CHAPTER ONE

INTRODUCTION

Contamination of commodities, feeds, foods and raw materials by toxic secondary metabolites of microorganisms is well documented (Rashedi *et al.,* 2012; Makun *et al.,* 2011; Rodrigues *et al.,* 2011). Mycotoxins, heavy metals contaminants and the depletion of nutritional content are some of the major threats associated with food materials sold in open markets across the globe. Discolorations, quality deterioration, reduction in commercial value and mycotoxins production are often associated with improper storage and handling of food materials (Dongo *et al.,* 2008). This appalling situation is made worse in the tropics where the warm and humid climate is stable for microbial (fungal) proliferation and subsequent establishment in numerous substrates. Most of these toxic contaminants that are also harmful to man and animals are mainly produced by fungi as low molecular weight natural product, hence fungi do not only cause direct losses but can threaten the health of both man and animals by producing poisonous substances called mycotoxins, which contaminate food and feeds. In other words, fungal growth causes direct losses in volume and quality of food and food materials, leaving behind some poisonous contaminants (mycotoxins). Therefore mycotoxins are group of secondary metabolites of fungi characterized by both diversity of chemical structures and biological activities (Abarca *et al.,* 2001). Mycotoxins are natural contaminants in food, raw materials and feeds. Fungal species that produce mycotoxins are extremely common, and they can grow on a wide range of substrates under a wide range of environmental conditions; they occur in agricultural products all around the world (Bennet and Klich, 2003).

Mycotoxins of significant importance that impact agricultural commodities include aflatoxin produced by *A.flavus* and *A.parasiticus,* Zearalenone and tricothecenes produced by

Fusarium spp, ochratoxin produced by *A. ochreaceus* and fumonisin produced by *Fusarium moniliforne*. Other toxigenic fungi frequently found on food materials are *Alternaria, Trichoderma, Chaetonium* and *Acremonium.* Many parasitic and saprophytic fungi infect growing crops and may continue to develop through post harvest processing (Mabbett, 2003; Vieira, 2003).

Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health and consequently its national economic implications (Bhat and Vashati, 1999). Risk of mycotoxin contamination of food, feed and raw material in Africa is increased due to environmental, agronomic and socio-economic factors. Environmental conditions especially high humidity and temperature favour fungal proliferation. Farming practices in Africa sustain fungal and toxin contamination of food and feed. The socio-economic and food security status of the majority of inhabitants of sub-Saharan Africa leaves them with little option in choosing good quality products (Hell *et al.,* 2010). Many individuals in developing countries are not only food insecure but chronically exposed to high levels of mycotoxins through their diets (Adetunji *et al.,* 2014). In many developing countries such as Nigeria, mycotoxins affect staple foods such as maize, groundnut, rice etc such that exposure is continuous and often at high levels. The exposures occur in towns, villages and communities that produce and consume their own food; hence regulatory measures to control exposure are largely ineffective (Wild and Gong, 2010). Moreso, tropical countries such as Nigeria, Ghana and Kenya have a high concentration of mycotoxins because their warm climatic condition supports the growth of mycotoxigenic fungi and consequently the production of mycotoxins (Rodrigues *et al.,* 2011). Therefore, the predisposing conditions for mycotoxin production relate mainly to poor hygienic practices during transportation and storage, high temperature, high moisture content and

heavy rains (FAO, 1991). These conditions are typically observed in different African countries. The demand for the storage of food substances has been increased due to the increasing population in African continent. However, improper storage, poor transportation system and inadequate processing facilities may facilitate fungal growth and subsequently lead to mycotoxin production and contamination of food and feedstuffs (Bhat and Vashati, 1999).

Unsafe food causes many acute and life - long diseases, ranging from diarrheal diseases to various forms of cancer. The monitoring of contaminants in food provides important information on risks associated with the consumption of foods and on the efficiency of control measures that are in place. The safety of foods and feeds for human and animal consumption should be of topmost priority with regards to the regulation of agricultural and food industries so that markets are not compromised by the sale of low quality or unsafe food (Conway and Toenniessen, 2003). Unfortunately, this is not the case in many parts of Africa as the limited availability of food far out-weighs other considerations such as food safety (Adetunji *et al.*, 2014). However, there is no doubt that high levels of exposure of people to food borne mycotoxins and high doses of metals (especially heavy metals) pose a significant food safety and public health risk (Cardwell *et al.*,2001). Exposure to mycotoxins may occur through inhalation, ingestion and dermal contact, and it is mainly accidental. Most cases of mycotoxicoses (animals and humans) result from eating contaminated food. Human exposure can be direct through cereals or indirect through animal products such as meat, milk and eggs (CAST, 2003). Children are the most sensitive to the effects of aflatoxin- contaminated food. The effects of chronic exposure to aflatoxin are common in Africa. Acute toxicity leading to death of humans has also been reported (Azziz--Baumgartner *et al.,* 2005). Some of the highest and most persistent human exposures to aflatoxin occur in West Africa, where nearly 99% of the children were positive for

an aflatoxin biomarker (Gong *et al.*, 2002, 2004). Mycotoxins producing fungi can infect and produce mycotoxins in food crops in the field, during growing stage, at harvest time, during storage and even at the market. As food materials progress through harvesting, storage, food processing and delivery to farm and markets, the level of mycotoxin contamination generally increases (Park, 2002).

Approximately 25% of the world's crops are affected by fungal growth, and commodities may be, both pre- and post-harvest contaminated with mycotoxins and heavy metals (Francesca and Chiara, 2012). Occurrence and concentrations of mycotoxins were variable by year, which is expected because of the annual variation in weather conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). Also mycotoxins that can be expected in food differ from location to location in relation to the different crops, agronomic practices and climatic conditions (Bryden, 2007). Since climate changes affect the growth of mycotoxigenic fungi, mycotoxin production is also influenced (Makun *et al.*, 2010). Hell *et al.* (2010) reported that food can be contaminated with storage fungi, some of which may develop as by-products mycotoxins that can be harmful to animal and human health. The study of Ahmad (1993), depicts that aflatoxin contamination in storage was dependent on the storage system and agro-ecological region.

Mycotoxins and heavy metals can cause a wide range of acute and chronic health effect in humans, although acute mycotoxin poisoning is rare, some mycotoxins can cause cancer in long run, certain toxins have specific effect like affecting the immune system, liver or kidney. Mycotoxins may cause liver cancer, suppressed immune systems, retarded growth and development by contributing to malnutrition (Egal *et al.*, 2005).

ʿEgusiʾ (*Citrullus colocynthis* (L.) Schrader), ʿOgbonoʾ(*Irvingia wombolu* Vermoesen) and cassava chips are important staple food in south eastern Nigeria (Akusu and Kiin-kabari, 2016). These food materials are susceptible to fungal infection both in the stores and in the growing field (Pereira *et al.,* 2014). Hence production of mycotoxins by fungi contaminating food and raw material is a fact that cannot be contested because mycotoxins are secondary toxic metabolites synthesized by fungi during their stationary phase of growth (Devi *et al.,*2009).

Ogbono (*Irvingia wombolu* Vermoesen) of the family Irvingiaceae popularly known as bush mango or wild mango is an indigenous forest tree belonging to the group of plant classified as non-timber forest products (Atangana *et al.,* 2001). *Irvingia* seeds constitute an important part of the rural diet in Nigeria (Ekpe *et al.*, 2007). The kernel serves as condiment used in thickening and flavouring soups. The more the ground kernel ‘draws’ in soup, the more acceptable it is (Atangana *et al.,*2001; Akusu and Kiin-kabiri, 2013).

Melon seed (*Citrullus colocynthis* (L.) Schrader) of the Cucurbitaceae family is commonly known in West Africa as ‘egusi’. It is a seed crop and the commonest soup condiment enjoyed in the south-east region of Nigeria. The fruit contains soft spongy pulp, with numerous oval-shaped, compressed, white or brown seeds (Ude *et al.,* 2002). The seeds contain about 53% oil, 28% protein and some other important mineral nutrients (Achinewhu *et al.,* 1996). Bankole *et al.,* (2006) reported that one major problem that besets melon seeds is that it deteriorates quickly in storage due to fungal infection which decreases the nutritive value, increase in the peroxide value and the production of mycotoxin.

Cassava chip is one of the products from cassava (*Manihot esculenta* Crantz). The conservation of cassava is always hampered by their easily perishable nature and the roots are highly contaminated by fungi and bacteria (Wareing *et al.*, 2001). One of the major ways to minimize post harvest losses and increase the shelf life of cassava is to process the tubers into dried cassava chips (Diop *et al.*, 1997).

Many plants are extensively used locally in traditional medicine for the treatment and control of disease (Okigbo and Igwe, 2007), these plants posses effective broad – spectrum antifungal activities in laboratory studies over the years (Wee yeow Chin, 1992). Their effectiveness has been confirmed by modern scientific studies. Among such plants are *Moringa oleifera* L. and *Chromolaena odorata* L. of the families Moringaceae and Anacardiaceae respectively.

1.1. Justification of the Study

World Health Organization (2002) estimated that about a quarter of the diseases facing human being today occur due to bio-accumulation of toxins and heavy metals in human system as a result of consumption of food materials that are improperly handled and processed. The notion that consumption of foods that are improperly handled is sometimes hazardous to human health cannot be overemphasized. Improper handling of food materials from the field, store to the market place is a common phenomenon in Nigeria. These food materials sold in the open market bio-accumulate heavy metals from the atmosphere (Rotich *et al.*, 2006; UNDP, 2006; USEPA, 2002). Therefore, food contaminants such as mycotoxins and heavy doses of metals can cause damage to health and even death. Human Exposure to multiple chemical combinations in food samples has led to series of human health disorder.

Heavy metals, moulds and aflatoxin contamination of food materials have been reported in Nigeria (Makun *et al.,* 2010; Bankole and Adebanjo, 2003) including their distribution (Atehnkeng, *et al.,* 2008), but there is a dearth of information on the distribution of heavy metals and mycotoxins in local food materials found in south eastern Nigeria as well as assessment of the risks associated with human exposure. Melon, Ogbono and Cassava chips are important food for people living in south eastern Nigeria. It is imperative and disturbing to note that between 2003 and 2013, Nigerian agricultural products mainly melon, groundnut, palm oil, beans, Ogbono (*Irvinga wombolu*) witnessed high presence of aflatoxin. This has resulted in the increasing rate of alert notifications under the Rapid Alert System for Food and Feed (RASFF) from the EU on some of Nigerian agricultural products with the attendant loss in export revenue annually (Awolowo, 2014). The high level of aflatoxin in melon and other oil seeds have been attributed to the poor pre and post harvest handling practices along the value chain especially at the harvesting and storage stages. Unfortunately, this is as a result of lack of information and absence of awareness on Good Agricultural Practices (GAP).

The use of chemicals has helped in the control of mycotoxigenic fungi but some identifiable problems such as chemical residues, biodegradation, phytotoxicity, pollution, development of resistance in target organism, high cost, not readily availability and hazard to man and his environment have rendered them either slow to adopt by farmers or farmers have totally failed to adopt them, for one cultural reasons or the other (Okigbo and Odurukwe, 2009). Hence alternative control methods are employed. Presently considerable efforts are directed at exploring the potentials of plant extracts as alternatives to synthetic chemicals. Plant extracts have the advantage of not only being readily available and affordable but are also sources of non-

phytotoxic and easily biodegradable alternative fungicides and antibiotics, hence environment friendly (Okigbo and Omodamiro, 2006; Okigbo and Nmeka, 2005; Akuesh *et al*., 2002).

Even though there is a high possibility of the development of mycotoxins and heavy metals in stored egusi, ogbono and cassava chips, especially the most potent aflatoxins, which are known to have serious health effects on livestock and humans, there has been very little research to document their occurrences in south eastern Nigeria (William, *et al*., 2004). Hence information on the microbial infestation and mycotoxin contamination of locally available foods is very scanty and where available, these data are related to only the most popular/common food materials (Glami and Wachukwu, 1997). It is glaring that the quality of food materials stored and sold in our local markets call for serious investigation, hence it is against the backdrop of the above scenario that this research is conducted. Therefore, the inference of this study will serve the purpose of alerting the public and consumers in particular on the dangers of consuming poorly stored/handled food materials as well provide a basis for decision making both to government, researchers and development organizations.

1.2. Aim of the study

The aim of this study is to assess the quality of local food materials sold in open markets in South Eastern Nigeria and to determine the effects of some plant extracts in controlling mycotoxigenic fungi.

1.3. Objectives of the study

The objective of this research work is to:

- Assess the post-harvest handling practices of ogbono (*Irvingia wombolu*), egusi (*Citrullus colocynthis*) and Cassava chips by local farmers in South Eastern Nigeria.
- Determine the level of aflatoxin and the fungi infestation of *Irvingia wombolu*, *Citrus colocynthis* and cassava chips sold in markets within South Eastern Nigeria.
- Evaluate the effect of location and weather on the heavy metals contamination of *Irvingia wombolu, Citrullus colocynthis* and cassava chips found in South Eastern Nigeria.
- Determine the antifungal potential of some plants in the control of mycotoxigenic fungi of *Irvingia wombolu, Citrullus colocynthis* and cassava chips

CHAPTER TWO

LITERATURE REVIEW

2.1. General description of the food system circumstances in South Eastern Nigeria

Agriculture is a long-standing occupation of the people of the zone. Farm size is small, about 1.5 hectares. Rainfed crop production is dominant. Cropping is based on fallow system on outlying farms while compound and other farms close to homesteads are continuously cropped. All crops are sold and a proportion is used for food. Industrial crops like cocoa, rubber and cotton are grown in minor quantities in the zone, but are cash crops. Yam (*Dioscorea* spp.), melon ʻEgusiʼ (*Citrullus colocynthis*), ʻogbonoʼ (*Irvingia wombolu*), cassava (*Manihot* spp.), cocoyam (*Colocassia* spp.), edible legumes, vegetables and rice (*Oryza sativa*) are grown extensively in some areas of the zone. Mixed cropping based on root/tuber crops is universally practised. Rice is grown as a sole crop in seasonal swamps.

The dominant tree crops are oil palm, citrus, banana, plantain, kola, coconut, mango and rafia palm. There are still pockets of food gathering, hunting and fishing, but deforestation has made it difficult to hunt regularly. Fishing is carried out in the riverine areas, such as Nkpologu and near Adani areas in Enugu State. Livestock farming (local cow, goat, pig, chicken, rabbit and fish) is practised in the Igbo culture area. In rural areas local chickens brood anywhere, yet there are well-established poultry farms in both rural and urban areas of the Igbo culture area. Many useful fruit trees are exploited from semi-wild conditions. These include breadfruit (*Treculia* sp.), African pear (*Dacroydes* sp.), *Irvingia* sp. and *Pentaclethra macrophylla* (oil bean seed), *Dialium* sp., *Parkia vitex* and *Chrysophyllum* sp. Wild and semiwild leaf vegetables of importance in the zone include *Pterocarpus* sp., *Pergularia* sp. and *Gnetum* sp.

2.2. Food commodities produced in south eastern Nigeria

2.2.1. Ogbono (*Irvingia wombolu*)

Ogbono (*Irvingia wombolu*) is a commercial and indigenous fruit tree of West and central Africa which has been identified as the most important tree for domestication (Nangue *et al*., 2011). The plant grows freely in the tropical rain forest (Joseph and Aworh, 1991). This indigenous forest tree also known as African mango belongs to the group of plants classified as non timber forest products. The seeds constitute very important soup condiment used in thickening and flavouring soups in Nigeria. Its high nutritional and socio-economic potential makes it stand-out amongst other food crops in Nigeria (Ogunbusola *et al.,* 2014). Natural geographical distribution of *Irvingia* species span through the humid forest zones of West and Central Africa, including Angola, Cameroon, Nigeria, Central African Republic, Congo, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Guinea-Bissau, Liberia, Senegal and Sierra Leone, Sudan, Uganda, Sao Tome and Principe (Kengni, *et al.* 2011).

2.2.1.1. Botany (Taxonomical Description) of Ogbono (*Irvingia wombolu*)

Irvingia gabonensis and *Irvingia wombolu* kernels are well known in the *Irvingiaceae* family of plants. However, *I. wombolu* also known as dika fruit, African bush mango, wild mango, sweet bush mango is known for its edible fleshy fruits, whereas the fruit of *I. wombolu* is bitter and not eaten but their kernels are used in local food preparations (Leakey *et al.*, 2003 , Ainge and Brown, 2001). *Irvingia fruit* is a broadly ellipsoid drupe; yellowish and having very juicy fibrous pulp when ripe. Its stony nut encases an oil rich dicotyledonous kernel wrapped inside a brown seed-coat (Ogunsina *et al*., 2008). The average length, width and thickness of the nut are 43.3×30.62×22.11 mm respectively (Ogunsina *et al.*, 2008).

2.2.1.2. Nutritional content and Health benefits of Ogbono

Ogbono is usually eaten as a delicacy with solid foods such as eba or foofo in the south eastern and south western part of Nigeria. Ainge and Brown (2001) reported that the defatted flour of *I. wombolu* is potentially useful as raw material in food products development. Based on its nutritional properties, the kernel oil and meal have been reported as potential base materials for confectioneries, edible fats, soaps and cosmetics (Ayuk *et al*., 1999; Joseph, 1995 and Agbor, 1994). Onimawo *et al.* (2003) reported the following as the proximate composition of *I. wombolu* kernel: moisture, crude protein, crude fat, mineral ash, crude fiber and carbohydrates. On the same hand, Leakey *et al.* (2005) also documented that the fat content of 151 *Irvingia* nut kernels from 24 *Irvingia* nut trees of Nigeria and Cameroon ranged from 37.5 to 75.5% while their fatty acid composition showed lauric acid (33.5-42.1%) and myristic acid (48.7-55.5%) as the major fatty acids. The juicy fruit pulp of *I. wombolu* is rich in vitamin C and is widely consumed as a dessert fruit or snack throughout Western and Central Africa (Ejiofor 1994, Leakey and Newton 1994). The kernel of the seeds is enclosed in a fibrous testa, and is especially valued for their fat and protein rich nuts which serves as a sauce thickening agent and oil (Matos *et al.*, 2009).

In addition to its nutritional and economic benefits, *I. wombolu* is highly valued for its health and medicinal benefits (Ndoye *et al.*, 1997; Duguma *et al.*, 1990), and agricultural potentials. Studies have shown that seed extract of *I. wombolu* caused a significant reduction in body weight among obese people (Ngondi *et al.*, 2005). Similarly, earlier studies have shown that consumption of *I. wombolu* reduces the risk of diseases such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (Vinson *et al.*, 1998; Ames *et al.*, 1993). This positive influence of *Irvingia* fruits in human health is attributed to natural antioxidant

phytochemicals inherent in them (Quideau *et al.,* 2011). Interestingly, Agbor *et al.,* (2005) showed that *I. wombolu* seeds possess high concentration of antioxidant phytochemicals.

2.2.1.3. Utilization and Economic benefits of Ogbono

The seeds constitute very important soup condiment used in thickening and flavouring soups in Nigeria. Its high nutritional and socio-economic value makes it unique amongst other food crops in Nigeria (Ogunbusola *et al*., 2014). Besides being valued for their wood and edible nuts, it ranks as the most important species for food and commercial value in Nigeria and other African countries like Cameroun, while the kernels (*ogbono*) are used as thickening agent in traditional soups and stews in West and Central Africa (Leaky *et al.,* 2003).They are also a source of oil for making soap, and for medicinal purposes (Abbiw, 1990). They, thus, provide opportunities for income diversification among farmers (Ayuk *et al.,* 1999). Falconer (1990) reported that the quantity of *Irvingia* kernel marketed in Nigeria annually is over 78, 000 tonnes. Processed kernels (ogbono*)* are traded within Nigeria and between countries in West and Central Africa, and to Europe and the United State (Lapido and Boland, 1994). Ogbono seed is very expensive in the Nigerian markets only the middle class and the upper class can buy enough quantity for soup preparation (Akusu and Kiin-Kabari, 2013).

2.2.1.4. Drying and Storage of Ogbono

Fresh ogbono seeds have relatively high moisture content (about 65% moisture) and high fat content (Ezeanya *et al*., 2012), which easily enhance mould growth and thus there is need for proper drying before storage. Being a seasonal crop, storage is inevitable; drying therefore enhances storage and ensures their availability during off season. Improper drying also favours mould growth on the seeds. Moreso, high relative humidity of tropical environment contribute to

greater fungal growth and thus lowers storability of the seeds of *irvingia wombolu*. Drying and preservation of ogbono seeds are traditionally achieved in many parts of Nigeria through sun drying and drying over heat from the fire place in the kitchen. These traditional methods produce low quality products, they are time consuming and the products are easily exposed to the attack of rodents, insects and dust. Apart from the traditional methods of drying, many researchers have used various mechanical device to dry ogbono seeds (Ezeanya *et al*., 2012, Awolu and Oluwafemi, 2013; Akusu and Kiin-Kabari, 2013). The report of Akusu and Kiin-Kabari (2003) on the effect of storage period on the functional properties of Ogbono seed showed that oil absorption capacity of the Ogbono flour decreases with increase in storage period (0.50g/g for week 0 to 0.44 g/g at week 4). The least gelation concentration for the stored Ogbono seed flour were found to significantly increased from 6.0% at week zero to 10% at week 4. Protein concentration especially globulin fraction and interactions between proteins, carbohydrate and lipids are responsible for the gelation capacity of legumes and oil seed protein. They further argued that most probably as the storage period increases, hydrolysis set in due to absorption of water and increase the number of hydrophilic group on the proteins thus increasing the gelation capacity of the Ogbono flour. Akusu and Kiin-Kabari (2013) concluded that as the storage time increases more Ogbono flour will be needed to thicken the soup which will increase the quantity of Ogbono needed for the soup preparation hence higher cost incurred.

2.2.1.5. Local and international trade for Ogbono

The market for ogbono products is said to be worth some US$ 50 million (Vandenbosch, 2006). Ladipo and Boland (1994) reported extensive local and regional trade within Nigeria and between countries in West and Central Africa such as Gabon and Cameroon. Processed kernels of *Irvingia* are also transported from Africa to the UK and the USA, particularly to areas where

African immigrants abound in large numbers. They are also sold in France (Parish) and Brussels. Further uses of ogbono may increase this market. For example it is suggested that the kernel oil could be of value in the binding of pharmaceuticals and have considerable industrial applications.

2.2.1.6. Pre-harvest operations that determine Ogbono (*Irvingia)* kernel quality

This is the cultural or field aspect of quality control. In the case of planted trees, this essentially involves the use of good planting materials and the application of good tree management techniques which will allow the full potential of the genotypes planted. It also involves good disease and pest control management. In the case of wild trees, the source of which constitutes 99% of produce presently marketed in West and Central Africa, it is the selective collection of fruits that matters so the kernels extracted can be of good quality.

2.2.2. Egusi (*Citrullus colocynthis*)

Citrullus colocynthis L. (Melon seed) commonly known in West Africa as'egusi' belongs to *cucurbitaceae* family, which usually consists of a large number of varieties that are generally known as melons. It is among the 300 species of melon found in tropical Africa**.** It is a seed crop and the commonest soup condiment enjoyed in the south-east region of Nigeria. Hence Egusi melon is among the popular African indigenous vegetable crops produced in Nigeria on a large scale.

2.2.2.1. Botany and the fruit maturity of Melon (Egusi)

The melon plant has smooth spherical fruits of the size of cucumber sometimes or as big as a small ball. The fruit contains soft spongy pulp, with numerous ovale-shaped, compressed,

white or brown seeds (Ude *et al.*, 2002). It is an annual, herbaceous, monoecious plant with a non-climbing creeping habit. After planting, they completely cover the soil surface within 3 weeks and flowering starts. Pollination is by insects. Often the fruits are ready to harvest 90-120 days (3-4 months) after sowing (Ng, 1993). The fruits are indehiscent smooth berries, very large and seedy and when sound can be removed, washed and dried.

Melon is a plant with an indeterminate growth, which continues to develop fruits progressively along the length of the stem. This means that melon fruits produced at the base of the stem are older, and continue to reduce in age, as they get closer to the apex of the stem. A typical melon seed lot is therefore usually composed of seeds whose individual ages vary widely. Seed age has however been reported, as been a major determinant of seed quality. (Demir *et al*., 2004; Oladiran and Kortse, 2002; Demir and Samit, 2001). Since the seeds are also derived from fruits at different positions on the mother plant therefore, the food composition of individual seeds in the lot also varies. While seeds from fruits harvested at the base may be fully filled, seeds from the apex fruits may not have had enough time to be fully filled before senescence. Fruit position on the mother plant has however, also been reported as having an effect on seed quality (K'Opondo, 2011; Alan and Eser, 2007). The plant has tremendous genetic diversity, extending to vegetative and reproductive characteristics (Ng, 1993). They also thrive in tropical, subtropical, arid deserts and temperate locations.

2.2.2.2. Origin and domestication of Egusi Plant

'Egusi' melon (*Citrullus*) is a native of Africa, which has probably been introduced to Asia, Iran and Ukraine (Schippers, 2000). It is a vegetable crop commonly cultivated in West

Africa (Van der Vossen *et al*. 2004), hence its cultivation is across the country (NAERLS-PCU, 2005).

2.2.2.3. Nutritional content and utilization (use) of Egusi

Egusi is mainly cultivated for its seeds, which are rich in oil (53%) and protein 28% (Nwokolo and Sim, 1987). In addition to oil and protein as the chief components the seed also contain some other important mineral nutrients. Bankole and Adebanjo (2003) acknowledged that melon seeds (*Citrullus colocynthis* L.) are an important indigenous oil seed consumed by many rural communities in West Africa. Dehulled seeds contain oil, protein, amino acids, and some amounts of vitamins in varying quantities.

They are consumed in 'egusi soup', melon ball snacks and ogiri, (a fermented condiment). The seed is high in edible oil, up to 50.2% on dry weight basis such that its use as a soup condiment masks the economic value as an oil-seed. Egusi seed-oil is clear, semi-drying and easily refine-able, suitable for cooking (edible), soap-making and pharmaceuticals (Adewusi *et al*., 2000) and so offers huge prospects for extraction in cottage vegetable oil mills and processing for industrial use. In other words, the oil extract from the seeds is mostly used for cooking purposes and could also be used for producing biscuits, margarines and soaps (Ajibola, 1990). A valuable vegetable oil is extracted from the seed while the ground seed is used to prepare various delicacies including cake and soup. In West Africa, a region where soups are integral to life, egusi melon (*Citrullus*) seeds are a major soup ingredient and a common component of daily meals. Nutritional details of egusi based on research studies have shown that the oil content in the seeds varies from one bioclimatic region to another, hence Olaofe, *et al.*,(1994) reported 50% oil in egusi seed, while Fokou *et al.*,(2004) recorded 42-57% oil and Achu *et al.*,(2005) documented 44-53% oil for seeds cultivated in different bioclimatic regions.

Moreso, Because of its creeping nature and ability to use its leaves to provide cover on the soil, farmers use it as weed suppressant in their mixed crop farms. According to Achigan-Dako *et al*. (2008), 'egusi' melons play vital roles in the farming system and in the well-being of West African rural dwellers as weed suppressants and for soil fertilization. Further studies by Achigan-Dako *et al*. (2008) summarized the socio-cultural cum economic uses of 'egusi' melons to include the provision of cash income, household food, gift to relatives and seeds. The seed kernels are rich in healthy fatty acids, minerals and proteins.

2.2.2.4. Economic benefits of Egusi

The report of Schippers (2000) revealed that Egusi is presently playing a vital role and occupying an important position in the income generation ability of subsistence Africans. The notion that egusi is produced for domestic consumption has been accepted for so long a time that its contribution to inter-regional trade is largely ignored. Ojo *et al.* (2002) reported a seed yield in Nigeria ranging between 131 to 1005kg /ha. About 5,000-7,000 metric tonnes (MT) seed were exchanged between Nigeria and countries in the Economic Community of West African States (ECOWAS) and North Africa in 2002, even as unrecorded trade in pulp and oil to African communities in Europe and North America probably takes place. Besides, almost 10-20% of seed produced in Nigeria is made into pulp for sale in the market (Van der Vossen *et al*., 2004). These outflows were hardly considered in the egusi output projections to meet human dietary and raw material needs for processing to vegetable oil and livestock feeds that assumed a 4.5% growth rate in 1990-2005 (FMAWRRD, 1989).

2.2.2.5. Storage and microbial contamination of Egusi

Bankole *et al.*(2005) reported that one major problem that besets melon seeds is that it deteriorates quickly in storage due to fungal infection which decreases the nutritive value,

increases the peroxide value and the production of mycotoxin. Microbial profile of stored egusi melon according to Oladimeji and Kolapo, (2008) showed the presence of *P. vulgaris, Bacillus licheniformis, A.niger* and *Rhizopus spp.* Kolapo and Sanni (2006) reported a general decrease of the proximate content of stored defatted egusi residues as a result of microbial activity of the organisms associated with the stored residues. Their study also depicted that there was a significant increase ($P<0.05$) in both the bacterial and fungal counts with storage. This may be due to an increase in the moisture content of the defatted residues compared to that of the whole seeds.

The microbial profile of the stored defatted residues is similar to those reported for stored soybean daddawa (Kolapo *et al*., 2007; Kolapo and Sanni 2006) and stored edible oil.They conclude by saying that from the public health point of view, the high levels of the associated microorganisms may not be particularly frightening as the majority of these organisms are saprophytes. However, the effect of the proliferation of these isolated organisms on the storage qualities of these defatted residues may not be farfetched as they may have been responsible for the reduction in the nutritive value of the stored residues. The report of Oladimeji and Kolapo, (2008) confirmed that prolonged storage negatively affects the proximate composition of egusi and this proximate content is the feature that made egusi to be highly valued for food and feed formulation.

2.2.3. Cassava (*Manihot esculenta*)

Cassava, a major staple food for many nations of tropical origin, America and Africa and the third largest source of food carbohydrate in the world (Wikipedia cassava) is gradually making its way up in the world market due to its high demand. Cassava can be consumed after

direct cooking or fermented into various products from which different dishes are made. In Nigeria, examples of such fermented products are gari and fufu.

2.2.3.1. Botany of Cassava

Cassava *(Manihot esculenta)* is a woody shrub native to South America- it is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root which is a major source of carbohydrate (Wikipedia, cassava).

2.2.3.2. Nutritional content and utilization of Cassava

In Africa about 70% of cassava production is used as food. However, to a large extent cassava is considered as food for the lower and middle class. Though the protein content is low but high in good quality starch, contains significant amount of vitamin C and minerals. Cassava accounts for a higher food calories per unit weight than yam. It is on record from literatures that 70% of cassava production and over 50% cereal produced mostly in Nigeria are processed into different forms which includes but not limited to gari, tapioca, abacha.

Cassava has over the years been used in industries for different purposes. It is used directly as cooked starchy food, custard and other forms, as thinner, filler, binders, stabilizers, in bakery product, in confectionery, as animal feed, adhesive and particle board in industries like textile, pharmaceuticals, foundry etc. The usage is mostly achieved by the conversion of cassava into starch for easy utilization. Recently Antekhai and Ozakhome (2005) were able to produce biscuits from composite flour (*Manihot esculenta* and Soybean *Glycine maxima*) with higher protein content as well as being nutritionally balanced. In addition, there has been an increase in the demand of cassava production in Nigeria due to Federal government policy to incorporate

this indigenous food into the food system of the populace , for example cassava flour is now been incorporated into the wheat flour to make composite bread.

2.2.3.3. Economic benefits of Cassava

Cassava is playing a major role in an effort to alleviate the African food crisis (FIIRO, 2006). Hence the socio-economic importance of cassava provides a large percentage of the caloric requirements to the people of the Sub-Saharan Africa. The Federal government of Nigeria attempted to boost the production of cassava and encourage its economy through the export of cassava. Now, many large populations in Nigeria live on farming of cassava as a means of livelihood and financial base (income) both within the urban and rural area.

2.2.3.4. Cassava chips

Cassava chips, a derived cassava product, are very popular in Africa where it forms the raw material for the bulk of cassava-based foods (Ugwu and Ay, 1992). The cassava processing cycle leading to their production has several steps, which include fermentation, drying and storage. At each of these steps, contamination by fungi may occur. To date, only tangential references have been made on the incidence of fungal flora in a limited number of derived cassava products and there is dearth of information on the quality deterioration, mycotoxins and heavy metals contamination of cassava chips sold in the market. Cassava chip is processed by harvesting cassava tubers, after which they are peeled, washed and cooked. These are then shredded into fine thin slices, and soaked overnight for fermentation so as to thoroughly reduce the starch and hydrogen cyanide from the cassava. The shredded and fermented cassava is again thoroughly washed the following day before drying it for 2-3 days.

2.2.3.5. Storage of Cassava chips

Food is stored by almost every human society and by many animals (Wikipedia, food storage). For human beings, food storage is important traditionally, domestically and industrially. Despite high levels of production and consumption of cassava in Nigeria, dependable methods for preservation have not been developed. A good number of producers do not practice the curing and storage processes that can extend the shelf life of the crop in a fresh form. To extend shelf life, farmers slice the roots into chips and dry them under the sun using different methods. These chips can be stored for several months, depending on moisture conditions and are milled into flour which can be utilised in different forms as food, feed or starch.

