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Occurrence and Distribution of Toxigenic Mycoflora and Nutritional Composition Content of Peanut Seeds Sold in Niger State, Nigeria

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Abstract

Despite the economic importance of Peanut (*Arachis hypogaea L.*), it suffers infestation from toxigenic fungi leading to its contaminations thereby reducing the nutritional contents. Therefore, this study investigated the associated toxigenic mycoflora and nutritional composition of Peanut seeds sold in ten (10) markets in Niger State, Nigeria. Thirty-six (36) samples of peanuts seeds were collected across the three Agricultural zones of Niger State, namely Bida, Mokwa (Zone I), Minna, Shiroro (Zone II), Kotongora, and Rafi (Zone III), respectively. Isolation of fungus species were done using dilution of 10^4 factor on PDA. Proximate composition analysis was done following standard procedures. Ninety-three (93) fungal isolates were identified, belonging to number of genera: *Aspergillus*, *Penicillium*, and *Fusarium* respectively. The highest was isolated from Bida samples (27.48%), while the least was obtained from Shiroro samples (7.27%). Significant difference ($P > 0.05$) was observed in the proximate composition of Peanut seeds across all the study areas. The highest percentages in moisture contents, Crude fat, Protein, and Carbohydrate were obtained, from Shiroro (4.64%), Mokwa (47.24%), Kotangora (22.15%) and Shiroro (29.27%) samples, respectively, while the least percentage was obtained from Minna (2.46%), Shiroro (41.6%), Kotangora (18.48%) and Rafi (23.37%) respectively. The result indicates that the majorities of fungi isolated from this research were mostly toxigenic moulds and may have a direct impact on its nutritional contents. Therefore, improved management of these oil-rich seeds will enhance the high-quality product and reduce the risk of health challenges that go with consuming contaminated peanut products.

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INTRODUCTION

Peanut (*Arachis hypogaea L.*), also known as groundnut, earthnut, monkey nut, and goobers, is a Fabaceae family self-pollinating allotetraploid legume crop (Janila *et al*., 2013). Peanut seeds are high in oil (35–56%), protein (25–30%), carbohydrates (9.5–19.0%), minerals (P, Ca, Mg, and K), and vitamins (E, K, and B) (Gulluoglu *et al*., 2016). The crop has a variety of industrial applications, including food, feed, paints, lubricants, and insecticides (Variath and Janila, 2017). The nutritive value of food is high as the groundnut is affordable and serves as good source of oil and protein. Groundnut seeds are acknowledged as a rich cheap source of vegetable protein with vitamins enrichment and 100 per gram high calories (Atasie *et al.*, 2009). Groundnut protein is increasingly becoming important as food and feed sources, especially in developing countries (Ayoola *et al.*, 2010).

These crops are susceptible to infection by *Aspergillus* spp., which are fungi that produce a group of toxins known as aflatoxins (Guchi 2015). Janardhan *et al*. (2011) found that *Asperigillus* is a common mould in tropical and sub-tropical countries and causes aflatoxin contamination as a result of moulding of badly stored commodities, such as peanut, cereal and cotton seeds. Peanut being an oil seed boosts the vigour of pathogenic fungi resulting in bio-deterioration by the production of lipase. The tropical climate with high temperature and high relative humidity along with unscientific storage conditions adversely affect the preservation of oilseeds, etc., which lead to the total loss of seed quality (Isalar *et al*., 2021). Intensive crop improvement programme has resulted in the development of large number of high yielding varieties in different crops and more so in peanut. Many of the varieties are in seed production chain in the organized sector. Thus, production and distribution of quality seeds to the farmers free from deterioration has become increasingly important (Pedro *et al*., 2022).

