FUNGITOXICITY OF SELECTED BIO-PESTICIDES ON THE STORAGE ROT DISEASES AND BIOCHEMICAL COMPOSITION OF YAM VARIETIES (*Dioscorea spp*) IN OWERRI.`

BY

MBA, PAUL CHIKEZIE B. Agric. Tech., M.Sc., (FUTO)

REG. NO. 20164998648

A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D.) DEGREE IN PLANT PATHOLOGY

JULY, 2021

CERTIFICATION

We certify that this work on "Fungitoxicity of selected bio-pesticides on storage rot diseases and biochemical composition of yam (*Diocorea spp*) in Owerri, Imo State" was carried out by Mba, Paul Chikezie (20164998648) of the Department of Crop Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri.

Prof. M.I. Nwufo Principal Supervisor	31 - 8 - 2021 **Date**
Prof. G.O. Ihejirika Co-Supervisor	24/08/2021 **Date**
Prof. (Mrs) O.P. Onyewuchi Co-Supervisor	31/08/2021 **Date**
Prof. G.C. Onyishi Head of Department	31/08/2021 **Date**
Prof. D.O. Ohajianya Dean, S.A.A.T	30/9/21 **Date**
Prof. C.C. Eze Dean, Postgraduate School	**Date**
Prof Emmanuel Hala Kwon-Ndung External Examiner	26th JULY 2021 **Date**

DEDICATION

This work is dedicated to God Almighty for His grace upon me throughout the duration, my beloved wife Lady Nwanyieze Mba who accommodated my disturbances during night vigils and financial problems to accomplish this arduous task of producing it.

ACKNOWLEDGEMENT

Glory to the Almighty God for His Grace and protection throughout the duration of this work. I am grateful to my supervisors, Professors M. I. Nwufo, G. O. Ihejirika and O. P. Onyewuchi whose constructive inputs and commitment helped in the success of this work.

I appreciate my Head of Department, Prof. G. C. Onyishi, my lecturers, Professors M. I. Nwufo, G. O. Ihejirika, O. P. Onyewuchi, A.A. Ngwuta, M. O. Ofor, I. I. Ibeawuchi, C. I. Duruigbo, I .J Ogoke, C. M. Agu, S. A. Dialoke, Dr. F. O. Ojiako, Dr. Mrs. C. P. Anyanwu, Dr. Mrs. Peter-Onoh, Dr. S. E. Okere, Dr. Mrs Lilian Okafor, Dr. K. O. Ogbedeh, Dr. Echereobia, Dr. N. C. Adikuru, Dr. E. M. Nwokeji, Dr. Mrs. Alagba R.A, Dr. Mrs. Cookey and all others too numerous to mention here. I will not forget the assistance of Miss Tessy Onyewuchi during the laboratory aspect of this work, she was so committed to my admiration. I want to sincerely thank my bosom friend Prof B. O. Esonu who compelled me with much pressure to actualize this dream, he was a major source of inspiration to me, Prof A. A. Ngwuta who guided me in doing the work at the early stages of it, Late Chief. Ukpai A. Ukpai Ph.D who was also a great support and an inspiration to the success of this work and HE Senator Adolph Wabara whose words of advice gingered me to complete this work.

I am indebted to my children and their families, Dr Mrs Adaeze Mba Uwakwe, Mr Prince Chijioke Mba (worked assiduously to produce this work), Engr Jasper Chika Mba and Barr Ezinne Mba Chibueze for their unprecedented encouragement during the stressful period. The company and assistance of my friends and classmates are hereby acknowledged. Mazi. Elekwachi I. Eme who contributed to produce this work from the beginning, Daniel Emerole, Miss Ugwunna Ifeoma Mercy, Mr. Okechukwu Kalu Agwu, Pastor Kelechi Ejike, Kingsley Chukwuemeka also assisted me to produce this work and others I can't remember immediately.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

ABSTRACT

The study was conducted at the laboratory of Crop Science and Technology, School of Agriculture and Agricultural Technology, Federal University of Technology Owerri between 2017 to 2019.This project evaluated the fungitoxicity of some bio-pesticides on the control of storage rot diseases of yam varieties (*D. rotundata* and *D. alata*) especially caused by *Aspergillus spp, Fusarium spp, Rhizopus stolonifer, Yeast* and *B. theobromae.* The fungitoxic activity of ethanol extracts of seven plant leaves (used as bio-pesticides); *Chromolaena odorata, Carica papaya, Vernonia amygdalina, Ocimum gratissimum, Azadirachta indica, Psidium guajava* and *Cymbopogon citratus* were tested in Potato Dextrose Agar (PDA) medium to determine their percentage inhibitions against the organisms isolated from the tubers. The layout for experiment was a Completely Randomized Design (CRD) with seven (7) treatments and three (3) replications. Data was collected on some chemical constituents on both yam varieties of (*D. rotundata* and *D. alata*) and seven (7) leaf extracts used for the experiment. Result revealed that *D. rotundata* recorded higher chemical constituents than *D. alata.* The results of the study showed that *Azadirachta indica* extracted with ethanol as bio-pesticides for the experiment was the most fungitoxic as it possessed the best level (%) of inhibition on all the organisms isolated from yam varieties of *D. rotundata and D. alata* this could be because it contains phytochemicals such as Alkaloids, Flavonoids, Tannins, Phenols in reasonable percentage more than other plant extracts used in this work. It substantially suppressed the growth of *A. niger*. Furthermore, *Azadirachta indica* was most fungitoxic on rot diseases of *A. niger* (9.8%), *Yeast* (11.13%) and *Rhizopus stolonifer* (11.53%) isolated from *D. alata* and *D. rotundata* while *Carica papaya* (12.0%) was found to be most effective in the control of rot diseases of *Fusarium oxysporium* during the investigation. The application of *Azadirachta indica* was able to limit the growth of all the microorganisms identified during pathogenicity test. On point of attack the varieties of *D. alata* and *D. rotundata* showed that the most susceptible point of attack on the tubers was the basal part. The losses due to pathogenic attack was significantly reduced mostly by *Azadirachta indica* followed by *Vernonia amygdalina* then *Carica papaya* when *Ocimum gratissimum* was the lowest and are therefore recommended in that sequence.

Key words: Fungitoxicity, Pathogen, Bio-Pesticides, Inhibition, *D. rotundata*, *D. alata*.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Yamis a plantof the genus *Dioscorea* that belongs to the family known as *Dioscoreceae* and order *Dioscoreales*, (Amusa *et al.*, 1999). Yams (*Dioscorea spp*.) are cultivated in many tropical and sub-tropical countries like Africa, the Caribbean, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania, and remain an important food crop for millions of people (Okigbo *et al.,* 2000). The International Institute for Tropical Agriculture (IITA, 2009); Nahanga *et al.*, (2014), have identified about 600 species out of which only about six are socially and economically important in terms of food, cash and medicine. Agwu *et al.,* (2005) in their studies of root and tuber crops in West Africa have found that *Dioscorea spp* are mostly cultivated and are ranked second only after cassava.

The six major edible species of yam that are cultivated and consumed are: White yam (*Dioscorea rotundata*), Water yam (*Dioscorea alata*), Bitter yam (*Dioscorea dumetorum*), Aerial yam (*Dioscorea bulbifera*), Chinese yam, (*Dioscorea esculenta*), and Yellow yam (*Dioscorea cayenensis*) {Zaknayiba *et al.*, 2013; Ebewore *et al.,* 2013; Lawal *et al*., 2014; Princewill-Ogbonna *et al*., 2015}.

Nigeria is said to be the world's largest producer of yam, accounting for over 70-76 percent of the world total output. Yams are also grown throughout Africa, including countries like Cameroon, Togo, Ghana, Nigeria and Ivory Coast (Frank *et al.,* 2014). According to FAO, (2008) and Kleith *et al.,* (2012), Nigeria has the highest production record of 38.92 million metric tonnes of yam tubers annually, while about 74 percent of the world yam production are produced by West and central African countries (IITA, 2007).

Yam is an important staple food in Nigerian and West African diets and provides some 200 calories of energy per capita daily (Onumadu *et al.,* 2008). A variety of palatable dishes are also prepared from yam tubers (Aluko *et al.*, 2003; Fasasi *et al.*, 2005; Oladebo *et al.*, 2010). Researchers have found that yam has very high food value and a major source of carbohydrate and minerals such as calcium, phosphorus, iron and vitamins including riboflavin, thiamine and vitamins B and C (Okigbo *et al.,* 2006; Okigbo *et al.,* 2010). Yams equally contain about 1-2 % dietary protein which is high when compared with other tropical root crops and tubers (Ekefan *et al.,* 1999) and also have considerable social and cultural significance among the people of Southeastern Nigeria (Sangoyomi, 2004). Eke-Okoro *et al.,* (2014), reported that another importance of yam is that it is also a source of food security and employer of labour in areas where it is cultivated. Recently, yams are reported to be economically and socially important for export purposes, medicine and food (IITA, 2009).

Sanusi *et al.,* (2006) have found that more than 90% of the total world output are produced in the area that is commonly referred to as the **"yam zone" or "yam belt"** consisting of Nigeria, Cote d' Voire, Ghana, Benin, Cameroon and Togo.

Many studies have identified some major problems that are associated with yam production. These include lack of access to farm inputs, high cost of inputs, poor producer prices, very high incidence of pests and diseases as well as inadequate storage facilities which negatively impact yam production (Zaknayiba *et al.,* 2013).Studies by (IITA, 1993; Cornelius, 1999; and Amusa *et al*., 2003) have also shown that fungal rot is the greatest cause of tuber losses in storage. Microbial attacks on yam result into dry rot, soft rot and wet rot (Ime *et al.,* 2012; Glover *et al.*, 2013 and Afiukwu *et al.*, 2013).

The principal species of microorganisms that cause severe losses resulting from the rot of yam in storage in Southern Nigeria as investigated by Onuegbu, (1999) and Okigbo *et al.,*

2015) included *Aspergillus niger, Rhizopus nodosus, Scerotia rulfii, Fusarium oxysporium* and *Botrydiplodia theobromae*. Other major microorganisms that cause rot diseases in yams including *Aspergillus flavus, Fusarium solani, Penicillium chrysogenum, Rhizoctoniaspp., Penicillium oxalicum, Trichoderma viride* were identified by many authorities (Okigbo *et al*., 2001; Okigbo, 2004; Aidoo, 2007). IITA, (1993) have reported that *theobromae* and *Penicilliumspp* cause dry rot of yam, while Amusa *et al*.. (2003) reported that *Rhizopusspp*. causes soft rot. Equally, fungal pathogens such as *Lasiodiplodia theobromae, Rhizoctonia solani* and *Selerotium rolfsii* are commonly associated rotting of yam tubers (Aideo, 2007). Other pathogens commonly implicated with the diseases of yam at various stages of growth have been found to include *Colletotrichum spp, Curvularia eragrostide Fusarium moniliforme*(Amusa *et al*., 2003; Gwa *et al*., 2015; Gwa *et al*., 2017; and Gwa *et al*., 2018)These pathogens reduce the growth, quantity and quality of yam (Arinze, 2005;Okigbo *et al.*, 2006; Okigbo *et al.*, 2009, Taiga, 2011, and FAO, 2013).

1.2 Problem Statement

A number of fungi pathogens are associated with the deterioration of yams in storage causing reduction in quantity and quality of yam tubers. These pathogens decimate yam tubers (*Dioscorea spp*) at all stages in storage causing severe losses to farmers, processors, marketers, and consumers. Chemical control of these fungal pathogens are effective but are costly and have side effects due to residues that accumulate in the produce, soil and environment as well as their effect on non-target organisms hence there is therefore the need to investigate the effectiveness of bio-pesticides as alternative to synthetic chemicals in reducing losses from rot pathogens.

1.3 Justification of the Research

Greater proportion of yams in storage (almost 70%) is often destroyed by rot pathogens causing substantial losses to farmers, marketers and consumers. Use of synthetic chemicals has largely proved to be inadequate in the control of rot pathogens due to their exorbitant costs, health challenges and ecological problems arising from their use. There was therefore the need for other methods of controlling the development and growth of rot pathogens of yam and this has generated considerable interest in the search for and application of natural plant-derived products as fungicides to control rot pathogens of yams in storage. Finally, the use of potential bioactive components isolated from selected plant parts will prove more cost effective, be easily available and safe for human beings and the environment by eliminating most of the hazards posed by the use of synthetic chemicals.

1.4 Aim and Objectives

The aim of this study was to evaluate the fungitoxicity of selected bio-pesticides on the storage rot diseases and biochemical composition of yam (*Dioscorea spp*) varieties. Consequently, the study was designed to achieve the following specific objectives:

i. Assessment of the efficacy of plant extracts (botanicals) from plant leaves of selected plants namely; *Azadirachita indica, Chromolaena odorata, Ocimum gratissimum, Psidium guajava, Vernonia amygdalina, Cymbopogon citratus and Carica papaya* in the control of rot diseases of yam varieties (*Dioscorea rotundata* and *Dioscorea alata*) in storage,

ii. Comparison of the effectiveness of the botanical extracts on the different pathogens on the yam varieties (*Dioscorea rotundata* and *Dioscorea alata*) and confirmation of the most efficacious on them, *in vitro*

iii. Qualitative screening of the phytochemical composition of the botanicals.

iv. Determination of point of easy attack by the pathogens on the two yam species

v. Assessment of the biochemical composition of the infected and healthy yam tubers in storage, viz; ash content, carbon, nitrogen, protein, fibre, and moisture contents.

1.5 Scope of the Study

This study evaluated the fungitoxicity of bio-pesticides derived from the plant leaves of *Azadirachta indica, Chromolaena odorata, Ocimum gratissimum, Psidium guajava, Vernonia amygdalina, Cymbopogon citratus and Carica papaya* in the control of rot diseases of yam varieties (*Dioscorea rotundata* and *Dioscorea alata*) in storage. The intent of this study was to isolate the rot pathogens of yam samples used in the study through laboratory evaluation and determine the potency of the bio-pesticides on the pathogens that are discovered during the study.

The study's focal point was to determine the bio-pesticide among the seven plant leaves that was most fungitoxic when applied to the pathogens isolated during the experiment and such will then be recommended for the control of rot pathogens of *Dioscorea rotundata and Dioscorea alata* by the researcher.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of Yam

Yam is most probably one of the oldest food crops known to man. The word 'yam' comes from Portuguese ***inhame*** or Spanish ***name,*** which both ultimately were derived from the Wolof word ***nyame,*** meaning 'to eat'. Yams are native to tropical regions throughout the world (CABI, 2016, and Barton H. 2014).Yam is the common name for members of the genus***Dioscorea,*** family*Dioscoreaceae, in the order of Dioscoreales.*According to Orkwor, (1998), the most important edible yams are believed to have originated in the tropical areas of South East Asia, South America and Africa.

The *Dioscorearotundata* (White yam) and *Dioscoreacayenensis* (Yellow yam) species are believed to have originated in West Africa and are among the relatively few truly West African domesticated plants (IITA, 1995) while *Dioscoreaalata* (Water yam) and *Dioscoreaesculenta (Lour.*) (Chinese yam) originated in Southern Asia and was introduced to West Africa during recent centuries (IITA, 1995). Researchers have also reported about species of wild yams in Africa that are harvested and consumed during periods of scarcity of edible yams

2.2 Yam Species

There is a controversy as to the exact number of yam species in the world. Jayasuriya, (1995) identified more than six hundred and thirty (630) yam species.

However, an authority had earlier found ten (10) species that were extensively used as food to include: the white Guinea yam (*Dioscorearotundata Poir*j, yellow yam (*Dioscoreacayenensis Lam*), water yam (*Dioscoreaalata*), trifoliate yam [*Dioscoreadumentorum (Kunth) Pax*],

aerial yam (*Dioscoreabulbifera*) Chinese yam (*Dioscoreaesculenta (Lour) Burk*], cush-cush yam (*Dioscoreatrifida L.*), cinnamon yam (*Dioscoreaopposita Thunb*), *Dioscoreajaponica Thunb* and *Dioscoreahispida* (Onwueme, 1982), Out of these yam varieties, *Dioscorea rotundata* (White yam), *Dioscorea dumetorum* (Bitter yam). *Dioscorea cayensis* (Yellow yam), *Dioscorea alata* (Water yam), *Dioscorea esculenta* (Chinese yam) and *Dioscorea bulbifera* (Aerial yam) are the most cultivated yam species in the world.

2.3 Botany of Yam

Yams are members of the flowering plantgenus*Dioscorea*. They are monocots, related to palms, grasses, and orchids.Studies by Cornelius, (1998) have shown that the tuber of yams, which is the only edible part, has a tremendous capacity to store food reserves. Yam plants have been described as monocots and members of the genus *Dioscorea*, family *Dioscoreaceae* and order *Dioscorales*. Although some level of controversy has centred on the nature of the yam tuber, it is currently accepted that the yam tuber is neither a stem structure nor a root structure. Rather it has its origins as a hypocotyl structure (Degras, 1993).

A more elaborate Scientific Classification of Yam is presented below:

i. Kingdom: *Plantae*

ii. Division: *Magnoliophyta*

iii. Order: *Diocoreales*

iv. Family: Diocoreaceae

v. Genus: *Dioscorea*

2.4 Major Yam Producers in the World

According to Okigbo *et al*., (2006), yams *(Dioscorea* spp.) are among the most important staple foods in the world, especially in many parts of the tropics and subtropics. African, Asian and South American countries are the major producers of yams in the world as

reported by Okigbo *et al.,* (2006) and FAO, (2013). In their study, Okigbo *et al.,* (2006) found that yam ***(****Dioscorearotundata****)*** production and consumption is mostly in West Africa, East Africa, the Caribbean, South America, India and South East Asia. This has been confirmed by FAO report in 2013. Nigeria has the highest production record of 38.92 million metric tonnes of yam tubers annually [FAO, (2008); Kleih *et al.*, (2012)].

FAO, (2005) had earlier reported that Nigeria produced about 66.6% (26.6 million metric tonnes) of total world's yam production, with Ghana producing 9.8% (3.9 million metric tonnes) every year. Ghana, however, is the leading exporter of yams in Africa. Although, Nigeria is the largest producer of yams, her exports remain considerable lower than that of Ghana for many reasons. These includes that harvested yams were mostly for self-consumption and not market-oriented, to meet the ever enlarging demand for food yams of the fast growing population of Nigeria, that yam tubers do not reach the full size for good sale and market prices are low (Fu *et al.,*2011).In addition, yams in storage are attachted by different pathogens of yam various complex and tuber rots that results into dry rot, soft rot and wrt rot that reduce their quality and quantities available for consumption and export. [Amusa,*et al.,* (1999), Opara,(1999)].The Food and Agriculture Organization (FAO) have estimated that over the past 15 years, world production of yams has been rising almost entirely due to increased production in Africa (FAO, 1994).

Recent statistics Food and Agricultural organization (FAO) indicate that more than 90% of the global yam production (40 million tons fresh tubers/year) is produced in West Africa with Nigeria accounting for about 35.02 million metric tons (up from 2005 figure of 26.6 million metric tonnes in 2004 (FAO, 2008).

