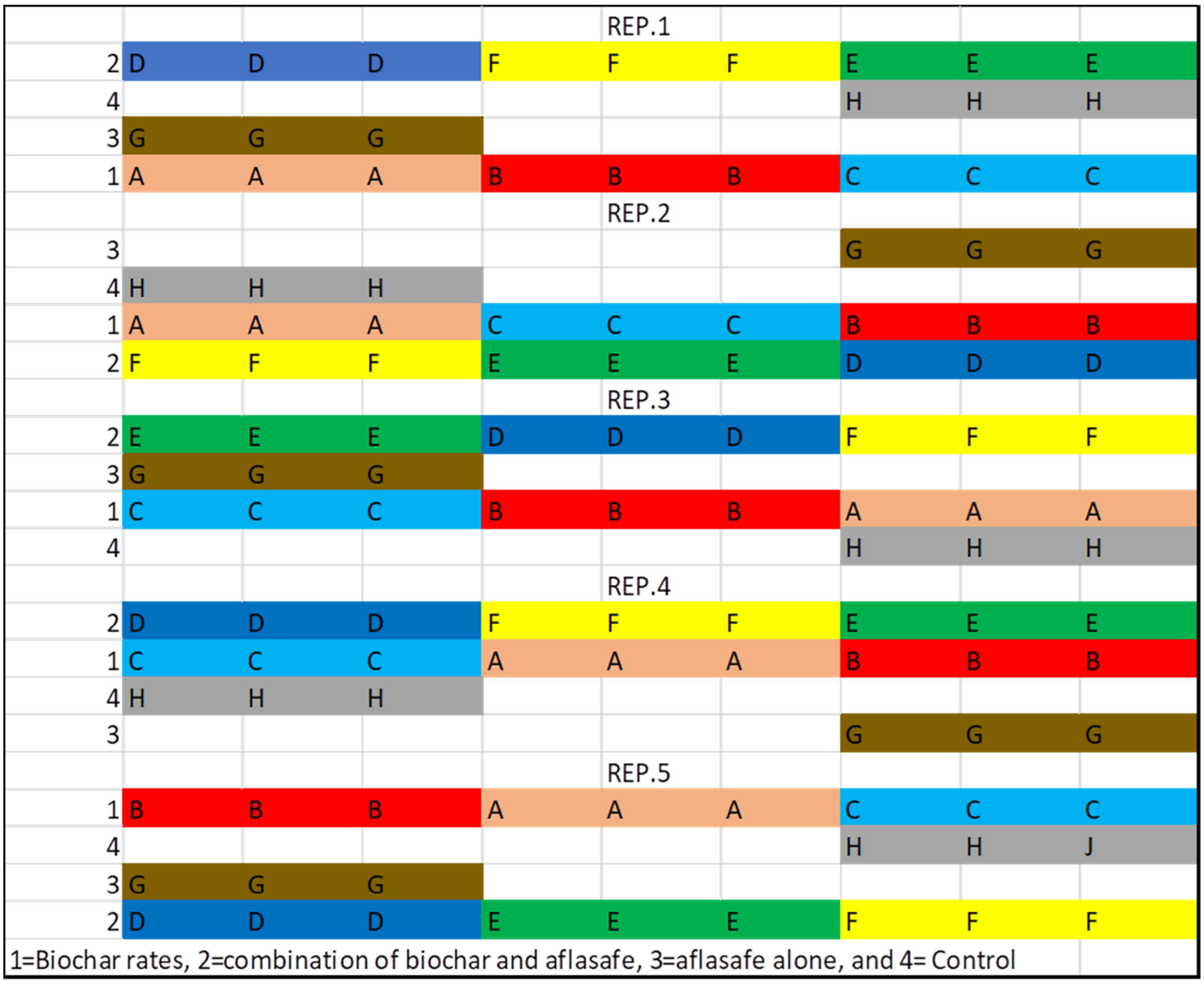
The inoculum of the S-type virulent strain of *A. flavus* isolates No. TGS 55-6 was cultured in the 90 mm Petri dishes containing half-strength Potato Dextrose Agar (PDA). The incubation was done at 30°C for seven days to allow the formation of infective spores. The inoculum was then harvested from its culture using distilled autoclaved water, and then twin 20 was added for dispersion. The concentration was adjusted to 2.5×10^6 spores/mL by using a hemocytometer.

