IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 16, Issue 11 Ser. I (November 2022), PP 09-17
www.iosrjournals.org

Effects of seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame in the southern guinea savannah of Nigeria

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Abstract
Background: *Sesame is grown in almost all tropical and sub-tropical regions of Africa for its seeds and oil from the seeds. Hence, Nigeria seeks to expand its production and explore other markets including making inroads into the European Union (EU) and Mediterranean markets. However, the stringency of the food safety standards of the developed countries has posed a stiff challenge to the participation in the world trade in agricultural produce. In Nigeria, sesame export has dwindled due to the detection of aflatoxin contamination levels above the United States and European Union aflatoxin regulatory standards. Hence the need to investigate the effects of variety, seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame in the southern guinea savannah agroecological zone of Nigeria.*
Materials and Methods: *Treatments comprised 2 sesame varieties (E-8 and Ex-Sudan), 3 seeding rates (3 kg* ha^{-1}*, 5 kg* ha^{-1} *and 7 kg* ha^{-1}*) and 3 threshing regimes (1, 2 and 3 weeks after harvesting – WAH). A 2 x 3 x 3 factorial combination of the treatments were laid out in Randomized Complete Block Design (RCBD) and replicated four times in both the early and late season plantings. Analysis of variance was carried out on data generated while t-test was used to compare differences between early and late season plantings.*
Results:*Results of statistical analyses revealed that in the early planting season, only threshing regime and the interaction between variety and threshing regime showed significant effect. In the late season planting, variety, seeding rate and their interaction were significant. Lower density plantings of Ex-Sudan at Akwanga gave rise to lower aflatoxin levels. Generally, delay in time of threshing favoured accumulation of aflatoxins in seeds of sesame. There were significant variety and threshing regimes interactions although the pattern varied across locations. At Akwanga and Tor Musa, there was a pattern of increase in aflatoxin level of seeds with delay in threshing in both varieties in the early season. In the late season, only E-8 showed this pattern atTor Musa while both varieties did not show any significant variation at Akwanga.*
Conclusion:*It was clearly observed that for all locations, irrespective of variety, seeding rate and threshing regime, aflatoxin contamination was higher in seeds of early planted sesame compared to the late planted sesame. It is suggestive from this study that aflatoxin load is influenced by crop genotype, seeding rate and threshing regime.*
Keywords: Sesame, Seeding rate, Threshing Regime, Aflatoxin contamination, Seed Quality

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Date of Submission: 01-11-2022 Date of Acceptance: 12-11-2022

I. Introduction

Sesame (*Sesamumindicum*L.) is one of the most ancient oilseed crops. It is well adapted and grown in almost all tropical and sub-tropical regions of Africa, Asia, Latin America and southern Europe for its seeds and oil from the seeds[1]. According to[2] about 65% of the annual sesame crop produced is processed into oil for domestic and industrial uses while 35% is consumed as whole seed. Sesame is also used for pharmaceutical purposes. The presence of some antioxidants (sesamum, sesamolin and sesamol) results in the oil being one of the most stable vegetable oils in the world. It is also rich in carbohydrates, calcium, phosphorus and protein[3]. The seed cake is rich in protein and is used in compounding protein-rich feeds for livestock[4].In the year 2000, it was estimated that up to 570 000 ha of land was under sesame cultivation, producing an estimated 24 945 metric tons. The contemporary global wave of knowledge and understanding of the health and nutritional attributes of sesame and other emerging crops[5] has resulted in an increase in world market demand thereby motivating farmers to expand their land areas of sesame cultivation[1]. By the year 2001, Nigeria became the main exporter to the world's largest importer of sesame seeds to Japan[6](. However, when compared to the productivity of other competing countries, Nigeria's sesame productivity is sub-optimal.

Nigeria produces high quality sesame, an attribute that has earned the country a position in the top 10 chart of world sesame producers. According to[7,8] the quality of sesame seeds is measured by the colour, with white – pearly white seeds that are used for confectionary purposes while black seeded varieties are mainly used in Asian culinary, In addition, seed size and weight (weight of 1000 seeds $\geq$ 3.0 g), oil content (40 – 50 %) and moisture content (< 6 %) are other notable quality characteristics assessed[7,8]. Hence, Nigeria seeks to expand its production and explore other markets including making inroads into the European Union (EU) and Mediterranean markets. However, the stringency of the food safety standards of the developed countries has posed a stiff challenge to the participation in the world trade in agricultural produce. In recent times, Nigeria sesame export has dwindled due to the detection of aflatoxin contamination levels above the United States and European Union aflatoxin regulatory standards[9].