2.5 Major Yam Species Produced in West Africa.

According to Aboagyne-Nuamah,*et al.*, (2005) yam is mainly cultivated as a staple crop across the humid and semi-humid tropics, especially in West Africa. The region of West Africa is responsible for about 90% of the total world yam production. Various studies conducted by Titteh and Saakwa, (1994); Elechi and Souzey, (1999) have shown that white yam (*Dioscorearotundata)* is the most predominant yam specie and constitutes about 80% of total yams produced worldwide.FAO, (2000) have reported that six (6) countries in West Africa namely - Cameroon, Nigeria, Benin, Togo, Ghana and Côte d'Ivoire within the yam production zone of West Africa called "the yam belt" lies between latitudes 25°N and 15°S. Yams are mostly cultivated in the coastal region in rain forests, wood savanna and southern savannahabitats.

Yams (*Dioscorea spp*.) has been described as an important food crop for millions of people in many tropical and sub-tropical countries in Africa, the Caribbean, the Northern and Central part of South East Asia including parts of China, Malaysia, Japan and Oceania (Okigbo,*et al.*, 2000). Researchers have established that a variety of palatable dishes are prepared from yam tubers (Aluko, *et al*., 2003; Fasasi,*et al.*, 2005; Oladebo,*et al.*, 2010). Yams may be consumed in a variety of ways. They can be roasted, boiled, fried in oil, pounded into fufu, smoked, or grilled. It can also be cooked or fried with rice, beans, plantain and other delicacies (IITA, 2004, Timothy, *et al*, 2009). Yam tubers can also be peeled and sliced into tiny pieces that can be milled into yam flour and flakes after drying. (Udoh,*et al.*, 2005).

White yam (*Dioscorea rotundata*) has organoleptic qualities that make them the preferred carbohydrate food, hence contributing to 350 dietary calories per person per day for millions of people in the major producing countries (IITA, 1993).

In Nigeria, for instance, white yam (*Dioscorea rotundata*) have been found to command the highest market value among the six major species produced within West Africa due to its superiority and preference by consumers as a source of food.Akanji *et al.,* (2003) in their studies have enumerated the major yam producing states in Nigeria. These states are Adamawa, Benue, Cross River, Delta, Edo, Ekiti, Imo, Kaduna, Kwara, Ogun, Ondo, Osun, Oyo, and Plateau.

The FAO statistics of 2008 indicated that yam production in Nigeria had nearly doubled since 1985, with the production of 35.017 million metric tonnes with equivalent market value of US$5.654 billion in foreign exchange (FAO, 2011). The record for world's second and third largest producers of yams, namely Côte d'Ivoire and Ghana, was 6.9 and 4.8 million tonnes of yams respectively in 2008. The International Institute of Tropical Agriculture, (IITA, 2011) also indicated that Nigeria accounted for about 70 percent of the world production amounting to 17 million tonnes from land area 2,837,000 hectares under yam cultivation. Varieties of yams that are in West Africa are therefore the most important source of food and income for millions of producers, processors and consumers in West Africa (Akinbo, *et al.,* 2016).

The Food and Agricultural Organization of the United Nations (FAO, 2017) have provided the yam production trends in Nigeria from the year 1961 to 2017 in the table below. The Table (1) shows that yam production in Nigeria grew from a meager 3,500,000 metric tonnes in 1961 to the current 47,942,712 metric tonnes in 2017. This was however a drop from the production level of 49,384,352 recorded for 2016. The country has sustained the output level of above 30m metric tonnes from 2004 to 2013 and reached the production threshold of 45,151,939 in 2014. This increase in yam production is being sustained till date.

2.6 Importance of Yam Consumption

According to Ogaraku,*et al*., (2008), yam is an important staple food in the tropics, being a major source of carbohydrates, vitamins and dietary fibers. Yam (*Dioscorea spp*.) is an economically important tuber crop in tropical and subtropical regions of the world (Adeniyan, *et al.,* 2008). Yam (*Dioscorea spp*) is a tuber crop belonging to the family *Dioscoreaceae.*The species of economic importance include *Dioscorea rotundata, Dioscorea alata, Dioscorea cayenensis, Dioscorea dumetorum, Dioscorea bulbifera* and *Dioscorea esculentus* (Akinbo, *et al.,* 2016). In West Africa, *Dioscorea rotundata* is also referred to as white yam or white guinea yam. *Dioscorea rotundata* is the most widely cultivated as well as the most important food and income source for millions of producers, processors and consumers in West Africa and 31.8% of the population in Nigeria and 26.2 % in Ghana largely depend on yams for food and income security, (Akinbo, *et al.,* 2016). The study by Honger, *et al.,* (2017) has also found that yam is also an important foreign exchange earner in these producer countries with about 60 million people involved in its production and marketing. The crop plays an encouraging role as a guarantee for household food security (FAO, 2013). It has also been reported by some authorities the many farmers essentially are involved in yam production for household production, production of planting materials for private uses, income from sale of yams,seed yams and that the superstition and rituals which are often associated with yam in West Africa is an indication of its antiquity (Frank and Kingsley, 2014).

CABI,(2017)discovered that yams are consumed in a variety of preparations, such as flour or whole vegetable pieces in Africa and in the form of fufu (which includesmashed and pounded yam flour) that are prepared by young African women. Yams of African species must be cooked to be safely eaten, because various natural substances in yams can cause illness if

consumed raw (Nweke*et al.,* 2013). The most common cooking methods in Western and Central Africaare by boiling, frying or roasting.

Norman,*et al*., (1995) have affirmed that ritual ceremonies and superstitions which often surrounded the utilization of yam in West Africa remains a strong indication of the antiquity of the use of this crop.

Although carbohydrate is the main constituent of the yam tuber, yam also contains an appreciable amount of proteins, vitamins and minerals as found by Babaleye, (2003). It serves as an important source of income to many people. The tuber is of economic value and is the most important part of the crop. It can be eaten in different forms such as roasted, boiled, fried or better still made as pounded yam or as flour which is a delicacy in Nigeria.

Arinze, (2005) have asserted that although yam is credited with a high profile nutritional status, medicinal and industrial uses, its production and effective long term storage have continued to remain a mirage due to a myriad of constraints. One of such is the attack by storage rot pathogens such as fungi and bacteria.

2.7 Nutritional Values of Yam

The nutritional values of Yam (Raw) in terms of energy, vitamins and minerals are presented in Tables 2.1-2.3.

Table 2.1: Nutritional value per 100g (3.5 oz)

Energy	494 kJ (118 kcal)
Carbohydrates	27.9g
Sugars	0.5g
Dietary fiber	4.1g
Fat	0.17g
Protein	1.5g

Source: USDA Nutrient Database (2018)

Table 2. 2: Vitamin Contents/Quantity% DV

Vitamins	Quantity% DV	
Vitamin A equiv	1%	7 µg
Thiamine (B1)	10%	0.112 mg
Riboflavin (B2)	3%	0.032 mg
Niacin (B3)	4%	0.552 mg
Pantothenic acid (B5)	6%	0.314 mg
Vitamin B6	23%	0.293 µg
Folate (B9)	6 %	6 µ
Vitamin C	21 %	17.1 mg
Vitamin E	2%	0.35 mg
Vitamin K	2%	2.3 µg

Source: USDA Nutrient Database (2018)

Table 2.3: Mineral Contents/Quantity% DV

Minerals	Quantity%	DV
Calcuim	2%	17 mg
Iron	4%	0.54 mg
Magnesium	6%	21 mg
Manganese	19%	0.397 mg
Phosphorous	8%	55 mg
Potassium	17%	816 mg
Zinc	3%	0.24 mg

Source: USDA Nutrient Database (2018)

2.8 Production of Yam

Zakari,*et al.,* (2014): have asserted that the production of yam requires a temperature of 25-30^0 C with moisture from well distributed rainfall of over 150 cm for normal growth. Yam is essentially a tropical plant for which loamy soil of high fertility that are rich in organic matter are best suited for its production. Depending on the species, yam grows for six to ten months and is dormant for two to four months, these two phases usually corresponds to the wet season and the dry season. For maximum yield the yam requires an annual rainfall of over 1,500 mm distributed uniformly throughout the growing season. White, yellow and water yams normally produce annually a single large tuber, often weighing 5-10 kg.

The FAO, in 2005 reported that the world production of yams in 2004 was 40 million metric tonnes of which about 92% were from West Africa, with Nigeria contributing about 66% of world total production. The average yield of yams in the yam-growing areas was about 9 tons per hectare cultivated (FAO, 2005). According to FAO, (2017) the worldwide production of yams in 2014 was 68.1 million tonnes. Nigeriawas the highest producer with 66% (44.95%) of the global total. Nigeria farmed yams on 5.4 millionhectares, 70% of the world land area of 7.8 million hectares devoted to yam farming. The world average annual yield of yams was 8.8 tonnes per hectare in 2014, with Ethiopia having the most productive farms of 29.7 tonnes per hectare (FAO, 2017).

Table 2.4: Statistics of yam production in Nigeria: 1961 -2017

S/N	Year	Value(t)
1	2017	47,942,712
2	2016	49,384,352
3	2015	45,677,939
4	2014	45,151,589
5	2013	35,618,420
6	2012	32,318,900
7	2011	32,134,172
8	2010	37,328,180
9	2009	29,091,980
10	2008	35,971,000
11	2007	31,136,000
12	2006	36,720,000
13	2005	34,000,000
14	2004	31,776,000
15	2003	29,697,000
16	2002	27,911,000
17	2001	26,232,000
18	2000	26,201,000
19	1999	25,873,000
20	1998	24,768,000
21	1997	23,972,000
22	1996	23,201,000
23	1995	22,818,000
24	1994	23,153,000
25	1993	21,632,000
26	1992	19,781,000
27	1991	16,965,000
28	1990	13,624,000
29	1989	9,609,000
30	1988	9,132,000

Table 2.4: Statistics of yam production in Nigeria: 1961 -2017 (Contd.)

31	1987	4,886,000
32	1986	5,209,000
33	1985	4,738,000
34	1984	4,600,000
35	1983	4,998,000
36	1982	5,385,000
37	1981	5,212,000
38	1980	5,248,000
39	1979	5,256,000
40	1978	5,780,000
41	1977	6,376,000
42	1976	6,556,000
43	1975	8,621,000
44	1974	7,160,000
45	1973	6,935,000
46	1972	6,900,000
47	1971	9,766,000
48	1970	12,033,000
49	1969	9,785,000
50	1968	8,233,000
51	1967	7,806,000
52	1966	6,987,000
53	1965	6,000,000
54	1964	5,000,000
55	1963	4,500,000
56	1962	3,500,000
57	1961	3,500,000

Source: FAO, (2017)

2.9 Methods of Yam storage and Implications

The work of Adamu *et al.,* (2014) have shown that the kind of storage structure used by farmers will depend on a number of factors which include: the design, construction, material available, amount of tuber produced, prevailing climatic condition of the area, purpose of tuber storage and the resource of the farmers. Another study done in Kwara State by Falola,*et al.,*(2017) indicated the awareness of and usage of various storage methods by farmers as indicated in Table 2.5.

Akangbe,*et al.,* (2012) in their study of yam storage strategy in Asa LGA of Kwara State identified six methods of yam storage in that area to include: Barn (47.3%), Indigenous plant materials (27.3%), Thatched–roof pit (9.1%). Ventilated store shed (4.3%), elevated store shed (7.3%) and Open side store (4.5%).

The common method of yam storage is the use of the traditional yam barn which consists of a vertical framework to which the yam tubers are tied with local materials such as raffia. The height of the poles may vary from l-2m made from vertical poles, which may be 5-10cm in diameter and lm apart. Degras, (1993), have found that barns are usually more effective for the storage of yams through the dry season but that once the rainy season sets in tubers tend to deteriorate rapidly.

IITA has developed an improvement upon the traditional barn method by using simple open-work shelving inside the barn. Its advantages include placing tubers on shelves with less time and labour. Also, damaged tubers can be more easily spotted and be removed (Hahn, *et al.*, 1995).

Table 2.5: Methods of Yam Storage and their Usage

Storage Method	Awareness (%)	Usage (Ranking)
Yam barn	89.4%	1st
Covered table	79.4%	4th
Elevated pole	80.6%	5th
Yam house	93.9%	3rd
Heap on the floor	94.4%	2nd
Underground structure	65.0%	6th
Open side shelves	44.4%	8th
Coating with termitarial soil	51.7%	7th
Cold storage	0.0%	-
Gamma Radiation	0.0%	-

Source:Falola, *et al.,* (2017)

Plate 2.1 Traditional Yam Barn using Live Stakes/Trees
(Source: Prince Odeh Jones Isaiah, (2017), m.facebook.com)

Plate 2.2 Thatched Roof Barn

(Source: Second Life Market Place (2016)

Plate 2.3: Open side shelves

(Source: Amponsah S.K, (2015)

Plate 2.4: Simple open –work shelve
(Source: IUSSD Working Group WRB, (2014)

Plate 2.5: Yam house
(Source: Anaadumba, P, (2014)

Plate 2.6: Improved yam house
(Source: Eric Lafforgue,(2020)

Plate 2.7: Elavated pole
(Source: Katie P, 2019)

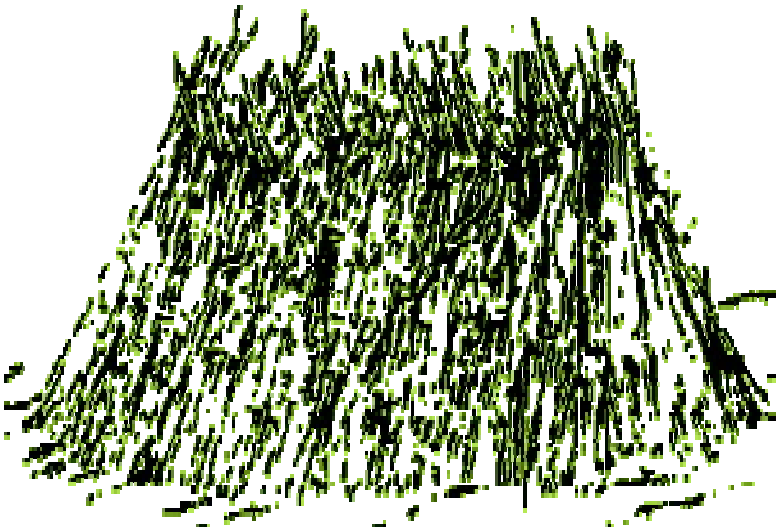


Plate 2.8:Heap on the floor covered with indigenous leaves
Source: Damien O'Connor (2019)

Plate 2.9: Three-sided vertical yam barn with tubers tied with local materials
Source: (www.wikipedia.org)

Plate 2.10:Open side shelves

(Source: Ogbo Frank C. and Agu Kingsley C, (2013) www.sciencedomain.org

Plate 2.11: Open side shelves

(Source: (Agu Diop,(1998), dictionary.cambridge.org)

Many advantages and disadvantages associated with the traditional methods of storage of yams have been recorded as in Table 2.6

Table 2.6: The advantages and disadvantages of yam storage methods.

Storage technique	Advantages	Disadvantages
A. Pit – Silo	The pit –Silo ensures cutting down of labour meant for transportation. It offers protection against weight loss owing to respiration and transportation.	Tuber rot is favoured by the absence of aeration and the direct contact established between tubers. Regular control is not possible.
B. Heap on the soil	This facility does not require supplementary funds and provides protection against heat.	It is not well aerated and does not enable necessary control. Yams usually rot in series.
C. Straw shelter	Minimal investment is required. Aeration is better as compared to heaps on the soil. Thus reducing damage.	The shelter is open to insect and rodent attack. Domestic animals foraging for food at times destroy the shelter. The quality of yams cannot be controlled.
D. Clay barn	This barn has a life span of at least 20 years if well catered for. Tubers piled inside are protected from rain and sun. clay and straw used as construction materials create a favourable temperature.	The construction of this barn requires a lot of work and is more expensive than the previous storage facilities. Aeration of tubers is not sufficient and their inspection is difficult. Yams easily rot in series.
E. Vertical tying	This is the most popular storage system in West Afica. It enables perfect aeration. Control and removal operations are easy.	This construction equally requires a lot of; work and is relatively expensive. Storage entails a lot of work Since each tuber is tied separately. Yams are not protected from insects and rodents. Tubers receive a lot of humidity and rot easily during the rainy season.

Source:(Ngue – Bissa, *et al.*,(2007)

2.10 Limitations of Yam production

Yam production is sometimes constrained by several factors; these include high cost of production,pests and diseases. For example, Nweke, *et al.*, (1991), in their studies found that just planting materials and labour accounts for about 50% and labour for over 40% of the cost of production. Additionally, large quantities of yam become unsuitable for use as planting seeds because of deterioration caused by pests and disease storage.

Several authorities have isolated the major species of microorganisms that are associated with yam rot in Nigeria to include; *Botryodiplodia theobromae Pat., Sclerotium Rolfsii Sacc, Aspergillus niger Van Tiegh* and *A. tamari* Kita (IITA, 1993; Amusa and Baiyewu, 1999; Amusa *et al*., 2003; Okigbo, 2005).

Other major problems that also tend to limit yam production are: high labour requirements, low yield per hectare when compared to crops such as cassava or sweet potato, and more so,the relatively large amount of planting materials that is required and it's long growing season.

The most critical of problem of yam production is labour requirement, which exceeds that of the other comparable crops. For these reasons and problems of storing harvested yam, the costs of yam production are high and yam is slowly losing ground as cassava. It has been estimated that the cost per 1,000 calories of yam is four times greater than those of cassava. But, despite these high costs the nutritional value of yam is sufficiently high to justify further work into its general improvement.

Different studies have identified many pathogenic micro-organisms including bacteria, fungi and viruses that attack yams at all stages of growth and during storage of tubers [Ogaraku, *et al.*,2008 Okigbo *et al.*, 2010; Ayanwuyi *et al.*, 2011; Okigbo *et al.*, 2005; Gwa *et al.*, 2016

and Gwa *et al.,* 2017]. In his study, Degras, (1993) found that one of the major production constraints in yam cultivation is damage from pests and diseases, such as insects, nematodes, fungi, bacteria and viruses, which singly or in combination have direct adverse effects on the yield. The investigation of yam pathogens by Sadiku *et al.,* (2011) has supports the above findings and added that rots caused by pathogenic organisms may be exacerbated due to challenges in climate change.

2.11 Different Pathogens of Yams.

Many diseases are usually associated with yams either during growth or in storage. Yam species, especially *Dioscorea rotundata* and *Dioscorea alata*, are often very susceptible to a variety of pests and diseases during growth and during postharvest storage. Microorganisms such as nematodes and yam virus are the most devastating causes of deterioration in yam tubers in storage. The major postharvest disease is tuber rots caused mostly by fungi (Opara, 2003). Some common diseases of yams are presented in the table below.

Table 2.7: Common diseases of yams and recommended control strategies.

Common Name	Organism	Incidence and` Characteristics	Control strategy
Yam virus Complex		Occurs throughout the West African yam zone. Reduces yields considerably	Use virus-free planting materials or resistant cultivars. Thermotherapy and meristem culture
Tuber rots	Soft rots caused by*Penicillium* spp., *Fusarium* oxysporum, and *Botrydiplodia theobromae.* Dry rots caused by *Rosselinia* and*Sphaerostilbe*. Other fungi*: Rhizopus nodosus. F.solani.*	Infection in the field can persist and lead to rotting during storage	Plant with disease-free material. Crop rotation. Minimise physical damage Of tuber during postharvest operations. Treat the sett or tuber with systemic fungicide or alkaline material such as Bordeaux mixture. Provide adequateaeration and inspect stored tuber regularly.