Experimental design

The screen house experiment was laid out in a completely randomized design with five replications and three observations per replication. A 2L plastic pots filled with sterilized soil were used to grow groundnuts whereby four seeds were sown and thinned to two plants per pot after two weeks. Treatments used were (i) Aflasafe 33ml ($2x10^6$) + 2.5% Biochar, (ii) Aflasafe 33ml ($2x10^6$) + 5% Biochar, (iii) Aflasafe 33ml ($2x10^6$) +7.5% Biochar, to check for the best combination that can increase aflasafe efficiency in the field condition. Another treatment was Biochar only at the rate of (iv) 2.5%, (v) 5%, and (vi) 7.5%, (vii) Aflasafe 33ml ($2x10^6$) applied in the soil without biochar, and the negative control was soil without Biochar. Twenty (20mls) with a concentration of $2x10^4$ of toxigenic *A. flavus* were used for injection in each pot for the whole experiment. Application of Biochar in each treatment was done before planting by mixing it with soil in each pot. All the treatments were assigned at random (Fig. 3), and irrigation was done regularly by using water.

Soil analysis

Before the application of Biochar, the soil was taken at TARI Uyole Centre Soil Laboratory for nutrient analysis to know the initial soil fertility. Then, after harvest, sampling of soil for post nutrient analysis was done at the same laboratory. The measurement of the pH and exchangeable cations was done in 0.01 M $CaCl_2$ with a 1:2.5 soil: solution ratio (Van Reeuwijk, 1992) read on a pH meter (Hanna, HI2210-01 Benchtop pH/mV Meter) and in a 1:5 soil: solution ratio (Richards, 1954) using a conductivity meter (Hanna, HI98312 DiST® 6 EC/TDS/temperature Tester) respectively.



					REP.1				
2	D	D	D	F	F	F	E	E	E
4							H	H	H
3	G	G	G						
1	A	A	A	B	B	B	C	C	C
					REP.2				
3							G	G	G
4	H	H	H						
1	A	A	A	C	C	C	B	B	B
2	F	F	F	E	E	E	D	D	D
					REP.3				
2	E	E	E	D	D	D	F	F	F
3	G	G	G						
1	C	C	C	B	B	B	A	A	A
4							H	H	H
					REP.4				
2	D	D	D	F	F	F	E	E	E
1	C	C	C	A	A	A	B	B	B
4	H	H	H						
3							G	G	G
					REP.5				
1	B	B	B	A	A	A	C	C	C
4							H	H	J
3	G	G	G						
2	D	D	D	E	E	E	F	F	F

1=Biochar rates, 2=combination of biochar and aflasafe, 3=aflasafe alone, and 4= Control

Fig. 3. Layout of the experiment done at NM-AST screen house, Arusha, Tanzania, during the year 2022

The same letter indicates observations per treatment. 1. Biochar ar the rate of A= 2.5%, B=5%, C=7.5%. 2. Integration of 2×10^6 Aflasafe and Biochar at D=2.5%, E=5% and F= 7.5%. 3 Aflasafe at the rate of G=2×10^6 and 4 Control (H=0 Biochar and 0 Aflasafe)

A spectrophotometric machine was used to read the available P after analyzing using the Bray 1 method; Total N was determined by the Kjeldahl method (Bremner and Mulvaney, 1982) and Organic matter by Walkley and Black's (1934) chromate reduction method. Exchangeable acidity (EA) and bases were determined by titration method (McLean, 1965) and Atomic Absorption spectrophotometer (AAS) Perkin Elmer Analyst 400, respectively. Silicon (Si) content was determined by XRF and Cation exchange capacity (CEC) by the leaching method (Rowell, 1994). Soil texture was determined by the hydrometric method

Harvesting

Decision to harvest was set at fourth months after the groundnut plants showed all symptoms of maturity by leaves yellowing. Harvesting was done by groundnut plants being separated with soil by pouring the pot contents on a clean table, and the pods were harvested by hands one by one. Shelling of the pods was done by hand. Shelled groundnut seeds were left dry to optimum moisture, packed and labelled properly as per treatment. The packed groundnut seeds were sent to ILRI laboratory Kenya for aflatoxin quantification.

