Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

Evaluating the Effects of *Curcuma longa* and *Azadirachta indica* on Albino Rat Growth and Groundnut Aflatoxin Contamination

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https://doi.org/10.62050/faic2025.bop25.010

Abstract
This study evaluated the effects of *Curcuma longa* and *Azadirachta indica* treatments on albino rat growth parameters and assessed aflatoxin contamination in stored groundnuts across Nasarawa State, Nigeria. A two-way ANOVA revealed significant weight variations in rats administered *C. longa* doses (100–5000 mg/kg) over 21 days ($P<0.001$), with no significant treatment-duration interaction ($P=0.494$). Similarly, *A. indica* treatments demonstrated significant dose- and time-dependent weight changes ($P<0.005$), though interactions remained non-significant. Biochemical analyses indicated elevated alanine aminotransferase in *A. indica* high-dose groups ($P<0.003$) and reduced total protein ($P<0.019$), highlighting dose-specific metabolic impacts. Concurrently, aflatoxin analysis revealed widespread contamination in stored groundnuts, with total aflatoxin levels exceeding international safety thresholds (≤20 µg/kg) in 51.3% of samples. Nasarawa North recorded the highest mean total aflatoxin (37.93 µg/kg), while Toto LGA exhibited the highest prevalence (78.3%). Aflatoxin B1 (AFB1) dominated contaminations, with 98.6% incidence in Nasarawa North. Moisture content (5.34±2.85% in Nasarawa North) showed no statistical correlation with aflatoxin levels ($P>0.05$). These findings underscore the efficacy of herbal treatments in modulating animal physiology and emphasize urgent public health concerns regarding aflatoxin contamination in Nigerian agricultural produce. Regulatory interventions and improved storage practices are critical to mitigate exposure risks. The study provides actionable insights for enhancing food safety and optimizing phytotherapeutic applications in animal husbandry.

Keywords: *Curcuma longa*, *Azadirachta indica*, aflatoxins, biochemical parameters, food safety.

Introduction

Aflatoxins are highly toxic secondary metabolites produced by specific fungal species, primarily *Aspergillus flavus* and *Aspergillus parasiticus* (Akharenegbe et al., 2025). These fungi flourish in warm, humid environments and frequently colonize agricultural commodities such as maize, groundnuts, cottonseed, and tree nuts. Although their mere presence does not confirm aflatoxin contamination, it significantly elevates the risk of toxin production (Kumar et al., 2017). These ubiquitous "weedy" molds can proliferate pre-harvest or during storage, particularly under high humidity or environmental stressors like drought (Nwankudu et al., 2020; Gallo et al., 2016). Among these commodities, groundnuts are especially vulnerable to

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contamination by *A. flavus*, leading to the production of highly carcinogenic aflatoxins that pose severe risks to both human and animal health (Titiya and Njoroge 2020). The use of plant-based therapies for managing health-related disorders dates back to the earliest human civilizations. Traditional healthcare systems worldwide have long relied on botanicals as primary sources of medicinal compounds (Asghar et al., 2022). Today, plant-derived therapeutics are integral to medical practices across both developed and developing nations. For instance, *Azadirachta indica* (Neem) is widely employed in modern medicine, homeopathy, Unani, and Ayurveda due to its diverse pharmacological properties. The neem tree contains over 140 biologically active compounds with complex structures and diverse chemical characteristics (Asghar et al., 2022). Notably, its leaves are prized for a range of bioactivities, including anticarcinogenic, antimutagenic, antioxidant, antiviral, antibacterial, antifungal, antimalarial, antiulcer, antihyperglycemic, anti-inflammatory, and immunomodulatory effects (Subapriya and Nagini, 2005). Similarly, the ethnomedicinal importance of *Curcuma longa* (turmeric) has been well established over centuries, with numerous studies documenting its potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer properties (Ashraf et al., 2018). Beyond traditional therapeutic uses, certain plant species have shown significant potential in mitigating dietary aflatoxin contamination and promoting detoxification processes. Although various botanicals have been investigated for their anti-aflatoxigenic properties such as *Curcuma longa* , Cinnamomum zeylanicum, Zingiber officinale, and Salvia officinalis (Mohammed et al., 2024) challenges persist, including the intermittent detection of aflatoxins in some medicinal herbs (Polp and Che, 2006; Rizzio et al., 1998; Arranz et al., 2006) and in dried fruits like figs (Bircan, 2009). Despite these advances, a significant research gap remains: few in vivo studies have rigorously evaluated the detoxification potential of these botanicals against aflatoxins. To address this gap, the present study investigates the use of neem leaves and turmeric powder to mitigate aflatoxin effects in albino rats, while also assessing their contamination levels in groundnut Nasarawa State. Given the severe health risks associated with aflatoxin exposure and the stringent regulatory standards imposed by bodies such as the European Union (Wu, 2008), this research is both timely and critical. It aims to contribute to the development of accessible, cost-effective plant-based interventions that not only lower aflatoxin levels in agricultural commodities but also harness their intrinsic detoxifying potential to enhance human health and boost the economic value of food exports.

Material and Methods

Sampling Sites

Nasarawa State is divided into three distinct agricultural zones. Each zone comprises five local government areas (LGAs), except for the western zone (Karu), which consists of three LGAs. A purposive survey was conducted to assess the population of farmers utilizing traditional mud barns, locally known as 'rumbu' in Hausa, for groundnut storage. This exercise was facilitated with the support of the local government agricultural department and traditional authorities.

Sample Size Determination

A total of 300 stored groundnut samples were collected from rumbu across the three agricultural zones of Nasarawa State. The sample size was determined using Yamane's (1967) formula: $n= N/ (1+N (e)^2)$

Where:

n= signifies the sample size.

N= signifies the population under study (1,170)

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e= Signifies the Margin of error (which was taken to be 0.05).

