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Aflatoxin levels in seeds of commonly grown groundnut varieties (*Arachis hypogaea* L.) in Ghana as influenced by storage method

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ABSTRACT

Quality seeds of improved local genotypes is an important input for increasing the productivity of groundnut in Ghana. The existing means of meeting groundnut seed requirements, especially by smallholder farmers, have serious challenges with timely supply and access to these improved genotypes as a result of the limited participation of the private sector and the self-pollinated nature of the crop. Smallholder farmers who take the initiative to store their own seeds, have challenges with storage fungi and aflatoxin contamination. Farmers' groundnut seed stocks have shown that improvement in seed quality and farmers' seed management requires maintaining healthy seed stock. Toxicogenic fungi and mycotoxins have been reported in several human and animal health disorders and are major contaminants of groundnut seeds during storage. Some level of success has, however, been achieved from earlier studies to evaluate the efficacy of some plant botanicals for preserving shelled groundnuts. However, for smallholder on-farm safety, such phyto-based preservation methods rather reduce groundnut seed embryo vigour and germination rates. Designing and developing economically appropriate storage solution (practices) for maintaining seed integrity for use requires evidence-based research and an eco-friendly approach. Thus, this study was aimed at assessing the efficacy of using jute bag (JB) and interlaced polyethylene jute bag (IPJB) combinations for the storage of groundnut seeds (varieties) against fungal infection and aflatoxin contamination under ambient storage conditions. The study was undertaken at the Department of Biochemistry, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Seeds were packaged and stored either in jute bags (JB) or interlaced polyethylene jute bags (IPJB) at ambient conditions over four months' period. All seeds of groundnut varieties were partly colonised by a range of *Aspergillus, Fusarium* and *Rhizopus* spp of fungi. Aflatoxins were detected in only Nkosour (148.21 ppb) while *Adepa and Kwame Danso* recorded elevated levels of aflatoxin B1 (45.918 ppb) and B2 (410.974 ppb) at four months after storage (MAS). Results indicate that, the IPJB packaging was effective for short-term storage only, while the level of pathogen infection and aflatoxin contamination recorded was low. However, none of the packages evaluated proved exceptionally efficient. Irrespective of the storage package used, *Mireku, Konkoma, Nkate Broni, Kumawu Local, Shitaochi, Azizivi, and Jenkaah* recorded biologically and economically insignificant levels of aflatoxins. Thus, planting these groundnut varieties by smallholder farmers may offer them some level of security from aflatoxin contamination and loss of seed quality.

Key words: Mycotoxin, storage pests, postharvest damage, groundnut, legumes, postharvest handling, fungi, Ghana

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INTRODUCTION

Groundnut is an important grain legume, widely cultivated both in the Guinea Savanna and Forest agro-ecological zones of Ghana. The forest zone is characterised by a long rainy period, high humidity and a short rainy period with low humidity. Groundnut is usually grown in both the major and minor seasons [1]. The Food and Agriculture Organisation (FAO, [2]) production estimates put groundnut in Ghana at 426,280 metric tonnes. Groundnut production in Ghana is, however, constrained by inadequate production of certified seeds partly traceable to limited private sector participation in the sector, leaving a few state-owned enterprises with the arduous task to produce and supply planting materials required across the country. The situation is compounded by the rapid loss of seed viability and low seed multiplication ratio [3]. However, due to the self-pollinating nature of the crop, some innovative farmers grow and maintain their own seed stocks at least for three years. This is, however, impeded by fungal infections and aflatoxin contamination which are strongly associated with the method of production, harvesting, and storage [4].

Aflatoxin is a natural secondary metabolite produced by fungi, mainly of the *Aspergillus (A)* group, such as *A. flavus*, *A. parasiticus* and *A. nomius*. These metabolites are carcinogenic compounds (mycotoxins) [5, 6] found in staple foods such as maize and groundnuts and acute exposure to humans causes aflatoxicosis [7]. A considerable number of people have suffered aflatoxicosic risks in India (1974) and Kenya with reported 233 deaths [8, 9]. There has been reported unsafe limits of aflatoxin contamination in Ghana [10]. In the European Union, mycotoxin standard limits aflatoxin B1 to 2 parts per billion (ppb), and total aflatoxin to 4 ppb [7]. This has been proven to reduce trade in commodities that are susceptible to aflatoxins between the producers and their target markets [11].

