COMPARATIVE STUDIES OF TOXIGENIC MYCOFLORA, PROXIMATE AND AFLATOXINS CONTENTS OF GROUNDNUT (ARACHIS HYPOGAEA L.) SEEDS AND CAKE IN NIGER STATE, NIGERIA

BY

MUSA, Muhammad Liman
MTech/SLS/2018/8228

DEPARTMENT OF PLANT BIOLOGY
SCHOOL OF LIFE SCIENCES
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA
NIGERIA.

FEBRUARY, 2022

COMPARATIVE STUDIES OF TOXIGENIC MYCOFLORA, PROXIMATE AND AFLATOXIN CONTENTS OF GROUNDNUT (ARACHIS HYPOGAEA L.) SEEDS AND CAKE IN NIGER STATE, NIGERIA

BY

MUSA, Muhammad Liman
MTech/SLS/2018/8228

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF TECHNOLOGY (M.TECH) DEGREE IN BOTANY (MYCOLOGY)

FEBRUARY, 2022

ABSTRACT

Despite economic importance of groundnut, it is vulnerable to contaminations by toxigenic mycoflora. Therefore, this study investigated the associated toxigenic mycoflora, proximate composition and aflatoxin contents of groundnut seeds and cakes (*Arachis hypogaea* L.) sold in ten (10) markets of Niger State, Nigeria. Eighteen (18) samples of groundnuts seeds and eighteen (18) samples of groundnut cakes were collected across each of the three agricultural zones of Niger State, namely Bida and Mokwa (zone 1), Minna and Shiroro (zone 2), Kotongora and Rafi (zone 3). Isolation of fungal species was done using dilution of 10^4 factor on PDA. Proximate composition and Aflatoxin content analyses were done following standard procedures. Ninety-three 93 isolates were obtained from groundnut seeds while 166 isolates from groundnut cake and were characterized into five genera: *Aspergillus, Fussarium, Penicillium, Rhizopus*, and *Alternaria*. In groundnut seeds, significant difference ($p < 0.05$) was observed in fungal isolates across the study areas. Significantly, highest isolates were obtained in samples from Bida (27.48 %) while samples from Shiroro (7.27 %) was the least. In groundnut cakes, significantly highest isolates was obtained in samples from Mokwa (25.20 %), while samples from Shiroro (8.33 %) was the least. Significant differences were observed in the proximate composition of groundnut seeds and cakes across all the study areas. The groundnut seeds had the highest moisture contents, crude fat, protein and carbohydrate in Shiroro (4.64 %), Mokwa (47.24 %), Kotangora (22.15 %) and Shiroro (29.27 %) respectively, while in the same order the least percentage was obtained from Minna (2.46 %), Shiroro (41.61 %), Minna (18.48 %), and Rafi (23.37 %) respectively. In groundnut cakes the highest moisture contents, crude fat, protein and carbohydrate in Shiroro (8.89 %), Bida (35.48 %), Minna (34.39 %), and Rafi (43.97 %) respectively, while in the same order the least percentage was obtained from Rafi (3.00 %), Kotangora (16.48 %), Rafi (21.02 %), and Bida (21.16 %) respectively. Correlation results indicated that the moisture content of groundnut cakes showed positive correlation with the protein and carbohydrate content of groundnut seed (5 %). From groundnut seed samples, the highest percentage of Aflatoxin (AfB1) Bida (77.65 µg/kg) and least in samples from Kotangora (1.57 µg/kg). In groundnut cakes samples, the highest (AfB1) (24.43 µg/kg) was obtained in samples from Bida while samples from Minna (9.67 µg/kg) were the least. The results indicate that fungi isolated from this study were toxigenic mould, and AfB1 content of the samples were above the tolerable limits. Therefore, improved management of groundnut seeds is essential in order to ensure a high quality product that will in turn reduce the health challenges associated with consuming contaminated groundnut products.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
EAC	East Africa Community
EU	European Union
FDA	Food and Drugs Authority
FAO	Food and Agriculture Organization
GPS	Global Positioning System
HCC	Hepatocellular Carcinoma
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometer
µg/Kg	Microgram per Kilogram (ppm)
Mg/Kg	Milligram per Kilogram (ppb)
MTL	Maximum Tolerable Limits
MT	Metric Tones
SPSS	Statistical Package for Social Science
SE	Standard Error
USA	United States of America
USAID	United States Agency for International Development
UV	Ultra Violet
SON	Standard Organization of Nigeria
NAFDAC	National Agency for Food and Drug Administration and Control

PPB	Part per billion
AOAC	Association of Official Analytical Chemists
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
IARC	International Agency for Research on Cancer
NACGRAB	National Centre for Genetic Resources and Biotechnology
NAERLS	National Agricultural Extension and Research Liaison Services
NBS	National Bureau of Statistics
USDA	United States Department of Agriculture
PDA	Potato Dextrose Agar
HPLC	High Performance Liquid Chromatography
%	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Groundnut (*Arachis hypogaea* L.), is also called peanut, monkey nut, earthnut, and goobers, is a self-pollinated allotetraploid leguminous crop belonging to family Fabaceae (Janila *et al*., 2013). Groundnut seeds are a rich source of 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 various industrial uses including products such as food, feed, paints, lubricants, and insecticides (Variath and Janila, 2017).

Groundnut cake (Kuli-kuli) constitutes an inexpensive source of protein, fat, minerals, and vitamins in the foods of rural communities, mostly children (Adjou *et al*., 2012). Groundnut cake (Kulikuli) is one of the most important foods supplements in the diet of the population, especially in rural areas (Honfo *et al*., 2010). It is made from groundnut after oil extraction. In the past, the crop was exported from Nigeria, but nowadays it is consumed locally and also used in the preparation of food (groundnut cake) and for oil extraction (Honfo *et al*., 2010). Groundnut cake plays vital role in the elimination of malnutrition in some African countries (Guimon and Guimon, 2012). Many reports have shown that the product is rich in protein and crude fat similar to its parent material, groundnut (Oladimeji and Kolapo, 2008).

However, despite the economic importance of groundnut products it is vulnerable to contamination by fungi such as *Aspergillus*, *Fussarium*, and *Penicillium* that secrete toxins (Sultan and Magan, 2010). The qualitative loss of groundnuts could be due to fungi contamination that may result in biochemical changes in protein, carbohydrates,

fatty acids, and vitamins (Waliyar *et al*., 2016). Aflatoxins are known to be the most carcinogenic among all of the mycotoxins (Singh *et al.*, 2018). Aflatoxins, of which the most important isomers are AFB1, AFB 2, AFG 1 and AFG2, are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (Do and Choi, 2007). International cancer studies classified aflatoxin B1 as the most toxic group 1 carcinogen (International Agency for Research on Cancer (IARC), 2010). Therefore, exposure to fungal contamination can cause serious health conditions such as cancer and liver cirrhosis, weakened immune systems (Wu and Khlangwiset, 2010).

1.2 Statement of the Research Problem

Over the years poor handling of groundnut and its products have been responsible for contaminations leading to varying health challenges. Due to improper production methods and inadequate storage facilities, they are exposed to air which results in fungal contamination and secretion of Mycotoxins (aflatoxin). Fungal contamination may lead to biochemical changes in fatty acids, protein, vitamins, and carbohydrates causing qualitative value loss of the products as well health challenge (Waliyar *et al*., 2016). The downside of groundnut consumption, however, is that the product is highly susceptible to the growth of mycotoxigenic fungi and hence it is prone to aflatoxin contamination (Liang *et al*., 2006). These toxins have received much attention due to the losses they cause in some food crops and their adverse effects on human and animal health (Negedu *et al*., 2011).

In 1990, Akano and Atanda reported the presence of toxigenic mycoflora and aflatoxins in groundnut cakes (Kuli-kuli) from Ibadan, Oyo State, Nigeria after the incidence of deaths resulting from consumption of aflatoxin-contaminated foods in Nigeria. Aflatoxins are regarded as the most carcinogenic among all of the Mycotoxins (Singh *et al.*, 2018), they pose potential threats to human and animal health. Thus, it affects

groundnut trades resulting in great financial loss. There is limited information about Aflatoxin contamination on groundnut products in the study area.

Hence, this research is aimed at comparing toxigenic mycoflora, proximate, and aflatoxin content of groundnut seeds and cakes (Kuli-kuli), sold in Niger State.

1.3 Aim and Objectives of the Study

The aim of this research is to compare toxigenic mycoflora, proximate, and aflatoxin content of groundnut (*Arachis hypogaea* L.) seeds and cakes (Kuli-kuli), in Niger State.

Objectives of the study are to;

i. isolate and identify toxigenic mycoflora associated with groundnuts seed and cake in samples collected from Niger state
ii. determine proximate composition of groundnut seed and cake samples collected from Niger state
iii. determine aflatoxin contents associated with groundnut seed and cake samples collected from the markets in Niger state
iv. determine the effect of fungal contamination on the nutritional composition of groundnut seed and cake samples collected from the markets in Niger state.

1.4 Justification for the Study

Despite groundnut cakes being one the cheapest and common sources of proteins that are readily available to both human and animal consumption, this protein-rich product is affected by aflatoxin, which is caused by toxigenic fungi contamination. However, groundnut cake (Kuli-kuli) is consumed by people of all social classes across the country, but only very few data are currently available on this food product safety in terms of aflatoxin contents. Interestingly, the available data focused on the nutritional attributes and functional characteristics of the products (Ezekiel *et al.*, 2011). The scarcity of data in regards to the aflatoxin contents of groundnut cake (Kuli-kuli) across Nigeria may be due to fact that the product is believed to be mostly consumed by the low-income populace and therefore not seen as a major food product.

Thus, Isolation and identification of the fungal species responsible for contamination of groundnut and its products will help in understanding the level of Aflatoxins present, in bid to enhance human health and secure food safety as well as public health enlightenment to the carcinogenic toxin. Therefore, there is a need to evaluate toxigenic fungal strains and aflatoxins contents of groundnut seeds and cakes (Kuli-kuli).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of Groundnut (*Arachis hypogaea* L.*)*

Groundnut is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft). As a legume, it belongs to the botanical family Fabaceae (also known as Leguminosae, and commonly known as the bean or pea family). Just like most other legumes, groundnuts harbor symbiotic nitrogen-fixing bacteria in their root nodules. The leaves are opposite and pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to 2¾ in) long and 1 to 3 cm (⅜ to 1 in) across, the leaves are nyctinastic; that is, they exhibit "sleep" movements, closing at night. The flowers are 1.0 to 1.5 cm (0.4 to 0.6 in) across, and yellowish-orange with reddish veining (Baughman *et al.*, 2015).

Groundnut are borne in axillary clusters on the stems above ground and last for just one day. The ovary is located at the base of what appears to be the flower stem but is a highly elongated floral cup. Groundnut pods develop underground, an unusual feature known as geocarpy. After fertilization, a short stalk at the base of the ovary (termed a pedicel) elongates to form a thread-like structure known as a peg. This peg grows down into the soil, and the tip, which contains the ovary, develops into a mature groundnut pod. Pods are 3 to 7 cm (1.2 to 2.8 inches) long, normally containing one to four seeds (Baughman *et al.*, 2015).

Groundnut is an annual crop and can either be an erect shrubby plant, 45 – 60 cm (18 – 24 inches) high with short branches or have a spreading form, 30 – 45 cm (12–18 inches) high with long branches that lie close to the soil. The stems are sturdy, hairy and bear pinnately compound leaves with two pairs of leaflets. The flowers are borne in the

axils of the leaves and have golden-yellow petals about 10 mm (0.4 inches) across. The oblong pods have rounded ends and are most commonly 25 – 50 mm (1 – 2 inches) long with two or three seeds; the pods are contracted between the seeds and have a thin, netted, spongy shell. The seeds vary from oblong to nearly round and have a papery seed coat that ranges in color from whitish to dark purple (Baughman *et al.,* 2015).

Groundnut crop is made up of five parts which are the Shell that is the outer covering, in contact with dirt, the Cotyledons (two) which is the main edible part, the Seed coat which is the brown paper-like covering of the edible part, the Radicle which is the embryonic root at the bottom of the cotyledon, which can be snapped off and the Plumule which is embryonic shoot emerging from the top of the radicle (Baughman *et al.,* 2015).

2.2 Groundnut Production

Nigeria remain the highest groundnut-producing country in West Africa, accounting for 51 % of production in the region and contributes (3,028,571 m.t and 1,130.1 kg ha^{-1}), 10 % of total global production and 39 % of that of Africa (Food and Agriculture Organization [FAO], 202). World production of groundnut is (44,041,913 m.t and 29,596,969 kg ha^{-1}) with China (16,685,915 m.t and 3,674.1 kg ha^{-1}), India (6.857.000 m.t and 1,182.2 kg ha^{-1}), Nigeria (3,028,571 m.t and 1,130.1 kg ha^{-1}), USA (2.578,500 m.t. and 4.118,6 kg ha^{-1}) and Sudan (1,826,000 m.t. and 788.8 kg ha^{-1}) are the highest groundnut-producing countries in the world (FAO, 2021). Groundnut is grown in nearly 100 countries on six continents between 40 ^{o}N and S of the equator on nearly 24.6 m ha, with a production of 44,041,913 m.t. and productivity of 1676 kg ha-1 during 2020 as shown in Table 2.1 below (FAO, 2021).

Table 2.1: The World Major Groundnut Producing Countries

S/N	Country	Production (tons)	Yield (kg/ha-1)
1	China	16,685,915	3,674.1
2	India	6,857,000	1,182.2
3	Nigeria	3,028,571	1,130.1
4	USA	2,578,500	4,118.6
5	Sudan	1,826,000	788.8
6	Myanmar	1,572,407	1,589.6
7	Chad	1,040,077	1,070.8
8	Argentina	1,001,113	2,928.6
9	Cameroon	747,677	1,647.5
10	Senegal	719,000	817

Source; (FAO, 2021).

However, the productivity of Asia and Africa (2217 $kgha^{-1}$ and 929 $kgha^{-1}$) is low when compared to the Americas (3632 $kgha-^{1}$) this may be due pest infestation, inadequate storage facilities, and poor farm managements (FAO, 2021). Asia, with (11.6 m ha) (47.15 %), and Africa, with (11.7 million ha) (47.56 %), hold the maximum global area under groundnut. Developing countries in Asia, Africa, and South America account for over (97 %) of world groundnut area and 95 % of total production (FAO, 2021).

Groundnuts grow best in light, sandy loam soil with a pH of 5.9 – 7.0, and their ability to fix nitrogen implies that, as long as they nodulate correctly, nitrogen-containing fertilizer enhances soil fertility little or not at all. Hence, they are valuable in crop rotations. Also, the yield of the groundnut crop itself is increased in rotations, through reduced diseases, pests, and weeds. For example, in Texas, groundnuts in a three-year rotation with corn yield 50 % more than non-rotated groundnuts (Baughman *et al.*, 2015). Adequate levels of phosphorus, potassium, calcium, magnesium, and

micronutrients are also necessary for good yields (Baughman *et al.*, 2015). To develop well, groundnuts need warm weather throughout the growing season. They can be grown with as little as 350 mm (14 inches) of water, but for best yields need at least 500 mm (20 inches) (Jauron, 2011).