Collection of Medicinal Plants

Medicinal plants, specifically neem (*Azadirachta indica*) and turmeric (*Curcuma longa*), were gathered from farms and bushes around Lafia, Nasarawa State. The collected plant specimens were identified at the Herbarium Unit of the Department of Plant Science and Biotechnology, Federal University Lafia, and cataloged under voucher numbers FUL/PLS/010 (*A. indica*) and FUL/PLS/011 (*C. longa*).

Preparation of Aqueous Leaf Extracts of *A. indica* and *C. longa*

The collected leaves were thoroughly washed with distilled water to remove dust and damaged parts, then air-dried in the shade. Once dried, the leaves were ground into a fine powder using a Binatone grinder (Blg 595). A total of 100 g of each powdered plant sample was mixed with 500 mL of distilled water and subjected to shaking at 100 RPM for 24 hours. The extract was passed through a No. 60 sieve, filtered using Whatman No. 1 filter paper, and further sterilized using a 0.2-µm membrane filter to eliminate microbial contamination (Singh et al., 2016). The filtrate was concentrated using a rotary evaporator, yielding a semi-solid residue that was subsequently dried into an aqueous extract (Jonathan, 2002).

Experimental Design for Evaluating Cytotoxic Effects of Aqueous Plant Extracts in Albino Rats

The study adhered to the National Institute of Health (NIH, 2011) guidelines for the ethical handling and use of laboratory animals. Albino rats were divided into three groups:

Group 1 (Control Group): Fed with a standard pellet diet and saline water.

Group 2 (Low-Dose Treatment Group): Administered 100 mg/kg body weight of *A. indica* and *C. longa* extracts.

Group 3 (High-Dose Treatment Group): Administered *A. indica* and *C. longa* extracts at 1500 mg/kg (moderate dose) and 5000 mg/kg (high dose) body weight.

The body weights of the animals were monitored daily. At the end of three weeks, the rats were sacrificed, and blood samples were collected from the heart chambers for biochemical analysis (Preetha et al., 2006).

Evaluation of Aflatoxin Detoxification Potential of Plant Extracts

Detoxification Studies

Forty-five albino rats (120–150 g) were housed in clean cages under standard conditions (23±2°C, 12-hour light/dark cycle). The animals were acclimatized for one week with unrestricted access to a standard pellet diet and water.

Experimental Design

The rats were allocated into five groups (n=9 per group):

1. Control Group: Received normal saline.
2. Aflatoxin-Induced Group: Administered 1 mg/kg body weight of aflatoxin B1 (AFB1).
3. Aflatoxin-Sorafenib Group: Treated with 1 mg/kg body weight of sorafenib (a cancer treatment drug).
4. Aflatoxin-Turmeric (*C. longa*) Group: Treated with *C. longa* extract at varying doses (100 mg/kg, 200 mg/kg, and 300 mg/kg body weight).

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5. Aflatoxin-Neem (*A. indica*) Group: Treated with *A. indica* extract at varying doses (1 g/kg body weight).

Except for the control group, all experimental groups received an intraperitoneal injection of 1 mg/kg AFB1, following the modified protocol of Preetha et al. (2006). One week post-AFB1 injection, plant extracts were administered orally at different doses for three weeks. Sorafenib was administered intramuscularly in the Aflatoxin-Sorafenib group, followed by a repeat dose 24 hours later. All animals were sacrificed four weeks after the experiment commenced. The liver, heart, and kidneys were immediately excised and weighed.

Serum Biochemical Assay

Blood samples were collected from the rats, and the serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were determined. Additionally, liver function was evaluated by measuring total proteins, albumin, and conjugated bilirubin (Wani *et al*., 2017).

Determination of Aflatoxin Concentration in Stored Groundnuts

Aflatoxins were extracted from groundnut samples using the method described by Sobolev and Dorner (2002). Twenty grams of groundnut samples were ground and mixed with 100 mL of 80% methanol. The mixture was shaken vigorously for 60 seconds in a 250-mL conical flask, followed by the addition of 40 mL of filtrate, 40 mL of 10% sodium chloride, and 25 mL of hexane. After shaking on an orbital shaker (Scientific Industries, Bohemia, NY) for 30 minutes at 400 RPM, 25 mL of dichloromethane was added and left for 60 seconds. The mixture was stratified, and the bottom layer was extracted into a beaker and evaporated to dryness over a bed of 20 g of anhydrous sodium sulfate (Estefan *et al.,* 2013).The extracts were dissolved in 1 mL of dichloromethane and transferred into an Eppendorf tube for scanning densitometry. Extracts (4 µL) were spotted alongside aflatoxin standards (Supelco, Bellefonte, PA) on aluminum-backed thin-layer chromatography (TLC) silica gel 60 F254 plates (Merck, Darmstadt, Germany). The TLC plates were scanned using a CAMAG TLC Scanner 3 at 365 nm. The intensity of each aflatoxin spot was analyzed using winCATS 1.4.2 software (Camag, AG, Muttenz, Switzerland), and aflatoxin concentrations were determined by comparing peak areas of the samples with those of the aflatoxin standards.

Statistical Analysis

Pearson's Chi-square test was used to compare the proportions of fungal species among different agricultural zones and local government areas (LGAs). The distribution of aflatoxin concentration data was assessed using the Shapiro-Wilk normality test, which indicated a non-normal distribution. Consequently, the data were log-transformed prior to conducting a one-way analysis of variance (ANOVA). Additionally, Chi-square tests were performed to evaluate the statistical significance of differences in mean aflatoxin concentrations as well as moisture content.

Result and Discussion

Mean Weights of Animals Based on Treatments and Number of Days

Table 1 shows the means and standard deviation of various weights of the albino rats treated with *Curcuma longa* . From the initial weight, day one of treatment to the final day of the study. A two-way analysis of variance was carried to statistically querythe various doses (low dose 100 mg/kg medium 1500 mg/kg and 5000 mg/kg) in relation to the durations of treatment. The effects of the various doses and control in relation to duration was statistically significant at $P< 0.001$ however compared to the effects of interaction between treatment and control was not statistically different ($P> 0.494$).