Source: (Adapted from Opara, 1999).

Rot is the major factor limiting the post-harvest life of yams and losses can be very high. It was estimated that rots could cause about 10-30 per cent losses to retailers and 10-40 per cent losses to exporters (Cornelius, 1998). Fungal pathogens on yams usually penetrate through wounds created on the tubers by insects, nematodes, rodents or poor handling during and after harvest and then infect the inner tissues (Arinze, 2005).

Adeniji, (1996); Amusa, (2001); Amusa *et al.*, (2002); Nweke *et al.*, (2008) have all reported that fungi constitute the main spoilage micro organisms associated with post harvest deterioration of plants and food materials. Micro organisms responsible for post-harvest rot of yam tubers have also been identified (Amusa *et al.*, 1999; Okigbo *et al.*, 2000; Khadijat, 2003; Yusuf and Okusanya, 2008; Okigbo *et al.*, (2013). A number of fungi pathogens are associated with storage deterioration of yams in storage causing reduction in quantity and quality of yams produced. These pathogens decimate yam species (*Dioscorea spp*) at all stages of growth in the field, transit or storage causing severe losses to farmers. There is therefore need to discover bio-pesticides to reduce losses resulting from rot pathogens as against the use of synthetic chemicals. In previous studies, some pathogens that have been implicated with diseases in yam at various stages of growth and storage included: *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Botryodiplodia theobromae, Colletotrichum spp, Curvularia eragrostide, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Rhizoctonia spp, Rhizopus nodosus, Penicillium expansum, Penicillium digitatum, Penicillium oxalicum, Penicillium purpurogenum* (*Markson, et al.,* 2012*;* Gwa *et al.,* 2015*;* Gwa *et al.,* 2017*;*and Gwa *et al.,* 2018).

Amusa *et al.*,(1999) had earlier found microorganisms associated with stored and marketed yam tubers obtained from the tropical forest region of South-western Nigeria and their pathogenicity as presented in Table 2.8.

Table 2.8: Pathogen, symptoms of infection and pathogenicity of rot diseases in yam

Pathogens	Symptoms of infection	Pathogenicity
Botryodiplodia Theobromae	Dry rot	***
Aspergillus Tamari	“	**
Penecillium oxalicum	“	***
P. cyclopium	“	***
P. italicum	“	***
Fusarium oxysporium	“	**
F. solani	“	**
Rhizopus nigricana	Soft rot	**
Sclerotium rolfsii	”	***
Muccor circinelloides	“	***
Trichoderma viridae	“	**
Erwinia carotovora	Wet rot	***

Key: Mildly pathogenic (< 10 < 50mm in diameter; *** = Highly pathogenic (>50mm in diameter) Source: Amusa and Baiyewu, 1999)

2.12 Categorization of Rot Diseases of Yam

According to Amusa and Baiyewu, (1999), storage diseases of yam can be broadly categorized into three, based on the symptoms and the causal agents.

These are: (i) Dry rot, (ii) Soft rot and (iii) Wet rot

Dry rot

Dry rot of yams has been identified by Amusa*et al.,* (2003) as the most devastating of all the storage diseases of yams that ultimately cause substantial reduction in the quantity and market value of yams. Symptoms of dry rot include varying colouration, depending on the invading pathogen. For example, IITA, (1993) has established that when tubers are infected with either *Penicillium oxalicum* or *Penicillium cyclopium*, the tubers turn brown and then become hard and dry, maintaining their integrity, except when the tisues were invaded by *Sphaerostilbe marcescens*. However, when tissues are invaded by *Sphaerostilbe marcescens* they are covered with the greenish mycelia of the fungus, while tubers infected with *Aspergillus niger* and *Aspergillus tamari* turn brown with yellowish margin. *Rosellina bunodes* and *B. theobromae* have also been reported to cause dry black rots. When yam tubers are infected by the two organisms, they first turn grey and then black. These tubers ultimately become pulverulent and break into small dry particles (IITA, 1993).

Soft rot

Soft rot has equally been identified by Amusa *et al* (2003) to be associated with yam decays. When yam tubers are infected by soft rot organisms they often turn brown, soft and become wet due to rapid collapse of cell walls of tissues. Fungi that are associated with this type of rot are *Rhizopus spp.***,** *Mucor circinelloides, Sclerotium rolfsii, Rhizoctonia solani and Armillariella mellea* (Amusa and Baiyewu, 1999)

Wet rot

Wet rot of yam tuber is however characterized by the oozing of out of infected tissues when pressed. This symptom is usually associated with the bacterium, *Erwinia carotovora* (IITA, 1993; Amusa and Baiyewu, 1999).

2.13 Control of Postharvest Pathogens of Yams

Researchers have reported on many methods that have been adopted for controlling losses due to post harvest diseases of yams. These strategies include the use of chemicals, biological methods of control, curing, and use of natural plant extracts (Amusa *et al.*, 2003).

Also, the use of synthetic chemicals such as sodium orthiophenylphenate and borax can significantly reduce storage rot in yam (Okigbo and Ikediegwu, 2000; Okigbo, 2004). But the synthetic chemicals have many disadvantages. First, most farmers lack the expertise to handle the chemicals safely. Secondly, the farmers cannot afford the prohibitive cost of these chemicals. Furthermore, these synthetic chemicals accumulate in the ecosystem and may induce pathogen resistance to pesticides (Okigbo and Ikediegwu, 2000; Okigbo, 2002; 2005). However, biological control is now being generally accepted as an alternative method of plant disease management in place of chemicals as it overcomes the above disadvantages (Amadioha and Obi, 1999).

2.14 Use of Bio-Pesticides to Control Rot Diseases of Yam

The use of plant extracts or bio-pesticides is now considered as safer, more economical and better alternative to the application of chemical pesticides. Plant extracts are composed of various bioactive compounds such as alkaloids, flavoniods, glucosides, phenols, saponins, sterols etc (Gwa, *et al*, 2017). It has also been reported that extracts from plant leaves/seedslike*Azardirachta indica, Carica papaya, Piper nigrum, Zingiber officinale,*

and*Nicotiana tabacum* have been effective in the control of yam rots (Gwa *et al,* 2018). Mba *et al.*,(2017)

have further reported that ethanol extracts of *Chromolaena Odorata, Carica papaya, Vernonia amygdalina, Ocimum gratissimum, Azardirachta indica, Psidium guajava,* and *Cymbopogon citratus* all possess inhibitory properties that can be utilized as post-harvest control to suppress rot-causing fungi of yams in storage at various degrees of potency.

Fungitoxic effect of ethanol and cold-water extracts of *Cymbopogon citratus, Chromolaena odorata,* and *Newbouldia laevis* were equally demonstrated against microbial pathogens of *Botryodiplodia theobromae, Aspergillus niger, Fusarium solani,Penicilliumspp* and *Rhizopus stolonifer*; although the antimycotic effect of the plant extracts varied (Okigbo and Ezebo, 2017). Their study further concluded that as the concentration of the extracts increased, the level of inhibition on the mycelia growth of the fungal increased in line with the reports of Okigbo and Igwe, (2007).

The work of Oyelana, *et al*.,(2011) on the leaf extracts of *Ficus thonningii, F. saussureana, F. exasperata* and *F. sur* screened for antimicrobial properties on eight (8) fungal species which included *Aspergillus flavus, A. niger, Botryodiploidia theobromae, Fusarium oxysporum, F. solani, Penicillium chrysogenum, P. oxalicum* and *Rhizopus stolonifer* and two (2) bacterial species viz; *Pseudomonasspp* and *Klebsiellaspp* that were isolated from the rot portions of tubers of *Dioscorea rotundata* proved thatthe extracts from the *Ficus* species had low antimicrobial effect at 25 and 50 mg mL^{-1} concentrations while a significant arrest of mycelia growth was observed at 75 and 100 mg mL^{-1} concentrations. Equally,the investigation on extract application of leaves of *Carica papaya* Lam (pawpaw), rhizomes of *Zingiber officinale* Rosc.(Ginger), *Piper guineense* Schumach,(Black pepper), *Azadirachtaindica* A. Juss. (neem), and leaves of *Nicotiana tabacum* Linn. (Tobacco) on

tubers before storage for five months showed high level of potency in controlling rot pathogens of yams.

Another study by Onuh *etal.,*(2015) on the aqueous extracts of nine plants: *Terminalia catapa*, *Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, and *Mangifera indica* of fresh and dry materialshowed that three pathogens, *Rhizopus stolonifera* (fungi), *Erwinia carotovora* and *Pseudomonas aeruginosa* (bacteria) isolated were completely inhibited each by a plant. The study also showed that *Passiflora edulis* had the best antimicrobial activity for both fungi and bacteria as it indeed inhibited completely *Rhizopus stolonifera* which was stubborn. Most of the other plantextracts of *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, and *Mangifera indica however* were also able to inhibitmost of the fungi but not completely.

Okigbo,*et al.,*(2009) in their investigation that used the extracts *Azardiractha indica* and *Chromolaena odorata* to control rot diseases of yams found that both were fungitoxc against the yam rot spoilage fungi that were tested. Although this study indicated that *Azardirachta indica* and *Chromolaena odorata* were able to suppress rot-causing fungi of yam, *Azadirachta indica* was found to inhibit organisms more than *Chromolaena odorata*. It has been demonstrated that solvent of extraction and concentration of the extracts always exert significant influence on the performance of the extracts against mycelia growth (Markson *et al.,* 2012).The studies have shown the antimicrobial potency of five crude plant extracts and chemical fungicide in vitro control of *Aspergillus flavus*, causal agent of white yam (*Dioscorea rotundata*) tuber rot.The findings have shown the potential of plants in the control of yam rotting fungus caused by *Aspergillus flavus*. The result revealed that plant extracts could be an alternative to toxic fungicides for controlling plant pathogens since they are composed of various bioactive compounds such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc. (Gwa VI, *et al.,* 2016).

2.15 Bioactive compounds in the plant leaves used as biopesticides

2.15.1 Bioactive compounds present in *Azadirachta indica* (Neem)

Azadirachta indica, commonly known as neem, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*, and is typically grown in tropical and semi-tropical regions. The Taxonomical or Scientific classification of the neem tree is shown below:

Taxonomical/Scientific Classification:

Kingdom: *Plantae*

Clade: *Tracheophytes*

Clade: *Angiosperms*

Clade: *Eudicots*

Clade: *Rosids*

Order: *Sapindales*

Family: *Meliaceae*

Genus: *Azadirachta*

Species: *A. indica*

Binomial name: *Azadirachta indica*

[Source: Royal Botanic Gardens, Kew. (2016)]

According to National Academics Press, US,(1992), Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–

25metres (66–82 ft). The neem tree is very similar in appearance to its relative, the Chinaberry (*Melia azedarach*).

Many studies have reported on the phytochemical screening of the extracts of *azadirachta indica* for secondary metabolites.Okigbo *et al.,* (2015) found that *azadirachta indica* contained bioactive compounds like alkaloids, tannins, flavanoids, saponins and steroids while Ravi *et al.* (2015) also found polyphenols and steriods in ethanolic extracts of *azadirachta indica*. The study by Timothy *et al.,*(2011) equally showed the presence of tannins, saponins, flavonoids, alkaloids, glycosides, reducing sugars and terpenes in the ethanolic leaf extract of *azadirachta indica*. Qualitative phytochemical screening of selected parts of neem plant by Ezeonu, *et al.,* (2019) also showed the presence of alkaloids, glycoside, flavanoids, carbohydrates, reducing sugar, steroids, tannins, and saponins. The presence of these phytochemical components may be responsible for the antimicrobial activity of the plant leaf extract of *Azadirachta indica* as observed in many studies.

Neem-coated urea is being used as an alternative to plain urea fertilizer in India. It reduces pollution, improves fertilizer's efficacy and soil health.

2.15.2Bioactive compounds present in*Cymbopogon Citratus* (Lemon Grass)

Shah, *et al.,*(2011), identified *Cymbopogon* as a genus of about 55 species, which are indigenous in tropical and semi-tropical areas of Asia and are cultivated in South and Central America, Africa and other tropical countries. These are described by Weiss, (1997), and Kumar *etal.,*(2000), as tufted perennial grasses with numerous stiff stems arising from a short, rhizomatous rootstock,as with citrus flavor, and can be dried and powdered or used fresh. The name *Cymbopogon* is derived from the Greek words “kymbe” (boat) and “pogon” (beard), referring to the flower spike arrangement (Plants data base, 2003).

TAXONOMICAL CLASSIFICATION

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Liliopsida*

Order: *Poales*

Family: *Poaceae*

Genus: *Cymbopogon Spreng*

Species: *citratus*

Parts Used:Leaves and whole plant.

Cymbopogon citratus, Stapf (Lemon grass) is commonly used in teas, soups and curries. It is also suitable for poultry, fish and seafood.

Shah *et al*., (2011), also reported that *Cymbopogon citratus*, (Lemon grass) is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in aromatherapy. The compounds identified in *Cymbopogon citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. The plant also contains phytoconstituents such as flavonoids and phenolic compounds. Asaolu *et al*.,(2009) in their study found that the ethanolic extract of *Cymbopogon citratus*contained alkaloids, saponins, tannins, athaquinones, steroids,phenols and flavonoids.

2.15.3Bioactive compounds present in*Ocimum gratissimum* (Mosquitoe Plant – ncheanwu)

Ocimum gratissimum, variously known as clove basil, African basil, and as wild basil in Hawaii, is a species of *Ocimum*. It is native to Africa, Madagascar, Southern Asia, and the Bismarck Archipelago, and naturalized in Polynesia, Hawaii, Mexico, Panama, West Indies, Brazil, and Bolivia (USDA Plants, (2009). According to Dzoyem *et al.*,(2017) the genus *Ocimum*is a member of the Lamiaceae family, comprising more than 150 species. It grows widely and is distributed throughout temperate regions of the world with the greatest number of species in Africa.

Scientific classification

Kingdom	*Plantae* – plantes, Planta, Vegetal, plants
Subkingdom	*Viridiplantae* – green plants
Infrakingdom	*Streptophyta* – land plants
Superdivision	*Embryophyta*
Division	*Tracheophyta* – vascular plants, tracheophytes
Subdivision	*Spermatophytina* – spermatophytes, seed plants, phanérogames
Class	*Magnoliopsida*
Superorder	*Asteranae*
Order	*Lamiales*
Family	*Lamiaceae* – mints, menthes
Genus	*Ocimum* L. – basil
Species	*O. gratissimum* L. – African basil
Binomial name	*Ocimum gratissimum*

Other common or local names for *Ocimum gratissimum*

- Ebe-amwonkho in Edo
- Tchayo in Fon
- Efirin in Yoruba
- Añyeba in Igala
- Daidoya in Hausa
- Nchuanwu also Arimu in Igbo
- Ntong in Ibibio, Efik
- Kunudiri in Okrika
- Nunum in Akan
- Nunu Bush in Jamaica (from the Akan language)
- Yerba di holé in Papiamento
- Fobazen in Haiti
- Scent leaves in Nigeria and in the African diaspora
- Mujaaja in Uganda

Afolabi *et al.*,(2007) in their investigation found that*Ocimum gratissimum* is rich in phytochemicals and also revealed that the extract from the leaves of *O. gratissimum* possessed good antioxidant potential presumablybecause of its phytochemical constituents. In the works of Mbata *et al.*,(2007), the phytochemical screening of the ethanolic extracts of *Ocimum gratissimum* contained alkaloids, resins, tannin, flavonoids, glycosides, saponin, cardiac gycosides, and steroidal terpenes at different concentrations.

2.15.4 Bioactive compounds present in *Psidium guajava* (Guava)

Psidium guajava also known as guava is an evergreen tree. It is a shrubby evergreen tree up to 10 meters in height. The stem is hard, woody and reddish brown in color. The bark scales off. It bears white flowers and numerous pear-shaped fruits that are edible by animals, birds, and humans (Emmanuel *et al.*,(2019). *Psidium guajava* (Myrtaceae) commonly known as guava is a native of tropical America and has long been naturalized in South East Asia. Different parts of the plant are used in the traditional system of medicine for the treatment of various human ailments such as wounds, ulcers, bronchitis, eye sores, bowels, diarrhoea, and cholera.

Scientific classification:

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass Rosidae

Order Myrtales

Family Myrtaceae – Myrtle family

Genus *Psidium* L. – guava P

The bioactive components of *Psidium guajava*leaves have been evaluated in many studies. For example, the qualitative analysis of ethanolic and aqueous extract of *Psidium guajava* leaves by Rajan, (2014) showed that tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, polyphenol and glycoside were present in both extracts whilethe quantitative determination of *Psidium guajava*leaves contained Phenol (9.33 mg/gm powder), Tannin (4.30 mg/gm powder), Flavonoids (6.42 mg/gm powder) and Saponin (3.67 mg/gm powder). Egga *et al*.,(2014), showed that the phytochemical screening of *Psidium guajava* revealed the presence of all metabolites and compounds tested for such as flavonoids, tannins, reducing sugar, terpenes, saponins, anthraquinones and alkaloids. Emmanuel *et al.,*(2019) the preliminary phytochemicals investigation found that most of the bioactive compounds tested of, were present in the ethanolic extract of *P. guajava* leaf of the plant. Saponins, glycosides, tannins, flavonoids, alkaloids, volatile oils and triterpenes were found to be present in the ethanol extract of the leaf of *P.guajava,* while phenolic compound happen to be the only bioactive compound absent in the ethanol extract of the leaf of *P. guajava.*

2.15.5 Bioactive compounds present in *Chromolaena odorata* (Elizabeth Plant)

Chromolaena odorata is a tropical and subtropical species of floweringshrub in the sunflower family. It is native to the Americas, from Florida and Texas in the United States south through Mexico and the Caribbean (*Nesom, 2006*) to South America. It has been introduced to tropical Asia, West Africa, and parts of Australia (King *et al.*, 2017).

Scientific Classification:

Kingdom - *Plantae*– Plants

Subkingdom - *Tracheobionta*– Vascular plants

Superdivision - *Spermatophyta* – Seed plants

Division - *Magnoliophyta* – Flowering plants

Class - Magnoliopsida – Dicotyledons

Subclass - Asteridae

Order - Asterales

Family -Aster family

Genus - *Chromolaena* DC.– thoroughwort P

Species - *Chromolaena odorata* (L.)– Jack in the bush P

[Source: R.M. King & H. Rob. (2017)]

Igboh *et al.*,(2009) in their study found that the phytochemical profile of *Chromolaena odorata* consisted of alkanoids, cyanogenic glycosides, flavonoids (aurone, csihatoone,) flavone, flavonol) phytates, saponins, and tannins. The result of the work of Usunomena *et al.,* (2016) showed that the extracts of *Chromolaena odorata* contained flavonoids, alkanoids, saponins, tanins and steroids while cardiac glycosides and anthroquinones were absent. Agaba *et al.,* (2016) equally established that the chemical constituents of *Chromolaena odorata* were alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins.

Okigbo and Ezebo, (2017)in their phytochemical screening of the leaf extracts of *Chromolaena odorata* also found the presence of saponins, alkaloids, tannins and flavonoids in the extracts of *Chromolaena odorata* but at different concentrations.

2.15.6 Bioactive compounds present in *Carica papaya* (Pawpaw)

Carica papaya (Pawpaw) is believed to be Native to Mexico and northern South America, papaya has become naturalized throughout the Caribbean Islands, Florida, Texas, California, Hawaii, and other tropical and subtropical regions of the world.