Groundnut is grown in (31 of the 37) states and FCT. Kano and Niger states account for about 19.6 % and 10.7 %, respectively, followed by Kaduna, Benue, Zamfara, Taraba, Bauchi, Borno, Katsina and Nasarawa as shown in Table 2.2.

Table 2.2: Groundnut Trend in Top Ten Producing States in Nigeria.

S/N	States	Production (tons)	Yield (kg/ha-1)
1	Kano	331,000	728
2	Niger	316,000	1365
3	Benue	335,000	1650
4	Zamfara	121,000	839
5	Taraba	177,000	1237
6	Bauchi	134,000	955
7	Borno	226,000	2067
8	Kastina	56,000	529
9	Nassarawa	77,000	1153
10	Others	443,000	580

Source: Calculated from NBS (NBS, 2018).

These top 10 producing states account for nearly 80 % of the total area of groundnut cultivation Nigeria. Some states achieved substantial increases by increasing land area for groundnut cultivation; examples Taraba, Borno, Katsina, Kaduna, and Bauchi; groundnut area for Kano, Zamfara and Niger showed declines ranging from (4.41 % to 2.27 %) (FAO, 2021). Traditional commercial groundnut-producing areas in Nigeria encompass the Sahel, Sudan and derived savanna, Northern Guinea, and most parts of the Southern Guinea vegetation zone. The major groundnut producing states are Kano,

Katsina, Kaduna, Jigawa, Sokoto, Zamfara, and Kebbi in the Northwest; Adamawa, Bauchi, Yobe, and Borno in the Northeast; and Benue, Plateau, Taraba, Nasarawa, FCT Abuja, Kogi, Niger and Kwara in the Central Zone (Ndjeunga *et al.,* 2010). Areas under groundnut cultivation and total production have shown marked increases during the period 1997– 2016 in Africa (FAO, 2020).

Nigeria recorded the top yield levels > 1000 kg/ha across the same years. In general, increased groundnut production in Africa emanated from the expansion of agricultural lands. Some reports indicated that groundnut yields of (1,700 – 2,500 kg per/ha) can be realized using elite/ improved varieties in Africa despite that farmers yet continue cultivating unimproved local varieties (Kebede and Tana, 2014).

Famer varietal choice of selection is regarded to be important means of enhancing and obtaining developed seed and boost adoption rate of advanced varieties in Africa (Monyo and Varshney, 2016). Despite the numerous benefits and roles, groundnut play at individual to the national level in Nigeria, pod yield from farmers' fields has remained low averaging 1082 $kgha^{-1}$ compared to (3000 $kgha^{-1}$ and 3500 $kgha^{-1}$) potential yield and those from developed countries respectively (Ndjeunga *et al.,* 2010). The large gap has been attributed to several factors such as poor soil fertility, continued use of poor yielding indigenous varieties, inappropriate crop management practices, pests, and diseases (Zekeri and Tijjani, 2013).

In most of the developing countries, the productivity levels are lower than in the United States of America, mainly due to a number of production constraints such as i.) the cultivation of the crop on marginal lands under rainfed conditions; ii.) Occurrence of frequent drought stress due to vagaries of monsoon; and iii.) higher incidence of disease

and pest attacks; iv.) low input-use and v.) factors related to socio-economic infrastructure (Ndjeunga *et al.,* 2010).

2.2.1 Constraints of groundnut production

Groundnut improves soil fertility through nitrogen fixation, thereby increasing the productivity of other crops when used in rotation or in a cereal cropping system. The poor productivity of groundnut cultivation in African countries may be attributed to a combination of factors such as unreliable rains, mostly non-irrigated nature of cultivation, traditional small-scale farming with little mechanization, outbreaks of pests and diseases, use of low-yielding varieties, increased and/or continued cultivation on marginal land, poor adoption of agronomic practices and limited extension services (Ajeigbe *et al*., 2014). Groundnut is one of the poorly stored foods. Storing seeds after harvest till the next cropping season without impairing the quality is of prime importance for successful seed production. Being an oilseed crop groundnut seed has a short life and loses viability quickly under ambient conditions (Ndjeunga *et al.,* 2013).

Aging in groundnut seed leads to increased lipid peroxidation, decreased activities of several free radical and peroxide scavenging enzymes (Rao *et al*., 2006). Groundnut seeds are more sensitive to storage conditions like high temperature; high seed moisture content and light exposure. The qualitative loss of seed can be attributed to biochemical changes in protein, carbohydrates, fatty acids, and vitamins (Waliyar *et al*., 2016). Oxidation of the lipid fraction of groundnut product is a major cause of deterioration in fatty groundnuts due to the high degree of fatty acid instauration (Talcott *et al*., 2005). Polyunsaturated fatty acids, specially linoleic and linoleic acid, are very susceptible to oxidation even under mild ambient conditions and are easily incorporated into the chain mechanism of lipid peroxidation, to yield free and peroxy radicals (Talcott *et al*., 2005).

Lipid oxidation is usually implicated as a primary cause of a decreased shelf life, adverse tastes loss of nutrients and generation of undesirable aromas during extended storage of peanut meals (Reed *et al*., 2002). In addition, groundnut tends to be contaminated with aflatoxin due to fungal growth. It is important to develop preservation methods for the peanut meal. Although recently a research successfully claimed for minimizing oil migration through coating with protein based isolate but its contribution to the overall long term preservation of peanut was found questionable also the effect of coating on further processing needs to be evaluated (Han *et al*., 2009). Aflatoxin contamination of groundnut prevents groundnut producers from accessing bigger western markets, increases dependency on foreign food aid, stifles economic opportunities, and adversely affects consumer health. According to FAO, developing countries account for approximately (95 %) of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of aflatoxin contamination (FAO, 2020).

Aflatoxin contamination and associated fungi in groundnut continue to attract worldwide attention and have been reported from various countries. Aflatoxin contamination can occur on pods and seeds in the soil near harvest, during harvest, and post-harvest in storage. Aflatoxin contaminates a vast array of food and agricultural commodities such as cereals, nuts, dried fruits, coffee, cocoa, spices oil seeds, dried peas, and beans and fruit (Reddy *et al*., 2011). *Aspergillus* species can grow on a variety of substrates under different environmental conditions (Reddy *et al*., 2011). Oilseeds are the most widely distributed food crops in the world and their contamination by these seed-borne fungi can lead to mycotoxin accumulation during the stages of growing, harvesting, storage, transporting, and processing. Aflatoxin contamination of foods and

feeds has gained global importance because of its deleterious effects on human as well as animal health (Okoli *et al*., 2006).

2.3 Harvest of Groundnut Seeds

Groundnuts plants often continue to produce flowers when pods are developing, despite that even they are ready for harvest some pods are immature. The timing of harvest is an important decision to ensure maximum yield. If it is too early, too many pods will be unripe. If too late many pods will snap off at the stalk and will remain in the soil. For harvesting, the entire plant, which includes most of the root, is removed from the soil. The fruit wrinkled shells are constricted between pairs of one to four (usually two) seeds per pod (Marsalis *et al.,* 2009). Groundnut is usually harvested and stored dry in different storage facilities, traditional and modern. Under such storage conditions, groundnuts are susceptible to attack by fungi, insects and other microorganisms (Aliyu and Kutama, 2007). Harvesting can occur in two stages: In mechanized systems, where a machine is used to cut off the main root of the groundnut plants by cutting through the soil just below the level of the groundnut pods (Marsalis *et al.,* 2009).

The machine lifts the "bush" from the ground and shakes it, then inverts the bush, leaving the plants upside down on the ground to keep the groundnut out of the soil. This will allow groundnut to dry slowly to a little less than a third of their original moisture level for three to four days. The second stage is the use of hand to pull groundnut from the soil and inverted by hand (Marsalis *et al.,* 2009). Depending on growing conditions and the cultivar of groundnut, harvest is usually 90 to 130 days after planting for subspecies *Arachis hypogaea.* fastigiate types, and 120 to 150 days after planting for subspecies *Arachis hypogaea* types. (Marsalis *et al.,* 2009). Subspecies *Arachis hypogaea* types yield more and are mostly preferred because the growing seasons take more time. The optimum air temperature for the growth and development of groundnut

is between 25 °C and 30 °C. Moreover, groundnut yield in the rainy season is lower than in the post-rainy season due to cloudy weather and the presence of diseases and insect pests (Marsalis *et al.*, 2009).

2.4 Groundnut Products

Groundnut consumption all over the world varies in large proportions hence the commercial products too are variant and generally localized. Groundnuts has been developed into the variety of products like roasted groundnuts, groundnut butter, groundnut oil, groundnut paste, groundnut sauce, groundnut flour, groundnut milk, groundnut beverage, groundnut snacks (salted and sweet bars) and groundnut cheese analog. Raw groundnuts are consumed all over the world. Roasted groundnuts are processed by heating the groundnuts up to temperature of 180 °C for around 12 - 15 minutes or at 160 °C for 40 - 60 minutes depending on the moisture content (Arya *et al*., 2015).

The influence of boiling, roasting and frying on the digestion of groundnuts in simulated gastric environment was studied and the results show that processing improved the gastric disintegration of groundnuts, and the disintegration rate was in an order of fried > roasted > boiled > raw groundnuts (Kang *et al*., 2017). Groundnut oil is obtained by different extraction methodologies and is mainly consumed in the Asian subcontinent especially India. Maximum amount of the groundnut production around the world is utilized for oil production. The world production of groundnut oil has risen from 4.53 million metric tons in 2000 to 4.91 in 2010. Production across the countries of the world, where China (44 %), Indian (20 %), and Nigeria (11 %) are the largest producers, is expected to account for almost (75 %) of the world's groundnut oil (United States Department of Agriculture-Foreign Agricultural Service [USDA-FAS], 2011).

The snacks of groundnut (salted) are widely enjoyed throughout Asia, especially in India. This is typically made by frying and coating the kernel of peanut (Kang *et al*., 2017), groundnut meal used in various dishes such as soup, biscuits and curries due to its emulsifying characteristics and as composite meal after extraction of oil is usually made by grinding the degraded groundnut meal (Ndjeunga *et al.*, 2013). It is used for meat coating. Peanut flour may be used to make composite meals with non-white cereal products as well as for the addition of protein-rich sources to its flour, such as legume meals, particularly in areas with inadequate wheat output (Stefano *et al*., 2011).

2.4.1 Groundnut cake (kulikuli)

Groundnut cake (Kuli-kuli) is one of the most important foods supplement in the diet of the population, especially in rural areas. It is made from groundnut after oil extraction. Previously, groundnut cakes (Kuli-kuli) were exported from northern part to the western part of Nigeria, but nowadays it is consumed both Northern and South west part of Nigeria and also used in the food supplements for oil extraction (Honfo *et al*., 2010). It has been reported to be rich in protein and crude fat similar to its parent material, groundnut (Oladimeji and Kolapo, 2008). Groundnut cake (Kuli-kuli) is an important source of food and constitutes an inexpensive source of protein, fat, minerals, and vitamins in the diets of rural populations, especially children (Adjou *et al*., 2012).

Groundnut kernels are often pressed to obtain groundnut oil, which is widely used in many rural and urban households of countries in West and Central Africa. In addition, groundnut and groundnut products are important snacks for travelers especially those on religious and tourist expeditions. The crop was useful in the elimination of malnutrition in some African countries (Guimon and Guimon, 2012). Although groundnut cake is consumed by humans across some West African states, only very few data are currently available on this food material in terms of its safety, nutritional status, and aflatoxin

content. Interestingly, the available data in Nigeria and have focused on the microbiological quality and nutritive value of groundnut cake.

2.4.2 Steps involved in production of groundnut cake (Kulikuli).

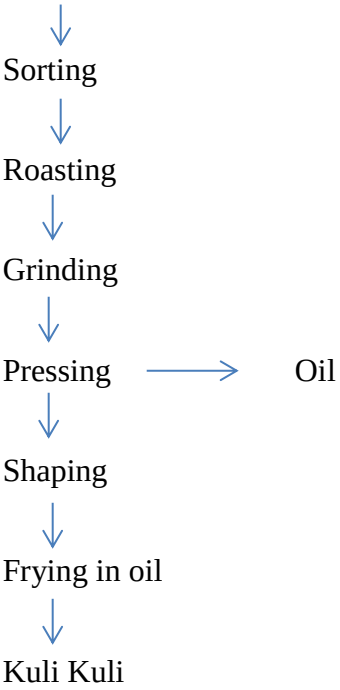


Figure 2.1. Processing of groundnut cake from groundnut seed in Niger state.

Source; (Adjou *et al*., 2012).

2.4.3 Economic importance of groundnut products

Agriculture remains a key component of the Nigerian economy and dominates the labor market by employing about half (48.19 %) of total workers (National Bureau of Statistics [NBS], 2018) and by the first quarter of 2016 contributes (19.17 %) to the country's Gross Domestic Product (NBS, 2018). It constitutes the single largest contributor to the wellbeing of the rural poor, sustaining 90 % of the rural labor force. In Nigeria, groundnut (*Arachis hypogaea* L.) is a major crop produced in almost all northern States including Kano, Niger, Jigawa, Sokoto, Katsina, Zamfara, Kaduna, Adamawa, Bauchi, Yobe, Plateau, Kebbi, Borno, Taraba, Gombe, and Nasarawa States

(National Agricultural Extension and Research Liaison Services [NAERLS], 2016). Groundnut also provides multiple benefits to smallholder farmers growing the crop. It serves as an inexpensive source of protein to families who cannot afford the more expensive animal-based diets (Rachier, 2005).

Varieties of contaminants are found naturally occurring in foods of these, mycotoxins are the major contaminants and 25 percent of foods are contaminated with mycotoxins. Among them aflatoxins are the major mycotoxins produced by toxigenic strains of *A. flavus and A. parasiticus* in the suitable environment (Reddy *et al*., 2011). Aflatoxins are the secondary metabolites produced by these fungi. Aflatoxins cause economic and trade problems at almost every stage of marketing of groundnut especially during export (Reddy *et al*., 2011).

Levels of mycotoxins acceptable in foods in developed countries have been lowered with the maximum limit for aflatoxin B1 in the European Union as 5 µg/kg and 10 µg/kg for the sum of Aflatoxin B1, B2, G1, and G2 in food (European Commission, 2010), while the limit set for total aflatoxin by United States Food and Drug Administration (FDA) is 20 µg/kg (FDA, 2011) and for East African Commission (EAC) the limit is 10 µg/kg (EAC, 2011). However, fungal contamination is the main problem in groundnut production. Fungi are the main spoilage agents both various plant pathogens and food. Fungal contamination caused plant infection not only seed contamination with mycotoxins but also results in a decrease in crop yield and significant economic losses of quality (Makun *et al.*, 2010).

Groundnuts are the main sources of human exposure to aflatoxin because it is immensely consumed worldwide (13.3 million tons of groundnuts were use up in 2001-2003 and expected consumption of 16.32 million tons in 2030) and unfortunately are the

most susceptible crop to aflatoxin contamination (Wu and Khlangwiset, 2010). For this reason, exposure to aflatoxin in groundnut represents a serious risk to the economy and health for many countries (Kumar *et al*., 2008; Guo *et al*., 2008).