Aflatoxins are a group of four mycotoxins (B_1, B_2, G_1 and G_2) which are secondary metabolites produced mainly by *Aspergillus*species, most notably *A. flavus*and *A. parasiticus*[10]. Several findings by local researchers of various producing countries have confirmed the presence of aflatoxin in sesame viz in Chinese sesame[11], in Sudanese sesame oil up to 9.8 microgram per kilogram of sesame[12] and in Nigerian sesame seeds (up to 25ppb)[13]although a recent report by[14] contradicted some of the earlier studies. The ubiquitous nature of this contaminant has assumed an important position among the issues of global concern due to the adverse toxicological consequences on the health of humans and animals. Thus, Nigerian agricultural produce has suffered rejection at the international market as a result of its inability to meet phytosanitary standards due to the presence of toxins (chemical or biological) and the presence of fungal growth in the consignments[15]. Consequently, the Nigerian sesame farmers do not attract premium prices from the industry.

Tackling the issue of quality and yield of the Nigerian sesame seed requires an examination of the causal factors and levels. Thequality and quantity of grain crops depend on soil, climate and time of cultural management practices[16]. Similarly, [17]reported the importance of timely harvest while[18] reported on the effects of time of planting and crop population density. Several other authors posited that field and storage conditions that induce stress on crops (including high temperature and humidity, drought, inadequate nutrition and excessive plant population, insect attack at fruiting stages and weeds) favors growth of fungi and induce aflatoxin contamination[19,20,21]. Althoughassociated with storage, [22]suggested that aflatoxin producing species of *Aspergillus* start infestation of crops from the field and field management practices that increase yields may serve as an early preventative intervention to prevent or reduce incidences of aflatoxin contamination. Better sesame prices can be obtained by production of high-quality sesame. Seeding rate and threshing regime might also play a role to reduce aflatoxin incidence. Hence the need to determine the effect of seeding rate and threshing regime on the incidence of aflatoxin contamination on grains of two early and late season planted sesame varieties in the southern guinea savanna of Nigeria

II. Material and Methods

Experimental trials were conducted at three different locations, namely; Makurdi, Tor-Musa and Akwanga. Two varieties of sesame were used for the experiments: E8 and Ex-Sudan sourced from National Cereal Research Institute (NCRI), Badeggi, Niger State, Nigeria.

Experimental design

The experimental design at each location was a 2 x 3 x 3 factorial arranged in a Randomized Complete Block Design and replicated four times. There were 2 varieties of sesame (E-8 an Ex-Sudan), 3 seeding rates (3 kg ha^{-1}, 5 kg ha^{-1} and 7 kg ha^{-1}), and 3 threshing regimes (threshing at one week after harvest (1WAH), 2WAH and 3WAH)), resulting in eighteen treatment combinations in all. The experiment was conducted as early and late season plantings.

Planting and cultural practices

Sowing was done by broadcasting the seeds. The seeds were calibrated for the three seeding rates (3 kg ha^{-1}, 5 kg ha^{-1} and 7 kg ha^{-1}), mixed with dry river sand to facilitate even distribution on the seedbed. The seeds were then raked lightly into the soil with the use of garden forks. A formulation of NPK fertilizer was applied by broadcasting method at the rate of 40:15:15 ai ha^{-1}, at three weeks after sowing (3WAS). Weeding was done manually at 3WAS and repeated at 6WAS to maintain a weed free field. An organic insecticide (Bio neem) was used to control insect attacks on all plots using recommended rates of 100 ml in a 20 l knapsack.

Data collection

Harvest and post-harvest operation

At 95 % maturity (i.e. 90 % leaf senescence), 13WAS, plants within an area of 1 m^2 quadrant were harvested with sickles, tied with a rope, labelled appropriately and stalked upright by experimental treatment units. Threshing was carried out at 1WAH, 2WAH and 3WAH for the appropriate treatment units. Threshing was done by turning the stacks upside down over a clean tarpaulin and beating it with a stick. In between plot threshing, the tarpaulin was carefully dusted to avoid seed mixture.