Aflatoxins are made up of a group of more than fifteen toxins and are the most important mycotoxins with the frequent occurrence, and toxicity for developing countries and influence on international commodity trade. Aflatoxins B1, B2, G1 and G2 have been detected in groundnut, pulses, and other agricultural commodities [12]. The presence of aflatoxins in seed potentially compromises seed quality as the pathogen, which metabolises the substance is transferrable to the progeny through a seed-to-seed transmission [13]. Consequently, consumers and smallholder farmers who are mostly unaware of the impact of aflatoxin on public health are the most exposed to the dangers of aflatoxins. It is established that *A. flavus* infection could occur before (pre-harvest), during harvest and postharvest stages [4].

Post-harvest infection by fungal pathogens and aflatoxin contamination of groundnuts are major constraints to the production of the crop in Ghana. Previously, attempts have been made using plant extracts and other packaging and storage methods to remedy the situation [13, 14]. Some level of success has been achieved from earlier studies to evaluate the efficacy of some plant botanicals for storing/preserving shelled groundnuts [13, 14]. However, for smallholder on-farm safety, such phyto-based storage methods are less economically prudent as they reduce seed embryo vigour and germination rate. A study on the use of on-farm storage method for safe seed-storage of groundnut is,

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therefore, critical to lessen storage costs and potential chemical damage to seed embryos. The study was aimed at assessing the efficacy of using *jute bag (JB) and interlaced polyethylene jute bag (IPJB) combinations* for the storage of groundnut seeds (varieties) against fungal infection and aflatoxin contamination under ambient storage conditions.

MATERIALS AND METHODS

Field and laboratory experiments

The study was conducted both on the field and in the laboratory. The field study was undertaken at the research fields of the Crops Research Institute (CRI-Ghana) of the Council for Scientific and Industrial Research (CSIR), Ghana, at Fumesua, Kumasi, between the period of May-August 2015, while laboratory analyses were conducted at the Aflatoxin Laboratory of the Department of Biochemistry, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in 2015.

Postharvest handling and curing

Groundnut pods of ten varieties that had been winnowed and inverted to sun curing for five days were used. The ten varieties were *Mireku, NkateBroni, Kumawu Local, Kwame Danso, Shitaochi, Konkoma* (all local varieties), *Adepa, Nkosour, Azizivi and Jenkaah* (improved varieties). These pods were sun-dried after they were stripped from the haulms and their moisture content determined before storage with Moisture analyser (SinarAP6060-001 AG, UK) at ambient temperature for 72 hours (approximately 3 days). The average moisture level for all the varieties was 8.83 % (Table 1). Clean pods from each of the 10 varieties were divided into six 3 kg lots each. Three of the 3 kg lots of each variety were placed in jute bag interlaced with polyethylene, while the remaining were placed in jute sacks only and sealed. All the sets of bags were stored in a non-sanitised ambient room to mimic the storage condition the smallholder farmer would otherwise have used on-farm. The study lasted approximately 5 months (August-December), usually the period when smallholder farmers wait for dormancy to break to use the seeds in subsequent seasons.

Sampling and climatic condition of the sampling environment

At 2 and 4 months after storage (MAS), seeds of each variety were sampled to obtain a representative sample using the manual hand halving method [15]. In this procedure, the seed lot from which the sample was to be taken was sub-divided into eight different subsamples. Subsamples one and seven, two and eight were bulked together. The same procedure was repeated for subsamples three and five, and four and six, respectively. A uniform sample was then hand-picked from each sub-sample with a clean spoon to form the working sample on which the test was run. Meteorological data for the study area during the entire growing season was obtained from the weather station at CRI-Ghana. All daily weather data for the study period were averaged; and thus, the average relative humidity and temperature for the study period were 77.07 % and 26.59 ^{o}C, respectively (Figure 1).

Aflatoxin determination

The extraction of aflatoxin from the test samples was done according to the methods of the Association of Official Analytical Chemists (AOAC) [16]. Aflatoxins standard

(AFB1, AFB2, AFG1 & AFG2) was purchased from Sigma Aldrich (Germany). Standard stock solutions (0.3 µg/ml for B1 and G1 and 0.1 µg/ml for B2 and G2) were prepared according to the AOAC methods [17]. A standard calibration curve of five solutions was prepared (5 µg/kg, 10 µg/kg, 15 µg/kg, 30 µg/kg and 50 µg/kg) to estimate aflatoxin contents of each B1, B2, G1, G2 using chrompass computer software [16].