2.4.4 Nutritional value of groundnut products

Groundnut is an important crop grown worldwide. Commercially it is used mainly for oil production but apart from oil, the by-products of groundnut contains many other functional compounds like proteins, fibers, polyphenols, antioxidants, vitamins and minerals which can be added as a functional ingredient into many processed foods (Arya *et al*., 2015). Groundnut is the 6th most important source of edible oil and the 3rd most important source of vegetable protein (Nigam, 2014). The chemical composition of groundnut per 100 g edible portion as reported by (United States Department of Agriculture [USDA], 2010) constitute moisture (6.5 g), carbohydrate (16.1 g), lipids (49.2 g), protein (25.8 g), dietary fibre (8.5 g), magnesium (168 mg), phosphorus (376 mg) and iron (4.6 mg). Groundnut kernels contain 40-50 % fat, 20-50 % protein, and 10-20 % carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, folacin, calcium, phosphorus, magnesium, zinc, iron, and potassium (USDA, 2010).

Protein, fats, and fiber are the major components that make up groundnut. All these components are present in their most beneficial forms. The protein is plant-based: the fat is unsaturated, and the fiber is complex carbohydrate, which are all proved the best for human nutrition (Arya *et al*., 2015). Groundnuts are essentially a legume and have a higher proportion of protein than any other nut than beans. The protein content of the cake might approach 50 percent after extraction of peanut oil (Zhu *et al.*, 2013). Groundnuts contain all 20 variable amino acids and are the main protein source named 'arginine' (USDA, 2010). Recently it has also revealed that groundnut are excellent source of compounds like resveratrol, phenolic acids, flavonoids and phytosterols that

block the absorption of cholesterol from diet (Arya *et al*., 2015). It is also a good source of Co-enzyme Q10 and contains all the 20 amino acids with highest amount of arginine. These bioactive compounds have been recognized for having disease preventive properties and are thought to promote longevity (Arya *et al*., 2015). The processing methods like roasting and boiling have shown increase in the concentration of these bioactive compounds.

Groundnut seeds are eaten raw, boiled, or roasted or used in the preparation of groundnut sauce mixed with onions, garlic, vegetables for vegetarians, for preparation of maafe (meat stew) in Mali, for preparation of nkate nkwan (groundnut butter soup) in Ghana, groundnut powder is also an important ingredient in the spicy coating of kebab in both Nigeria and Ghana; it is used in making biodiesel fuel, laxatives, dye, shampoo, insecticides, glue, and explosives. The extracted oil can be used for cooking, for margarine, vegetable ghee, salads, for deep-frying, for shortening in pastries and bread (Prasad *et al.*, 2009).

The cake produced from groundnut after extraction of oil can be used as a feed supplement for livestock, as fertilizer, and for the preparation of kuli-kuli (traditional recipe in Nigeria) (Olayinka *et al*., 2013). Groundnut can also be processed into Yaji (roasted meat pepper), Sisipelebe or Gudigudi, Donkwa, Kunungeda, groundnut chin-chin, kulikuli, roasted groundnut, boiled groundnut and groundnut soup (Obeepa-Yoruba, Nkatieenkuwn-Ibo, Miyanyakuwa-Hausa, Omiisagwe-Benin) (Olayinka *et al*., 2013). Groundnut has good digestibility in both raw and roasted forms of consumption and the energy value is generally slightly higher in the roasted form than the raw form. Ayoola and Adeyeye (2010) reported that groundnut seeds (raw, sun-dried, and roasted) for proximate composition and some nutritionally valuable minerals and found that the

roasted groundnut can be considered as a good source of valuable minerals, while the raw groundnut is a good source of protein with high nutrition value.

2.5 Diseases of Groundnut Plant and Deterioration of Groundnut Products

2.5.1 Diseases of groundnut plant

Groundnut is affected by several diseases, such as late leaf spot (*Phaeoisariopsis personate* Berk and Curt), early leaf spot (*Cercospora arachidicola* Hori), collar rot (*Aspergillus niger*), rust (*Puccinia arachidis* Speg), and bud necrosis (bud necrosis virus (BNV). Groundnut rosette disease causes more severe yield losses than any of the groundnut viral diseases in the region (Okello *et al*., 2010). Early and late leaf spots caused 100 % yield loss in Ghana (Gaikpa *et al*., 2015). Groundnut is attacked by 50 genera of fungal, 1 bacterial, 16 nematodes, 15 viruses and 2 phanerogamic parasites (Naikoo *et al*., 2013). *Aspergillus spp*. is the most important fungal pathogen of tropical as well as temperate countries (Elwakil and El-Metwally, 2001).

Groundnut is usually harvested and stored dry in different storage facilities, traditional and modern. Under such storage conditions, groundnuts are susceptible to attack by fungi, insects and other microorganisms (Aliyu and Kutama, 2007). The extent of deterioration depends on the condition of the groundnut. These conditions range from maturity of the crop in the field, completeness of shell to the type of storage facility used (Aliyu and Kutama, 2007). Most of the diseases of the groundnuts crop are caused by seed borne fungi that can easily survive in infected groundnut seeds (Magnoli *et al*., 2006).

Mycotoxigenic molds have direct economic losses by spoiling seed, which can result in lowered export earnings by most of the developing countries that cannot comply with the stricter lucrative markets' regulations (Hell *et al*., 2005). Commodities contaminated

with aflatoxins have a lower market value and often utilized locally since they cannot be exported (Hell *et al.,* 2005). At the farm level, animals fed with aflatoxin-contaminated seeds have lower productivity and slower growth resulting in serious economic problems (Ting, 2010). Aflatoxin contamination caused by the fungus *Aspergillus flavus* and *A. parasiticus* is an important biotic factors affecting groundnut products quality and sustainable groundnut production in Africa (Guchi, 2015; Njoroge *et al*., 2017). Groundnuts are frequently contaminated by the fungal species *Aspergillus flavus*, which can produce the aflatoxin, this infection can occur during transportation or storage of groundnut. Aflatoxins are highly toxic and carcinogenic secondary metabolites of concern in food safety (Achar *et al*., 2009).

Aflatoxins are a group of structurally related toxic polyketide-derived secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Waliyar *et al*., 2009). Groundnut infection and aflatoxin levels can be linked to soil stress during pod-filling when soil temperatures are nearly optimum for *A. flavus*. These links may form the basis of a decision-building system for predicting the risk of aflatoxin contamination in groundnut (Craufurd *et al*., 2006).

2.5.2 Deterioration of groundnut products

Aspergillus flavus is one of the important storage fungi producing aflatoxin, which have carcinogenic potentials on human and animals. Aflatoxin contamination is a major problem worldwide, which reduces the quality of food and feed especially in storage conditions (Hell and Mutegi, 2011). *A. flavus* is responsible for some common clinical syndromes such as granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infection, osteomyelitis following trauma and inoculation; it also acts as an agent of *otitis*, *keratitis*, pulmonary and systemic infections in immune-compromised patients (Hedayati *et al*., 2007).

Morphological identification of *Aspergillus* section Flavi is usually based on the microscopic structures, such as the uni- or biseriate conidial heads, production of dark-colored sclerotia by certain species, and yellow-green to brown shades conidia. *Aspergillus* section Flavi includes 33 species, and most of them are natural producers of aflatoxins (Frisvad *et al*., 2019). Members of this section can exist in the soil as sclerotia or conidia, or mycelia in plant tissue. Sclerotia of *A. flavus* and *A. parasiticus* (Horn *et al*., 2009) can also be produced naturally in crops by an asexual or sexual stage and are dispersed onto the soil during harvest. Sclerotia can survive under severe environmental conditions in the field and germinate into mycelia, followed by the formation of the conidiophores and conidia when the condition becomes favorable (Horn *et al*., 2014). Horn *et al*. (2016) have described the mechanism of A. flavus sexual reproduction in a natural environment, which includes the fertilization in soil and crops.

The exchange of genetic materials during sexual recombination results in the high genetic diversity in the *A. flavus* population. Thus, the morphology, mycotoxin production, and vegetative compatibility groups (VCGs) in *A. flavus* are more diverse as compared to other species in section Flavi. The normal process of cooking (Kumar *et al*., 2017) cannot destroy food products contaminated with aflatoxins. Enzymes of the liver can break down aflatoxins and the breakdown products intercalate DNA and cause genomic damage during cell division that causes cancer where these breakdown products accumulate in the liver to create liver cancer. It is also estimated that due to the harmful effect of aflatoxins, approximately 25 % of agricultural products are damaged worldwide (Yard *et al.*, 2013).

Optimum growth of *Aspergillus parasiticus* was analyzed at 35 °C in the ranges 17 – 42 °C temperature with varying combinations of 0.90 – 0.99 water activity (aw), that stimulate the regulatory genes' (aflR/aflS) expression levels and production of aflatoxin

in *A. flavus* and *A. parasiticus* (Schmidt-Heydt *et al*., 2010). *A. flavus* can survive at a temperature ranging from 12 °C to 48 °C (Hedayati *et al*., 2007). Water activity (aw) and optimum temperature have remarkable effects on species of *Aspergillus* and the production of aflatoxins (Sanchis and Magan, 2004). The growth and production of AFB1 of *A. flavus* decrease under the temperature to 37 °C during water stress. It was reported that growth of fungal biomass and AFB1 production was highest at 28 °C temperature and 0.96 water activity, while no prominent fungal growth and AFB1 production detected at 20 °C with the dried state condition at 0.90 and 0.93 water activity (Gallo *et al*., 2016; Kumar *et al*., 2017).

2.6 Aflatoxins Contamination

Aflatoxins are common contaminants of foods particularly in the staple diets of many developing countries. Aflatoxins are produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* as secondary metabolites when the temperatures are between 24 ^{0}C and 35 ^{0}C. They form in many commodities in conditions of excess moisture during harvest and storage. Aflatoxins are regarded by the United States Food and Drug Administration to be unavoidable contaminants of foods (FDA, 2011).

Aflatoxins are a group of closely related compounds with small differences in chemical composition. There are four main aflatoxins – B_1, B_2, G_1, and G_2, with aflatoxin B_1 being the most prevalent. To date, there are18 known analogs of aflatoxins with three series being significantly important from a food safety perspective: B-series (AFB1 and AFB2), G-series (AFG1 and AFG2), and M-series (AFM1 and AFM2). *A. flavus* and *A. parasiticus* are the major producers of aflatoxins, whereby the *A. flavus* produces B-series aflatoxins, while *A. parasiticus* produce both B- and G-series. The “B” and “G” refer to the blue and green fluorescence colors produced under UV light, while the

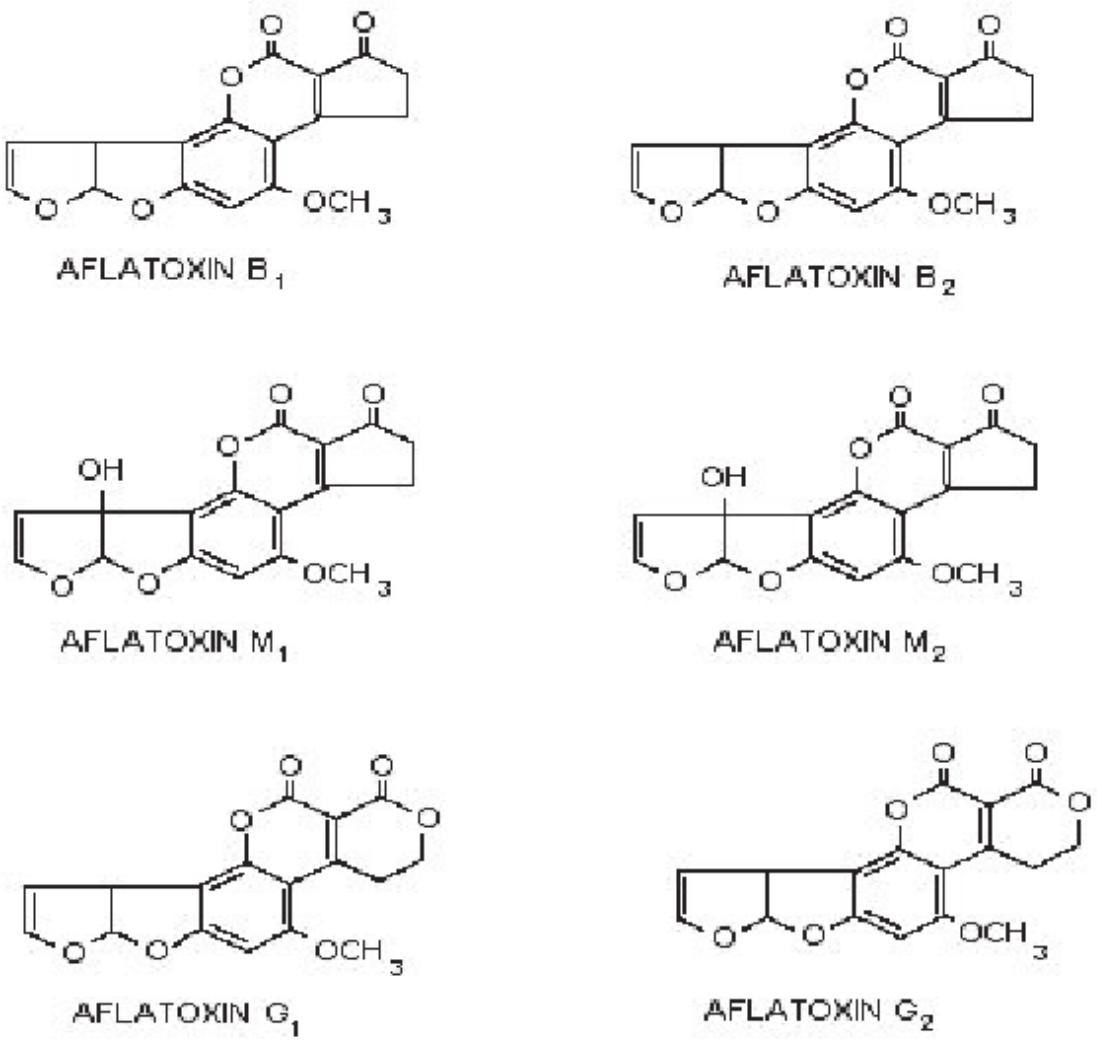
subscript numbers indicate major and minor compounds, respectively (Dhanasekaran *et al*., 2011).

AFB1 is classified as a Group 1 carcinogen by the IARC (2010) due to the sufficient evidence of its involvement in cancer development in humans. Upon ingestion of the contaminated feeds by the animals, AFB1 and AFB2 are then metabolized in the body, thereby causing milk produced by the animals to be contaminated with their hydroxylated derivatives known as AFM1 and AFM2 as shown in Table 2.3 (Dhanasekaran *et al*., 2011).

Table 2.3: Types and Sources of Aflatoxins

Type	**Source**
Aflatoxin B_1 and B_2	*Aspergillus flavus* and *A. parasiticus*
Aflatoxin G1 & G2	*Aspergillus parasiticus*
Aflatoxin M1	A metabolite of Aflatoxin B1 is found primarily in the milk of humans and animals

Source; (Dhanasekaran *et al*., 2011).



Figures 2.2: Chemical structure of the aflatoxins, (Source: FDA, 2011).