Determination and analysis of aflatoxin from sesame grain samples

The Enzyme Linked Immunosorbent Assay (ELISA) method for Aflatoxin analysis was used as described by[23].

Sample Preparation

One hundred grams of sesame seeds were collected from each treatment and blended separately in a Waring commercial blender. After each blending operation, the blender was washed and rinsed thoroughly with sodium hypochlorite (NaOCI) to prevent cross contamination between samples. Twenty grams (20 g) each of the blended sample was further ground into fine powder and titrated with 100 ml of 70% methanol (v/v 70 ml absolute methanol in 30 ml distilled water) containing 5 g potassium chloride to enhance homogeneity. The extract was transferred into a labelled 250 ml flat bottom flask and shaken on a Benchmark orbital shaker (Model ORBI-Shaker) for 30 minutes. Filtration was done using Whatman filter paper number 41 after which the filtrate was diluted in 1:10 phosphate buffer saline in tween-20 (1 ml of extract and 9 ml of buffer) and left standing for 10 hours after which analysis of each sample commenced.

Sample analysis

AFB_1- BSA antigen was coated unto an ELISA plate. Specific antibodies available in the sample or standard competed with the bound AFB_1-BSA antigen with help of immune-globulins. Para nitro phenyl phosphate substrate was added to facilitate colour development. With the use of a spectrophotometer (Jenway 6305), which produced optical density values at 405 nm wavelength, AFB_1 levels were ascertained. A standard curve was extrapolated with a known correlation coefficient thereby giving AFB_1 concentrations in parts per billion.

Statistical Analysis

All the collected data were organized for analysis using Microsoft Excel package and subjected to Analysis of Variance (ANOVA) using GenStat (V.17) statistical package. Significant means were separated using Tukey test ($p \leq 0.05$).

III. Results

Variety, seeding rate and threshing regime on aflatoxin of early and late-planted sesame at Akwanga

The result of analysis of variance showing the effect of variety, seeding rate and threshing regime (V×SR×TR) for early and late-planted sesame atAkwanga is presented in Table 1 below. The result shows that V×SR×TR interaction significantly influence aflatoxin contamination in the early season at Akwanga location. In the late season, result shows that V×SR×TR interaction did not significantly influence aflatoxin content at Akwanga. Also, V×TR interaction as well as SR×TR interaction did not significantly influence aflatoxin content. However, the effect of V×SR interaction was found to significantly influence aflatoxin content. Whereas the main effect of threshing regime on late-planted sesame at Akwanga showed no significant differences in aflatoxin content, the study reveals significant main effect of V and SR on aflatoxin content.

Table 1: Probability values for Variety, Seeding rate and Threshing Regimes on aflatoxin contamination of early and late planted sesame at Akwanga

SOV	Aflatoxin (µg kg^{-1}) early	Aflatoxin (µg kg^{-1}) late Season
Variety (V)	0.001**	0.000**
Seeding Rate (SR)	0.000**	0.000**
Threshing Regime (TR)	0.000**	0.989ns
V x SR	0.000**	0.000**
V x TR	0.000**	0.518ns
SR x TR	0.006**	0.593ns
V x SR x TR	0.000**	0.138ns
CV	6.10	18.70

Key: * = significant. ** = highly significant. ns = not significant.

Effect of variety, seeding rate and threshing regime interaction on aflatoxin content of early planted sesame at Akwanga

The effect of V×SR×TR interaction on aflatoxin contamination of early planted sesame in Akwanga Location is presented in Table 2. The result reveals that significant variations exist with respect to aflatoxin contamination.The result of delayed threshing of variety E-8 planted at 3 kg ha^{-1}seeding rate at three weeks after harvest shows a comparatively higher level of seed contamination (7.01 μg kg^{-1}). Threshing variety E-8 planted at 3 kg ha^{-1}rates after one or two weeks after harvest was found to significantly reduce aflatoxin rates to 2.21μg $kg^{-1}$and 3.56 μg kg^{-1} respectively (Table 2). Similar trend was observed for variety E-8 planted at 5 kg ha^{-1}. Delay in threshing shows that variety E-8 planted at 5 kg ha^{-1}seeding rate recorded higher aflatoxin contamination (7.15 μg kg^{-1}), significantly higher than aflatoxin rates of 3.19 μg kg^{-1} and 3.70μg kg^{-1} recorded when variety E-8 planted at 5 kg $ha^{-1}$was threshed after one and two weeks respectively (Table 2). The result of variety E-8 planted at 7 kg ha^{-1} also recorded higher aflatoxin contamination of 7.58μg kg^{-1} when threshing was delayed for three weeks. Statistically lower aflatoxin content (3.31 μg kg^{-1} and 4.59 μg kg^{-1}) were obtained when threshing was done at one and two weeks after harvesting (Table 2).

