EXPANDING EXPORT OF SESAME SEED AND SHEANUT/BUTTER THROUGH IMPROVED SPS CAPACITY BUILDING FOR PUBLIC AND PRIVATE SECTOR

DEVELOPMENT OF A SIMPLE PREDICTIVE MODEL FOR MOULD GROWTH AND AFLATOXIN PRODUCTION IN THE NIGERIAN SESAME PRODUCTION CHAIN

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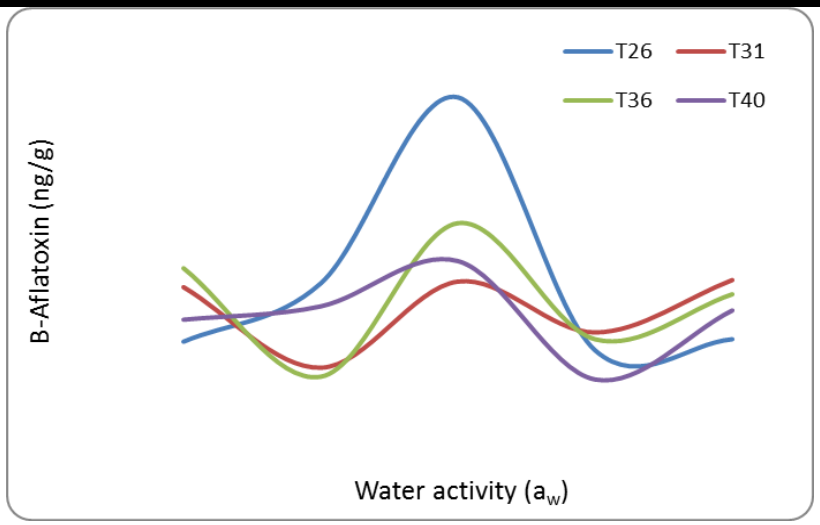

STDF Project 172, co-financed by NEPC and STDF, implemented by NEPC, and supervised by ITC, 2014

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1.1. INTRODUCTION

Mould infestation and subsequent aflatoxin contamination has been observed in a variety of agricultural commodities, and particularly oil seeds and nuts have been implicated as highly susceptible to aflatoxin contamination by various researchers. Aflatoxin contamination of agricultural products depends on environmental conditions, particularly temperature and water activity, and posts-harvest handling practices. Thus, failure to comply with GPAs requirements had resulted in aflatoxin contamination of products, leading to serious export problems (Table 1).

European Commission established a standard in 1999 for maximum level of 4 µg/kg for total aflatoxins and 2 µg/kg for aflatoxin B_1 in seeds, nuts and products for direct human consumption, or as ingredients in food products, and 10 µg/kg for total aflatoxins and 5 µg/kg aflatoxin B_1 in nuts, subject to sorting. According to AGMARK standard of agricultural products in India, maximum % by weight of moisture content for special sesame seeds is 5%, 6% for good quality and 7% for general. High levels of aflatoxins have been found in groundnuts, (5000 µg/kg) (Kumar *et al*., 2008), cotton seeds; (200,000-300,000 µg/kg), (Smith and Moss, 1985), Brazil nuts; (15 µg/kg of B_1), (Oslen *et al*., 2008), shea-nuts; up to 600 µg/kg (Kershaw, 1982), and up to 45 µg/kg in sesame paste (Feng-Qin-Li *et al*., 2009).

Commodities that have received international attention most recently due to unsafe aflatoxin levels include sesame and melon seeds, which received Japanese and EU delegations in 2012 to collaborate with export authorities in addressing aflatoxin levels in these export commodities (Abt Associates Inc, 2012). Aflatoxin contamination of crops is more acute in the tropics (within major exporting countries) due to favourable fungal invasion conditions (e.g, high temperature and humidity, and poor agricultural practices). However, no work has been undertaken to determine the relationship between mould growth and aflatoxin production across the sesame value chain under varying conditions of water activity, relative humidity and temperature.

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Table 1. Exibit 5: EU Alerts of border rejections, detainment of Nigeria originating import due to aflatoxin contamination (2007-2012)

Year	Peanut	Kukuli	G.nut Oil	Maize	Cereal Products	Melon	Ogbono	Dorum Beans	Spices	Ginger	Ehuru Seeds	Total
2007	3	2				16	4	8	1			34
2008	2	1	1			9	2		1		1	17
2009	1					7	3					11
2010	1				2	8	3	1		1		16
2011	1			1		8	1					11
2012		1		1	1	10						13

Source: EU notifications were provided by Mrs Folasade of NAFDAC's Laboratory on 8/30/12. Published by Abt Associate Inc. 2012. Notes: (1) Groundnut/peanut paste, (2) Melon seeds are very popular in stews and have received a high level of EU attention this year for high aflatoxin levels: (3) Ogbono is a mangolike seeed commomly used in soups and stews: (4) Ehuru seeds are sold as spices.

1.2. MATHEMATICAL MODELLING OF MOULD GROWTH AND TOXIN PRODUCTION

Mathematical modelling of mould growth and toxin production under different physical conditions (temperature, relative humidity, pH and water activity) has been a useful tool in predictive mycology. This has been used to predict the extent of mould growth and invasion in foodstuffs as a function of environmental conditions. Mould growth and aflatoxin production as well as other mycotoxins have in recent times been modelled. Vaamonde *et al*., (2006) worked on the effect of water activity and temperature on production of aflatoxins and cyclopiazonic acid by *A. flavus* in pea-nuts. Monila and Giannuzi (2002), were able to model aflatoxin production by *A. parasiticus* in a solid medium at different temperatures, pH and propionic acid concetrations. Also, growth of *Fusarium langsethiae* and consequent T2 and HT-2 toxin contamination of oats under different growth conditions have been modelled by Mylona and Magan (2011).

Modelling mould growth in corn (Samapundo *et al*., 2007), considered the individual and combined effects of water activity and temperature on the radial growth of *A. parasiticus* and *A. flavus* on corn. Likewise, Galatia and Giannuzia (2011) conducted similar studies on Argentinian flint maize. However, modelling of aflatoxin production in the sesame seeds production chain is a novel area and the result will help to establish the hazard analysis critical control points in the value chain of the crop.

Hence, the objectives of the study are to:

Conduct a survey to identify the existing stages of production, and collect samples of sesame seeds at the different identified stages in the sesame production chain from 3 major sesame-producing states in Nigeria

To identify *Aspergillus spp* associated with the different stages in the sesame production chain

To determine the relationship between water activity, temperature, relative humidity and aflatoxin production in the sesame production chain in order to establish the critical control points

To develop a predictive model for aflatoxin production in the sesame production chain.