2.6.1 Aflatoxin contamination of groundnut products

Aflatoxins are mostly toxic secondary fungal metabolites that are derived from certain strains of fungi such as species of *Aspergillus*, specifically *Aspergillus flavus*, *Aspergillus parasiticus* (Kumar *et al*., 2008) and that can quickly absorb by blood cells in the human body if consume any aflatoxin-contaminated food. Aflatoxins are known as potent and harmful groups of mycotoxins. Aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2 are found mostly in nature and more than 20 types of aflatoxin are identified (Giray *et al*., 2007). The most important foods such as groundnuts, rice, wheat, dried fruit, maize, pearl millet, tree nuts (almonds, pecans, walnuts), black pepper, coriander,

turmeric, zinger cocoa beans, etc. are mostly contaminated by aflatoxins (Bbosa *et al*., 2013; Smith *et al*., 2015).

Mycotoxins contamination intensity in food crops varies geographically and groundnut is the main source of mycotoxins. Groundnut seed is predominantly infected with *Aspergillus flavus* and *Aspergillus niger* (Gebreselassie *et al.,* 2014). The reported outbreak s of aflatoxicosis in man was due to the consumption of aflatoxin-contaminated food and feed products (Reddy and Raghavender, 2007). The economic consequences of mycotoxin contamination are profound, as the crops contaminated with elevated levels of mycotoxin are often destroyed (Fakruddin *et al*., 2015).

Due to the potential health risks of aflatoxins, many countries have established maximum limits (MLs) or levels for key agricultural commodities including groundnut products. These regulations single out aflatoxin as the most regulated mycotoxins underlining public and private sector concerns for food safety in both developing and developed economies. For example, the MLs set by the EU vary between 2 - 12 µg/kg for AfB1 and 4 - 15 µg/kg for total aflatoxins (FAO, 2020). The MLs of the US Food and Drug Administration is 20 ppb (= 20 µg/kg) for total aflatoxins in all foods except milk (Bediako *et al*., 2019). The MLs set by the Standard Organization of Nigeria, (SON) (SON, 2006) for groundnut kernel are 20 and 4 µg/kg for Kuli-kuli - a by-product of groundnut.

However, in Africa, large proportions of groundnut and groundnut-based products contain aflatoxins exceeding MLs. Between 22 to 54 % of groundnut samples collected in Mali during the 2009 and 2010 cropping seasons, contained AfB1 contamination levels above 20 µg/kg (Waliyar *et al*., 2015). In Nigeria, between (30 to 90 %) of marketed and/or stored kernels of groundnuts were contaminated by aflatoxin of which

between (25 to 83 %) exceeded the Nigerian and EU MLs of 20 and 4 µg/kg, respectively (Ezekiel *et al*., 2012). Average aflatoxin concentrations were reported to be between 43 and 118 µg/kg for AfB1 respectively in Southwestern Nigeria (Ezekiel *et al*., 2012). Similar reports from the Kaduna and Port Harcourt cities in North and South Nigeria respectively, showed that between 14 and 25 % of groundnut kernel and groundnut-based products exceeded the US and Nigeria MLs of 20 µg/kg.

Aflatoxin contamination affects groundnut trade resulting in financial losses estimated at US dollars ($) 750 million per annum in Sub-Saharan Africa (Kamika and Takoy, 2011). Aflatoxin contamination of groundnut prevents groundnut producers from accessing larger western markets, increases dependency on foreign food aid, stifles economic opportunities, and adversely affects consumer health. According to FAO, developing countries account for approximately 95 % of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of aflatoxin contamination (FAO, 2020).

These types of fungi are commonly found in stored agricultural commodities such as groundnut, corn, millet, sesame seeds, sorghum, sunflower seeds, and different types of spice for keeping in improper storage. If this infected food (with aflatoxins containing fungi) is processed, then aflatoxins can enter the processed food, and that is harmful to human health and animals by affecting several problems. Children are frequently suffered from exposure to aflatoxins, which results in stunted growth, delayed growth and development (Voth-Gaeddert *et al*., 2018), liver damage, and finally liver cancer. Adults are capable to tolerate a higher level of aflatoxins exposure, but they should be conscious. The symptoms of severe aflatoxicosis include hemorrhagic necrosis of the liver, bile duct proliferation, edema, lethargy, and death observed (Kumar *et al*., 2008).

Aflatoxin contaminates a vast array of food and agricultural commodities such as groundnut, cereals, dried fruits, coffee, cocoa, spices oil seeds, dried peas, and beans and fruit (Turner *et al*., 2009; Reddy *et al*. 2011). *Aspergillus* species can grow on a variety of substrates under different environmental conditions (Reddy *et al*., 2011). Oilseeds are the most widely distributed food crops in the world and their contamination by these seed-borne fungi can lead to mycotoxin accumulation during the stages of growing, harvesting, storage, transporting, and processing (Okoli *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Total of Thirty-six (36) samples of groundnut seeds and cake, (50 g each) were collected from 10 markets in three major groundnut utilization areas cutting across all the agricultural zones of Niger State, namely; Bida-Mokwa (zone 1), Minna-Shiroro (Zone 2), Kotongora and Rafi (zone 3). The global positioning system (GPS) was used to take coordinates of sample locations.

Table 3.1: Location (Local Government Areas) where Samples were Collected in Niger State.

S/N	Local Government Areas (town)	Location coordinates
1	Bida	N9º.04’49.58 E6º.00’35.64
2	Mokwa	N9º17’41.35 E5º03’14.83
3	Minna	N9º.36’54.86 E6º.32’51.94
4	Shiroro (Kuta)	N9º.52’47.3 E6º.42’25.7
5	Kotangora	N10º.40’71.7 E5º.47’07.3
6	Rafi (Kagara)	N10º.24’98.5 E6º.24’88.9

3.2 Preparation of Media

3.2.1 Potato dextrose agar (PDA)

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

3.3 Isolation and Identification of Fungi Associated with Groundnut Seeds and Cakes

One gram (1 g) of pounded composite samples groundnut seeds and cakes each was aseptically suspended into 9 ml of sterile distilled water in a test tube and vortexed properly. 1ml was serially diluted up to the fourth fold 10^4. From the fourth dilution fold test tube 10^4, 1 ml was transferred into a sterile Petri plate. Twenty milliliters (20 ml) of Potato Dextrose Agar (PDA), to which 1ml of streptomycin was added, and then poured into the Petri dish 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 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. Subculture was made at regular intervals. The fungal isolate was identified using the fungal family of the world mycological monograph (Adebola and Amadi, 2012; Sarah *et al*., 2016). The percentage frequency of occurrence of the toxigenic mycoflora was obtained using the formula below, according to Chukunda *et al*. (2015).

$$\% \text{ frequency} = \frac{\text{samples containing fungi}}{\text{total number of samples assessed}} \times 100 \qquad \text{Equation (1)}$$

3.4 Proximate Composition of Groundnut Seeds and Groundnut Cakes

Proximate composition of groundnut seeds and cakes (KuliKuli) samples was carried out in triplicates to test the moisture content, fat, crude protein, ash, and carbohydrate percentages using Association of Official Analytical Chemist (AOAC, 2012) methods.

3.4.1 Determination of ash content

Ash content was determined using incineration at 600 °C in a muffle furnace, according to the method described by Opega *et al*. (2016). Two grams of each grounded sample were weighed into a crucible and ignited tarred crucible (w1). The crucible and weighed sample were placed on a hot plate inside a fume cupboard to prevent smoke accumulation, the remaining residue was transferred to a preheated muffle furnace and maintain at 600 °C for 6 hours until the sample is reduced to light ash, the crucible was removed, placed in the desiccators, cooled and weighed (w_2) and the ash content was calculated in equation (1) as follow:

$$\% \text{ ash } = \frac{W2-W1}{2.0 \text{ g}} \times 100 \qquad \text{Equation (2)}$$

3.4.2 Determination of fat content

Fat content was determined using the soxhlet extraction method according to Opega *et al*. (2016). Two grams (2 g) of the sample was weighed into a thimble (w1), a dry and cool boiling flask was weighed, filled with 300 mL petroleum ether (w_2), and boiled at 60 °C with the extraction thimble in soxhlet apparatus, which was allowed to reflux for 6 hours. The thimble was carefully removed, while the extracted oil in the petroleum ether flask was dry between 105 - 110 °C for 1 hour. It was then be transferred from the oven to the desiccators, allowed to cool, weighed, and calculated in equation 3 as follow;

$$\% \text{ fat} = \frac{W2-W1}{\text{weight of the sample (2.0 g)}} \times 100 \qquad \text{Equation (3)}$$

3.4.3 Determination of crude protein content

The crude protein was determined using the micro–Kjeldahl method described by Prabhavathi *et al*. (2017). Two grams (2 g) were weighed along with 20 mL of distilled water into a micro – Kjeldahl digestion flask. It was shaken and allowed to stand for some time. One tablet of selenium catalyst and 20 mL tetra Oxo sulphate (VI) acids (H_2SO_4) was added. The flask was heated on the digestion block at 100 °C for 4 hours until the digest became clear. The flask was removed from the block and allowed to cool and the content was transfer into a 50 mL volumetric flask and diluted to the mark with water. Nitrogen in the distillate was determined by titrating with 0.014 M of H_2SO_4; the endpoint was obtained when the color of the distillate changed from green to pink. $\%\ \text{Crude protein} = \frac{\text{actual titre value} - \text{titre value of blank} \times 0.1 \times 0.014 \times conversion\ factor}{\text{weight of food sample}} \times 100$

Equation (4)

3.4.4 Determination of moisture content

The moisture content of each sample was determined as described by Khalifa *et al*. (2017) using the vacuum oven method. Two grams of the grounded sample was rapidly weighed into a pre-weighed dried dish (w_1) and weighed with the dish (W_2), It was dried to a constant weight at 100 °C at a pressure that will not exceed 100 mHg for 5 hours. When the drying procedure was completed, the dish was placed in the desiccators to cool and reweighed (W3) and the recorded loss in weight, was the moisture. The percentage moisture was calculated as below;

$$\%\ \text{moisture} = \frac{W1+W2}{W3-W1} \times 100 \qquad \text{Equation (5)}$$

Where;

W_1 = Initial weight of the empty crucible

W_2 = Weight of the crucible plus (+) the sample before drying

W_3 = Final weight of crucible + sample after drying

% total solid (dry matter) = 100 % moisture. Equation (6)

3.4.5 Determination of crude fiber content

A non-enzymatic method (Prabhavathi *et al*., 2017), was used to determined crude fiber content. Two grams of the dry sample was defatted with petroleum ether and boiled under reflux for 30 minutes with 200 mL of a solution containing 1.5 g of H_2SO_4 /100 mL of the solution. The solution was filtered through linen on a fluted funnel and wash with boiling water until the washing is no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes in 200 mL of a solution containing 1.25 g of carbonate-free NaOH per 100 mL. Final residue was filtered through a thin but closed pad of washed and ignited asbestos in a porcelain crucible. It was dry in an electric oven, weighed, incinerated, cooled, and reweighed;

The loss in the weight after incineration x 100 was calculated as the percentage (%) of the crude fiber.

$$\% \text{ crude fibre} = \frac{\text{loss in weight (g)}}{\text{original mass (2.0)}} \times 100 \quad \text{Equation (7)}$$

3.4.6 Determination of carbohydrate content

Carbohydrate content was determined as described by Khalifa *et al*. (2017) where the total proportion of carbohydrate in the sample was obtained by calculation, using the percentage weight method by subtracting the % sum of food nutrients: (% protein, % crude fiber, fat % and % ash %) from 100 %. Where, percentage (%) of carbohydrates (=) (CF + CP + F + A + M – 100 %) where; CF = Crude Fibre, CP= Crude Protein, M = Moisture, F = Fat and A = Ash.

Note: Triplicate values were obtained for each sample.

3.5 Detection of Aflatoxin Content from Groundnut Seeds and Cakes

The extraction and purification of aflatoxins concentration from groundnut seeds and groundnut cake (Kuli Kuli) samples were determined using the High-Performance Liquid chromatography Technique (HPLC) (AOAC, 2012).

3.5.1 High performance liquid chromatography (HPLC) technique procedures

Twenty-five-gram (25 g) groundnut seeds and cakes with 5 g salt (NaCl) was weighed and place in the blender jar, 125 mL methanol: water (70:30) was added to the jar, then the blender was cover and blend at high speed for 1 minute. The cover was removed from the jar and pour extract into fluted filter paper. The filtrate was collected in a clean vessel. Twenty milliliters (20 mL) of the filtered extract was Pipetted into a clean vessel. The extract was diluted with 20 mL of purified water and mix well. The dilute extract was Filtered through glass microfiber filtered into a glass syringe barrel using markings on the barrel to measure 10 mL. Ten milliliters (10 mL) of filtered diluted extract (10 mL = 1 g sample equivalent) and completely was pass through the AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through the column. 10 mL of purified water was passed through the column at a rate of about 2

drops/second. Elute affinity column by passing 1.0 mL HPLC grade methanol through the column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette. 1.0 mL of purified water was eluated and Injected into 20 µL onto HPLC. Detectors were used to determine the separated compounds by Ultra Violet light absorption, the response signals from ultra violet light were recorded by computer software in form peak and purity of the samples.

3.6 Data Analysis

Data were expressed as mean ± standard error. The data obtained were subjected to an analysis of variance (ANOVA) test to determine whether there were significant differences, and Duncan's multiple range test (DMRT) was used to separate the means where there were significant differences. The Pearson's linear correlation was used to show relationships among various parameters.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Isolated toxigenic mycoflora from groundnut seeds and cakes

Total of 93 fungal colony count were obtained from groundnut seeds and 166 from groundnut cakes belonging to five genera. (Table 4.1); *Aspergillus, Fussarium, Penicillium, Rhizopus,* and *Alternaria*. In groundnut seeds, the percentage frequency ranged from (4.30 – 30.11 %). *Aspergillus niger* had the highest percentage frequency of occurrence (30.11 %), followed by *A. flavus* (21.51 %), *P. chrysogenum* (12.90 %) and the least was *Alternaria* (4.30 %).

The general order of occurrence was *A. niger* (30.11 %) > *A. flavus* (21.51 %) > *P. chrysogenum* (12.90 %) > *A. parasiticus* (11.82 %) > *Fussarium* spp. (7.52 %) > *A. fumigatus*. (6.45%) > *Rhizopus* spp. (5.38%) > *Alternaria* spp. (4.30%), while in cakes, the percentage frequency ranged from (4.82 – 27.11 %). *A. niger* (27.11 %) was the highest followed by *A. flavus* (19.88 %), *P. chrysogenum* (16.87 %) and *Fussarium* (4.82 %) was the least. The general order of occurrence was *A. niger* (27.11 %) > *A. flavus* (19.88 %) > *P. chrysogenum* (16.87 %) > *A. parasiticus* (11.45 %)> *Rhizopus spp.* (10.84 %) > *A. fumigatus*. (9.03 %) > *F. oxysporum* (4.82 %) respectively as shown in Table 4.1.

Table 4.1: Percentage Frequency of Fungi Isolated from Groundnut Seed and Cake (Kuli-kuli) Samples.