A statistically similar trend in aflatoxin contamination was observed with delayed threshing for Ex-Sudan sesame variety (Table 2). When planting of Ex-Sudan variety was done at 3 $kgha^{-1}$ seeding rate, threshing at three weeks after harvesting showed that higher aflatoxin content (7.41 μg kg^{-1}) produced, significantly different from the reduced levels of contamination (2.52μg kg^{-1} and 4.61μg kg^{-1}) recorded at one and two weeks after threshing (Table 2). Planting at seeding rate of 5 kg ha^{-1} also showed similar result as delayed threshing (three weeks after harvest) produced high aflatoxin (6.88 μg kg^{-1}), significantly more than those produced when threshing was done at one and two weeks after harvesting (2.42μg kg^{-1} and 5.48μg kg^{-1} respectively). The result of aflatoxin contamination in Ex-Sudan variety planted at 7 kg ha^{-1} also showed that delayed threshing (3 weeks after harvesting) produced significantly higher contaminations (7.20μg kg^{-1}), greater than the amount of aflatoxin (2.96μg kg^{-1} and 4.95μg kg^{-1}) obtained when threshing was done at one and two weeks after harvesting (Table 2).

Table 2: Variety, seeding rate and threshing regime interaction on aflatoxin content of early planted sesame at Akwanga

VARIETY	SEEDING_RATE	THRESHIM_REGIME	Aflatoxin (μg kg^{-1}) early Season
E8	3kg	1WAH	2.21 f
E8	3kg	2WAH	3.56 d
E8	3kg	3WAH	7.01 a
E8	5kg	1WAH	3.19 de
E8	5kg	2WAH	3.70 d
E8	5kg	3WAH	7.15 a
E8	7kg	1WAH	3.31 d
E8	7kg	2WAH	4.59 c
E8	7kg	3WAH	7.58 a
EX SUDAN	3kg	1WAH	2.52 ef
EX SUDAN	3kg	2WAH	4.61 c
EX SUDAN	3kg	3WAH	7.41 a
EX SUDAN	5kg	1WAH	2.42 f
EX SUDAN	5kg	2WAH	5.48 b
EX SUDAN	5kg	3WAH	6.88 a
EX SUDAN	7kg	1WAH	2.96 def
EX SUDAN	7kg	2WAH	4.95 bc
EX SUDAN	7kg	3WAH	7.20 a
S.E.D			2.21

Key: Means within the same column with similar alphabet are not significantly different at 95% level of probability.

Effect of Variety x Seeding Rate interaction on aflatoxin content of late-planted sesame at Akwanga

The effect of V×SR as shown in Table 3 reveals significant interactions for aflatoxin contamination only in the late season planted sesame. Although a generally low aflatoxin content was observed in E-8 variety, its production at 3 kg ha^{-1}seeding rate resulted in very low aflatoxin contamination (1.05μg kg^{-1}), significantly different from the 1.88μg kg^{-1} observed at a higher seeding density of 7 kg ha^{-1}but statistically similar to that recorded at 5 kg ha^{-1}seeding rate with contamination level of 1.32μg kg^{-1}. In variety E-8, aflatoxin contamination increases with increased seeding rate. Similarly in Ex-Sudan variety, aflatoxin contaminations were significantly higher at both 5 kg and 7 kg ha^{-1}seeding rates (4.42μg kg^{-1} and 4.38μg kg^{-1} respectively) than when planting was done at 3 kg ha^{-1}seeding rate.

Table 3: Variety x Seeding Rate interaction on seed quality of early and late planted sesame at Akwanga

Variety	**Seeding Rate**	**Aflatoxin (μgkg^{-1}) Late Season**
E-8	3kg	1.05 d
E-8	5kg	1.32 cd
E-8	7kg	1.88 bc

EX-SUDAN	3kg	2.02 b
EX-SUDAN	5kg	4.42 a
EX-SUDAN	7kg	4.38 a
S.E.D		0.19

Key: Means within the same column with similar alphabet are not significantly different at 95% level of probability.

Seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame at Makurdi

Analysis of variance showing the effect of V×SR×TR on aflatoxin contamination of early and late planted sesame in Makurdi is presented in Table 4 below. The result of the study shows that aflatoxin content of sesame planted during the early season was not significantly influenced by V×SR×TR interaction. Similarly theinteractions of V×SR and V×TR as well as SR×TR showed no significant effect on early planted sesame in Makurdi location. While the effects of variety and seeding rate on early planted sesame in Makurdi showed no differential response for aflatoxin contamination, the effect of threshing regime was found to significantly influence aflatoxin content in early grown sesame at Makurdi.

In the late-planted sesame, result shows no significant V×SRxRT interactions for aflatoxin contamination (Table 4). Similarly, V×SR interaction as well as SR×TR interaction did not significantly influence aflatoxin content. However, variation in aflatoxin contamination was significantly influenced by V×TR interaction. Whereas threshing regime showed differential response in aflatoxin contamination, main effects of variety and seeding rate did not produce any differential response on aflatoxin in late sesame cultivation at Makurdi as shown in Table 4.

Table 4: Probability values for variety, seeding rate and threshing regimes on aflatoxin contamination of early and late-planted sesame at Makurdi

SOV	Aflatoxin (μgkg^{-1}) Early Season	Aflatoxin ($\mu g\ kg^{-1}$) Late Season
Variety (V)	0.648ns	0.540ns
Seeding Rate (SR)	0.104ns	0.343ns
Threshing Regime (TR)	0.000**	0.000**
V x SR	0.389ns	0.510ns
V x TR	0.969ns	0.024*
SR x TR	0.416ns	0.983ns
V x SR x TR	0.121ns	0.299ns
CV	25.30	59.73

Key: * = significant. ** = highly significant. ns = not significant.

Effect of threshing regime on aflatoxin content of early planted sesame in Makurdi location

The differential response of aflatoxin to effect of threshing regime in early-planted sesame at Makurdi location (Table 5) shows that significant variation exist. Result showed that aflatoxin contamination increased significantly with increasing time of threshing. The amount of aflatoxin recorded at 3WAH (4.21$\mu g\ kg^{-1}$) was significantly greater than that recorded at 2 WAH (1.95 $\mu g\ kg^{-1}$) and at 1WAH (0.98 $\mu g\ kg^{-1}$).

Table 5: Effect of threshing regime on aflatoxin content of early planted sesame in Makurdi location

Threshing regime	Aflatoxin ($\mu g\ kg^{-1}$) Early Season
1WAH	0.98 c
2WAH	1.95b
3WAH	4.21a
S.E.D	0.35

Key: Means within the same column with similar alphabet are not significantly different at 95% level of probability.

Effect of variety x threshing regime interaction on aflatoxin content of late-planted sesame in Makurdi location

Table 6 shows the effect of V×TR interaction on aflatoxin contamination of late-planted sesame at Makurdi. The effect of V×TR interaction on amount of aflatoxin present in late-planted sesame at Makurdi shows that in both varieties, the amount of aflatoxin increased significantly with every increase in threshing time. In Variety E-8, the amount of aflatoxin varied from 0.97$\mu g\ kg^{-1}$ in seeds of threshed sesame plants at one week after harvest to 2.07$\mu g\ kg^{-1}$ at two weeks after harvest and ultimately to 4.01 $\mu g\ kg^{-1}$ in seeds of threshed plants at three weeks after harvest. Similar result was observed in Ex-Sudan variety showing increasing aflatoxin contamination with increased duration between harvest and threshing time.

Table 6: Variety and threshing regime interaction on aflatoxin content of late-planted sesame at Makurdi

Variety	Threshing regime	Aflatoxin (µg kg^{-1}) Late Season
E8	1WAH	0.97 c
E8	2WAH	2.07 b
E8	3WAH	4.01 a
Ex Sudan	1WAH	0.98 c
Ex Sudan	2WAH	1.83 b
Ex Sudan	3WAH	4.41 a
S.E.D		0.161

Key: Means within the same column with similar alphabet are not significantly different at 95% level of probability.

Seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame at Tor Musa

Probability estimates showing the effect of variety, seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame at Tor Musa is presented in Table 7 below. The result shows that the interaction of V×SR×TR was found to significantly influence aflatoxin contamination in sesame planted both during the early and late season at Tor Musa. In fact, all the main effects and interaction effects were found to be significant in sesame planted during the late season.

Table 7: Probability values for variety, seeding rate and threshing regimes on aflatoxin contamination of early and late planted sesame at Tor Musa

SOV	Aflatoxin (µg kg^{-1}) Early Season	Aflatoxin (µgkg^{-1}) Late Season
Variety (V)	0.100ns	0.000**
Seeding Rate (SR)	0.000**	0.000**
Threshing Regime (TR)	0.000**	0.002**
V x SR	0.095ns	0.000**
V x TR	0.000**	0.000**
SR x TR	0.041*	0.000**
V x SR x TR	0.000**	0.000**
CV	6.70	16.10

Key: * = significant. ** = highly significant. ns = not significant.

Effect of Variety x Seeding Rate x Threshing Regime interaction on aflatoxin of early and late planted sesame at Tor Musa

The effect of V×SR×TR interaction on aflatoxin contamination of early and late planted sesame in Tor Musa Location is presented in Table 8.The effect of V×SR×TR interaction on the degree of aflatoxin contamination is highly varied.During the early planting, the effect of V×SR×TR interaction on the amount of aflatoxin showed that aflatoxin contamination was highest when threshing was delayed (3WAH) in both varieties planted at all seeding rate. In variety E-8 planted at 3 $kgha^{-1}$ seeding rate, aflatoxin content was highest (6.85µg kg^{-1}) when threshing was delayed up to 3 WAH, significantly different from lower amounts of aflatoxin (2.44 µg $kg^{-1}$and 3.64µg kg^{-1}) recorded when threshing was done at 1 WAH and 2WAH respectively (Table 8). Similar trend was observed at 5 kg ha^{-1} seeding rate of variety E-8, as delayed threshing (3WAH) resulted in high amount of aflatoxin (7.38 µg kg^{-1}), statistically different from lower amounts of aflatoxin (3.49µg $kg^{-1}$and 3.94µg kg^{-1}) found in seeds threshed at 1 WAH and 2WAH respectively (Table 8). The result of variety E-8 planted at 7 kg ha^{-1} seeding rate also followed similar pattern, with delayed threshing (3WAH) recording the highest amount of aflatoxin content (7.32µg kg^{-1}), significantly different from lower amounts of aflatoxin (3.11µg $kg^{-1}$and 4.52µg kg^{-1}) observed for threshing at 1WAH and 2WAH respectively (Table 8).

In Ex-Sudan variety planted at 3 $kgha^{-1}$ seeding rate, aflatoxin content was highest (7.08µg kg^{-1}) when threshing was delayed up to 3 WAH. This amount was differed significantly from lower amounts of aflatoxin (2.66 µg $kg^{-1}$and 3.88µg kg^{-1}) recorded when threshing was done at 1 WAH and 2WAH respectively (Table 8). Similar trend was observed at 5 kg ha^{-1} seeding rate of Ex-Sudan variety. Delayed threshing (3WAH) resulted in high amount of aflatoxin (6.84 µg kg^{-1}), statistically different from lower amounts of aflatoxin (2.34µg $kg^{-1}$and 5.30µg kg^{-1}) found in seeds threshed at 1 WAH and 2WAH respectively (Table 8). The result of Ex-Sudan variety planted at 7 kg ha^{-1} seeding rate also followed similar pattern, with delayed threshing (3WAH) recording the highest amount of aflatoxin content (7.79 µg kg^{-1}), significantly different from lower amounts of aflatoxin (3.27µg $kg^{-1}$and 4.67µg kg^{-1}) observed for threshing at 1WAH and 2WAH respectively (Table 8).