2.3. Fungal and Mycotoxin contamination of food commodities

Fungal and mycotoxin contaminations of food and feed commodities constitute a worldwide serious problem, due to their implications relating to both health and the economy. There is an increasing knowledge and understanding of the role played by moulds in food spoilage. The discovery of mycotoxin production in foods has highlighted the importance of moulds in food quality. It is, however, only within the last 5-10 years that major progresses have been made towards the prevention of spoilage caused by moulds. This is due to recent international agreements on taxonomy and analytical methods for foodborne moulds, which has led to the discovery, that a specific, very limited fungal (mycobiota) is responsible for the spoilage of each kind of food. This is called the associated or critical funga and has been shown to consist of less than ten species (Filtnborg *et al.,* 1996). Fungal and mycotoxin contamination of food and feed commodities constitute a worldwide serious problem due to their implications relating to both health and the economy. Generally, the fungi associated with mycotoxins production belong mainly to the *Aspergillus, Penicillum* and *Fusarium* genera and these

contaminate and compromise food and feed safety and quality (Pitt, 2000). In otherwords, these three potential mycotoxin producers could be considered the most significant toxigenic fungi growing in processed and stored foods. Due to their capability to develop in a wide range of environmental conditions, fungi in the genus *Aspergillus* are comparatively more widespread than others. Consequently, special care is to be devoted to them, especially as they could play an important role in food decay and mycotoxin formation under certain storage conditions.

According to Wagacha and Muthomi (2008), among the numerous species of fungi, only about 100 species appear to produce mycotoxins as the results of their secondary metabolism. These producers of toxic metabolites can be classified into two groups which are: field fungi and storage fungi (CAST, 2003). Field fungi colonize plant and produce mycotoxins before harvest and proliferate better when rainfall is in abundance, while storage fungi infect crops right after harvest and during their period of storage under improper conditions (Friswad *et al.*,2006).

2.3.1. Fungi

2.3.1.1 *Aspergillus* spp

Aspergillus constitutes a large part of storage fungi (approximately 200 species) found in all environments rich in oxygen especially in soil and hay. Some species including *A.flavus*, *A.fumigatus* and *A.niger* are known to be the most common and dangerous to human and animals (Richard and Payne, 2003). *Aspergillus flavus* is a filamentous fungus with yellow-green colour and gold or red underneath (Pitt, 2000). *A.flavus* and *A.parasiticus* are mostly found in grains stored in environments with high moisture contents and high temperature levels, where they multiply and produce mycotoxins such as Aflatoxins (Bennett and Klich, 2003).

These conditions also permit *Aspergillus* species to decrease the quality of crops. For example, the cause of ear and kernel rots in maize can be due to *Aspergillus flavus* (Pitt, 2000)

even though it is normally regarded as saprophytes. *Aspergillus fumigates* known as the most temperature tolerant fungi, can survive between 20-55^{0}C. It has also been reported that *A.fumigatus* together with *A.flavus* are the fungi most responsible for producing the spores which are harmful to humans in causing the disease, aspergillosis (Pitt, 2000). *Aspergillus fumigates* can be identified with its blue-green or grey colour on its surface. It has also been reported to produce numerous immunosuppressive mycotoxins such as gliotoxin (Kamei and Watanabe, 2005). *Aspergillus niger,* another Aspergillus species is more abundant in nature as it grows mostly in a variety of substances and can also be found on damp wall of houses (Pitt, 2000). It appears black on the surface and white or yellow underneath and often found associated with crops like maize, rice and groundnut, where it can produce mycotoxins such as Ochratoxin A (Ting, 2010; Bennett and Klich, 2003).

2.3.1.2. *Fusarium* spp

The Fusarium species are filamentous field fungi, which are mostly found in the soil (Miller, 1995). They are regarded as one of the most important toxigenic fungi infecting all types of plants in the field, especially under favourable conditions. The *Fusarium* spp, especially *F.verticillioides* and *F.graminearum* are likely to be found in maize, but can also be associated with other commodities such as rice and wheat grown in sub-tropical and tropical areas (Shepherd *et al.,* 2005) as they grow and proliferate better under elevated temperature conditions.

2.3.1.3. *Penicillium* spp

Penicillium species are soil filamentous fungi found both in tropical and temperate regions, where they proliferate and produce mycotoxins such as ochratoxins known to be produced mainly by *P.verrucosum.* The ability of these fungi to grow on grains and other stored

foods depends on their propensity to thrive in low humidity conditions and to colonize rapidly by aerial dispersion, while the seeds are sufficiently moist. The most common species of Penicillium are *P. citrinum, P.chrysogenum* and *P.purpurogenum* (Pitt, 2000). These fungi have been found in different food commodities such as maize, cassava and groundnut from different African countries such as Cameroon (Njobeh *et al.,* 2009), Nigeria (Makun *et al.*,2007) and South Africa (Marasas *et al.*,2004).

2.3.2. Factors influencing microbial development in storage

The invasion of food materials by microbial pathogens, especially fungi is considered the most critical factor in food spoilage. Early work on fungi associated with post-harvest rot of food materials in Nigeria include but not limited to those of Ugwuanyi and Obeta (1996) and Onuegbu (1999). A number of fungal organisms have been implicated in the storage spoilage of food. These fungi pathogens may cause infections either singly or in combination with several other (Sangoyomi, 1995). Fungi on their own cannot penetrate intact tubers, husk, corms and cormels, hence wounding agents such as pests through their feeding activities and more importantly mechanical damages arising during harvesting, storage, transportation and handling are sufficient to provide entry points for these fungal pathogens (Sangoyomi, 2004).

Environmental factors such as temperature and relative humidity are also very important in the rate of disease development in storage. Chelule *et al.* (2001) reported that the growth of *A. flavus* and *A. parasiticus*, and subsequent aflatoxin production in storage, are favoured by high humidity (>85%), high temperature (>25 ◦C) and insect or rodent activity and all these conditions are prevalent in the humid tropics including Nigeria. Adeniji (1970) studied the extent of decay in root and tuber crops caused by three storage fungi *Penicillium oxalicum, Aspergillus niger* and *Botryodiplodia theobromae.* There was negligible decay in the tubers inoculated with

these fungi stored for four weeks at 15^0C. *Penicillium oxalicum* was less aggressive above 25^0C but *Aspergillus niger* produced greater decay at 35^0C. However, Ogundana *et al*.,(1970) reported that *P. sclerotigenum* appeared to be more pathogenic at lower storage temperature while *B. theobromae* and *A. niger* were more aggressive at higher temperature below 10-12^0C and were detrimental to root and tuber crops in storage (Coursey, 1968).

Humidity influences the degree of disease development during storage. Uncured tubers stored showed over 50% loss mainly due to infection by *B. theobromae* whereas in another experiment, tubers stored negligible at a relative humidity of 30% showed negligible decay after 95 days (Noon and Colhoum, 1979). Excessive heat leads to tuber deterioration by raising internal temperature of the tuber to a harmful level. When tubers are exposed to excess sunlight, they lose weight and rot much quickly than those kept in the shade as the ambient temperature.

2.3.3. Mycotoxins

2.3.3.1. Types of mycotoxins

There are numerious mycotoxins produced in agricultural crops in Nigeria (Bankole and Adebanjo, 2003). These mycotoxins are produced by different genera and species of mycotoxigenic fungi.

2.3.3.1.1. Aflatoxins

Aflatoxins are a group of mycotoxins produced by *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus* (Martins *et al.*, 2001). The four major aflatoxins are B1, B2, G1 and G2. Aflatoxins M1 and M2 are hydroxylated metabolites of aflatoxins B1 and B2, respectively in animals. Exposure to aflatoxin is widespread and currently, more than 55

billion people worldwide suffer from uncontrolled exposure to aflatoxin (Li and Wu, 2010). Although aflatoxin contamination poses a global problem, the impact of the problem is higher in tropical climatic regions, between 40° North and 40° south of the equator, including the entire African continent (ECOWAS, 2013).

Many substrates support fungal growth and aflatoxin production by aflatoxigenic moulds. Diener (1987) documented that natural contamination of cereals, figs, oilseed, nuts and a long list of other commodities produced in tropical countries is a common occurrence. The genetic ability to make aflatoxin contamination is highly variable; therefore Klich (2002), reported that sometimes crops become contaminated with aflatoxin in the field before harvest, where it is usually associated with drought stress, even more problematic is the fate of crops stored under conditions that favor mold growth. According to Wilson and Payne (1994), the most important variables in storage are the moisture content of the substrate and the relative humidity of the surroundings.

2.3.3.1.2. Fumonisins

Fumonisins are phytotoxic mycotoxins which are synthesized by various species of the fungal genus *Fusarium* such as *Fusarium verticillioides* and *Fusarium proliferatum.*The most important field fungi of food materials in Africa and worldwide are *Fusarium* spp, and they are known to produce over 100 secondary metabolites that can adversely affect human and animal health. *F. verticillioides* (sny *F. moniliforme*) has been found to be the most widespread. Many studies in Nigeria have found this fungus to be the most frequent in preharvest and stored maize (Essien, 2000; Ekpo and Banjoko, 1994;). This fungus is so intimately associated with maize that it was frequently observed in symptomless maize kernels in Nigeria. In addition to corn or corn-based foods, the occurrence of fumonisins has also been reported to include some products such

as beans, rice, sorghum, wheat noodles and curry. Soriano and Dragacci (2004), reported the presence of fumoonisins in cereal between 1997 and 2002.

There is not enough conclusive evidence of the human health hazards associated with fumonisin contaminated food, though human health risks associated with fumonisin are possible (FDA, 2001). However, some correlation studies have suggested a link between the consumption of maize with high incidence of *F. verticillioides* and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of Africa and China (Anon, 2000; Yoshizawa *et al*., 1994). In a survey conducted from 2000 to 2001 in Nigeria, fumonisin B1 was detected in 55 of the 108 maize samples (Bankole *et al*., 2003). The concentrations of fumonisin B1 in the Nigerian samples varied from 65 to 1830 g/kg with mean levels in positive samples of 390 g/kg. Linear correlation analysis showed a significant positive correlation between the number of samples positive to fumonisin B1 and those infected by *F.verticillioides*. It was found that the fumonisin level in samples did not correlate with the extent of visible mouldiness in samples (Bankole *et al*., 2003). The level of insect damage in grains influences the extent of fumonisin contamination. Avantaggio *et al*. (2002) found that insect damage of food materials is a good predictor of *Fusarium* mycotoxin contamination, and can serve as early warning of fumonisin contamination. Insects carry the spores of *Fusarium* from plant surfaces to the interior of the stalk or kernels or create infection wounds due to the feeding of the larvae on stalks or kernels (Munkvold and Hellminch, 2000).

2.3.3.1.3. Ochratoxin A

Ochratoxin A (OTA) is a secondary toxic metabolites produced mainly by different species of *Aspergillus* and *Penicillium*, though it was first isolated from cultures of *Aspergillus ochraceus*. These species can grow in different climates. According to W.H.O. (2002) *Aspergilus*

are found in tropical countries whereas *Penicilia* are found in temperate regions, and can grow when the temperature is as low as 5^0C, hence *Aspergillus* species are mainly responsible for the production of ochratoxin in Nigeria. Generally, Ochratoxin A formation occurs mainly after harvesting on insufficient dried cereal and cereal products. Factors influencing ochratoxin A formation include environmental conditions, such as temperature and water activity, and also in the type of the seed, hence Madhyastha *et al.*,(1990) extensively documented that *Aspergillus ochraceaus* grows better in oil seeds (groundnut and soy bean) than in grain crops such as wheat and corn, whereas *Penicillium verrucosun* may grow better in wheat and corn.

Furthermore, a wide variety of nutritional based biotic factors may affect the production of Ochratoxin A biosynthesis, hence Abdelhamid *et al.* (2009) pointed out that different carbon sources including glucose, sucrose, galactose or xylose appear to repress Ochratoxin A production in *Aspergillus ochraceus* whereas other compounds such as lactose and organic nitrogen such as urea and amino acids induces its production.

OTA is found as natural contaminants in many foodstuffs including cereals, dried fruits, cocoa, wine poultry eggs and milk. It was concluded by the Committee on Toxicity of Chemicals in Food, Consumer Products and Environment (COT) that OTA is a genotoxic carcinogen, and proposed that levels in foods be reduced to the lowest level that can be technologically attained. The joint expert Committee on Food Additives of the WHO and FAO set a provisional maximum intake of 100 ng/kg body weight (bw), while the Scientific Committee on Food of the European Union proposed that the maximum daily intake of OTA should not exceed 5 ng/kg bw (WHO, 1996). Sedmikova *et al*. (2001) found that ochratoxin A can increase the mutagenic ability of aflatoxin B1 in the case of the two simultaneously occurring in the same crop. It is frequently associated with crops grown in semi-arid and temperate regions, and it is not

considered a major problem under the tropical climate. However, OTA has been found as a contaminant in tiger nut (Adebajo, 1993), while Kpodo (1996) reported the detection of OTA in five out of 20 samples of fermented maize dough at levels less than 6.1 g/kg. OTA has also been established to be a problem in cocoa beans exported from West Africa. The EU is presently contemplating on introducing regulatory limits in cocoa and cocoa products, and industries have been mandated to implement preventive measures to reduce OTA (CABI, 2001).

2.3.3.2. Factors affecting the incidence of mycotoxigenic fungi and mycotoxins in Nigeria

According to Atanda *et al.,* (2013) numerous classifications are often adopted in categorizing the factors that affect the incidence of mycotoxins and mycotoxigenic fungi in the food chain. Some authors categorize these factors as extrinsic and intrinsic, some as physical, chemical and biological factors while others classify them as ecological, environmental and storage factors (Zain, 2011). Irrespective of the form of classification, Lacey (1986) identified the key elements involved in stating that the type and amount of mycotoxin produced is always determined by the fungi, substrate and environmental factors. Atanda *et al.,* (2013) group these factors into 15 types as outlined below.

2.3.3.2.1. Climatic conditions

Probably the two most important environmental components favouring mold growth and mycotoxin production are hot and humid conditions. Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of moulds. Although they can also be found in temperate zones, tropical climates such as those existing in Nigeria have been found to be quite conducive for mould growth and mycotoxin production. Mycotoxigenic fungi are most abundant in the tropics and as such, are major food spoilage agents in these warmer climates. Although the optimum temperature and moisture content for growth and toxin production for the

various toxigenic fungi vary, many of them achieve best growth and toxin synthesis between 24^{o}C and 28^{o}C and seed moisture content of at least 17.5% (Ominski *et al*., 1994). These conditions approximate the ambient climatic conditions in most parts of Africa and hence also account for the high prevalence of the toxins on the continent. Drought conditions actually constitute stress factors to plants rendering them vulnerable to mould infection with ensuing increase in toxin production. An indelible sign that droughts prop up toxin contamination is the fact that these conditions preceded the fatal outbreak of acute human aflatoxicosis that occurred in Kenya in 2004 (CDC, 2004). Edema and Adebanjo (2000) and Makun *et al.*, (2009) recorded higher mycotoxigenic fungal contamination during the rainy season than in the dry harmattan season among produce in Nigeria.

2.3.3.2.2. Availability of nutrients and conditions for mould growth

The fact that a strain of mould has the genetic potential to produce a particular mycotoxin is not enough for it to do so. There must be enough nutrients to encourage mould growth and the level of mycotoxin production would in part be influenced by the nutrients available to the mould (Atanda *et al.,* 2013). Typically, moulds require a source of energy in the form of carbohydrates or vegetable oils in addition to a source of nitrogen either organic or inorganic, trace elements and available moisture for growth and toxin production. Substrate may also play a role in selecting for or against toxin producing strains of a given species, e.g., there is a high proportion of toxin-producing strains of *A. flavus* isolated from peanuts and cottonseed than from rice or sorghum. It has also been found that strains of ochratoxin and citrinin producing *P. viridicatum* isolated from meat were more unstable than those isolated from grain and rapidly lost toxin-producing ability. Field fungi like *Fusarium* and *Alternaria* contaminate grains before or during harvest. The storage fungi (e.g. *Penicillium* and *Aspergillus*) are capable of growing at

lower water content than the field fungi and they tend to contaminate the grains in silos and other storage places. It is known that aflatoxin production is favoured by prolonged end of season drought and associated elevated temperatures (Rachaputi *et al*., 2002). Moulds can grow and produce mycotoxins under a wide temperature range with optima generally between 20 to 30^{o}C. However, temperatures optimum for toxin production need not correspond to those optimum for growth: *Fusarium tricinctum* grows well at 25^{o}C but produces T-2 toxin best near freezing temperatures. *Penicillium martensii* produces penicillic acid rapidly at 20-30^{o}C, but considerably more toxin eventually accumulates between 4 to 10°C.

2.3.3.2.3. Farming systems and agricultural techniques

A number of farming techniques have been shown in various reports as stimulating mould growth in agricultural produce. For example produce harvested from land on which groundnut has been planted the previous year were infested more by *Aspergillus flavus* and contained more aflatoxin than crops grown on land previously planted with rye, oats, melon or potatoes indicating that crop rotation influences mycotoxigenic mould growth. Likewise, previously fungicide-treated soil has been shown to reduce incidence of *A. flavus* in groundnuts to very low levels (Atanda *et al.,* 2013).

2.3.3.2.4. Soil types and soil conditions

Soil is a natural factor that exerts a powerful influence on the incidence of fungi. Crops grown in different soil types may have significantly different levels of mycotoxin contamination. For example, peanuts grown in light sandy soils support rapid growth of the fungi, particularly under dry conditions, while heavier soils result in less contamination of peanuts due to their high water holding capacity which helps the plant to prevent drought stress (Codex Alimentarius Commission, 2003).

2.3.3.2.5. Pre-harvest conditions

Genotypes, drought, soil type, plant density, fertilization level, and insect activities are important components in determining the likelihood of pre-harvest contamination (Cole *et al*., 1995). However, the most important factor appears to be high night time temperatures, which favour fungal growth and toxin production at a time when the plant is deprived of its usual energy source and thus least able to resist fungal attack (Abbas *et al*., 2007).

2.3.3.2.6. Time of harvesting

Harvest is the first stage in the production chain where moisture content becomes the most important parameter in terms of the management and protection of the crop. It also marks a shift from problems caused by plant pathogenic fungi, like Fusarium, to problems caused by storage fungi, like *Penicillium verrucosum.* Ideally, grains will always be harvested after a spell of dry weather when it is at'safe' moisture content, so that immediate drying is not necessary. However, this is not always possible hence inappropriate harvest time is a risk factor in Nigeria. Another important control measure at harvest will be visual examination of the grain for symptoms of disease, and the segregation of diseased batches from healthy grain. Early harvesting reduces fungal infection of crops in the field and consequent contamination of harvested produce. Even though majority of farmers in Africa are well aware of the need for early harvesting, lack of storage space, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Bankole and Adebanjo, 2003). Kaaya *et al*. (2006) observed that aflatoxin levels increased by about 4 times by the third week and more than 7 times when maize harvest was delayed for 4 weeks. However, if products are harvested early, they have to be dried to safe levels to stop

fungal growth. Rachaputi *et al*. (2002) reported lower aflatoxin levels and higher gross returns of 27% resulting from early harvesting and threshing of groundnuts.

2.3.3.2.7. Pest infestation

Insects are the chief causes of deterioration and loss of grains and seeds. Their invasion of cereals decreases the quality, grade and market value of these agricultural products which in most instances are rendered unsafe for human and animal consumption. Pest infestation is largely due to improper post-harvest and storage conditions and the level of insect damage influences the extent of mycotoxin contamination. Avantaggio *et al*. (2002) found that insect damage of maize is good predictor of *Fusarium* mycotoxin contamination. Insects carry spores of mycotoxin-producing fungi from plant surfaces to the interior of the stalk or kernels or create infectious wounds through their feeding habits (Munkvold, 2003).

2.3.3.2.8. Post-harvest handling

The post-harvest stages are those stages following harvest and leading up to primary processing such as milling. This will typically involve drying (if required), storage and transportation steps. Post-harvest movement of food/feed commodities can be complex, passing as it may between a number of intermediaries such as traders and intermediate processors, who may be situated at different geographical locations. In the simplest case, produce may remain on-farm in store or buffer storage for short periods of time before being passed directly onto the processor. In more complex cases it may pass through the hands of merchants or third party drying facilities (if harvested wet e.g. grains) and held in storage for periods of time before finally arriving at the processors. At all times the produce can become susceptible to fungal contamination and mycotoxin production if the storage conditions are not strictly controlled.

2.3.3.2.9. Drying conditions and duration

Rapid drying of agricultural products to low moisture level is critical as it creates less favourable conditions for fungal growth, proliferation, and insect infestation. It helps keep products longer (Lanyasunya *et al*., 2005). Ayodele and Edema (2010) evaluated the Critical Control Points (CCP) in the production of dried yam chips with a view to reducing mycotoxin contamination and identified the drying stage as a CCP. Aflatoxin contamination can increase 10 fold in a 3-d period, when field harvested maize is stored with high moisture content (Hell *et al*., 2008). The general recommendation is that harvested commodities should be dried as quickly as possible to safe moisture levels of 10 – 13 %. Achieving this through simple sun-drying under the high humidity conditions of many parts of Africa, such as the humid southern Nigeria is very difficult. Even, when drying is done in the dry season, it is not completed before loading grains into stores as observed by Mestre *et al*. (2004) and products can be easily contaminated with aflatoxins. During storage, transportation and marketing, low moisture levels should be maintained by avoiding leaking roofs and condensation arising from inadequate ventilation.

2.3.3.2.10. Storage factors

Mycotoxin contamination of foods or feeds may result from inadequate storage and/or handling of harvested products. To preserve quality in storage, it is necessary to prevent biological activity through adequate drying to less than 10% moisture, elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres (Lanyasunya *et al*., 2005). Several field and storage fungi have been reported in Nigeria and the post-harvest contamination is normally characterized by the activities of the 'storage' fungi, typically *Aspergillus* and *Penicillium* species that are able to grow in relatively dry conditions (Atanda *et al.*, 2013).

2.3.3.2. 11. Sanitation

Basic sanitation measures such as removal and destruction of debris from previous harvest would help in minimizing infection and infestation of produce in the field. Sorting out physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in aflatoxins levels (Atanda *et al.,* 2013).

2.3.3.2.12. Traditional processing methods

A study conducted in Benin by Fandohan *et al*. (2005) to determine the fate of aflatoxins and fumonisins through traditional processing of naturally-contaminated maize and maize based foods, demonstrated that sorting, winnowing, washing, crushing combined with dehulling of maize grains were effective in achieving significant mycotoxins removal. Similar results have been reported by Park (2002). This approach is based on separation of contaminated grains from the bulk grains and depends on heavy contamination of only a small fraction of the seeds, so that removing those leaves a much. Wet and dry milling processes as well as heat in the cooking process have been shown to reduce mycotoxin production in foods. Heating and roasting can significantly decrease aflatoxin content in corn. Grain cleaning and further processing in mills can divert mycotoxins to various mill streams, and further processing such as baking may reduce mycotoxin levels. A review of several studies, however, suggested that processing and pasteurization of milk do not completely destroy mycotoxins (Manorama and Singh, 1995).

2.3.3.2.13. Presence of previous contaminants

The presence of other microorganisms either bacteria or fungi may alter elaboration of mycotoxins on food materials. When *A. parasiticus* was grown in the presence of some bacteria; *Streptococcus lactis* and *Lactobacillius casei,* aflatoxin production was reduced (Atanda *et al.,* 2013). Meanwhile, fungal metabolites such as rubratoxins from *Penicillium purpurogenum;*

cerulenin from *Ephalosporium caerulens* and *Acrocylindrium oryzae* enhance aflatoxin production even though they repress growth of aflatoxin-producing fungi. This type of positive interaction between fungi in the same food matrix with regards to aflatoxin synthesis coupled with multi-occurrence of mycotoxins from the different fungi could have additive or synergistic effect on the health of the host and worsen the aflatoxin plight in Nigeria because such simultaneous co-occurrence of fungi and mycotoxins in African agricultural commodities is a very common phenomenon as indicated by many workers such as Makun *et al*. (2007) and Makun *et al*. (2009).

2.3.3.2.14. Substrate types and properties

Certain Agricultural produce have been observed to permit the growth of some moulds over others. For example, maize allows the growth of aflatoxins and fumonisins producing moulds above others, while groundnuts have been found to be excellent substrate for aflatoxin contamination (Bankole and Adebanjo, 2003). Other food products for which mycotoxin contamination has been reported in Nigeria are dried yam chips, tiger nut, melon seeds and stored herbal plants. Cereal grains, peanuts, cottonseed and some forages appear to be commonly contaminated with foods and feed substances that may be contaminated with mycotoxins. Similarly, intake of *Fusarium* toxins, such as trichothecenes and fumonisins, is almost solely due to consumption of cereals).

2.3.3.2.15. Lack of awareness

Lack of awareness of the dangers posed by mycotoxin contamination of produce is a major factor responsible for its high incidence in Nigeria. Majority of farmers produce and food handlers and/or processors are illiterate with virtually no knowledge of the implications of toxigenic mould growth. The Mycotoxicology Society of Nigeria has done a lot to reverse this

trend. The stake holders believe that the powdery substance can be easily dusted off or rinsed with water before the food material is eaten or processed for consumption with no associated risks. The contaminated, mould infested produce in most cases are proudly displayed on market stalls for sale out of ignorance (Atanda *et al.*, 2013).

2.3.3.3. Incidence of mycotoxins in agricultural crops and food stuffs in Nigeria

In Nigeria, a report showed that 33% of maize samples from different agro-ecological zones were contaminated with AFs (Udoh, *et al.*,2000). A survey was conducted on the natural occurrence of AFs and FUMs in preharvest maize from fields in southwestern Nigeria. AFB1 was detected in 18.4% of samples, while AFs B2, G1 and G2 were present in 7.8%, 2.9% and 1% of the samples, respectively, in contaminated samples. FUMB1 was the predominant toxin detected in 78.6% of samples, while FUMB2 was detected in 66% of samples (Bankole and Mabekoje, 2004). In another report, AFs were detected in 21 rice samples, at total AF concentrations of 28–372 μg/kg. OTA was found in 66.7% of the samples, also at high concentrations (134–341 μg/kg). ZEA (53.4%), DON (23.8), FB1 (14.3%) and FB2 (4.8%) were also found in rice, although at relatively low levels (Makun *et al.*,2011). In regard to the mycotoxin contamination of the home-made weaning food in Nigeria, it was reported that AFM1 contents ranged from 4.6–530 *n*g/m*l* (Oluwafemi and Ibe, 2011).

Udoh *et al.* (2000) reported that 33% of maize samples from different ecological zones of Nigeria were contaminated with aflatoxins. Preharvest aflatoxin production in maize is dependent on weather conditions during crop maturations. The risk of aflatoxin contamination before harvest is highest when environmental conditions are characterized by soil moisture stress with elevated temperatures (Payne, 1992).

Groundnuts cultivated in Northern Nigeria were contaminated with aflatoxin levels up to 2000 μg/kg. Akano and Atanda (1990) found aflatoxin B1 concentrations in the range of 20-455μg/kg in groundnut cake ('kulikuli') purchased from markets in Ibadan, Oyo State, Nigeria. Adebajo *et al.,* (1994) reported that most of the corn-groundnut snack ('donkwa') contained aflatoxins above 30μg/kg immediately after preparation. Yameogo and Kassamba (1999) reported that seeds of groundnuts from Burkina Faso inoculated with *A.flavus* excreted all the four major aflatoxins, which peaked at 170 ppb after 6 days. Aflatoxin formation in groundnut is favoured by prolonged end of season drought and associated elevated temperature (Rachaputi *et al*., 2002).

Aflatoxin was detected in 98% of samples of dried yam chips surveyed in Benin with levels ranging from 2.2 to 220μg/kg and a mean value of 14μg/kg (Bassa *et al*., 2001). Aflatoxin B1 was detected in 22% of yam chips in Ogun and Oyo States of Nigeria (Bankole and Mabekoje, 2003), while in a larger survey conducted later, 54.2% of dried yam chips were contaminated with aflatoxin B1 (4– 186 μg/kg; mean = 23μg/kg), 32.3% with aflatoxin B2 (2- 55 μg/kg), while 5.2% were positive for aflatoxin G1 (4-18μg/kg), and two samples tested positive for aflatoxin G2 (Bankole and Adebanjo, 2003). Adebajo (1993) reported the presence of aflatoxins in tiger nut (*Cyperus esculentus*) at toxicologically unsafe levels. Bankole and Eseigbe (1996) detected aflatoxins in 35% of tiger nut with concentrations ranging from 10-120μg/kg collected from different parts of Nigeria, and the incidence of *A. flavus* and aflatoxin contamination was found to be correlated. It was documented that fungal contamination of herbal drug plants stored for sale in Ibadan, and demonstrated the mycotoxin producing ability of the isolates on artificial medium. The problem with mycotoxin contamination in herbal plants is that they are consumed directly, unlike other products such as maize and groundnuts, which may undergo some

processing before eating. In a recent survey, 27% of melon seed samples from farmers' stores contained aflatoxin B1 with mean levels of 14μg/kg in the forest and 11μg/kg in the savanna of Nigeria (Bankole *et al.,* 2004). Rice, which is widely consumed in the country, has also been reported by various authors to favour aflatoxin production.

2.3.3.4. Implication of Mycotoxin Contamination

Since mycotoxins are often present at significant amounts in food commodities, especially in those consumed almost in daily basis, there is great risk of increased human exposure to these mycotoxins (Bennett and Klich, 2003). Subsequently, there are several possible problems associated with such exposures. The implications of mycotoxin contamination of food and feed commodities are many and classified in terms of the economy and health related problems.

2.3.3.4.1. Economic Implications

Economic loss caused by mycotoxin is mainly due to the reduction in quality and quantity of the commodities as well as reduced animal performance and even death in severe circumstances, leading to reduced profit margin. Estimates suggest that up to 25% of the world's food crop are affected to some degree by mycotoxin contamination annually (Smith and Anderson, 1991), this gives a loss of about one billion metric tons of agricultural commodities and food products (David, 2011). In Africa, aflatoxins decreases the quality of commodities leading to rejection of some commodities in the international market or reduction of their prices, leading to a significant economic loss (Coulibaly *et al.*,2008).

Alysalwa and Anwer (2009) extensively documented that the occurrence and level of mycotoxin contamination in foods varies among commodities, year and regions. As a result high levels of mycotoxin contamination and resulting economic losses are more likely to occur in

years where environmentally stressful conditions such as extreme drought exist (Sternberg, 1994). However, even in such years, affected food materials may be randomly scattered through the environmentally stressed region making estimation of aggregate damage and economic losses difficult. Essien (2000) pointed out that besides environmental factors, mycotoxin levels are also affected by economic factors and their impact on cultural practices such as harvesting, storage technology and the use of insecticide and fungicides. In addition to these factors, agricultural price supports and government farm programs can also have a major impact through their influences on production pattern and cultural practices.

2.3.3.4.2. Health Implications

Consumption of large amounts of toxin in a short period of time will cause acute toxicity leading to death, while small doses over long time will result in chronic effects to the consumer. Mycotoxins bind to DNA and disrupt genetic coding, thus promoting carcinogenesis (Darwish *et al.*,2014). Mycotoxins exert adverse health effects in human and animals (Njobeh *et al.*,2010; Shephard *et al.*,2005; Farombi, 2005). Since mycotoxins have different chemistries, they are likely to exhibit different toxicities in both animal and man (Njobeh, *et al.*,2010; Tanya, 2010). The degree with which these adverse effects are exhibited is not only determined by the type and the level of toxins present in the commodity consumed, but also on the duration of exposure (James and Marion, 2007). There is a striking association between AFs and impaired growth in children (Egal, *et al.*, 2005; Gong *et al.*,2003). The acute cases of aflatoxicosis within a short time are sometimes characterized by Jaundice, abdominal pain, vomiting and high faecal fat content which sometimes may lead to death (Pitt, 2000; Bennett and Klich, 2003; Ting, 2010). A study in Nigeria found that blood and semen AF levels ranged from 700–1,393 *n*g/m*l* and 60–

148 *n*g/m*l*, respectively, in infertile men and were significantly higher than that in fertile men (Uriah, *et al.,* 2001).

2.3.3.5. Safe level of mycotoxins in food

Mycotoxins occur with varying severity in agricultural products all around the world (Desjardins, 2006). The estimate usually given is that one quarter of the world's food is contaminated to some extent with mycotoxin (Okoli *et al.*,2006), therefore the question that arises is: what is the safe mycotoxin level present in food and food materials to man and animals? Some of the factors that make diagnosis difficult also contribute to the difficulty in establishing levels of safety. These factors include lack of research, sensitivity difference by animal species, imprecision in sampling and analysis, the large number of potential mycotoxins, interaction with other mycotoxins and interaction with the stresses of environment and production. Furthermore, mycotoxin effects are also moderated by factors such as gender, age, diet and duration of exposure. In addition Desjardins, (2006) concluded that it is therefore impossible to provide general guidelines on worldwide basis about definite levels of mycotoxins that will result in mycotoxicoses; hence recommendations that provide mycotoxin levels of concern are intended to present the lower levels of mycotoxins that have been associated with mycotoxicoses.