S/N	Fungi isolated	Percentage frequency (%)	
		G/SEED	G/Cake
1	***Aspergillus flavus***	21.51	19.88
2	***A. parasiticus***	11.82	11.45
3	***A.niger***	30.11	27.11
4	***A. fumigatus***	6.45	9.03
5	***Fussarium* species**	7.52	4.82
6	***Penicillium* species**	12.90	16.87
7	***Rhizopus* species**	5.38	10.84
8	***Alternaria* species**	4.30	-
9	**Total**	100	100

4.1.1.2 Percentage frequency of fungi isolated at different sampled locations

Significant differences ($P < 0.05$) were observed in frequency of occurrence across all the study areas. In groundnut seeds, highest fungal isolates (27.48 %) was obtained in samples from Bida and it was significant different from those other locations, followed by samples from Kotangora (22.43 %), while samples from Shiroro (7.27 %) was the least. For cakes significantly ($P < 0.05$) higher fungal isolates was obtained in samples from Mokwa (25.20 %), follow by samples from Kotangora (24.60 %) and are significantly different ($P < 0.05$) from each other while samples from Shiroro (8.33 %) was the least shown in Table 4.2.

Table 4.2: Percentage Frequency of Fungi Isolated at different Sampled Locations

Location	G/SEEDS	G/CAKES
Bida	27.48 ± 0.48^{f}	19.14 ± 0.14^{d}
Mokwa	15.03 ± 0.03^{c}	25.20 ± 0.10^{f}
Minna	10.38 ± 0.38^{b}	13.20 ± 0.05^{c}
Shiroro	7.27 ± 0.27^{a}	8.33 ± 0.10^{a}
Kotangora	22.43 ± 0.15^{e}	24.60 ± 0.10^{e}
Rafi	16.07 ± 0.07^{d}	9.02 ± 0.02^{b}

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

4.1.1.3 Characteristics features of isolated fungi

Aspergillus niger: Rapidly growth colonies on PDA with abundant submerged mycelium, Carbon black/ deep brownish-black conidial heads. Non-Branched conidiophore with bulb end carries conidia like sun rays. Pin like black growth to pale yellow conidial on reverse Petri dish with initial globose and then radiate well-defined columns (Plates I and II).

Aspergillus flavus: Moderate to rapid growth colonies on PDA with Pin-like green growth, Yellow/greyish green. Non-Branched conidiophore with bulb end carries conidia (Plates I and II).

***Fussarium* spp:** Colonies appear brown in the center & with white edges, a White cottony colony with dense growth on PDA short crescent conidiophores, septate hyphae with abundant micro-conidia, Spindle-like conidia, and multi-cellular (Plates I and II).

Penicillium chrysogenum: Colonies are usually gradually to fast-growing, Green or Green-greyish color colonies with a white ring at the margin, sometimes white, mostly consisting of dense conidiophores. Brush-like conidiophore carries conidia, Conidiophores is hyaline, erect, branched, and penicillate at the apexes with 2-3 metula, 3-4 verticilate phialides, and catenulate conidia in each phialide, forming rather compact cylindrical (Plates I and II).

Alternaria* spp.*: colonies grow slowly from white to Dark green deeply grown colonies, oil-drop like colony when seen upside down the Petri- dish. Pineapple-like conidia multi-cellular, septated horizontally & vertically, arrange in chains (Plate I).

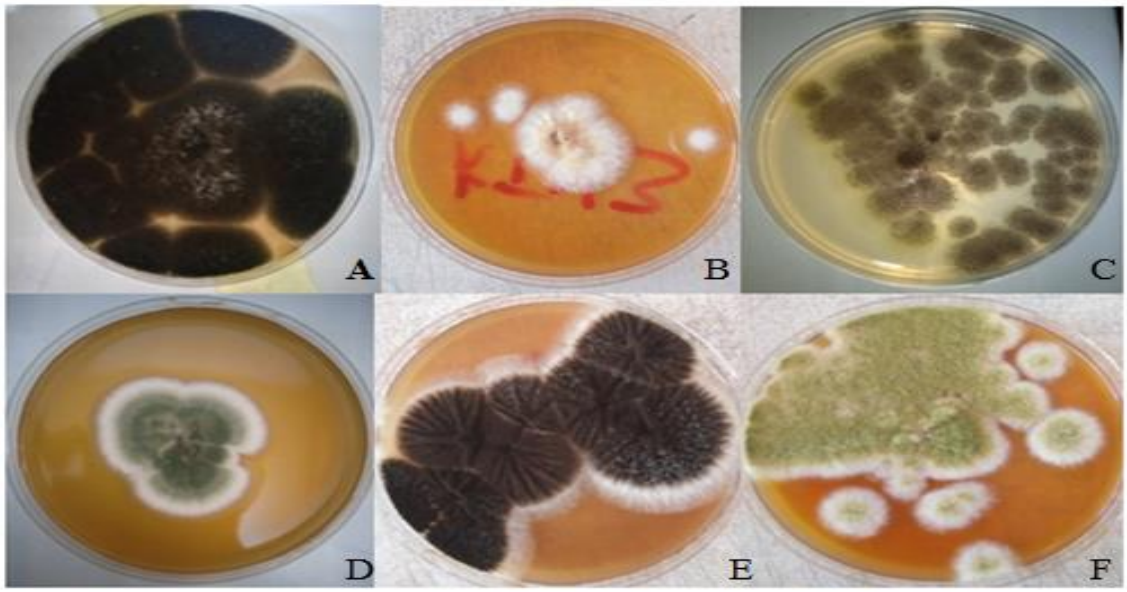


Plate I: Pure culture plates of isolated Toxigenic mycoflora on PDA. *A.Alternaria* spp. *B. Fusarium* spp. C. *A.niger*. D. *P. chrysogenum.* E. *A. fumigatus.* F. *A. flavus*.

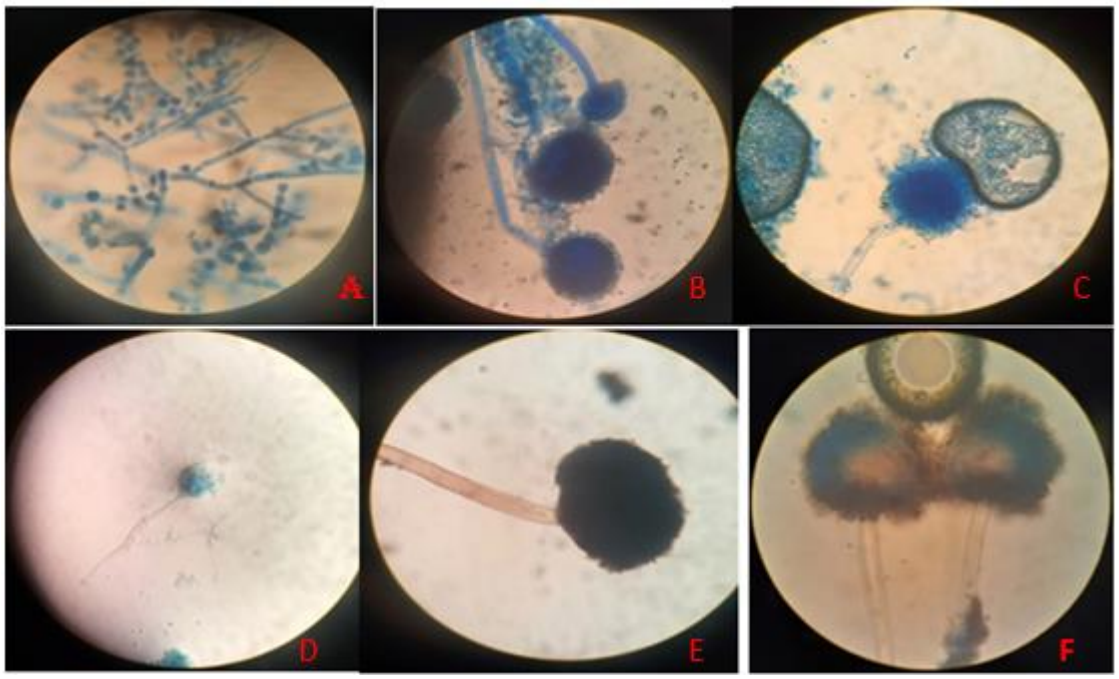


Plate II: Photomicrograph of isolated Toxigenic mycoflora (×100) *Fussarium* species: B. *A. parasiticus.* C. *P. chrysogenum.* D. *A. flavus.* E. *A. niger.* F. *A. fumigatus.*

4.1.2 Proximate composition of Groundnut seeds and cakes

4.1.2.1 Proximate composition of groundnut seed samples

The moisture contents of the seed samples obtained from this study ranged from (2.80 – 4.46 %). 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 %). Ash content significant highest was observed from Shiroro samples (3.07 %) while Rafi (2.02 %) were the least. There were no significant differences ($p < 0.05$) in ash content 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 samples respectively. Crude fat obtained from this study showed that samples from Mokwa (47.24 %) recorded highest, while samples from Shiroro (41.61 %) were least. Significant difference ($p < 0.05$) was observed within the sampled locations.

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 %). Significant differences ($p < 0.05$) were observed between Rafi (25.96 %) and other samples. In terms of crude fibre and 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 Crude Fibre samples from Bida (1.96 %), Mokwa (1.91 %), Minna (1.93 %) and Shiroro (2.02 %) were the same but slight difference ($p < 0.05$) from Kotangora (1.78 %) samples, while Rafi (1.33 %) was significantly ($p < 0.05$) low with others. For carbohydrate significant highest was obtained from Shiroro (29.18 %) samples, while samples from Rafi (23.37 %) was the least Table 4.3.

Table 4.3: Proximate Composition of Groundnut Seed Samples in Niger State

Sampled site	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 $P < 0.05$ DMRT.

4.1.2.2 Proximate composition of groundnut cake samples

The Moisture (8.89 %) and Ash (6.90 %) content obtained in samples from Shiroro and Rafi were the highest while, samples from Rafi (3.00 %), and Shiroro (3.46 %) were the least. Significant differences ($p < 0.05$) were observed in moisture content between all the study areas. The Ash content obtained from this study observes significant difference ($p < 0.05$) between all the study areas at ($p < 0.05$), but with exception of samples from Mokwa (4.94 %) and Kotangora (5.06 %). In Crude fat and crude protein, Bida (35.48 %, and 29.33 %) recorded highest, while Kotangora (16.48 %) and Rafi (21.02 %) observed the least values respectively.

All samples observe a significant difference at ($p < 0.05$). Significant highest Crude fibre and carbohydrate were recorded in samples from Rafi (4.56 % and 43.97 %) while samples from Shiroro (2.28 %) and Bida (21.16 %) were the least. Significant difference ($p < 0.05$) was observed in crude fibre between all the study areas. Significant difference ($p < 0.05$) was observed in carbohydrates content between all the study areas, but samples from Mokwa, Shiroro, and Kotangora were the same Table 4.4.

Table 4.4: Proximate composition of groundnut cake samples from Niger State

Sampled site	Moisture	Ash	Crude fat	Crude protein	Crude fibre	Carbohydrate
Bida	7.43±0.01^{d}	3.98±0.02^{b}	35.48±0.02^{f}	29.33±0.35^{c}	2.62±0.02b	21.16±0.29^{a}
Mokwa	4.48±0.01^{c}	4.94±0.04^{c}	19.84±0.29^{b}	27.28±0.04^{b}	3.26±0.03c	40.22±0.17^{c}
Minna	3.35±0.08^{b}	5.32±0.20^{d}	26.35±0.03^{e}	34.39±0.68^{d}	3.51±0.13d	27.09±0.25^{b}
Shiroro	8.89±0.02^{f}	3.46±0.01^{a}	23.27±0.00^{d}	22.83±1.12^{a}	2.28±0.01a	39.28±1.15^{c}
Kotangora	8.06±0.05^{e}	5.06±0.06^{c}	16.48±0.02^{a}	26.25±0.20^{b}	3.34±0.04cd	40.81±0.33^{c}
Rafi	3.00±0.02^{a}	6.90±0.06^{e}	20.56±0.06^{c}	21.02±0.09^{a}	4.56±0.04e	43.97±0.02^{d}

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

4.1.2.3 Correlation of proximate composition of groundnut seed and cake samples

The moisture content of groundnut cakes was significant and positively correlated with protein and carbohydrate content of groundnut seeds (0.83 and 0.89) at P > 0.05. The moisture content of groundnut seeds was significantly correlated with the carbohydrate of groundnut seeds (0.90) at (P > 0.05). Ash content of groundnut cake showed perfect correlation (1.00) with Fibre content of groundnut cake at (P > 0.05), and negative correlation with Ash groundnut seed (-0.89), Protein content of groundnut seed (-0.81) at p > 0.05.

Ash groundnut seed was significant and perfectly correlated with Fibre groundnut seed (1.00) at p > 0.01 and high negatively correlated with Fibre groundnut cake (-0.89) at p > 0.05. Fat groundnut cake observed a high negative correlation carbohydrates of groundnut cake (-0.91) at p > 0.05. Fibre content of groundnut cake is significant and showed negative correlation with Fibre content of groundnut seed (-0.89) at p > 0.05. Protein content of groundnut seed is significant and negative correlated with Fibre of groundnut cake (-0.82) at p > 0.05 (Table 4.5).

Table 4.5: Correlation of Proximate composition of Groundnut Seed and Cake samples

	Moisture_C	Miosture_S	Ash_C	Ash_S	Fat_C	Fat_S	Protien_C	Protien_S	Fibre_C	Fibre_S	CHO_C	CHO_S
Moisture_C	1.00											
Miosture_S	.761	1.00										
Ash_C	-.803	-.594	1.00									
Ash_S	.529	.387	-.892*	1.00								
Fat_C	.105	-.392	-.440	.395	1.00							
Fat_S	-.623	-.629	.283	.028	.246	1.00						
Protien_C	-.204	-.493	-.184	.542	.452	.625	1.00					
Protien_S	-.839*	-.666	.816*	-.779	-.032	.236	-.227	1.00				
Fibre_C	-.803	-.607	1.000**	-.895*	-.421	.293	-.180	.820*	1.00			
Fibre_S	.535	.387	-.892*	1.000**	.394	.029	.545	-.786	-.896*	1.00		
CHO_C	-.077	.465	.428	-.529	-.910*	-.397	-.760	.201	.412	-.531	1.00	
CHO_S	.897*	.908*	-.685	.521	-.284	-.592	-.224	-.880*	-.696	.526	.225	1.00

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

_C (Cakes)

_S (Groundnut Seed)

4.1.3 Aflatoxin contents of groundnut seeds and cakes

4.1.3.1 Aflatoxin content of groundnut seed samples

The AfB_1 contents of groundnut seed obtained from this study (Table 4.6) showed that samples from Bida (77.65 µg/kg) have the highest contents, while samples from Kotangora (1.57 µg/kg) were the least. These results were significantly different (P > 0.05) from one another and from the AfB1 contents obtained from other study areas samples. AfB_2 was less in concentration, and it appears only in samples from Kotangora (0.78 µg/kg). In AFG_1, significant highest were (p > 0.05) obtained in samples from Bida (21.77 µg/kg) while samples from Kotangora (3.59 µg/kg) had the least. Minna and Shiroro (0 %) obtained zero (0) concentration (0), AfG1 (Table 4.6).