During the late planting season, result reveals that significant variations exist with respect to aflatoxin contamination (Table 8). The result of delayed threshing of variety E-8 planted at 3 kg ha^{-1}seeding rate at three weeks after harvest shows a comparatively higher level of seed contamination (Table 8). Threshing after one or two weeks after harvest recorded reduced aflatoxin rates in variety E-8 planted at 3 kg ha^{-1}rates. A generally lower degree of contaminations below 3 μg kg^{-1} were observed when variety E-8 was planted at 5 kg ha^{-1}seeding rate, decreasing with increased threshing time. Thus, threshing Variety E-8 planted at 5 kg ha^{-1}seeding rate any time after one week is recommended for low aflatoxin production. This is not much different from planting at 7 kg ha^{-1}seeding rate.Irrespective of threshing time, Ex-Sudan variety planted at 3 kg ha^{-1}seeding rate recorded acceptable levels of aflatoxin production at Tor Musa. These however were observed to produce critically low grain yields. The varied time of threshing did not influence the observed high aflatoxin contamination in Ex-Sudan variety planted at 5 kg ha^{-1}seeding rate. Similar result was also observed in Ex-Sudan variety planted at 7 kg ha^{-1}seeding rate (Table 8).

Table 8: Variety x seeding rate x threshing regime interaction on aflatoxin contamination of early and late-planted sesame at Tor Musa

Variety	Seeding Rate	Threshing Regime	Aflatoxin (μgkg^{-1}) Early Season	Aflatoxin (μgkg^{-1}) Late Season
E-8	3kg	1WAH	2.44 gh	1.04 bc
E-8	3kg	2WAH	3.64 e	1.85 bc
E-8	3kg	3WAH	6.85 b	4.48 a
E-8	5kg	1WAH	3.49 ef	1.31 bc
E-8	5kg	2WAH	3.94 de	1.14 bc
E-8	5kg	3WAH	7.38 ab	0.93 c
E-8	7kg	1WAH	3.11 efgh	1.83 bc
E-8	7kg	2WAH	4.52 cd	2.02 b
E-8	7kg	3WAH	7.32 ab	2.08 b
Ex-Sudan	3kg	1WAH	2.66 fgh	1.80 bc
Ex-Sudan	3kg	2WAH	3.88 de	1.75 bc
Ex-Sudan	3kg	3WAH	7.08 ab	1.95 bc
Ex-Sudan	5kg	1WAH	2.34 h	4.18 a
Ex-Sudan	5kg	2WAH	5.30 c	4.21 a
Ex-Sudan	5kg	3WAH	6.84 b	3.68 a
Ex-Sudan	7kg	1WAH	3.27 efg	4.21 a
Ex-Sudan	7kg	2WAH	4.67 cd	4.21 a
Ex-Sudan	7kg	3WAH	7.79 a	3.90 a
			0.23	0.30

Key: Means within the same column with similar alphabet are not significantly different at 95% level of probability.

IV. Discussion

Lower aflatoxin levels at lower seeding rates makes choice of Ex-Sudan particularly attractive especially as it was evident that higher seeding rates increased aflatoxin contamination. All these may have some bearing with environment of growth as the above scenario was obtainable at Akwanga. From another perspective, it could be inferred that the higher aflatoxin levels at higher planting densities of Ex-Sudan is due to higher plant to plant transmission of toxigenic fungi at high plant density. Variety E-8 is therefore to be preferred for high density planting at Akwanga when aflatoxin contaminations are a serious concern for production of good seed quality.In Corn, it was observed that high plant densities resulted in increased aflatoxin content[24]. Another author[25], also reported significant higher levels of mycotoxins in harvested corn when agronomic techniques including higher seeding densities were employed.In this study, it was generally evident that aflatoxin accumulation was favored by delay in time of threshing the sheaves. For instance, relatively high aflatoxin levels as witnessed at Makurdi when E-8 was threshed 3WAH points to a disadvantage of delayed threshing. For high seed yield and superior quality, sesame sheaves may not need to be dried beyond 15 days[26].In a study[27] reported different levels of aflatoxin contamination, attributing toxicity of aflatoxin in sesame to environmental factors such as rainfall, temperature and relative humidity. Since infection of plants by mycotoxin-causing organisms is influenced by environmental factors[21,27], leaving the harvested crop on the field for a long time before threshing implies higher level of infection due to such influence. It has been noted that the practice in Africa of leaving harvested produce for a long time on the field prior to storage, helps to promote fungal and insect attack[28].