2.3.3.6. Mycotoxins regulations in Africa

In Africa, fifteen countries were known to have specific mycotoxin regulations. These countries cover approximately 59 percent of the inhabitants of the continent. For the majority of the African countries, specific mycotoxin regulations (probably) do not exist. The fact that countries have no specific regulatory limit for mycotoxins does not mean that the problem is ignored. Several of these countries recognize that they have problems due to mycotoxins and that

regulations should be developed, and they indicated this in their responses to the inquiry. The mycotoxin issue in Africa needs to be viewed, however, in the overall context of local food safety, health and agricultural issues (Shephard, 2004). The establishment of mycotoxin regulations will have limited effects in terms of health protection in those countries where many farmers grow agricultural produce for their own consumption (subsistence farming), which is the case in many African countries. Most of the existing mycotoxin regulations in Africa concern the aflatoxins. Morocco had the most detailed mycotoxin regulations.

2.3.3.7. Control/Management of Mycotoxin Problems in Nigeria

Control of mycotoxin in Nigeria and Africa by extension is a matter of importance not only for health implications, but also for improvement of its economy. Thus, a number of strategies for reduction and control of mycotoxins have been considered in different African countries. The control of mycotoxins in Africa involves: (a) prevention of mould growth in crops and other feedstuffs; (b) decontamination of mycotoxin-contaminated foods as a secondary strategy; and (c) continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food.

2.3.3.7.1. Prevention of mould growth in crops*:*

This aim could be achieved by following strict hygienic precautions during harvesting, storage and processing of agricultural crops and feedstuffs. Hygienic agricultural practices include early harvesting of the crops. Early harvesting of groundnuts resulted in lower AF levels and higher gross returns of 27% as compared to delayed harvesting (Rachaputi *et al.,* 2002). Proper drying and storage of the crops are considered effective tools for reduction of mould

growth and mycotoxin production. A trial focused on thorough drying and proper storage of groundnuts, and this achieved a 60% reduction in mean AF levels (Turner *et al.,* 2005, 2009).

2.3.3.7.2. Decontamination of mycotoxin-contaminated foods*:*

Physical approaches, such as sorting, washing and crushing combined with de-hulling of maize grains, were effective in achieving significant Aflatoxins and Fumonisins removal (Fandoham *et al.*, 2005). Chemical approaches are considered as the most effective method for mycotoxin decontamination despite some of the implications on food safety. Fumonisins contamination could be reduced by application of fungicides that have been used in control of *Fusarium* head blight, such as prochloraz, propiconazole, epoxyconazole, tebuconazole cyproconazole and azoxystrobin. On the other hand, application of fungicides has been shown to effectively control the AF-producing *Aspergillus* species (Ni and Streett, 2005). Chemical reduction of FUM toxicity can be achieved through the use of allyl, benzyl and phenyl isothiocyanate in model solution and in food products. The BEA reduction varied from 10% to 65% in wheat flour and was dose-dependent with allyl isothiocyanate (Meca *et al.*,2012).

Chemoprotection of AFs has been used with the use of a number of chemical compounds, such as oltipraz and chlorophylin. Dietary substances, such as broccoli sprouts and green tea that either increases animal's detoxification processes (Kensler *et al.,*2004) or prevents the production of the epoxide that leads to chromosomal damage, are considered as effective tools for reducing the health hazards caused by various mycotoxins. AF-induced changes in the liver of mice were significantly reduced with co-treatment of black tea extract (Jha *et al.,* 2012).

Using of calcium hydroxide and hydrogen peroxide during the washing step of white pepper effectively reduced AFB2 and AFG2 by 68.5% and 100%, respectively (Jalili and Jinap, 2012). Additionally, ozone has the ability to control aflatoxigenic fungal growth, and thus, it is considered as is an important alternative for peanut detoxification. Gamma radiation is also effective in reduction of total mould counts in a dose-dependent fashion.

Biological approaches are based on developing atoxigenic fungi that compete with toxigenic ones in the field environment. Introduction of atoxigenic strains of *A. flavus* and *A. parasiticus* to soil of developing crops has resulted in 74.3% to 99.9% reduction in AF contamination in peanuts in the USA (Dorner *et al.,* 1998). The use of the yeast *Saccharomyces cerevisiae* reduced the AFB1 concentration in peanuts by 74.4% (Armando *et al.,* 2012). Control of FUM-producing fungi by endophytic bacteria has also been reported. *In vitro* inhibition of OTA production by *A. ochraceus* by three yeasts (*Pichia anomala, P. kluyveri* and *Hanseniaspora uvarum*) was also reported (Masoud and Kaltoft, 2006). The usage of *Trichosporon mycotoxinivorans* as an OTA deactivator in broiler feeds has been recently reported. Lactic acid bacteria, such as *Bifidobacterium bifidum* and *Lactobacillus rhamnosus,* could be a promising biological control strategy for PAT in aqueous solutions. Fungal strains of *Trichoderma* have also been demonstrated to control pathogenic fungi through mechanisms, such as competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, mycoparasitism, biofertilization and the stimulation of plant-defense mechanisms (Benitez *et al.,* 2004).

2.3.3.7.3. Continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food*:*

In addition, education of African populations about mycotoxins, their health hazards and ways to protect against such toxins remains a matter of significance. Additionally, holding seminars, workshops and media announcements addressing this problem are very important strategies for controlling mycotoxins in African countries (Fandoham *et al.*, 2005). However, these continuous efforts are impeded by limitations in these areas, the foremost of which being the shortage of research funding and technology in many institutes and universities that facilitate this surveillance in various African countries. The second problem is the lacking experience of individuals working on this issue. Thus, in order to overcome mycotoxin contamination in African countries, all international efforts must gather to help address this issue (Adetunji *et al.*, 2014).

2.3.3.7.4. Inactivation and detoxification of aflatoxins

Aflatoxins can be inactivated by physical methods, including cooking, roasting, frying, spray drying, and baking. Irradiation of unrefined groundnut oils by UV light destroys up to 85% of aflatoxins in 18 h and 40% of aflatoxins in 2 h (Choudhary and Kumari, 2010). Bright sunlight and gas-filled tungsten lamps also destroy aflatoxins in unrefined groundnut oils. Chemical detoxification by ozonation at various concentrations, temperatures, and times of exposure is also effective. AFB1 was reduced in groundnuts by 77% with 10 min of ozonation at 75 °C (Proctor *et al.*, 2004). Ammonization also decontaminates produce containing aflatoxin when exposed for a long time at high temperature and high pressure Sodium bisulphate, potassium bisulphite, and sodium chloride have also been reported to be effective chemical detoxifiers (Njobeh *et al.*, 2010).

2.3.3.7.5. Filtration technique

Aflatoxins in oil can easily be separated by filtration. A single filtration can eliminate up to 90% of toxin from crude groundnut oil (Choudhary and Kumari, 2010). Basappa and Sreenivasamurthy (1979) developed a filter-pad system that can be adopted in oil mills to remove aflatoxins from crude oil.

2.3.3.7.6. Physical separation

Kaaya and Warren (2005) showed that uprooting groundnuts with hand hoes results in considerable damage to shells and kernels, thus predisposing them to fungal infection during storage. Approximately 80% of aflatoxin contamination can be attributed to small, shrivelled seeds, mouldy and stained seeds (Fandohan *et al.*, 2005; Turner *et al.*, 2005, 2009), and damaged seeds. Hence, sorting of kernels to remove discoloured or damaged/shrivelled pods is often recommended to minimise aflatoxin levels (Afolabi *et al.*, 2006; Fandohan *et al.*, 2005; Park, 2002). Low-quality groundnuts have higher aflatoxin levels than high-quality groundnuts (Mutegi *et al.*, 2007). Sorting can be done by physical characteristics (colour, size, density) and by near-infrared reflectance (DeMello and Scussel, 2009). Floating and density segregation also reduces aflatoxins in storage units; kernels that float in tap water contain up to 95% aflatoxins. Electronic sorting and hand-picking methods are also practiced to bring down aflatoxin levels significantly in shelled groundnuts. Advances in sorting technologies, for example, infrared and UV sorting coupled with colour-detection technology, are now available to enable inspection of aflatoxin-contaminated products on a large scale (Womack *et al.*, 2014).

2.3.3.7.7. Smoking and chemical fumigation

Moisture content of grains can be reduced and mould infestation thereby minimised effectively by smoking grains during storage (Hell and Mutegi, 2011). The efficacy of smoking

in reducing aflatoxin contamination of grains in farm storage was reported by Udoh *et al.* (2000). Ethylene oxide and methyl bromide are commonly used chemical fumigants that significantly reduce toxigenic moulds However, some of the common fumigants also have adverse effects on human health. Some evidence has shown that inhalation exposure to ethylene oxide can increase the rate of miscarriage in female agricultural workers (ATSDR, 1990). Methyl bromide has also been phased out of many agricultural processes because of health concerns.

2.3.3.7.8. Antifungal compounds

Spray application of chemicals onto freshly harvested groundnut pods under field conditions reduces *A. flavus* invasion and aflatoxin contamination in kernels during storage. Spraying 5% sodium ortho-phenylphenate (SOP) solution on moist in-shell groundnuts under field conditions and in bags effectively controlled the external fungal growth. However, SOP application was not effective for reducing aflatoxin contamination because it could not penetrate into the kernels (Fonseca *et al.*, 1994). Spraying antifungal materials from natural sources and chemical preservatives is a viable practice to prevent post-harvest aflatoxin contamination in groundnuts (Onyeagba *et al.*, 2004). Popular plant derivatives, such as cinnamon and clove oils, have shown significant inhibitory effects on growth and toxin production of *A. flavus* under experimental conditions. Application of eugenol, the main antifungal active compound of clove, can be expensive. However, methyleugenol (4-allyl-1,2-dimethoxybenzene) can be a cost-effective derivative of eugenol and can be applied for post-harvest protection of groundnut pods and kernels from *A. flavus* and aflatoxin contamination when sprayed at 0.5% concentration (Sudhakar *et al.*, 2009).

2.3.3.7.9. Management options for post-harvest aflatoxin contamination

Community-based approaches through adoption of post-harvest intervention measures can be good options for minimising exposure to aflatoxins. Identification of low-cost technologies for post-harvest management of aflatoxins is a pre-requisite for such interventions. Use of low-technology approaches at the subsistence-farm level in SSA can substantially reduce the disease caused by aflatoxin exposure and its carcinogenic effects. Some of the important factors that lead to high aflatoxin risk in Africa are (1) lack of political commitment to mycotoxin research; (2) shortage of trained personnel and infrastructure; (3) limited awareness of both smallholder farmers and consumers on the negative effects of aflatoxins and the available strategies and technologies for mitigating aflatoxin contamination; and (4) prevailing climatic conditions (Hell and Mutegi, 2011). Possible intervention strategies for mycotoxin management in Africa can be broadly categorised into: (1) prevention of exposure to toxins; (2) decontamination; and (3) continuous surveillance and monitoring of moulds in contaminated food/feed. Preventive measures include the adoption of certain good agricultural practices (GAPs) in the field, during harvesting, storage, transportation, marketing, processing, and legislation etc. The GAPs include early harvesting, proper drying of produce, physical separation, sanitation, proper storage, insect management, use of biocontrol agents, appropriate use of pesticides, decontaminants, and resistance breeding, etc. (Wagacha and Muthomi, 2008).

2.4. Storage methods of food materials

In Africa, smallholder farmers traditionally store seeds/commodities in containers that are usually made of wood, bamboo, thatch, or mud placed on raised platforms and covered with thatch or metal roofing sheets. New storage practices, such as use of metal or cement bins offer an improvement over traditional storage methods. However, high cost and access to improved

materials remain major constraints for their adoption by small-scale farmers (Hell and Mutegi, 2011). Polypropylene bags are now being used, but because these are not airtight, seeds/commodities are still susceptible to fungal and aflatoxin contamination (Hell *et al.*, 2000; Udoh *et al.*, 2000). A major precaution in bag storage is to ensure that bags are clean when reusing them, especially when used for maize, rice, sorghum, beans, or cocoa. This is because reused bags often contain *A. flavus* spores (Hell *et al.*, 2000). Grain moisture content, mould growth, aflatoxins, and free fatty acid content were significantly higher in pods stored in jute bags than in those stored in polyethylene-doubled jute bags (Bulaong and Dharmaputra, 2002). The use of hermetic triple-layer bags (PICS, Purdue Improved Crop Storage) for grain storage of several crops is gaining favour given their advantages over traditional storage devices (Hell *et al.*, 2010; Murdock *et al.*, 2003). These triple-layer bags are now being marketed in Africa (Ben *et al.*, 2009). Hermetic packaging could protect groundnuts from moulds and aflatoxin contamination (Paramawati *et al.*, 2006). Preliminary studies have indicated the efficacy of hermetic storage of groundnut pods using triple-layer polyethylene bags in minimising aflatoxins. Use of small-scale metal silos also results in better-quality grains and less pesticide usage. Better storage allows farmers to set higher prices, particularly when grain is sold during the off-season, and directly contributes to income, rural development, and poverty reduction in South Saharan Africa (Kimatu *et al.*, 2012).

2.4.1. Use of desiccants in storage

The use of desiccants to prolong seed viability during storage is also a good practice. Calcium chloride ($CaCl_2$) and silica gel are the most commonly used desiccants that help to maintain low seed moisture content, lower sugar content, and enhance seed germination. Other

beneficial effects include increased field emergence and pod yield of the ensuing crop (Basave and Nanja, 2008).

2.4.2. Storage conditions

Moisture and temperature are the main factors that influence post-harvest contamination of stored commodities by *A. flavus* (Hell and Mutegi, 2011). Because groundnut is an oilseed crop and hygroscopic, the seeds absorb moisture from the surrounding storage environment and lose viability. Awuah and Ellis (2002) reported that groundnuts dried to 6.6% moisture levels are free of fungi for 6 months regardless of the storage protectant used. These safe moisture levels are applicable to both unshelled and shelled groundnuts. The maximum moisture content for storage of unshelled groundnuts is 9%, higher than that for shelled groundnuts (7%). At these moisture levels, if the relative humidity is maintained at 70% and temperature at 25-27 °C, groundnuts can be stored for 1 year (Waliyar *et al.*, 2007, 2008). Community-based intervention studies in Guinea, West Africa, with an emphasis on proper drying and storage conditions, achieved a significant reduction in mean aflatoxin levels in the villages where the intervention occurred (Turner *et al.*, 2005). It is also essential to maintain low moisture levels during storage, transportation, and sales by avoiding other moisture sources such as leaking roofs and condensation arising from inadequate ventilation (Wagacha and Muthomi, 2008). Biological activity during storage should be minimized to preserve grain quality by adequate drying to <10% moisture, elimination of insect activity (which increases moisture content through condensation of moisture resulting from respiration), low temperature, and inert atmosphere . Storing dry pods in airy, dry, and clean rooms reduces aflatoxin accumulation (Rahmianna *et al.*, 2007). Sanitation by cleaning storage units prior to loading new produce can result in reduced aflatoxin levels in grains (Hell *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the South-Eastern Nigeria. South-Eastern Nigeria with a population of 30 million people (Census 2006 estimate- NPC, 2006) has an area of 16,000 sq mi (40,000 km2) and is located within the latitude 5-7 degrees north and longitude 6-8 degrees east. The crops commonly grown in this region include but not limited to yam, cassava, Ogbono, egusi, cocoyam and various vegetables.

Geographically, South-Eastern Nigeria extends from latitudes 4° 40^{I} to 7° 20^{1} north latitude, and 6° 00^{1} to 8° 20^{1} east longitude. The culture area occupies about 50 000 km^2 of Nigeria's total area of 923 768 km^2.

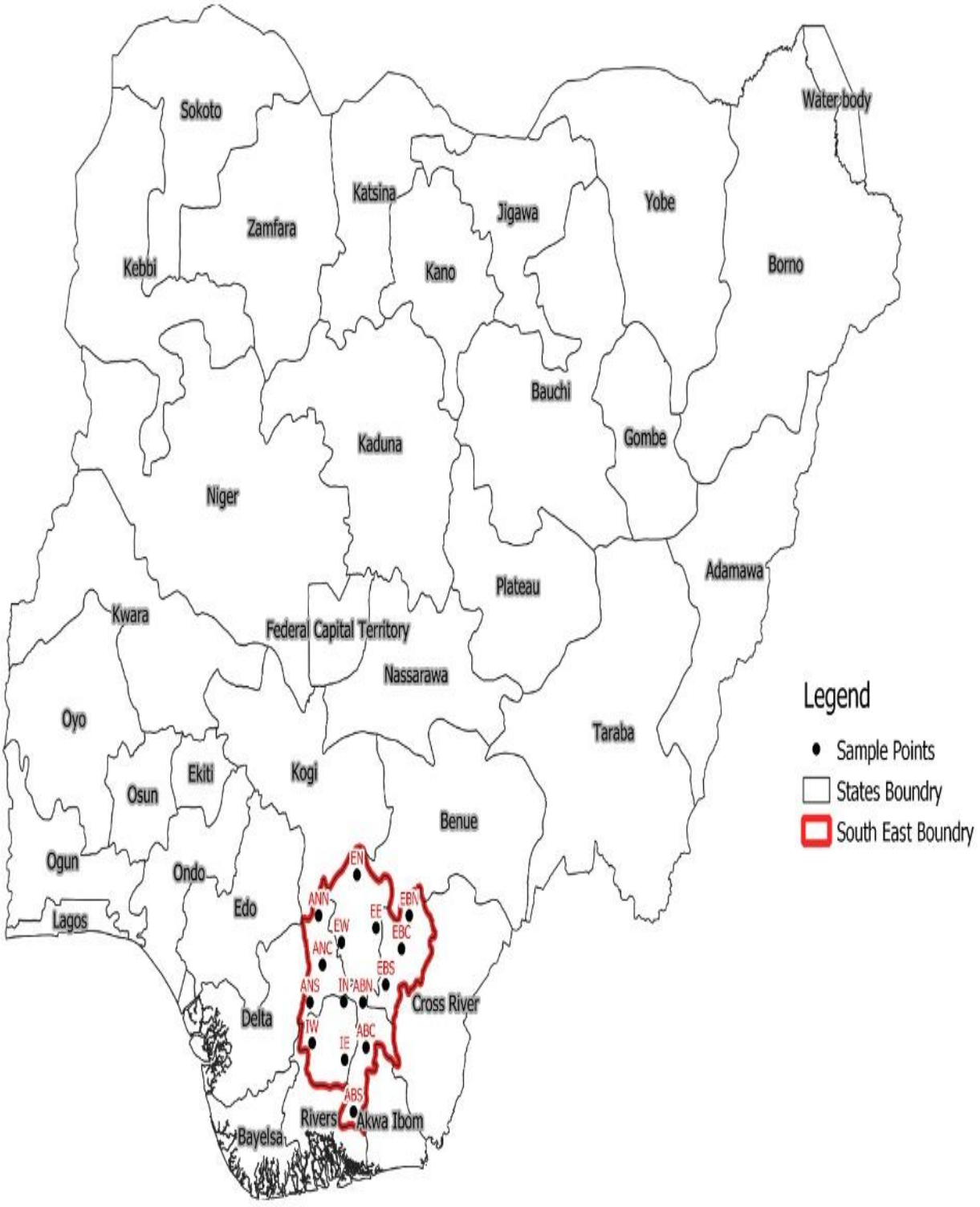
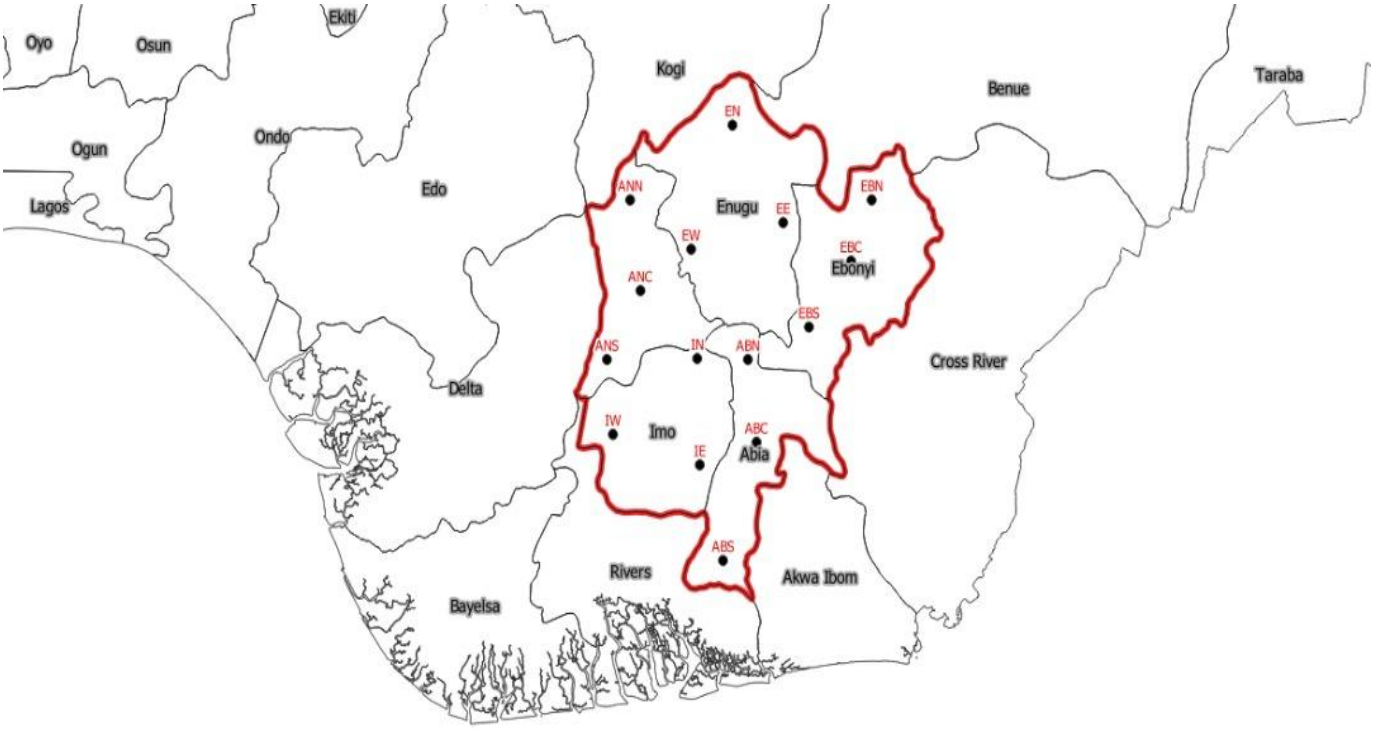


Fig 1: South eastern State of Nigeria with sampling points



Legend:

ANN: Anambra North
ANC: Anambra Central
ANC: Anambra South
IN: Imo State
IW: Imo West
IM: Imo East
EN: Enugu North
ES: Enugu South
EW: Enugu West
ABN: Abia North
ABC: Abia Central
ABS: Abia South
EBN: Ebonyi North
EBS: Ebonyi South
EBC: Ebonyi Central
_____South east boundary
_____State boundary

- **Sampling points**

Fig 2: South eastern Nigeria region with senatorial zones in each of the states

3.2 Ethno-study

Survey was conducted between February, 2015 and November, 2016 in five states of South Eastern Nigeria. The states were Enugu, Ebonyi, Abia, Anambra and Imo. A well structured questionnaire on post harvest handling practices of egusi (*Citrullus colocynthis*), ogbono (*Irvingia wombolu*) and cassava chips was designed and administered randomly to six hundred and seventy five (one hundred and thirty five from each state) local farmers/traders within the study area (Appendix 1). The questionnaire was made up of two sections: the first section was made up of information about the respondent while the second section which focused on the post-harvest handling practices included six major questions (items); this is to find out the practices that may likely predispose these food materials in the study area to aflatoxins, heavy metal contamination and fungal infestation (Appendix 1).

3.3. Design of the study

This experiment was a factorial experiment laid in a Complete Randomized Design (CRD). The independent variables were wave of collection/ sampling period, plant materials and location. The dependent variables include fungal occurrence, aflatoxin content, heavy metal concentration and phytochemical constituent.

3.4 Sample collection

The method of Adetunji *et al.* (2014) was adopted in sampling food materials; the food materials sampled were *I. wombolu, C. colocynthis* and cassava chips. The objective of the sampling was to obtain a small quantity of food material that represents the whole. Food samples that were locally sourced, sun dried, not sorted and had been stored for at least four months in their respective state of sampling were collected in dry season (February- April), wet season (May- August) and Harmattan (November- January) from designated markets and stores in the

three senatorial districts of each state. Using simple random sampling technique a total of three (3) markets from each senatorial zone were sampled, 50 g each of sample was collected from three (3) different sampling points from each market. Each of the plant materials purchased from all senatorial zones within a state were pooled together to form a single bulk, from where the secondary sample was made. Two hundred grams (200 g) working sample was drawn at random from the mixed samples. These working samples represented plant materials from the states in which they were collected. Hence a total of five samples per plant materials at each analysis time (dry season, wet season and harmattan) were used for the experiment. The plant materials that were used for microbial inhibition (*Moringa Oleifera* L. and *Chromolaena odorata* L.) were collected from the research quarters of the National Root Crops Research Institute Umudike.These plant materials were identified by Prof. R. N. Okigbo and authenticated by Mr Tochukwu Egboka of the Botany Department, Nnamdi Azikiwe University, Awka. The specimens were labeled, numbered and annotated with the date of collection and locality.

3.5. Sample Preparation

This was done according to the method of Anon (2002). The samples were air dried in their respective state of sampling. They were later weighed with weighing machine and ground to a powderd form. The samples were divided into three parts as follows: mycotoxin (40 g), fungal isolation (20 g) and Mineral (30 g). Each of the samples were labeled, packaged in polythene bag and taken to the laboratory for analysis.

3.6 Preparation of Culture Media

Potato Dextrose Agar (PDA) was the culture media used for microbial growth and maintenance. The preparation of the media was according to the specifications of the manufacturer as thus: Thirty nine gram (39 g) of PDA powder was dissolved in one litre of

distilled water in a flat bottom flask. This was swirled and melted by boiling in a heater, then sterilized with the aid of autoclave at a temperature of 121^0C for 15 minutes. The medium formed was cooled to a temperature of 47^0C and later poured into sterile Petri dishes where it was allowed to gel.

3.7. Isolation of Fungi from Milled Food Samples

One gram (1 g) from each sample was weighed on a sensitive metre scale. A test-tube plastic rack was arranged with 9ml of sterile test-tubes each containing 9 ml of sterile distilled water (SDW). A ten fold serial dilution (Fasole and Oso, 1988) was carried out by dispersing 1g sample into the first test-tube (10^{-1}) shaken together. 1ml was again taken from (10^{-1}) dilution and transfer to the next test tube (10^{-2}). The dilutions continue to (10^{-9}). Each test tube was shaken vigorously before transfer.

3.8. Inoculation

A pour plate method (Fasole and Oso, 1988; modified by Okigbo *et al.,* 2015) was used in plating all the samples. One mill (1ml) from dilution (10^{-9}) was dispensed into sterile Petri dish with the aid of a sterile pipette. A molten Potato Dextrose Agar was poured into the plates (about 10 ml). The plates were swirled for easy mix up of the sample and the media. All plates were allowed solidification on the bench. Each plated sample was duplicated.

3.9. Incubation

All inoculated plates were transferred into an incubator at 25^0C for 3-5 days. All plates were examined daily for mycelia growth.

3.10. Subculturing and purification

A flamed surgical blade was used for sub-culturing the myelia from Potato Dextrose Agar plates (PDA) into a newly prepared PDA plates for purification. All plates were incubated at 25^0C for 3-5days (Okigbo *et al*., 2012).

3.11. Identification of purified cultures

Macroscopic examination was done by physical characteristics of the mycelia-like structure and colour of the mycelia. Microscopic characteristics through the morphological structure according to (Mathur and Kongsdal, 2003; Bernette and Hunter, 1987) was employed. A wet mount method (Fasole and Oso, 1988) was done before viewing the isolates under ^40 compound microscope. The morphological structures viewed include septate or non-septate mycelia, presence of sporangiosphores, fruiting bodies and special organs like rhizoids. Each morphological structure of each isolates was matched with a mycology atlas for identification.

3.12. Determination of percentage of fungal occurrence

This was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from the three plant materials collected from five states in three waves. The number of occurrence for each of the isolates in each of the samples (plant materials) were recorded and calculated as a ratio of the total number of occurrence and was then expressed as a percentage. It was given by the formula below;

Percentage occurrence= x/n X 100/1

x= Total number of each organism in all the samples.

n = Total number of the entire organism in all the samples screened.

3.13. Analysis of Aflatoxin

The samples were screened and analyzed for aflatoxin B1, B2, G1 and G2. The Thin Layer Chromatographic method of the Association of Analytical Chemistry (AOAC) (1996) was adopted for the analysis.

3.13.1. Extraction of aflatoxin from cassava chips

Cassava chips samples were carefully ground with commercial waring blender (Waring blade series L HP blender, made in USA) and thoroughly mixed. Twenty gram (20g) of the ground sample was randomly picked and weighed out (in 3 replications) for extraction purpose. Twenty gram (20g) ground working sample was thoroughly mixed with 100 ml of 80% methanol for three minutes using waring blender. Blended mixture was poured into a 250 ml Pyrex conical flask, and the flask was sealed with parafilm. Mixture from the step above was shaking vigorously using orbit shaker at 4 x 100 rpm for 30 minutes. Thereafter, it was filtered into a clean conical flask (that had been previously rinsed with absolute methanol) using No 1 quantitative Whatman filter paper, 185 mm. The filtrate (40 ml) was poured into a separating flask. Twenty mills (20 ml) of distilled water was added. Twenty five mills **(**25 ml) of dichloromethane was also added. The separating flask containing the mixture was hand-shook gently and left for few minutes to separate (i.e. allow mixture to separate into top and bottom phases). The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker. To the remaining mixture in the separating flask, 10 ml of dichloromethane was added. This was hand-shook gently and allowed to separate. The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker (that contains the first extract). The extract was allowed to stay in the

fume hood to dry overnight. Dried extract was reconstituted with 1 ml of dichloromethane and transferred into a 1.5 ml eppendorf tube and left in the hood to dry overnight.

3.13.2. Extraction of aflatoxin from *I. wombolu* and *C. colocynthis*

Ogbono (*I. wombolu*) and melon (*C.colocynthis*) samples were carefully ground with commercial waring blender and thoroughly mixed. Twenty gram (20 g) of ground Ogbono or melon was randomly picked and weighed out (in 2 replications) for extraction purpose. Twenty gram (20 g) working sample of ogbono or melon was thoroughly mixed with 100 ml of 80% methanol for three minutes using waring blender. Blended mixture was poured into a 250 ml Pyrex conical flask, and the flask was sealed with parafilm. Mixture from step 4 above was shaking vigorously using orbit shaker at 4 x 100 rpm for 30 minutes. Thereafter, it was filtered into a clean conical flask (that had been previously rinsed with absolute methanol) using No 1 quantitative Whatman filter paper, 185 mm. The filtrate (40 ml) was poured into a separating flask. Fourty mill (40 ml) of 10% Sodium chloride was added. Twenty-five mill (25 ml) of hexane was also added. The mixture was shaking vigorously by hand for 1 minute and allowed to separate. The extract or the bottom phase was drained into a 250 ml conical flask and what remained in the separating flask was discarded. At this point, the filtrate was poured back into the cleaned separating flask. Twenty five mill (25 ml) of dichloromethane was added. The separating flask containing the mixture was hand-shook gently and left for few minutes to separate (i.e. allow mixture to separate into top and bottom phases). The extract or bottom phase was drained through a bed of 20 g *anhydrous sodium sulphate* into a 150 ml white plastic beaker. To the remaining mixture in the separating flask, 10 ml of dichloromethane was added. This was hand-shook gently and allowed to separate. The extract or bottom phase was drained through a bed of 20 g *anhydrous sodium sulphate* into a 150 ml white plastic beaker (that contains the first

extract). The extract was allowed to stay in the fume hood to dry overnight. Dried extract was reconstituted with 1 ml of dichloromethane and transferred into a 1.5 ml eppendorf tube and left in the hood to dry overnight.

3.13.3. Procedure for Quantitative assay of extracts

Reconstituted dried extract was dissolved. One ml dichloromethane and vortex to homogenize the mixture. High Performance Thin Layer Chromatography (HPTLC) plate to be used for spotting was calibrated according to the standard format. A cleaned micro-capillary tube was inserted into a bulb assembly through silicone tip and made sure it was firm. Carefully and gently the capillary tube was cleaned with acetone solution in three changes. Carefully, 4 ul of aflatoxin **G** and **I** standards was spotted on 6^{th} and 8^{th} marked spots respectively on calibrated HPTLC. Carefully, 4ul of each sample extract was spotted on remaining 1cm interval marked spots on the HPTLC plate. Spotted and air-dried plate was developed in a solution of diethyl ether, methanol and distilled water in ratio 96:3:1. The developed plates were viewed under the ultraviolet light- box (wavelength = 365 nm) to see whether each extract fluoresces or not. Those with fluorescence and those without are compared with the standards. Quantitatively, extracts were subjected to quantitative analysis to ascertain total amount aflatoxins (B_1, B_2, G_1 and G_2) in each of the samples. This was done with the aid of *CAMAG* Thin Layer Chromatography scanner 3; which enables quantitative evaluation of densitometric data to be generated.