Aflatoxin quantification using High-performance liquid chromatography (HPLC)

Sample preparation

Groundnut seeds were powdered using a heavy-duty blender before 1g of the powder was taken and mixed with 5 ml of 70% methanol (v/v).

Afterwards, the mixture was vortexed for 5 minutes and incubated at room temperature with shaking for 60 minutes. Thereafter, ten minutes of Centrifugation at 3000×g was done to get the supernatant, which was used for the quantification of aflatoxin.

Extraction of aflatoxin in groundnut

A 50ml polypropylene centrifuge tube was used to measure 5.0g of the sample, and then 1.0g of NaCl salt was added to it. The weighing spatula was sterilized with 70% ethanol and wiped dry with a paper towel after each sample. 25ml of 70% methanol was added into the 50-mL Falcon tube containing 5g of milled peanut and NaCl, then shaken at room temperature for 20 minutes at 250 rpm. 20 minutes later, samples were removed and allowed to stand undisturbed for 15 minutes. After that, samples extracted 1:1 were diluted with 1 % Acetic acid into 2 ml Eppendorf tubes, caped and vortexed for at least 10 seconds, and filtered through a GHP 0.2 µm syringe filter into a UPLC sample vial. The vial was caped and loaded into the UPLC autosampler for analysis. The concentration of aflatoxins standard AFG1, AFG2, AFB1 and AFB2 were 50, 15, 50 and 15ng/mL. The column used was Phenomenex Synergi 2.5u Hydro – RP 100mm x 3.00mm. The mobile phase was Water: Methanol (60:40), and the flow rate was 0.4 ml/min. A standard calibration curve from a plot of peak areas against the known concentration of the injected volume was established using LabSolutions data analysis software. The injection volume was 20 µL for each. The retention time of the chromatographic peak of the target compound in the test sample and that of the corresponding standard chromatographic peak was used to identify the analyte of interest. The calibration curve was used to determine the concentration of the test solution. The values outside the linear range of the standard curve were re-analysed after being diluted and loaded into the UPLC autosampler. Note: Total aflatoxin was the sum of the individual aflatoxins.

Data collection

Aflatoxin content data

Data on Aflatoxin content in the test sample concentrations were calculated according to the formula below:

$$\mathrm{X\ (ng/g)} = \frac{\mathrm{C \times V \times F \times 100}}{\mathrm{W \times R}}$$

Where,

X- The overall content of distinct aflatoxin in the test sample, ng/g

C- Aflatoxin concentration in the examined sample (ng/mL),

V- Extraction volume (mL)

F- Dilution factor

100- Recovery Percentage

W- Test sample weight (g)

R - Recovery factor from spike recovery experiment

Soil nutrient contents and soil reaction data

Data on soil analysis before planting and after harvesting for N, P, K, Ca, Mg, Bo and Si were collected in mg/kg of soil. pH and CEC in Cmol/Kg of the soil were also measured.

Data analysis

Data on Aflatoxin and soil nutrient contents were checked for normality (Shapiro–Wilk's test). Homogeneity of variances (Levene's test) and analysed using analysis of variance (ANOVA), followed by mean separation test using Tukey's honest significant difference test ($P < 0.05$) using the JAMOVI statistical package version 2.3.2(2022). Correlation analysis was done to measure the strength of the linear relationship between treatments and Aflatoxin content and their association.

Results and discussion

The effect of biochar and integration (Biochar and atoxigenic A. flavus) on aflatoxin contamination in groundnuts

The analysis of different levels of Biochar and Biochar-Aflasafe integration showed a significant difference ($P < .001$) in reducing Aflatoxins B1, B2, G1, and G2. The Mean Separation test on Biochar levels and Biochar-Aflasafe integration indicated that all treatments were able to reduce aflatoxins and differed significantly ($P < .001$) with negative control (Table 1).