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Mean Weights of Animals Based on Treatments and Number of Days

The average weights and standard deviation of the rats from the initial day and final day treatment of *Azadirachta indica* is shown in Table 2. Two-way analysis of variance was used to compare weights of animals based on treatments and number of days, the rows with different numeric superscripts are statistically significant as $P< 0.005$. Also, the columns with different alphabetical superscript are also statistically significant $P< 0.005$. However, the comparison was further tested between the interactions of the treatment and control and it was not significant.

Table 1: Mean Weights of Animals Based on Treatments of *Curcuma longa* and Number of Days

	Dose of *Curcuma longa* administered					
	Control[1]	Low dose[1]	Medium dose[2]	High dose[2]		
Duration	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	***P-value***
Initial[a]	153.00 ± 2.65	154.00 ±2.37	155.50 ±5.00	157.67 ±4.0	13.68[†]	**<0.001[•]**
Day 1[a]	154.33 ± 2.08	156.00 ±2.19	158.00 ± 5.09	159.00 ± 2.65	417.48[‡]	**<0.001[§]**
Day 7[b]	160.66 ± 2.08	161.33 ± 3.14	163.67 ± 3.80	164.77 ± 2.86	0.97[*]	0.494[#]
Day 8[b]	162.67 ± 2.51	162 .23 ± 33	164.17 ± 3.54	167.44 ± 2.86		
Day 14[c]	173.66 ± 2.08	173.33 ± 1.96	177.00 ±3.90	176.44 ±2.80		
Day 21[d]	185.67 ± 2.00	185.50 ±1.24	190.83 ± 4.27	187.11 ± 2.47		
Final[d]	187.00 ±1.53	187.33 ±1.21	191.33 ± 4.27	188.33 ± 3.0		

[†]F-value for treatments
[‡]F-value for number of days
[*]F-value for interaction between treatments and number of days
[•]*P-value* for treatments
[§]*P-value* for number of days
[#]*P-value* for interaction between treatments and control
Rows with different numeric superscripts are significantly different at $P <0.05$
Columns with different alphabetical superscripts are significantly different at $P<0.05$
Significant difference are expressed in boldface

Mean Biochemical Parameters of Animals Based on Treatments of *C. longa* and Duration

Table 3 shows the result of means of various biochemical parameters of albino rats that were subjected to *Curcuma longa* treatments. The levels of alanine aminotransferase in the control, low and medium doses were higher compared to the high dose. This pattern is consistent with other parameters with the exception of total bilirubin and albumin. A one- way analysis of variance using Turkey's multiple comparison test was used to compare the biochemical parameters and as shown there was no statistical difference in the interaction between the biochemical parameters and the various levels of doses.

Mean Biochemical Parameters of Animals Based on Treatments of *A. indica* and Duration

Levels of alanine aminotransferase (ATP) in Table 4 shows elevated levels of 92.56 ± 1.03 high doses compared with other treatment of control (76.23 ± 5.53), low dose (68.80 ± 7.64), and medium dose (68.95 ± 8.40)P-value < 0.003 the test showed significance statistically in

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

the elevated levels of ATP. Another biochemical parameter showed a decreased level (total protein) in the high (dose 62.88 ± 4.62) compared to the control (68.93 ± 1.15) and the high dose (62.88 ± 4.62), this test showed that total protein was statistically significant, while the other parameters the high dose showed high elevated levels compared to the control and were not statistically different.

Table 2: Mean Weights of Animals Based on Treatments and Number of Days

	Dose of *Azadirachta indica* administered					
	Control[1]	Low dose[2]	Medium dose[3]	High dose[3]		
No of days	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	*P-value*
Initial[a]	153.00 ±2.64	159.33 ±1.97	161.17 ±2.60	160.55 ± 3.17	134.87[†]	**<0.001**[•]
Day 1[a]	154.33 ±2.08	161.33 ±1.80	163.33 ± 2.17	162.66 ±2.91	819.29[‡]	**<0.001**[§]
Day 7[b]	160.67 ± 2.08	172.67 ±1.21	174.00 ± 1.54	173.55 ±2.69	1.00[*]	0.464[#]
Day 8[b]	162.67 ± 2.51	174.00 ± 1.55	176.33 ±2.60	174.44 ± 4.00		
Day 14[c]	173.66 ±2.08	184.50 ±1.37	186.50 ±3.34	186.11 ± 2.61		
Day 21[d]	185.00 ± 2.00	195.50 ± 2.90	199.50 ±5.00	198.55 ± 1.70		
Final[d]	187.00 ± 2.00	197.85 ±1.20	200.66 ± 2.50	200.22 ± 1.10		

[†]F-value for treatments
[‡]F-value for number of days
[*]F-value for interaction between treatments and number of days
[•]*P-value* for treatments
[§]*P-value* for number of days
[#]*P-value* for interaction between treatments and control
Rows with different numeric superscripts are significantly different at $P < 0.05$
Columns with different alphabetical superscripts are significantly different at $P < 0.05$
Significant difference are expressed in boldface

Range and Mean Distribution of Aflatoxin Types in Stored Groundnuts from the Three Agricultural Zones in Nasarawa State.

A wide variation of aflatoxins concentration in relation to mean and range of stored groundnut was observed across the three agricultural zones as shown in Table 5. It spreads across aflatoxin B1 which ranged from the limit of detection (LOD)1 - 1791 µg/ Kg followed by aflatoxin B2 (LOD-508) then aflatoxin G1, LOD - 312 while the least range was recorded in aflatoxin G2 LOD – 121. No statistical differences was observed in the mean for total aflatoxin across the agro-zone. Nasarawa north had the highest mean value of total aflatoxin 37.93 µg/ Kg in contrast to Nasarawa west and Nasarawa south 37.75 µg/ K and 25.19 µg/ Kg respectively. A slight difference was observed for aflatoxin B1 as Nasarawa south had 32.97 µg/ Kg compared to Nasarawa west 21.86 µg/ Kg and Nasarawa north 32.40 µg/Kg, regardless of these differences, there was no statistical significance at $p > 0.05$.