3.14. Determination of Elements

3.14.1. Sample Digestion

One gram (1g) of each prepared sample was weighed into a 100ml beaker. 30mls of aqua-regia (a mixture of nitric acid and hydrochloric acid in the ratio 1:3) was measured in a 100ml measuring cylinder and added into the weighed samples. Ten (10) drops of hydrogen

peroxide was added to each of the preparation to increase the complexing power of the mineral acids. The samples in beakers were placed on a digital laboratory heating mantle under fume cupboard and heated at 1000^0C until the samples completely digest.

Each digest was allowed to cool and diluted with 50ml of distilled-deionised water. They were filtered into 100ml volumetric flask using whatman filter paper (125mm). The digests were made up to the 100ml mark using distilled-deionised.

3.14.2. Metals Determination

The digested samples were analyzed for metals (lead, copper, zinc and iron) using Atomic Adsorption Spectrophotometer (AAS) manufactured by Buck Scientific, 210VGP, USA. Values were reported in milligram per gram.

3.15. Preparation of plant extracts

Cold solvent extraction method of Doughari *et al.*,(2007) was used. The leaves of *Moringa oleifera* and *Chromolaena odorata* were washed with sterilized distilled water (SDW) and were air dried for 7 days. The dried samples were milled (separately) in a labouratory mill after which the ground samples were sieved to obtain powdered processed sample used for the extraction. Twenty five gram (25g), Fifty gram (50g), Seventy five gram (75g) and one hundred gram (100g) portion of each processed sample was mixed with 100 ml of water and 100ml of ethanol separately in a bottle to with corresponding concentrations of 25g/100ml, 50g/100ml, 75g/100ml and 100g/100ml concentrations respectively. The extracts were sieved through sterile cheese cloth and stored in a sterile cornical flask, which was later used for fungal mycelia growth inhibition.

3.16. Effect of plant extracts on fungal growth

Food poisoning technique method of Sangoyomi, (1995) modified by Okigbo *et al.,* (2009) was used to determine the effect of plant extracts on the mycelia growth of the six test fungi. One milliliter of each plant extract concentration (25g/100ml, 50g/100ml, 75g/100ml and 100g/100ml) was dispensed per Petri dish and 9ml of the media was added to each of the Petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA-extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10% extract concentration. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 5mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the test fungi. Plates dispensed with molten PDA and one ml of mancozeb inoculated with each test fungi served as the positive control. Blank agar plate (no extract) inoculated with the test fungi served as the negative control.

All the plates were incubated at 28 ± 2^{0}C for 7 days and observed daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Okigbo *et al.* (2012):

Percentage inhibition = R1-R2/R1*100

Where R1 is the farthest radial distance of pathogen in control plate while R2 is the farthest radial distance of pathogen in extract incorporated agar plates.

3.17. Phytochemical screening of plant extracts

To ascertain the presence of phytochemicals of interest in the plant extract, quantitative tests were conducted using different standard methods, the presence of Glycosides, Phytosterols, Flavonoid, Phenol, Alkaloid, Saponin, Tritepernoids and Tannins was determined.

3.17.1. Sample Preparation for phytochemical screening

Fresh leaf samples of *Chromolaena odorata* and *Moringa oleifera* were collected from the research quarters of the National Root Crops Research Institute Umudike and air dried under shade for one week. The dried leaf samples were pulverized using a blender and sieved using a sieve size 250µm. Samples were stored in reagent bottles. Twenty grams (20g) of prepared samples each were soaked in 100ml ethanol and distilled media separately for 24 hours and liquid extracts were collected in separate amber colored specimen bottles and stored in refrigerator prior to analysis.

3.17.2. Qualitative Analysis

3.17.2.1. Alkaloids

(a) Dragendorff's test: Two mills (2 ml) of the ethanolic / aqeous extract was mixed with 5 ml of distilled water, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

(b) Hager's test: Two mills (2 ml) of the ethanolic aqeous extract was taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow ppt confirms the presence of alkaloids.

3.17.2.2. Flavonoids

Shinoda's test: Two mills (2 ml) of ethanolic / aqeous extract was measured in a test tube, 10 drops of dilute hydrochloric acid (10%) followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.

3.17.2.3. Terpenoids: Liebermann - Burchard's test

Two mills (2 ml) of ethanol / aqueous extract was introduced into a test tube, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a pink colour indicates the presence of terpenoids.

3.17.2.4. Saponins

In a test tube containing about 5 ml of an ethanolic / aqueous extract, a drop of sodium bicarbonate solution (10%) was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicates the presence of saponins.

3.17.2.5. Steroids

(a) Liebermann-Burchard's test: Two mills (2 ml) of ethanolic / aqueous extract in test tube, 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicates the presence of steroids.

(b) Salkowski reaction: Two mills (2 ml) of ethanolic / aqueous extract was shaked with chloroform in a test tube, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

3.17.2.6. Glycosides

Molisch's test: Two mills (2 ml) of ethanolic / aqueous extract, 2-3 drops of Molisch's reagent was added, mixed and 2 ml of concentrated sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appear, indicating the presence of glycosides.

3.17.2.7. Tannins

Two mills (2 ml) of the ethanolic / aqueous extract, few drops of 5% w/v $FeCl_3$ solution was added. A green colour indicated the presence of gallotannins, while brown colour indicates the presence of pseudotannins.

3.17.3. Quantitative Analysis

3.17.3.1. Alkaliods

Alkaloid was determined according to the method of Harborne (1973) modified by Okigbo *et al.,* (2014). Two grams (2 g) of the sample powder was weighed into a 250 ml beaker. 100 ml of 10% acetic acid in ethanol was added (10 ml of acetic acid in ethanol) and allowed to stand for four hours. The mixture was shaked in a rotatory shaker during this period. After this period, the mixture was filtered. Ammonium hydroxide was added drop wise until complete precipitation. It was allowed to precipitate over night after which it was filtered using a weighed filter paper. The precipitate collected in the filter paper is the alkaloid. The filter paper was dried in hot air oven at 60^0C and allowed to cool. The filter paper was weighed.

$$\% \text{ Alkaloid} = \frac{(\text{wt of filter paper and precipitate}) - (\text{wt of empty filter paper})}{\text{wt of sample used}} \times 100$$

3.17.3.2. Flavonoids

Flavonoid was determined according to the method of Soni and Sosa, (2013). Two grams (2 g) of the sample powder was weighed into a 250 ml beaker and extracted three times with 100 ml of 80% aqueous methanol for one hour each. The whole solution was filtered through

whatman No.1 filter paper. The filtrate was poured into a weighed beaker and heated to dryness in water bath at 100^0c.

$$\% \text{ Flavonoid} \quad \frac{(\text{wt of beaker and extract}) - (\text{wt of empty beaker})}{\text{wt of sample used}} \times 100$$

3.17.3.3. Terpenoids

Terpenoids was determined according to the method of Ferguson, 1956 modified by Obadoni and Ochuko (2001).. Two grams (2 g) of the sample powder was weighed into a conical flask and soaked with 50ml of ethanol for 24 hours. The solution was filtered and filtrate introduced into a 250 ml separating funnel. The filtrate was washed with petroleum ether. The ether extract was poured into a weighed beaker and heated to dryness at 100^0C.

$$\% \text{ Terpenoid} = \frac{(\text{wt of beaker and extract}) - (\text{wt of empty beaker})}{\text{wt of sample used}} \times 100$$

3.17.3.4. Saponin

Saponin composition was determined using the gravimetric method of Nweze and Nwafor, (2014), 200 ml of 20% aqueous ethanol (40 ml of ethanol and 160 ml of distilled water) was added to 2g of the sample powder and stirred using a magnetic stirrer for 4 hours at 55° C. The solution was filtered using Whatman No 1 filter paper and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extract was reduced to 40 ml in water bath at 100^0C. 40 ml Diethyl ether was added in a separating funnel and shaken vigorously. The ether layer, which is the upper layer, was discarded while the pH of the aqueous solution was adjusted to 4.5 by adding 0.1M NaOH. 60 ml of n-butanol was finally used for extraction. The lower portion was discarded. The Butanol extract (the upper layer) were washed twice with 10ml

of 5 % NaCl (the lower layer was discarded). The upper layer was finally washed with distilled water twice. The upper layer was poured into a weighed 250 ml beaker and evaporated to dryness at 100^0c in a fume cupboard to give a crude saponin which was weighed.

$$\%\text{Saponin} = \frac{(\text{Wt of beaker and extract}) - (\text{Wt of empty beaker})}{\text{Wt of sample used}} \times 100$$

3.17.3.5. Glycosides

Method of Harbone (1973) modified by Edeoga *et al.*, (2005) was adopted, two grams (2 g) of sample was weighed into a 250 ml beaker and soaked with 100ml of distilled water for three hours. The solution was filtered and 1ml of the filtrate was measured into a clean glass test tube. Two mills (2ml) of dinitrocylsalic acid (DNS) was added and warmed for five minutes in water bath. The test tube was allowed to cool and absorbance read in ultra violet spectrophotometer at 540nm. The concentration was determined using a standard glycoside graph.

$$\%\ \text{Glycoside} = \text{concentration} \times \text{dilution} \times 100$$

That is;

$$\%\ \text{Glycoside} = y/2g \times 2g/100ml \times 100$$

3.17.3.6. Tannins

Spectrophotometric method of Oludoru *et al.* (2012) was used to determine tannin. Two grams (2g) of the sample extract was weighed into a conical flask. 20ml of 50% aqueous methanol was added to it and placed in a water bath at 77^0c for an hour and shaked. The solution was filtered with a whatman No.1 filter paper. 0.5ml of sample filtrate was added to 0.5ml ferric

solution in alkaline medium and allowed to stand for 30 minutes for colour development. The absorbance of the solution was read at a wave length of 760 nm using ultra violet spectrophotometer. The concentration of tannin was determined a tannic acid standard graph.

% Tannin = concentration × dilution × 100

That is;

$$\% \text{ Tannin} = y/2g \times 2g/20ml \times 100$$

3.18. Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test (1955) at 0.05 probability level to determine significant differences among the samples obtained from the different locations and periods. Descriptive statistics such as frequency counts and percentiles were used to describe the data collected for the survey.

CHAPTER FOUR

RESULTS

4.1. Ethno-study

4.1.1. Methods of drying food materials

With respect to the methods of drying food materials, in Enugu state, 45 respondents representing 33.3% stated that they sun dry Cassava chips, while 35% representing 29.9% stated that they sun dry egusi (*C. colocynthis*) and ogbono (*I. wombolu*), only 3 respondents representing 2.2% affirmed that they oven dry ogbono while 10 and 7 respondents representing 7.4% and 5.2 stated that they use crib in drying *C. colocynthis* and *I. wombolu* respectively, crib was not used by any of the respondents to dry cassava chips. In all, 115 respondents representing 85.1% sun dry food materials while 3 respondents (2.2%) oven dry food materials, 17 respondents (12.6%) use cribs for drying food materials. In Anambra State, 45 respondents representing 33.3% stated that they sun dry all the food materials while oven and cribs were not used by respondents from the state, hence all the 135 respondents (99.9%) from Anambra state sun dry food materials. In Imo state, 45 respondents (33.3%) stated that they sun dry cassava chips while 31 and 38 respondents representing 23.0% and 28.1% respectively stated that they sun dry *C. colocynthis* and *I. wombolu* respectively. Seven respondents (5.2%) each stated that they oven dry equsi, while respondents from Imo state do not oven dry *I. wombolu* and cassava chips. Respondents from Imo state do not use crib in drying cassava chips while seven respondents representing 5.2% use crib in drying *C. colocynthis* and *I. wombolu*. A total of 114 respondents (84.4%) in Imo state sun dry food materials while 7 respondents representing 5.2% oven dry their food materials, 14 respondents (10.4%) use crib.

For Abia state, 45 respondents each representing 33.3% said that they sun dry *I. wombolu* and cassava chips while 34 respondents (25.2%), respondents from Abia state that they do not oven dry or use crib for drying *I.wombolu* and cassava chips while for *C.colocynthis* 9 respondents (6.7%) oven dry it and 2 respondents (1.5%) use cribs for drying egusi in Abia state. In all 114 respondents (84.4%) sun dry food materials, 7 respondents (5.2%) oven dry while 14 respondents representing 10.4% use crib.

In Ebonyi state, 27 respondents representing 20% sun dry *C.colocynthis*, while 22 respondents and 20 respondents representing 16.3% and 14.8% sun dry *I.wombolu* and cassava chips respectively. Nine respondents (67%) use oven for drying *C.colocynthis* while 11 and 12 respondents respectively representing 8.1% and 8.9% use oven for drying *I.wombolu* and cassava chips. Nine respondents (6.7%) use crib, while 12 and 13 respondents representing 8.9% and 9.6% use crib for drying *I.wombolu* and cassava chips. In all, 69 respondents (51.1%) sun dry food material, while 32 and 34 respondents representing 23.7% and 25.2 use crib for drying *I.wombolu* and cassava chips (Table 1).

Table 1: Methods of drying food materials

States	Food samples	Drying Method					
		Sun drying		Oven drying		Use of cribs	
		Freq	**%**	**Freq**	**%**	**Freq**	**%**
Enugu	*C. colocynthis*	35	25.9	0	0.0	10	7.4
	I.wombolu	35	25.9	3	2.2	7	5.2
	Cassava chips	45	33.3	0	0.0	0	0.0
	Total	**115**	**85.1**	**3**	**2.2**	**17**	**12.6**
Anambra	*C. colocynthis*	45	33.3	0	0.0	0	0.0
	I.wombolu	45	33.3	0	0.0	0	0.0
	Cassava chips	45	33.3	0	0.0	0	0.0
	Total	**135**	**99.9**	**0**	**0.0**	**0**	**0.0**
Imo	*C. colocynthis*	31	23.0	7	5.2	7	5.2
	I.wombolu	38	28.1	0	0.0	7	5.2
	Cassava chips	45	33.3	0	0.0	0	0.0
	Total	**114**	**84.4**	**7**	**5.2**	**14**	**10.4**
Abia	*C. colocynthis*	34	25.2	9	6.7	2	1.5
	I.wombolu	45	33.3	0	0.0	0	0.0
	Cassava chips	45	33.3	0	0.0	0	0.0
	Total	**124**	**91.8**	**9**	**6.7**	**2**	**1.5**
Ebonyi	*C. colocynthis*	27	20.0	9	6.7	9	6.7
	I.wombolu	22	16.3	11	8.1	12	8.9
	Cassava chips	20	14.8	12	8.9	13	9.6
	Total	**69**	**51.1**	**32**	**23.7**	**34**	**25.2**

4.1.2. Duration of drying food materials before storage

For the period of drying food materials before storage, in Enugu state, 25 respondents (18.5%) dry *I.wombolu* for 1 day while 22 and 14 respondents representing 16.3% and 10.4% respectively dry *C. colocynthis* and cassava chips for just 1 day. Twenty seven respondents representing 20.0% dry cassava chips for two days while 14 and 9 respondents representing representing 10.4% and 6.7% respectively dry *I.wombolu* and *C. colocynthis* for 2 days. Eight respondents (5.9%) stated that they dry egusi for 1 week before they store it, 5 respondents (3.7%) confirmed that they dry *I.wombolu* for 1 week while 4 respondents representing 3.0% asserted that thay dry cassava chips for 1 week. None of the respondents from Enugu state stated that they dry cassava chips for more than 1 week, 6 (4.4%) respondents agreed that they dry *C. colocynthis* for morethan 1 week; just 1 respondent from Enugu stated that *I.wombolu* was dried for morethan 1 week. In all, 61 (45.2%) respondents from Enugu state dry food materials for just 1 day, 50 (37.1%) dry food materials for 2 days before storage, 17 (12.6%) stated that they dry food materials for 1 week before storage while 7 respondents representing 5.1% stated that they dry food materials for morethan 1 week before storage. In Anambra state, 22 respondents (8.9%) agreed that they dry cassava chips for just 1 day, while 13 and 12 respondents representing 9.6% and 8.9% agreed that they dry *I.wombolu* and *C.colocynthis* for 1 day. With respect to two days of drying 19 respondents (14.1%) dry *I.wombolu* for 2 days while 17 respondents (12.6%) dry *C. colocynthis* for two days and 12 respondents (8.9%) dry cassava chips for 2 days. With respect to 1 week of drying, 12, 11 and 9 respondents representing 8.9%, 8.1% and 6.7% respectively dry *C.colocynthis*, cassava chips and *I.wombolu* for 1 week before they store it. Respondents from Anambra state do not dry cassava chips for morethan 1 week while 5 and 3 respondents representing 3.7% and 2.2% stated that they dry food materials for morethan 1 week, In all 47

respondents (34.8%) dry food materials for just 1 day, 48 respondents (35.6%) dry food materials for 2 days, while 32 (23.7%) and 8 (5.9%) respondents dry food materials for 1 week and morethan 1 week respectively. In Imo state, 15 respondents each represent 11.5% dry *C.colocynthis* and cassava chips for 1 day while respondents (6.9%) dry *I.wombolu* for just 1 day. With respect to 2 days of drying 29 respondents representing 22.3% dry *I.wombolu* for 2 days while 20 respondents representing 15.4% dry cassava chips for 2 days, with respect to 1 week of drying, 15 respondents representing 11.5% dry *C.colocynthis* for 1 week, while 3 respondents representing 2.3% dry cassava chips for 1 week before storage. Respondents from Imo state, do not dry egusi and ogbono for more than 1 week while only 2 respondents (1.5%) dry cassava chips for morethan 1 week before storage. In Abia state, 38 respondents (28.1%) dry cassava chips for just 1 day, while 36 and 31 respondents representing 26.7% and 23.0% respectively dry *I.wombolu* and *C.colocynthis* for just 1 day. With respect to 2 days of drying 9 respondents (6.7%) agreed that they dry egusi for just 2 days, while 2 and 7 respondents representing 1.5% and 5.2% respectively affirmed that they dry *I.wombolu* and *C.colocynthis* for 2 days. Respondents from Abia do not dry cassava chips for 1 week, while 7 and 5 respondents representing 5.2% and 3.7% respectively dry *I.wombolu* and *C.colocynthis* for 1 week. All the respondents from Abia state stated that they do not dry any of the food materials for morethan 1 week. In all 105 respondents representing 77.8% from Abia state stated that they dry food materials for just 1 day, 18 and 12 respondents representing 13.4% and 8.9% respectively agreed that they dry food materials for 2 days and 1 week respectivey. In Ebonyi state, 36 repondents representing 26.7% *I.wombolu* for just 1 day while 31 and 22 respondents representing 23% and 16.3% dry *C.colocynthis* and cassava chips for just 1 day. Five (3.7%) respondents each stated that they dry *C.colocynthis* and *I.wombolu* representing for 2 days while 13 respondents

representing 9.6% dry cassava chips for 2 days. With respect to1 week of drying, 9 respondents representing 6.7% stated that they dry *C. colocynthis* and cassava chips for 1 week each while *I.wombolu* was not dried for 1 week. Respondents from Ebonyi state do not dry food materials for morethan 1 week except *I.wombolu* with only 5 respondents representing 3.7% affirmed that they dry for morethan 1 week. In all, 89 (66.0%) respondents from Ebonyi dry food materials for just 1 day, 23 (17.0%) dry food materials for 2 days, 18 (13.4%) respondents dry food materials for 1 week while only 5 respondents representing 3.7% dry food materials for morethan 1 week (Table 2).

Table 2: Duration of drying food materials before storage

State	Food samples	Drying Period							
		1day		2 days		1 week		More than 1 week	
		Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
Enugu	*C. colocynthis*	22	16.3%	9	6.7	8	5.9	6	4.4
	I.wombolu	25	18.5%	14	10.4	5	3.7	1	0.7
	Cassava chips	14	10.4%	27	20.0	4	3.0	0	0.0
	Total	**61**	**45.2%**	**50**	**37.1**	**17**	**12.6**	**7**	**5.1**
Anambra	*C. colocynthis*	13	9.6%	17	12.6	12	8.9	3	2.2
	I.wombolu	12	8.9%	19	14.1	9	6.7	5	3.7
	Cassava chips	22	16.3%	12	8.9	11	8.1	0	0.0
	Total	**47**	**34.8%**	**48**	**35.6**	**32**	**23.7**	**8**	**5.9**
Imo	*C. colocynthis*	15	11.5	15	11.5	15	11.5	0	0.0
	I.wombolu	9	6.9	29	22.3	7	5.4	0	0.0
	Cassava chips	15	11.5	20	15.4	3	2.3	2	1.5
	Total	**39**	**29.9**	**64**	**49.2**	**25**	**19.2**	**2**	**1.5**
Abia	*C. colocynthis*	31	23.0	9	6.7	5	3.7	0	0.0
	I.wombolu	36	26.7	2	1.5	7	5.2	0	0.0
	Cassava chips	38	28.1	7	5.2	0	0.0	0	0.0
	Total	**105**	**77.8**	**18**	**13.4**	**12**	**8.9**	**0**	**0.0**
Ebonyi	*C. colocynthis*	31	23.0	5	3.7	9	6.7	0	0.0
	I.wombolu	36	26.7	5	3.7	0	0.0	5	3.7
	Cassava chips	22	16.3	13	9.6	9	6.7	0	0.0
	Total	**89**	**66.0**	**23**	**17.0**	**18**	**13.4**	**5**	**3.7**

4.1.3. Sorting out of contaminated food materials

With respect to sorting out of contaminated food materials, In Enugu state only a few respondents stated that they sort out contaminated food materials based on visual assessment,13 respondents (9.6%) sort out *I.wombolu* while 7 (5.2%) respondents sort *C.colocynthis*, majority of the respondents (23-27 representing 17% and 20%) in Enugu do not always sort out contaminated food materials. In Anambra State, many of the respondents (45 for cassava chips, 30 for *I.wombolu* and 20 for *C. colocynthis* representing 33.3%, 22.25 and 14.8% respectively) stated that they do not sort out these food materials while 20 (14.8%) respondents and 15 (11.1%) respondents affirned that they sort *C.colocynthis* and *I.wombolu* respectively. In Imo state most of the respondents (23-40 representing 17% -29.6%) do not sort out contaminated food materials, while 13 (9.6%) and 17 (12.6%) respondents stated that they sort out *I.wombolu* and *C.colocynthis* respectively, 5 respondents from Abia state representing 3.7% stated that they do not always sort out *C.colocynthis, I. wombolu* and cassava chips. In Abia state, 13 (9.6%), 6 (4.4%) and 17 (12.8%) stated that they sort out *C.colocynthis*, *I. wombolu* and cassava chips respectively, while 23 (17.0%), 34 (25.5%) and 28 (20.7%) respondents from Anambra state affirmed that they do not sort out *C.colocynthis*, *I. wombolu* and cassava chips respectively. Majority of the respondents from Ebonyi state, do not sort out contaminated food materials, 31 respondents representing 23% stated that they do not sort out cassava chips, 22 (16.3%) respondents do not sort out contaminated *C.colocynthis* while 14 (10.4%) respondents do not sort out contaminated *I.wombolu*. Only 20 (14.8%), 24 (17.8%) and 9 (6.7%) of the respondents agree that they sort out contaminated *C.colocynthis*, *I. wombolu* and cassava chips respectively before marketing (Table 3).

Table 3: Sorting out of contaminated food materials

States	Food sample	Sorting					
		Yes		No		Not Always	
		Freq	**%**	**Freq**	**%**	**Freq**	**%**
Enugu	*C. colocynthis*	7	5.2	15	11.1	23	17.0
	I.wombolu	13	9.6	5	3.7	27	20.0
	Cassava chips	0	0.0	45	33.3	0	0.0
	Total	**20**	**14.8**	**65**	**48.1**	**50**	**37.0**
Anambra	*C. colocynthis*	20	14.8	20	14.8	5	3.7
	I.wombolu	15	11.1	30	22.2	0	0.0
	Cassava chips	0	0.0	45	33.3	0	0.0
	Total	**35**	**25.9**	**95**	**70.3**	**5**	**3.7**
Imo	*C. colocynthis*	13	9.6	27	20.0	5	3.7
	I.wombolu	17	12.6	23	17.0	5	3.7
	Cassava chips	0	0.0	40	29.6	5	3.7
	Total	**30**	**22.2**	**90**	**66.6**	**15**	**11.1**
Abia	*C. colocynthis*	13	9.6	23	17.0	9	6.7
	I.wombolu	6	4.4	34	25.2	5	3.7
	Cassava chips	17	12.6	28	20.7	0	0.0
	Total	**36**	**26.6**	**85**	**62.9**	**14**	**10.4**
Ebonyi	*C. colocynthis*	20	14.8	22	16.3	3	2.2
	I.wombolu	24	17.8	14	10.4	7	5.2
	Cassava chips	9	6.7	31	23.0	5	3.7
	Total	**53**	**39.3**	**67**	**49.7**	**15**	**11.1**

4.1.4. Storage materials

For storage materials, in Enugu, *C. colocynthis* is mainly stored in basket (17 respondents representing 12.6%), *I.wombolu* is mainly stored in Polythene bag (17respondents representing 12.6%) while cassava chips is manly stored in the basket (26 respondents representing 19.3%). In all food materials fromEnugu are mainly stored in basket and jutr bag container with 56 and 33 respondents representing 41.5% and 24.4% respectively agreeing to this, In Anambra state, *C. colocynthis* is mainly stored in plastic container and jute bad (13 and 11 respondents representing 9.6% and 11% respectively), *I.wombolu* is mainly stored in jute bag and basket (15 and 16 respondents representing 11.1% and 11.9% respectively), cassava chips is mainly stored in jute bag (22 respondents representing 16.3%). In all, food materials from Anambra are mainly stored in jute bage and in basket with values of 48 and 28 respondents representing 35.5% and 20.8 respectively.In Imo state, all the food materials are mainly stored in basket (22-36 respondents representing 16.2% - 26.5% respectively), in all 85 respondents representing 62.6% store food materials in basket, 20 (14.7%) respondents stated that they store food materials in polythene bag while 12 respondents representing 8.9% affirmed that they store food materials in plastic container. In Abia state, *C. colocynthis* is mainly stored in Jute bag and basket (14 and 13 respondents representing 10.4% and 9.6% respectively) agreed to this, *I.wombolu* is mainly stored in polythene bag and jute bag (13 respondents each representing 9.6%) while cassava chips is mainly stored in basket (23 respondents representing 17%). In all, majority of the respondents from Abia state stated that they store food materials in basket and jute bag, 48 and 44 respondents representing 35.5% and 32.6% agreed that they store food materils in basket and jute bag respectively. In Ebonyi state, *C. colocynthis* is mainly stored in basket (16 respondents representing 11.9%) while *I.wombolu* is mainly stored with polythene bag (20 respondents

representing 14.8%), cassava chips is mainly stored in jute bag (15 respondents representing 11.1%), in all, majority of the respondents from Ebonyi state, stated that they polythene bag and in basket, 43 (31.8%) and 34 (25.2%) respondents affirmed that they store food materials in polythene bag and basket respectively (Table 4).

Table 4: Storage materials

State	Food Sample	Storage materials							
		Polythene Bag		Plastic Container		Jute Bag		Basket	
		Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
Enugu	*C. colocynthis*	9	6.7	7	5.2	12	8.9	17	12.6
	I.wombolu	17	12.6	0	0.0	15	11.1	13	9.6
	Cassava chips	13	9.6	0	0.0	6	4.4	26	19.3
	Total	**39**	**28.9**	**7**	**5.2**	**33**	**24.4**	**56**	**41.5**
Anambra	*C. colocynthis*	9	6.7	13	9.6	11	8.1	12	8.9
	I.wombolu	9	6.7	5	3.7	15	11.1	16	11.9
	Cassava chips	11	8.1	12	8.9	22	16.3	0	0.0
	Total	**29**	**21.5**	**30**	**22.2**	**48**	**35.5**	**28**	**20.8**
Imo	*C. colocynthis*	9	6.6	5	3.7	9	6.6	22	16.2
	I.wombolu	9	6.6	5	3.7	5	3.7	27	19.9
	Cassava chips	2	1.5	2	1.5	5	3.7	36	26.5
	Total	**20**	**14.7**	**12**	**8.9**	**19**	**14.0**	**85**	**62.6**
Abia	*C. colocynthis*	9	6.7	9	6.7	14	10.4	13	9.6
	I.wombolu	13	9.6	7	5.2	13	9.6	12	8.9
	Cassava chips	5	3.7	0	0.0	17	12.6	23	17.0
	Total	**27**	**20.0**	**16**	**11.9.**	**44**	**32.6**	**48**	**35.5**
Ebonyi	*C. colocynthis*	13	9.6	7	5.2	9	6.7	16	11.9
	I.wombolu	20	14.8	5	3.7	9	6.7	11	8.1
	Cassava chips	10	7.4	13	9.6	15	11.1	7	5.2
	Total	**43**	**31.8**	**25**	**18.5**	**33**	**24.5**	**34**	**25.2**

4.1.5. Storage Period before marketing

For storage period before marketing, in Enugu majority of the respondents (27 representing 20%) store *C. colocynthis* between 4-6 months while just 5 (3.7%) respondents stated that they store egusi over nine months, *I. wombolu* and cassava chips also, were stated by most of the respondents that they store between 4-6 months. In all majority of the respondents from Enugu state (71 representing 52.6%) store food materials for 4-6 months, while only 12 respondents (8.9%) store food materials for morethan 9 months before marketing. In Anambra state, *C. colocynthis* is stored for over 9 months before marketing by most of the people interviewed (17 respondents representing 12.6%), *I. wombolu* and cassava chips are mainly stored for 7-9 months by most of the respondents, with 24 (17.8%) and 12 (8.9) respondents agreeing that they store *I.wombolu* and cassava chips for 7-9 months before storage, in all majority of the respondents (49 representing 36.3%) stores food materials for 7-9 months before marketing, while 40 (29.6%) and 30 (22.2%) store food materials for 4-6 months and above 9 months respectively before marketing. In Imo state, all the food materials (*C.colocynthis*, *I. wombolu* and cassava chips) are mainly stored by most of the respondents (17 representing 12.6%) for 1-3 months, across all the food samples, 51 respondents representing 37.8% store food materials for 1-3 months while 34 (25.1%) and 33 (24.5%) store food materials for 4.6 months and 7-9 months respectively, only 17 respondents (12.6%) stated that they store food materials above 9 months. In Abia state, majority of the food materials are stored between 7-9 months before marketing 22 respondents agreed to this representing 16.3%, 7 (5.2%) respondents each stated that they store *C.colocynthis*, *I. wombolu* and cassava chips for just 1-3months another 7 (5.2%) respondents said that they stores all these food materials for morethan 9 months before marketing, while 9 respondents representing 6.3% said that they store these food

materials for 4-6months before marketing. In Ebonyi state, most of the food materials are stored 1-3 months before marketing, 22 respondents representing 16.3% each agreed that *C.colocynthis, I. wombolu* are stored for 1-3 months while 34% representing 25.2% stated that *C.colocynthis* is stored for just 1-3 months before marketing, total across all the food samples depicted that 78 (57.8%) of respondents from Ebonyi state store food materials for 1-3 months, while 33 (24.5 respondents) store food materials above 9 months before marketing, just 6 (4.5%) respondents said that they store food materials for 4-6 months (Table 5).

Table 5: Storage Period before marketing

States	Food Samples	Storage Period							
		1-3 Months		**4-6 Months**		**7-9 Months**		**Above 9 Months**	
		Freq	%	Freq	%	Freq	%	Freq	%
Enugu	*C. colocynthis*	8	5.9	27	20.0	5	3.7	5	3.7
	I.wombolu	10	7.4	25	18.5	5	3.7	5	3.7
	Cassava chips	17	12.6	19	14.1	7	5.2	2	1.5
	Total	**35**	**25.9**	**71**	**52.6**	**17**	**12.6**	**12**	**8.9**
Anambra	*C. colocynthis*	10	7.4	5	3.7	13	9.6	17	12.6
	I.wombolu	1	0.7	7	5.2	24	17.8	13	9.6
	Cassava chips	5	3.7	28	20.7	12	8.9	0	0.0
	Total	**16**	**11.8**	**40**	**29.6**	**49**	**36.3**	**30**	**22.2**
Imo	*C. colocynthis*	17	12.6	11	8.1	12	8.9	5	3.7
	I.wombolu	17	12.6	12	8.9	9	6.7	7	5.2
	Cassava chips	17	12.6	11	8.1	12	8.9	5	3.7
	Total	**51**	**37.8**	**34**	**25.1**	**33**	**24.5**	**17**	**12.6**
Abia	*C. colocynthis*	7	5.2	9	6.7	22	16.3	7	5.2
	I.wombolu	7	5.2	9	6.7	22	16.3	7	5.2
	Cassava chips	7	5.2	9	6.7	22	16.3	7	5.2
	Total	**21**	**15.6**	**27**	**20.1**	**66**	**48.9**	**21**	**15.6**
Ebonyi	*C. colocynthis*	22	16.3	2	1.5	5	3.7	16	11.9
	I.wombolu	22	16.3	2	1.5	9	6.7	12	8.9
	Cassava chips	34	25.2	2	1.5	4	3.0	5	3.7
	Total	**78**	**57.8**	**6**	**4.5**	**18**	**13.4**	**33**	**24.5**

4.1.6. Use of Pesticides

With respect to the use of pesticides all the respondents across the states and food samples stated they do not apply pesticides on these food materials.