1.3. MATERIALS AND METHOD

1.3.1. Survey and sample collection of sesame seeds in Nigeria

A survey was conducted in 3 sesame producing states in Nigeria namely: Kogi, Benue, and Nasarawa States. In each state, samples were collected from at least 3 Local Government Areas (LGA). In Kogi State, samples were collected from five LGAs (Okene, Itobe, Anyigba, Lokoja, Ankpa), four LGAs in Benue State (Makurdi, Apa, Guma, Otukpo) and three LGAs (Agwantashi, Doma, Obi) in Nassarawa State. In each LGA samples were collected from at least three farmers who planted sesame during the previous season. About 2-4 Kg of sesame seeds were collected at different processing stages namely: farm gate stage (FG), cleaning stage (CS) and stored sesame seed (SS). A total of 62 sesame samples (3 varities: Ex-sudan, E8 and black variety) were collected and transported to Pathology Unit IITA and stored at 4 ºC. The different stages of sesame processing were identified as shown in figure 1. The temperature and relative humidity at the time of sampling was collected using a Thermo Hygrometer.

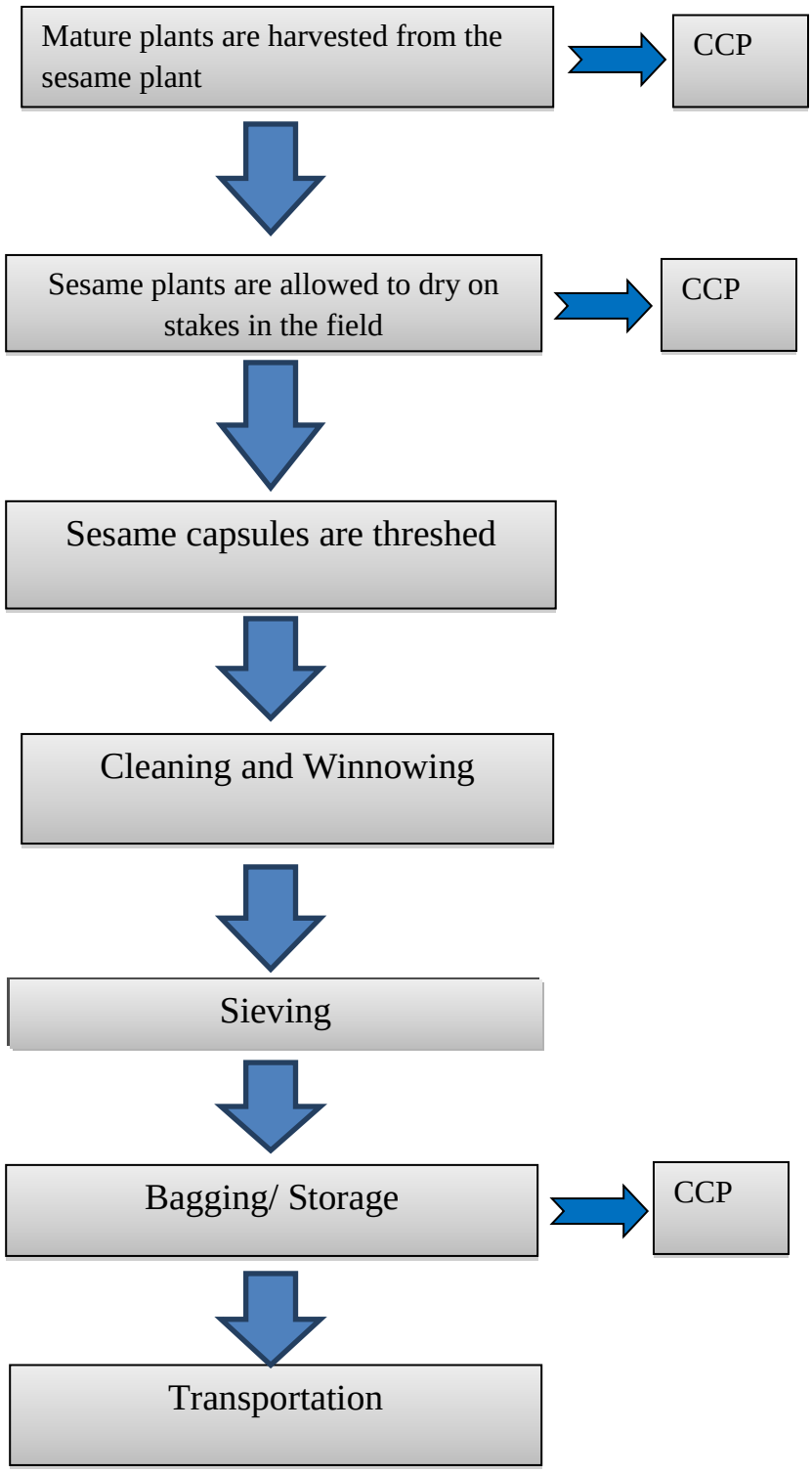
1.3.2. Sample preparation

One hundred grams of each sample was weighed out and milled in a laboratory blender (Waring Commercial Laboratory Blender). The milled samples were kept in sterile sample bags and stored in the cold room. These sub-samples were used for Isolation and colony forming unit (CFU/g) counts, aflatoxin analysis and determination of the free fatty acid content of the samples.

1.3.3. Isolation of *Aspergillus* spp from samples

The Isolation of *Aspergillus* was carried as described by Atehnkeng *et al.,* (2008). Where 1 g of each ground sample was aseptically (in a laminar flow hood) weighed into 10 ml sterile distilled water in a 40 ml sterile vial. This was mixed on a vortex shaker for 1 minute. 50, 100, 150, 200 and 500 µl were each plated on Modified Rose Bengal Agar (MRBA) medium (a seletive medium for *Aspergillus spp,* composition: (0.3% sucrose, 0.3% $NaNO_3$, 0.075% KH_2PO_4, 0.025% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.05% KCl, 1% NaCl, 1.3% EM agar, Rose Bengal, Dichloran, chloramphenicol and streptomycin). The inoculated plates were incubated upright at 31 ºC for three days.

Fig 1. Flow chart for sesame production



1.3.4. Enumeration of Aspergillus species on MRBA

The enumeration of Aspergillus colonies with distinct characteristics on MRBA medium were performed using a colony counter for each of the volumes plated, and the Colony Forming Units (CFU/g) estimated. Petri Plates with an uncountable number of colonies were further diluted and plated again. During transfers of isolates, only plates with less than 10 colonies were transferred and all the colonies were transferred to avoid bias. Finally, 20 isolates were picked up in each of the samples.

1.3.5. Identification of Aspergillus isolates

Aspergillus isolates were identified macro-morphologically by plating on 5/2 agar medium. After 5 days of incubation (unilluminated, 31° C), isolates were classified into species and strains by observing colony characteristics, and conidial morphology as described previously (Cotty, 1989). Isolates that produced small sclerotia (average sclerotial diameter < 400 µm) on 5/2 were identified as having the S morphology (i.e. as being either S_{BG} or S-type A. flavus), while those with a smooth conidial surface and either an average sclerotial diameter > 400 µm or without sclerotia were identified as L-type A. flavus. Isolates that had dark green colonies on 5/2 and produced rough conidia were considered A. parasiticus.