The loss of product quality is due to biochemical changes in protein, carbohydrates, fatty acids, and vitamins (Makun *et al*., 2010). The rate of peanut seed aging is often determined by genotype, moisture, and temperature (Waliyar *et al*., 2016). However, Peanut seeds are susceptible to contamination by toxin-secreting fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* (Sultan and Magan, 2010). Hence, this research studied the occurrence and distribution of toxigenic mycoflora and proximate compositions in Peanut seeds sold in some markets in Niger State since it remain one of the primary crop cultivated across the agricultural zones of Niger State.

MATERIALS AND METHODS

Collection of samples

Thirty-six (36) samples of Peanut seeds, weighing 50g each from four major producing areas collected from 10 markets across each of the three agricultural zones in Niger State, namely Bida, Mokwa (Zone 1), Minna, Shiroro (Zone2), Kotongora, and Rafi (Zone 3) respectively, were individually homogenized. The residents and population based the Peanut seed collection on the rate of utilization. The four major peanut producing areas of Niger State are Kotangora, Mokwa, Lavun and Wushishi. The choice of Bida and Minna in this study was due to its market size and geographical location.

Preparation of Potato Dextrose Agar (PDA)

Thirty-nine (39) grams of PDA were suspended in 1000 ml distilled water and heated to dissolve the powder completely. The medium was sterilized by autoclaving at 121°C for 15 minutes (Manufacturer's guide).

Isolation and Identification of fungi contaminating Peanut seeds

One gram (1g) of homogenized Peanut seed was aseptically suspended into 9ml of sterile distilled water in a test tube and vortexed properly. 1 ml was serially diluted up to fourth fold 10^4. 1 ml from the fourth dilution fold test tube (10^4) was transferred into a sterile Petri plate. Twenty (20 ml) of Potato Dextrose Agar (PDA), to which 1ml of streptomycin was added, and then poured into the petri dish and incubated at 28±2°C for 3 days. After the third day, a single conidium was picked up with a sterile needle and viewed under microscopic observation, transferred individually to PDA plates, and incubated at ambient temperature (Subramanian *et al*., 2013). The monoculture was prepared and stored on PDA slants at 40±2°C. The fungal isolate was identified using the fungal family of the world mycological monograph (Musa *et al.*, 2022; Sarah *et al*., 2016; Adebola and Amadi, 2012).

Proximate analysis of Peanut seeds

Proximate composition of the various Peanut seeds were carried out in triplicate to test the moisture content, fat, crude protein, ash, and carbohydrate percentages using AOAC (2012) methods.

Determination of Ash Content

Ash content was made by a 600 °C incineration in a muffle furnace, as stated by the technique of (Opega *et al*., 2016). A crevassable and ignited tarred crevasses weighed two grams of each sample (w1). The samples were weighted and placed on a hot plate inside the fumigation cupboard to avoid the accumulation. The rest was transported to a pre-heated stomach furnace and kept at 600°C for 6 hours to ash, until the sampling was reduced to light ash.

$$\%\text{ ash } = \frac{W_2 - W_1}{2.0\text{ g}} \times 100$$

Determination of Fat Content

The soxhlet extraction method was used for fat content determination following the method of Opega *et al.* (2016). Where A thimble (w1) weighing two grams of the seed were measure and dried. Cool boiling flask filled with petroleum ether of 300 mL (w2) was used to boil the seed with the soxhlets at 60°C, allowing for 6 hours to reflux. The thimble was carefully removed and the extracted oil was dried for 1 hour between 105-110°C in the petroleum ether flask.

$$\%\text{ fat} = \frac{W_2 - W_1}{\text{weight of the sample (2.0 g)}} \times 100$$

Determination of Crude Protein

The micro–kjeldahl apparatus was used for Protein content following the procedure described by Prabhavathi *et al*. (2016). A micro-Kjeldahl digestion flask weighed two grams together with 20 mL of distilled water. It was shaken and allowed to stand. A tablet of the Oxo Sulfate (VI) acids and the selenium catalyst 20 mL tetra (H_2SO_4) were added. The flask was heated to 100 °C for 4 hours on the digestion block, until the digest is clear. The bottle was removed from the block and cool down. The content was transferred into 50 mL volumetric bottle and watered onto the mark. The distillate's titrated nitrogen is 0.014 M of H_2SO_4. When the color of the distillation changed from green to rose, the endpoint was achieved.