4.2. Frequency of occurrence of isolated fungi

A total of six fungi (*Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Fusarium oxysporium, Penicillium spp* and *Trichodema viride*) were consistently isolated from the three plant samples. All the six fungi were present in samples collected during Dry Season. For harmattan all the fungi were isolated except *Rhizopus stolonifer* and *Penicillium spp. Aspergillus niger* and *Aspergillus flavus* had the highest frequency of occurrence of 30.27% and 27.55% respectively while *Penicillium spp* had the least frequency of occurrence of 4.04% (Table 6).

Table 6: Frequency of occurrence (%) of isolated fungi from food samples.

Fungal Isolate	% occurrence			
	Wave of collection			
	DS	WS	HM	Mean
Aspergillus niger	30.2	33.33	27.27	30.27
Aspergillus flavus	21.3	25	36.36	27.55
Rhizopus spp	15.15	8.33	-	7.83
Fusarium spp	12.12	25	27.27	21.46
Penicillium spp	12.12	-	-	4.04
Trichoderma spp	9.09	8.33	9.09	8.84

Key: DS= Dry Season, WS= wet Season, HM= harmattan

The number of fungi isolated varies across the food samples, state of collection and wave of collection. More fungi were isolated from *I. wombolu* and *C. colocynthis* than from *M. esculenta* (Cassava chips). With respect to the wave of collection, more fungi were isolated during Wet Season and Dry Season than during Harmattan. *A.niger* and *A.flavus were* isolated from 24 and 17 samples respectively, while *Rhizopus* spp and *Trichoderma* spp were isolated from 5 samples each, *Penicillium* spp was isolated from only 3 samples(Table 7).

Table 7: Number of fungi isolated from each sample

Wave of collection	State	Food sample	Number of fungi isolated
DS	Abia	*C. colocynthis*	1
DS	Abia	Cassava chips	1
DS	Abia	*I.wombolu*	2
DS	Enugu	*C. colocynthis*	1
DS	Enugu	Cassava chips	2
DS	Enugu	*I.wombolu*	2
DS	Ebonyi	*C. colocynthis*	2
DS	Ebonyi	*I.wombolu*	4
DS	Ebonyi	Cassava chips	2
DS	Anambra	*C. colocynthis*	3
DS	Anambra	*I.wombolu*	3
DS	Anambra	Cassava chips	1
DS	Imo	*C. colocynthis*	2
DS	Imo	*I.wombolu*	3
DS	Imo	Cassava chips	1
WS	Abia	*C. colocynthis*	2
WS	Abia	Cassava chips	ND
WS	Abia	*I.wombolu*	2
WS	Enugu	*C. colocynthis*	2
WS	Enugu	Cassava chips	1
WS	Enugu	*I.wombolu*	1
WS	Ebonyi	*C. colocynthis*	1
WS	Ebonyi	*I.wombolu*	1
WS	Ebonyi	Cassava chips	1
WS	Anambra	*C. colocynthis*	3
WS	Anambra	*I.wombolu*	4
WS	Anambra	Cassava chips	1
WS	Imo	*C. colocynthis*	1
WS	Imo	*I.wombolu*	1
WS	Imo	Cassava chips	ND
HM	Abia	*C. colocynthis*	2
HM	Abia	Cassava chips	ND
HM	Abia	*I.wombolu*	ND
HM	Enugu	*C. colocynthis*	2
HM	Enugu	Cassava chips	1
HM	Enugu	*I.wombolu*	2
HM	Ebonyi	*C. colocynthis*	1
HM	Ebonyi	*I.wombolu*	1
HM	Ebonyi	Cassava chips	1
HM	Anambra	*C. colocynthis*	2
HM	Anambra	*I.wombolu*	1
HM	Anambra	Cassava chips	1
HM	Imo	*C. colocynthis*	1
HM	Imo	*I.wombolu*	1
HM	Imo	Cassava chips	ND

Key: DS= Dry Season, WS= Wet Season, HM= Dry harmattan, ND= Not Detected

4.3. Concentration of Aflatoxins in food samples

For the comparison of aflatoxins in food samples, there was significant difference in the concentrations of AFB1 across all the food samples, the highest value of AFB1(0.154±0.012 parts per billion (ppb) was observed from *I.wombolu*, next to this was 0.090±0.038 ppb recorded from *C. colocynthis* and the least concentration (0.018±0.063 ppb) was observed from cassava chips. For AFB2, the highest value was detected in *C. colocynthis* (0.136±0.001ppb) this was significantly higher than 0.014±0.056ppb and 0.034±0.073ppb recorded from cassava chips and *C. colocynthis* respectively. *I. wombolu* has the highest concentration of AFG1 (0.073±0.018 ppb), the least in AFG1 was cassava chips (0.001±0.003 ppb). AFG2 was not detected *I. wombolu*, while very small quantities of 0.0067±0.045 ppb and 0.0004±0.000 ppb were observed in cassava chips and *C. colocynthis* respectively. For total aflatoxins, there was no significant difference between the values recorded from *C. colocynthis* (0.234±0.278 ppb) and *I. wombolu* (0.262±0.274 ppb), the least concentration of total Aflatoxin was recorded from cassava chips (Table 8).

Table 8: Comparison of Aflatoxins in the Food Samples

Food Samples	Aflatoxin Concentration (ppb)				
	B1	B2	G1	G2	Total
cassava chips	0.018±0.063^{c}	0.014±0.056^{b}	0.001±0.003^{b}	0.0067±0.045^{a}	0.040±0.091^{b}
C.colocynthis	0.090±0.038^{b}	0.136±0.001^{a}	0.008±0.019^{b}	0.0004±0.000^{b}	0.234±0.278^{a}
I. wombolu	0.154±0.012^{a}	0.034±0.073^{b}	0.073±0.018^{a}	0.0000±0.000^{c}	0.262±0.274^{a}
p-value	0.000	0.000	0.002	0.395	0.000

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

4.3.1. Concentrations of Aflatoxins in cassava chips

There was no significant difference in the concentration of AFB1 in cassava chips across the states except in Imo State where AFB1 was not detected, for concentrations of AFB2 in cassava chips, the values of 0.040±0.105ppb and 0.022±0.067ppb were detected in samples collected from Enugu state and Abia state respectively, these were significantly higher than 0.009±0.012ppb recorded from samples collected from Ebonyi state, AFB2 was not detected in Anambra and Imo state. AFG1 not detected in all the samples across the states except in Ebonyi state with a value of 0.003±0.008ppb, AFG2 was also not detected in all the states except in Anambra State (0.033±0.100ppb). For total aflatoxin, the highest values was recorded from Anambra state (0.086±0.142ppb), this was significantly (P>0.05) higher than 0.047±0.140ppb and 0.044±0.021ppb recorded from samples collected from Abia state and Enugu state respectively, the least concentration of 0.023±0.014ppb was obtained from Ebonyi state, while no aflatoxin was detected from Imo state. With respect to the wave of collection, there was no significant difference in the values of AFB1 and AFB2 recorded across the wave of collection, although the highest value of AFB1 (0.026±0.082ppb) and AFB2 (0.01±0.0240ppb) were recorded during the dry season but these values were not significantly (P>0.05) higher than values recorded from samples collected during harmattan and Wet season. AFG1 and AFG2 were not detected from samples collected across the waves of collection except during harmattan with values of 0.002±0.006ppb and 0.020±0.077ppb for AFG1 and AFG2 respectively. For total aflatoxin there was significant difference in the values obtained, the highest values of 0.056±0.136ppb was recorded during the wet season, this was significantly higher than 0.036±0.028ppb recorded during harmattan at P>0.05 level of significance while the least value of 0.017±0.028 was observed during dry season. There was significant difference in total

aflatoxins across the wave of collection, 0.056±0.136ppb was recorded during wet season this was significantly (P>0.05) higher than 0.036±0.028ppb observed during dry season, the least value of 0.027±0.077ppb was recorded during harmattan (Table 9).

Table 9: Concentrations of Aflatoxins in Cassava chips

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	(Total)	0.024±0.073[a]	0.022±0.067[a]	0.000±0.000[b]	0.000±0.000[b]	0.047±0.140[b]
Anambra		0.050±0.120[a]	0.000±0.000[c]	0.000±0.000[b]	0.033±0.100[a]	0.086±0.142[a]
Ebonyi		0.010±0.011[a]	0.009±0.012[b]	0.003±0.008[a]	0.000±0.000[b]	0.023±0.014[c]
Enugu		0.004±0.009[a]	0.040±0.105[a]	0.000±0.000[b]	0.000±0.000[b]	0.044±0.021[b]
Imo		0.000±0.000[b]	0.000±0.000[c]	0.000±0.000[b]	0.000±0.000[b]	0.000±0.000[d]
Total	HM	0.003±0.005[a]	0.002±0.005[a]	0.002±0.006[a]	0.020±0.077[a]	0.027±0.077[c]
	DS	0.026±0.082[a]	0.01±0.0240[a]	0.000±0.000[b]	0.000±0.000[b]	0.036±0.028[b]
	WS	0.014±0.052[a]	0.041±0.105[a]	0.000±0.000[b]	0.000±0.000[b]	0.056±0.136[a]

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different ($p>0.05$)
HM is Harmattan
DS is Dry season
WS is Wet season

4.3.2. Concentrations of Aflatoxins in *Citrullus colocynthis*

For the concentrations of AFB1 in *C.colocynthis* sampled across the states, samples collected from Anambra State and Enugu State with values of 0.184±0.153ppb and 0.180±0.208ppb were significantly (P>0.05) higher than values recorded from other states, the least value of 0.015±0.018ppb was observed in sample collected from Abia state, for AFB2, the highest concentration was detected from sample collected from Anambra state with value of 0.447±0.147ppb this was significantly (P>0.05) higher than 0.129±0.198ppb obtained from samples collected from Enugu State, lower values of 0.024±0.024ppb, 0.053±0.116ppb and 0.024±0.055ppb were recorded from Abia, Ebonyi and Imo state respectively, these values were not significantly different from each other. For AFG1, the values recorded across the states ranged between 0.002±0.004ppb and 0.017±0.033ppb although there was no significant difference in the concentrations across the state but the highest value was recorded from Abia state while the least value was recorded from Anambra state. AFG2 was not detected in all the samples across the state of collection except in Anambra state (0.002±0.004ppb). For total aflatoxin, there was significant difference in values obtained from samples across the states, the highest value of 0.636±0.078ppb was recorded from Anambra state, next to this was 0.323±0.352ppb obtained from sample collected from Enugu state, then 0.086±0.038ppb from Ebonyi state while a significantly lower values of 0.056±0.047ppb and 0.068±0.073ppb observed from Imo and Abia state respectively, all the values were significantly (P>0.05) different from each other except the values from Ebonyi and Enugu that were not significantly different from eachother, total aflatoxin was not detected in Imo state. With respect to the wave of collection, there was no significant difference in the concentration of AFB1 across the wave of collection, although the highest value of 0.026±0.082ppb was obtained during dry season while

the least value of 0.003±0.005ppb was obtained during harmattan, on the same hand, there was no significant difference in the concentration of AFB2 across the wave of collection but the highest value was detected during the wet season (0.041±0.105ppb) while the least during harmattan, AFG1and AFG2 were not detected in samples collected across the waves except during harmattan with values of 0.002±0.006ppb and 0.020±0.077 respectively. For total aflatoxin there was significant difference in *C. colocynthis* across the wave of collection, the highest value of bnn0.346±0.354ppb was obtained during the harmattan season this was significantly (P>0.05) higher than 0.0179±0.260ppb and 0.0176±0.210ppb recorded from samples collected during dry season and wet season respectively (Table 10).

Table 10: Concentrations of Aflatoxins in *Citrullus colocynthis*

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia		0.015±0.018[b]	0.024±0.024[d]	0.017±0.033[a]	0.000±0.000[b]	0.056±0.047[c]
Anambra		0.184±0.153[a]	0.447±0.147[a]	0.002±0.004[a]	0.002±0.004[a]	0.636±0.078[a]
Ebonyi	(Total)	0.029±0.017[b]	0.053±0.116[c]	0.004±0.013[a]	0.000±0.000[b]	0.086±0.038[c]
Enugu		0.180±0.208[a]	0.129±0.198[b]	0.014±0.021[a]	0.000±0.000[b]	0.323±0.352[b]
Imo		0.040±0.069[b]	0.024±0.055[d]	0.004±0.013[a]	0.000±0.000[b]	0.068±0.073[c]
	HM	0.142±0.190[a]	0.196±0.222[a]	0.008±0.022[b]	0.000±0.000[b]	0.346±0.354[a]
Total	DS	0.041±0.070[c]	0.122±0.220[b]	0.017±0.023[a]	0.000±0.000[b]	0.179±0.260[b]
	WS	0.086±0.114[b]	0.089±0.152[c]	0.000±0.000[c]	0.001±0.003[a]	0.176±0.210[b]

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

HM is Harmattan

DS is Dry season

WS is Wet season

4.3.3. Concentrations of Aflatoxins in *Irvingia wombolu*

For the concentration of AFB1 in *Irvingia wombolu*, there was a significant difference in the values obtained across the states, values of 0.221±0.140ppb and 0.367±0.150ppb recorded from Abia and Anambra were not significantly different from eachother but significantly higher than values recorded from other states at P>0.05 level of significance, next to this was 0.118±0.110ppb obtained from sample collected from Enugu state while the least in AFB1 was 0.017±0.032ppb obtained from Imo state. There was significant difference in the values of AFB2 in *Irvingia wombolu* across the states, relatively higher values of 0.093±0.036ppb and 0.046±0.057ppb were recorded from samples collected from Anambra state and Enugu state respectively these were not significantly different from eachother but significantly higher that values obtained from other states at P>0.05 level of significance, next to these were 0.021±0.013ppb and 0.011±0.033ppb recorded from Abia and Ebonyi respectively, AFB2 was not detected in *I. wombolu* collected from Imo state. For AFG1, values of 0.180±0.047ppb and 0.152±0.084ppb were not significantly different from eachother but significantly higher than values recorded from other states, next were 0.029±0.048ppb obtained in samples collected from Imo state, lower concentrations of 0.001±0.002ppb and 0.005±0.007ppb were recorded from Enugu and Ebonyi respectively. AFG2 was not detected in *I. wombolu* across all the states. For total aflatoxin, Anambra with value of 0.612±0.019ppb and Abia with value of 0.412±0.058ppb were significantly higher than values obtained from other states at P>0.05 level of significance, Ebonyi with a value of 0.074±0.011ppb was next, relatively lower values of total aflatoxin were obtained from Enugu (0.165±0.032ppb) and Imo state (0.047±0.063ppb). With respect to wave of collection, there was no significant difference in the concentration of AFB1 across the wave of collection, although the highest value of AFB1 was detected from sample collected during wet

season with value of 0.222±0.055ppb while the least in AFB1 was from sample collected during dry season (0.107±0.017ppb), on the same hand, there was no significant difference in the concentration of AFB2 recorded from the samples across the waves of collection, the values ranged between 0.030±0.031ppb and 0.037±0.004ppb, the highest concentration of AFG1 was recorded from sample collected during harmattan (0.097±0.026ppb) this was not significantly higher than 0.081±0.015ppb obtained during dry season while least value of AFG1 (0.041±0.047ppb) was obtained during wet season, AFG2 was not detected from *I. wombolu* in all the samples collected across the waves. For total aflatoxin values of 0.300±0.005ppb and 0.268±0.044ppb recorded during wet season and harmattan respectively were not significantly different from eachother but significantly higher than 0.218±0.083ppb obtained during dry season at P>0.05 level of significance (Table 11).

Table 11: Concentrations of Aflatoxins in *Irvingia wombolu*

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	(Total)	0.221±0.140[a]	0.011±0.033[b]	0.180±0.047[a]	0.000±0.000[a]	0.412±0.058[a]
Anambra		0.367±0.150[a]	0.093±0.036[a]	0.152±0.084[a]	0.000±0.000[a]	0.612±0.019[a]
Ebonyi		0.049±0.016[c]	0.021±0.013[b]	0.005±0.007[c]	0.000±0.000[a]	0.074±0.011[b]
Enugu		0.118±0.110[b]	0.046±0.057[a]	0.001±0.002[c]	0.000±0.000[a]	0.165±0.032[c]
Imo		0.017±0.032[d]	0.001±0.000[c]	0.029±0.048[b]	0.000±0.000[a]	0.047±0.063[c]
Total	HM	0.134±0.145[a]	0.036±0.070[a]	0.097±0.026[a]	0.000±0.000[a]	0.268±0.044[a]
	DS	0.107±0.017[a]	0.030±0.031[a]	0.081±0.015[a]	0.000±0.000[a]	0.218±0.083[b]
	WS	0.222±0.055[a]	0.037±0.004[a]	0.041±0.047[b]	0.000±0.000[a]	0.300±0.005[a]

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

HM is Harmattan

DS is Dry season

WS is Wet season

4.4. Concentrations of heavy metals in food samples

For the comparison of metals in food samples, the highest concentration of lead was detected in *C. colocynthis* (0.039±0.006 mg/g) this was significantly (P>0.05) higher than 0.009±0.005 mg/g and 0.008±0.0004 mg/g recorded from cassava chips and *I. wombolu* respectively. There was no significant difference in the concentration of Zinc across all the food samples, although the values recorded ranges between 0.163±0.025 mg/g-0.223±0.022 mg/g, for copper the concentration was higher in *C. colocynthis* (0.199±0.02 mg/g) and *I. wombolu* (0.11±0.054 mg/g), Iron was lowest in *I. wombolu* (0.218±0.093 mg/g) this was significantly lower than values recorded in other food materials at P>0.05 level of significance, the highest in Iron was Egusi with value of 0.596±0.025 (Table 12).

Table 12: Comparison of Heavy Metals in the Food Samples

Food Samples	Metal Concentration (mg/g) LEAD	ZINC	COPPER	IRON
Cassava chip	0.009±0.005[b]	0.163±0.025[a]	0.038±0.004[c]	0.281±0.926[b]
C.colocynthis	0.039±0.006[a]	0.218±0.085[a]	0.199±0.020[a]	0.596±0.025[a]
I. wombolu	0.008±0.004[b]	0.223±0.022[a]	0.110±0.054[b]	0.218±0.093[b]
p-value	0.022	0.526	0.008	0.042

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

4.4.1. Concentration of heavy metals in cassava chips

For the concentration of metal cassava chips, lead was not detected in all the samples across the states except in Enugu (0.042±0.098mg/g). For zinc, there was significant difference in its concentration across the states, the highest concentration was detected in Enugu state (0.305±0.332mg/g) this was significantly higher than values recorded from other states, next was Abia state (0.252±0.071mg/g), values recorded from Anambra (0.057±0.011mg/g) and Ebonyi (0.050±0.033mg/g) were significantly lower than values obtained from other schools. Copper was not detected from samples collected from Ebonyi and Imo state, the highest value of copper was detected from Enugu (0.148±0.081mg/g) this was significantly higher than 0.01±0.0270mg/g and 0.031±0.094mg/g recorded from Abia and Anambra states. The highest concentration of iron was recorded from sample collected from Abia state (0.735±0.010mg/g), this was significant different from 0.214±0.063mg/g obtained from Enugu, relatively lower concentration of iron was recorded in samples collected from Anambra (0.116±0.018mg/g) and Imo state (0.151±0.006mg/g). With respect to the wave of collection, lead was not detected during dry season but was detected during harmattan (0.022±0.077mg/g) and wet season (0.003±0.013mg/g), zinc was highest during wet season with a value of 0.294±0.306mg/g, this was significantly (P>0.05) higher than 0.144±0.075mg/g observed during harmattan which is on the same hand significantly higher than 0.049±0.039ppb recorded during dry season. Copper was relatively higher during harmattan (0.063±0.038mg/g) and wet season (0.047±0.014mg/g), there was significant difference in the concentration of iron across the wave of collection, the highest value of 0.442±0.092 mg/g was recorded during harmattan, next to this was 0.207±0.041 mg/g observed during dry season while the least concentration of 0.195±0.047mg/g was recorded during wet season (Table 13)

Table 13: Concentration of heavy metals in cassava chips

States	Wave of Collection	Average Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia		0.000±0.000[b]	0.252±0.071[b]	0.01±0.0270[b]	0.735±0.010[a]
Anambra		0.000±0.000[b]	0.057±0.011[d]	0.031±0.094[b]	0.116±0.018[d]
Ebonyi		0.000±0.000[b]	0.050±0.033[d]	0.000±0.000[c]	0.191±0.037[c]
Enugu		0.042±0.098[a]	0.305±0.332[a]	0.148±0.081[a]	0.214±0.063[b]
Imo		0.000±0.000[b]	0.148±0.141[c]	0.000±0.000[c]	0.151±0.006[d]
	HM	0.022±0.077[a]	0.144±0.075[b]	0.063±0.038[a]	0.442±0.092[a]
Total	DS	0.000±0.000[c]	0.049±0.039[c]	0.004±0.009[c]	0.207±0.041[b]
	WS	0.003±0.013[b]	0.294±0.306[a]	0.047±0.014[b]	0.195±0.047[c]

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

HM is Harmattan

DS is Dry season

WS is Wet season

4.4.2. Concentration of heavy metals in *Citrullus colocynthis*

With respect to state of collection, lead was only detected in Enugu state with a value of 0.193±0.233mg/g. Zinc was detected in samples from all the states highest in Abia with a value of 0.373±0.055mg/g, although this was not significantly different from 0.315±0.003mg/g and 0.242±0.234mg/g detected from Enugu and Imo state respectively, lower value of zinc was detected from Anambra (0.081±0.093mg/g) and Ebonyi state (0.080±0.053mg/g).Copper was more in Abia (0.150±0.040mg/g) and Ebonyi state (0.103±0.057mg/g) and least in Imo state (0.005±0.011mg/g). For iron, more concentrations were detected from samples collected from Enugu state with a value of 2.362±1.920mg/g, this was significantly (P>0.05) higher than concentrations of iron recorded from other states, Abia state was next in iron (0.137±0.212mg/g) while the least concentration of iron was detected from Anambra and Ebonyi state with concentrations of 0.086±0.040mg/g and 0.084±0.077mg/g respectively. With respect to wave of collection, lead was detected from all the samples across the waves, highest during dry season (0.067±0.176mg/g) and lowest during wet season (0.005±0.021mg/g), the highest concentration of zinc was obtained during wet season 0.448±0.053mg/g, next to this was0.159±0.096mg/g obtained during harmattan and the least concentration of zinc was detected from samples collected during dry season 0.047±0.021mg/g. Copper was detected more in sample collected during harmattan and wet season with values of 0.256±0.039mg/g and 0.240±0.032mg/g respectively these were not significantly different from eachother but significantly higher than 0.102±0.0219mg/g recorded during dry season, there was no significant difference in the concentration of iron across the wave of collection, although the highest values of 0.709±0.106mg/g was recorded during harmattan while the least value of 0.499±0.198mg/g was obtained during wet season (Table 14).

Table 14: Concentration of Heavy Metals in *Citrullus colocynthis*

States	Wave of Collection	Average Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia		0.000±0.000[b]	0.373±0.055[a]	0.150±0.040[b]	0.137±0.212[c]
Anambra		0.000±0.000[b]	0.081±0.093[b]	0.053±0.099[c]	0.086±0.040[d]
Ebonyi		0.000±0.000[b]	0.080±0.053[b]	0.103±0.057[b]	0.084±0.077[d]
Enugu	(Total)	0.193±0.233[a]	0.315±0.003[a]	0.685±0.004[a]	2.362±1.920[a]
Imo		0.000±0.000[b]	0.242±0.234[a]	0.005±0.011[d]	0.309±0.291[b]
	HM	0.044±0.129[b]	0.159±0.096[b]	0.256±0.039[a]	0.709±0.106[a]
Total	DS	0.067±0.176[b]	0.047±0.021[c]	0.102±0.021[b]	0.579±0.237[b]
	WS	0.005±0.021[a]	0.448±0.053[a]	0.240±0.032[a]	0.499±0.198[b]

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

HM is Harmattan

DS is Dry season

WS is Wet season

4.4.3. Concentration of heavy metals in *Irvingia wombolu*

Lead was not detected in any of the states except in Enugu (0.042±0.068mg/g). The values of zinc were not significantly different from eachother across the state except in Abia state with value of 0.078±0.082mg/g this was significantly lower than values from other states. For copper, 0.394±0.451mg/g was obtained from Enugu; this was significantly higher than 0.077±0.139mg/g and0.058±0.118mg/g recorded from Anambra and Ebonyi state respectively, samples from Abia state and Imo state gave a lower concentration of copper with values of 0.011±0.021mg/g and 0.011±0.023mg/g. Iron was more from sample collected from Enugu state (0.430±0.047mg/g) next were 0.255±0.061mg/g and 0.235±0.032mg/g recorded from Imo and Abia state respectively, Ebonyi was the least in iron with the concentration of 0.053±0.059mg/g. With respect to wave of collection, there was significant difference in the concentration of lead across the wave of collection, the highest value of lead was detected harmattan (0.023±0.006mg/g) this was significantly higher than 0.003±0.010mg/g recorded during dry season, lead was not detected during wet season, the value of zinc across the wave of collection ranged between 0.073±0.016mg/g and 0.303±0.081mg/g but there was not significant difference among the values at P>0.05 level of probability, high concentrations of copper were detected during harmarttan and wet season with values of 0.186±0.025mg/g and 0.138±0.079mg/g respectively, these were significantly higher than 0.007±0.019mg/g obtained during dry season. There was significant difference in the concentration of iron across the three waves of collection; the highest value of iron was recorded during wet season with values of 0.371±0.075mg/g while the least in iron was dry season (0.078±0.002mg/g) (Table 15).

Table 15: Concentrations of Metals in *Irvingia wombolu*

States	Wave of Collection	Average Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia		0.000±0.000[b]	0.078±0.082[d]	0.011±0.021[c]	0.235±0.032[b]
Anambra		0.000±0.000[b]	0.394±0.001[a]	0.077±0.139[b]	0.118±0.033[c]
Ebonyi	(Total)	0.000±0.000[b]	0.141±0.048[c]	0.058±0.118[b]	0.053±0.059[d]
Enugu		0.042±0.068[a]	0.289±0.071[b]	0.394±0.451[a]	0.430±0.047[a]
Imo		0.000±0.000[b]	0.210±0.036[b]	0.011±0.023[c]	0.255±0.061[b]
	HM	0.023±0.006[a]	0.303±0.081[a]	0.186±0.025[a]	0.207±0.046[b]
Total	DS	0.003±0.010[b]	0.073±0.016[c]	0.007±0.019[c]	0.078±0.002[c]
	WS	0.000±0.000[c]	0.291±0.041[b]	0.138±0.079[b]	0.371±0.075[a]

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

HM is Harmattan

DS is Dry season

WS is Wet season

4.5. Effects of plant extracts on the inhibition of test organisms

The inhibitory effects of the extracts on the organisms tested varied with the type of plant material, extraction medium, concentration and the test organism. Colony diameter of the inhibition decreased as the concentration of the extract increased with 7.5% and 10.0% being more potent than 2.5% and 5.0% extract concentration.

Ethanol extract of *M. oleifera* at 7.0 and 10% extract concentration showed complete inhibition of *A. flavus* (100% inhibition) next to this was aqueous extract of *M. oleifera* at 7.0 and 10% concentration with values of 78% inhibition, aqueous extract of *C.odorata* at 10% extract concentration gave the least inhibitory effect of 0% on *A.flavus*, the synthetic fungicide used (mancozeb) inhibited the growth of *A. flavus* completely (Fig 3).

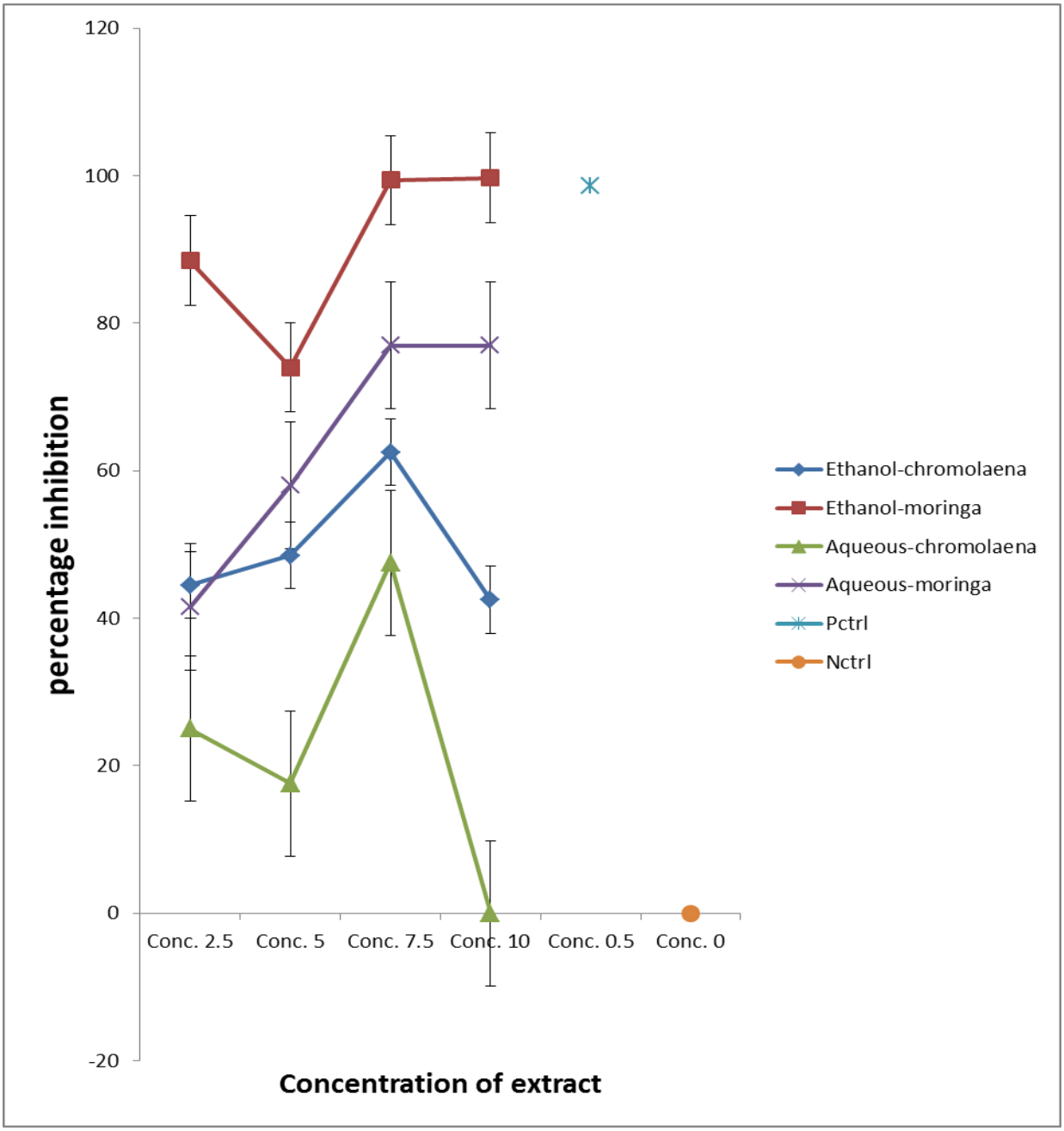


Figure 3: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Aspergillus flavus*

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

For *F.oxysporium*, Ethanol extract of *M.oleifera* at all concentrations gave inhibitory effects that were significantly better than other extracts; at 10% concentration it gave the highest inhibitory effect with value of 98%. Ethanol extract of *C.odorata* and aqueous extract of *M. oleifera* at 7.5 and 10% respectively gave the next inhibitory value of 63%. Aqueous extract of *C.odorata* at 10% extract concentration did not inhibit the growth of *F.oxysporium.* Mancozeb (positive control) showed 100% inhibition (Figure 4).