According to Boning, (2006) *Carica papaya* (Pawpaw) is believed to be from southern Mexico, Central America, and northern South America, the papaya is now cultivated in most tropical countries. In cultivation, it grows rapidly, fruiting within 3 years. It is, however, highly frost-sensitive, limiting its production to tropical climates. Temperatures below −2 °C (29 °F) are greatly harmful if not fatal. In Florida, California, and Texas, growth is generally limited to southern parts of the states. It prefers sandy, well-drained soil, as standing water will kill the plant within 24 hours.

Scientific Classification:

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Super division Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass Dilleniidae

Order Violales

Family Caricaceae – Papaya family

Genus *Carica* L. – papaya P

Species *Carica papaya* L. – papaya P

[Source: National Plant Germplasm System (2017)]

Various studies have been conducted on the phytochemical constituents of *Carica papaya*. For instance, Yusha'*et al.*,(2009) in their found that the results of phytochemical screening indicated the presence of alkaloids, flavonoids, steroids and tannins in either ethanol extract, fraction(s) or both. Enyiukwu *et al.*,(2017) established that the plants extracts of *Carica papaya* contained a broad spectra of phytochemicals (secondary metabolites) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, phlobatannins, polyphenols and steroids and that these secondary metabolites constitute plants' weaponry

against pests and pathogens invasion. These groups of phytochemicals possess wide ranging chemical functional groups; by which they establish contact with and bind to sites on target pathogens to in effectuate them.

2.15.7 Bioactive compounds present *Vernonia amygdalina* (Bitter Leaf)

Vernonia amygdalina, a member of the daisy family, is a small shrub that grows in tropical Africa. *V. amygdalina* typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Ejike, 2011). *V. amygdalina* is commonly called Congo Bololo in D. R. Congo, bitter leaf in English because of its bitter taste. African common names include grawa (Amharic), ewuro (Yoruba), etidot (Efik), onugbu (Igbo), ityuna (Tiv), oriwo (Edo), Awnwono (Akan), chusar-doki (Hausa), mululuza (Luganda), labwori (Acholi), olusia (Luo), ndoleh (Cameroon) and olubirizi (Lusoga) (*Egedigwe,* 2010; Kokwaro, 2009); Appiah, 2018). The scientific classification is shown below:

Scientific Classification:

Kingdo: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Asterales

Family: Asteraceae

Genus: *Vernonia*

Species: *V. amygdalina*

Botanical name: *Vernonia amygdalina*

Usunomena *et al.*,(2016) in their investigation found *Vernonia amygdalina* contained phytochemicals such as alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, cardiac glycosides, and reducing sugar.

2.16 Traditional Methods used to Control Yam Diseases

The adoption of some of the modern yam rot control methods are made impossible because of the educational standard and income levels of most peasant yam farmers and dealers in West Africa who dwell mainly in rural communities. Such farmers, therefore, use traditional methods of storage in their attempt to control tuber rots (Cornelius, 1998).

Improved storage methods, curing under jute sheet or jute sacks, pre-storage treatments such as: pit curing, keeping freshly harvested yams in shade for a few days and the application of wood ash, lime, wet earth, vegetable oils and extracts from some local plants on the wounded surfaces of the tubers are included as othertraditional methods of reducing storage rot diseases (FAO, 1990).

2.16.1Nutritional composition of yam tubers

According to Onwueme,*et al.*, (1991) yam tubers that are freshly harvested consist of about 70% water, 25% starch, 1-2% protein and traces of vitamins. In their study, Nwufo,*et al* (2016), have reported on Moisture, Ash, Crude fibre, Crude protein, Fat, NFE (CHO), Nitrogen and Carbon contents of *Dioscorea rotundata*, respectively.According to a study by Knoth, (1993), most yams are essentially composed of water, starch, small quantities of protein and other minor constituents as shown in Table2.9:

Table 2.9: Composition of species of yams % wb.(Knoth, 1993).

Variety	Moisture Content	Carbohydrate	Fats	Crude Protein
D. alata	65 – 73	22 – 29	0.10.3	1.1 -2.8
D.rotundata	58 – 80	15 – 23	0.1 0.2	1.1 -2.0
D. esculenta	67 – 81	17 – 2		1.3 - 1.9
D. bulbifera	63 – 67	27 – 33	0.1	1.1 - 1.5

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site:

This study was carried out at the Crop Science and Tech. laboratory of the Federal University of Technology, Owerri, Imo State, located on Latitude 5° 30' 01" N and Longitude 7° 01' 44" E in the tropical rainforest region of Nigeria.

The different plants for the leaf extracts were obtained from the plants in Generic Resource unit of the Department of Crop Science Technology, Federal University of Technology, Owerri.

3.2 The Study Plan:

This research was carried out for a period of specific twenty four (24) months with proper supervision and in different laboratories to generate results that are competitive.

The collection of plant materials and rotten yam tuber samples was carried out within a month.The morphological identification of fungi was performed macroscopically and microscopically.The morphological identification was based on Barnett and Hunter, (1998) and Muthar and Kongsdal, (2003)

Table 3.1:Gantt chart: Showing the Study Plan/work Plan

Quarter	Q1			Q2			Q3			Q4		
Activities	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct
Collection of materials												
Visual identification of rots on the tuber												
Isolation & Identification of micro organism												
Bio – pesticide preparation												
Efficacy test of bio-pesticides												
Investigation of molecular compounds of Bio-pesticides												
Point of attack on the yam tubers												
Biochemical composition												

3.3 Experimental Design:

Completely randomized design (CRD) with seven treatments in three replications on two yam species respectively wasused for this investigation .This research was carried out for a period of twenty four (24) months.

Healthy and diseased yam tuber samples were collected from the National Root Crop Research Institute,(NRCRI)Umudike within two months.

3.4 Pathogenicity Studies

Isolation and identification of fungal species from the rotten yam tuber varieties was carried out - 2 months according to Mathur and Kongsdal (2003) and Barnett and Hunter, (1998).

The following experiments were carried out as follows:

3.4.1 Experiment 1: Preparation of bio-pesticides using Ethanol Extraction Method – 2 months.

Pathogenicity test: Healthy looking yam tubers of the *Dioscorea rotundata and Dioscorea alata* was used for the test as described by Okigbo and Ikediegwu, (2000) and Mathur and Konsdal, (2003) - four (4) months.

The efficacy/ inhibiting effects of the bio-pesticides were tested for their fungicidal activities in controlling post-harvest diseases and calculated by using Koch's postulate, (2003) – two (2) months.

3.4.2 Experiment 2 Comparison of the efficacy of the botanical extracts on the different pathogens on the yam varieties (*Dioscorea rotundata* and *Dioscorea alata*) and confirmation of the most efficacious on them, *in vitro*

3.4.3 Experiment 3 Qualitative screening of the phytochemicals composition of the seven (7) botanicals.

3.4.4 Experiment 4: Assessment of the site of infection on the yam tubers and point of easy attack – (2) months.

3.4.5 Experiment 5: Determination of biochemical composition of the infected and healthy yam tubers in storage, viz; ash content, crude protein, crude fibre, Ether Extract (Fat) and moisture content.

3.5 Source of plant materials:

Two yam varieties that were used for this experiment namely *Dioscorea rotundata*, and *Dioscorea alata*, were sourced from NRCRI (National Root Crop Research Institute) Umudike.

Plate 3.13a: *Dioscorea rotundata*

D. rotundata tubers

Plate 3.13b: *Dioscorea alata*

D. alata tubers

3.6 Source of the plant materials used as extract

The different plants used for leaf extracts were obtained from the plants in Genetic Resource Unit of the Department of Crop Science and Technology, Federal University of Technology Owerri, Imo State.The seven (7) plant leaves used for the experiment are: *Azardirachta indica* (Neem leaves), *Carica papaya* (Pawpaw leaves), *Chromolaena odorata* (Elizabeth Plant leaves), *Cymbopogon citratus* (Lemon Grass leaves), *Ocimum gratissimum* (Ncheanwu leaves), *Psidium guajava* (Guava leaves), *Vernonia amygdalina* (Bitter leaves). The plant specimen used for the experiment were collected fresh from the Genetic Resource

Unit of the Department of Crop Science and Technology, Federal University of Technology Owerri, Imo State.

Plate 3.14: *Azadirachta indica*

Plate 3.15: *Carica papaya*

Plate 3.16*: Chromolaena odorata*

Plate 3.17: *Cymbopogon citratus*

Plate 3.18*: Ocimum gratissimum*

Plate 3.19: *Psidium guajava*

Plate 3.20: *Vernonia amygdalina*

3.7 Physical Identification of the Diseases that Infected the Yams in Storage

Physical investigation of five tubers each of *Dioscorea rotundata and Dioscorea alata* infected yam tubers was conducted by virtual assessment.

3.8 Isolation of Micro Organisms

A portion of each fungal colony which developed was picked using a sterile inoculating needle and aseptically sub-cultured onto fresh potato dextrose agar (PDA) plates. The plates were kept as stock cultures for subsequent use. Pieces of diseased tissues cut from the periphery of rotten yam tubers with a sterilized knife were surface-sterilized in 5% sodium hypochlorite solution for 5 minutes. The surface-sterilized diseased tissues were washed three times using sterile distilled water. The tissues were allowed to dry in a sterile Lamina flow chamber. The dried diseased tissues were plated on a potato dextrose agar (PDA) medium (Manufacturer: Mearek). Five days after incubation, mycelia that grew from the plated yam tissueswere sub-cultured into fresh PDA. Further sub-culturing was carried out until pure culture of single species isolates was obtained. From these pure cultures, inocula of the different fungal species isolates were obtained for the pathogenicity tests.

3.9 Identification of Fungal Isolates

Fungal isolates were examined macroscopically and microscopically using the needle mounts method. A wet mount slide was made by transferring a small amount of the culture with a dissecting needle to a drop of lactophenol cotton blue stain on a grease –free clean glass slide, covered with a cover glass and examined under low power objects.Characteristics of fungal isolates from rotten yam tubers such as pigment production, colony texture, spore or conidia-producing structures and spore shapes were documented. The characteristics were observed from fungal growth for five days. Spore and mycelium was viewed usingthe

compoundmicroscope. Their characteristics was studied and used in identifying the fungal organism to the species level, following the standards described by Mathur and Kongsdal, (2003) and Barnett and Hunter, (1998).

3.10 Preparation of Bio-Pesticides:

Ethanol extraction method was used for the preparation of the botanical extracts. Fresh leaves of *Azadirachta indica* (Neem plant leaf), *Chromolaena, Odorata* (Elizabeth plant leaf), *Ocimum gratissimum* (Mosquito plant leaf- ncheanwu), *Psidium guajava* (Guava), *Vernonia amygdalina* (Bitter leaf), *Cymbopogon citratus* (Lemon grass) *and Carica papaya* (Pawpaw plant leaf) were washed thoroughly with water. These were further blended into a fine paste separately for each botanical with a blender (Binatone, BLG-401, Hong Kong) at a speed of 4000 r.p.m. for five to ten minutes after drying in an oven at a temperature of 121^{O} C. Extract concentrate of 60% (w/v) was obtained by adding 40mls of sterile distilled water to 60g each botanical paste with vigorous stirring. The efficacies of the botanical extracts were tested for their fungicidal activity in controlling yam tuber post-harvest diseases.

Plate 3.21a:Preparation of bio-pesticides

Plate3.21b:Samples of prepared leaf extract

3.11 Control of Yam Tuber Diseases in Storage with Bio-Pesticides

3.12 Ethanol Extraction of Rot and Rust from the Diseased Yam Tubers

Ethanol extraction method was used for the preparation of the botanical extracts. Fresh leaves of *Azadirachta indica* (Neem plant leaf), *Chromolaena, Odorata* (Elizabeth plant leaf), *Ocimum gratissimum* (Mosquito plant leaf- ncheanwu), *Psidium guajava* (Guava), *Vernonia amygdalina* (Bitter leaf), *Cymbopogon citratus* (Lemon grass) *and Carica papaya* (Pawpaw plant leaf) were washed thoroughly with water. These were further blended into a fine paste separately for each botanical with a blender (Binatone, BLG-401, Hong Kong) at a speed of 4000 r.p.m. for five to ten minutes after drying in an oven at a temperature of 121^{O}C. Extract concentrate of 60% (w/v) was obtained by adding 40mls of sterile distilled water to 60g each botanical paste with vigorous stirring. The efficacies of the botanical extracts were tested for their fungicidal activity in controlling yam tuber post-harvest diseases.

3.13 Anti-fungal Activity of Bio-Pesticides (Percentage Inhibition)

Ethanol Extraction Method

70g each of the leaves was heated respectively in 200ml ethanol at 35% concentration. After 48 hours, the ethanol was evaporated on a hot plate after decanting. An aliquot of 0.1ml of each plant extract was introduced in a Petri dish after which it was inoculated with pure cultures of the organisms identified with the following diseased conditions (Rot and Rust respectively).

An aliquot of 0.1ml of each of these plant extracts was used to determine their inhibiting capacity on the identified organisms.

To establish the efficacy of Bio-Pesticides in controlling post-harvest diseases, seven plant leaves were used namely *Azadirachta indica, Chromolaena odorata, Ocimum gratissimum,*

Psidium guajava, Vernonia amygdalina, Cymbopogon citratus and *Carica papaya*. Extraction was done through ethanol extraction. The efficacy of these Bio-Pesticides was tested for their fungicidal activities in controlling post-harvest diseases. The inhibiting effects of the Bio-Pesticides was thereafter observed after five (5) days by weighing the difference between the initial and final weights and calculated with Koch's Postulate, (2003) thus:

Percentage Inhibition = Wi-WoX $\frac{100}{1}$

Where;

Wo = Initial weight before the treatment

Wi = Final weight after the treatment

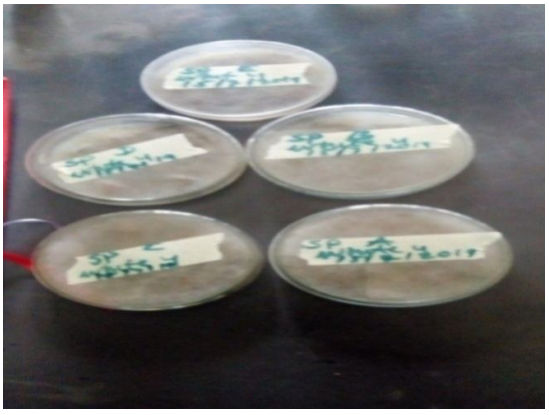


Plate 3.22a Inoculated diseased *D.rotundata*

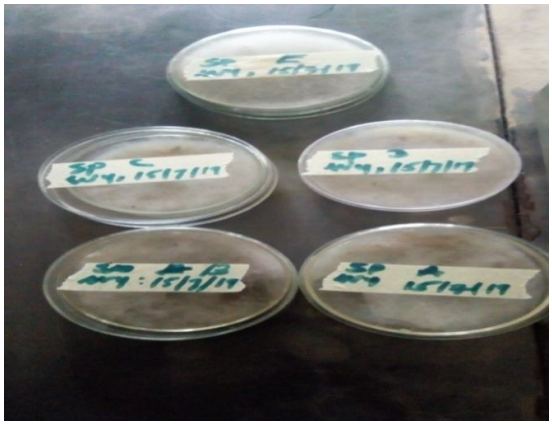


Plate3.22b Inoculated diseased *D. alata*

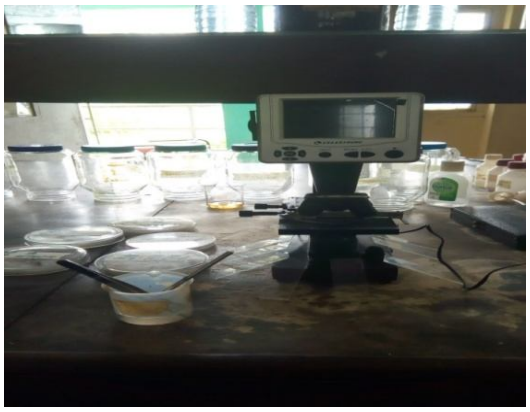


Plate 3.22c Identification of the microorganisms from the microscope

3.14 Pathogenicity Test:

The method of Okigbo and Ikediugwu, (2000) was used. Healthy-looking yam tubers of the variety *Dioscorea rotundata* and *Dioscorea alata* was thoroughly rinsed in sterilized water and further sterilized with 70% ethanol. Thereafter, cylindrical cores of 1cm deep were removedfrom various spots of each yam tuber sample with 5mm cork borer that was sterilized by dipping in ethanol followed by flaming. Then the yam tubers were inoculated with the fungal isolates that were identified in section 3.9. One week old pure cultures of the fungal isolates obtained from the yam tubers produced on PDA were the source of inocula for the pathogenicity studies. The five-millimeter diameter cork borer was used to cut plugs from the one week old cultures of the isolates to be tested. These fungal plugs were put in the holes created in the yam tubers after which the removed yam tuber disc was used to plug the holes. Melted candle wax from burning candle was used to seal the edges of the replaced yam disc. This process will prevent any external influence on the positioned inocula. Each fungal isolate was replicated three times (on ten tubers five each of *Dioscorea rotundata* and *Dioscorea alata*). Control was set up in which the sterile cork borer which was used to remove five-millimeter diameter tuber tissue. This disc was used to plug the hole and its edges sealed with melted wax. In the control, no fungal organism/plant extract was placed in the hole. These activities were carried out inside a sterile hood. To re-confirm the various pathogens previously identified and used as inocula in the test, pure cultures were plated from different colonies for three days and viewed on the microscope using the slide and blue phenol solution or distilled water. The pathogenicity test showed growth in all the yam tuber samples but at various degrees. Fungalspecies implicated as the rot pathogens of *Dioscorea rotundata*and *Dioscorea alata*yam tuber samples were studied.