Table 1. ANOVA table showing the sole Effect of different levels of biochar and biochar-aflasafe integration on aflatoxin contamination *in vivo*

TREAT'S	AFL.B1	AFL.B2	AFL.G1	AFL.G2	TOT.AFL
BCH 5AFL	0.32^{a}	0.13^{a}	0^{a}	0.18^{a}	0.44^{a}
BCH7.5AFL	0.42^{a}	0.47^{a}	0.24^{a}	0.19^{a}	1.14^{a}
BCH2.5AFL	1.47^{a}	0.07^{a}	0.02^{a}	0.13^{a}	1.56^{a}
AFL.	4.7^{a}	2.44^{a}	1.48^{a}	1.04^{a}	8.63^{a}
BCH 7.5	8.6^{a}	12.64^{b}	8.1^{a}	12.12^{a}	29.34^{b}
BCH 5	12.94^{a}	3.04^{a}	8.95^{a}	1.07^{a}	24.93^{b}
BCH 2.5	92.91^{b}	83.14^{d}	75.17^{b}	35.62^{b}	251.22^{c}
CTR.	153.99^{c}	33.59^{c}	198.54^{c}	156.07^{c}	386.12^{d}
cv(%)	17.6	20	19.5	33.6	8.3
lsd	7.791	4.373	9.21	11.18	9.37
p-value	<.001	<.001	<.001	<.001	<.001
df	32	32	32	32	32

BCH 2.5AFL= combination of biochar at 2.5% and aflasafe, BCH 5AFL= combination of biochar at 5% and aflasafe, BCH 7.5AFL= combination of biochar at 7.5% and aflasafe, AFL= Aflasafe (2 x 10^{6}), BCH 2.5= Biochar at the level of 2.5%, BCH 5= Biochar at the level of 5%, BCH 7.5= Biochar at the level of 7.5% and CTR= Control, CV= Coefficient of variance, LSD= least significant difference, DF= Degree of freedom, AFL.B1= Aflatoxin B1, AFL.B2= Aflatoxin B2, AFL.G1= Aflatoxin G1, AFL.G2= Aflatoxin G2, TOT.AFL= Total aflatoxin. The same letter shows no significant difference between the treatments

Table 2. Kendall's rank correlation coefficient between the combination (biochar and aflasafe) and aflatoxin

AF. B1	1.0000					
AFL.B2	0.2988	1.0000				
AFL.G1	0.3635	0.2484	1.0000			
AFL.G2	0.2440	0.0867	0.3156	1.0000		
COMB	-0.6993	-0.4550	-0.2380	-0.2390	1.0000	
TOT.AFL	0.6689	0.4155	0.5289	0.4682	-0.6410	1.0000
	AFL.B1	AFL.B2	AFL.G1	AFL.G2	COMB	TOT.AFL

AFL.B1=Aflatoxin B1, AFL.B2=Aflatoxin B2, AFL.G1=Aflatoxin G1, AFL.G2=Aflatoxin G2, TOT.AFL= Total aflatoxin

Table 3. Kendall's rank correlation coefficient between biochar and aflatoxin contamination in groundnuts

AFL.B1	1.0000					
AFL.B2	0.3951	1.0000				
AFL.G1	0.4783	0.3284	1.0000			
AFL.G2	0.3622	0.0870	0.3920	1.0000		
BCH	-0.5671	-0.1051	-0.0183	-0.0280	1.0000	
TOT.AFL	0.8311	0.5460	0.6692	0.5940	-0.3530	1.0000
	AFL.B1	AFL.B2	AFL.G1	AFL.G2	BCH	TOT.AFL

AFL.B1=Aflatoxin B1, AFL.B2=Aflatoxin B2, AFL.G1=Aflatoxin G1, AFL.G2=Aflatoxin G2, TOT.AFL= Total aflatoxin

Based on individual treatment effect, integration of 5% Biochar and 2 x 106 atoxigenic *A. flavus* was the most effective and showed a 99.97% aflatoxins reduction efficiency compared to the positive control (Aflasafe), which has 90% and Biochar, which had 85.6% (Table 1). This result implies that Biochar has the ability to reduce *A. flavus* competitive ability. A similar result was reported by Iacomino *et al.* (2022), demonstrating that Biochar was capable of reducing soil-borne fungal pathogens by 86%. Several authors reported the positive effect of Biochar reducing fungal pathogens growth or diseases in crops (de Medeiros *et al.*, 2021; Frenkel *et al.*, 2017a; Jaiswal *et al.*, 2014; Medeiros *et al.*, 2021; Poveda *et al.*, 2021). These findings could be attributable to the Biochar's high surface area and many macro and micro-pores (Fig. 2), owing to the property of absorbing cell-wall biodegrading enzymes released by *A. flavus* as an infection tool (Khashi *et al.*, 2022) hence the success of atoxigenic strains in competition.