$$\%\text{Crude protein} = \frac{\text{ATV} - \text{TVB} \times 0.1 \times 0.014 \times \text{CF}}{\text{Weight of food sample}} \times 100$$

ATV: Actual titre value. TVB: Titre value of blank CF: Conversion factors

Determination of Moisture Contents

Each sample was made to moisture content according to the method of Khalifa *et al*. (2017) using the vacuum oven method. Two pre-weighed dried dishes (w1) and a weighted dish (W2) were quickly weighed. At 100 ^{o}C it was dried at a pressure of 100mHg no more than five hours. The desiccators was cooled and re-weighed (W3) with a registered weight loss of moisture after drying the sample. The moisture percentage was determined as shown:

$$\%\text{ Moisture} = \frac{\text{W1} + \text{W2}}{\text{W3} - \text{W1}} \times 100$$

Determination of Crude Fiber

The fiber content was measured using an enzyme-free technique as reported by Prabhavathi *et al*. (2016) 1.5 g of H_2SO_4 /100 mL solution was distorted by 2g of the dry petroleum ether sample and cooked under a 200 mL reflux for 30 minutes. The system was filtered through a fluted funnel lined with linen and then washed with hot water until the acidity was eliminated. The residue was transmitted to a beaker and boiled at a solution of 1.25g carbonate-free NaOH per 100 mL in 200 mL for 30 minutes. With a thin but closed pad of washed and inflamed asbestos, the final residue was filtered into a porcelain crust. For the last processes such as dry and burn, an electric oven was employed.

The percentage (percent) of the fibers following incineration x 100 was computed to weight loss.

$$\%\text{ Crude fibre} = \frac{\text{Loss in weight (g)}}{\text{Original mass (2.0)}} \times 100$$

Determination of Carbohydrate Content

Carbohydrate value was determined according Khalifa *et al*. (2017). When calculated using the percent of weight method, the total proportion of carbohydrates was derived from the percentage of food nutrient sum: 100 % protein, percent crude fiber, percent fat and percent ash percent. Where, the percentage of carbohydrates (=) (CF + CP + F + F + A + M– 100 %); where CF, = Crude Fiber, CP, = Crude Protein; M= Moisture, F= Fat, and, A=Ash.

Data analysis

Data generated was subjected to analysis of variance (ANOVA) using Statistical Package for social science (SPSS version 24). Duncan Multiple Range Test (DMRT) was used to separate the means and test for the level of significance at (5%).

RESULTS AND DISCUSSION

Isolated and Identified Toxigenic Mycoflora

Ninety-three (93) fungal species were isolated and identified belonging to three genera: they include *Aspergillus, Fusarium* and *Penicillium* species respectively. *Aspergillus niger* had the highest percentage of occurrence (30.11%) and the least was *A. fumigatus* (6.45%). A significant highest was observed in Bida (27.48%) while Shiroro (7.27%) was the least. The colonies were identified based on color, morphology, and shape on PDA Table 1 and Figure 1 respectively. The fungi isolate obtained from this study is in line with the work of Pedro *et al.* (2022) who isolated eight fungus species from stored groundnut seed in Nasarawa State, Tobin-west *et al*. (2018) who isolated *Aspergillus spp., Mucor spp., Rhizopus spp., Penicillium spp.*, and *Fusarium spp.*, from raw groundnut in River State, while Musa *et al*. (2022) who isolated *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp. *Mucor* spp. and *Aspergillus fumigatus* in groundnut cakes. However, the differences observed among the sampled location could be due to geographical location, method of storage and climatic conditions (such as temperature, rainfall) as they occupy different geographical positions as reported by El-Maghraby *et al*. (2020).