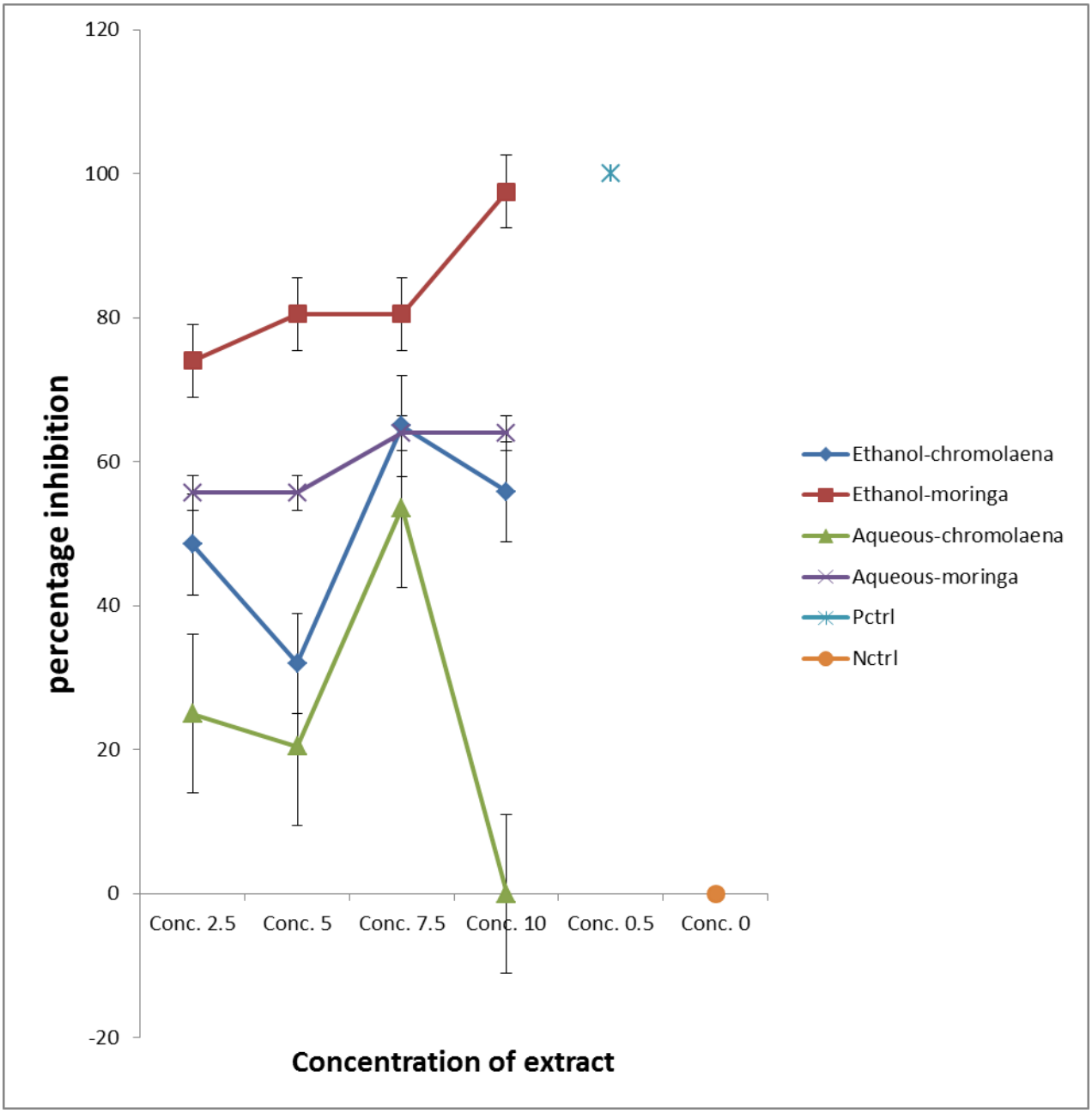


Figure 4: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Fusarium* spp

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

For *Penicillium* spp, ethanol extract of *M. oleifera* at all concentrations was more potent than other extracts at all concentrations, with respect to concentration; the highest inhibition was recorded at 7.5% extract concentration. The next inhibitory effect was observed from ethanol extract of *C. odorata* and aqueous extract of *M. odorata at* 7.5% concentration. Aqueous extract of *C. odorata* gave the least inhibitory effects across the concentrations; no inhibition was recorded at 10% concentration while 35% inhibition was recorded at 7.5% concentration (Fig 5).

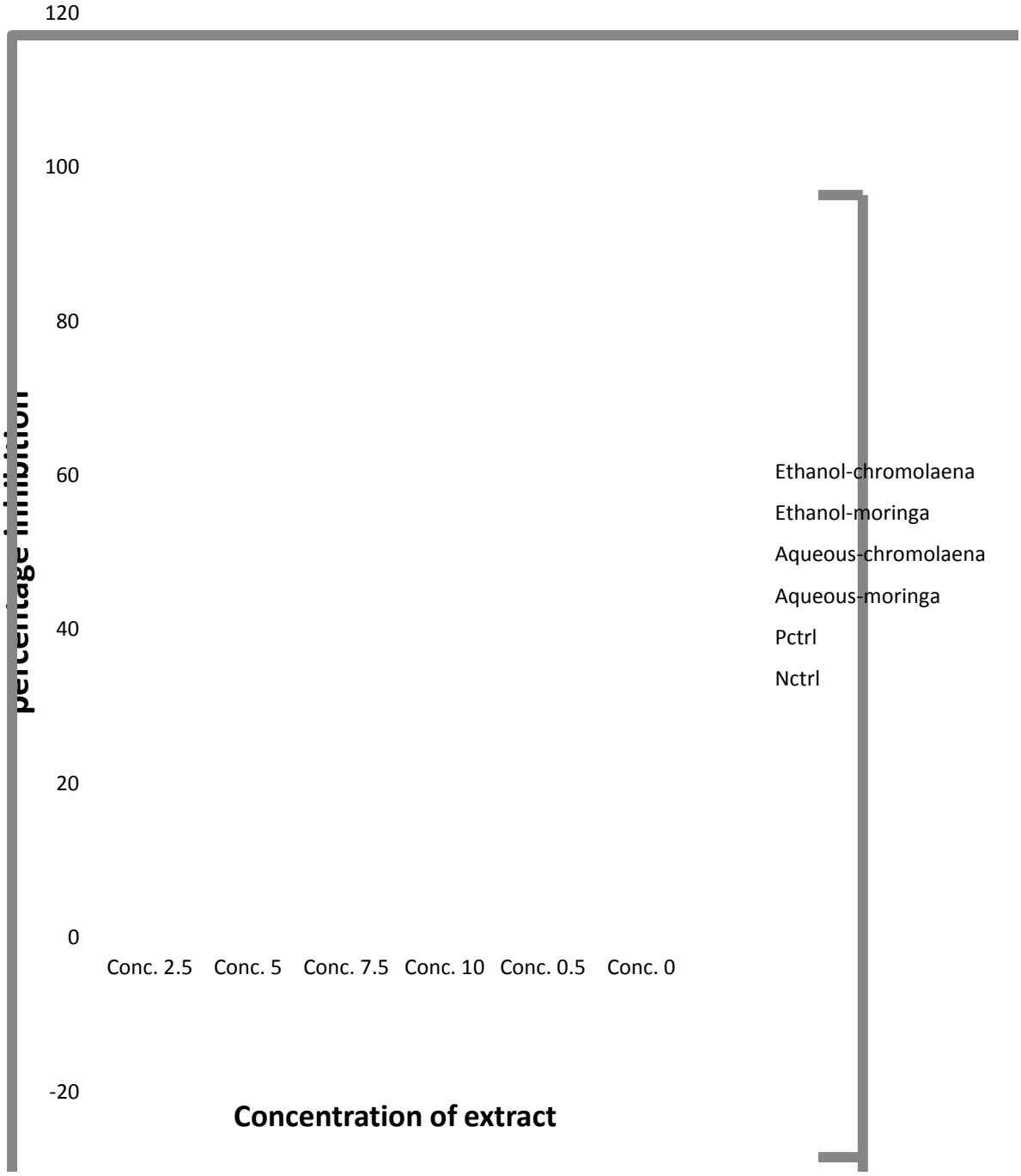


Figure 5: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Penicillium* spp

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

For *A.niger,* values recorded from Ethanol extract of *M. oleifera* across all concentrations was significantly better than other plant extracts at all concentrations with the highest values 94% and 100% recorded at 7.5% and 10% extract concentration. Ethanol extract of *C. odorata* at 7.5% extract concentration was next with a value of 68% inhibition. The least inhibition was recorded from aqueous extract of *C. odorata* across all concentrations, the inhibition increased with increase in concentration with a value of 50% inhibition observed from 10% extract concentration. The positive control used (Mancozeb) gave an inhibitory effect of 98% this was significantly higher than the values recorded from other interractions except *M. oleifera* at 7.5 and 10% extract concentration (Figure 6).

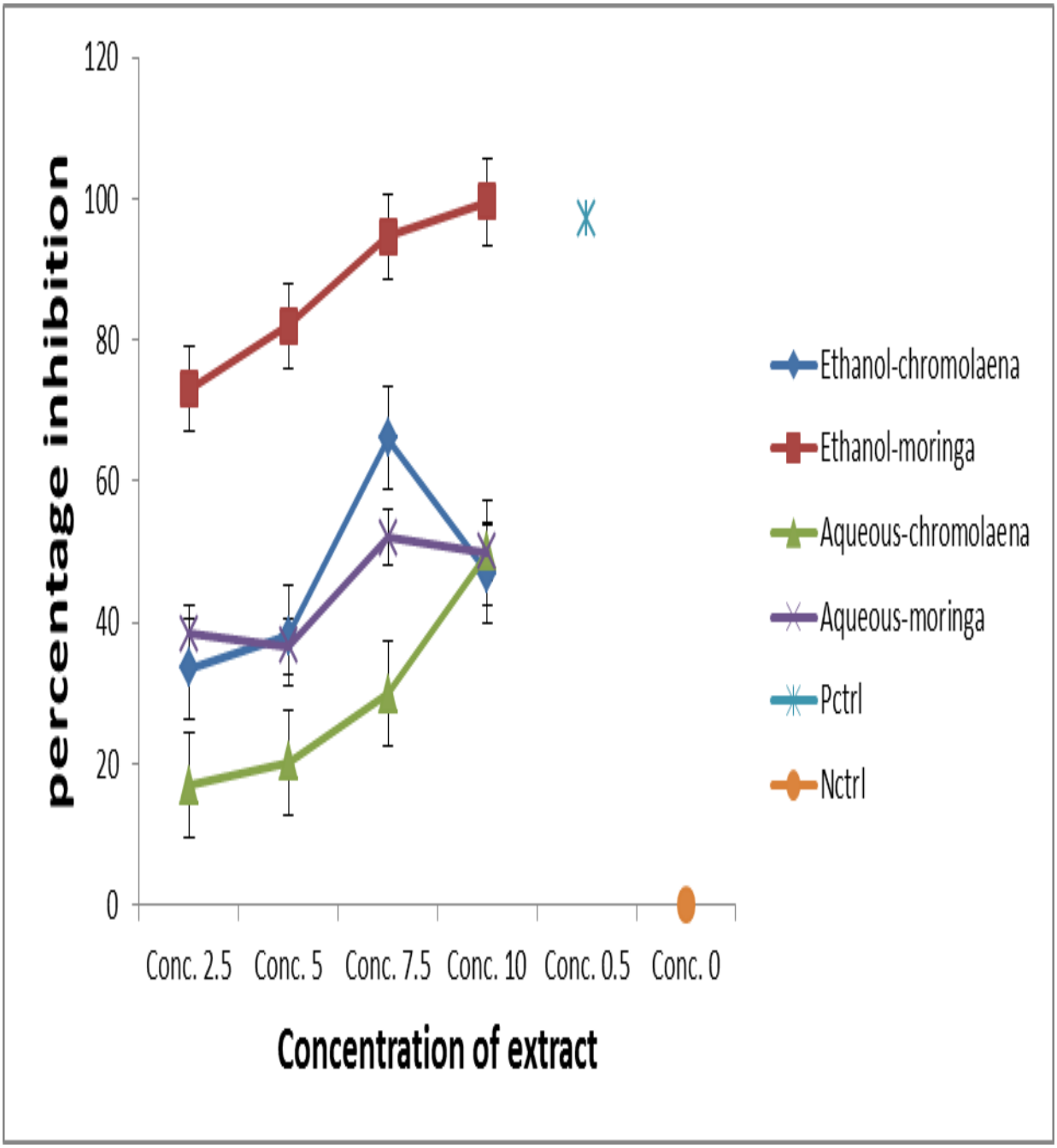


Figure 6: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Aspergillus niger*

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

Ethanol extract of *M. oleifera* at 7.5 and 10% completely inhibited the growth of *T.viride*, this was significantly higher than the values observed from other plant extracts at all concentrations. Aqueous extract of *M.oleifera* at 7.5% and 10% and ethanol extract of *M. oleifera* at 7.5% gave an inhibition percentage of 65%. Aqueous extracts of *C. odorata* was least in inhibition but better at 7.5% extract concentration with value of 52% Mancozeb inhibited the growth of *T. viride,* a value of 98% was recorded (Fig 7).

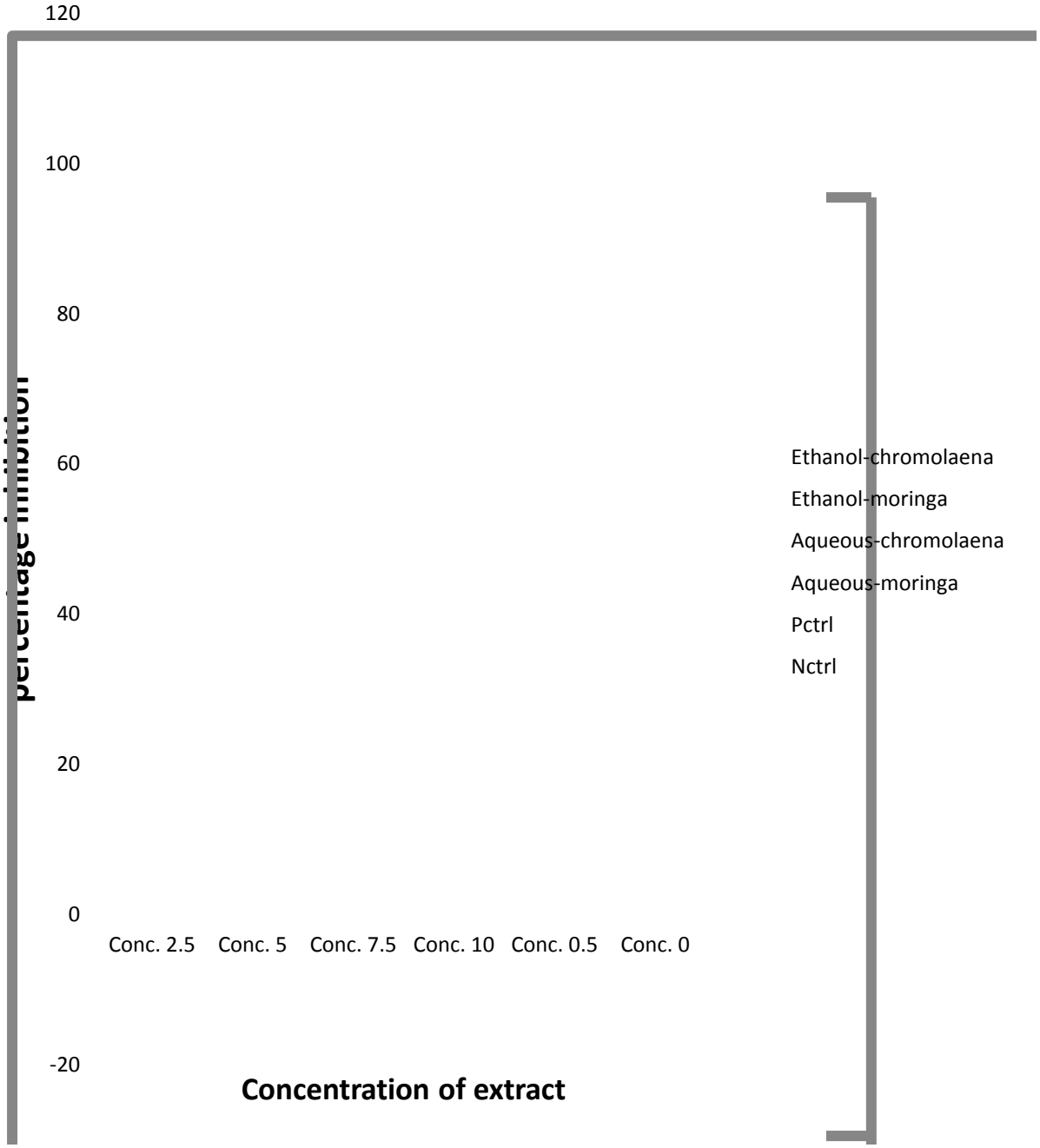


Figure 7: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Trichoderma viride*

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

For *R.stolonifer,* ethanol extract of *M.oleifera* at all concentrations except 2.5% gave high inhibitory effects with values ranging between 85-90%. Ethanol extract of *C.odorata* at 7.5% was next with inhibitory value of 72%. Aqueous extract of *C.odorata* and aqueous extract of *M. oleifera* at all concentrations slightly inhibited the growth of *R.stolonifer* with values ranging between 0-10% and and 16-18% for aqueous extract of *M. oleifera* and aqueous extract of *C.odorata* respectively. The synthetic fungicide used (positive control) moderately inhibited the growth of *R. stolonifer* (Figure 8).

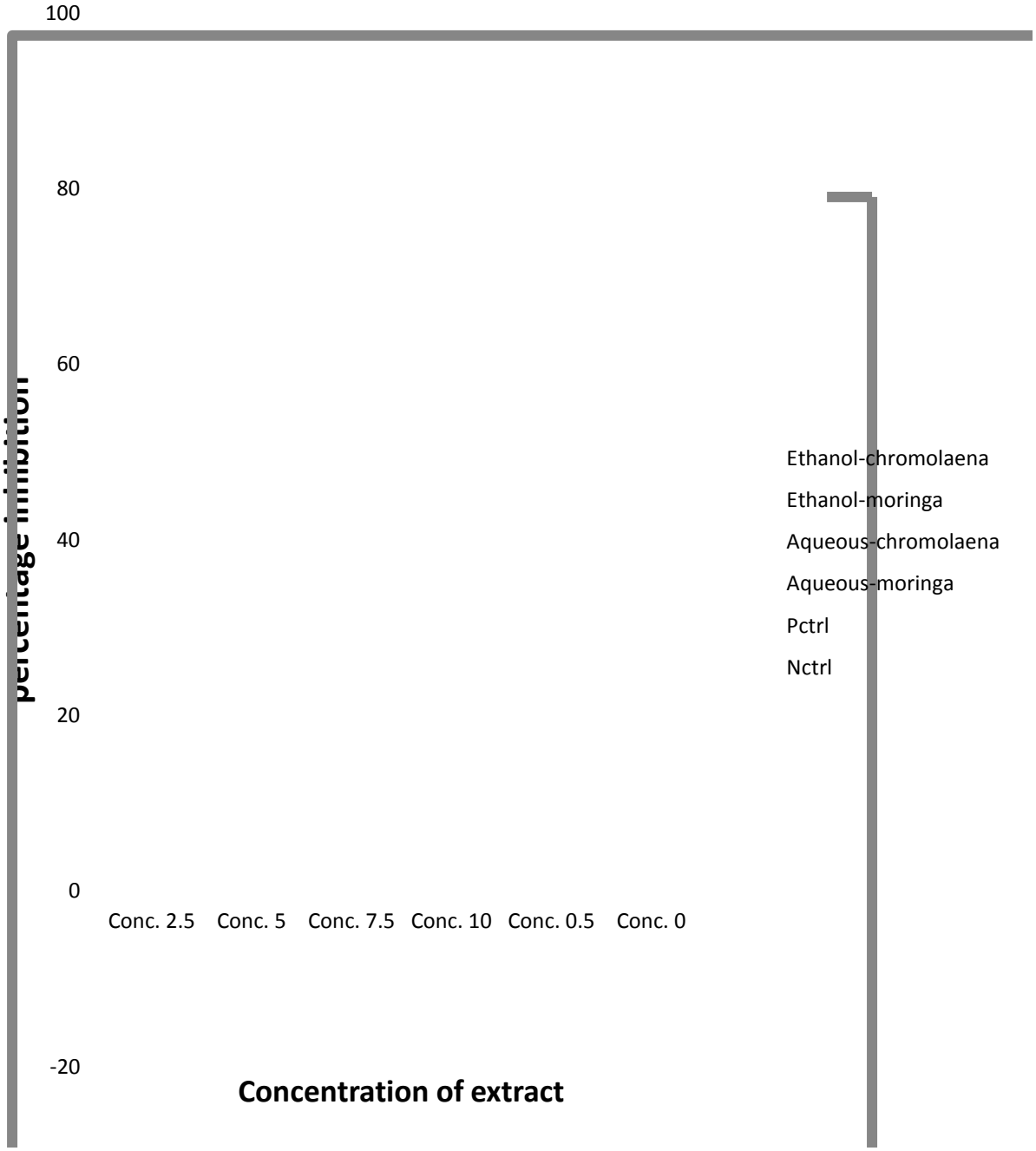


Figure 8: Effects of ethanol and aqueous extracts of *Chromolaena odorata and Moringa oleifera* on the inhibition of *Rhizopus stolonifer*

Key: Ethanol-chromolaena is ethanolic extract of C*hromolaena odorata*
Ethanol-moringa is ethanolic extract of *Moringa oleifera*
Aqueous-chromoleana is aqueous extracts of *Chromolena odorata*
Aqueous-moringa is aqueous extract of *Moringa oleifera*
Pctrl is Positive control (synthetic fungicide: Mancozeb)

4.6. Phytochemical Analysis

4.6.1. Qualitative Phytochemical Screening

Screening of plants materials for phytochemicals of interest revealed that all the plants test positive to all the phytochemicals except Tanins that is negative in *Chromolaena odorata*. These phytochemicals were extracted in these plant materials using three solvents (Ethanol, Water and Ethylacetate) with molecular weights far apart from each other.

All the phytochemicals except Tannins and Tritepernoids were detected in *Chromolaena odorata* extracted with water and Ethylacetate, while none of the phytochemicals except Saponin, Alkaloids and Tritepernoids were detected in Ethanol (Table 16). *Moringa oleifera* tested positive to all the phytochemicals in Ethylacetate solvent, Tannins was negative in water while Phytosterols was also negative in Ethanol solvent (Table 17).

Table 16: Qualitative phytochemical screening of *Chromolaena odorata*

PARAMETER	ETHANOL	WATER	ETHYLACETATE
Saponins	+	+	+
Alkaloids	+	+	+
Tanins	-	-	-
Glycosides	-	+	+
Phytosterols	-	+	+
Flavonoids	-	+	+
Phenols	-	+	+
Tritepernoids	+	-	+

Table 17: Qualitative phytochemical screening of *Moringa oleifera*

PARAMETER	ETHANOL	WATER	ETHYLACETATE
Saponins	+	+	+
Alkaloids	+	+	+
Tanins	+	-	+
Glycosides	+	+	+
Phytosterols	-	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tritepernoids	+	-	+

4.6.2. Quantitative Phytochemical Assay

For Quantitative phytochemical analysis of plant samples, *Moringa oleifera* proved to contain relatively higher quantity of all the phytochemicals than *Chromolaena odorata.* The highest quantitative yield of Alkaloid was observed in *Moringa oleifera* (5.66%) as against 3.16% recorded from *Chromolaena odorata*. The quantities of Saponin, Cardiac glycosides, flavonoids, Phenols and Tritepernoids in *Moringa oleifera* are 3.81%, 0.53%, 4.17%, 0.21% and 7.28% respectively while the quantitative yields of Saponin, Cardiac glycosides, flavonoids, Phenols and Phytosteroids in *Chromolaena odorata* is 2.28%, 0.19%, 0.75%, 0.13% and 2.64% respectively. For Tannis, 0.36% was recorded in *Moringa oleifera* while 0% was observed in *Chromolaena odorata.* Quantitative analysis of the two plant materials showed that they contain 0% of Tritepernoids (Figure 9).

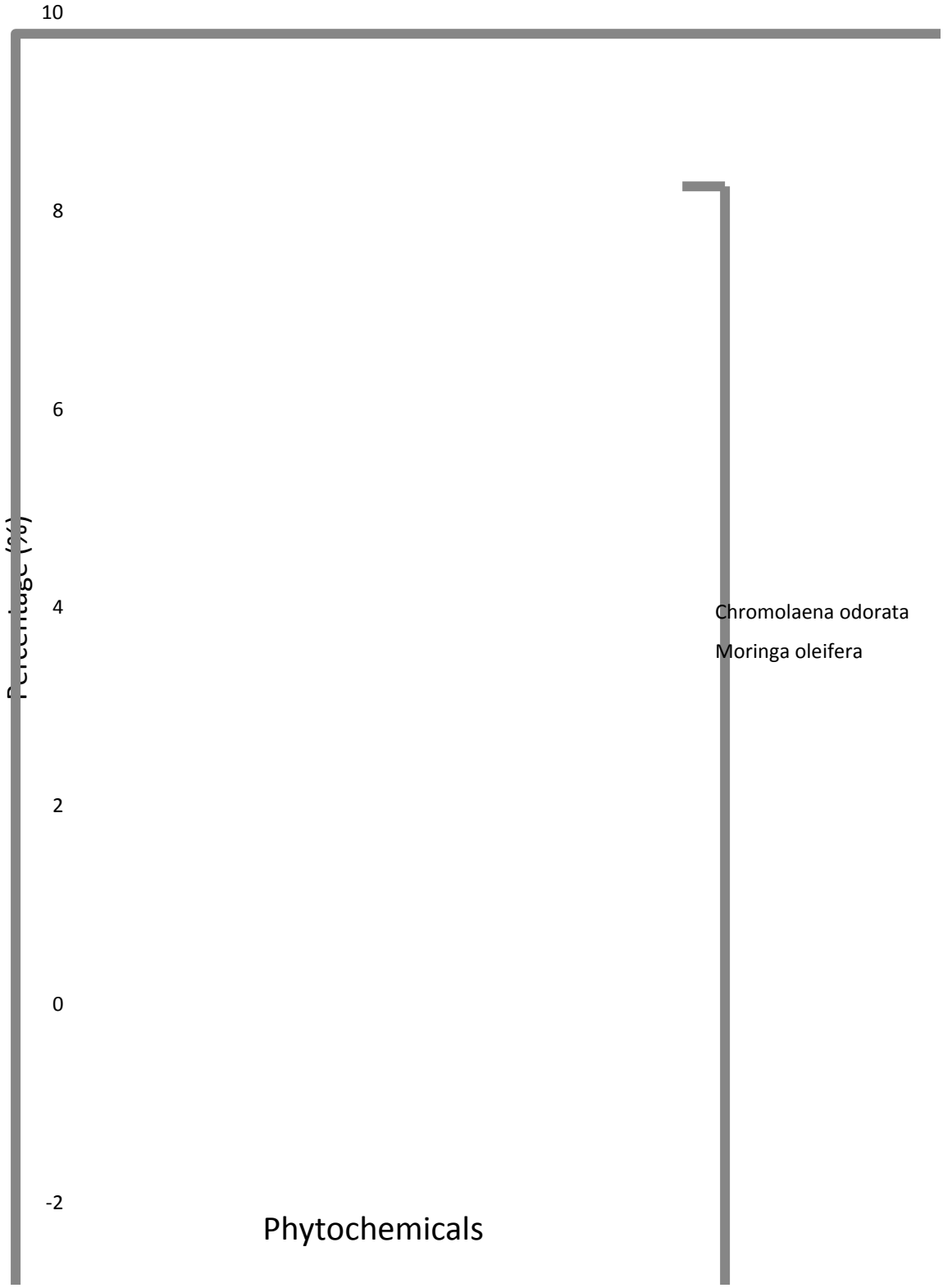


Fig 9: Quantitative phytochemical analysis of plant samples

CHAPTER FIVE

DISCUSSION

5.1. Ethno-study

The respondents across the states in south eastern Nigeria had fairly uniform ways of handling food materials. During the survey, it was established that farmers/food handlers/food processors sun dry food materials (Table 1) for two days (Table 2). This implies that these food materials are not properly dried, hence the possibility of high moisture content that encourages the growth and proliferation of toxin producing organisms. A comparative study on the methods of drying food materials carried out by several researchers confirmed that oven and smoke dried foodstuffs had the lowest incidence of disease, contaminants, moisture content and level of *Aspergillus* spp infestation while sun drying introduces contaminants (Bankole *et al.,* 2005). Therefore, the implication of the result of this study is that the perception and practice of methods of drying food materials in south eastern Nigeria encourages contamination of food materials.

In the current study, storage of food materials before marketing was reported to last as long as 7-9 months in some of the states studied, such an extended storage period promotes insect/rodents and mould infestation and can lead to discolouration and change in flavor (Kaaya and Eboku, 2010). Bankole *et al.,* (2005) reported that one major problem that besets food materials especially *C.colocynthis* is that it deteriorates quickly in storage due to fungal infestation. Storage of food materials for a long period of time decreases its nutritive value, increases the perioxide value and the production of mycotoxins. On the same hand, the result of a study conducted by Kolapo and Sanni (2006) confirmed that prolonged storage negatively affect the overall quality of foodstuffs such as its proximate composition.

It was also confirmed in this study that food handlers in the region do not use pesticides to control pests and they do not sort out bad ones, these are likely to be among the main factors that predispose foodstuff from this region to contaminants. Reports of some researchers such as Hell *et al.* (2000) affirmed that pests and rodents contribute significantly to food contamination, shortage and spoilage hence the need for the use of pesticide in controlling them. Another report from an empirical research conducted by Hell *et al.* (2010) stated that the use of pesticides reduced the risk of fungal infestation and aflatoxin contamination, while Bankole *et al.* (2005) remarked that *A.flavus* contamination was strongly correlated with high densities of weevils and unsorted foodstuff.

The practices of food processing and handling in south eastern Nigeria make the food materials susceptible to contamination, this is because several drying and storage factors that may help to reduce aflatoxin levels and contaminants in stored food materials are grossly ignored and neglected, these factors are: control of storage insect pests through the sorting out of damaged ones/parts from undamged ones, the use of appropriate storage pesticides and ``awareness'' of the farmers on the risk that pests, heavy metals and toxins present in stored foodstuffs (Bankole *et al.*,2005).

5.2. Fungal Infestation

Fungi isolated from these stored food materials are *Aspergillus niger, Aspergillus flavus, Fusarium oxysporium, Penicillium spp, Trichoderma spp* and *Rhizopus spp* (Table 7). This is in tandem with the result of a similar experiment conducted by Bankole *et al.*, (2004) except that *Penicillium spp, Trichoderma spp* and *Rhizopus spp* were not isolated but differs with the report of Oyedele and Fatoki (2017) and Etebu and Bawo (2012) who in addition to *Aspergillus* species also isolated *Rhizopus oryzae*, *Mucor racemosus* and *Saccharomyces cerevisiae* from various

food materials sold in various markets in Bayelsa state, Nigeria. Most of the organisms isolated from this research work are storage fungi that have been at various times implicated in the spoilage of fruits and vegetables. Members of the fungal group micromycetes which include all the fungi isolated from the present research work have at various times implicated as being responsible for the spoilage of stored fruits of the family Cucurbitaceae to which *C.colocynthis* belongs (Aboloma *et al.,* 2009). Some of the fungal isolates such as *Penicillium* and *Aspergillus* are known to be producers of mycotoxins which are secondary metabolites that are known to cause a lot of harmful effects when ingested in food by man (Oyeleke, 2003). The high occurrence of *Aspergillus* species in the food samples agrees with the report of so many workers who reported *Aspergillus* species as one of the most frequent organisms associated with seed rot of melon (Aboloma and Ogunbusola, 2012; Oyeleke and Olaniran, 2002). These *Aspergillus* fungi, especially *A. flavus* and *A. niger*, produce aflatoxins which are a major health threat to man and livestock because of their acute and chronic health effects. It is on record that Aflatoxins pose the greatest risk to health in tropical Africa because of their widespread prevalence and high toxicity (Manjula *et al.*, 2008) and have been known to be carcinogenic, suppresses the immune system (Jiang *et al.*, 2005, Turner *et al.* 2005) and are anti-nutritional contaminants in many food commodities (Williams *et al.,* 2004) and even causes death. Since chronic diseases in developing countries are under reported, the actual health impacts of aflatoxins maybe higher than what is known. These food materials from where these fungi were isolated (ogbono and egusi) are purchased, ground in the market and the ground seed taken home to make soup, while cassava chips is taken home without grinding. These fungi if mycotoxigenic, can lead to diseases in due course. If the fungi are mycotoxigenic, the mycotoxins will be swallowed along with food since they cannot be destroyed by heat or

processing. Most of the fungi are storage fungi whose spores may have attached to the seeds during storage/processing or handling. All the fungi species except *Penicillium* were isolated during the wet Season, this can be attributed to poor drying during storage, This is because rain/drizzles may fall on the drying products and predispose them to fungal growth as drying is often done in the open and unprotected areas, hence the possibility of these food materials been contaminated by fungi such as *Aspergillus, Fusarium* and *Penicillium*.