High performance liquid chromatography (HPLC) conditions for aflatoxin analyses

The Mobile Phase consisted of water, acetonitrile, and methanol in the ratio (60:20:20) to a volume of 1 litre. Additionally, 120 mg of potassium bromide and 350 ul of nitric acid were utilised per litre of mobile phase. The column was 30 cm long and 4.6 mm wide and had Supelco C-18 detector 5 um in thickness: Fluorescence detected was excited to a wavelength of 365 nm and an emission wavelength of 435 nm. The volume of the sample injected per test was 100 ul and the flow rate was 0.8 ml/min.

The standard blotter method [15] was used to detect a wide range of fungi species including *Aspergillus flavus, Aspergillus parasiticus, Fusarium* spp*, Rhizopus* and other undefined range of fungi that grow on seeds in the presence of humidity. Four hundred untreated pure seeds from each of the samples were plated on moistened blotters (Whatman No. 1) in 9 cm diameter Petri dishes at the rate of 20 seeds per dish and the seeds were incubated for 7 days at 20-25^{o}C under alternating cycles of 12 hours near ultraviolet light and 12-hour darkness. Seeds were examined under Astereo-microscope for the presence or absence of fungi. Identification was confirmed by examining for the presence of mycelium and/or conidia under a compound microscope.

Data analyses

Data on seed moisture content were analysed using analysis of variance (ANOVA) procedure (Genstat, ver.12, 2006) and means compared using LSD at 5 % significance level. For aflatoxin data, samples were grouped based on aflatoxin content as: <4 ppb, >4 ppb to 10 ppb and >10 ppb. Samples above 4 ppb are rejected by the European Union which is the biggest target market for most groundnuts produced in Ghana.

RESULTS AND DISCUSSION

Fungal pathogens which were detected in the seeds before storage include *Aspergillus* spp (50.34 %), *Fusarium* spp (35.67 %), *Rhizopus* spp (28.33 %) and other undefined spp (15.00 %) were also detected from the seed lots after storage (Table 1 & 2). The fungi were ubiquitous in all the samples regardless of the packaging method employed. The initial percentage infection was 20 % but increased over the first two months (Table 2). There was a marginal increase in the infection rates compared with the increase between the start of storage and two months into storage. Relative humidity, temperature and rainfall are key factors in post-harvest management and, therefore, influence pathogen build-up in storage environments as evidenced by the average relative humidity (%), temperature (^{0}C) and rainfall (mm) for the study period (Fig 1). Rainfall was generally fluctuating, which might have instigated the sharp decline in relative humidity of the storage environment.

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Hell and Mutegi [18] recommended that harvested grains and cereals should be dried as quickly as possible to 10-13 % safe moisture levels to prevent the growth of mycotoxigenic fungi and reduce insect infestations. Hayma [19] also reported that grains with high moisture content have a high rate of respiration, while Lee [20] predicted a direct positive correlation between temperatures in grains with moisture content. However, in our current study, the initial moisture content of the groundnut seeds were found to be within limits for safe storage of the seeds. Various species of fungi initially detected in the seeds four MAS (Table 1) may be attributed to the changing relative humidity levels in the storage environment (Figure 1). Dharmaputra *et al*. [21] noted that, kernel infection by fungi is largely due to moisture content and relative humidity of storage environment and that, species of *Aspergillus* from various seeds produced aflatoxins on samples examined. Malaker *et al*. [22] observed that *Aspergillus* spp is more invasive than other species, which might have accounted for the higher levels of *Aspergillus* spp than any other fungi sp.