1.3.6. Determination of free fatty acid (FFA) content of samples

This was carried out according to method described by IOCCC (1996) for determination of FFA in crude oils. Five grams of extracted oil were melted and dissolved in 50 mL of diethylether and 95% ethanol mixture (1:1, v/v), and were titrated against standardized 0.1 N alcoholic NaOH solution using 1 mL phenolphthalein as indicator. A faint pink colour that persisted for 15 seconds indicated the end-point. FFA (oleic acid %) was calculated using the formula below:

$$\text{FFA (Oleic acid \%)} = 0.564 \times V$$

where, V = Volume of NaOH used in the titration

1.3.7. Determination of total aflatoxins in sesame samples

Extraction and purification of total aflatoxins in samples was carried out by the BF-method for aflatoxins in peanuts and peanut products AOAC (1990) with slight modifications, using high performance, thin-layer chromatography (HP-TLC). Sesame samples were extracted with 100 ml methanol/water (80:20 v/v) and 40 ml n-hexane. The samples were blended at high speed for 3 minutes. The extract was shaken for 30 minutes and filtered through Whatman No 1 filter paper. The purification of aflatoxins was done with 10% NaCl, and 30 ml n-hexane and finally into 25 ml dichloromethane.

The extracts were allowed to dry in plastic cups and the detection of aflatoxins was done by spotting on HP-TLC plates and developed in diethyl ether/methanol/water (96:3:1). Aflatoxins were quantified using a scanning densitometer and accompanying software (TLC Scanner 3, with Wincats 1.4.2 software, Camag, Muttenz, Switzerland). The minimum detection limit was 0.1 ng/g aflatoxin and the recovery rate was 89.5%.

1.3.8. Measurement of water activity of sesame samples

Water activity of samples was determined using the isopiestic method decribed by Sablani *et al.,* (2001) using saturated salt solutions. In this method, 1.6 g of starch (standard material) was dried in the oven at 70 ºC for 24 hours using $CaCl_2$ as the absorbent. Starch standard was equilibrated with saturated salt solutions: KI, KCl, NaCl, $NaNO_3$, $(NH4)_2SO_4$, and $MgCl_2$ in dessiccatiors at 31.52 ºC to establish different relative humidities, which were measured with Hobo data loggers. After equilibration (usually after 12-25 days), the moisture contents of the standard over the different salt solutions were determined by a halogen moisture analyzer. A graph of moisture content and equilibrium relative humidity (water activity) of salt solution was plotted and the standard curve was modelled by the GAB equation (Bell and Labuza, 2000). Same mass of standard was then equilibrated over 20 g of sample in a dessicator. The moisture content of the standard was determined after 48 hours. The water activity of the samples was estimated from the standard curve. Water activity of standard is equal to water activity of sample at equilibrium.

1.3.9. Rehydration of sesame samples and determination of water activity levels

Ten grams of whole sesame seeds were weighed into 30 ml vials and sterilized at 121 ^oC for 20 minutes to kill the spores of organisms that may have been present. Different volumes of sterile water (100-2000 µl) was added and thoroughly mixed together on a vortex mixer to obtain a homogeneous mixture. The vials were stored at 4 ^oC for 72 hours for equilibration to take place. The moisture contents (MC) of the equilibrated samples was thereafter determined by the oven method at 103 ^oC. the MC values obtained were fitted into the absorption isotherm curve of sesame seeds developed by Kumar and Balasubrahmanyam (1986), which was modelled using the GAB equation (Bell and Lazuba, 2000) to obtain the corresponding water activity levels. The water activity levels chosen for inoculation were 0.881, 0.919, 0.948, and 0.979. The MC for each of the water activity levels is 9.8%, 10.8%, 11.8% and 13.0% respectively.

1.3.10. Inoculation and incubation of sesame samples

Ten grams of autoclaved sesame seed samples in 30 ml vials with known water activity levels were inoculated with 500 µl of 10^6 spore suspension of toxigenic *A flavus* L previously isolated from the samples and confirmed to be toxigenic. The inoculation was replicated 4 times for each water activity level. The inoculated samples at the 4 water activity levels were incubated at 25, 31, 36 and 40 ^oC along with an un-inocualted control sample for each water activity level. The samples were incubated over glycerol / water solutions adjusted to the water

activity levels of the samples inside sealed dessiccators / polythene bags for 7 days. Samples at water activity 0 (dry state) were also inoculated and incubated along with the hydrated samples at the 4 temperature regimes.

1.3.11. Enumeration of fungal spores on inoculated sesame samples after incubation

After 7 days of incubation of the inoculated shea samples, colonization by A flavus *at different temperature and water activity levels was estimated by counting the number of spores of* A. flavus *with a turbidimeter. Samples with high spore concentrations were diluted appropriately for accurate estimation of the number of spores.*

1.3.12. Extraction and quantification of total aflatoxins in inoculated sesame samples

Ten grams of inoculated sesame samples were extracted with 50 ml, 80% methanol and 25 ml n-hexane, which were blended at hi-speed for m minutes, shaken for 30 minutes and filtered with Whattman no 1 filter paper. The extract was further purified with h-hexane and NaCl solution and toxins were partitioned into 25 ml dichloromethane. The dried extract was spotted on HP-TLC plates and quantified using scanning densitometer, CAMAG TLC Scanner 3 with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described previously (Suhagia *et al*., 2006).

1.3.13. Statistical analysis

Data on fungal incidence aflatoxin production model was summarized and analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC), and means were separated using Fisher's protected least significant difference (LSD) test to determine significant differences among the samples. Means for Aflatoxin levels, free fatty acids, and degree of colonization were separated using descriptive statistics.

Polynomial equations for the amount of spores produced, as well as toxin production with regard to the incubation at different temperatures and water activity, were obtained by forward stepwise regression using the SAS 9.2 package. These equations included both linear effects of a_w and temperature as well as their interactions.

1.4. RESULTS

1.4.1. Survey to collect shea nut samples.

The different locations where sesame samples were collected are shown on the Nigerian map (Fig. 2) with green dots. The relative humidity at the time of the sampling ranged from 65% in Lokoja in Kogi State to 84% in Makurdi, Benue State. Temperature also ranged from 26.0 ºC for Makurdi in Benue State to 33.7 ºC in Lokoja in Kogi State (Table 2).