Plate 3.23a Pathogenecity test on *D. rotundata*

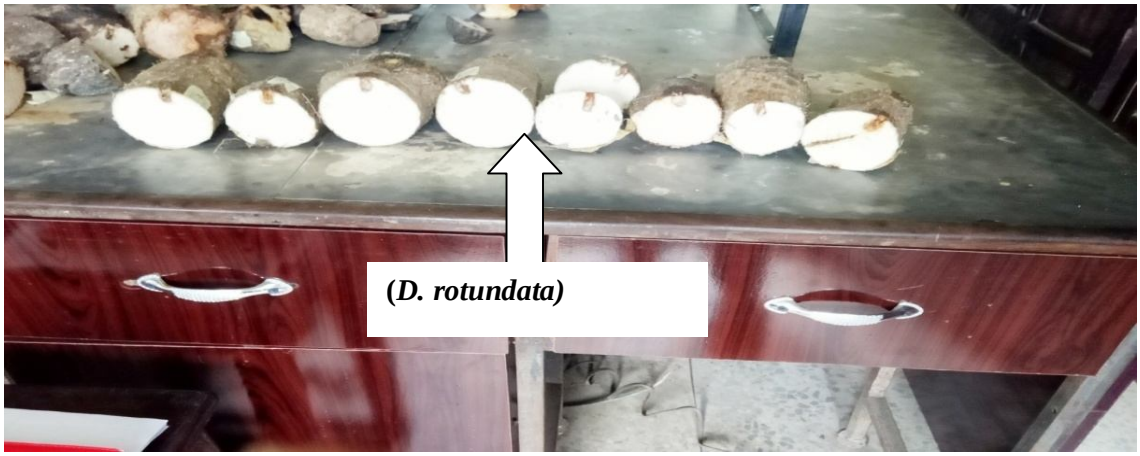
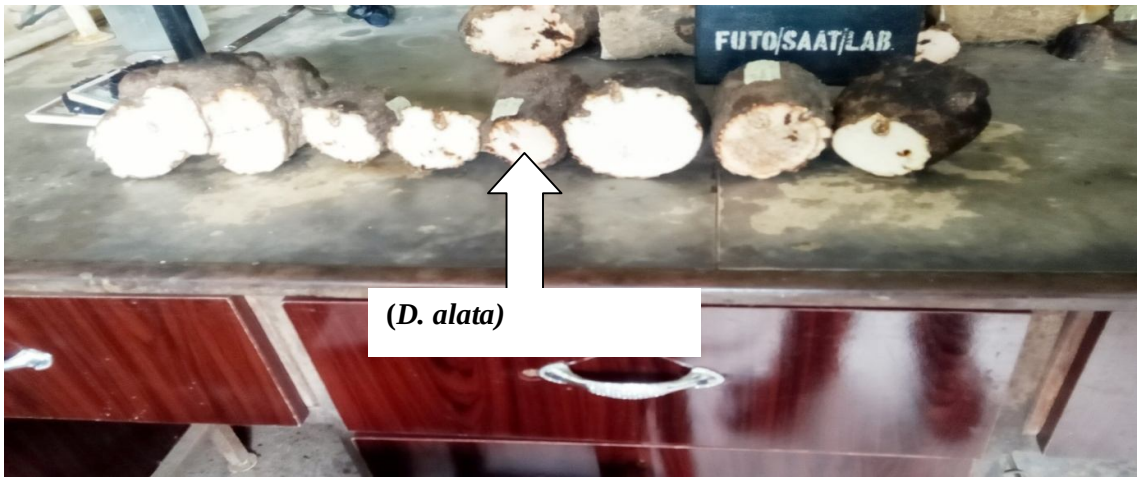
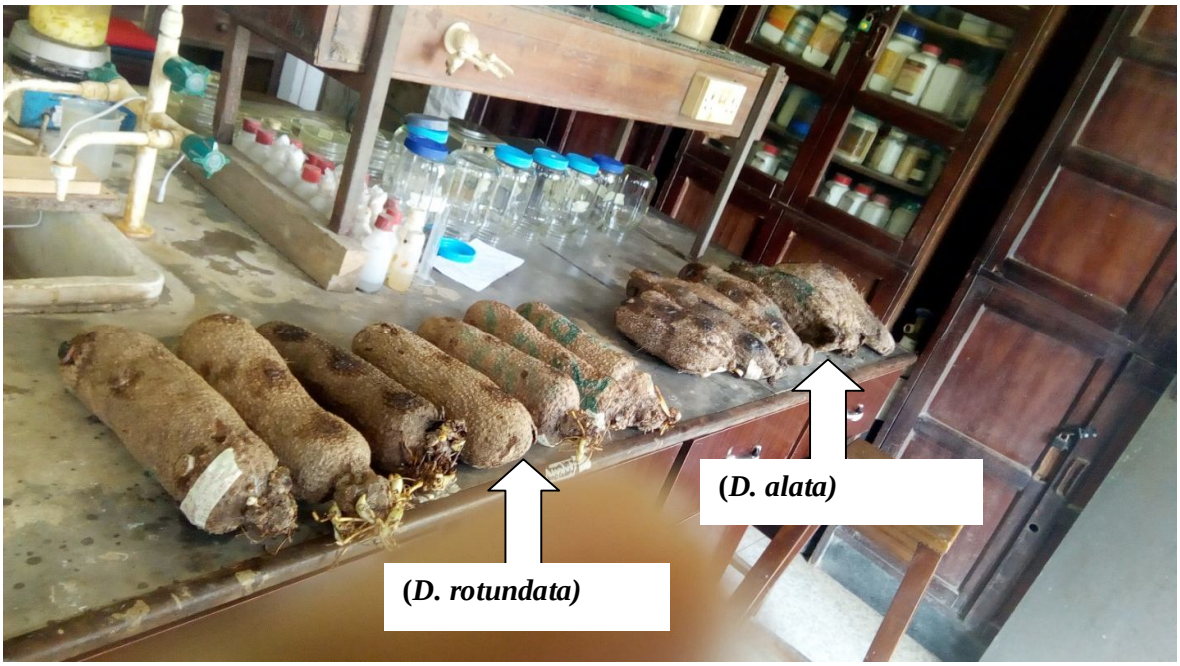


Plate 3.23b Pathogenicity test on *D.alata*



3.15: Determination of depth of pentration

Plate 3.24: Determination of depth of penetration



D. rotundata* and *D. alata

3.16: Determination of point of Easy attack

Plate 3.25: Determination of point of easy attack.

D. alata

D. rotundata

3.17. Determination of the nutritional Composition of the rotten and healthy yam tubers

3.17.1Moistusre content:

Drying Methods: These involved the measurement of the mass loss due to the evaporation of water at or near the boiling point. Although such methods are frequently used as they can give accurate result when considered on a comparative basis, it should be borne in mind that the value obtained may not be true measure of the water content of the samples. For example, volatile oil may be lost at drying temperatures such as 100^{o}C. The loss in mass may also depend on other factors, including particle size and mass of samples used, type of dish and temp variations in the oven from shelf to shelf. Ovens which are mechanically ventilated by means of an internal fan give more consistent results and an increased rate of drying.

Procedure: The container was washed and dried in the oven, tranfered to the desicator, weighed first,2g of the sample was weighed,dried in the oven at a temp of 105ºc for sometime. The container and the sample was reweighed, then taken to the oven and dried, put in the desicator and weighed again. The process continued until a consistent result was obtained.

Calculation: % Moisture content= $\frac{W1}{W2} - \frac{W2}{W} X \frac{100}{1}$

Where:

W_1 =Mass of container + samples before drying

W_2= Mass of container + samples after drying

W = Mass of container.

3.17.2 Ash content:

The ash content of a material is the residue remaining after ignition at 575 $\pm 25^{0}$C for 3 hours or longer to burn off all the organic matter or carbon. It is a measure of mineral content in the

sample but is not necessarily quantitatively equal to them as there may be losses due to volatilization. The ash content or figure can be regarded as a general measure of quality.

Procedure: The silica (or platinum) dish or a crucible was washed and dried, then cooled in a desicator. The crucible was weighed and 2g of the sample put in the crucible. The crucible and the contents placed in a muffle furnace temp regulated at 575± 25ºC until it carbonized, then calculated until the black particles were no more. The furnace was switched off and allowed to cool somewhat, then the crucible and the content was placed in a desicator and weighed.

Calculation: % Ash Content $=\frac{W1}{W2} - \frac{W2}{W} X \frac{100}{1}$

Where:

W_1 =Mass of crucible.

W_2 = Mass of crucible + sample before ignition

W_3 = Mass of crucible + Ash after ignition.

$W_2 - W_1$ = Mass of sample taken for ignition.

3.17.3 Crude Fibre

Crude Fibre is the combustible organic residue that is left after other biomolecules like proteins have been removed by successive treatments with boiling acid and alkalis, alcohols and ether. This empirical treatment provides a crude fibre consisting largely of the cellulose content together with a proportion of the lignin and hemicelluloses content of the samples. The amounts of these substances in the crude fibre will vary with the condition employed, so for consistent results a standardized procedure must be rigidly followed.

Procedure: Two (2)g of the sample was weighed into a beaker and placed in a hot 200 ml of 1.25% H_2S0_4 and boiled for 30 minutes. It was then filttered through a buckner

funnelequipped with muslin cloth and held firm with elastic band. The funnel was made hot by pouring boiling water on to it. Hot acid sample solution was filtered. The residue was washed with boiling water which removed the acid from it. The residue was returned to 200ml boiling 1.25% Na0H and boiled for 30 minutes then filtered, removed progressively and washed with boiling water, 1% HcL and boiling water to remove acid from it. The residue was washed twice with alcohol and three times with petroleum ether using small quantities. The residue was drained and transferred completely to a porclain crucible and dried in the oven to a constant mass. It was cooled and weighed, incinerated at 600ºc for 2hrs in a muffle furnace. The crucible was weighed and the content cooled in a desicator. The loss on incineration is the mass of crude fibre.

Calculation: % Crude Fibre $= \frac{W3}{W2} - \frac{W4}{W1} X \frac{100}{1}$

Where:

W_1 =Mass of beaker.

W_2 = Mass of beaker + sample

W_3 = Mass of crucible + residue after drying

W_4 = Mass of crucible + Ash after incineration.

3.17.4 Crude Fat (Ether Extract)

The fatty constituents of foods/feed sample consist of a number of lipid substances. The fat content (Sometimes called the ether extract or crude fat), which may be considered as consisting of the free lipid constituents is that which can be extracted by the less polar solvent such as light petroleum fractions, diethyl ether, whereas the bond lipid constituents require more polar solvents such as alcohols for their extraction. The bond lipids can be broken down by hydrolysis or other chemical treatment to yield free lipid. Hence the amount

of extracted lipid found in a food stuff/feed sample will depend on the method of analysis used.

Direct solvent extraction methods: The free lipid content, which consists essentially of neutral fats (triglycerides) and free fatty acids, can be conveniently determined in foods (samples) by extracting the dried and ground material with a light petroleum fraction of diethyl ether in a continuous extraction apparatus. A useful procedure for extracting fat from semi-solid and wet foods which avoids the initial drying stage is to mix the sample with calcium sulphate, anhydrous sodium sulphate or vermiculite. When the mixture becomes powdery and dry it can be transferred to a soxhlet thimble in an extraction apparatus.

Extraction in the presence of alcohols causes the release of lipids bound to proteins and carbohydrates: hence maximum extraction is obtained by a mixture of polar and non-polar solvents.

Procedure: A known quantity of the samples was weighed into a thimble of filter carefully wrapped and tied with a thread. The flask was washed, dried and then weighed. The thimble or filter paper and contents were placed in the soxhlet extractor column and extracted for about 6 hours. When the solvent becomes clear in the column, the fat must have been extracted. The defeated sample was carefully removed and the solvent was recovered. The flask and oil was oven dried until all the solvent was gone. Then the flask and its content were reweighed.

Calculation: % Crude Fat = $\frac{W2-M1}{W3} X \frac{100}{1}$

Where:

M_1 = Mass of the flask

M_2 = Mass of flask + fat

M_3 = Mass of the sample.

3.17.5 Crude Protein: (The Kjeldahl method or procedure)

The Kjeldhahl method is based on the wet combustion of the sample by heating with Concn H_2SO_4 in the presence of metallic and other catalysts to effect the reduction of organic nitrogen in the sample to ammonia, which is retained in soln as is distilled or steam distilled to release the ammonia which is trapped and titrated. Reduction in the digestion time has also been achieved by the addition of Potassium or sodium sulphate which raises the digestion temperature. Metal catalysts are conveniently available in tablet form compounded in a Potassium sulphate base. Also the addition of hydrogen peroxide significantly accelerates digestion and decreases foaming. Traditionally, the ammonia liberated from the digest having been made alkaline, is distilled into a standard quantity of dilute acid which is finally titrated with standard alkali to give the organic nitrogen content of the sample. More popular nowadays is to distil into (2%) 4% boric acid soln and titrate the ammonia direct with standard H_2SO_4 or HCl.

Procedure: A small quantity of 0.20g of the sample was weighed and carefully transferred to a kjeldal flask containing boiling chips. A mixture of copper and sodium sulphate using spatula was added and it raised the temperature of boiling. About 20ml of conc H_2SO_4 was added to the flask to assist the oxidation process. The mixture was heated until it became clear, then cooled to room temperature. It was then transferred quantitavely to a 10ml volumetric flask and rinsed with water. It was made up to mark. Blank experiment was also carried out with same procedure. About 20ml of the digest was pipetted and transferred to the distillation flask. About 10ml of 2% Boric acid was measured into a receiver (small beaker) and two drops of methyl red indicator added,further more,35ml of 40% Na0H was added to the distillation flask and quickly replaced with plug. The mixture was distilled until 30ml of the distillate was collected. Some procedure was carried out for the blank experiment.

Titrated against standard 0.IN HcL.

Calculation:

% Crude Protein $= = \frac{(T-B)x\ 6.25\ xvol.madex\ 0.00014}{Aliqot\ x\ mass\ of sample\ used} X \frac{100}{1}$

Where:

T = Titre value of the sample

B = Blank titre value

NHCL = Normality of HCL used

Aliquot = Sample aliquot (volume) taken

The volume was made up to = $100cm^3$

From your results, calculate the NFE.

State the precautions taken in each parameter as you report.

To determine the biochemical composition of the yam tuber species before carrying out the experiment, the biochemical composition (Moisture content, Ash, Fat, Protein, Fibre, Ether extract and Carbon content) of the diseased yam tubers was determined using proximate analysis according to AOAC (2016) Standard. For example:

(i) % N $= \frac{\text{Crude protein}}{6.25}$

(ii) NFE(CHO) = Moisture + Ash + Crude Fibre + Crude Protein + Ether Extracts (Fat)

3.18 Determination of anti-fungal Activity of Bio-Pesticides (Percentage Inhibition)

An aliquot of 0.1ml of each of these plant extracts was used to determine their inhibiting capacity on the identified organisms.

To establish the efficacy of Bio-Pesticides in controlling post-harvest diseases, seven plant leaves was used namely *Azadirachta indica, Chromolaena odorata, Ocimum gratissimum, Psidium guajava, Vernonia amygdalina, Cymbopogon citratus* and *Carica papaya*. Extraction was done through ethanol extraction. The efficacy of these Bio-Pesticides was

tested for their fungicidal activities in controlling post-harvest diseases. The inhibiting effects of the Bio-Pesticides was thereafter was observed after five (5) days by weighing the difference between the initial and final weights and calculated thus, using Koch's postulate,(2003):

Percentage Inhibition = = $\frac{Wi-Wo}{Wo}$ X $\frac{100}{1}$

Where;

Wo = Initial weight before the treatment

Wi = Final weight after the treatment

3.19 Qualitative screening of the phytochemicals in the plant extracts.

This is a series of tests to determine the presence or absence of certain substances present in a plant. Chemical tests were carried out by using standard procedure to identify the preliminary phytochemical screening following the methods described by Rasal, (2005) and Kokota, (2003).

Test for alkaloids

1. Mayer's test (Evans, 1997): To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.
2. Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.
3. Each extract was boiled (15 minutes) in HCl (25.0 ml, 1%). Equal volumes of the resulting suspension were filtered into two test tubes (A and B). To A, 5 drops of freshly prepared Dragendorrf's reagent was added. Formation of a precipitate indicated the presence of alkaloids. To confirm the results, B was treated with

saturated sodium carbonate solution until a drop of the solution turned the Universal Indicator paper blue, (pH 8-9). The resulting solution was dissolved in CHCl3 (4 ml) and allowed to stand. The aqueous layer was collected and acetic acid added to it drop wise, until the solution turned Universal Indicator paper yellow-brown (pH 5).

Test for Polyphenols

1. Ethanol (10.0 ml) was added to each extracts and the resulting solution (3.0 ml) was transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly prepared ferric cyanide solution were added to the extract solution. Formation of a blue green colour indicated the presence of polyphenols.
2. Yellow precipitates were obtained by the addition of 3 drops of lead acetate solution (5%) indicated the phenolic compounds.
3. ml of 0.1% of gelatin solution was added to 5ml of ethanolic extract. Precipitation indicated.

Test for Tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids:

1. Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, (1993), 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A

yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.
3. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for flavonols and flavones 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols and flavones

Test for phytosterol:

1. Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.
2. The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Conc. H_2SO_4. Appearance of bluish green colour showed the presence of phytosterol.

Test for Terpenoids (Salkowski test):

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4(3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for triterpenoids:

Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H_2SO4. Formation of reddish violet colour indicates the presence of triterpenoids.

3.20 Data analysis

Data generatedwere subjected to statistical analysis using analysis of variance (ANOVA) at 5% level of probability and the treatment means were separated as described by Obi (2002).

CHAPTER FOUR

RESULTS AND DISCUSIONS

4.1 RESULTS

4.1.1 Phytochemical screening of the seven plant extracts

The result obtained from the evaluation of the phytochemical screening of the plant extract in ethanol is presented in Table 4.1. The result revealed that alkaloids were highly present in *O.gratissimum,*while in *A.indica and C. odorata* were moderately present, but in *V.amygdalina and P. guajava* were slightly present but absent in *C.papaya* and *C.citratus*. Also flavanoids were absent in *P.guajava,* but moderately present in *A.indica* and *C.odorata* but slightly present in *V. amygdalina, O. gratissimum, C. papaya* and *C. citratus*.However, Glycosides were present in *O.gratissimum,* and *A. indica* while absent in *V.amygdalina, C.odorata, P.guajava, C. papaya and C.citratus.* The result further revealed that Tannins were moderately present in *O.gratissimum, A. indica, C.odorata* and slightly present in *V.amygdalina,P. guajava, C. papaya* and *C. citratus*, while Terpenoids were absent in *V.amygdalina, C.odorata, P.guajava, C.papaya* and *C.citratus,* but slightly present in *O.gratissimum*and *A. indica*. However, saponins were absent in *O.gratissimum*and *C.citratus*but slightly present in *P.guajava* and *C.papaya* but moderately present in *C. odorata* while Phenols were moderately present in *A.indica* and slightly present in *V.amygdalina, O. gratissimum* and *P. guajava* but phenols were not present in *C. odorata, C. papaya* and *C. citratus.* However, steroids were slightly present in *V.amygdalina, O. gratissimum* and *A. indica,* but absent in *C. odorata, P. guajava.C.papaya* and *C.citratus.*

Table 4.1: Phytochemical screening of the plant extracts in ethanol

	Plant extract						
Phytochemical	***O. gratissimu***	***A. indica***	***V. amaygdalina***	***C. odorata***	***P. guajava***	***C. papaya***	***C. cittratus***
Alkaloids	+++	++	+	++	+	-	-
Flavanoids	+	++	+	++	-	+	+
Glycosides	+	+	-	-	-	-	-
Tannins	++	++	+	++	+	+	+
Terpenoids	+	+	-	-	-	-	-
Saponins	-	+	+	++	+	+	-
Phenols	+	++	+	-	+	-	-
Steroid	+	+	+	-	-	+	+

Key: + =slightly present, ++= moderately present, +++=highly present

4.1.2 Evaluation of the percentage occurrence of different diseases on the yam tubers from *D. rotundata* and *D. alata*

The result obtained from the evaluation of the percentage occurrence of the different types of diseases on tubers of two yam species *(Dioscorea rotundata* and *Dioscorea alata)* ispresented in Table 4.2.The result showed that 8 and 16% of yam tubers of sample A from *D. rotundata* were infected with yam rust and rot, 3 and 14% of the tubers from sample B were equally infected with yam rust and rot. However 6 and 18% of tubers from sample C were also infected with yam rust and rot. Furthermore, 5 and 13% of the tubers from sample D were also infected with yam rust and rot, while 4 and 13% of yam sample E were equally infected with both rust and rot diseases of yam.