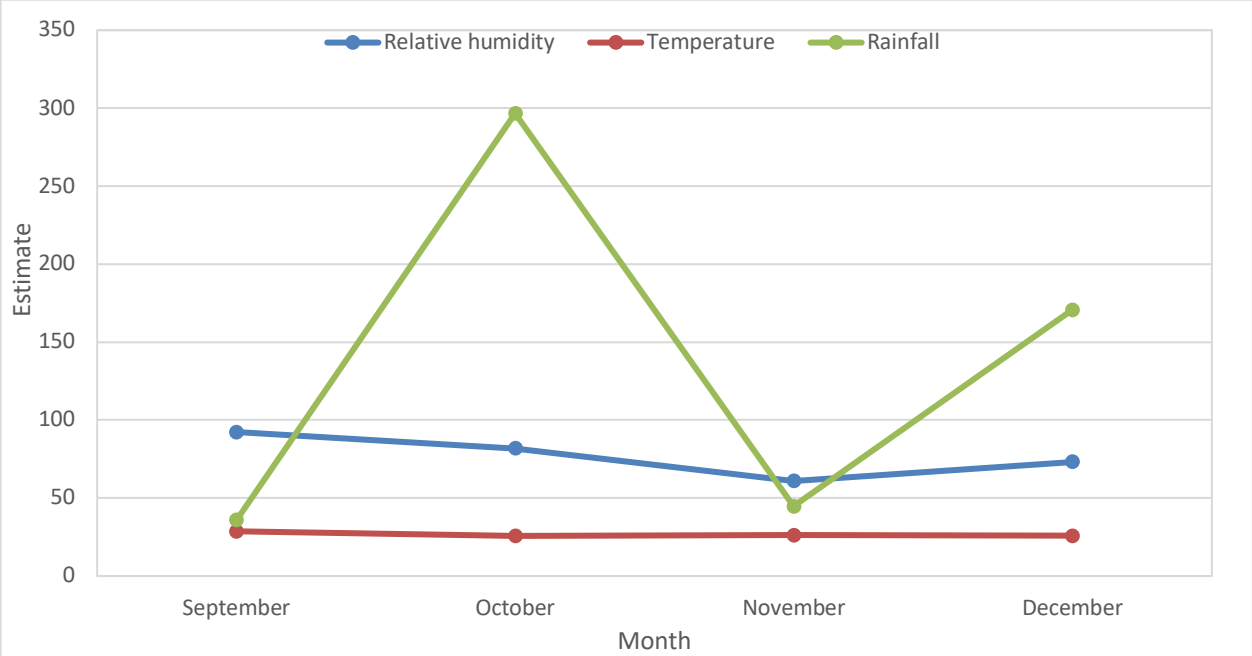

Figure 1: Average relative humidity (%), temperature (ºC), and rainfall (mm) of the study environment

Before the seeds were packaged for storage, four varieties (*Mireku, Konkoma, Adepa and Azizivi*) recorded varying levels of aflatoxins (Tables 1 & 2). The amount recorded ranged from as low as 0.013 ppb for Adepa to as high as 0.070 ppb for *Konkoma* (Tables 1 & 2). The remaining varieties recorded no aflatoxins. At two MAS, while there was no aflatoxin detected in *Kumawu Local, Shitaochi, Adepa, Azizivi and Jenkaah* under jute bag only, Nkosour recorded the highest aflatoxin content of 148.21 ppb for jute bag. With polyethylene and jute bag combinations, *Mireku, Konkoma, NkateBroni, Kumawu Local, Shitaochi and Azizivi* had no aflatoxins detected (Tables 2 & 3).

Varying storage time has been implicated in groundnut aflatoxin damage [16, 21, 22]. There is evidence that storage methods can facilitate fungal proliferation and aflatoxin

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contamination in groundnut [22, 23, 24]. Prior to storage, aflatoxins were detected in four of the varieties studied (*Mireku, Adepa, Azivivi, Konkoma*); though the presence of aflatoxin-producing fungi such as *A. flavus* and *A. parasiticus* in the seed lots was confirmed. Awuah and Ellis [14] made a similar observation when mouldy and pathologically-cleaned samples were both found to have some amount of aflatoxins despite the suspicion that the clean samples in polyethylene storage could inhibit aflatoxin contamination compared to those of the jute bag storage. Dorner *et al.* [25] suggested that percentage fungal infections of groundnut was a poor metric of aflatoxin contamination as this does not indicate the level of growth of the fungus on seeds. Thus, both mouldy seeds and visibly clean seeds may contain aflatoxin. However, after establishing weak correlations between infection percentages and aflatoxin contaminations, Cole *et al*. [26] noted that fungal infection and growth are separate events which occur independently. In this study, there was a seemingly gradual decrease in the amounts of aflatoxins detected in some of the seeds. This may be ascribed to the enzymes released by competing pathogens especially *Fusarium* spp to degrade aflatoxins already produced on the seed lots [27].