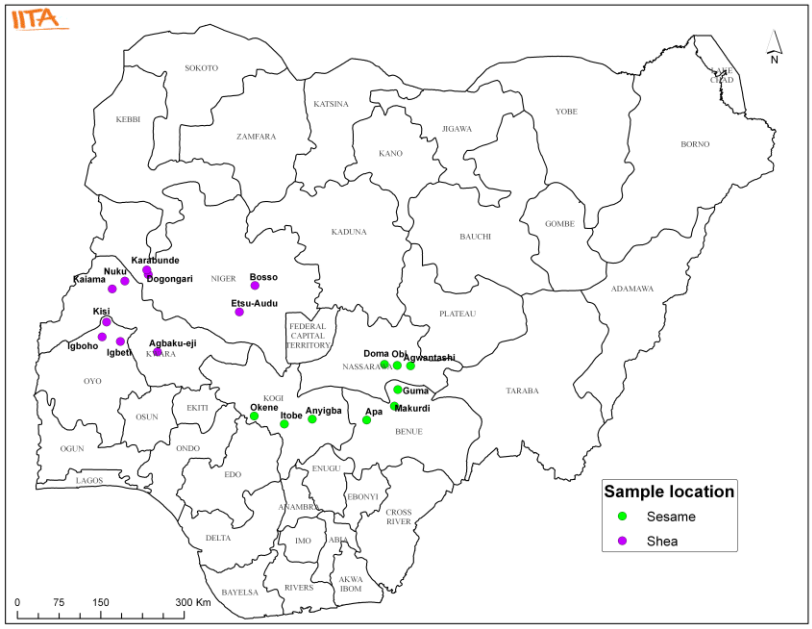


Fig. 2 Map of Nigeria showing locations where the sheanut and sesame samples were collected

Table 2. Locations visited during survey and number/type of samples collected

State	Location	No of sample	Variety of sample collected	Type of sample	RH (%)	Temp (^{o}C)
Benue	Makurdi	8	Ex-sudan, E8	FG, CS, SS	84.1	26.7
	Guma	8	Ex-sudan	FG, CS, SS	69.2	30.6
	Apa	1	E8	SS	70.8	29.6
	0tukpo	5	Ex-sudan	CS	72.4	27.3
Nasarawa	Agwantashi	9	Ex-sudan, E8, black	CS, SS	75.0	28.5
	Doma	8	E8, black variety	CS, SS	77.9	27.8
	Obi	7	Ex-sudan	FG, CS, SS	76.1	27.9
Kogi	Okene	7	Ex-sudan	FG, CS, SS	71.3	28.4
	Anyigba	1	E8	SS	77.8	27.4
	Itobe	1	Ex-sudan	SS	74.8	32.0
	Lokoja	1	E8	SS	65.1	33.7
	Ankpa	6	Ex-sudan	FG, CS	69.6	27.9

FG- farm gate stage, CS- cleaning stage, SS- storage stage

1.4.2. Sources of contamination of sesame seeds in the field and during storage

Delayed harvesting of sesame capsules after maturity may lead to splitting of the capsules exposing the seeds to contamination by aflatoxigenic fungi. Also, improper drying methods after harvest can create openings for aflatoxigenic fungi to contaminate the seeds (Fig 3). Inadequate drying to safe moisture (<12%) content before storage, poor hygienic condition, rewetting, and poor aeration can enhance colonization by aflatoxigenic fungi

leading to aflatoxin contamination in sesame seeds Fig 4. The processing stages were evaluated, and critical control points were determined (Table 3).

1.4.3. Distribution of Aspergillus species in sesame samples

Generally*, Aspergillus flavus L* was the most predominant isolate across the sesame value chain in all the states. Strain S_{BG} occurred across the value chain though in low percentages, but were significant because of large volumes of aflatoxins produced by these strains. The presence of *A. parasiticus* in CS samples from Benue and FG samples from Nasarawa indicate that initial contamination by these species may be from the field during harvesting or drying and then may be transferred through the other stages of production. Other *Aspergillus* species like *A. tamari and A. niger* were observed, but they are not of significance because they are not implicated in aflatoxin production (Table 4).

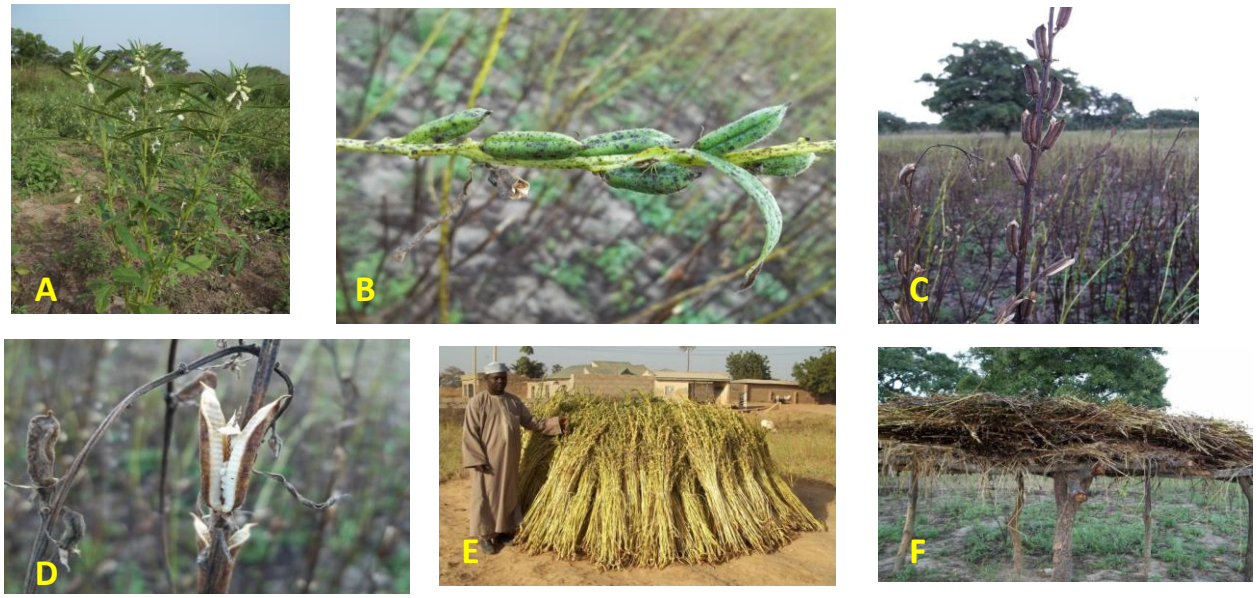


Fig. 3. Sesame plant at flowering stage (A) matured sesame capsules are sometimes colonized by mycotoxigenic fungi before they are harvested (B) delayed harvesting of dry/mature sesame capsules in the field can also lead to contamination (C, D) poor drying and storage conditions after harvest may lead to contamination by aflatoxigenic fungi (E, F)

Fig. 4. Poor storage (A), poor hygienic conditions (B), re-wetting during storage (C) or during retail in the markets (D) could lead to colonization by aflatoxigenic fungi resulting to aflatoxin contamination

Table 3. Different stages of processing and Critical Control Points (CCP) in the sesame production chain