Yam samples from *D.alata* also were infected at varying degrees with both diseases. The result revealed that 5 and 15% of the sample A(*D.alata*)were infected with yam rust and rot, 3 and 13% of sample B (*D.alata*) were equally infected with yam rust and rot.Also 6 and 16% of samples C (*D.alata*) were infected with these same diseases, while 4and 15% of the tubers from sample D(*D.alata*) were equally infected with yam rust and rot. 2 and 14% of sample D from *D.alata* specie were infected with yam rust and rot.The rot disease was prevalent on both the yam tuber samples of *Dioscorea alata* and *Dioscorea rotundata* used in this experiment.

Table 4.2 Percentage occurrence of different diseases(physical assessment)on the yam tubers from *D. rotundata* and *D.alata*(%)

White yam(*D. rotundata*) samples	**Rust**	**Rot**	**Water(*D. alata*) yam samples**	**Rust**	**Rot**
A	8.00	16.00	A	5.00	15.00
B	3.00	14.00	B	3.00	13.00
C	6.00	18.00	C	6.00	16.00
D	5.00	13.00	D	4.00	15.00
E	4.00	13.00	E	2.00	14.00
Mean	5.20	14.80		4.0	14.60
LSD (p=0.05)	**0.816**	**0.816**		**0.816**	**0.816**

4.1.3 Isolation and identification of fungi from the diseased yam samples

The result obtained from the isolation and identification of fungi from the two yam species is presented in Table 4.3. The result revealed that the fungi species isolated and identified from *D. rotundata* are namely: *Aspergillus* species, yeast, *Rhizopus species* and *B.theobromea*, while the fungi species isolated and identified from *D.alata* includes: *Aspergillus* species, yeast, *Rhizopus* species and *Fusarium species.*The result further revealed that *Aspergillus* specie and yeast were very prevalent as they were encountered in all the five samples from the two yam species under investigation. However, *Rhizopus* species were encountered in only three samples from the two yam samples. *Botryodiplodia* specieswere encountered in only two samples from *D.rotundata* only while *Fusariumspecies* were encountered in only three samples from *D.alata.*

Table 4.3 Identification of fungi isolated from the diseased yamtubers (*D. rotundata*and *D.alata*)

Present samples	Yam specie	Cultural description	Microscopy	Probable genera
A,B,C,D, E	*D.rotundata* and *D.alata*	Black/brown colouration in both front and reverse view of culture plate	Aseptate hyphae with rough head of pigmented oval shaped spores	*Aspergillus spp*
A,B,C,D,	*D.rotundata* (absent in E)*D.alata*	Grow on yeast malt,yeast extract-peptone,peptone dextrose and negative growth on vitamine-free medium and 50% glucose	Oval,spherical or cylindrical shaped, light/cream colouration,smooth, elongate absence of ascospore.	*Yeast spp*
A,E,C	*D.rotundata*(absent in B and D) *D.alata*(absent in C)	Growing colonies fade from white to dark as they produce spores	Broad hyphaescarcely septate, rhizoids and stolons present, sporangiophores	*Rhizopus spp*

			brown, sporangia round,	
B,C	*D. rotundata* (absent in samples A,D E)	Dark black pycnidia arranged either on the periphery, edge or centered or scattered in the colonies.	Raised, uniform , cottony to grayish black, sporulate and fluffy colony growth	*Botryodiplodia* specie
A, C,D	*D.alata* (absent in samples B, E)	Grow on a range of carbon source, tolerance to inhibitors especially copper sulphate and malachite green.	Produces both macro and microconidia from slender phialides, macroconidia are hyaline, fusiform to sickle-shaped with enlongated apical cell.	*Fusarium species*

Plate 4.1 Yeast species

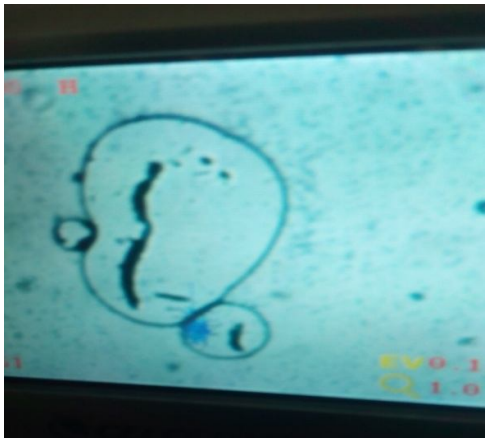


Plate 4.2a*Aspergillus*species

Plate 4.2b *Aspergillus* species

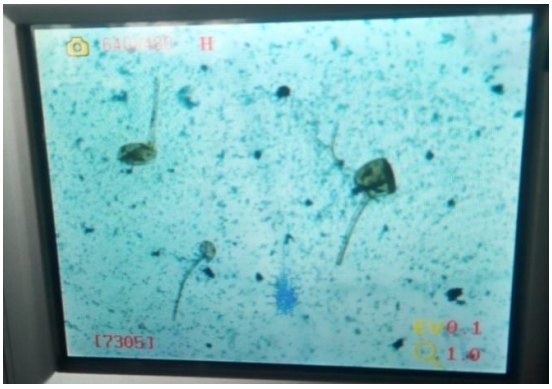


Plate 4.3a *Botryodiplodia* species

Plate 4.3b *Botryodiplodia* species

Plate 4.4*Rhizopus* species

Plate 4. 4b *Rhizopus* species

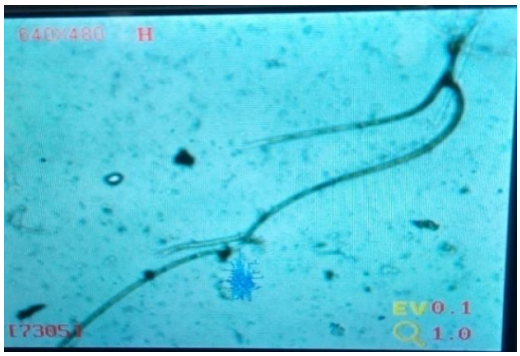
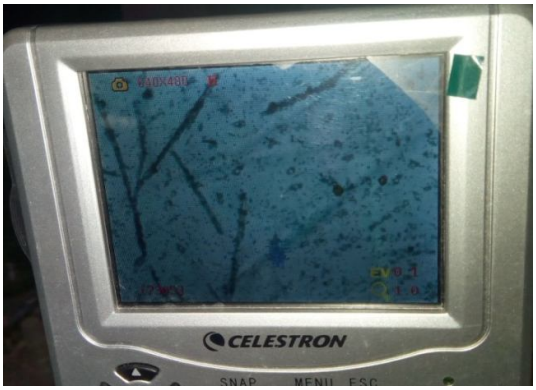
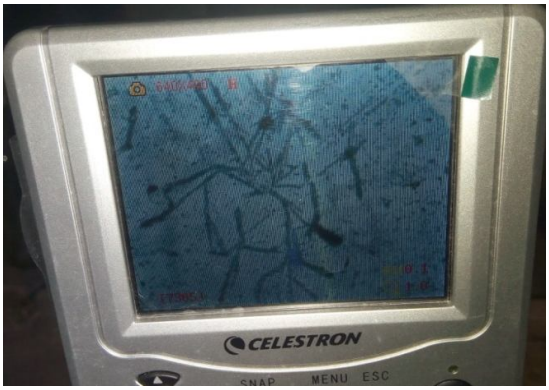


Plate 4.5a *Fusarium* species

Plate 4.5b*Fusarium* species





4.1.4.0 Assessment of percentage inhibition of pathogens isolated from *D.rotundata* and *D.alata*

4.1.4.1 Percentage inhibition of pathogens isolated from *D.rotundata*

The result obtained from the assessment of percentage inhibition of the pathogens isolated from the two yam species by the plant extracts is presented in Table 4.4.The result presented showed that *Cymbopogon citratus* had lowest level of inhibition of 37.3% on *Aspergillus spp*, followed by *Ocimum gratissimum* at 33.3%, *C. odorata* at 31.3% and *Psidium guajava* at 27.1%, while the highest level of inhibition of 19.5% obtained from *Azadirachta indica* on *Aspergillus* specie was most potent followed by *Carica papaya, Vernonia amygdalina* and *Psidium guajava* at 20.8%, 22.0% and 27.1% respectively. Also *Azadirachta indica* at 10% inhibition was also most potent in the control of *Rhizopus spp*, followed by *Vernonia amygdalina* at 18.7%, *Cymbopogon citratus* at 21.9% while *Ocimum gratissimum* at 26.2% had the lowest inhibition followed by*Carica papaya* at 24.7% and *Psidium guajava* at 24.7%. However , the following percentage inhibition of 13.7, 8.0, 28.0, 25.3, 27.3 18.0 and 26.7% on yeast was obtained from *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively, furthermore 27.7, 11.3, 26.0, 26.7, 22.7, 19.3 and 36.4% inhibition on *Botryodiplodiaspp* was obtained from *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively which were significantly different.

Azadirachta indica showed highest level of inhibition on all the pathogens isolated from *D. rotundata* including Yeast and *Botrydiplodia spp* of 8.0% and 11.3% respectively.

4.1.4.2 Percentage inhibition of pathogens isolated from *D.alata*

The percentage inhibition of 24.9, 9.8,18.0,22.0,26.7, 31.5 and 21.7% on *Aspergillus* specie isolated from *D.alata* was obtained from *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively. However, 43.1, 11.5, 24.0,27.3, 30.7,15.1 and 15.5% inhibition on *Rhizopus* species was obtained from *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively. Further assessment of the result of the inhibition revealed that 24.0, 11.1, 16.4, 21.3,27.5, 23.1 and 28.9% inhibition on yeast were obtained from *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively, while 23.5, 18.7,15.5,22.1, 28.3, 12.0 and 31.2% inhibition on *Fusarium* species were obtained from*Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* respectively which were also significantly different.

4.1.4.3 Comparison of the percentage inhibition between the pathogens isolated from different yam species (*D. rotundata* and *D. alata*)

The result presented in Table 4.4 further revealed that *Ocimum gratissimum* had the lowest percentage inhibition of 43.1% on *Rhizopus species* isolated from *D. alata* followed by 33.3% inhibition on *Aspergillus* species isolated from *D.rotundata* while the highest inhibition of 13.7% was gotten on Yeast isolated from *D.rotundata*. *A.indica* had 19.5, 10.0, 8.0 and 11.3% on *Aspergillus* species, *Rhizopus* species, yeast and *B. theobromae* specie isolated from *D.rotundata*, but it had 9.8, 11.5,11.1, and 18.7% inhibition on the *Aspergillus* species, *Rhizopus* species, yeast and *fusarium* species respectively on *D. alata*.. The highest

inhibition was on Yeast and the lowest was on *Aspergillus*spp both isolates from *D.rotundata.* Also *Vernonia amygdalina* had 22.0,18.7, 28.0 and 26.0% inhibition on *Aspergillusspp,Rhizopus* species , yeast and *Botriy* species isolated from *D.rotundata*, 18.0, 24.0, 16.4 and 15.5% inhibition on *Aspergillus* species, *Rhizopus* species, yeast and *Fusarium* species isolated from *D.alata* respectively. The highest inhibition was on yeast isolated from *D. rotundata* and *D. alata* respectively. Also *C. odorata* had 31.3, 34.7,25.3 and 26.7% inhibition on *Aspergillus* species, *Rhizopus* species, yeast and *Botry* species isolated from *D. rotundata* respectively, while 22.0, 27.3, 21.3 and 22.1% inhibition on *Aspergillus* species, *Rhizopus* species , yeast and *Fusarium* species isolated from *D.alata* respectively. The lowest inhibition of *C. odorata* was on *Rhizopus* species at 34.7% isolated from *D. rotundata,* while the highest was on yeast at 21.3% isolated from *D. alata*. Furthermore,*P. guajava*had 22.1, 24.7, 27.3 and 22.7% inhibition on *Aspergillus, Rhizopus,* yeast and *Botry.* Species isolated from *D.rotundata* respectively, while 26.7, 30.7,27.5 and 28.3% inhibition on *Aspergillus, Rhizopus*, yeast and *Fusarium* species obtained from *D.alata* respectively. The lowest inhibition was on *Rhizopus* species at 30.7% isolated from *D. alata* while the lowest inhibition was on *Botry.* Species at 22.7% isolated from *D.rotundata*. *Carica papaya* had 20.0, 24.7, 18.0 and 19.3% inhibition on *Aspergillus, Rhizopus*, yeast and *Botry* species respectively while 31.5, 15.1, 23.1 and 12.0% inhibition on *Aspergillus, Rhizopus,* yeast and *Fusarium* species isolated from *D.alata* respectively. The lowest inhibition was on *Aspergillus species* at 31.5%while the highest was on *Fusarium* species at 12.0% isolated from *D.alata*. Also *C.citratus* had 37.3, 21.9, 26.7 and 36.4% inhibition on *Aspergillus, Rhizopus,* yeast and *B. theobramae* species respectively, while 21.7, 15.5, 28.9 and 31.2% inhibition on *Aspergillus, Rhizopus,* yeast and *Fusarium* species respectively. The lowest inhibition was on *Aspergillus spp* at 37.3% isolated from *D. rotundata* and thehighest was on *Rhizopus spp* at 15.5% isolated from *D. alata*

Table 4.4 Percentage Inhibition of Pathogens Isolated from *D.rotundata* and *D.alata*

	Organisms							
Plant extracts	***D.rotundata***				***D. alata***			
	Aspergillus spp	***Rhizopus spp***	***Yeast***	***Botryodiplodia spp***	***Aspergillus spp***	***Rhizopus spp***	***Yeast***	***Fusarium spp***
Ocimum gratissimum	33.0	26.2	13.7	24.7	24.9	43.1	24.0	23.5
Azadirachta indica	19.5	10.0	8.0	11.3	9.8	11.5	11.1	18.7
Vernonia amaygdalina	22.0	18.7	28.0	26.0	18.0	24.0	16.4	15.5
Chromolaena odorata	31.3	34.7	25.3	26.7	22.0	27.3	21.3	22.1
Psidium guajava	27.1	24.7	27.3	22.7	26.7	30.7	27.5	28.3
Carica papaya	20.0	24.7	18.0	19.3	31.5	15.1	23.1	12.0
Cymbopogon citratus	37.3	21.9	26.7	36.4	21.7	15.5	28.9	31.2
Mean	24.7	22.9	21.0	23.9	22.1	23.9	21.8	21.6
LSD(p=0.05)	**0.816**	**0.776**	**0.817**	**0.816**	**0.816**	**0.816**	**0.816**	**0.835**

4.1.5.0 Depth of penetration of the micro-organisms isolated from the diseased tubers of *D. rotundata* and *D. alata*

4.1.5.1: Depth of penetration of pathogens on samples from *D. rotundata*

The result obtained from the evaluation of the depth of penetration of the pathogens on both *D.rotundata* and *D. alata* is presented in Table 4.5. The result revealed that yeast species had 1.8, 2.5,3.0, 2.20 and 1.0cm penetration in samples A,B,C,D and E (*D. rotundata*) respectively while *Aspergillus species* penetrated 2.5,2.4, 3.0,4.0,4.0 and 1.1cm in samples A,B,C,D and E respectively. Also *Rhizopus* species on samples A,B,C,D and E had 2.5,1.5,1.8,3.4 and 1.0 cm penetration respectively on *D. rotundata* , while *Bothryodiplodia* specie on samples A,B,C,D and E had 1.5, 2.2,1.8,1.6 and 1.0cm penetration which were significantly different.

4.1.5.2: Depth of penetration of the pathogens on samples from *D. alata*

The result presented in Table 4.5 further showed that yeast specie had 2.0, 2.3,0.9, 2.5 and 1.1cm penetration on samples A,B,C,D and E from *D.alata* respectively, while *Aspergillus* species penetrated 2.2, 2.0,2.8,1.5 and 1.0 cm into samples A,B,C,D and E respectively. However, *Rhizopus* species had 3.0, 2.7,1.8,2.6 and 1.0cm penetration into samples A,B,C,D and E on *D.alata* respectively. *Bothryodiplodia* species on samples A,B,C,D and E penetrated 1.4, 2.6,1.9,1.5 and 1.0 cm respectively. These results were highly significantly different.

Table 4.5 Depth of penetration of the micro-organisms isolated from the diseased tubers (*D. rotundata* and *D. alata)*

	Depth of penetration (cm)								
	D.rotundata					***D. alata***			
Tuber samples	***Yeast*spp**	***Aspergillus* spp**	***Rhizopus* spp**	***Botryodiplodia*spp**	**Tuber samples**	***Yeast*spp**	***Aspergillus* spp**	***Rhizopus* spp**	**Botryodiplodia spp**
A	1.80	2.50	2.50	1.50	A	2.00	2.20	3.00	1.40
B	2.50	2.40	1.50	2.20	B	2.30	2.00	2.70	2.60
C	3.00	3.00	1.80	1.80	C	0.90	2.80	1.80	1.90
D	2.20	4.00	3.40	1.60	D	2.50	1.50	2.60	1.50
E	1.00	1.10	1.00	1.00	E	1.10	1.00	1.00	1.00
Mean	2.10	2.60	2.04	1.62	Mean	1.76	1.90	2.22	1.68
LSD(p=0.05)	**0.081**	**0.081**	**0.081**	**0.081**	**LSD(p=0.05)**	**0.081**	**0.081**	**0.081**	**0.081**

4.1.5.3 Comparison of the depth of penetration among the pathogens on the samples from *D. rotundata* and *D.alata*

Further evaluation of the result presented in Table 4.5 revealed that *Aspergillus* and *Rhizopus* species had the highest level of penetration on sample A (*D.rotundata*) at 2.5cm while *Rhizopus* species had the highest depth of penetration on sample (*D.alata*) at 2.5cm depth too. Again yeast had the highest depth of penetration on sample B (*D.rotundata*) at 2.5cm depth while, *Rhizopus* species had the highest depth of penetration on sample B (*D.alata*) at 2.7cm depth. Also, Yeast and *Aspergillus* species had the highest penetration on sample C (*D. rotundata*) at 3.00cm respectively, while *Aspergillus* species had the highest depth of penetration on sample C from *D.alata* at 2.8cm depth. Furthermore, *Aspergillus* species had the highest depth of penetration on sample D (*D.rotundata*) at 4cm depth while, *Rhizopus* species had the highest penetration on sample D on *D.alata* at 2.6cm depth. However, penetration on sample E from both yam species were very low, though *Aspergillus* had the lowest at 1.1cm depth on sample E (*D.rotundata*) while yeast had the highest of 1.1cm on sample E on *D.alata* as samples E on *D. rotundata* and *D. alata* were controls.

4.1.6 Evaluation of different sections of the tuber foreasy penetration by the pathogens on *Dioscorea alata* and *Dioscorea rotundata*(cm). Point of Easy Attack

The result obtained from the evaluation of the different sections of the tuber that are easily penetrated by the pathogens is presented in Table 4.6. The result revealed that yeast had 1.7, 2.0 and 4.60cm penetration on the head, middle and basal part of *D.rotundata, Aspergillus* had 2.0, 2.03 and 2.10 cm depth of penetration on the head, middle and basal part of the tuber respectively. Also *Rhizopus* species had 1.6, 2.10 and 4.20 cm penetration on the head, middle and basal part of *D. rotundata* respectively. Boty specie had 1.0, 1.0 and 2.40cm depth of penetration on the head, middle and basal part of *D.rotundata* respectively.