Mean Distribution and Range of Aflatoxin Types in Stored Groundnuts from the Local Government Areas in Nasarawa State.

A wide mean variation was identified in Table 6 for aflatoxin B1 and total aflatoxin from the Nasarawa Eggon (76.29±14.39 µg/ Kg, 87.31±15.64 µg/ Kg), Toto (69.47±4.97 µg/Kg, 81.75±5.57 µg/Kg) and Lafia (51.12±15.36 µg/Kg, 59.30±17.82 µg/Kg). In contrast with lower means and standard deviation observed in other locations sampled. Lafia LGA had the highest levels of aflatoxin B1 (1791 µg/Kg) and total aflatoxins (2564 µg/Kg) followed by Keana LGA

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

aflatoxin B1 (1790 µg/Kg) and total aflatoxins (2577 µg/Kg) while Toto LGA had the least range of aflatoxin B1 (567 µg/Kg) and Total aflatoxin (818 µg/Kg).

Table 3: Mean Biochemical Parameters of Animals Based on Treatments of *C. longa* and Duration

	Dose of *Curcuma longa* administered					
	Control	Low dose	Medium dose	High dose		
Biochemical parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	*P-value*
Alanine aminotransferase (IU/L)	76.23 ± 5.53	82.43±2.27	80.36±9.08	71.76±1.05	0.449	0721
Aspartate aminotransferase (IU/L)	106.69±2.73	118.25±3.69	116.33 ± 5.70	115.83±3.39	0.053	0.983
Total protein(g/L)	68.93±1.50	68.83±3.32	63.06±6.97	67.76±9.96	0.660	0.587
Total bilirubin(µmol/L)	21.60±4.51	20.40±3.70	21.33±5.23	22.33±5.10	0.154	0.926
Conjugated bilirubin (µmol/L)	7.33±2.64	7.93±1.51	7.15±2.52	6.600±2.29	0.430	0.734
Albumin(g/L)	34.45±5.88	31.89 ±3.88	29.67± 3.59	31.46±3.51	1.075	0.382
Alkaline Phosphatase (IU/L)	283.10±2.78	289.43±5.91	291.10±5.29	288.18±4.94	0.18	0.997

Total Aflatoxin Distribution in Stored Groundnuts by Category from the Agricultural Zones in Nasarawa State.

The distribution of total aflatoxin contents in the agro-zone were categorized as shown in Table 7, All agro zones had non-detectable levels of aflatoxins, the lowest aflatoxin levels was recorded in Nasarawa North (10.3 %) for stored groundnut sample within 20 µg/Kg whereas the highest was obtained in Nasarawa South (20 %) for total aflatoxin samples within 20 µg/Kg.

Table 4: Mean Biochemical Parameters of Animals Based on Treatments of *A. indica* and Duration

		Dose of *Azadirachta indica* administered				
	Control	Low dose	Medium dose	High dose		
Biochemical parameters	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	***P-value***
Alanine aminotransferase (IU/L)	76.23 ± 5.53^{ab}	68.80 ± 7.64^{a}	68.95 ± 8.40^{a}	92.56 ± 1.03^{b}	6.40	**0.003**
Aspartate aminotransferase (IU/L)	106.60 ± 2.74	104.43 ± 3.34	109.85 ± 3.19	130.21 ± 2.89	1.20	0.335
Total protein(g/L)	68.93 ± 1.15	71.68 ± 7.50	70.17 ± 4.34	62.88 ± 1.62	4.20	**0.019**
Total bilirubin(µmol/L)	21.60 ± 4.52	20.03 ± 5.33	20.07 ± 5.58	22.49 ± 4.70	0.41	0.751
Conjugated bilirubin (µmol/L)	7.13 ± 2.64	6.62 ± 1.45	6.68 ± 2.15	8.46 ± 2.57	1.13	0.361
Albumin(g/L)	34.37 ± 5.88	33.00 ± 5.23	34.65 ± 1.64	29.11 ± 3.82	1.05	0.393
Alkaline Phosphatase (IU/L)	283.10 ± 3.79	285.08 ± 3.72	284 ± 4.66	285.33 ± 3.73	0.002	1.000

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

Table 5: Mean Distribution and Range (µg/ Kg) According to Aflatoxin Types in the Three Agricultural Zones in Nasarawa State

Types of aflatoxin	Nasarawa North	Range	Nasarawa West		Nasarawa South		Total		
	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min – Max	Mean ± SD	F	*P*
AFB1	32.40 ± 14.64	LOD - 1595	21.86 ± 9.86	LOD - 1570	**32.97 ± 12.05**	**LOD– 1791**	183.49 ± 332.90	0.86	0.426
AFB2	**44.68 ± 5.37**	LOD – 462	21.65 ± 5.32	LOD - 487	35.92 ± 6.18	LOD – 508	46.78 ± 94.54	2.41	0.09
AFG1	12.58 ± 2.17	LOD – 44	12.41 ± 3.61	LOD - 213	**17.50 ± 5.25**	LOD – 312	12.75 ± 36.77	0.88	0.417
AFG2	3.75 ± 2.16	LOD – 12	3.94 ± 3.35	LOD – 87	**5.70 ± 4.43**	LOD - 121	3.40 ± 12.53	1.16	0.319
Total aflatoxin	**37.93 ± 16.61**	LOD - 1918	25.19 ± 11.13	LOD - 2349	37.75 ± 13.66	LOD - **2577**	246.21 ± 460.18	0.79	0.457

LOD= Limit of detection, Limit of detection = 1, Min =Minimum, Max=Maximum, Percentage recovery = 80, AFB1= Aflatoxin B1, AFB2= Aflatoxin B2, AFG2= Aflatoxin G2, AFG1 =aflatoxin G1.