Stage	Practices/Purpose	Possible risk	Preventive measure	CCP
Harvesting of mature sesame capsules	By cutting the stem of mature capsules with knives and tying together for drying		Use of harvesters	
Drying of seeds	By staking the branches bearing the capsules together in windrows on bare farm land	Inadequate drying as safe moisture level cannot be measured on farm land Contamination of seeds by toxigenic *Aspergillus sp* from the soil	Use of dryers that will dry seeds to specific safe moisture level. Use of mats or polythene to cover the ground on which sesame capsules are staked for drying	CCP
Threshing	Threshing by beating with sticks	Mechanical damage to seeds which becomes entry points for toxigenic moulds	Mechanization	
Winnowing	Winnowing with large trays and sieves	Remove contaminated grains that will be sources of contamination during storage	Mechanization	
Sieving	With different sizes of sieves to remove stones, sand and other particles			
Bagging/ packaging	Bagging in poly-propylene bags and keeping in market stores ready to be sold to retailers	rewetting of seeds in store, which may lead to caking, mould growth and mycotoxin contamination	Packaging in air-tight, sealed containers	CCP
Transportation	Transport the produce in a cool covered van	High Temp. could induce fungal growth/spoilage at low Wa leading to increased contamination	Transport in a covered van with a cooling system	CCP

Table 4. **Distribution of *Aspergillus* isolates in sesame seed samples collected at different processing stages in some states in Nigeria**

State	Sample type	No isolated	*A. flavus (%)*	*Strain S_{BG} (%)*	*parasiticus (%)*	*A. tamarii (%)*	*A. niger (%)*
Benue	FG	120	99.0	1.0	0.0	0.0	0.0
	CS	220	86.8	7.4	0.5	5.3	0.0
	SS	80	77.1	2.5	0.0	20.5	0.0
Nasarawa	FG	80	88.2	3.9	1.4	7.9	0.0
	CS	255	91.6	4.2	0.0	2.8	0.0
	SS	120	79.5	18.7	0.0	1.8	0.0
Kogi	FG	118	93.7	0.9	0.0	5.4	0.0
	CS	120	88.4	2.5	0.0	9.1	0.0
	SS	77	85.7	0.0	0.0	0.0	14.3

FG- farm gate stage, CS- cleaning stage, SS- storage stage.

A. flavus = L-strain of *Aspergillus flavus*; Strain S_{BG} = unnamed taxon of *Aspergillus flavus*; *A. parasiticus* = *Aspergillus parasiticus*; *A. tamarii* = *Aspergillus tamarii*, *A. niger* = *Aspergilus Niger*

1.4.4. Aflatoxin concentration in sesame samples

Generally, all the aflatoxins occurred in all the sesame samples. The highest mean value was of 11.93 ng/g in FG samples from Nasarawa State, and overall FG samples had the highest aflatoxin concentration across the value chain. This indicates that the major source of contamination of the sesame seeds is on the field either at harvest through soil contact or during drying so these stages are critical stages in the sesame production chain. This correlates with the high frequency of *Aspergillus CFU* at the FG stage Table 5. The aflatoxin concentration in the CS stage reduced significantly, probably due to cleaning ad sieving of the sesame seeds, which can be supported by the fact that sorting of produce can reduce aflatoxin contamination by 70%. The least mean value was observed in SS samples from across the states. This shows that once sesame seeds are dried to a safe moisture level and properly cleaned before storage, colonization by moulds and subsequent aflatoxin contamination may be prevented.

1.4.5. Colony forming units for sesame samples.

The CFU count of FG and SS sesame samples was high across the 3 states sampled; this is also evident in the relatively high aflatoxin contents of the FG samples (Table 6). The presence of sand, stones, plant debris and other particles in the FG samples may contribute to the CFU count in this processing stage. High CFU count in the SS sesame sample can be explained as the result of bad storage practices by the farmers in spite of reduction in the CFU counts at the CS stage through cleaning and sorting; if the seeds are poorly stored (FG. 2), mouldiness and aflatoxin levels can be increased.

1.4.6. Free Fatty Acids (FFA) in sesame samples

Free Fatty Acid (FFA) is a function of fat hydrolysis by lipase enzymes produced in the crop itself and by moulds. The FFA values obtained for most samples across the value chain were higher than the acceptable limit of (1-2% max) for export of sesame seeds in Nigeria. The highest mean occurrence of FFA was at the FG for Benue state (5.7%) with a range of 3.2 – 8.2% while the least occurrence was at the cleaning stage at Kogi State (2.6%) with a range of 1.2 – 3.7%) (Table 7).

Table 5. Aflatoxin concentration (ng/g) in sesame samples collected at different processing stages in some states in Nigeria

Location	Type of sample	Range Mean (n)	Aflatoxin concentration (ng/g)			
			B_1	B_2	G_1	G_2
Benue	FG	Range	1.3 – 19.0	0.0 – 15.7	0.0 – 24.7	0.0 – 14.1
		Mean (n = 6)	8.5	3.8	14.4	8.6
	CS	Range	0.0 – 31.4	0.0 – 7.0	0.0 – 23.9	0.0 – 12.8
		Mean (n = 12)	4.2	2.7	4.9	3.2
	SS	Range	0.7-7.9	0.0- 1.1	0.0 - 0.0	0.0 - 0.0
		Mean (n = 4)	2.5	0.7	0.0	0.0
Nasarawa	FG	Range	0.0 – 19.6	0.0 – 12.7	0.0 – 9.1	0.0 – 4.4
		Mean (n = 4)	11.9	3.5	2.9	2.8
	CS	Range	0.0 – 20.9	0.0 – 10.9	0.0 – 35.5	0.0 - 10.6
		Mean (n = 14)	5.2	3.3	6.6	2.3
	SS	Range	0.6-8.1	0.0 - 5.5	0.0 - 2.6	0.0 - 2.0
		Mean (n = 6)	3.6	1.2	1.2	1.1
Kogi	FG	Range	2.0 – 17.6	1.9- 33.1	0.0 – 21.0	0.0 - 19.3
		Mean (n = 6)	5.8	9.7	12.5	6.4
	CS	Range	0.0 – 3.5	0.0 – 1.6	0.0 – 14.8	0.0 – 4.4
		Mean (n = 6)	1.6	0.8	5.9	2.5
	SS	Range	0.0 - 2.3	0.0-3.1	0.0 – 2.0	0.0 - 1.6
		Mean (n= 4)	1.0	1.1	0.8	1.0

FG- farm gate stage, CS- cleaning stage, SS- storage stage

Table 6: Colony forming units (CFU/g) of Aspergillus spp in sesame samples collected at different processing stages in some states in Nigeria

State	Sample Stage	No. of samples	Mean CFU/g of *Aspergillus spp*	Range
Benue	FG	6	2083	700 – 4000
	CG	12	550	100 – 3900
	SS	4	390	180 – 720
Kogi	FG	6	5908	2750 – 8800
	CG	6	3517	2000 – 8300
	SS	4	6455	20 – 25500
Nasarawa	FG	4	5150	2600 – 7100
	CG	14	464	50 – 1650
	SS	6	2627	60 – 13300

FG- farm gate stage, CS- cleaning stage, SS- storage stage

Table 7: Free fatty acid content of sesame seed samples collected at different processing stages in some states in Nigeria

State	Sample type	Number of samples	Mean FFA (%)	Range (%)
Benue	FG	6	5.7	3.2 – 8.4
	CS	12	3.0	1.5 – 5.9
	FG	4	5.1	3.7 – 5.9
	CS	14	3.3	1.9 – 6.0
	SS	6	5.7	3.7 –7.6
Kogi	FG	6	4.1	3.1 – 4.8
	CG	6	2.6	1.2 – 3.7
	SS	4	5.6	4.3 – 8.1

FG- farm gate stage, CS- cleaning stage, SS- storage stage. FFA = Free Fatty Acids

1.4.7. Measurement of Water activity of the sesame samples

A graph of moisture content and equilibrium relative humidity (water activity) of salt solution was plotted (Fig. 5 and 6). The standard curve was modelled by the GAB equation shown below:

$$\mathbf{M_w} = \frac{Mm\ YKa_w}{(1-Ka_w)(1-Ka_w + YKa_w)}$$

where M_m = Monolayer moisture (Kilogram water/Kilogram dry solid)

a_w = water activity

M_w = Moisture content (d.b)

Y, K, Mn = three free sorption parameters characterizing the sorption properties of the material.