4.1.3.2 Aflatoxin content of groundnut cake (Kuli-Kuli) samples

The AfB_1, AfB_2, and AfG_2 contents of groundnut cakes obtained from this study (Table 4.6), showed that significant highest were obtained in samples from Bida (24.43, 23.01 and 22.60 µg/kg, respectively), while samples from Minna with the value of (9.67, and 19.87 µg/kg) for AfB1 and AfB2 as well as (7.44 µg/kg) for AfG2 in samples from Mokwa had the least. For AfB_1 no significant difference (P > 0.05) was observed between samples from Shiroro (11.91 µg/kg), Kotangora (13.13 µg/kg), and Rafi (12.78 µg/kg), while significantly difference (P > 0.05) was observed between a sample from Bida and other study areas. There were no significant different (P > 0.05) in AfG1 and AfG2 between Rafi (8.00 µg/kg), Mokwa (7.44 µg/kg), and Kotangora (7.77 µg/kg). For AfG_2 Significantly differences (P > 0.05) were observed between all the study areas. Shiroro 6.29 µg/kg was the highest while, Rafi (1.30 µg/kg) were the least. (Table 4.6).

Table 4.6: Aflatoxin Content of Groundnut Seed and Cake (Kuli-kuli) Samples

	AfB1		AfB2		AfG1		AfG2	
	G/SEED	**G/CAKES**	**G/SEED**	**G/CAKES**	**G/SEED**	**G/CAKES**	**G/SEED**	**G/CAKES**
Bida	77.65±0.5^{d}	24.43±0.5^{d}	00.00±0.0^{a}	23.01±0.25^{c}	21.77±0.28^{e}	22.60±0.2^{d}	00.00±0.00^{a}	1.41±0.25^{c}
Mokwa	10.75±0.57^{b}	16.43±0.54^{c}	00.00±0.00^{a}	0.00±0.00^{a}	00.00±0.00^{a}	7.44±0.25^{b}	00.00±0.00^{a}	2.42±0.25^{d}
Minna	9.55±0.55^{b}	9.67±0.34^{a}	00.00±0.00^{a}	19.87±0.25^{b}	00.00±0.00^{a}	0.00±0.00^{a}	00.00±0.00^{a}	0.00±0.00^{a}
Shiroro	00.00±0.00^{a}	11.91±0.55^{b}	00.00±0.00^{a}	0.00±0.00^{a}	9.30±0.30^{d}	9.10±0.10^{c}	1.76±0.04^{b}	6.29±0.25^{e}
Kotangora	1.57±0.23^{a}	13.13±1.01^{b}	0.78±0.11^{b}	0.00±0.00^{a}	3.59±0.12^{b}	7.77±0.70^{b}	4.16±0.28^{d}	0.00±0.00^{a}
Rafi	35.57±1.00^{c}	12.78±0.55^{b}	00.00±0.00^{a}	0.00±0.00^{a}	6.85±0.19^{c}	8.00±0.20^{b}	0.55±0.30^{c}	1.30±0.30^{b}

Values are mean ± standard error of mean. Values followed by different superscripts along the same column are significantly different at $P < 0.05$ DMRT.
Af (Aflatoxin)
B (Blue color)
G (Green color)

4.1.3.3 Correlation of aflatoxin content of groundnut seed and cake samples

AfB1 of groundnut cake was significant and showed very high positive correlation with AfG1 groundnut cake (0.93) at ($p < 0.01$), and AfB1 groundnut seeds (0.83) at ($p < 0.05$). AfB1groundnut seeds were significantly and positively correlated with AfG1 groundnut seeds (0.81), AfG1 groundnut seed (0.82) at ($p < 0.05$). AfB2 groundnut seed was significantly high positively correlated with AfG2 groundnut seed (0.911) at ($p < 0.05$). AfG1 groundnut cake was significantly very high positive correlated with AfG1 groundnut seed (0.93) at ($p < 0.01$) (Table 4.7).

Table 4.7: Correlation of Aflatoxin Contents of Groundnut Seed and Cake Samples

	AfB1_C	AfB1_S	AfB2_C	AfB2_S	AfG1_C	AfG2_S	AfG2_C	AfG1_S
AfB1_C	1.00							
AfB1_S	.834*	1.00						
AfB2_C	.422	.609	1.00					
AfB2_S	-.149	-.343	-.315	1.00				
AfG1_C	.927**	.814*	.312	-.092	1.00			
AfG2_S	-.292	-.483	-.502	.911*	-.106	1.00		
AfG2_C	-.040	-.216	-.378	-.399	.151	-.027	1.00	
AfG1_S	.778	.824*	.450	-.200	.931**	-.143	.200	1.00

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).
_S (Groundnut Seeds)
_C (Cakes)
Af (Aflatoxin)
B (Blue color)
G (Green color)

4.2 Discussion

4.2.1 Isolated toxigenic mycoflora

The presence of *Aspergillus* spp, *Fussarium* spp, *Penicillium* spp. and *Rhizopus* spp. in groundnut seed and cake screened sample is likely to be contaminated with Mycotoxin since they all produce Toxins. This is in line with the work of Salau *et al.* (2017) who isolates eight fungus species from groundnuts products in Sokoto State. Tobin-west and Baraka (2018) also isolated *Aspergillus* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and *Fussarium* spp., from raw groundnut seeds in River State. The findings in this work are also related to the work of Vikas and Mishra (2010) who isolates nine species of fungi from the seeds of different varieties of groundnut seeds during one storage year.

The result is also in line with Chavan (2011) who reported the species of *Aspergillus, Penicillium, Fussarium, Rhizopus* and *Alternaria* as the commonly occurring postharvest molds in storage conditions, Fagbohun and Faleye (2012) isolated *Aspergillus niger*, *Aspergillus flavus, Rhizopus* spp. *Mucor* spp. and *Aspergillus fumigatus* in sundried groundnut seed. The result obtains from this study corroborates with the work of Odeniyi *et al*. (2019) who isolated *Aspergillus, Fussarium, Penicillium,* and *Rhizopus* species from groundnut cake and other groundnut based products in Niger state. The presence of *Rhizopus* species from this study corroborates the work of Boli *et al*. (2013) who reported *Rhizopus* species as a common saprophyte of many foods both at pre-harvest and post-harvest stages of groundnut butter from Benin.

The presence of different fungi isolates from this study could be attributed to improper production, packaging, and storage facilities. This is in line with the findings of Mupunga *et al*. (2017). Who reported that fungal contamination of groundnut products

occurs during storage, product preparation, packaging, or storage of finished products. The presence of *Aspergillus* species (*A. flavus* and *A. niger)*, *Fussarium* species, *Penicillium* species, and *Rhizopus* in the groundnut cake samples from this study may pose a toxicological threat to the consumers since the majority of the strains of these fungal species are toxigenic which corroborate with the work of Jimoh and Kolapo (2008) and Makun *et al*. (2010). The presence of *Rhizopus* in this study corroborates the findings by Ezekiel *et al*. (2011) who reported *Rhizopus* to liberate a metabolite rhizonin A.

The previous finding by Akano and Atanda (1990), reported the presence of these fungi and aflatoxins in groundnut cake from Ibadan, Oyo State, Nigeria, after the incidence of deaths resulting from consumption of aflatoxin-contaminated foods in Nigeria. The contaminating fungus species of groundnut cake samples in this study were found to be involved in the utilization of the nutrients inherent in this food material. The presence of *Alternaria* species from this study may indicate that probably groundnut seeds have been infected from the field as it has been reported by Mohamed and Ki (2017) to be a field pathogen.

4.2.2 Proximate composition of groundnut seeds and cakes

4.2.2.1 Proximate composition of groundnut seed samples

The moisture contents of groundnut seed obtained in this study were within the range (6.5 %) reported by USDA (2010), (5 %) Odeniyi *et al.* (2019*)* 3 %. Contrary to these results, Oyedele *et al*. (2017) reported (6.48 – 7.05 %) for groundnuts from different agro-ecological zones of Nigeria. Also, Emelike and Akuso (2018), Ekhuemelo and Abu (2018) reported higher moisture content of (6.31 – 8.35 %, 8.10 – 9.37 %), respectively from different studies. The difference in these results might be attributed to the level of dryness of the groundnuts, as it has not yet undergone any form of

processing. Ash content from this study is in agreement with the findings of Odeniyi *et al*. (2019) (4 %), who report similar results on groundnut products, Ekhuemelo and Abu (2018) (2.10 % – 2.59 %). but slightly different from the findings of Emelike and Akuso (2018) who reported the Ash contents of groundnut seeds to be within (3.19 – 4.63 %). The difference could be due to moisture content and the variety of seeds used in this study. The protein value from this study does not corroborate the work by Ekhuemelo and Abu (2018) who reported the protein level of groundnut to be (25.38 % – 29.11 %) and does not corroborate with USDA (2010) of (25 %). The difference observed from previous findings may be attributed to the type of variety used, and high protein content and low moisture content from this study as compared to previous findings.

4.2.2.2 Proximate composition of groundnut cake (Kuli-kuli) samples

The moisture content in this study is within the range reported by Odeniyi *et al.* (2019) that reported similar results of (4.55 - 5.31 %) in samples from Niger, Kano, Kaduna, and the Sokoto States respectively. The observed ash content observed from this study agreed with the work of Odeniyi *et al.* 6.17 % (2019) that report similar results from kuli-kuli samples from Niger, Kano, Kaduna, and Ibadan, Oyo State. The result also corroborates the findings by Tobin-west and Baraka (2018) who reported a similar range of groundnut products stored for 24 weeks. The fat, Protein, and Carbohydrates content observed in this present study corroborate the findings of Odeniyi *et al.* (2019), Tobin-west and Baraka (2018), Oko *et al*. (2015), and Ezekiel *et al*. (2012).

The low value of carbohydrates and high values of protein and fibre reported in this study showed that groundnut cake is not a good source of carbohydrate rather protein and fibre. This is an indication that groundnut cake will probably serve as a good source

of raw material for the formulation of food for diabetic patients and health-conscious individuals.

4.2.3 Aflatoxin contents of groundnut seeds and cakes

4.2.3.1 Aflatoxin content of groundnut seed samples

The Aflatoxin content obtained from this study corroborates the findings by Modupeade *et al.* (2018) that reported a ranged of 29.00 – 33.78 µg/kg AfB_1 in groundnut products samples from Lagos state. Oyedele *et al.* (2017), (216.1 μgkg^{-1} and 250 μgkg^{-1} respectively) in Nigeria reported high aflatoxin content of groundnut seeds. Ezekiel *et al*. (2012) reported that average aflatoxin concentrations were between 43 and 118 µg/kg for AfB1 in Southwestern Nigeria. Contrary to the finding by Ekhuemelo and Abu (2018) who reported AfB_1 ranged from 5.92–11.02 µg/kg. Similarly in another report by Ousman (2015) AfB_1 from six markets in the central region of Ghana was 20 µg/kg set by the Ghana Bureau of Standards, using HPLC analysis.

The results of samples from Mokwa, Minna, Shiroro, and Kotangora were within the limit set by Standard organization of Nigerian (SON) of (20 µg/kg) while, samples from Bida and Rafi were above the limit set by SON (2006), the European Commission (2010), FDA (2011) and EAC (2011). The total of aflatoxin B_1, B_2, G_1, and G_2 concentration of groundnut seed detected from this study (178 µg/kg) is above the 20 µg/kg limit established by the Nigerian Standard Organization, (SON). National Food and Drug Management and Control Agency sets a maximum limit of 20 µg/kg on the level of aflatoxin B1 in foodstuffs (FAO, 2020). European Union as 5 µg/kg and 10 µg/kg for the sum of Aflatoxin B_1, B_2, G_1 and G_2 in food (European Commission, 2010), while the limit set for total aflatoxin by United States Food and Drug Administration is 20 µg/kg (FDA, 2011) and for East African Commission, the limit is 10 µg/kg (EAC, 2011).

4.2.3.2 *Aflatoxin content of groundnut cake (Kuli-Kuli) samples*

The finding from this study is in agreement with Vabi *et al*. (2020) who reported AfB_1 ranged of (12.3 – 18.98 %) in the groundnut cake sampled from Kano and the Kastina States. Odeniyi *et al.* (2019) reported 18.47 µg/kg in groundnut cake sample from Niger State. On the contrary to other findings by Odeniyi *et al*. (2019) (90.47 µg/kg) reported a higher value in samples from Ibadan Oyo State, The difference observed from the previous findings may be attributed to high fungi contamination, poor handlings, poor storage facilities, and environmental conditions since the study area occupy a different geographical position from the previous study area.

Aflatoxin concentration of all samples from this study was high (9.67 - 24.43 µg/kg) when compared to European Union as 5 µg/kg and 10 µg/kg for the sum of Aflatoxin B_1, B_2, G_1, and G_2 in food (European Commission, 2010). While the limit set for total aflatoxin by the United States Food and Drug Administration is 20 µg/kg (FDA, 2011). National Agency for Food and Drug Administration and Control recommends 20 µg/kg as the maximum limit for aflatoxin B_1 concentration in foods (FAO, 2021). The high aflatoxin contents recorded from this study may be attributed to the high incidence of fungi strains of *Aspergillus flavus* and *A. parasiticus* associated with groundnut cake and, this can be of serious health concern when consumed. Since the biochemical contents of groundnut cakes are reduced by the presence of these toxigenic fungi causing varying health challenges ranging from cancer to liver problems as reported by Waliyar *et al*. (2015).

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATION AND CONTRIBUTION OF RESEARCH TO KNOWLEDGE

5.1 Conclusion

The findings of this study revealed that groundnut seeds and cakes (Kuli-kuli) are highly contaminated with toxigenic molds and the most abundant among them were *Aspergillus niger*, *Aspergillus flavus*, and *P. chrysogenum*, their percentage occurrence has a direct influence on its food qualities.

The proximate composition revealed that groundnut seed and cake samples were more of protein, and fibre than starch since the protein and fibre level was higher than the carbohydrate level in the samples. The Aflatoxin content revealed that the total aflatoxin B1, B2, G1, and G2 in the study were above the permissible limits (20 ppb) set by SON for total Aflatoxin content in foods.

Based on data obtained on aflatoxin contents of food samples, there is enough evidence to support the prevalence of fungi contaminant contributed to aflatoxin levels observed. Thus, an indication of poor processing techniques.

5.2 Recommendations

1. The use of modern technologies for hygienic storage and proper sanitary measures are needed to be put in place.
2. Public awareness should be carried out to inform the producers and consumers on the effects of consuming foods associated with high aflatoxin contents.
3. More research work should be carried out from time to time to monitor the aflatoxin content of this protein-rich food crop.

5.3 Contribution of Research to Knowledge

The thesis established the level of fungi frequency, proximate and aflatoxin contents of groundnut seeds and cakes varied from products and locations. Bida has the highest fungal isolates in seeds (27.48 %). While Mokwa had the highest fungal isolates, for cakes (25.20 %). Highest moisture and carbohydrate contents in groundnut seeds were observed in Shiroro (4.64%, 29.27 % respectively). However, for groundnut cakes the highest moisture content was observed in Shiroro (8.89 %); whereas, Rafi (43.97 %) had the highest carbohydrate contents. Bida had the highest aflatoxin contents both in groundnut seed and in cake with the value of 77.65 µg/kg and 24.43 µg/kg respectively. It further revealed that all isolates were toxigenic mould, and aflatoxin B1 contents were above the tolerable limits (SON, 20 µg/kg, Nigeria).

REFERENCES

Achar, P. N., Hermetz, S. R., Apkarian, R. & Taylor, J. (2009). Microscopic Studies on the *Aspergillus flavus* Infected Kernels of Commercial Peanuts in Georgia. *Ecotoxicology Environmental Safety*, 72, 2115–2120.