The correlation between Biochar and aflatoxin content indicated that there was a negative correlation between Biochar and all types of aflatoxins (Table 3). There was a strong negative relationship between Biochar-Aflasafe integration and aflatoxin B1 and total aflatoxin (Table 2). This might be due to the Biochar effect on toxigenic A. flavus competitive ability due to their narrow growth pH range (Reddy *et al.*, 1971). Toxigenic *A. flavus* has been stated to work efficiently in acidic conditions of pH ranging between 3.5 and 4.5 (Gallo *et al.*, 2015). High pH was reported to overpower aflatoxin biosynthesis gene expression that weakens toxigenic *A. flavus* competitive ability (Ivanova *et al.*, 2016). The synonymous result was reported by Scala (2015), Frenkel *et al.* (2017), and Rogovska *et al.* (2017) that Biochar promotes soil microbiome in favour of natural enemies.

Table 4. Regression table showing the influence of biochar to aflatoxin contamination

Parameter	Estimate	S.E.	T (16)	T PR.
Constant	552.2	21.2	26.09	<.001
%5B	-107.24	5.99	-17.58	<.001
%7_5B	-68.18	3.99	-17.08	<.001
%2_5B	-95.7	12	-9	<.001

The regression between Biochar and aflatoxin showed a significant variation among Biochar levels, with 5% being the most effective. The results show that a unit increase of 5% Biochar reduces aflatoxin content by 107.24 ppb at a constant of 552.2 ppb (Table 4). Biochar contains benzoic acid, ethylene glycol, propylene glycol, hydroxy propionic acid, hydroxybutyric acid and phenols which function as plant immunity inducers by increasing plant phytoalexin production that stimulates systemic resistance (Elad *et al.*, 2011; Hou *et al.*, 2022; Hou, Pugazhendhi *et al.*, 2022; Lehmann *et al.*, 2011; Yang *et al.*, 2022). Kochanek *et al.* (2022) reported that Biochar has been traditionally used as a pesticide. Contradicting results were reported by Cong *et al.* (2023), who stated the increase in plant diseases when Biochar was used in high concentrations. This paradox might be due to a plethora of small and large organic molecules obtained in Biochar that may independently or in combination have hormone-like or phytotoxic actions that are dose-dependent (Frenkel *et al.*, 2017). These hormones play a substantial role at low dosages both as a plant growth promotor and in promoting plant defenses against stresses (de Medeiros *et al.*, 2021; Frenkel *et al.*, 2017; Jaiswal *et al.*, 2014; Medeiros *et al.*, 2021; Poveda *et al.*, 2021).

Studies project an increase in aflatoxin contamination due to climate change, soil degradation and its biome, which could lead to an increase in crop stresses (Ehrlich, 2014). Biochar might be an important mitigating option as it reduces the impact of climate change by capturing Carbon, increasing soil stability, reducing crop stress due to its properties of reducing pests and diseases and sequestering Carbon dioxide (Desk, 2019; Egamberdieva *et al.*, 2016; Jaiswal and Graber, 2017; Kapoor *et al.*, 2022). Moreover, Biochar can remain active in the soil for a long time (more than 1000 years) without degrading (Atkinson *et al.*, 2010; Obia *et al.*, 2020). In this context, the integration of Biochar and Aflasafe reduced Aflatoxin contamination in groundnut. Over space and time, the long-term positive effects of Biochar potentially might offer options for scaling up aflatoxin control at pre-harvest crop growth and development stages.