The graph was then used to determine the water activities of the samples (Table 8). Most storage moulds do not grow at a water activity level below 0.80 (Mannitoba, Canada, 2011); however, their spores still remain on the substrate in a passive state.

The highest water activity level was obtained in the FG samples (range: 0.715 - 0.73) across the tates sampled while SS sesame samples had the lowest water activity values across the States (range: 0.60-0.651) this shows that drying of the seeds to safe moisture level may not be achieved on the field; drying continues through all the other processes in the production chain until storage. The minimum water activity requirement for growth and aflatoxin production by *A. flavus and A. parasiticus* are 0.82 and 0.83-0.87 respectively (Mannitoba Canada, 2011).

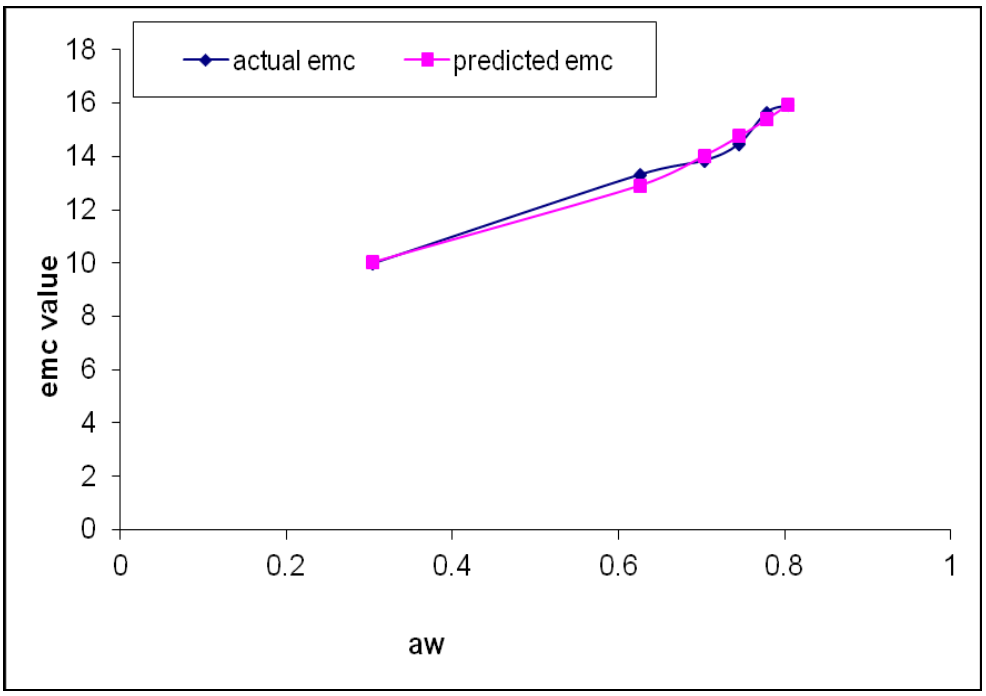


Fig 5: Graph of equilibrium moisture content versus water activity of standard as modelled by the GAB equation.emc= equilibrium moisture content, a_w = water activity

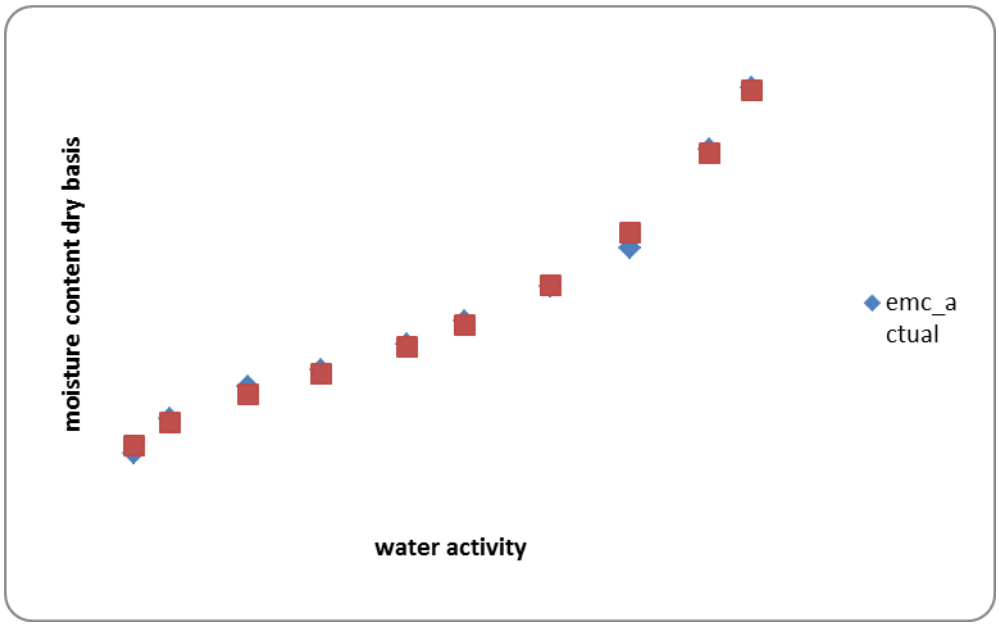


Fig 6: Graph of absorption isotherm of sesame seeds developed by Kumar and Balasubrahmanyam (1986), which was modelled by the GAB equation to obtain the predicted a_w values

Table 8. **Water activity (a_w) values of sesame seed samples collected at different processing stages in some states in Nigeria**

State	Sample type	EMC (%)	ERH (%)	Water activity
Benue	FG	6.89	72.0	0.720
	CG	6.59	69.5	0.695
	SS	5.62	60.0	0.600
Nasarawa	FG	6.81	71.5	0.715
	CG	6.00	64.1	0.641
	SS	5.88	62.0	0.620
Koggi	FG	7.02	73.0	0.730
	CG	6.53	69.0	0.690
	SS	6.10	65.1	0.651

EMC - Equilibrium Moisture content, ERH - Equilibrium Relative humidity, a_w - ERH/100