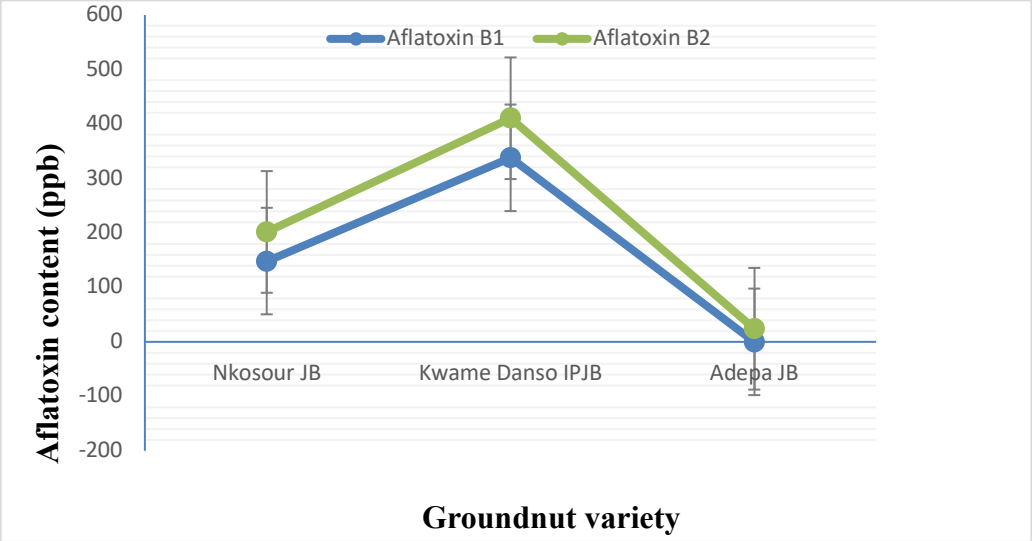

Figure 2: Comparison of varieties with high aflatoxin B1 and B2 contents stored in Jute bag (JB), and Interlaced polyethylene jute bag (IPJB). Error bars indicate standard errors

Only Adepa recorded a significant amount (45.918 ppb) of aflatoxins detected in them in the jute bag storage at 4MAS. Regardless of the package method used at 4MAS; *Mireku, Konkoma, NkateBroni and Jenkaah* recorded no aflatoxins. At 4MAS, the aflatoxin level of *Kwame Danso* in polyethylene and jute bag combinations was significantly higher (410.974 ppb; $p < 0.05$) compared with those in all other varieties (Figure 2). While *Nkosour* had 148.304 ppb for aflatoxin B1 and 53.459 ppb for aflatoxin B2, *Adepa* recorded 24.029 ppb of aflatoxin B2 but no aflatoxin B1 (Figure 2). Conversely, *Kwame Danso* in jute bag and polyethylene package combination recorded the highest levels of aflatoxin B1 (337.87 ppb) and B2 (72.614 ppb) (Table 2). Poor storage facilities and conditions of storage environment have been reported to predispose stored groundnuts (seeds) to insect infestations and fungal contaminations [28]. The

aflatoxin levels detected in the seeds may have been partly predisposed by the type of packaging materials and storage methods used. Hell *et al.*[24], Udoh *et al*. [29], Guo *et al*. [30] and Danso *et al.* [32] reported that 'storage methods can facilitate fungal proliferations and aflatoxin contamination in maize'.

Three varieties, *Adepa, Nkosour and Kwame Danso* recorded aflatoxin levels with significant economic and health implications (Figure 2). This observation may be attributable to the presence of *Aspergillus* and other identified mycotoxin-causing organisms in the seed lots of these varieties. This is in consonance with the report by Awuah and Ellis [14], where mouldy groundnut samples were found to contain aflatoxin irrespective of the storage method used. This outcome indicates the potential of packaging and storage methods to inhibit fungal infection and aflatoxin contamination during long-term storage. However, once the fungal infection starts in the field, management practices aimed at mitigating mycotoxin build-up may not achieve desired results. The duration at which biologically significant levels of aflatoxins were detected was inconsistent with reports by Mutegi *et al.* [31], Danso *et al.* [32] and Atehnkeng *et al.* [33], who recommended that smallholder groundnut farmers store seeds before the new growing season six months after harvest. Again, the IPJB packaging was effective for short-term storage only as the level of pathogen infection and aflatoxin contamination recorded was low. However, none of the storage packages (JB & IPJB) evaluated proved exceptionally efficient as irrespective of the storage package employed, *Mireku, Konkoma, Nkate Broni, Kumawu Local, Shitaochi, Azizivi and Jenkaah* may have biologically and economically insignificant levels of aflatoxin. Planting these varieties, therefore, may insure farmers against significant loss of seed quality and other economic losses associated with aflatoxins contamination.