Effect of biochar on soil nutrient in relation to aflatoxin contamination

There was a significant (P <0.001) increase in all nutrients after Biochar application as compared to initial soil nutrient content and control (Table 5). Biochar was remarkably higher in K, Ca, P, Mn, B and CEC as compared to experimental soil (Fig. 4). Soil P, K, Ca, Mg, Mn, Bo, and Zn were significantly increased (P <0.001) in Biochar treated soils (Table 5). High mineral content in Biochar was attributed to high Biochar ash content (28.5%). These results corroborate those of Ringer *et al.* (2022), who found 18.5% ash content in Biochar pyrolyzed at 500oC. Hou *et al.* (2022) documented the increase in available soil nutrients after the addition of Biochar to the soil. This might be due to raised pH (Fig. 4), as soil with low pH tends to fix plant nutrients (Ehrlich, 2014), or it was due to nutrients supplied directly by ash content to the soil (Kapoor *et al.*, 2022).

Table 5. ANOVA table showing the effect of different levels of biochar in soil nutrients and soil reaction in NM-AIST, Arusha, Tanzania during season 2022/2023

Treat.	PH	Bo	Ca	CEC	K	Mn	P	Si	N
BCH.	10.38^{a}	1.9^{a}	10.4^{a}	9.8^{a}	0.21^{a}	11.3^{a}	6.1^{a}	13.6^{a}	0.02^{bc}
BCH.7.5	7.64^{b}	1.54^{ab}	8.23^{b}	7.62^{b}	0.168^{ab}	8.93^{b}	2.294^{d}	9.38^{b}	0.056^{c}
BCH.5	6.34^{c}	1.32^{bc}	5.664^{c}	6.84^{c}	0.154^{bc}	8.4^{c}	3.64^{b}	8.46^{c}	0.064^{bc}
BCH.2.5	5.06^{d}	1.18^{c}	3.65^{d}	5.164^{d}	0.136^{c}	6.32^{d}	3.206^{bc}	6.12^{d}	0.096^{bc}
BCH.0	4.72^{d}	1.1^{cd}	1.16^{de}	3.84^{d}	0.022^{d}	2.95^{f}	1.744^{d}	4.37^{e}	0.124^{ab}
I.S. N	4.3^{d}	0.86^{d}	2.4^{e}	4.8^{e}	0.04^{d}	3.9^{e}	2.6^{e}	5.8^{d}	0.21^{a}
CV (%)	6.2	8.4	11.9	3.6	9.3	3.3	9.3	4.8	34.3
P-VALUE	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
DF	16	16	16	16	16	16	16	16	16

BCH=Biochar, BCH 2.5= Biochar at the rate of 2.5%, BCH 5= Biochar at the rate of 5%, BCH 7.5= Biochar at the rate of 7.5%, BCH.0=Soil without Biochar, I.S. N= Initial Soil Nutrient, CV= Coefficient of variation, DF= Degree of freedom, Bo= Boron, Ca= Calcium, CEC= Cation exchange capacity, K= Potassium, Mn= Manganese, P= Phosphorus, Si= Silicon, N= Nitrogen. The same letter shows no significant difference between the treatments

These elements increase the aptitude of plants to fight against soil-borne diseases and toxins (Atkinson *et al.*, 2010; de Medeiros *et al.*, 2021; Gupta *et al.*, 2017; Hou *et al.*, 2022; Mullen *et al.*, 2010; Quilliam *et al.*, 2012). Calcium ions (Ca2+) are important for sustaining plant cell walls and cell membranes as well as enhancing plant growth and metabolism (Yang *et al.*, 2022, Bonanomi and Scala, 2015; Davis, 2017; Luo *et al.*, 2022; Wang *et al.*, 2020). Yang *et al.* (2022) reported a 90% reduction of Aflatoxin content in groundnut grains harvested from the soil supplemented with Ca2+.

Potassium ions (K+) function as the element for strengthening plants' cell walls and make it difficult for *A. flavus* penetration and infection (Cong *et al.*, 2023). Furthermore, K is one of the important elements in improving the performance of multiple plant enzymes responsible for plant resistance induction (Evans *et al.*, 2017). Potassium regulates the accumulation of inhibitory amino acids, phytoalexins, phenols, and auxins in plants (Gupta *et al.*, 2017). Specifically, the same authors observed a 70% decrease in fungal disease incidence when potassium was incorporated into the soil.