FG- farm gate stage, CS- cleaning stage, SS- storage stage

1.4.8. Predicting aflatoxin production for sesame

i. Modelling of the spore count (CFU/g) produced during incubation of sesame

The experiment was to evaluate the effect of temperature and water activity on the growth and sporulation of *Aspergillus* species in sesame. A linear model was obtained by forward stepwise regression for the effect of the temperature and water activity on Colony Forming Units (CFU/g). The spore count regression model was derived from SAS 9.3 regression model; only the terms a_w, t and t^2 were significant (P<0.01). The terms a_w.t, a_{w2} and $a_w^2t^2$ were dropped as shown in Table 8 below since their p-values were not significant (p > 0.1). All the actual values as derived in equation (1) are represented in equation (2).

log CFU = $C_0 + C_1 .a_w + C_2 .t - C_3.t^2$ (1)

log CFU = $-4.99 + 1.725a_w + 0.741t - 0.012t^2$ (2)

where a_w = water activity and t = temperature in degrees Celsius

ii. Effect of temperature and water activity on sesame fungal colony forming units counts

The logarithmic mean of *Aspergillus* CFU count at the five water activity levels over four temperatures are as shown in (Fig. 7 A and B). Highest spore count of *A. flavus L.* was observed at the wet conditions of 0.923 to 0.944 and 0.967 even though spores were also present at all the other water activity levels, and the lowest spore count was observed in the base line samples (samples at storage), since the sesame samples were very dry at this level.

High CFU count was also observed at temperatures 25-31 ^oC, which supports the claim that this temperature range is optimum for the growth of *Aspergillus flavus* by various researchers. At temperatures 36 and 40 ^oC CFU counts reduced significantly at all the water activity levels.

High spore count of *A flavus L.* was observed from a_w 0.885 and remained relatively constant up to a_w 0.958, and started to reduce at the wettest condition of 0.993, which shows that this condition may have been too wet for proliferation of fungal spores Fig. 6A.

Table 9: Parameter estimate ± S.E and corresponding *p*-values for stepwise regression analysis for the effect of water activity and temperature on spore concentration

Variable	Estimated value ± S.E	p-value	$A_{w.2}t^2$ dropped Est. value ± S.E	P-value	a_{w2} dropped est. value ± S.E	p-value	$a_w.t$ dropped est. value ± S.E	p-value
Intercept[a]	34.085 ± 25.233	0.1872	-7.558 ± 5.398	0.1717	-7.747 ± 3.327	0.0266	-4.991 ± 1.956	0.0157
a_w[b]	-60.183 ± 39.643	0.1398	4.314 ± 10.812	0.6927	4.779 ± 3.021	0.1238	1.725 ± 0.473	0.0009
T[c]	-0.926 ± 1.047	0.3836	0.824 ± 0.146	<.0001	0.824 ± 0.143	<.0001	0.741 ± 0.118	<.0001
$a_w.t$[d]	1.848 ± 1.153	0.1199	0.092 ± 0.091	0.322	0.089 ± 0.092	0.314	0	0
a_w^2	20.456 ± 13.418	0.1382	0.282 ± 6.272	0.9645	0	0	0	0
t^2	0.002 ± 0.009	0.7844	-0.012 ± 0.002	<.0001	-0.012 ± 0.002	<.0001	-0.012 ± 0.002	<.0001
$A_w.^2t^2$	-0.018 ± 0.011	0.1023	0	0	0	0	0	0

Intercept[a] = constant, a_w[b] = water activity, **T**[c] = temperature, $a_w.t$[d] = water activity and temperature interaction, a_w^2 is the square of the water activity, t^2 is the square of temperature

The lowest spore counts were observed in the base line samples, since the shea kernels were very dry at this level. High CFU counts were also observed at temperatures 25-31 ^{o}C and gradually reduced at 36 ºC and then 40 ^{o}C in Fig. 6B, which supports the fact that *Aspergillus flavus L* grows at an optimum temperature of 27-31 ^{o}C. This implies that water activity significantly influences the degree of *Aspergillus* colonization in shea nut and subsequent aflatoxin formation.

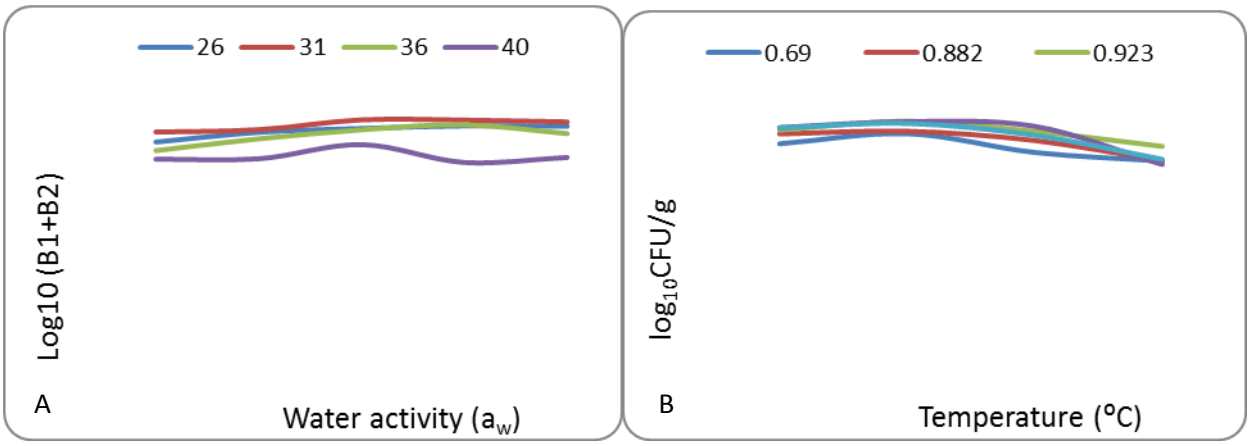


Fig 7: plot of log_{10} of *A. flavus* spores produced at 5 water activity levels (A) and 4 temperatures after 7 days of colonization by *Aspergillus flavus L.* (B)

1.4.9. Effect of temperature and water activity on B-aflatoxin production in inoculated sesame samples over a seven-day incubation period.

The sesame samples were dehydrated and the water activity adjusted, inoculated with the toxigenic strains of *Aspergillus flavus* and incubated at 4 temperature regimes. The sum of aflatoxins produced over the 5 water activity levels at 4 temperatures is shown in figure 10. Highest amounts of aflatoxin were produced at water activity 0.944 at all temperatures except for 31 ^{o} C, which showed fluctuations in toxin production at all the water activity levels, whereas at the wettest condition of 0.967, there is an obvious decline in toxin production at all the temperatures, indicating that water activity 0.944 at 25 ºC may be the optimum for aflatoxin production in sesame seeds. At temperatures 36 and 40 ^{o}C aflatoxins was produced in very small quantities.