The use of planting techniques and harvesting methods was advised by[29]in order to avoid damage to kernel, which will result in fungal infection and aflatoxin contamination. This might have informed enactment of the law of “Food Hygiene Control Regulations Article IV” by the Peoples’ Republic of China which states that rural and state-owned farms should be guided to harvesting in time, threshing, drying, removing impurities to prevent food mildew pollution during harvesting process[30]. The findings of this study reveal the possibility of increasing aflatoxin contamination for every delay in threshing. Thus, to avoid aflatoxin growth and multiplication, it is advisable to shorten the period between harvesting and threshing. Low amount of aflatoxin contamination is a desirable trait for consumption and market acceptability[31].

Interestingly, when E-8 variety was planted at 3 $kgha^{-1}$ and threshed 3WAH at Tor Musa, it resulted in very high levels of aflatoxin contamination. There was a change however when seeding rate was changed to 5 $kgha^{-1}$. Such observation may appear difficult to explain, but obviously local factors may have come into operation to produce observed results. It is however important to state here that all the levels of aflatoxin encountered in this location lies within the safe limit for human consumption (4 – 30 μgkg^{-1}) [32]. Values obtained were less than 10 μgkg^{-1}, which were higher only with respect to the European Union strict limits of 2 – 4 $\mu g\ kg^{-1}$ [33]. It can therefore be stated that sesame produced in this location is safe for human consumption.

It is worth pointing out that in all the locations, significant variety and threshing regimes interactions were noticeable. However, the pattern was not exactly the same across locations. At Akwanga and Tor Musa, there was a clear pattern of increase in aflatoxin level of seeds with delay in threshing in both varieties during the early season. In the late season, only E-8 showed this pattern at Tor Musa. At Akwanga, both varieties did not show any significant variation in aflatoxin content in the late season as threshing time varied. Thus particularly at this location, Ex-Sudan and E-8 can be cultivated with some flexibility in threshing schedule without the risk of accumulating excessive amounts of aflatoxins. It was however evident that Ex-Sudan had substantially higher amounts of aflatoxin than E-8, an observation that seemed to apply only to Akwanga. This demonstrates that choice of varieties with respect to aflatoxin sensitivity could be location specific.

At Tor Musa, only Ex-Sudan remained indifferent to threshing regime. One author[27]have already made a case for adoption of aflatoxin minimizing strategies which among others includes use of varieties with lower risk of contamination. In our context, E-8 and Ex-Sudan can be suitably cultivated at Akwanga during the late season since delayed threshing did not result in higher contamination levels. Variety E-8 may however still be preferred at this location due to significantly lower levels of the toxin compared to Ex-Sudan. On the other hand, at Tor Musa, only Ex-Sudan can be so cultivated at the late season. At Makurdi however, the two varieties behaved similarly in both seasons. That is there was an increase in aflatoxin contamination with progressive delay in threshing. It is likely that the more humid nature of Makurdi may account for this peculiarity.

An interesting aspect of this study is the observation that for all locations, irrespective of variety, seeding rate and threshing regime, early-planted sesame had higher aflatoxin levels than the late crop. This may be attributed to the differences in the total rainfall between the early and late season across the various locations. Extrapolation from the meteorological data presented in Tables 3.1, 3.2 and 3.3 showed a higher total rainfall of 811.8 mm, 1285.9 mm and 903.9 mm at Makurdi, Tor-Musa and Akwanga locations, respectively. It has already been stated that environmental factors have a strong bearing on crop infection by aflatoxin causing organisms[21]. Since the early crop matures in the peak of the rainy season when moisture conditions are high, it is likely to provide a more conducive environment for these organisms to thrive. The situation may become worse if threshing after harvesting is delayed, as it typically happens under the traditional production systems. The late crop matures in the dry season, with prevailing low humidity conditions and hence low aflatoxin levels. Thus to produce high quality seed (in terms of aflatoxin content), it is preferred to go for late season cropping. It is however heartening that even with higher aflatoxin levels associated with early season sesame, values are still within the safe limits as earlier indicated[32].

V. Conclusion

It is recognizable that certain varieties may have greater ability to produce higher aflatoxin levels than others at given plant population levels. In addition, late season sesame has been found to be lower in aflatoxin load than the early season crop. It is also obvious from this study that recommendations for production of sesame have to be specific, bearing in mind genetic, environmental and agronomic factors as they relate to product yield and quality.

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Orbunde, Abigail Kashimana, et. al. "Effects of seeding rate and threshing regime on aflatoxin contamination of early and late-planted sesame in the southern guinea savannah of Nigeria."*IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 16(11), (2022): pp 09-17.