Table 6.: Mean Distribution and Range in relation to Aflatoxins types from the Local Government Areas in Nasarawa State

LGAs	N	AFB1(µg/ kg)		AFB2(µg/ kg)		AFG1(µg/ kg)		AFG2(µg/ kg)		Total Aflatoxins (µg/ kg)	
		Mean ± SD	Min - Max	Mean ± SD	Min – Max	Mean ± SD	Min – Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max
Nas Eggon	23	**76.29±14.39**	LOD-1595	39.90±4.59	LOD-306	9.16±1.60	LOD-15	2.73±2.30	LOD-8	**87.31±15.64**	LOD-1914
Akwanga	23	21.01±18.73	LOD-600	90.84±4.42	LOD-255	19.37±2.15	LOD-41	4.15±1.63	LOD-7	25.17±22.39	LOD-839
Wamba	23	21.23±10.25	LOD-1400	26.68±6.80	LOD-462	12.61±2.78	LOD-44	8.06±1.39	LOD-12	24.82±11.79	LOD-1918
Karu	23	11.49±9.15	LOD-871	13.60±4.19	LOD-106	5.35 ±3.74	LOD-42	2.63±2.38	LOD-8	13.04±10.17	LOD-973
Keffi	23	13.73±12.22	LOD-674	32.34±6.30	LOD-285	32.27±2.44	LOD-67	3.04±2.66	LOD-14	15.65±14.27	LOD-945
Kokona	23	26.08±12.78	LOD-1562	32.34±5.66	LOD-487	16.78±4.31	LOD-213	7.13±6.08	LOD-87	31.43±14.77	LOD-2349
Nasarawa	23	19.99±9.21	LOD-1570	15.40±5.74	LOD-367	9.07±3.74	LOD-76	4.97±4.19	LOD-23	22.30±10.17	LOD-2035
Toto	23	69.47±4.97	LOD-567	22.78±5.42	LOD-200	13.42±2.51	LOD-49	3.12±2.17	LOD-12	81.75±5.57	LOD-818
Awe	23	30.15±9.49	LOD-890	15.30±6.26	LOD-289	7.83±3.68	LOD-67	2.90±2.99	LOD-24	35.83±10.60	LOD-1270
Doma	23	37.29±8.87	LOD-903	39.00±6.88	LOD-356	12.27±8.13	LOD-167	**23.24±3.34**	LOD-78	41.24±10.10	LOD-1475
Keana	23	22.72±13.84	LOD-1790	44.21±8.35	LOD-467	**60.87±2.76**	LOD-231	8.92±5.16	LOD-89	25.72±16.09	**LOD-2577**
Obi	23	28.85±16.39	LOD-1711	33.77±4.56	LOD-456	12.28±6.03	LOD-231	2.96±2.54	LOD-12	32.47±18.15	LOD-2008
Lafia	24	51.12±15.36	**LOD-1791**	**104.42±3.83**	**LOD-508**	35.71±4.49	**LOD-312**	8.69±5.98	**LOD-121**	59.30±17.82	LOD-2564

Min= Minimum, Max= Maximum, LOD=Limit of Detection, Limit of Detection=1 µg/ kg, Percentage recovery = 80, AFB1= Aflatoxin B1, AFB2= Aflatoxin B2, AFG2= Aflatoxin G2, AFG1 =aflatoxin G1.

Total Aflatoxin Distribution in Stored Groundnuts by Category from Local Government Areas in Nasarawa State.

Table 8 showed that all samples from Nasarawa Eggon, Nasarawa and Wamba LGA's were within detectable limits (< 0.99 µg/Kg). Nasarawa Eggon (26.1 %) had the lowest aflatoxins

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

in the stored groundnut samples within 20 µg/Kg for total aflatoxin levels while Keffi, Keana and Obi LGA's had the highest stored groundnut samples within 20 µg/Kg.

Table 7: Total Aflatoxin Distribution in Stored Groundnuts by Category from the Agricultural Zones in Nasarawa State.

Total Aflatoxin category (µg/ Kg)	Nasarawa North	Nasarawa west	Nasarawa South	Total (%)
	N (%)	N (%)	N (%)	N (%)
ND	**1 (0.3)**	11 (3.6)	20 (6.7)	32 (10.7)
1 -4	23 (7.6)	35 (11.6)	31 (10.3)	89 (29.7)
5 – 20	7 (2.3)	9 (3)	9 (3)	25 (8.3)
≤ 20	31 (10.3)	55(18.3)	**60(20)**	146(48.7)
> 20	38 (12.6)	60 (20)	56 (18.6)	154(51.3)

ND = Not detected; limit of detection=1 µg/Kg; ≤4=European Union Standard and National Agency for Food and Drug Administration and Control; ≤20= United States Food and Drugs Administration >20=Not fit for human consumption.