Yeast on the other hand had 2.1, 1.9 and 2.0 cm penetration on the head, middle and basal part of *D.alata* respectively, *Aspergillus* species equally had 2.10, 2.0 and 2.6cm depth of penetration on the head, middle and basal part of *D.alata* respectively. However, the depth of penetration on the head, middle and basal part by *Rhizopus* species on *D. alata* was 2.0, 2.10, and 2.6 cm respectively. *Fusarium* species equally had 2.60, 1.10, and 2.60 cm depth of penetration on the head, middle and basal part of *D.alata* respectively. The result further revealed that the highest depth of penetration was on the basal part followed by the middle sections while the section with the lowest depth of penetration was the head from the two yam species under investigation. These results were highly significantly different.

Table 4.6 Evaluation of Different Sections of the Tuber for Easy Penetration by the Pathogens on the *Dioscorea alata* and *Dioscorea rotundata*(cm)

	D.rotundata			***D. alata***			
Pathogen	**Head**	**Middle**	**Basal part**	**Pathogen**	**Head**	**Middle**	**Basal part**
Yeast spp	1.70	2.00	4.60	*Yeast*	2.10	1.90	2.00
Aspergillus spp	2.00	2.03	2.10	*Aspergillus*	2.10	2.00	2.60
Rhizopus spp	1.60	2.10	4.20	*Rhizopus*	2.00	2.10	2.60
Botry. Spp	1.0	1.00	2.40	*Fusarium spp*	2.60	1.00	2.60
Mean	1.46	1.63	2.86	Mean	1.96	1.60	2.24
LSD(p=0.05)	**0.372**	**0.084**	**0.635**	**LSD(p=0.05)**	**0.372**	**0.520**	**0.520**

4.1.7.0 Evaluation of the effect of diseases on the nutritional composition of yam

4.1.7.1 Effects of diseases on the nutritional composition of *D. rotundata*

The result obtained from the evaluation of the effects of the pathogen on the nutritional composition of the two yam species is presented in Table 4.7. The result showed that sample A, B,C,D,E and F(control) from *D. rotundata* had 61.5,71.9,68.1,65.2, 54.6 and 54.4% moisture content, the highest moisture content of 71.9% was obtained from sample B while the lowest was obtained from the control sample which is the healthy yam sample. Also samples E had the highest (1.71%) ash than all the other samples followed by the control which had 1.31%, while the lowest ash of 0.33% was obtained from sample D. The control had the highest crude fibre of 2.50% than all the other yam samples followed by samples A and B which had 1.50% each, the lowest ash of 1.0% was obtained from sample D and E each.Furthermore, the control had the highest crude protein of 5.60% when it was compared with the other yam samples, followed by sample C(4.50%), while the lowest crude protein was obtained from sample E (3.47%). The control had 0.70% fat higher than all the other samples followed by sample B and E which had 0.6% each while the lowest percent fat (0.33%) was obtained from sample D. Also the control had 41.7% higher carbohydrate than all the other samples, followed by sample E which had 33.4%, while the lowest value of 21.6% was obtained from sample B. Further evaluation of the results revealed that the control also had the highest percentage nitrogen of 0.84% when it was compared with the other yam samples followed by sample C(0.60%),the lowest was obtained from sample B which had 0.39%. Again the control had the highest percentage carbon of 4.90% when it was compared with the other yam samples, the lowest was obtained from sample B(2.50%). These results were significant.

4.1.7.2 Effects of diseases on the nutritional composition of *D. alata*

The result presented in Table 4.6 also showed that the highest moisture content of 65.5% was obtained from the control from *D.alata*, followed by 50.3% from sample A, while the lowest 39.4% was obtained from sample C. The control equally had the highest percentage ash of 0.70% than all the other samples evaluated, while the lowest value of 0.23% was obtained from sample C. The highest crude fibre of 1.50% was obtained from the control and the lowest value of 0.40% was obtained from sample E. However, 1.90, 2.10, 1.50, 1.30, 1.70 and 2.50% crude protein were obtained from sample A,B,C,D,E and F(control) respectively, the highest value was obtained from the control . The result of the crude fat further showed that samples A, B,C,D,E and F had 0.07, 0.04,0.02,0.09,0.03 and 0.11% fat respectively, the highest value of 0.11% was equally obtained from the control. Also the control had the highest value of carbohydrate (31.7%) than all the other yam samples under investigation followed by sample E(25.9%), while the lowest value of 20.17% was obtained from sample D. However the control had the highest value of nitrogen (0.5%) when it was compared with the other yam samples with levels of disease infection, followed by sample D with 0.47%, while the lowest value of 0.34% was obtained from sample B. Again the control also had the highest value of carbon(3.90%) than all the other samples, while the lowest was obtained from sample B which had 2.67% carbon. These results were highly significantly different.

Table 4.7 Evaluation of the Effect Diseases on the Nutritional Composition of yam

	Parameters															
	D. rotundata								***D.alata***							
Yam samples	**MC (%)**	**Ash (%)**	**CF (%)**	**CP (%)**	**Fat (%)**	**CHO (%)**	**N (%)**	**C (%)**	**MC (%)**	**Ash (%)**	**CF (%)**	**CP (%)**	**Fat (%)**	**CHO (%)**	**N (%)**	**C (%)**
A	61.5	1.09	1.50	4.40	0.50	29.1	0.41	3.00	50.30	0.40	0.50	1.90	0.07	23.13	0.45	2.79
B	60.9	1.04	1.20	3.70	0.60	21.6	0.39	2.50	41.40	0.39	0.70	2.10	0.04	20.83	0.34	2.67
C	68.1	0.74	1.50	4.50	0.40	22.2	0.60	2.60	39.47	0.23	0.50	1.50	0.02	27.53	0.40	3.38
D	60.2	0.33	1.00	4.20	0.33	27.1	0.50	3.30	40.26	0.37	0.60	1.30	0.09	20.17	0.44	2.49
E	54.6	1.71	1.00	3.47	0.60	33.4	0.53	2.80	43.50	0.29	0.40	1.70	0.03	25.90	0.39	3.24
F(Healthy)	65.3	1.31	2.50	5.60	0.70	41.7	0.84	4.90	65.50	0.70	1.50	2.50	0.11	31.77	0.52	3.90
LSD(p=0.05	**0.874**	**0.091**	**1.01**	**0.168**	**0.077**	**3.24**	**0.516**	**0.342**	**0.975**	**0.047**	**0.082**	**0.082**	**0.008**	**0.767**	**0.016**	**0.09**

Key:

MC-Moisture content

CF-Crude fiber

CP-Crude protein

CHO-Carbohydrate

N-Nitrogen

C-Carbon

4.2.0 Discussions

4.2.1 Phytochemical screening of the seven plant extracts

The result presented showed that the major phytochemicals such as alkaloid, flavonoid, tannins, saponins and phenols were present at varying levels in all the plant extracts evaluated. However *A.indica* unlike the plant extracts contains higher levels of these bioactive compounds. This result suggests that *A. indica* will be more effective as a bio-pesticide than all the other extracts evaluated. This result is in agreement with the findings of Anyanwu and Dawet, (2005) as they reported that these constituents are known to have anti protozonal, antibacterial and antifungi activities. This result further supports the findings of Akol, *et al*., (2001) as they reported that registered neem pesticides formulationshave been found to be effective against pests of vegetables but safe to their natural enemies. This result also supports the findings of many authors like Joachin, *et al.,* (2012), Udochukwu, *et al.,* (2016) who have worked on the phytochemical screeing of these plant extracts.

4.2.2 Evaluation of the percentage occurrence of different diseases on the yam tubers from *D. rotundata* and *D. alata*

The result presented revealed that yam rust and rot were the most prevalent diseases affecting yam cultivation and storage for both *D.rotundata* and *D. alata*. This result supports the report of Amusa and Baiyewu, (1999) and Amusa, *et al.,* (2003) who categorized storage diseases of yam to include dry rot, soft rot and wet rot. They implicatedthe following pathogens for the deterioration of tubers of *Dioscorea rotundata* as *Aspergillus niger,* Yeast, *Rhizopus stolonifer* and *Botryodiplodia theobromae* while the organisms that attacked *Dioscorea alata* were *Aspergillus niger,* Yeast, *Rhizopus stolonifer* and *Fusarium oxysporium.* This result is further in agreement with works of Amusa, *et al.,* (2003) and Okigbo (2005) and also supports the findings of Markson,*et al.,* (2012), Gwa, *et al.,(* 2015), Gwa, *et al.,*(2017), Mba, *et al.,* (2017) and Gwa, *et al.,* (2019).

4.2.3 Isolation and identification of fungi from the diseased yam samples

The result presented showed that *Aspergillus*, yeast, *Rhizopus* and *Botryodiplodia* and *Fusarium* species were all isolated and properly identified from all the samples from the two species as the causative agents in the deterioration of yam tubers in storage. This result is also in agreement with works of Amusa, *et al.,* (2003) and Okigbo (2005) and also supports the findings of Markson, *et al.,* (2012), Gwa, *et al.,(* 2015), Gwa, *et al.,* (2017), Mba, *et al.,* (2017) and Gwa, *et al.,* (2019).

4.2.4. Assessment of thepercentage inhibition of pathogens isolated from *D.rotundata* and *D.alata*

The result presented revealed that all the plant extracts used in this study inhibited the isolated pathogens at different rates. This finding could be attributed to the presence of various phytochemicals in the plant extracts which were present at different levels. Extracts of *Azardirachta indica* was found to be the most fungitoxic on rot diseases of *Aspergillus niger,Rhizopus stolonifer* and Yeast isolated from *Dioscorea alata* while *Carica papaya* was most effective *on Fusarium oxysporium* micro-organism isolated from *Dioscorea alata*. Generally, the application of *Azardirachta indica* was able to prevent or slow down all the micro-organisms identified during the pathogenic test. On the control of*Aspergillus niger, Vernonia amygdalina, Cymbopogon citratus, Chromolaena odorata, and Ocimum gratissimum* had more effect on *Dioscorea alata* while Carica *papaya* had more effect on *Dioscorea rotundata* than on *Dioscorea alata*. This result could be attributed to the high level of phytochemicals such as alkaloids, flavoniod, glucoside, phenols, saponinis etc present in the plant extract. This finding is in agreement with the findings of Gwa, *et al*., 2017 and 2018. The effect of *Cymbopogon citratus, Azadirachta indicia Carica papaya* and *Chromolaena odorata*, *Psidium guajava* and *Vernonia amygdalina* too but between

Cymbopogon citratus on the inhibition of *R.stolonifer* isolated from *D. alata* while *Ocimum gratissimum* and *Psidium guajava* did not significantly inhibit *R. stolonifer*.

On the *R. stolonifer* isolated from *Dioscorea rotundata*, the effect of *Azadirachta indica* and *Cymbopogon citratus ,Chromolaena odorata, Carica papaya, Psidium guajava* and *Ocimum gratissimum, Vernonia amygdalina* on the inhibition of this organism were highly significant which could be attributed to the high level of the phytochemicals present. Thesefindings agreed with the findings of Markson, *et al*., (2012) and Okigbo, *et al.*, (2009). The result further is in agreement with the findings of Oyelana, *et al.*, 2011 who worked on the effects of these plant extracts on *R.stonlonifer*.The interaction of the two yam species (*Dioscorea alata* and *Dioscorea rotundata*) on *Rhizopus stolonifer* on the plant extracts used for the experiment were all significant the interaction of the seven plant extracts on *Dioscorea alata* shows that all were significant while they all had significant difference on *Dioscorea rotundata.*

The effect of the following plant extracts namely *Azardirachta indica, Cymbopogon citratus, Chromolaena odorata, Ocimum gratissimum* and *Vernonia amygdalina* significantly inhibited yeast isolated from the two yam species except *Psidium guajava* that did not significantly inhibit yeast in this study. This result again could be attributed the concentrations of the active ingredients in plant extracts such as the phytochimicals used in this investigation as different plant extract contain varing levels of phytochemicals (Oyelana, *et al.*, 2011)

The effects of the seven different plant extracts on *Fusarium oxysporium* and *Botryodiplodia theobromae* isolated from *Dioscorea alata* and *Dioscorea rotundata* respectively revealed that the organisms were significantly inhibited which may be due to the presence of different bioactive compounds.

4.2.5 Depth of penetration of the micro-organisms isolated from the diseased tubers of *D. rotundata* and *D. alata*

The result obtained from this study showed that all the plant extracts inhibited all themicroorganisms isolated from the two yam species namely *D. alata*and *D. rotundata*. Thisresult showed that these extract contain varying levels of bioactive compounds that could beutilized effectively as bio-pesticides such as alkaloids, flavonids, glucosides, phenols, saponins, sterols etc in the cultivation of residue-free plant materials for consumption by man and livestock. This result is in agreement with the report of Gwa, *etal.*,(2017). In addition this result also is in agreement with the findings of Mba, *et al*., (2017) and Oyelana, *et al*., (2011) in which they reported that plant extract from *A. indica, Carica papaya, Zingiber officinale* etc are effective in the control of yam rots and possessed inhibitory properties that can be utilized as post harvest control to suppress rot causing fungi of yams in storage at various degree of potency.

The result further showed that *R.stolonifer* had the highest penetration on D. alata while *A.niger* had the highest penetration on *D. rotundata*. This result could be attributed to the high level of the enzyme produced by these microorganisms which enable them to invade the tubers more aggressively unlike the other microorganisms. This result agrees with the findings of Baggio, *et al.,* (2016) who reported the effect of penetration of *R.stolonifer* on stone fruits.

4.2.6 Evaluation of different sections of the tuber for easy penetration by the pathogens on *Dioscorea alata*and *Dioscorea rotundata* (cm). Point of Easy Attack.

The result presented on the evaluation of the different sections of the yam tuber that are easily attacked by the various organisms shows that the tail sections of *Dioscorea rotundata* and *Dioscorea alata* was the point of easy attack by all micro-organisms investigated. This could be attributed to the fact that tissues at the basal part are still young and yet to be consolidated unlike the other parts of the tuber with high degree of curticular tissues and wax which made penetration difficult.Only *Fusarium oxysporium* was able to attack the basal part and head regions of *Dioscorea alata*. The next susceptible section for attack by micro-organisms was the middle part of the yam tuber samples of both *Dioscorea rotundata* and *Dioscorea alata* as confirmed by this study. The head section was the least susceptible to pathogenic attacks by the micro-organisms which could be attributed to the high level of consolidation of plant tissues at this region especially curticle and wax. This could be the first account of this discovery.

4.2.7 Evaluation of the effect of diseases on the nutritional composition of yam

The result presented showed significantly lower nutritional composition when it was compared with the healthy samples in all the nutritional parameters evaluated. These findings could be attributed to the utilization of nutrients by the invading pathogens as it is their major reason why they attack and invade plants. This does not only reduce the nutritional composition but also reduce the market value of the crop. This result is in agreement with the reports of Sahore, *et al.*,(2007), as they reported that the reduction in moisture content, carbohydrate and protein in yam tubers infected with yam rot.Again the result obtained from this study supports the findings of Omadamiro, *et al.*, (2015) who reported the effects of *Fusarium* species infection on the chemical composition of flour from two yam species.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This work has identified botanicals or plant extracts with inhibiting properties that was used for yam tubers of*D. rotundata*and*D. alata* after harvest. Additionally, the postharvest rot pathogens that decimate yam tubers were isolated, identified and confirmed through pathogenicity test. They include *Aspergillus, Rhizopus,*Yeast, *Fusarium* and *Bothryodiplodia* species.

This investigation has shown that the seven plant extracts used in this study namely *Chromolaena odorata, Carica papaya, Vernonia amygdalina, Ocimum gratissimum, Azadirachta indica, Psidium guajava* and *Cymbopogon citratus*contain different levels of phytochemicals or bioactive compounds such as alkaloids, flavanoids, tannins, saponins, steroids, triterpenoids, glycosides and phenols which are toxicto all the pathogens isolated and identified in this study .

The inhibitory effect of *Azadirachta indica* was very high on all the organisms isolated from *D. rotundata* and *D. alata* while the other six (6) plants extracts showed high level of fungitoxity on the pathogens isolated at varying degrees.The investigation on “Point of easy attack” on the tubers showed that the basal part of the tuber was the most susceptible for both *D. rotundata* and *D. alata* attack.

Finally, the proximate analysis on the yam varieties showed that yam diseases significantly reduced the nutritional composition of the rotten *D. rotundata* and *D.alata* when they were compared with healthy yam tubers.

5.2 RECOMMENDATIONS

It is therefore recommended that the plant extracts derived from the leaves of *Azadirachta indica, Vernonia amygdalina, Carica papaya,* and *Ocimum gratissimum* be applied in the storage of yam tubers of *D. rotundata* and *D. alata* as they showed high level of inhibition on the growth of *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporium, Yeast and B. theobromae* and consequently be regarded as bio-pesticides as alternatives to synthetic chemicals. This is also because they are more environmentally friendly, readily available, and easily affordable by the farmers and other end users.

Applying the potent bio-pesticides on the basal part of the yam tubers immediately after harvest will go a long way in preventing attack by rot pathogens and increase the storage life of the tubers which is more money to the farmers

However further studies are strongly recommended especially in the quantitative screening of the phytochemical composition of these plant extracts and also in the molecular identification of the pathogens that are responsible for yam rot.

5.3 CONTRIBUTIONS TO KNOWLEDGE

(1) This research has been able to put on record that *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina, Chromolaena odorata, Psidium guajava, Carica papaya* and *Cymbopogon citratus* contain different levels of phytochemicals or bioactive compounds which can be utilized as bio-pesticides in the control of rot pathogens of yam such as *Yeast, Aspergillus niger, Rhizopus stolonifer, Botryodiplodia theobromae* and *Fusarium oxysporium.*

(2) This research has also been able to establish that *Yeast, Aspergillus niger, Rhizopus stolonifer, Botryodiplodia theobromae* and *Fusarium oxysporium* are also responsiblefor the decay and deterioration of yam tubers during storage.

(3) This research have been able to put on record that the basal part of the yam tuber is the point at which pathogens easily attack and penetrate the yam tubers. This could be the first account of this discovery.

(4) This researchhas also been able to establish that pathogens reduce the nutritional composition of yam tubers under storage.

REFERENCES

Aboagye-Nuama, F., Offei, S. K., Cornelius, E. U., and Bancroft, R. D. (2005): Severity of Spoilage storage rots of white yam (*Dioscorea rotundata Poir*). *Annals of Applied Biology*, 147: 183-190.

Adamu, A., Adie., D.B and Alika, U.A, (2014): The antibacterial activity, antioxidant and selectivity index of leaf extracts of South African tree species used in ethnorenerinary medicine to treat helminth infections. BMC Vet Res 10, 52 (2014). https://doi.org/10.1186/1746-6148-10-52.

Adeniji, M. O. (1996). Fungi associated with the deterioration of gari. Niger. J. Plant Prot .2:74– 77.

Adeniyan, B.O., Ojeniyi, S.O. and Awodun, M.A. 2008. *Relative Effect of Weed Mulch Types on Soil Properties and Yield of Yam inSouthwest Nigeria*.J .Soil .Nature. 2(3): 01-05

Afiukwu, C. A., Ogah, O., Ugwu, O. P. C., Oguguo, J. O., Ali, F. U. &Ossai E. C. (2013): Nutritional and Anti-nutritional Characteristics of Two Wild Yam Species from Abakaliki, Southeast Nigeria. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 4, 840-848.