5.3. Aflatoxin Contamination of Food Samples

Aflatoxin was detected in all samples, highest in *C. colocynthis* and lowest in *M. esculenta* (cassava chips), although the levels of contamination in this study were lower than the NAFDAC and Codex Alimantarius Commission (CAC) maximum permissible levels of aflatoxins of 10 µg/kg and 4ug/kg or 4ppb respectively in the food samples (Atanda *et al.,* 2011; Marco *et al.*, 2008) (Table 9). This agrees with the reports of Muzanila *et al.*, (2000) and Chiona *et al.* (2014) who recorded a very low aflatoxin contamination in cassava chips, but differs with the study of Salau *et al.,* (2017) who reported that the aflatoxins level contained in food materials were above the Nigerian (10 µg/kg) limits and the European Union tolerance level of 2µg/kg for AFB1 and 4µg/kg for Total Aflatoxin. The result of this research work also differs with the documentations of Okigbo *et al.,* (2015) who reported that there were more concentrations of aflatoxins in *I. wombolu* than *C. colocynthis*. Even though the levels of Aflatoxins detected in this work is below the maximum permissible level, the frequent contamination of these food materials at a reasonable concentration by these potent carcinogen especially AFB1 and AFB2 that were detected in relatively higher quantities call for serious concern. Moreso, these toxins however small when consumed in food materials bio-accumulates in the body with serious health effects. Human Exposure to multiple chemical combinations in food samples has led to series of

human health disorder (Rotich *et al.,* 2006; UNDP, 2006; USEPA, 2002). The occurrence of all the different types of aflatoxins in the food materials points to the diversity of fungal species that colonized the food materials from the field to the market (Adetunji *et al.,*2014). The significant differences in the concentrations of aflatoxins detected in the food materials observed in this study can be linked to the method of drying these food materials across the states of sampling (Atanda *et al.,*2011, Turner *et al.,*2005), hence cassava chips has the least concentration because of its dry nature.

The concentration of aflatoxins in the food materials with respect to the states of collection increases with increase in temperature and rainfall of the states except for Abia State (temperature) and Imo State (Rainfall). Among all the states in south eastern states of Nigeria, Anambra State (37°C) and Enugu State (32.5°C) have a relatively higher mean annual temperature than the other three states (Annual Abstract of Statistics, 2012). From this research, the highest concentration of aflatoxin was detected in Anambra and Enugu except in *I. wombolu*, this can be attributed to the slight difference in the climatic conditions of these states from other states in south eastern Nigeria, this does not agree with the report of Atanda *et al.,* (2013) who stated that more toxins accumulates and are produced between the temperature of 4 to 10°C.

The concentrations of aflatoxins in the food materials with respect to wave of collection was more during wet season in all the samples (except in *C. colocynthis*), then in harmattan and the least was in dry Season, this agrees with the result of Makun *et al.,* (2007) who reported that the quantity of aflatoxin detected from samples collected during the wet season were significantly higher than those detected during harmattan and Dry Season in all the food materials. The growth and proliferation of mycotoxigenic fungi is influenced by water (Atanda *et al.,* 2013), hence the higher concentration of aflatoxins detected during wet season. This vital

condition is adequately fulfilled more during rainy seasons and in wetter areas than in drier seasons and places with resultant higher fungal contamination of food materials in wet conditions than dry ones. This explains why fewer fungi incidences and less mycotoxin were detected and recorded in dry seasons and in wet season. On the other hand, in *C.colocynthis*, more mycotoxins were detected during harmattan; this can be linked to the fact that more fungi were isolated from *C. colocynthis* during harmattan.

5.4. Anti-fungal potentials of plant extracts against mycotoxigenic fungi

This study revealed that antifungal agents were present in *C.odorata* and *M. oleifera,* since they prevented the growth of all the fungi pathogens tested.This is partially in tandem with the report of Omorusi *et al.* (2014) who stated that *C.odorata* and *M.oleifera* inhibited the growth of rot inducing pathogens of food materials. The reports of Omorrusi *et al.* (2014) and Okigbo and Nnadiri (2017) also stated that extract of *C.odorata* at all concentrations was more efficacious than extract of *M.oleifera* in the control of *A. niger, F.oxysporium* and *T.viride* responsible for cocoyam rot, this area is in contrast with the inference of this research work. Although all the extract showed varying degrees of antifungal efficacy, ethanol extract proved to be more potent. Results from this research indicate that *M.oleifera* was more effective than *C. odorata* on all the test organisms. *C. odorata* especially at low concentrations was relatively less effective against the pathogens. This does not agree with the findings of Uyi *et al.* (2014) whose report was in sharp contrast with the findings of this research work.

Moreso, This study has shown that *R. stolonifer, T.viride, A. flavus, Penicillium spp, F. solani* and *A. niger* associated with the post harvest contamination of *C. colocynthis, I. wombolu* and cassava chips were inhibited by the ethanolic extracts of *M. oleifera* and *C.odorata* at all the concentrations *in vitro*. These results also agree with the reports of other workers on the

inhibitory action of plant products employed on the mycelia growth and spore germination of other pathogenic fungi responsible for the pre and post-harvest spoilage of other food materials (Uzuegbu and Okoro, 1999; Bankole and Adebanjo, 1995; Okigbo *et al.* 2009; Okoi *et al.* 2015). The inference of this study suggests that these plant materials especially *M. oleifera* are capable of checking the spread of many fungal diseases of food crops. A comparative study of the phytochemical constituents of the plant materials depicted that *M.oleifera* contains more quantity of all the phytochemicals tested (Alkaloid, flavonoid, saponin, tritepernoids, glycosides, Tannins and phenol) than *C.odorata* (Fig. 7). This suggests that the high inhibitory efficacy of *M. oleifera* could be as a result of its phytochemicals content. Although the plant extracts effectively inhibited the growth of the fungal pathogens *in vitro*, it is essential to conduct further experiments to confirm the efficacy of their stem and other plant parts to control storage pests of food materials *in vivo*. The antifungal attribute of the two plant extracts on the fungal isolates of post harvest storage of *C colocynthis*, *I. wombolu* and cassava chips (*in vitro*) observed in this work, can further be developed pharmacologically for the control of mycotoxigenic fungi.

Hence, it is glaring from the result of this study that the the two tropical plants used in this study can be used as bio-fungicide in the control of mycotoxigenic fungi of food materials. Moreso, there is need for field trials of these plant materials especially *M.oleifera* to confirm the findings of this study.

5.5. Metal Concentration

All the metals tested were detected in all the food materials sampled at different concentrations. Some were present at concentrations above the permissible levels while some at concentrations below the permissible level (Table 13). The concentration of lead in all the food samples were not significantly different from each other but they were all higher than the

maximum permitted concentration (0.002mg/g) of lead (CAC, 2003) in food materials consumed by man. This is in tandem with the results of some researchers, Nkansan and Amoako (2010) reported that there was high level of lead in food materials (spices) sampled from markets. Chukwujindu *et al.*, (2008) recorded that lead concentration in food materials were above the guideline value for lead. The results of some other researchers do not agree with the findings of this research, for instance Aiwonegbe and Ikhuoria, (2007) reported that the lead concentration in food materials tested fell under safe range of concentration (below 0.02ug/g), while the result of a similar study carried out on locally produced wild rice in Kaduna by Umar and Wunzani, (2013) showed that the mean lead (Pb) content of the wild rice samples was below the permissible level. Concentrations of Iron, Zinc and copper observed in this research work were all below the permissible level, this is in contrast to the documentation of some researchers who carried out similar work, Izah *et al.* (2017) reported that metal concentrations in food samples, including iron, mercury, tin, antimony, cadmium, zinc, copper, chromium, lead, and manganese, seldom exceed the maximum contaminant level recommended by the Standard Organization of Nigeria (SON) and the World Health Organization (WHO), these differences could be linked to the different handling technique and other climatic and environmental factors such as air pollution.

The concentration of lead in the food materials also varies across the state, with Enugu State having the highest concentration. The knowledge of concentrations of metals (both heavy and essential) in food is of significant scientific interest because some of these elements are essential for human health (e.g., Cu, Fe, Mn, Zn) while others, if present even at low concentrations, can be toxic (e.g., Cd, Pb, Hg, As) (Onianwa *et al.*,1999; Soliman, and Zikovsky, 1999). The so-called essential metals can be toxic depending on their concentration.

Therefore the high level of lead from Enugu State might have resulted from accumulation of lead through air pollution and from industrial emission. Zukowska (2008) reported that Air, soil, and water pollution are contributing to the presence of harmful elements, such as cadmium, lead, and mercury in foodstuff. The occurrences of heavy metals-enriched ecosystem components, firstly, arise from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings. These agents have led to metal dispersion in the environment and, consequently, impaired health of the population by the ingestion of victuals contaminated by harmful elements. In this research, the high concentration of lead in the sampled food materials from Enugu that violated the permissible limits of lead set by WHO is of public health concern. Among possible target organs of heavy metals, are soft tissues such as the kidney and liver and the central nervous system appear to be specially sensitive (Apostoli, 2002). Lead exposure has been shown to cause severe anemia, permanent brain damage, neurological disorders, reproductive problems, diminished intelligence and a host of other diseases. According to the Agency for Toxic Substances and Disease Registry, a division of the U.S. Public Health Service, the major exposure of lead to the general population in food is through foods such as fruits, vegetables and grains (McNamara, 2008). Thus regular monitoring of these toxic heavy metals from foods sold in markets is essential, to prevent their excessive build-up in the food chain.

5.6. Phytochemical constituents of the Plant Materials

The high inhibitory effects of the two plant materials used on test fungi could be as a result of the presence of diverse biological active ingredients (phytochemicals) detected. This buttresses the inference of a research conducted by Srinwasan *et al.*,(2001) who stated that the presence of diverse biological active ingredients in plants confer resistance to plant pathogens (fungi, bacteria, nematodes and pests).

5.7. CONCLUSION

The occurrence of aflatoxins and heavy metals in food samples from south eastern Nigeria even though at a low concentration indicates that the regions population can be at risk of cancer and aflatoxicoses because these food materials are staple foods in the region. Although values obtained for heavy metals and aflatoxins were not above the maximum permissible limit except for lead, but long term exposure to low levels of these toxins in the food supply system may bio-accumulate in the human system and consequently cause various health challenges (USDA, 2005. The implication of the findings of this research is that most of the food materials presently on sale in our markets are partially acceptable for human consumption. Since these food materials are also distributed from south eastern Nigeria to other parts of Nigeria, it is possible that even more people are consuming contaminated foodstuffs in the country. Hence there is need for consistent sensitization of consumers in South Eastern Nigeria and awareness campaign on dangers/possibility of fungal infestation, heavy metals and aflatoxin contamination of various food materials exposed in open for sale in the market.

Though the amount of aflatoxins obtained in the foodstuff analyzed fall below the 10μg/kg limit set in Nigeria for unprocessed food products by NAFDAC. However, it is crucial to devise natural means of preventing the survival of aflatoxin-producing species in food products prior to consumption in south eastern Nigeria, especially during storage where they are proned to be colonised by these toxin-producing pathogenic organisms due to the prevalent environmental conditions in many of the storage facilities used by commercial retailers. Intrestingly, the results from this research work indicate that extract of *Moringa oleifera* and *Chromolaena odorata* could be exploited for the control of fungal contaminants of cassava chips, *Citrullus colocynthis* (Egusi) and *Irvingia* wombolu (Ogbono).

5.8. RECOMMENDATIONS

This research has elucidated so many facts about the quality of foodstuffs sold in the open markets in south eastern Nigeria and by reason of these glaring facts; suggestions have been proffered on how these anomalies could be corrected, hence the following recommendations:

- There is need for consistent sensitization of the masses in South Eastern Nigeria and awareness campaign on dangers of microbial infestation, heavy metal and aflatoxin contamination of food materials sold in the open markets in the region. In addition to this, food handlers should also be sensitized on good food handling/processing procedures with respect to storage and drying techniques/duration. Some aspects of Sanitary and Phyto-sanitary (SPS) measures can also be discussed with the food handlers to enlighten them (participants) on the safe level, monitoring and enforcement of the relevant regulatory and trade provisions, thus maintaining international market access for Nigerian Ogbono, Egusi and Cassava chips.

- It is therefore important that both the food processors and the marketers take necessary precautions in preventing contamination of *C. citrullus*, *I. wombolu* and cassava chips sold in the open market to reduce possible contamination and hence reduce the risk of aflatoxin and other mycotoxins that are deleterious to human health. *C. colocynthis* and *I. wombolu* are important soup thickners in south eastern Nigeria and in the entire Nigeria in general while cassava chips is also an important meal in the region mainly consumed by rural dwellers. In otherwords, these food materials are important foods, so there is the need therefore to adopt good handling and processing techniques so that the masses can

consume wholesome food. These food handlers should be conscious of the fact that proper handling of food stuff to avoid contamination is imperative.

- Government should subsidize agro-chemicals such as pesticides to enable farmers and food handlers to afford it.

- Government should provide good storage facilities to prevent post harvest losses and contamination of these food materials

- Information concerning the level of mould infestation, heavy metal and aflatoxin contamination of foodstuffs sold in the open market should be made available to the masses by relevant agencies such as the Research institutes and the Federal Ministry of Health, this information should be dessiminated at all levels of the society in the country by extension workers.

- The study and construction of localized mechanical drying equipments with suitable and adaptable technology that could be used in hot humid climate like South Eastern Nigeria.

- Government/relevant agencies should fund research centered on detailed study of fungal ecology and development of relatively cheaper biological control and plant based agents.

However, the prospect of Nigerian crops/foodstuffs remaining relevant in the international market is tied to the above recommendations. This is the only path to greater expectations of substantial contribution of these food materials to food supply and thereby to the economy of Nigeria.

5.9. SUGGESTIONS FOR FURTHER STUDY

Below are suggestions for further study

1. Descriptive research or Survey should be conducted on the perception and practice of food handling by marketers in other regions, geopolitical zones or agro-ecological zones in Nigeria
2. Research should be conducted on the quality of *C. colocynthis*, *I. wombolu* and Cassava chips sold in the open market in other regions or agro-ecological zones of the country.
3. Investigations of other organic/natural compounds that could inhibit fungal growth and influence aflatoxin synthesis.
4. Further investigations can also combine more and other plant extracts for possible synergistic effect.
5. More research involving *in vivo* and *in vitro* assay would be needed to investigate the fungistatic effects of these plant extracts on the mycotoxigenic fungi that are not included among the test fungi in this research work.
6. Research should be designed and conducted on the isolation of bioactive molecules and development of simple formulation technique is important for large scale implementation.

5.10 CONTRIBUTIONS TO KNOWLEDGE

1. There was limited information on the quality of Egusi, Ogbono and Cassava chips sold in South eastern Nigeria, but the findings of this study has added useful information on the quality of these food materials.
2. The inference of this study has shown that Egusi, Ogbono and Cassava chips sold in open markets in south eastern Nigeria are contaminated with aflatoxins but at a level that is below the maximum permissible limit of 4ppb set by NAFDAC.
3. The findings of this research also showed that extracts of *Moringa oleifera* and *Chromolaena odorata* are good inhibitors of mycotoxigenic fungi, even better than commercial fungicide.
4. It was also established that the extracts of *Moringa oleifera* and *Chromolaena odorata* contain antifungal substances that can be extracted by industries in the production of some antifungal materials.
5. The inference of this study is of great importance to the food handlers, extension workers, chefs, kitchen managers, school cooks, the government and all other stakeholders involved in handling of food samples.

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APPENDICES

Appendix 1:

Plate 1: Sample of Ogbono used for the analysis

Plate 2: Sample of Egusi used for the analysis

Plate 3: Sample of cassava chips used for the analysis

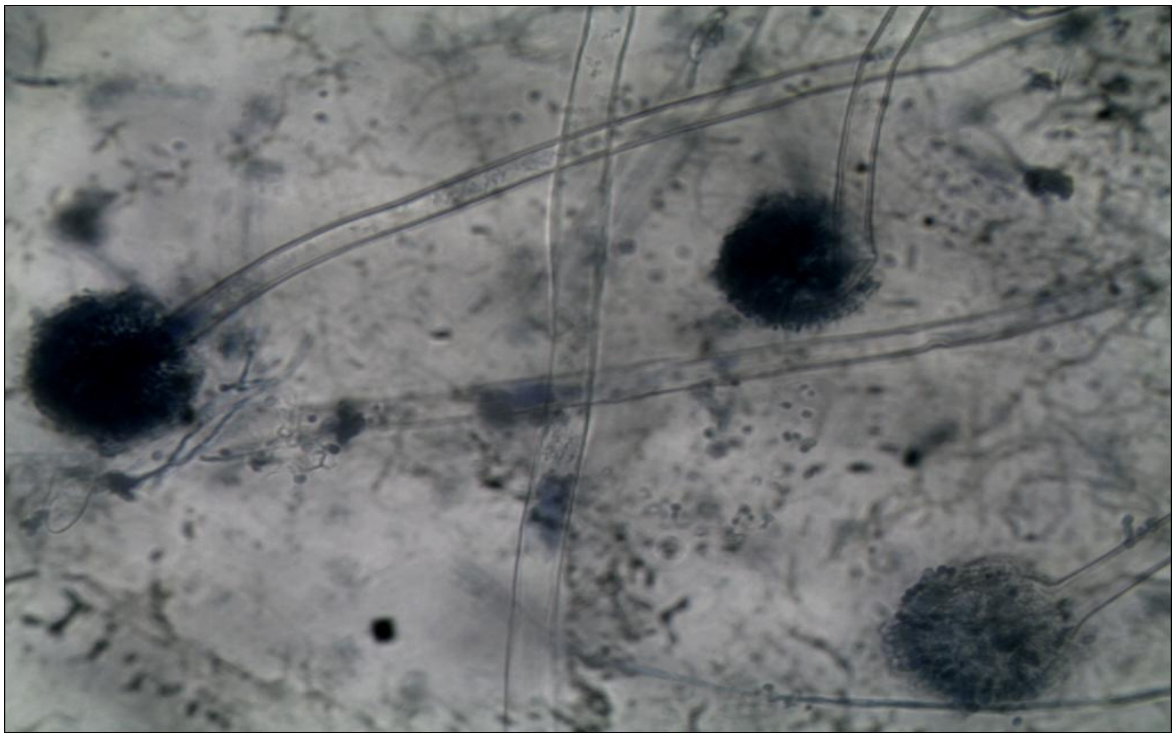


Plate 4: Spores of *Aspergillus niger* viewed under microscope

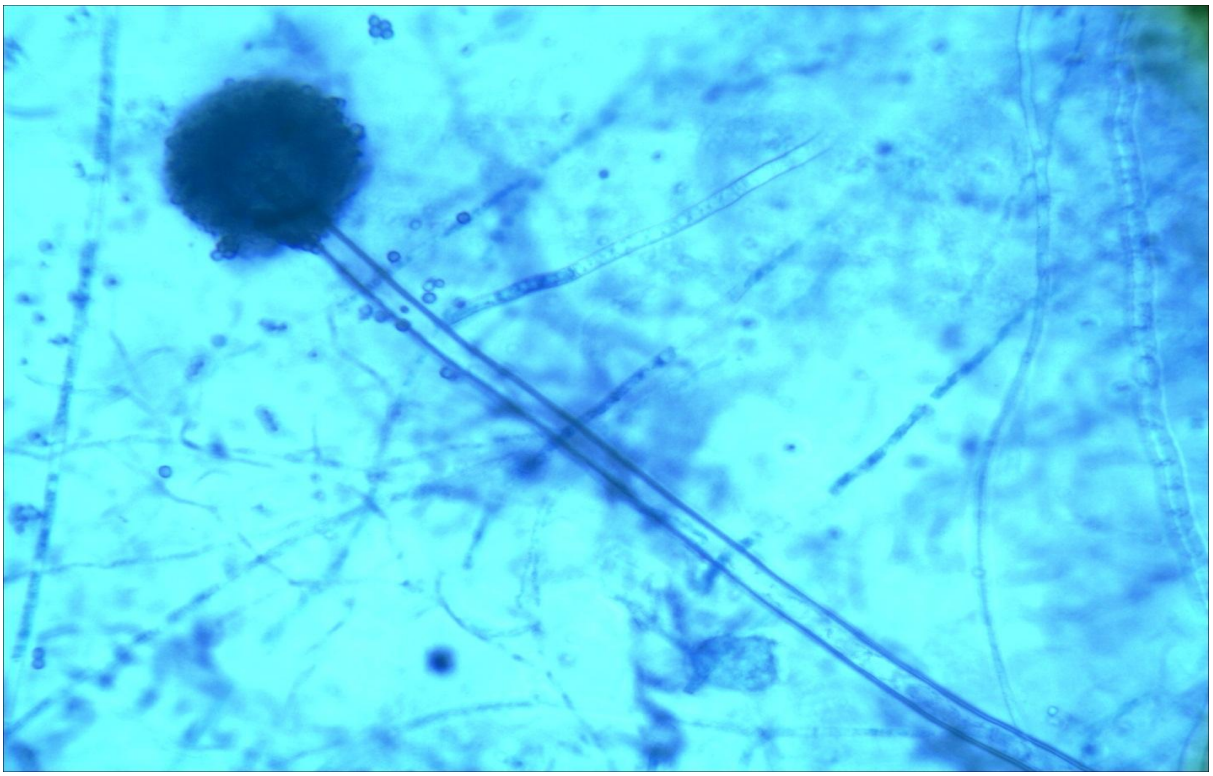


Plate 5: Spores of *Aspergillus flavus* viewed under microscope

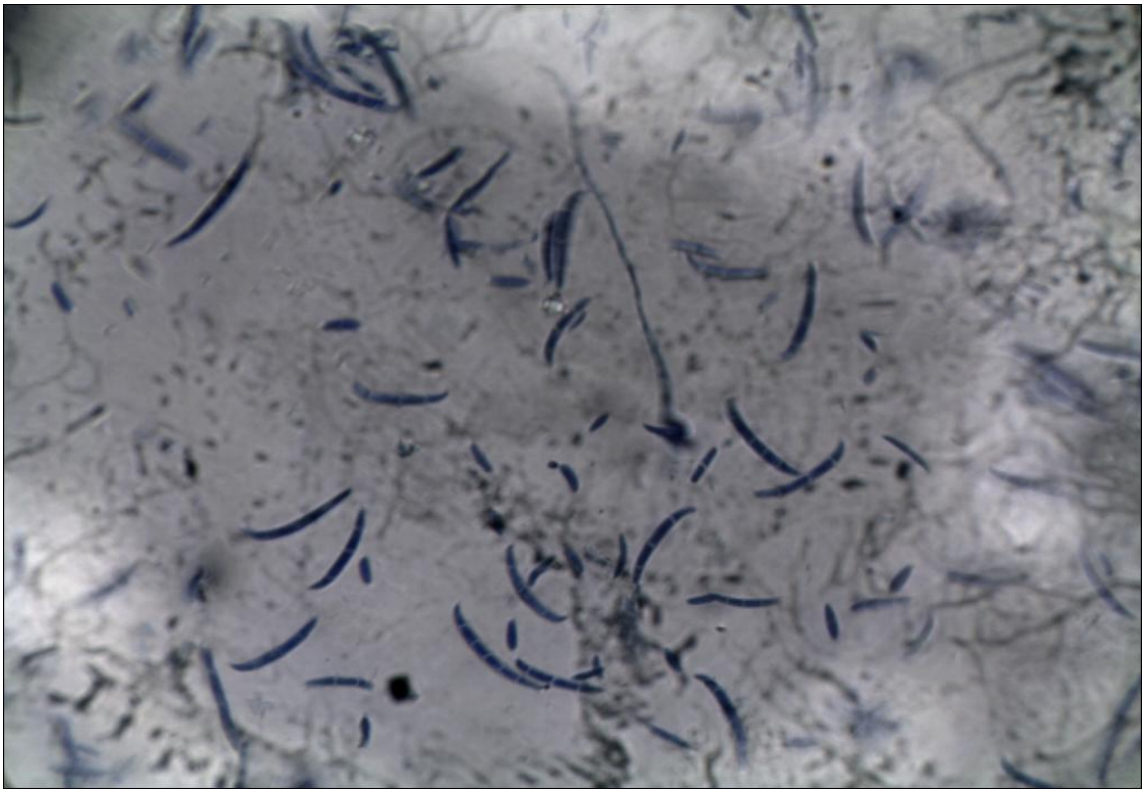


Plate 6: Spores of *Fusarium oxysporium* as viewed under microscope

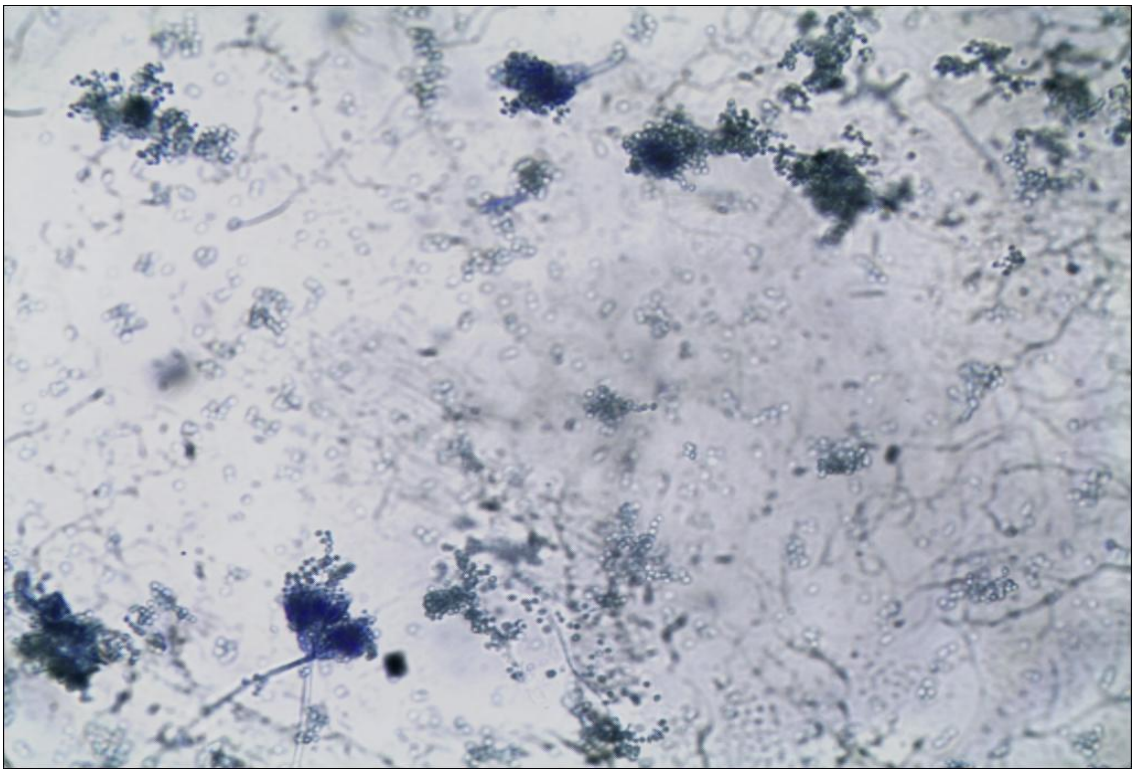


Plate 7: Spores of *Penicillium* spp as viewed under microscope

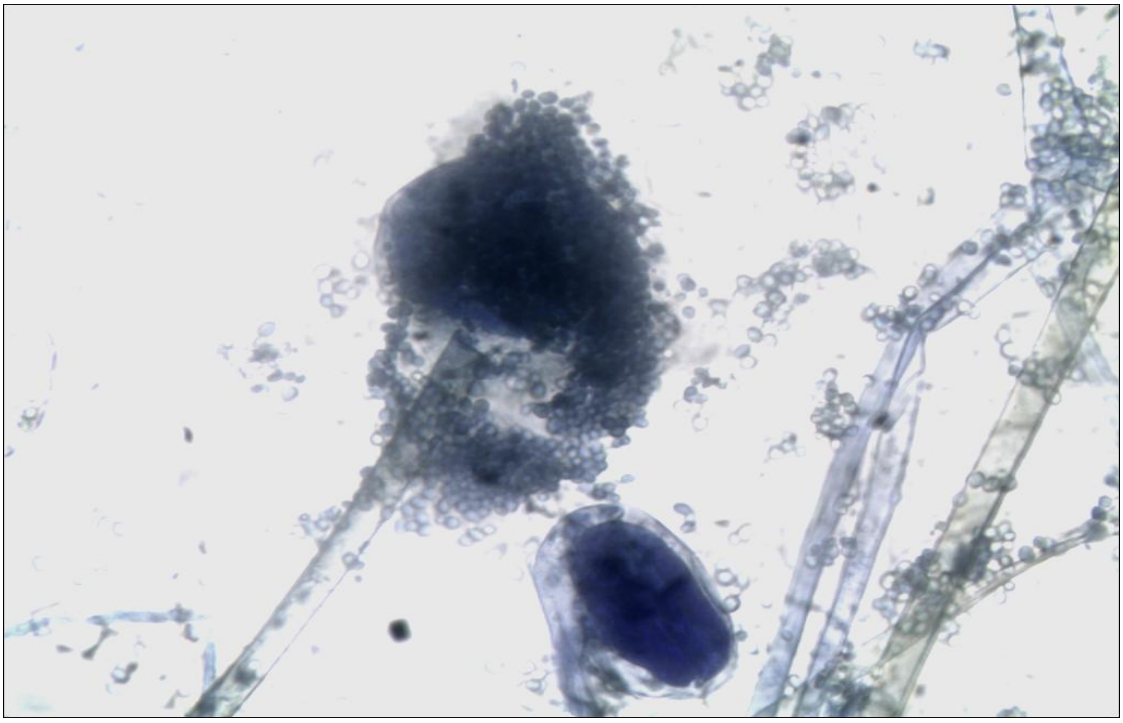


Plate 8: Spores of *Rhizopus stolonifer* as viewed under microscope

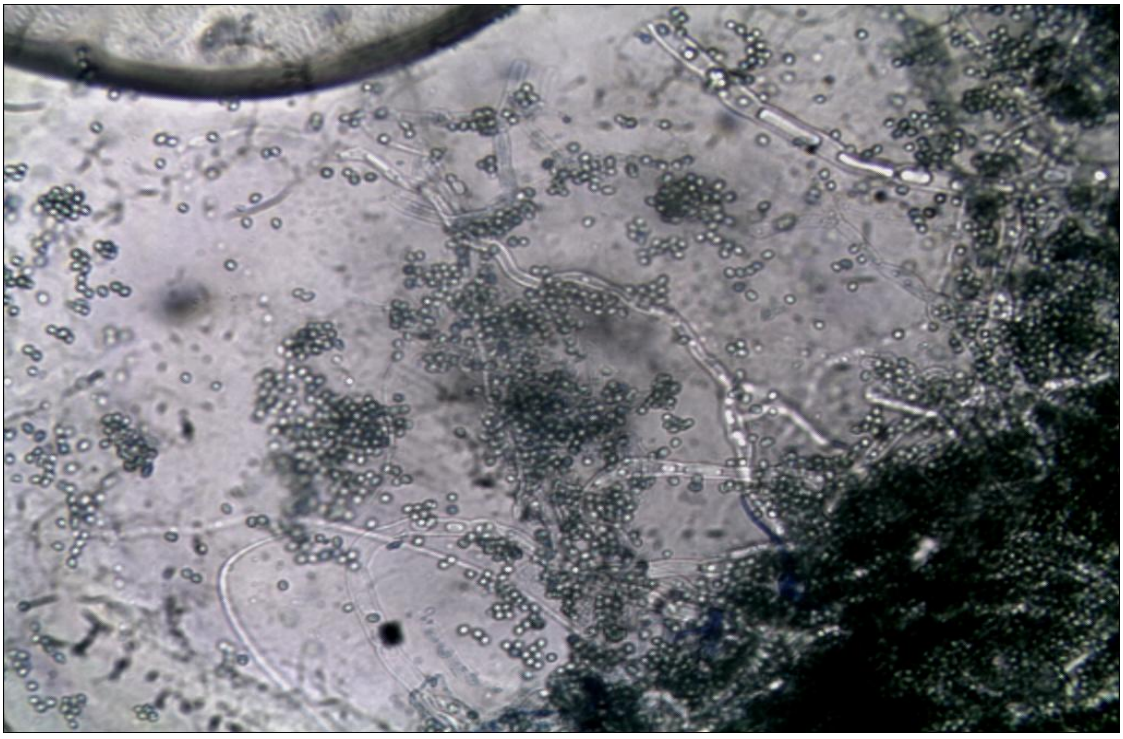


Plate 9: Spores of *Trichoderma viride* as viewed under microscope

Appendix 2:

RESEARCH QUESTIONNAIRE

Department of Botany,
Nnamdi Azikiwe University,
Awka,
Anambra State.
9th February 2015.

Sir/Madam,

RESEARCH SURVEY

This research survey entitled "Assessment of aflatoxin contamination of some foods and antifungal potential of some plant extracts in south-eastern Nigeria" is undertaken with a view to assessing the factors predisposing egusi, ogbono and cassava chips to fungal infestation and mycotoxin contamination.

The information you will supply will be treated with serious confidentiality and used strictly for academics purposes. Therefore, kindly be honest and objective in answering the questions raised in the questionnaire.