Table 8: Total Aflatoxin Distribution in Stored Groundnuts by Category from Local Government Areas in Nasarawa State

LGA's	Not Detected	1-4	5-20	≤ 20	>20	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Nas Eggon	**0(0.0)**	6(26.1)	0(0.0)	**6(26.1)**	17(73.9)	23 (100)
Akwanga	1(4.3)	9(39.1)	2(8.7)	12(52.2)	11(47.8)	23(100)
Wamba	**0(0.0)**	8 (34.7)	5(21.7)	13(56.5)	10(43.5)	23(100)
Karu	1(4.3)	10(43.5)	1(4.3)	12(52.2)	11(47.8)	23(100)
Keffi	3(13.0)	10(43.5)	1(4.3)	**14(60.9)**	9(39.1)	23(100)
Kokona	3(13.0)	7(30.4)	1(4.3)	11(47.8)	12(52.2)	23(100)
Nasarawa	**0(0.0)**	7(30.4)	3(13.0)	10(43.5)	13(56.5)	23(100)
Toto	4(17.4)	1(4.3)	3(13.0)	8(34.8)	15(65.2)	23(100)
Awe	1(4.3)	5(21.7)	1(4.3)	7(30.4)	16(69.6)	23(100)
Doma	6(26.1)	4(17.4)	3(13.0)	13(56.5)	10(43.5)	23(100)
Keana	4(17.4)	8(34.7)	2(8.7)	**14(60.9)**	9(39.1)	23(100)
Obi	6(26.1)	7(30.4)	1(4.3)	**14(60.9)**	9(39.1)	23(100)
Lafia	3(12.5)	7(29.2)	2(8.3)	12(50.0)	12(50.0)	24(100)

ND = Not detected; limit of detection=1 µg/Kg; ≤4=European Union Standard and National Agency for Food and Drug Administration and Control; ≤20= United States Food and Drugs Administration (USFDA) >20=Not fit for human consumption

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

Incidence of Aflatoxin Types in Stored Groundnuts from the Agricultural Zones of Nasarawa State.

The incidence of aflatoxin types in stored groundnuts from the three agro-zones of Nasarawa state is presented in Table 9. The incidence of AFB1 was highest in Nasarawa North 68/69 (98.6 %), also for AFB2 - 42/69 (60.9 %), AFG1 - 34/69 (49.3 %) and AFG2 – 29/69 (42.0 %) while Nasarawa South had the least incidence of AFB1 - 96/116 (82.8 %), AFB2 - 54/116 (46.6 %), AFG1- 40/116 (34.5 %) however, Nasarawa West recorded the least incidence for AFG2 – 31/115 (27 %).

Table 9: Incidence of Aflatoxin Types in Stored Groundnuts from the Agricultural Zones of Nasarawa state

Agro-zones	AFBI Incidence (%)	AFB2 Incidence (%)	AFG1 Incidence (%)	AFG2 Incidence (%)
Nasarawa north	**68/69 (98.6)**	**42/69 (60.9)**	**34/69 (49.3)**	**29/69 (42.0)**
Nasarawa west	104/115 (90.4)	59/115 (51.3)	40/115 (34.8)	**31/115 (27.0)**
Nasarawa south	**96/116 (82.8)**	**54/116 (46.6)**	**40/116 (34.5)**	34/116 (29.3)
Total	268/300 (89.3)	155/300 (51.7)	114/300 (38.0)	94/300 (31.3)

AFB1= Aflatoxin B1, AFB2= Aflatoxin B2, AFG2= Aflatoxin G2, AFG1 =Aflatoxin G

Prevalence of AFB1 in Stored Groundnut Samples aboveNAFDAC and EU Standards in the Study Area.

Figure 1 showed the least prevalence of 47.8 % and 56.5 % from Obi and Keffi Local Government Areas respectively of aflatoxin B1 in stored groundnuts and Toto Local Government Area that had the highest prevalence of 78.3 %, while Figure 2 showed the lowest prevalence of aflatoxin B1 in Nasarawa south (62.9 %) while the least prevalence was recorded in Nasarawa North (69.6 %).

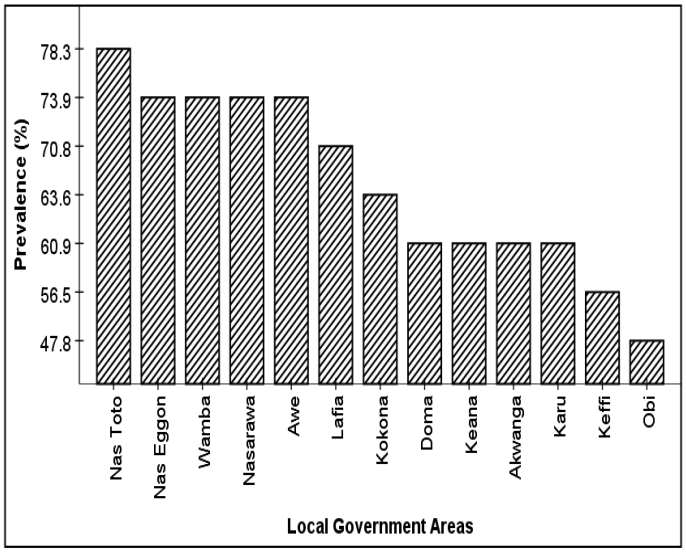

Figure 1: Prevalence of AFB1 in Stored Groundnut Samples above2 µg/kg across the LGAs in Nasarawa State

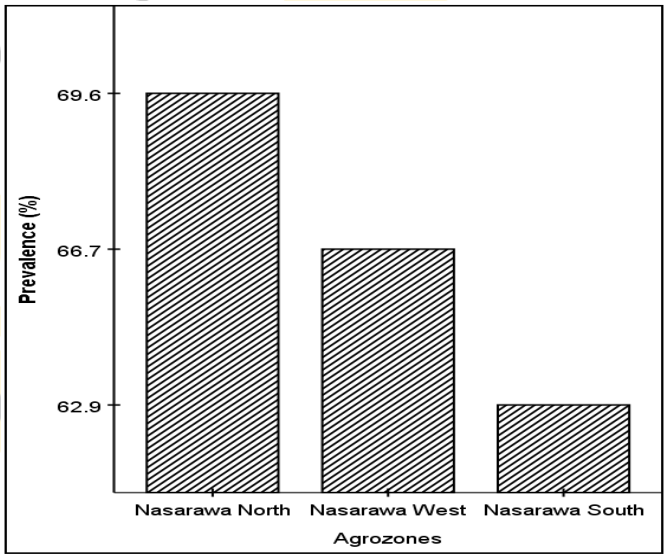

Figure 2: Prevalence of AFB1 in Stored Groundnut Samples above 2 µg/kg across the Agro-Zones.