The presence of *Aspergillus flavus, A. Parasiticus, Fusarium* and *Penicillium* from this study could imply that sample is likely to contaminated with mycotoxin as they have been reported to produce toxins (Aflatoxin, Fumonism and Ochratoxin) this corroborates the findings of El-Maghraby *et al*. (2020), Also Isalar *et al*. (2012) who revealed six fungal

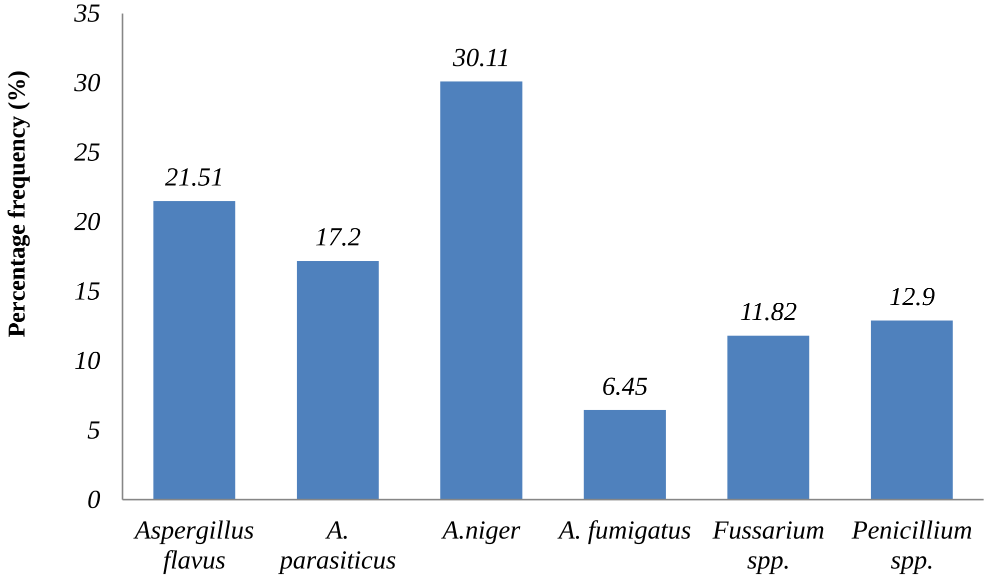

Figure 1. Percentage and frequency of fungi isolated Peanut seeds samples collected in Niger state.

Table 1. Percentage frequency of fungi based on Sampled location

Location	Peanut Seeds
Bida	27.48±0.48^{f}
Mokwa	15.03±0.03^{c}
Minna	10.38±0.38^{b}
Shiroro	7.27±0.27^{a}
Kotangora	22.43±0.15^{e}
Rafi	16.07±0.07^{d}

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at P < 0.05 as tested by DMRT

organisms viz *Aspergillus tamarii, Lasiodiplodia iranensis, Macrophomina phaseolina*, *Penicillium citrinum*, *Aspergillus oryzae*, and *Aspergillus pennicillioides* of which *Penicillium citrinum* produce mycotoxin.

Proximate composition of Peanut seed samples

The moisture contents of the seed samples obtained from this study ranged from (2.80 – 4.46%). A significant difference (P < 0.05) was observed between samples from Shiroro (4.64%) and samples from Minna (2.46%), Rafi (2.80%), and Bida (3.05%). The moisture content (3 %) recorded from this study corroborates with the findings of Odeniyi *et al.* (2019) who reported similar finding in groundnut seeds samples in northern part of Nigeria. The lower moisture content recorded from this study is good for shelf-life free from short time deterioration by fungi this is in line with Remi (2023) who reported that high moisture content could lead to short time deterioration. However, the difference observe between the sample location maybe attributed to method of processing, sanitary of the marked and materials used for storage as reported by Vabi *et al*. (2020) that attributed the deterioration of groundnut seed to high moisture and fat contents.