Manganese (Mn) is an important element in the biosynthesis of lignin and phenol compounds (Evans *et al.*, 2017), hence a difficult environment for A. flavus hyphae penetration. Additionally, Mn and Bo are both important for the activation of systemic acquired resistance (SAR) mechanisms of the plants, which further interact with salicylic acid and activate the defense mechanisms in groundnut plants (Evans *et al.*, 2017). Zhang *et al.* (2021) found reduced fungal invasion in leguminous crops that contain a high amount of Zn due to enhanced antioxidative enzyme activity (Zhang *et al.*, 2021).

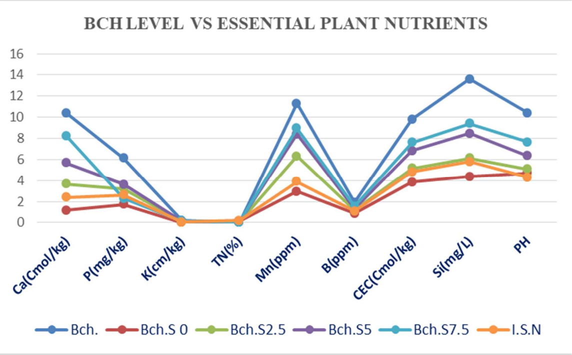


Fig. 4. Effect of biochar levels on soil nutrients under controlled experiment

Bch= Biochar, Bch.S 0= Soil without Biochar, Bch.S2.5= Soil amended with 2.5% Biochar, Bch.S5=Soil Amended with 5% Biochar, Bch.S7.5= Soil amended with 7.5% Biochar and I.S. N=Initial Soil Nutrient. Bo=Boron, Ca=Calcium, CEC=Cation exchange capacity, =K=Potassium, Mn=Manganese, P=Phosphorus, Si=Silicon, N=Nitrogen

The low aflatoxin contamination observed in this study might also be attributed to the high Silicon content observed in the soil amended with biochar (Fig. 4). Silicon, apart from not being among the plant's essential elements, has the properties of accumulating at the sites of hyphal penetration during fungal infection (Rizwan *et al.*, 2018).

Tubana *et al.* (2016) reported a higher accumulation of Si more than 3 times at the site of infection during a pathogen attack compared to unsuccessful infection sites. The continued supply of Si to the agricultural soils provides disease protection. Gupta *et al.* (2017) reported a tremendous increase in phenolics (plant defense hormone to pathogen attack) after the application of Si at the sites of fungal infection compared to the control. Silicon is considered as a chemical barrier to pathogen entry in the host plants (Pozza *et al.*, 2015).

Biochar application also significantly increased (P <0.001) soil CEC to approximately more than two times compared to the initial CEC (Fig. 2). Similar results were reported by Yeboah *et al.* (2020) who documented the increase of soil CEC after application of rice husk Biochar. High soil CEC was stated to increase plant health and vigour and raise plant resistance against biotic and abiotic stresses (Wu *et al.*, 2021). In this essence, Biochar could be used as soil amendment material due to its multiphase properties.

Concussion

The results of the present study demonstrate that Biochar was the best soil amendment material due to its properties of positively affecting soil physical and chemical properties such as increase of nutrient content, water holding capacity, redox activity, absorption of toxic substances released by A. flavus, increase of soil pH, and increase of soil microbiome diversity. Biochar is beneficial at low quantities but detrimental at high quantities of more than 5%. This finding tells us that biochar can increase the Aflasafe efficacy and suppress toxigenic *A. flavus* competitive ability at low dosages. Integration between Biochar and Aflasafe at 5% and 2 x 106, respectively, has the potential to be recommended as the suitable rates as aflatoxin management is concerned. The results validate that biochar amendment reduces aflatoxin contamination in groundnuts by affecting host susceptibility, fungal pathogenicity and soil environment. Finally, the long-run effect of biochar as an aflatoxin management practice needs to be investigated. Also, knowledge of the biochar-dose effect on plants is still scanty; hence, there is a need for further investigation.

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