Adebola, M. O. & Amadi, J. E. (2012). The Efficacy of *Paecilomyces* species and *Penicillium digitatum* on Black pod Disease Pathogen on the Field. *European Journal of Applied Science*, 4(3), 101-104.

Adjou, S. E., Boniface, Y., Codjo, M. S., Mohamed, M. S. & Comlan, A. S. (2012). Occurrence of Mycotoxins and Associated Mycoflora in Groundnut Cake Product (Kuli Kuli) Marketed in Benin. *African Journal of Biotechnology*, 78, 14354-14360.

Ajeigbe. H. A., Waliyar, F., Echekwu, C. A., Ayuba, K., Motagi, B. N., Eniayeju, D. & Inuwa, A. (2014). A Farmer's Guide to Groundnut Production in Nigeria. Patancheru 502 324, Telangana, India: International Crops Research Institute for the Semi-Arid Tropics. 36. Retrieved on 26 September, 2021 at http://oar.icrisat.org/8856/1/2015084%20Groundutnut%20Production%20in%20Nigeria.pdf.

Akano, D. A. & Atanda, O. (1990). Present Level of Aflatoxin in Nigerian groundnut Cake (Kuli-kuli'). *Letters in Applied Microbiology*, 4, 187-189.

Aliyu, B. S., & Kutama, A. S. (2007). Isolation and Identification of Fungal Flora Associated with Groundnut in Different Storage Facilities. *Science World Journal*, 2, 34-36.

Association of Official Analytical Chemist (AOAC). (2012). Official Methods of Analysis. 19TH Ed. Association of Official Analytical Chemists, Washington DC. Retrieved on 22nd January, 2021 at http://www.Sciepub .com/reference/177396.

Arya, S. S., Chauhan, S. & Salve, A. (2015). Peanuts as Functional food: A Review. *Journal of Food Science and Technology*, 53(1), 121 – 128. DOI,10.1007/s13197-015-2007-9.

Ayoola, P. & Adeyeye, A. (2010). Effect of Heating on the Chemical Composition and Physicochemical Properties of *Arachis hypogaea* (Groundnut) Seed Flour and Oil. *Pakistan Journal of Nutrition*, 9, 751-754.

Baughman, T., Grichar, J., Black, M., Woodward, J., Porter, P., New, L., Baumann, P. & McFarland, M. (2015). Texas Groundnut Production Guide (PDF). Texas A&M University. Retrieved August 26, 2021 at http://texasgroundnut board.com/wp-content/uploads/groundnutproductionguide.pdf.

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W. & Kyegombe, D. B. (2013). *Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems*, in: Razzaghi-Abyaneh, M. (Ed.), Aflatoxins - Recent Advances and Future Prospects, United Kingdom, IntechOpen Access Publisher, 239–265.

Boli, Z. A., Zoue, L. T., Alloue-Boraud, W. A. M., Kakou, C. A. & Koffi-Nevry, R. (2013). Proximate Composition and Mycological Characterization of Groundnut Butter Sold in Retail Markets of Abidjan (Côte d'Ivoire). *Journal of Applied Biosciences, 72, 5822-5829.*

Chavan, A. M. (2011). Nutritional Changes in Oilseeds due to *Aspergillus* species. *Journal of Experimental Sciences,* 2, 29-31.

Chukunda, F. A., Emri, U. N. & Ukoima, H. N. (2015). Studies of Seed-Borne Mycoflora of Castor Bean (*Ricinus communis* L.). *Academia Journal of Microbiology Research*, 3(1), 084-087.

Craufurd, P. Q., Prasad, P. V. V., Waliyar, F. & Taheri, A. **(**2006). Drought, pod Yield, Pre-harvest *Aspergillus* Infection and Aflatoxin Contamination on Peanut in Niger Field. *Crops Research,* 98 (1), 20-29.

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N. & Panneerselvam, A. (2011). Aflatoxins and Aflatoxicosis in Humans and Animals, in: Guevara-Gonzalez, R.G. (Ed.), Aflatoxins Biochemistry and Molecular Biology, United Kingdom, IntechOpen Access Publisher, 221- 256.

Do, J. H. & Choi, D. (2007). Aflatoxins: Detection, Toxicity, and Biosynthesis. *Biotechnology Bioprocess Engineering*, 12, 583–593.

East African Community (EAC) (2011). Final Draft East African Standards FDEAS No. 46/2011 of 2011 Specifying Requirements and Methods of Sampling and Test for Dry Common Beans (*Phaseolus vulgaris* L.) Intended for Human Consumption. Retrieved on 25 June, 2020 at https://law.resource.org/pub/ eac/ibr/eas.46.2011.html.

Ekhuemelo, C. & Abu, S. O. (2018). Evaluation of the Aflatoxin Contamination and Proximate Composition of Groundnut (*Arachis hypogaea* L.) Infected by *Aspergillus* species. *Nigeria Journal of Biotechnology,* 2, 130-138.

Elwakil, M. A. & El-Metwally, M. A. (2001). Seed-Borne Fungi of Peanut in Egypt: Pathogenicity and Transmission. *Pakistan Journal of Biological Sciences*, 4, 63-68.

Emelike, N. J. T. & Akusu, M. O. (2018). Proximate Composition and Sensory Properties of Kuli-Kuli Produced from the Blends of Groundnut and Cashew Kernel. *International Journal of Food Science and Nutrition Engineering*, 1, 1-4.

European Commission (2010). Setting Maximum Levels for certain Contaminants in Food Stuffs as Regards Aflatoxins. *Official Journal of the European Union*, 50, 8 – 12.

Ezekiel, C. N., Anokwuru, C. P., Fari A., Olorunfemi, M. F., Fadairo, O., Ekeh, H. A., Ajoku, K., Gbuzue, N. & Akinsanmi, F. (2011). Microbiological Quality and Proximate Composition of Groundnut cake (Kulikuli) in Nigerian Markets. *Academia Arena*, 4, 103-111.

Ezekiel, C. N., Kayode, F. O., Fapohunda, S. O., Olurunfemi, M. F., & Kponi, B. T. (2012). Aflatoxigenic Moulds and Aflatoxins in Street-Vended Snacks in Lagos, Nigeria. *International Journal of Food Safety,* 14, 88-92.

Fagbohun, E. D. & Faleye, O. S. (2012). The Nutritional and Mycoflora Changes During Storage of Groundnut (*Arachis hypogaea*). *International Journal of Agronomy and Agricultural Research,* 2(6), 15-22.

Fakruddin, M., Chowdhury, A., Hossain, M. N. & Ahmed, M. M. (2015). Characterization of Aflatoxin Producing *Aspergillus flavus* from Food and Feed Samples. *Springer Plus*, 4, 159 - 167.

Food and Agriculture Organization of the United Nations (FAO) (2020). FAOSTAT Database. Retrieved on 20 May, 2020 at http://www.faostat.fao.org.

Food and Agriculture Organization of the United Nations (FAO) (2021). FAOSTAT Database. Retrieved on 20 May, 2021 at http://www.faostat.fao.org.

Food and Drug Administration (FDA) (2011). Guidance for Industry: Action Levels for ` Poisonous and Deleterious Substances in Human Foods and Animal Feeds. FDA-1998-N 0050. Retrieved on 1st January, 2021 at https://www.fda.gov/regulatory-information/ search-fda-guidance.

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S. B., Nováková, A., & Chen, A. J. (2019). Taxonomy of *Aspergillus* Section Flavi and their Production of Aflatoxins, Ochratoxins and other Mycotoxins. *Student Mycology*, 93, 1–63.

Gaikpa, D. S., Akromah, R., Asibuo, J. W., Appiah-Kubi, Z. & Nyadanu, D. (2015). Evaluation of Yield and Yield Components of Groundnut Genotypes under *Cercospora* Leaf spots Disease Pressure. *International Journal of Agronomy Agricultural Resources*, 3, 66–75.

Gallo, A., Solfrizzo, M., Epifani, F., Panzarini, G. & Perrone, G. (2016). Effect of Temperature and Water Activity on Gene Expression and Aflatoxin Biosynthesis in *Aspergillus flavus* on Almond Medium. *International Journal of Food Microbiology*, 217, 162-169.

Gebreselassie, R., Dereje, A. & Solomon, H. (2014). On Farm Pre-Harvest Agronomic Management Practices of *Aspergillus* Infection on Groundnut in Abergelle, Tigray. *Journal of Plant Pathology & Microbiology,* 5, 2-8.

Giray, B., Girgin, G., Engin, A.B., Aydın, S. & Sahin, G. (2007). Aflatoxin Levels in Wheat Samples Consumed in some Regions of Turkey. *Food Control*, 1, 23–29.

Guchi, E. (2015). Stakeholders' Perception About Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) Along with the Value Chain Actors in Eastern Ethiopia. *International Journal of Food Contamination,* 2, 10-16.

Guimon, J. & Guimon, P. (2012). How Ready-to Use Therapeutic Food Shapes a New Technological Regime to Treat Child Malnutrition. *Technological Forecasting and Social Change.* 79, 1319-27.

Gulluoglu, L., Basal, H., Onat, B., Kurt, C. & Arioglu, H. (2016). The Effect of Harvesting on some Agronomic and Quality Characteristics of Groundnut

Grown in the Mediterranean Region of Turkey. *Field Crops Resources*, 21, 224–232.

Guo, B., Chen, P. X., Dang, B. T., Scully, X., Liang, C. C., Holbrook, J. Yu, & Culbreath, A. K. (2008). Groundnut Gene Expression Profiling in Developing Seeds at Different Reproduction Stages during *Aspergillus Parasiticu*s Infection. *BMC Development Biology* 8, 12.

Han, J., Bourgeois, S. & Lacroix, M. (2009). Protein-Based Coatings on Peanut to Minimize Oil Migration. *Food Chemistry*, 115, 462-468.

Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P. & Denning, D. W. (2007). *Aspergillus flavus*: human pathogen, allergen, and mycotoxin producer. *Microbiology*, 6, 1677– 1692.

Hell, K. & Mutegi, C. (2011). Aflatoxin Control and Prevention Strategies in Key Crops of Sub-Saharan Africa. *African Journal of Microbiology Research*, 5 (5), 459-466.

Hell, K., Fandohan, P., Bandyopadhyay, R., Kiewnick, S., Sikora, R. & Cotty, P. J. (2005). Pre- and Post-harvest Management of Aflatoxin in Maize: An African Perspective. In: Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. (Edited by Leslie, J.F. Bandyopadhyay, R. and Visconti, A.), *Cromwell press, Townbridge*, UK. 219 – 229. Retrieved on 24th September, 2020 at https://www.cabi. org/cabebooks/ebook/20083189575.

Honfo, F. G., Hell, K., Akissoe, N., Dossa, R. A. M. & Hounhouigan J. D. (2010). Diversity and Nutritional Value of Foods Consumed by Children in Two Agro-Ecological Zones of Benin. *African Journal of Food Science,* 4, 184-191.

Horn, B. W., Gell, R. M., Singh, R., Sorensen, R. B., & Carbone, I. (2016). Sexual Reproduction in *Aspergillus flavus* Sclerotia: Acquisition of Novel Alleles from Soil Populations and Uniparental Mitochondrial Inheritance. PLoS One 11(1), e0146169.doi:10.1371/journal.Pone.0146169. Retrieved on 2nd December, 2020 at https://doi.org/10. 1371/journal.pone.0146169.

Horn, B. W., Moore, G. G. & Carbone, I. (2009). Sexual reproduction in *Aspergillus flavus*. *Mycologia*, 101, 423–429.

Horn, B., Sorensen, R., Lamb, M., Sobolev, V., Olarte, R. & Worthington, C. (2014). Sexual Reproduction in *Aspergillus flavus* Sclerotia Naturally Produced in Corn. *Phytopathology*, 104, 75–85.

International Agency for Research on Cancer (IARC) (2010). Agents Classified by the IARC Monographs, 1, 100. Retrieved on 24th December, 2021 at https://monographs.iarc.who. int/list-of-classifications/

Janila, P., Nigam, S. N., Pandey, M. K., Nagesh, P. & Varshney, R. K. (2013). Groundnut Improvement: Use of Genetic and Genomic Tools. *Frontiers Plant Science*, 4, 1–16.

Jauron, R. (2011). Growing Groundnuts in the Home Garden Horticulture and Home Pest News. Retrieved on 30th May, 2019 at http://www. *Ipm.iastate.edu*.

Jimoh, K. O., & Kolapo A. L. (2008). Mycoflora and Aflatoxin Production in Market Samples of some Selected Nigerian Foodstuffs. *Research Journal of Microbiology*, 3, 169–174.

Kamika, I., & Takoy, L. L. (2011). Natural Occurrence of Aflatoxin B1 in Groundnut Collected from Kinshasa, Democratic Republic of Congo. *Food Control*, 11, 1760-1764.

Kang, E. K., Sirma, A. J., Murithi, G., Mburugu-Mosoti, C. K., Ouko, E. O., Korhonen, H. J., Nduhiu, G. J., Mungatu, J. K., Joutsjoki, V. & Lindfors, E. (2017). Occurrence of Mycotoxins in Food, Feed, and Milk in two Counties from different Agro-Ecological Zones and with Historical Outbreak of Aflatoxins and Fumonisins Poisonings in Kenya. *Food Quality Safety*. 1, 161–170.

Kebede, A. & Tana, T. (2014). Genotype by Environment Interaction and Stability of pod Yield of Elite Breeding Lines of Groundnut (*Arachis hypogaea* L.) in Eastern Ethiopia. *Science Technology Arts Resources Journal,* 3, 43–46.

Khalifa, A., Alfalluos, H., Soheal, A., Wesam, A., Kollab, F. A. & Salem, M. E. (2017). Qualitative and Quantitative phytochemical Analysis and Antimicrobial Activity of Retama" Extract Grown in Zliten Libya. *International Journal of Medical Science and Clinical Inventions*, 4, 2861-2866.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K. & Kang, S. G. (2017). Aflatoxins: a Global Concern for Food Safety, Human Health and their Management. *Frontiers in Microbiology*, 7, 2170.

Kumar, V., Basu, M. S. & Rajendran, T. P. (2008). Mycotoxin Research and Mycoflora in some Commercially Important Agricultural Commodities. *Crop Protection*, 6, 891–905.

Liang, X Q., Luo, M. & Guo, B. Z. (2006). Resistance Mechanisms to *Aspergillus flavus* Infection and Aflatoxin Contamination in Peanut (*Arachis hypogaea*). *Plant Pathology Journal*, 5, 115–124.

Magnoli, C., Astoreca, A., Ponsone, L., Fernández-Juri, M. G., Chiacchiera, S. & Dalcero, A. (2006). Ochratoxin A and the Occurrence of Ochratoxin A-producing Black Aspergilli in Stored Peanut Seeds from Córdoba, Argentina. *Journal of the Science of Food and Agriculture*, 86, 2369-2373.

Makun, H. A., Anjorin, S. T., Moronfoye, B., Adejo, F., Afolabi, O. A., Fagbayibo, G., Balogun, B. O. & Surajudeen, A. A. (2010). Fungal and Aflatoxin Contamination of some Human Food Commodities in Nigeria. *African Journal of Food Science*, 4, 127-135.