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

Prevalence of Total Aflatoxin in Stored Groundnut Samples in the Study Area.
Figure 3 showed Toto LGA having the highest prevalence (78.3 %) of total aflatoxin over and above other LGA's, the least prevalence of total aflatoxin was recorded in Obi (43.5 %), while Nasarawa North (65.2 %) had the highest prevalence of total aflatoxin and Nasarawa South (56 %) had the lowest prevalence of total aflatoxin across the zones as shown in Figure 4.

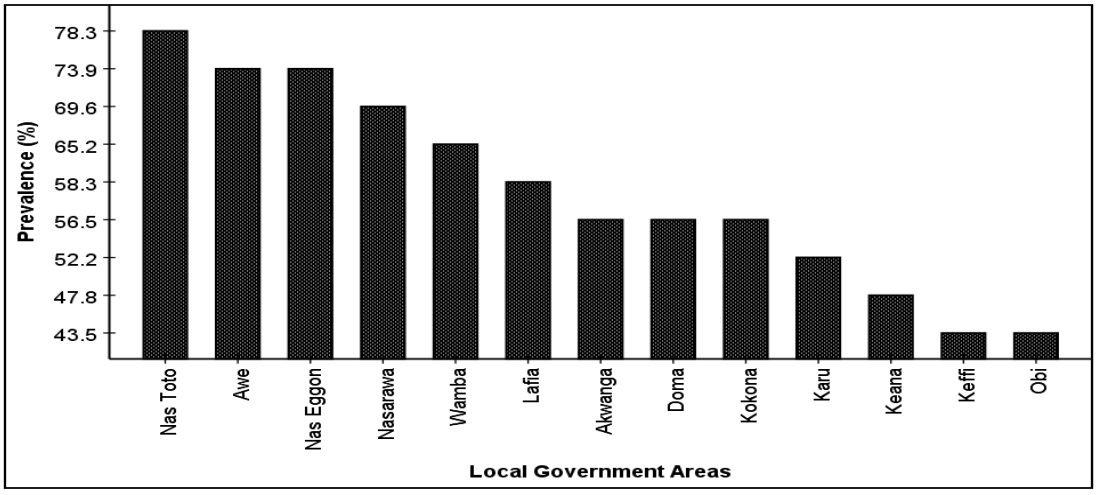

Figure 3: Prevalence of Total aflatoxin in Stored Groundnut Samples above 4µg/kg in Nasarawa State.

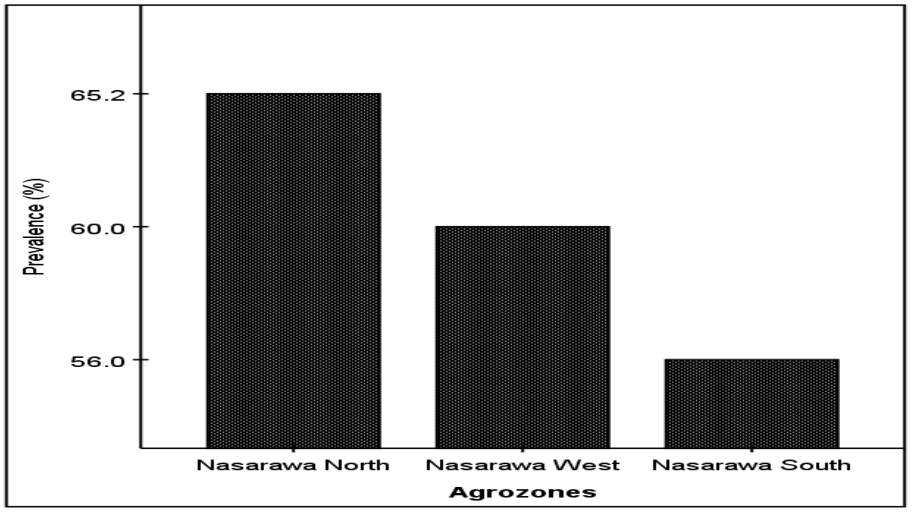

Figure 4: Prevalence of Total aflatoxin in Stored Groundnut above 4 µg/kg across the Agricultural Zones.

Moisture Content of Stored Groundnut in Relation to the LGA's and Agricultural Zones.

Nasarawa north (5.34 ± 2.85 %) had the highest mean and standard deviation of the moisture from Nasarawa State as made known in Table 10, and Nasarawa west (4.74 ± 5.41 %) had the least. However, the means were not statistically significant at P>0.05. In Table 11, the moisture content levels in Nasarawa Eggon (6.25 ± 2.48 %) was the highest compare to the least moisture

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

content recorded in Nasarawa LGA (3.66 ± 3.21 %). Despite these wide variations in the mean of moisture content across the zones, there was no significant difference (P>0.05)

Table 10: Means of Moisture Content from stored Groundnuts According to Agricultural Zones.

Agro-zone	Moisture content (%)		
	Mean ± SD	F	*P*
Nasarawa North	5.34 ± 2.85	2.11	0.123
Nasarawa West	4.08 ± 3.08		
Nasarawa South	4.74 ± 5.41		

Table 11: Moisture Content in Stored Groundnuts from to the LGAs in Nasarawa State

LGAs	Moisture content (%)		
	Mean ± SD	F	*P*
Nas Eggon	6.26 ± 2.48		
Doma	5.83 ± 8.95		
Lafia	5.26 ± 4.86		
Akwanga	4.94 ± 2.90		
Wamba	4.81 ± 3.02		
Keana	4.38 ± 0.91		
Awe	4.37 ± 3.16	0.81	0.644
Kokona	4.34 ± 4.05		
Karu	4.23 ± 2.31		
Toto	4.13 ± 2.51		
Keffi	4.03 ± 3.27		
Obi	3.83 ± 4.07		
Nasarawa	3.66 ± 3.21		