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Author Contribution Statement

AY conceived, designed and conducted the study. AY and JKA analysed the data. AY, SAN and JKA wrote and edited the manuscript. All authors have read and approved the manuscript.

Competing Interests

We declare that no competing interests exist.

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Table 1: Fungal species detected, percentage infection and seed moisture content (%) before storage

Groundnut	**Species of fungi detected, percentage infection and moisture content**					
Variety	*A. flavus (%)*	*A. parasiticus (%)*	*Fusarium* spp *(%)*	*Rhizopus* spp *(%)*	*Undefined* spp *(%)*	*Seed moisture content (%)*
Mireku	30.00±1.51^{d}	16.67±0.85^{c}	36.67±1.35^{d}	26.67±1.01^{c}	20.00±1.21^{b}	8.88±0.08^{b}
Kwame Danso	26.67±1.60^{e}	20.00±0.78^{b}	33.33±1.42^{e}	30.00±0.95^{b}	6.67±2.10^{f}	8.83±0.08^{b}
Konkoma	23.33±1.71^{f}	23.33±0.72^{a}	26.67±1.58^{g}	36.67±0.86^{a}	16.67±1.33^{c}	8.62±0.09^{c}
NkateBroni	26.67±1.60^{e}	20.00±0.78^{b}	40.00±1.29^{c}	23.33±1.08^{d}	10.00±1.72^{e}	9.23±0.08^{a}
Kumawu Local	20.00±1.85^{g}	16.67±0.85^{c}	50.00±1.16^{a}	20.00±1.17^{e}	20.00±1.21^{b}	9.25±0.08^{a}
Shitaochi	46.67±1.21^{a}	23.33±0.72^{a}	40.00±1.29^{c}	30.00±0.95^{b}	10.00±1.72^{e}	8.73±0.08^{c}
Adepa	23.33±1.71^{f}	13.33±0.95^{d}	20.00±1.83^{h}	36.67±0.86^{a}	13.33±1.49^{d}	8.45±0.09^{c}
Azizivi	43.33±1.26^{b}	23.33±0.72^{a}	30.00±1.49^{f}	30.00±0.95^{b}	20.00±1.21^{b}	8.99±0.08^{b}
Nkosour	33.33±1.43^{c}	23.33±0.72^{a}	36.67±1.35^{d}	23.33±1.08^{d}	23.33±1.12^{a}	8.70±0.08^{c}
Jenkaah	33.33±1.43^{c}	16.67±0.85^{c}	43.33±1.24^{b}	26.67±1.01^{c}	10.00±1.72^{e}	8.61±0.09^{c}
Mean	30.67	19.67	35.67	28.33	15.00	8.83
SE (0.05)	2.76	1.16	2.72	1.74	1.81	0.08

SE = standard error

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Table 2: Aflatoxin levels in seed samples at various durations of storage

Variety	Aflatoxin content BS (ppb)	Aflatoxin content 2MAS (ppb)		Aflatoxin content 4MAS (ppb)	
		JB	IPJB	JB	IPJB
Mireku	0.023±0.14	0.0001	0.0001	0.0001	0.0001
Kwame Danso	0.0001	0.025±0.57	0.147±0.18	0.025±0.56	410.9±8.27
Konkoma	0.070±0.08	0.010±0.88	0.0001	0.0001	0.0001
NkateBroni	0.0001	0.040±0.44	0.0001	0.0001	0.0001
Kumawu Local	0.0001	0.0001	0.0001	0.0001	0.023±0.95
Shitaochi	0.0001	0.0001	0.0001	0.0001	0.057±0.53
Nkosour	0.0001	148.2±6.09	0.0001	0.040±0.47	0.035±0.53
Adepa	0.013±0.19	0.0001	0.049±0.31	45.92±3.91	0.146±0.96
Azizivi	0.023±0.14	0.0001	0.0001	0.0001	0.021±0.42
Jenkaah	0.0001	0.0001	0.0001	0.0001	0.0001
Mean	0.01	14.83	0.02	4.60	41.12
SE (0.05)	0.19	1.97	0.32	6.42	19.22

BS = before storage; MAS = month after storage; JB = jute bag, IPJB = interlaced polyethylene jute bag; SE = standard error

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