Marsalis, M., Puppala, N., Goldberg, N., Ashigh, J., Sanogo, S. & Trostle, C. (2009). New *Mexico* Groundnut Production *(*PDF*)*. Circular-645. New Mexico State University. Retrieved October 16, 2020 at https://aces.nmsu.edu/pubs/_ circulars/CR645/.

Modupeade, C. A., Ogechi, P. A., Ngozi, P. A., Olusegun, O. A. & Mulunda, M. (2018). Microbiological Quality and Risk Assessment for Aflatoxins in

Groundnuts and Roasted Cashew Nuts Meant for Human Consumption. *Hindawi Journal of Toxicology,* 13, 81-88.

Mohamed, M. & Ki, D. K. (2017). Influence of Temperature and Water Activity on Deleterious Fungi and Mycotoxin Production during Grain Storage. *Mycobiology*, 4, 240-254.

Monyo, E. S. & Varshney, R. K. (2016). Seven Seasons of Learning and Engaging Smallholder Farmers in the Drought-Prone Areas of Sub-Saharan Africa and South Asia through Tropical Legumes, 2007–2014. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),* Patancheru, ISBN 978-92-9066-568-7.

Mupunga, I., Mngqawa, P. & Katerere, D. R. (2017). Groundnuts, aflatoxins and under nutrition in children in Sub-Saharan Africa. *Nutrients*, 9, 1287-1299.

Naikoo, A., Wani, M., Bhat, N. A., Dar, M. S. & Tak, M. A. (2013). Effect of Seed Borne Mycoflora on the Quality of Three Varieties of *Arachis hypogea* L. *International Journal of Agricultural Science and Research,* 3, 35- 41.

National Agricultural Extension and Research Liaison Services (NAERLS) (2016). Agricultural Survey for 2011 Wet Seasons. National Agricultural Extension Research and Liaisons Services. Ahmadu Bello University, Zaria, Nigeria. Retrieved on 11 September, 2021 at https://orr.naerls.gov.ng/national-agricultural-extension-and-research-liaison-services-naerls/resources?page=22&rdr=1.

National Bureau of Statistics (NBS) (2018). Labour Force Statistics Employment by Sector Report. Q3 2017 Vol. 2. The Federal Republic of Nigeria, Garki, Abuja, Nigeria. Retrieved on 10th September, 2020 at https://www.nigerianstat.gov.ng/.

Ndjeunga, J., Ntare, B. R., Ajeigbe, H., Echekwu, C. A., Ibro, A. & Amadou, A. (2013). Adoption and Impact of Modern Groundnut Varieties in Nigeria. Retrieved on 30th December, 2020 at http://www.grainlegumes.cgiar.org.

Ndjeunga, J., Ntare, B. R.., Abdoulaye, A., Ibro, A., Zarafi, M. A., Cisse, Y., Moutari, A., Kodio, O., Echekwu, C. A., Mohammed, S. G. & Micko, I. (2010). Farmer Preferences for Groundnut Traits and Varieties in West Africa: Cases of Mali, Niger and Nigeria, Working Paper Series no. 27. Patancheru 502 324, Andhra Pradesh, India: *International Crops Research Institute for the Semi-Arid Tropics*. 32. Retrieved on 25th March, 2021 at http://oar.icrisat.org/221/.

Negedu, A., Atawodi, S. E., Ameh, J. B., Umoh, V. J. & Tanko, H. Y. (2011). Economic and Health Perspectives of Mycotoxins: a Review. Contamination *Journal of Biomedical Sciences*, 5, 5–26.

Nigam, S. N. (2014). Groundnut at a Glance. 121. Retrieved on 11th September, 2021 at http://oar.icrisat.org/8455/1/Groundnut%20at%20a%20Glance.pdf.

Njoroge S. M. C., Matumba, L., Kanenga, K., Siambi, M., Waliyar, F., Maruwo, J., Machinjiri, N. & Monyo, E. S. (2017). Aflatoxin B1 Levels in Groundnut Products from Local Markets in Zambia. *Mycotoxin Resources,* 33, 113.

Odeniyi, O., Ojo, C., Adebayo-Tayo, B. & Olasehinde, K. (2019). Mycological, Toxigenic, and Nutritional Characteristics of Some Vended Groundnut and Groundnut Products from Three Northern Nigerian Ecological Zones. *African Journal of Biomedical Research*, 22, 65- 71.

Okello, D. K., Biruma, M. & Deom, C. M. (2010). Overview of Groundnuts Research in Uganda: Past, Present and Future. *African Journal of Biotechnology*, 9, 6448–6459.

Oko, J. O., Abriba, C., Audu, J. A., Kutman, N. A. & Okeh, Q. (2015). Bacteriological and Nutritional Analysis of Groundnut Cake Sold in an Open Market in Samaru, Zaria-Kaduna State. *International Journal of Scientific and Technology Research*, 5, 224-227.

Okoli, I. C., Nweke, C. U., Okoli, C. G. & Opara, M. N. (2006). Assessment of the Mycoflora of Commercial Poultry Feeds Sold in the Humid Tropical Environment of Imo State, Nigeria. *International Journal of Environmental Science & Technology*, 1, 9-14.

Oladimeji, G. R. & Kolapo, A. L. (2008). Evaluation of Proximate Changes and Microbiology of Stored Defatted Residues of some Selected Nigerian Oil Seeds. *African Journal of Agricultural Research*, 2, 126–129.

Olayinka, B. U., Abdulrahaman, A. A., Andrauwus, Z. D., Aluko, T. A., Adebola, M. O. & Oladele, F. A. (2013). Traditional Preparations and Uses of Groundnut in Nigeria. *Annals of Food Science and Technology*, 1, 29-34.

Opega, J. L., Orishagbebmi, C. O., Yusufu, P. A. & Ishaka, N. A. (2016). Proximate Composition, Mineral and Phytochemical Contents of Some Leafy Vegetables Native to Igala Kingdom Kogi State Nigeria. *International Journal of Biochemistry Research & Review*, 4, 1-11.

Ousman, S. D. (2015). Studies of *Aspergillus flavus* and Aflatoxin Contaminantion of Groundnut (*Arachis hypogaea L.*) from Six Markets in the Central Region, Ghana. Master of Health Philosophy Thesis in Seed Science Technology, University of Cape Coast, Ghana.124. Retrieved on 25th July, 2021 at https://www.semanticscholar.org/paper/Studies-of Aspergillus-flavus-and-Aflatoxin-of-l)Dorley/5bee217336484fd0ae5561b76ca320209 771e11 e#references.

Oyedele, O. A., Ezekiel, C. N., Sulyok, M., Adetunji, M. C., Warth, B, Atanda, O. O. & Krska, R. (2017). Mycotoxin Risk Assessment for Consumers of Groundnut in Domestic Markets in Nigeria. *International Journal of Food Microbiology*, 251, 24-32.

Prabhavathi, R. M., Prasad, M. P. & Jayaramu, M. (2017). Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. Pelagia Research Library. *Advances in Applied Science Research*, 4, 11-17.

Prasad, V. P., Kakani, G. V. & Upadhyaya, H. D. (2009). Growth and Production of Groundnut in Soils, Plant Growth, and Crop Production, (Ed. Willy H.Verheye), in Encyclopedia of Life Support System (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK. Retrieved on 9th

September, 2021 at http:// oar.icrisat.org /5776/1/ UNESCO_encylopedia_Growth_2010.pdf.

Rachier, G. O. (2005). Groundnut production, Research Status and Future Prospects in Western Kenya. Pages 13–21 in Proceedings of Groundnut Stakeholders Meeting held Tuesday, 14 June 2005 at KARI Headquarters Conference hall. Retrieved on 23rd July, 2021 at https://www.mdpi.com/2073-4395/10/5/704/htm.

Rao, N, Sastry K. & Bramel, P. J. (2006). Effects of Shell and Low Moisture Content on Groundnut Seed Longevity. *Peanuts Science,* 29, 122-125.

Reddy, B. N & Raghavender, C. R. (2007). Outbreaks of Aflatoxicoses in India. *African Journal of Food, Agriculture, Nutrition and Development,* 7, 5 - 12.

Reddy, E. S., Sudhakar, C. & Reddy, N. E. (2011). Aflatoxin Contamination in Groundnut Induced by *Aspergillus flavus* type Fungi: a Critical Review. *International Journal of Applied Biology* and *Pharceutical Technology*, 2, 0976-4450.

Reed, K. A., Sims, C. A., Gorbet D. W. & O'Keffe S. F. (2002). Storage Water Activity Effects of Flavor Fade in High-and Normal Oleic Peanuts. *Food Research International*, 35, 769-774.

Salau, I. A., Shehu, K. & Kasarawa, A. B. (2017). Morphological Characterization of Mycotoxigenic Fungi Contaminating Groundnut Products in Sokoto State. *International Journal of Research in Pharmacy and Biosciences,* 4, 1-7.

Sanchis, V. & Magan, N. (2004). Environmental Conditions Affecting Mycotoxins, in: N. Magan, N. and Olsen, M. (Eds.), Mycotoxins in Food: Detection and Control, Woodhead Publishing Limited., Cambridge, England, 174–189. Retrieved on 15th June, 2021 at https://link.springer.com/article/10.100 7/s11046-006-0057-2.

Sarah, K., Catriona, H., Helen, A. & David, E. (2016). Descriptions of Medical Fungi. Third edition (Revised November 2016) Retrieved on 15th September, 2021 at http://www.mycology.adelaide.edu.au.

Schmidt-Heydt, M., Rufer, C. E., Abdel-Hadi, A., Magan, N. & Geisen, R. (2010). The Production of Aflatoxin B1 or G1 by *Aspergillus parasiticus* at Various Combinations of Temperature and Water Activity is Related to the Ratio of aflS to aflR Expression. *Mycotoxin Research*, 4, 241–246.

Singh, P., J. March & P. J. Cotty (2018). *Aspergillus texensis*: A Novel Aflatoxin Producer with S Morphology from the United States. *Toxins*, 10, 513.

Smith, L. E., Prendergast, A. J., Turner, P. C., Mbuya, M. N. N., Mutasa, K., Kembo, G. & Stoltzfus, R. J. (2015). The Potential Role of Mycotoxins as a Contributor to Stunting in the Shine Trial. *Clinical Infectious Diseases*, 7, 733–737.

Standards Organization of Nigeria (SON) (2006). Standards for Groundnut (in-Shell and Kernels), *Nigeria Industrial Standard,* 491. Retrieved on 21st December, 2021 at https://studylib.net/doc/5498396/nigerian-industrial-standards-with-specifications-for.

Stefano, C, A., Bertazzo, Carlo, V. L., Costa, G. A. & Lopezm, M. M. (2011). Assessment of Protein Quality in Food by Calculating the Amino Acids Score Corrected by Digestibility. *Nutrition and Hospitality,* 21(1), 47-51.

Subramanian, G., Murugesan, R. & Lydia, O. (2013). Spot Diagnosis of Fungicide (*Carbendazim*) Resistance in Rice Using PCR with Reference to *Pyricularia oryzae*. *International Journal of Science, Environment and Technology*, 2(5), 1039-1059.

Sultan, Y, & Magan, N. (2010). Mycotoxigenic Fungi in Groundnuts from Different Geographic Regions of Egypt. *Mycotoxin. Resources*, 26, 133–140.

Talcott, S. T., Duncan, C. E., Pozo-Insfran, D. D. & Gorbet, D. W. (2005). Polyphenolic and Antioxidant Changes during Storage of Normal, Mid, and High Oleic Acid Peanuts. *Food Chemistry,* 89, 77-84.

Ting, Z. (2010). Mycotoxins in Food Safety and their Control Strategies. *Journal of Food Agriculture and Environment*, 4, 163 – 182.

Tobin-West, M. D. & Baraka, R. E. (2018). Effects of Methods of Processing Groundnut (*Arachis hypogaea* L.) on the Nutritional Composition and Storage Life of the Seed. *International Journal of Agriculture and Earth Science,* 6, 2489-0081.

Turner, N. W., Subrahmanyam, S. & Piletsky, S. A. (2009). Analytical Methods for Determination of Mycotoxins: a Review. *Analytica Chemistry Acta,* 2, 168-180.

United States Department of Agriculture (USDA) (2010). National Agricultural Library Nutrient Database. Retrieved on 13 July, 2021 at http://www.fas.usda.gov.

United States Department of Agriculture-Foreign Agricultural Service (USDA-FAS) (2011). Groundnut Area, Yield, and Production. Table 13. Retrieved on 13 July, 2021 at http://www.fas.usda.gov.

Vabi, M. B., Ajeigbe, H. A., Diama, A. & Affognon, H. (2020). Cook's Guide to Groundnut Delicacies: Favorite Recipes from Northern Nigeria. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)*, 86527, 00-4.

Variath, M. T. & Janila, P. (2017). Economic and Academic Importance of groundnut. In: Varshney R, Pandey M, Puppala N, Editors. The groundnut genome. Compendium of plant genomes. *Chemical Springer*, 3, 111 – 118. doi:10.1007/978-3-319-63935.

Vikas, P. U. & Mishra, U. S. (2010). Effects of Temperature on Dynamics of Storage Fungi of Oil Seeds. *International Journal of Plant Resources*, 23, 9-14.

Voth-Gaeddert, L. E., Stoker, M., Torres, O. & Oerther, D. B. (2018). Association of Aflatoxin Exposure and Height- for-Age among Young Children in Guatemala. *International Journal of Environmental Health Research*, 3, 1-13.

Waliyar, F., Kumar, K. V. K., Diallo, M., Traore, A., Mangala, U. N., Upadhyaya, H. D. & Sudini, H. (2016). Resistance to Pre-Harvest Aflatoxin Contamination in

ICRISAT's Groundnut Mini core Collection. *European Journal Plant Pathology*, 145, 901–913.

Waliyar, F., S. V. Reddy. & P. L. Kumar. (2009). Review of Immunological Methods for the Quantification of Aflatoxins in Groundnut and other Foods. *Groundnut Science,* 36, 54-59.

Waliyar, F., Umeh, V. C., Traore, A., Osiru, M., Ntare, B. R. & Diarra, B. (2015). Prevalence and Distribution of Aflatoxin Contamination in Groundnut (*Arachis hypogaea* L.) in Mali. West Africa. *Crop Protection,* 70, 1–7.

Wu, F. & Khlangwiset, P. (2010). Health Economic Impacts and Cost-Effectiveness of Aflatoxin Reduction Strategies in Africa: Case Studies in Biocontrol and Postharvest Interventions. *Food Addition Contamination Annals,* 27, 496-509.

Yard, E. E., Daniel, J. H., Lewis, L. S., Rybak, M. E., Paliakov, E. M., Kim, A. A., Montgomery, J. M., Bunnell, R., Abud, M. U., Akhwale, W., Breiman, R. F & Sharif, S. K. (2013). Human Aflatoxin Exposure in Kenya, 2007, a Cross-Sectional Study. *Food Additives Contaminants: Part A,* 30(7), 1322-31.

Zekeri, M. & Tijjani, I. (2013). Resource Use Efficiency of Groundnut Production in Ringim Local Government Area of Jigawa State, Nigeria. *Agro-search,* 2, 42-50.

Zhu, Z., Liu, G., Chen, Y. & Cheng, J. (2013). Assessment of Aflatoxins in Pigmented Rice using a Validated Immunoaffinity Column Method with Fluorescence HPLC. *Journal* of *Food Composition Analysis,* 31, 252–258.