Key: Nas Eggon= Nasarawa Eggon

The findings from this study reveals the widespread aflatoxin contamination in stored groundnuts across Nasarawa State, Nigeria, alongside exploratory data on the biochemical effects of *Curcuma longa* and *Azadirachta indica* in animal models. These results highlight urgent public health risks and underscore the need for targeted interventions to mitigate mycotoxin exposure in food systems. The study revealed alarming levels of aflatoxin contamination, with 51.3% of samples exceeding the USFDA threshold of 20 µg/kg (Table 7). Notably, Toto LGA exhibited the highest total aflatoxin prevalence (78.3%), while Obi LGA showed the lowest (43.5%) (Figures 1–2). Such spatial variability aligns with regional differences in post-harvest practices and storage conditions, which are critical determinants of *Aspergillus* proliferation (Hell et al., 2000). The dominance of AFB1 (89.3% incidence; Table 9) is particularly concerning, given its classification as a Group 1 carcinogen (IARC, 2002). The elevated AFB1 levels in Nasarawa North (98.6%) compared to Nasarawa South (82.8%) suggest localized agroecological stressors, such as humidity or soil composition, favouring *Aspergillus flavus* strains (Klich, 2007). Only 29.7% of samples complied with the stringent EU/NAFDAC limit of ≤4 µg/kg (Table 7), indicating systemic non-compliance with

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

international safety standards. These results mirror findings from sub-Saharan Africa, where aflatoxin contamination frequently exceeds regulatory thresholds due to inadequate drying and storage infrastructure (Waliyar et al., 2015). The detection of AFG1 and AFG2 in Nasarawa North (49.3% and 42.0%, respectively) further implicates *Aspergillus parasiticus*, which thrives in humid environments (Frisvad *et al.,* 2019). The mean total aflatoxin concentrations varied across agro-zones, with Nasarawa North (37.93 µg/kg), Nasarawa South (37.75 µg/kg), and Nasarawa West (25.19 µg/kg) showing no statistically significant differences ($P > 0.05$; Table 5). However, extreme values in Lafia LGA (AFB1: 1791 µg/kg; total aflatoxins: 2564 µg/kg) and Keana LGA (total aflatoxins: 2577 µg/kg) highlight "hotspots" of contamination (Table 6). These extremes likely reflect poor storage practices, such as using non-hermetic containers, which exacerbate fungal growth (Mutegi *et al*., 2013). The high AFB1 range (LOD–1791 µg/kg) underscores the urgent need for region-specific biocontrol strategies, such as deploying atoxigenic *Aspergillus* strains (Bandyopadhyay *et al*., 2016).Despite variations in moisture content across LGAs (3.66–6.26%), no significant correlation ($P > 0.05$) was observed with aflatoxin levels (Tables 10–11). This contrasts with studies identifying moisture >7% as a major driver of *Aspergillus* growth (Mutegi *et al*., 2013). The absence of significance here may reflect the relatively low moisture range in this study, which fell below the threshold for optimal fungal activity. However, other factors such as temperature fluctuations, insect damage, and prolonged storage likely synergized to elevate contamination risks (Latham et al., 2016). Future studies should employ multivariate models to disentangle these interactions

The animal model data suggest limited efficacy of *Curcuma longa* in modulating biochemical parameters. For instance, high-dose *C. longa* reduced alanine aminotransferase (71.76 ± 1.05 IU/L vs. control: 76.23 ± 5.53 IU/L), but differences were not statistically significant ($P = 0.721$; Table 3). In contrast, *Azadirachta indica* at high doses significantly elevated alanine aminotransferase (92.56 ± 1.03 IU/L vs. control: 76.23 ± 5.53 IU/L; $P < 0.003$) and reduced total protein (62.88 ± 1.62 g/L vs. control: 68.93 ± 1.15 g/L; $P < 0.019$; Table 4). These findings suggest that while *A. indica* may possess bioactive compounds, high doses could induce hepatotoxicity, aligning with reports of neem extract-induced liver stress in rodents (Biswas et al., 2002).

Conclusion

The findings from this study underscore the pervasive and severe aflatoxin contamination in stored groundnuts across Nasarawa State, Nigeria, with over 50% of samples exceeding the USFDA safety threshold of 20 µg/kg. Notably, AFB1 a potent carcinogen dominated contamination profiles, reaching alarming levels (e.g., 1791 µg/kg in Lafia LGA). Spatial variability in contamination (e.g., Toto LGA: 78.3% vs. Obi LGA: 43.5%) highlights the role of suboptimal post-harvest practices, regional agroecological conditions, and inadequate storage infrastructure. Despite variations in moisture content (3.66–6.26%), no significant correlation with aflatoxin levels was observed, suggesting that other factors (e.g., insect damage, prolonged storage) drive fungal proliferation. Concurrently, exploratory animal trials with *Curcuma longa* and *Azadirachta indica* revealed limited detoxification efficacy, with high-dose *A. indica* inducing hepatotoxicity, as evidenced by elevated liver enzymes and reduced total protein. These results emphasize the urgent need for multi-sectoral interventions to mitigate aflatoxin exposure while cautiously exploring plant-based mitigation strategies. It is advised to promote the use of airtight storage solutions (e.g., Purdue Improved Crop Storage bags) to inhibit fungal growth and toxin production. Additionally, deploying atoxigenic *Aspergillus* strains (e.g., Aflasafe™) validated for Nigerian agroecologies can effectively suppress toxigenic fungi. Farmers should also be trained in post-harvest best practices including timely drying, efficient sorting, and moisture control to reduce contamination risks.

Print ISSN: 1595-6237 E-ISSN: 1595-6229
ISBN: 978-978-774-671-4

Finally, integrating aflatoxin surveillance into national food safety frameworks, in alignment with Codex Alimentarius standards, is strongly recommended

Conflict of Interest

Authors declare that no conflict of interest exists.

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