Ash content significant highest were obtained from Shiroro samples (3.07%) while Rafi (2.02%) recorded the least. There were no significant differences (P < 0.05) in ash between samples from Bida (2.97%), Mokwa (2.90%), Minna (2.93%), and Shiroro (3.07%), while significant difference (P < 0.05) was observed between samples from Rafi (2.02%) and other sample locations respectively this is in line with Joseph *et al*. (2023) (3%) who reported low ash content of raw, roasted groundnut seed.

Crude fat obtained from this study showed that samples from Mokwa (47.24%) obtained the highest, while Shiroro (41.61%) obtained the least. A significant difference (P < 0.05) was observed within the sampled locations .The reduction in fat content is in agreement with the work of (Udo *et al*., 2021) who reported the fat content of raw, boiled, and fried groundnut seeds to be 39.32%, 32.76%, and 46.36%. Crude protein obtained from this study ranged from (18.48% – 22.15%). There were no significant difference (P < 0.05) in crude protein between samples from Bida (20.18%) and Mokwa (20.89%). A significant difference (P < 0.05) was observed between Rafi (25.96%) and Bida, Mokwa, Minna, Shiroro and Kotangora respectively. The protein content is in line with Joseph *et al*. (2023) who reported similar range (19-23%) in raw, roasted and fried groundnut seed. In addition, it was observed that the

Table 2. Proximate composition of Peanut seed samples in Niger State

Sample	Moisture	Ash	Crude Fat	Crude Protein	Crude Fibre	Carbohydrate
Bida	3.05±0.07[b]	2.97±0.01[c]	45.95±0.08[c]	20.18±0.04[b]	1.96±0.00[c]	25.90±0.06[b]
Mokwa	3.78±0.22[c]	2.90±0.10[c]	47.24±0.44[c]	20.89±0.67[b]	1.91±0.05[c]	26.35±0.50[b]
Minna	2.46±0.02[a]	2.93±0.05[c]	46.30±0.02[c]	22.15±0.17[c]	1.93±0.04[c]	24.24±0.09[a]
Shiroro	4.64±0.09[d]	3.07±0.06[c]	41.61±0.72[a]	19.40±0.04[a]	2.02±0.04[c]	29.27±0.50[c]
Kotangora	3.95±0.02[c]	2.69±0.07[b]	43.93±0.31[b]	18.48±0.09[a]	1.78±0.05[b]	29.18±0.08[c]
Rafi	2.80±0.04[a]	2.02±0.01[a]	44.53±0.02[b]	25.96±0.39[d]	1.33±0.01[a]	23.37±0.31[a]

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at $P<0.05$ as tested by DMRT.

result reported was almost the same as the result reported by Udo *et al*. (2021) for raw, boiled, and fried groundnut seeds (20.38%, 23.86%, and 25.64%). Carbohydrate content, samples from Shiroro (2.02% and 29.27%) obtained the highest while, samples from Rafi (1.33%, and 23.37%) were least. For carbohydrate significant highest was obtained from Shiroro (29.18%) samples, while samples from Rafi (23.37%) obtained the least (Table 2).

The Carbohydrate content obtained in this present study corroborate the findings of Odeniyi *et al.* (2019) who all reported similar findings (25 %) in groundnut seed sold in Kaduna State. also Joseph *et al*. (2023) who reported similar finding (20-30%) for groundnut seed and its products in raw and roasted groundnut. This result agrees with the result obtained by (Udo *et al*. 2021) who reported the highest carbohydrate content (30%) in boiled groundnut.

CONCLUSION AND RECOMMENDATION

The result indicates that the widely distributed fungi-affecting peanut from the sample locations were mostly post-harvest pathogens, of which *Aspergillus* species were predominant. Majorities of fungi isolated from this research were mostly toxigenic moulds producing mycotoxins and may have direct impact on its nutritional contents. Therefore, improved management such as the use bio fumigated (Jute and Polypropylene bag) of these oil-rich seed will enhance the high-quality product and reduce the risk of health challenges that go with consuming contaminated peanut products.

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