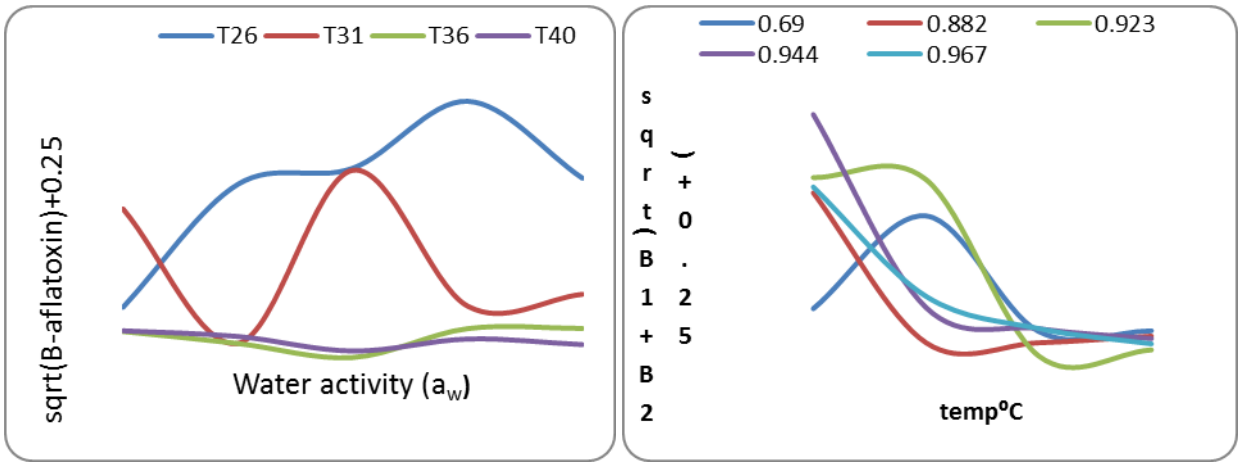


Fig. 10: Graph of aflatoxin $\sqrt{(B_1+B_2)}$ at 5 water activity levels and 4 temperature regimes

1.4.10. The predictive equation for aflatoxin production in sesame

The aflatoxin data generated at different water activities and temperature were subjected to SAS regression and used to derive this model below. Water activity and temperature were highly statistically significant as seen in table 10 below ($p<0.001$). This implies that optimum temperature and water activity play major roles in the colonization and aflatoxin accumulation in sesame.

Table 10. Parameter estimate ± S.E and corresponding p-values for stepwise regression analysis for the effect of water activity and temperature on aflatoxin

Variable	Estimated value ± S.E	p-value
Intercept	-3323.038 ± 857.491	0.0002
a_w	5573.578 ± 1375.190	0.0001
t	136.903 ± 35.769	0.0003
$a_w.t$	-176.114 ± 40.237	<.0001
a_{w2}	-1545.045 ± 476.505	0.0018
t^2	-0.972 ± 0.303	0.0021
$A_w.^2t^2$	1.524 ± 0.375	0.0001

The final equation derived from the table above is as follows.

Hence the model $(\sqrt{B_1}+B_2) = C_0 + C_1.a_w + C_2.t + C_3.a_w.t + C_4.a_w^2 + C_5.t^2 + C_6.a_w^2.t^2$.

The actual values for the 7 coefficients in the model are spelt out below

where a_w = water activity and t = temperature in degrees Celsius

$(\sqrt{B_1}+B_2) = -3323.038 + 5573.578a_w + 136.903t - 176.114a_w.t - 1545.045a_w^2 - 0.972t^2 + 1.524a_w^2.t^2$

To obtain the above model, the data was square root transformed for uniformity $(\sqrt{B_1}+B_2) + 0.25$ before subjecting to SAS regression model.

1.5. DISCUSSION

Contamination of sesame seeds by aflatoxigenic fungi across the sesame value chain from the farm gate to storage stage poses a major threat to food safety and the health of consumers. The high occurrence of *A. flavus* at all stages of sesame production indicates that initial contamination by these species may be from the field during harvesting or drying and then it is being transferred to the stores.

The high CFU count of *Aspergillus spp* observed in the farm gate (FG) samples across the states suggests that the FG stage is a critical control point during which fungal spores infest the crop, contaminate sesame seeds in

the field and consequently aflatoxins. This may also be from poor handling during harvesting or during sun drying.

The highest level of aflatoxin B_1 observed in sesame seed was 31.4 ng/g, which is above the allowable limit for oils seeds and nuts in the EU countries; however, over 75% of the total samples had below the stipulated 4 ppb. Chronic exposure by humans to minute quantities of aflatoxins should not be neglected.

However, when the water activity of the sesame seed samples was gradually increased, the aflatoxin level increased proportionately implying that water activity is significant in grain colonization by *Aspergillus* species, and increases aflatoxins concentration during drying and poor storage. Thus drying to safe moisture content within the shortest possible time before storage will reduce the rate of aflatoxin accumulation.

Overall, this study showed that a_w was the most important factor affecting sporulation and aflatoxin contamination in sesame. The effect of temperature was found also to be significant in aflatoxin accumulation in sesame. This study is similar to the results obtained by Medina and Magan (2011) who studied the effects of temperature and water activity effects on production of T-2 and HT-2 by *Fusarium langsethiae.* The models developed can be used to predict the safe environmental storage boundaries for sesame and conditions, which represent a high risk for aflatoxin accumulation in sesame. These models are also useful to determine the storage conditions for grain for export, and those involved holding sesame prior to processing.

1.6. CONCLUSIONS AND RECOMMENDATIONS

This study showed that water activity and temperature are the most important factors affecting sporulation and aflatoxin contamination in sesame. Farmers are advised to plant on time to avoid moisture stress during crop growth and drying of sesame to save moisture content (<12%) within the shortest possible time. This is a critical control point because prolonged or inadequate drying increases the risk of contamination by mycotoxigenic fungi; therefore, use of driers, determination of the moisture, and proper storage pending transportation is highly recommended.

High levels of aflatoxins were detected at all stages of sesame processing from farm gate to the store. Highest amounts of aflatoxin was produced at water activity 0.944 at all temperatures except for 31 ^{o}C which showed fluctuations in toxin production at all the water activity levels, whereas at the wettest condition of 0.967, there is an obvious decline in toxin production at all the temperatures, indicating that water activity 0.944 at 25 ^{o}C may be the optimum for aflatoxin production in sesame seeds.

Highest spore count of *A. flavus L.* was observed at a_w 0.944 even though spores were also present at all the other water activity levels, and the lowest spore count was observed in the base line samples since the sesame samples were very dry at this level. High CFU count was also observed at temperatures 25-31 ^{o}C which supports the claim that this temperature range is optimum for the growth of *Aspergillus flavus* by various researchers. At temperatures 36 and 40 ^{o}C CFU counts reduced significantly at all the water activity levels.

Bagging and storage is another critical control point, because most often, storing in improper moist condition increases the moisture content and growth of mycotoxigenic fungi. These mycotoxigenic fungi are capable of growing at very low water activity as observed in the CFU data where the isolate inoculated produced a large number of spores at very low water activity. In addition, aflatoxin concentration may increase if rewetting occurs in the storage facility. Hence, packing in air tight sealed bags may reduce insect infestation and subsequently aflatoxin contamination in the store.

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