Thank you,

Yours faithfully,

Anukwuorji Chidozie, A

Appendix 3

Questionnaire (Ethno-study)

Tick in the appropriate place

State: (a) Enugu ☐ (b) Anambra ☐ (c) Imo ☐ (d) Abia ☐ (e) Ebonyi ☐

Language: (a) Igbo ☐ (b) Igala ☐ (c) Hausa ☐ (d) Yoruba ☐

Age: (a) 10-20 ☐ (b) 21-30 ☐ (c) 31-40 ☐ (d) 41-50 ☐ (e) 51 and above ☐

Sex: (a) Male ☐ (b) Female ☐

Tick one or more of the options below

CASSAVA CHIPS				**EGUSI**				**OGBONO**			
(1) Drying method				**Drying method**							
Sun drying	Oven drying	Use of cribs	Others	Sun drying	Oven drying	Use of cribs	Others	Sun drying	Oven drying	Use of cribs	Others
(2) Drying period				**Drying period**				**Drying period**			
2 days	1 wk	2 wks	More than 2 weeks	2 days	1 wk	2 wks	More than 2 weeks	2 days	1wk	2wks	More than 2 weeks
(3) Storage method before marketing				**Storage method before marketing**				**Storage method before marketing**			
Polythene bag	Plastic container	Jute bag	Others	Polythene bag	Plastic container	Jute bag	Others	Polythene bag	Plastic container	Jute bag	Others
(4)Sorting out bad ones				**Sorting out bad ones**				**Sorting out bad ones**			
Yes	No	Not always		Yes	No	Not always		Yes	No	Not always	
(5) Storage period				**Storage period**				**Storage period**			
1-3 months	4-6 months	7-9 months	More than 9 months	1-3 months	4-6 months	7-9 months	More than 9 months	1-3 months	4-6 months	7-9 months	More than 9 months
(6) Do you use pesticide during storage				**Do you use pesticide during storage**				**Do you use pesticide during storage**			
Yes		No		Yes		No		Yes		No	
Gave the name				Gave the name				Gave the name			

Appendix 4: Concentrations of Aflatoxins in Cassava chips

States	Wave Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	DHM	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a
	HDS	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a
	RS	0.073±0.127b	0.067±0.115b	0.000±0.000a	0.000±0.000a	0.140±0.242b
Anambra	DHM	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.100±0.173b	0.100±0.173b
	HDS	0.030±0.052b	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.030±0.052a
	RS	0.120±0.208c	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.127±0.202b
Ebonyi	DHM	0.008±0.010a	0.010±0.000b	0.008±0.013b	0.000±0.000a	0.032±0.003a
	HDS	0.010±0.017a	0.015±0.022b	0.000±0.000a	0.000±0.000a	0.025±0.019a
	RS	0.011±0.010a	0.003±0.005a	0.000±0.000a	0.000±0.000a	0.014±0.012a
Enugu	DHM	0.000±0.000a	0.003±0.006b	0.000±0.000a	0.000±0.000a	0.003±0.006b
	HDS	0.012±0.013b	0.117±0.176b	0.000±0.000a	0.000±0.000a	0.030±0.029c
	RS	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a
Imo	DHM	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a
	HDS	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a
	RS	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.000±0.000a

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different (p>0.05)

Appendix 5: Concentrations of Aflatoxins in Egusi

States	Wave Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	DHM	0.012±0.016a	0.000±0.000a	0.027±0.046b	0.000±0.000a	0.006±0.006a
	HDS	0.023±0.023a	0.033±0.031b	0.023±0.040b	0.000±0.000a	0.080±0.066b
	RS	0.010±0.017a	0.040±0.000b	0.000±0.000a	0.000±0.000a	0.050±0.017b
Anambra	DHM	0.200±0.173a	0.467±0.115a	0.000±0.000a	0.000±0.000a	0.667±0.058a
	HDS	0.130±0.137a	0.533±0.118a	0.007±0.006a	0.000±0.000a	0.670±0.101a
	RS	0.223±0.193a	0.341±0.174a	0.000±0.000a	0.006±0.005b	0.570±0.036a
Ebonyi	DHM	0.045±0.000b	0.124±0.196c	0.013±0.023b	0.000±0.000a	0.074±0.015b
	HDS	0.020±0.000a	0.033±0.058b	0.000±0.000a	0.000±0.000a	0.053±0.058b
	RS	0.022±0.025a	0.003±0.006a	0.000±0.000a	0.000±0.000a	0.025±0.023a
Enugu	DHM	0.450±0.087b	0.383±0.104b	0.000±0.000a	0.000±0.000a	0.593±0.469b
	HDS	0.021±0.002a	0.000±0.000a	0.041±0.001b	0.000±0.000a	0.063±0.003a
	RS	0.068±0.012b	0.005±0.008a	0.000±0.000a	0.000±0.000a	0.073±0.003a
Imo	DHM	0.004±0.000a	0.008±0.006a	0.000±0.000a	0.000±0.000a	0.012±0.006a
	HDS	0.008±0.000a	0.008±0.014a	0.013±0.022b	0.001±0.001b	0.029±0.019a
	RS	0.107±0.095a	0.057±0.098b	0.000±0.000a	0.000±0.000a	0.163±0.021a

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)

Appendix 6: Concentrations of Aflatoxins in Ogbono

States	Wave Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	DHM	0.270±0.047b	0.000±0.000a	0.040±0.006a	0.000±0.000a	0.310±0.074a
	HDS	0.083±0.012a	0.033±0.058b	0.407±0.052c	0.000±0.000a	0.523±0.086b
	RS	0.3100±0.00b	0.000±0.000a	0.093±0.012b	0.000±0.000a	0.403±0.012b
Anambra	DHM	0.123±0.062a	0.073±0.027b	0.433±0.079c	0.000±0.000a	0.630±0.100b
	HDS	0.333±0.066a	0.040±0.035b	0.000±0.000a	0.000±0.000a	0.373±0.086a
	RS	0.643±0.061b	0.167±0.008c	0.023±0.039b	0.000±0.000a	0.833±0.041b
Ebonyi	DHM	0.035±0.006a	0.016±0.006a	0.014±0.003b	0.000±0.000a	0.064±0.011a
	HDS	0.054±0.010b	0.031±0.015b	0.000±0.000a	0.000±0.000a	0.085±0.006b
	RS	0.058±0.021b	0.016±0.015a	0.000±0.000a	0.000±0.000a	0.073±0.006a
Enugu	DHM	0.240±0.113b	0.09±0.085c	0.000±0.000a	0.000±0.000a	0.330±0.062a
	HDS	0.058±0.020a	0.045±0.007b	0.000±0.000a	0.000±0.000a	0.103±0.027a
	RS	0.055±0.046a	0.002±0.00a	0.004±0.000b	0.000±0.000a	0.061±0.046a
Imo	DHM	0.002±0.001a	0.002±0.002b	0.000±0.000a	0.000±0.000a	0.004±0.003a
	HDS	0.007±0.006a	0.000±0.000a	0.000±0.000a	0.000±0.000a	0.007±0.006a
	RS	0.043±0.051b	0.000±0.000a	0.087±0.040b	0.000±0.000a	0.130±0.017b

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)

Appendix 7: Concentration of metals in Cassava chips

States	Wave Collection	Average Heavy Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia	DHM	0.000±0.000a	0.261±0.372a	0.027±0.047a	1.081±0.093b
	HDS	0.000±0.000a	0.023±0.011a	0.003±0.005a	0.717±0.540a
	RS	0.000±0.000a	0.471±0.066a	0.000±0.000a	0.407±0.061a
Anambra	DHM	0.000±0.000a	0.047±0.050a	0.094±0.062b	0.208±0.077b
	HDS	0.000±0.000a	0.022±0.034a	0.000±0.000a	0.030±0.010a
	RS	0.000±0.000a	0.103±0.079b	0.000±0.000a	0.109±0.029b
Ebonyi	DHM	0.000±0.000a	0.083±0.013c	0.000±0.000a	0.279±0.003a
	HDS	0.000±0.000a	0.058±0.009b	0.000±0.000a	0.173±0.019a
	RS	0.000±0.000a	0.010±0.009a	0.000±0.000a	0.120±0.009a
Enugu	DHM	0.110±0.065b	0.091±0.006a	0.194±0.053b	0.312±0.045b
	HDS	0.000±0.000a	0.080±0.016a	0.016±0.014a	0.034±0.058a
	RS	0.025±0.035b	0.771±0.085b	0.195±0.010b	0.345±0.064b
Imo	DHM	0.000±0.000a	0.241±0.122b	0.000±0.000a	0.328±0.008b
	HDS	0.000±0.000a	0.060±0.070a	0.000±0.000a	0.083±0.005a
	RS	0.000±0.000a	0.143±0.232b	0.000±0.000a	0.043±0.004a

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

Appendix 8: Concentration of Metals in Egusi

States	Wave Collection	Average Heavy Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia	DHM	0.000±0.000a	0.09±0.0450a	0.229±0.038b	0.075±0.024a
	HDS	0.000±0.000a	0.052±0.015a	0.034±0.031a	0.047±0.010a
	RS	0.000±0.000a	0.977±0.068b	0.187±0.063b	0.289±0.057b
Anambra	DHM	0.000±0.000a	0.156±0.031a	0.133±0.053b	0.088±0.035a
	HDS	0.000±0.000a	0.051±0.044b	0.026±0.031a	0.054±0.047a
	RS	0.000±0.000a	0.036±0.055b	0.001±0.002a	0.115±0.005b
Ebonyi	DHM	0.000±0.000a	0.073±0.054a	0.127±0.020b	0.131±0.070b
	HDS	0.000±0.000a	0.032±0.017a	0.000±0.000a	0.027±0.047a
	RS	0.000±0.000a	0.135±0.013b	0.181±0.057b	0.093±0.000b
Enugu	DHM	0.219±0.244b	0.304±0.077b	0.790±0.033b	2.997±0.036b
	HDS	0.333±0.289b	0.051±0.009a	0.445±0.034a	2.71±0.034b
	RS	0.027±0.046a	0.589±0.086b	0.820±0.094b	1.38±0.0606a
Imo	DHM	0.000±0.000a	0.173±0.032b	0.000±0.000a	0.255±0.008b
	HDS	0.000±0.000a	0.050±0.009a	0.006±0.010b	0.056±0.022a
	RS	0.000±0.000a	0.502±0.019c	0.010±0.018b	0.617±0.013c

Results are in Mean ± Standard Deviation

Means with the same letter in a column are not significantly different ($p>0.05$)

Appendix 9: Concentrations of Metals in Ogbono

States	Wave Collection	Average Heavy Metal Concentrations (mg/g)			
		Lead	Zinc	Copper	Iron
Abia	DHM	0.000±0.000a	0.053±0.026b	0.016±0.028b	0.194±0.035a
	HDS	0.000±0.000a	0.008±0.012a	0.000±0.000a	0.137±0.037a
	RS	0.000±0.000a	0.172±0.066b	0.016±0.028b	0.375±0.007b
Anambra	DHM	0.000±0.000a	0.301±0.045b	0.197±0.006b	0.078±0.055a
	HDS	0.000±0.000a	0.042±0.038a	0.034±0.036b	0.033±0.005a
	RS	0.000±0.000a	0.84±0.061c	0.000±0.000a	0.244±0.022b
Ebonyi	DHM	0.000±0.000a	0.312±0.022b	0.104±0.008b	0.031±0.031b
	HDS	0.000±0.000a	0.046±0.02a	0.000±0.000a	0.002±0.003a
	RS	0.000±0.000a	0.066±0.03a	0.070±0.002b	0.127±0.005c
Enugu	DHM	0.113±0.081b	0.500±0.070b	0.615±0.534b	0.276±0.079b
	HDS	0.013±0.023a	0.042±0.013a	0.000±0.000a	0.019±0.033a
	RS	0.000±0.000a	0.326±0.411b	0.568±0.042b	0.994±0.006c
Imo	DHM	0.000±0.000a	0.349±0.013b	0.000±0.000a	0.455±0.025b
	HDS	0.000±0.000a	0.229±0.050b	0.000±0.000a	0.197±0.046a
	RS	0.000±0.000a	0.053±0.010a	0.034±0.031b	0.114±0.006a

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)

Tests of Between-Subjects Effects

Dependent Variable: Drying Method

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	58.030[a]	14	4.145	13.011	.000
Intercept	1095.704	1	1095.704	3439.273	.000
State	43.837	4	10.959	34.400	.000
Food Sample	4.234	2	2.117	6.645	.001
State * Food Sample	9.959	8	1.245	3.907	.000
Error	210.267	660	.319		
Total	1364.000	675			
Corrected Total	268.296	674			

a. R Squared = .216 (Adjusted R Squared = .200)

Tests of Between-Subjects Effects

Dependent Variable: Drying Period

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	25204.641[a]	14	1800.331	24.501	.000
Intercept	281048.731	1	281048.731	3824.817	.000
State	17240.044	4	4310.011	58.655	.000
Food_Sample	2281.284	2	1140.642	15.523	.000
State * Food_Sample	5634.267	8	704.283	9.585	.000
Error	48129.604	655	73.480		
Total	356092.000	670			
Corrected Total	73334.245	669			

a. R Squared = .344 (Adjusted R Squared = .330)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	36.889[a]	14	2.635	7.635	.000
Intercept	2408.333	1	2408.333	6978.293	.000
Food_Sample	.560	2	.280	.811	.445
State	20.948	4	5.237	15.175	.000
Food_Sample * State	15.381	8	1.923	5.571	.000
Error	227.778	660	.345		
Total	2673.000	675			
Corrected Total	264.667	674			

a. R Squared = .139 (Adjusted R Squared = .121)

Tests of Between-Subjects Effects
Dependent Variable: Storage Materials

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	95.897[a]	14	6.850	5.380	.000
Intercept	5187.629	1	5187.629	4074.587	.000
Food_Sample	8.936	2	4.468	3.510	.030
State	53.012	4	13.253	10.410	.000
Food_Sample * State	34.084	8	4.261	3.346	.001
Error	841.563	661	1.273		
Total	6127.000	676			
Corrected Total	937.460	675			

Appendix 14

Three Way ANOVA for AFBI. between samples, state and wave collection

Dependent Variable: AFB1

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2.394[a]	44	.054	4.861	.000
Intercept	1.026	1	1.026	91.673	.000
Wave	.093	2	.046	4.147	.019
State	.567	4	.142	12.670	.000
Wave	.421	2	.211	18.807	.000
State	.426	8	.053	4.758	.000
Plant materials	.106	4	.026	2.363	.059

Wave * State	.434	8	.054	4.851	.000
Wave * Plant materials	.347	16	.022	1.937	.027
Error	1.007	90	.011		
Total	4.428	135			
Corrected Total	3.402	134			

a. R Squared = .704 (Adjusted R Squared = .559)

Appendix 15:

Three way ANOVA for AFB2. Between food samples, wave collection and state

Dependent Variable: AFB2

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2.052[a]	44	.047	8.823	.000
Intercept	.509	1	.509	96.336	.000
Wave	.023	2	.012	2.179	.119
State	.538	4	.135	25.442	.000
Plant materials	.381	2	.191	36.038	.000
Wave * State	.109	8	.014	2.584	.014
Wave * Plant materials	.072	4	.018	3.414	.012
State * Plant materials	.679	8	.085	16.043	.000
Wave * State * Plant materials	.250	16	.016	2.957	.001
Error	.476	90	.005		
Total	3.037	135			
Corrected Total	2.528	134			

a. R Squared = .812 (Adjusted R Squared = .720)

Appendix 16

Three way ANOVA for AFG1. Between food samples, wave collection and state

Dependent Variable: AFG1

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1.024[a]	44	.023	3.771	.000
Intercept	.101	1	.101	16.394	.000
Wave	.013	2	.006	1.027	.362
State	.091	4	.023	3.669	.008

Plant materials	.144	2	.072	11.679	.000
Wave * State	.193	8	.024	3.899	.001
Wave * Plant materials	.015	4	.004	.588	.672
State * Plant materials	.176	8	.022	3.564	.001
Wave * State * Plant materials	.394	16	.025	3.986	.000
Error	.556	90	.006		
Total	1.681	135			
Corrected Total	1.580	134			

a. R Squared = .648 (Adjusted R Squared = .476)

Appendix 17

Three way ANOVA for AFG2. Between food samples, wave collection and state

Dependent Variable: AFG2

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	.029[a]	44	.001	1.000	.489
Intercept	.001	1	.001	1.137	.289
Wave	.001	2	.001	.936	.396
State	.003	4	.001	1.119	.353
Plant materials	.001	2	.001	.937	.396
Wave * State	.005	8	.001	.945	.484
Wave * Plant materials	.003	4	.001	1.036	.393
State * Plant materials	.005	8	.001	.944	.485
Wave * State * Plant materials	.011	16	.001	1.032	.432
Error	.060	90	.001		
Total	.090	135			
Corrected Total	.089	134			

a. R Squared = .328 (Adjusted R Squared = .000)

Appendix 18

Three way ANOVA for Total AF. Between food samples, wave collection and state

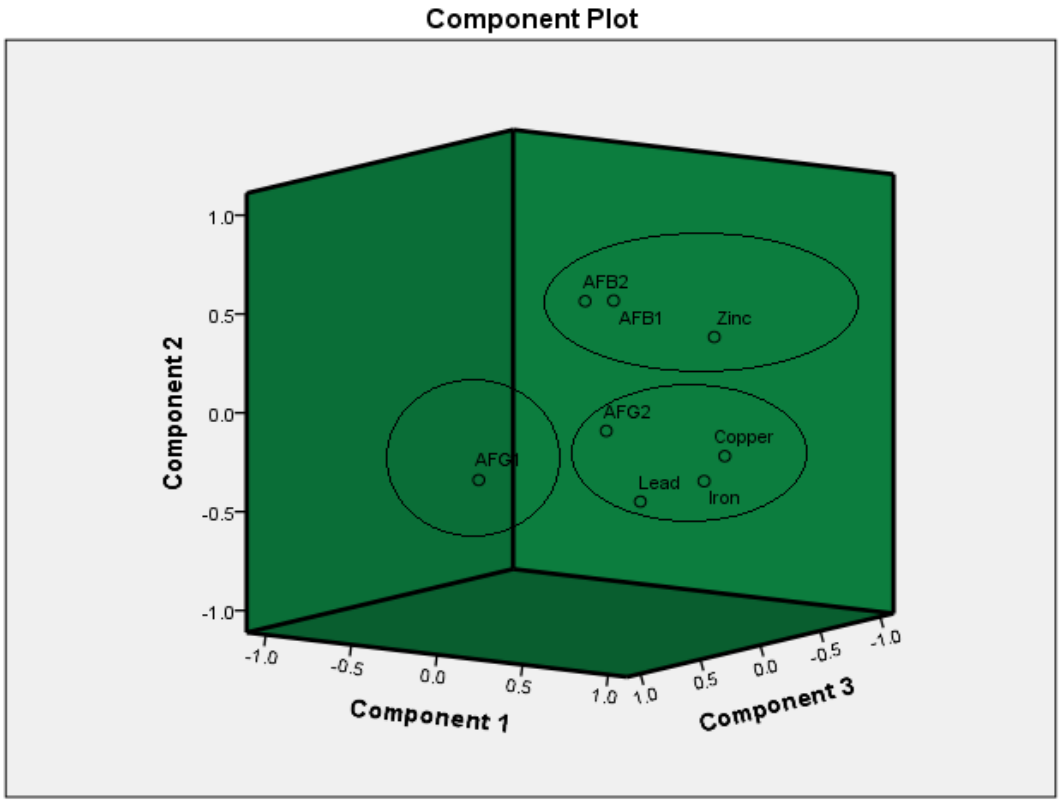
Dependent Variable: TOTAL AFLATOXIN

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	6.973[a]	44	.158	10.201	.000
Intercept	3.809	1	3.809	245.179	.000
Wave	.063	2	.031	2.021	.138
State	2.918	4	.729	46.958	.000
Plant materials	1.288	2	.644	41.440	.000
Wave * State	.539	8	.067	4.338	.000
Wave * Plant materials	.086	4	.022	1.387	.245
State * Plant materials	1.542	8	.193	12.408	.000
Wave * State * Plant materials	.537	16	.034	2.160	.012
Error	1.398	090	.016		
Total	12.180	135			
Corrected Total	8.371	134			

R Squared = .833 (Adjusted R Squared = .751)

Appendix 19

PCA Ordination of Aflatoxin and Heavy Metals in food samples



Appendix 20: Univariate Analysis of Variance Between-Subjects Factors (*A.flavus*)

CHEMICAL2	Chemical			Value Label	N
ETHANOL	2.5 ethanol	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
	5.0 ethanol	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
	7.5 ethanol	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2

	10 ethanol	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
AQUEOUS	2.5 aqueous	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
	5.0 aqueous	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
	7.5 aqueous	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	2
			5	P-CTRL (CH)	2
			6	N-CTRL	2
	10 aqueous	plant_extract	1	*Moringa*	2
			2	P-CTRL(MO)	2
			3	NCTRL	2
			4	*Chromolaena*	4
			5	P-CTRL (CH)	2

tiple Comparisons

Homogeneous Subsets

A.flavus

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

		Subset			
plant_extract	N	1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		44.5000		
P-CTRL(MO)	2			56.8500	
Moringa	2				88.5000
P-CTRL (CH)	2				96.0000
Sig.		1.000	1.000	1.000	.206

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5.521.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		46.5000		
P-CTRL(MO)	2		56.8500	56.8500	
*M*oringa	2			66.7000	
P-CTRL (CH)	2				96.0000
Sig.		1.000	.182	.213	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 9.734.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3
NCTRL	2	.0000		
N-CTRL	2	.0000		
P-CTRL(MO)	2		56.8500	
Chromolaena	2		62.5000	
P-CTRL (CH)	2			96.0000
Moringa	2			99.4000
Sig.		1.000	.523	.874

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 6.891.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		42.5000		
P-CTRL(MO)	2			56.8500	
P-CTRL (CH)	2				96.0000
Moringa	2				99.7000
Sig.		1.000	1.000	1.000	.830

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 6.771.

CHEMICAL2=AQUEOUSchemical=2.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		25.0000		
Moringa	2			41.5000	
P-CTRL(MO)	2				97.6000
P-CTRL (CH)	2				98.6000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 9.083.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		43.7000		
Moringa	2			57.0000	
P-CTRL(MO)	2				97.6000
P-CTRL (CH)	2				98.6000
Sig.		1.000	1.000	1.000	.999

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 7.563.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		47.5000		
Moringa	2			77.0000	
P-CTRL(MO)	2				97.6000
P-CTRL (CH)	2				98.6000
Sig.		1.000	1.000	1.000	.824

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .483.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	2	3
Chromolaena	4	.0000		
Moringa	2		77.0000	
P-CTRL(MO)	2		77.0000	
NCTRL	2			97.6000
P-CTRL (CH)	2			98.6000
Sig.		1.000	1.000	.845

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .823.

Post Hoc Tests

plant_extract

Homogeneous Subsets

Fusarium

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		48.5000		
moringa	2			74.0000	
P-CTRL (CH)	2				95.4000
P-CTRL(MO)	2				96.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5.470.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		32.0000		
moringa	2			80.5000	
P-CTRL (CH)	2				95.4000
P-CTRL(MO)	2				96.0000
Sig.		1.000	1.000	1.000	.999

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 2.137.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		64.9500		
moringa	2			80.5000	
P-CTRL (CH)	2				95.4000
P-CTRL(MO)	2				96.0000
Sig.		1.000	1.000	1.000	.998

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.411.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

plant_extract	N	Subset 1	2	3
NCTRL	2	.0000		
N-CTRL	2	.0000		
Chromolaena	2		55.8500	
P-CTRL (CH)	2			95.4000
P-CTRL(MO)	2			96.0000
moringa	2			97.5000
Sig.		1.000	1.000	.448

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .804.

CHEMICAL2=AQUEOUSchemical=2.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		20.4500		
moringa	2			55.7000	
P-CTRL(MO)	2				98.2000
P-CTRL (CH)	2				1.0000E2
Sig.		1.000	1.000	1.000	.962

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 3.698.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		17.6000			
moringa	2			58.0000		
P-CTRL(MO)	2				98.2000	
P-CTRL (CH)	2					1.0000E2
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .053.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		53.6000		
moringa	2			64.0000	
P-CTRL(MO)	2				98.2000
P-CTRL (CH)	2				1.0000E2
Sig.		1.000	1.000	1.000	.973

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4.320.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
Chromolaena	4	.0000			
moringa	2		64.0000		
P-CTRL(MO)	2		64.0000		
NCTRL	2			98.2000	
P-CTRL (CH)	2				1.0000E2
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4.62E-028.

Post Hoc Tests

plant_extract

Homogeneous Subsets

trichoderma

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

plant_extract	N	Subset 1	Subset 2
NCTRL	2	.0000	
N-CTRL	2	.0000	
P-CTRL (CH)	2	30.2500	30.2500
Chromolaena	2	37.0000	37.0000
P-CTRL(MO)	2		62.5000
moringa	2		64.4000
Sig.		.143	.187

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 107.558.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	Subset 2	Subset 3
NCTRL	2	.0000		
N-CTRL	2	.0000		
P-CTRL (CH)	2		30.2500	
Chromolaena	2		57.0000	57.0000
P-CTRL(MO)	2			62.5000
moringa	2			67.6000
Sig.		1.000	.067	.705

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 37.558.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	Subset 2	Subset 3	Subset 4
NCTRL	2	.0000			
N-CTRL	2	.0000			
P-CTRL (CH)	2		30.2500		
P-CTRL(MO)	2			62.5000	
Chromolaena	2			63.0500	
moringa	2				99.0000

Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 37.172.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

		Subset			
plant_extract	N	1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
P-CTRL (CH)	2		30.2500		
Chromolaena	2		49.5000	49.5000	
P-CTRL(MO)	2			62.5000	
moringa	2				99.5500
Sig.		1.000	.213	.532	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 37.188.

CHEMICAL2=AQUEOUSchemical=2.5 aqueous

Scheffe

		Subset				
plant_extract	N	1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		29.5000			
moringa	2			50.0000		
P-CTRL(MO)	2				56.7000	
P-CTRL (CH)	2					97.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .083.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

		Subset				
plant_extract	N	1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		28.0000			
P-CTRL(MO)	2			56.7000		
moringa	2				58.0000	
P-CTRL (CH)	2					97.0000

Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 6.73E-029.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		56.5000		
P-CTRL(MO)	2		56.7000		
moringa	2			64.0000	
P-CTRL (CH)	2				97.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .750.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
Chromolaena	4	.0000			
NCTRL	2		56.7000		
moringa	2			64.0000	
P-CTRL(MO)	2			64.0000	
P-CTRL (CH)	2				97.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.15E-028.

Rhizopus

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		17.5000			
P-CTRL(MO)	2			52.5000		
P-CTRL (CH)	2				66.8000	
moringa	2					88.8500
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4.411.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		20.0000		
P-CTRL(MO)	2			52.5000	
P-CTRL (CH)	2				66.8000
moringa	2				76.5000
Sig.		1.000	1.000	1.000	.071

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5.087.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
P-CTRL(MO)	2		52.5000		
P-CTRL (CH)	2			66.8000	
Chromolaena	2			74.0000	74.0000
moringa	2				85.2500
Sig.		1.000	1.000	.413	.114

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
P-CTRL(MO)	2		52.5000		
P-CTRL (CH)	2			66.8000	
Chromolaena	2			74.0000	74.0000
moringa	2				85.2500
Sig.		1.000	1.000	.413	.114

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 8.728.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		44.0000			
P-CTRL(MO)	2			52.5000		
P-CTRL (CH)	2				66.8000	
moringa	2					89.9000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 2.420.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1
NCTRL	2	.0000
P-CTRL (CH)	2	.0000
N-CTRL	2	.0000
moringa	2	11.5000
P-CTRL(MO)	2	15.0000
Chromolaena	2	17.5000
Sig.		.366

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1
NCTRL	2	.0000
P-CTRL (CH)	2	.0000
N-CTRL	2	.0000
moringa	2	11.5000
P-CTRL(MO)	2	15.0000
Chromolaena	2	17.5000
Sig.		.366

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 46.167.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1
NCTRL	2	.0000
P-CTRL (CH)	2	.0000
N-CTRL	2	.0000
moringa	2	11.5000
P-CTRL(MO)	2	15.0000
Chromolaena	2	17.5000
Sig.		.366

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 46.167.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	Subset 2
Chromolaena	4	.0000	
P-CTRL (CH)	2	.0000	
moringa	2	6.0000	6.0000
P-CTRL(MO)	2	12.0000	12.0000
NCTRL	2		15.0000
Sig.		.057	.172

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	Subset 2
Chromolaena	4	.0000	
P-CTRL (CH)	2	.0000	
moringa	2	6.0000	6.0000
P-CTRL(MO)	2	12.0000	12.0000
NCTRL	2		15.0000
Sig.		.057	.172

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10.286.

Homogeneous Subsets

Penicillium

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		32.3000		
P-CTRL (CH)	2			67.3500	
moringa	2			75.5000	
P-CTRL(MO)	2				98.0000
Sig.		1.000	1.000	.180	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5.988.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		35.0000			
P-CTRL (CH)	2			67.3500		
moringa	2				81.5000	
P-CTRL(MO)	2					98.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .891.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3
NCTRL	2	.0000		
N-CTRL	2	.0000		
Chromolaena	2		62.5000	
P-CTRL (CH)	2		67.3500	
P-CTRL(MO)	2			98.0000
moringa	2			99.0000

Sig.		1.000	.194	.991

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 2.224.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

		Subset			
plant_extract	N	1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		37.9500		
P-CTRL (CH)	2			67.3500	
P-CTRL(MO)	2				98.0000
moringa	2				99.0000
Sig.		1.000	1.000	1.000	.980

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.542.

CHEMICAL2=AQUEOUSchemical=2.5 aqueous

Scheffe

		Subset				
plant_extract	N	1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		19.5000			
moringa	2			44.0000		
P-CTRL (CH)	2				85.7000	
P-CTRL(MO)	2					96.4000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .417.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

		Subset				
plant_extract	N	1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		29.5000			
moringa	2			50.5000		
P-CTRL (CH)	2				85.7000	
P-CTRL(MO)	2					96.4000

Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .167.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		35.0000			
moringa	2			65.5000		
P-CTRL (CH)	2				85.7000	
P-CTRL(MO)	2					96.4000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 2.083.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
Chromolaena	4	.0000			
moringa	2		52.5000		
P-CTRL(MO)	2		52.5000		
P-CTRL (CH)	2			85.7000	
NCTRL	2				96.4000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.286.

Post Hoc Tests

plant_extract

A.niger

CHEMICAL2=ETHANOLchemical=2.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		33.5000		
moringa	2			73.0000	
P-CTRL (CH)	2				95.0000
P-CTRL(MO)	2				1.0000E2
Sig.		1.000	1.000	1.000	.735

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 9.083.

CHEMICAL2=ETHANOLchemical=5.0 ethanol

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		38.1500		
moringa	2			82.0000	
P-CTRL (CH)	2				95.0000
P-CTRL(MO)	2				1.0000E2
Sig.		1.000	1.000	1.000	.447

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4.541.

CHEMICAL2=ETHANOLchemical=7.5 ethanol

Scheffe

plant_extract	N	Subset 1	2	3
NCTRL	2	.0000		
N-CTRL	2	.0000		
Chromolaena	2		66.1000	
moringa	2			94.6500
P-CTRL (CH)	2			95.0000
P-CTRL(MO)	2			1.0000E2
Sig.		1.000	1.000	.593

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 7.238.

CHEMICAL2=ETHANOLchemical=10 ethanol

Scheffe

plant_extract	N	Subset 1	2	3
NCTRL	2	.0000		
N-CTRL	2	.0000		
Chromolaena	2		47.0000	
P-CTRL (CH)	2			95.0000
moringa	2			99.5000
P-CTRL(MO)	2			1.0000E2
Sig.		1.000	1.000	.071

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.347.

CHEMICAL2=AQUEOUSchemical=2.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		17.0000		
moringa	2			38.5000	
P-CTRL(MO)	2			43.3000	
P-CTRL (CH)	2				97.2000
Sig.		1.000	1.000	.399	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 3.750.

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		20.3000		
moringa	2			36.6500	
P-CTRL(MO)	2			43.3000	
P-CTRL (CH)	2				97.2000
Sig.		1.000	1.000	.265	1.000

CHEMICAL2=AQUEOUSchemical=5.0 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
NCTRL	2	.0000			
N-CTRL	2	.0000			
Chromolaena	2		20.3000		
moringa	2			36.6500	
P-CTRL(MO)	2			43.3000	
P-CTRL (CH)	2				97.2000
Sig.		1.000	1.000	.265	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5.164.

CHEMICAL2=AQUEOUSchemical=7.5 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4	5
NCTRL	2	.0000				
N-CTRL	2	.0000				
Chromolaena	2		29.8500			
P-CTRL(MO)	2			43.3000		
moringa	2				52.0000	
P-CTRL (CH)	2					97.2000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.334.

CHEMICAL2=AQUEOUSchemical=10 aqueous

Scheffe

plant_extract	N	Subset 1	2	3	4
Chromolaena	4	.0000			
NCTRL	2		43.3000		
moringa	2			50.0000	
P-CTRL(MO)	2			50.0000	
P-CTRL (CH)	2				97.2000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 1.15E-028.