Afolabi, C Akinmoladun; Ibukun, E. O., Afor, Emmanuel; Obuotor, E. M. and Farombi, E.O. (2007): Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. M Scientific Research and Essay Vol. 2 (5), pp. 163-166, May 2007. ISSN 1992-2248 © 2007 Academic Journals

Agaba T.A and Fawole, B. (2016): Phytochemical constituents of siamweed (*Chromolaena odorata*) and African custard Apple (*Annonasenegalensis*) International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277209X

Agu Diop, (1998): Open sides shelves

Agwu, A. E. and Alu, J. I. (2005): Farmers perceived constraints to yam production in Benue State, Nigeria. Proceedings of the 39th Annual Conference of the Agriculture society of Nigeria; pp 347-350.

Aidoo, K. A. (2007). Identification of yam tuber rot fungi from storage systems at the Kumasi Central market. A dissertation submitted to Faculty of Agriculture, K.N.U.S.T.; pp 18-19

Akangbe, J. A; Oluruntoba, O. O; Ibrahim, F.A and Komolafe, S. E: (2012): Analysis of yam storage strategy to promote food security in Asa LGA of Kwara State, Nigeria. Vol.5 No.4: supplement, doi:10.4314/ejesm.v514.515.

Akanji, B. O., Akpokodje, G. and Ogundele, O. O. (2003): Labour use pattern on farms. In NISER Annual Survey of Crop Production Condition in Nigeria, 2000. NISER, Ibadan, Nigeria, Pages 34–46.

Akinbo, O.K., Opara, E. U., and Ikoro, A. I. (2016): Isolation and Characterization of Soft Rot pathogen of Yam (*Dioscorea spp*) in Nigeria - A Review. Journal of Agriculture and Sustainability ISSN 2201 – 4357 Volume 9, Number 2, 2016, 198 – 209

Akol, A. M., Njagi, P. G. N., Sithanantham, S. and Mueke, J. M. (2001):Effects of two neem insecticide formulations on the attractiveness, acceptability and suitability of diamondback mothlarvae to the parasitoid, Diadegmamollipla (Holmgren) (Hym., Ichneumonidae). The International Centre of Insect Physiology and Ecology, Nairobi, Kenya; and Department of Zoology,Kenyatta University, Nairobi, Kenya

Aluko, O., Banjoko, I. O. and Adeoya, A. S. (2003).Effect of sprouting on the quality attributes of yam flour fufu. In: Proceedings of the 27thAnnual Nigeria Institute of Food, Science and Technology (NIFST) Conference13th– 17th October, 2003, Kano, Nigeria. pp. 197 – 199.

Amadioha AC, Obi VI (1999). Control of anthranose disease of cowpeaby *Cymbopogon citratus* and *Ocimum gratissimum.*Acta.Phytopathologiaet Entomological Hungarica. 34(1-2): 83-89.

Amponsah, S.K, (2015): Open side shelves. International Journal of Reasearch in Agriculture Forestry, Vol. 2 issue 10. ISSN 2394 – 5907, October 2015.

Amusa, N. A., and Baiyewu, R. A. (1999): Storage and market diseases of yam tubers in Southwestern Nigeria. *Ogun* Journal of research (Nigeria) 11: 211-225

Amusa, N. A. (2001). Fungi associated with yam chips in storage and the effects on the chip nutrient composition. Moor J. Agric. Res. (Nig.) 2:35-39.

Amusa, N.A, Kehinde, I.A, Ashaye O.A. (2002): Bio-deteriortion of breadfruit (Artocarpuscommunis) in storage and its effects on the nutrient composition. African Journal of Biotechnology vol. 1 No. 2 (2002) Doi: 10.5897/AJB2002.000

Amusa, N. A., Adegbite, A. A., Muhammed, S., and Baiyewu, R. A. (2003): Yam Diseases and its Management in Nigeria. *Afr. J. Biotecnol. 2* (12): 497-502.

Anaadumba, P, (2014): Yam house

Anyanwu, G. I., Dawet, A. (2005): Pharmacological and pytochemical screening of Hyptissuaveolenspoit (Lamiaceae) for bioactivity in rodents. Nigerian J. Botany, 18: 190 - 196

Ayanwuyi E, Akinboye A.O, Oyetoro JO, (2011): Yam Production in Orire Local Government Area of Oyo State, Nigeria: Farmer's Perceived Constraints. World Journal of Young Researchers 1(2): 16-19

AOAC, (2016): Official methods of analysis of AOAC International 20th edition, 2016. Book by AOAC International, 2016 editor: Dr. George W. Latimer, Jr. www.aoac.org>Forms. Pp 8

Appiah, Kwame (2018):"Medicinal Plants Used in the Ejisu-Juaben Municipality, Southern Ghana: An Ethnobotanical Study". Medicines.**6** (1): 1–27. Doi:10.3390/medicines6010001. PMC 6473417.PMID 30577439.

Arinze, A. E. (2005): Plant pathology and post -harvest food loss. An InauguralLecture Series 43: 29-72.

Asaolu, M. F., Oyeyemi, O. A., and Olanokun J.O., (2009): Chemical composition, phytochemical constituents and in vitro biological activity of cymbopogon citratus. Pakistani Journal of Nutrition. 8(12): 1920-1922, 2009

Babaleye, T. (2003): Raising the status of the yam, a major food crop in West Africa, ANB – BIA Suppl Issue/Ed 463:1 – 3

Baggio, J.S., Concalues, F.P, Lourenco, S.A., Tanaka, F.A.O., Pascholati, S.F and Amorim, L (2016). Direct penetration of *Rhizopus stolonifer* into stone fruits causing *Rhizopus* rot. Plant pathology, 65pp 633-645.

Barnett, H. L. and Hunter, B.B. (1998): Illustrated genera of imperfect fungi, 4th edn

Barton H. (2014): Origin and Development of Yam. In Smith C. (eds) Encyclopedia of Global Archaeology. Springer, New York, NY. htpps://doi.org/10.1007/978-1-4419-0465-2_2193.

Boning, Charles R. (2006). Florida's Best Fruiting Plants: Native and Exotic Trees, Shrubs, and Vines. Sarasota, Florida: Pineapple Press, Inc. pp. 166–167.

CABI, (2016): White yam (*Dioscorea alata.).* Center for Agriculture and Biosciences International (CABI). 2016. Retrieved 5 December 2016.

CABI, (2017); Centre for Agriculture and Bioscience International. *Centre for Agriculture and Biosciences International. http://. Retrieved 5 December 2018.*

Cornelius, E. W. and Oduro, K. A. (1999): Storage diseases of white yam (*Dioscorea rotundata*), causes, varietal susceptibility and control. *Journal of the Ghana Science Association,* 345-352.

Cornelius, E.W. (1998): Causes and control of tuber rots of white yam (Dioscorea rotundata Poir) varieties Araba, Asana and Puna (MPhil thesis.) University of Ghana.Pp137

Degras, L. (1993): The Yam, a tropical Root Crop. Macmillan, London. Pp xv + 408

Damien O.Connor, (2019): Heap on the floor covered with indigenous leaves. ISBN:978-1-98-859467-5

Dzoyem, J.P, Adebayo, S.A, Shai, L.J, Laham, S.A and Al-fade, F.M (2017): Anti-inflammatory and Anti-ceptive activities of African Medicinal Spices and vegetables. Medicinal spices and vegetables from Africa.Therapeutic potential against metabolic, inflammatory, infectious and systemic diseases. Page 239-270.

Ebewore, O. O., Egbodion, J., & Oboh, O. O. (2013): Profitability Analysis of Yam Production in Ika South Local Government Area of Delta State, Nigeria. *Journal of Biology, Agriculture and Health Care*. Pp 2224-3208

Egedigwe CA (2010): Effect of dietary incorporation of *Vernonia amygdalina* and *chromolaena odorata* on blood lipid profile and relative organ weights in albino rats (Thesis). Department of Biochemistry, MOUAU, Nigeria.

Egga, E.S., Adeyanju, O., and Agyeno, O.E (2014): Preliminary Phytochemical, Antimicrobial and Proximate Analysis of Tender Leaves of Psidium guajava L in Jos, Plateau State, Nigeria. Asian Review of Environmental and Earth Sciences ISSN: 2313-8173 Vol. 1, No. 2, 35-38,

Ejike, Ijeh II; CECC (2011)."Current perspectives on the medicinal potential of *Vernonia amygdalina* Del". J Med Plant Res. **5** (7): 1051–1061

Ekefan, E. J., Simon, S. A., Nwankiti, A. O., & Peter, J. C. (1999): Effect of Intercropping on the Incidence of Fioler Anthyacnose and Tuber Yield Susceptible *Dioscorea alata;* Yam in Nigeria. *Journal of Plant Protection in the Tropics.*12(2): 80-90

Eke-Okoro. O. N., Njoku, D. N., Mbe, J. O., Amanze, N. J., & Eke-Okoro, O. C. (2014): Contribution of Root and Tuber Crops in the Agricultural Agenda in Nigeria. *ASPN Journal of Agriculture and Biological Sciences.*Vol.9.No.8 p276.

Elechi, B. O and Souzey, J. A. (1999): Inhibition of bio-deterioration of yam tuber *(Dioscorea rotundata* Poir) in storage with phenolic extracts of *Acalyphahispida* burm F. leaves. J Stored Prod Res 35:127-134

Emmanuel,A., Kubmarawa, D., Sara, G. Y. and Wahu, A. (2019): Phytochemical Screening, Antioxidant and Antimicrobial Activities of the Essential Oils and Ethanol Extract of Psidium guajava Leaf. Asian Journal of Physical and Chemical Sciences 7(4): 1-8. ISSN: 2456-7779

Enyiukwu D. N., Awurum A. N., Ononuju C. C. and Nwaneri J. A. (2017): Significance of characterization of secondary metabolites from extracts of higher plants in plant disease management. International Journal of Advance Agricultural research

Eric Lafforgue, (2020): Improved Yam House

Evans, W. C. (1997): Trease and Evans Pharmacology. 14 edn. Harcourt Brace and Company, Asia. Pvt. Ltd. Singapore, 226p.

Ezeonu, C. S., Tatah, V. S., Imo, C., Mamma, E., Mayel, M. H., Kukoyi, A. J. and Jeji, I. A. (2019).Inhibitory Effect of Aqueous and Ethanolic Extracts of Neem Parts on Fungal Rot Disease of *Solanum tuberosum* Pakistan Journal of Biological Sciences.ISSN 1028-8880. DOI: 10.3923/pjbs.2019.206.213

Falola, A; Salami, M.F., Bello. A.A; Olaoye. T.A. (2017): Effect of yam storage techniques usage on farm in Kwara State, Nigeria. University of Ilorin, Ilorin, Nigeria. National

Centre for Agricultural Mechanization, Ilorin, Nigeria *Agrosearch (2017) 17 No. 1: 54– 65*

FAO, (1990): Indigenous knowledge systems for storage of yams in Nigeria, *www.sciencepub.net.*

FAO, (1994): The state of food and agriculture. Food and agricultural organization Rome, 1994. (FAO Agricultural Series, no 27) ISBN 92-5-103550-4

FAO, (2000): Food and Agriculture Organization. FAO Production Yearbook. FAO, Rome. *www.faostat.fao.org. Retrieved 17 June 2019*

FAO, (2005): Food and Agricultural Organization of the United Nations. FAOSTAT, Statistical Database, FAO, Rome. *https://www.fao.orgpublications>sofa Retrieved 17 June 2019.*

FAO, (2008): Food and Agriculture Organization, Production Year Book, FAO, Rome. *https://www.fao.orgpublications>sofa Retrieved 17 June 2019*

FAO, (2011): The methodology of the FAO study: Global food losses and food waste-extent, causes and prevention

FAO, (2013): Food and Agricultural Organizationof the United Nations.https://www.fao.org publications>sofa*Retrieved 17 June 2019*

FAO, (2017): The second report on the state of the world's plant genetic resources for food and agriculture (pp. 1-370). Retrieved from http://www.fao.org/faostat/en/data/QC.

Fasasi, A. R. & Fasina, O. O. (2005): Resource use efficiency in yam production in Ondo State, Nigeria: Agricultural Rebirth for improved production in Nigeria. In: Proceedings of the 39th Annual Conference of Agricultural Society of Nigeria (ASN), held at the University of Benin, Benin City, Nigeria, 9th– 13th October, 2005. Pp. 184 – 186.

Frank, C. O., and Kingsley C.A., (2014): Proximate composition, physiological changes during storage and shelf life of some Nigerian varieties of yam (*Dioscorea species).* Journal of Scientific Research and Reports, 3(4): 553-562. Doi: 10.9734/JSRR/2014/7013.

FU, Regina H. Y., Hidehiko Kikuno and Makoto Maruyama (2011): Research on yam production, marketing and consumption of Nupe farmers of Niger State, Central Nigeria. African Journal of Agricultural Research Vol. 6(23), pp. 5301-5313.

Glover, A. M., Quansah, J. and Peget, F. M. (2013): Performance and Acceptability of Legume-Fortified Yam Flours. *Food Science and Quality Management*. 17. 14-18.

Gwa V I, and Akombo RA (2016) Studies on the Antimicrobial Potency of Five Crude Plant Extracts and Chemical Fungicide in in vitro Control of *Aspergillus flavus,* Causal Agent of White Yam (*Dioscorea rotundata*) Tuber Rot. Journal of Plant Sciences and Agricultural Research 1(1): 1-8.

Gwa, V. I. and Nwankiti, A. O. (2017a): Efficacy of some plants extracts in in-vitro control of *colletotrichum species,* casual agent of yam (*Dioscorea rotundata* Poir) tuber rot. *Asi J Plant Sci and Res* 7: 8-16.

Gwa, V. I. and Richard I. B. 1 (2017): Susceptibility of White Yam (*Dioscorea rotundata* Poir) Tuber to Rot Fungi and Control with Extracts of *Zingiber officinale* Rosc. *Azadirachta indica* A. Juss.And *Piper guineense* Schumach.Plant Pathology & Microbiology Journal of Plant Pathology & Microbiology pp: 2157-7471

Gwa, V. I., Bem, A. A, and Okoro, J. K. (2015): *Yams (Dioscorea rotundata Poir and D. alata Lam.)* fungi etiology in Kastina-Ala Local Government Area of Benue State, Nige J Phytopathology and Plant Health 3: 38-43.

Gwa, V. I., Nwakiti, A. O. and Ekefan, E. J. (2018): Antifungal effect of five aqueous plant extracts on mycelia growth of *Penicillium expamsum* isolated from rotted yam tubers in storage, Acta Scientific Agri 2: 65-70.

Gwa, V.I., and Ekefan E.J., (2019): Fungal organisms isolated from rotten yam (*Dioscorea rotundata)* Tubers and Antagonistic potential of Trichodermaharzianum against colletotrichum species. Agricultural Research & Technology, Open Access Journal Vol 10. ISSN: 2471-6774. Doi: 10.19080/ARTOA/.2019.10.555787.

Hahn, S.K., Osiru, D.S.O., Akoroda, M.O and Atoo, J.A. (1995): Production of yam, present role and future prospectus. A Research Guide Of International Institute Of Tropical Agriculture, Guide 46. Pp 36

Honger, J. O; Brentu, C; Opoku, N. and Dzidzienyo, D. (2017): Identification and molecular phylogenetics of *Lasiodiplodiaparv a*associated with white yam*(Dioscorea rotundata*.) in Ghana. Ghana J. Sci.57: 23-33

Igboh M. Ngozi; Ikewuchi C. Jude and Ikewuchi Cathrine (2009): Chemical profile of *Chromolaena odorata* L.(King and Robinson) leaves Pakistan Journal of Nutrition 8 (6) 521-524

IITA, (1995): International Institute of Tropical Agriculture. Annual Report – CG Space

IITA, (2011): Annual Report 2011 - International Institute of Tropical Agriculture. IITA annual report 2011 (p. 97).Ibadan IITA.

Ime, J., Onimisi, M. Y., and Jonah, S. A. (2012): Effects of Irradiation on Sprouting of Water Yam (*Dioscorea alata*) Using Different Doses of Gamma Radiation. *American Journal of Chemistry*.2, 137-141.

International Institute for Tropical Agriculture (IITA), (2007): Yam research for development. *Assessed 12/02/2018*

International Institute for Tropical Agriculture (IITA), (2009): Yam (*Dioscorea species*). IITA Publications.www.iita.org>cropsnew>dioscoria. *Assessed 12/12/2018*

International Institute for Tropical Agriculture (IITA). Crop improvement Division/ Tuber root improvement program. Archival reports (1989-1993). Part III yam (*Dioscorea spp*) Ibadan, Nigeria. Fito Pathologia Bras.1993; 8:1-7.

International Institute for Tropical Agriculture (IITA, 2004): Annual Report, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Pp 8-10

IUSS working group WRB (2014): Simple Open-work shelves. World Reference Base for Soil Resources, 2014.Edited by Schad P, Van Huyssteen C, Micheli E. World Soil Resources Reorts No. 106. FAO, Rome. 189. ISBN – 978-92-5-108369

Jayasuriya AHM (1995): *Diosoreaceae* in MD. Dissanayake (Ed.), A revised handbook to the flora of Ceylon (Vol. IX: 47 – 80).

Joachin D. Gbenuo, Judith F. Ahaunou, (2012): Pytochemical composition of *Cympobogon citratus*and *Eucalptus* essential oil and their anti-inflammatory and analgesic properties on wister rats. Molecular biology reports 40 (2) Doi 10.1007/s11033-0212-2155-1. .

Katie P, (2019): Elevated Pole

Kew, (2016): Royal Botanical Gardens. Taxonomical and Scientific classifications

Khadijat, A. A. (2003). Fungi associated with the spoilage of yam (*Dioscorea sp*.) Irish potato (*Solanum spp*) and sweet potato (*Ipomea spp*.) tubers during storage. Proceedings of the 27th Annual Conference of the Nigerian Institute of Food Science and Technology (NIFST), held in Kano 13th– 17th October, 2003 pp. 101 – 102.

King, R. M. and Robinson, H (2017): *Chromolaena odorata* (L.) (Asteraceae). In Biological Control of Tropical Weeds Using Arthropods; Muniappan, R., Reddy, G.V.P., Raman, A., Eds.; Cambridge University Press: Cambridge, UK, 2009. 31.

Kleith, U; Phillips, D; Ogbonna, M. and Siwoku, B. (2012): Nigeria-Scoping Yam Value Chain Analysis. YamImprovement for Income and Food Security in WestAfrica. ABK Publishers pp: 1-58.

Knoth, J. (1993): Traditional Storage of Yams and Cassava and its Improvement. *Deutshe Gesellchaf fur Techinishe Zusammenaribit (GTZ) Postharvest Project*, Hamburg, 9 – 43.

Koch's (2003): Koch's postulate is used for calculation and determination of Anti-fungal activity of Bio-pesticides (percentage inhibition)

Kokate C.K, (2003): Practical Pharmacognosy. Pune: Vallabh Prakashan; 2003.

Kokwaro, John (2009): Medicinal Plants of East Africa 3rd ed. Nairobi, Kenya: University of Nairobi Press. ISBN 978-9966-846-84-6.

Kumar S, Dwivedi S, Kukreja AK, Sharma JR, and Bagchi GD (2000).editors.*Cymbopogon*: The Aromatic Grass Monograph. Lucknow, India: Central Institute of Medicinal and Aromatic Plants;

Lawal, B., Ossai, P., Shittu, O., Abubakar, A., & Ibrahim, A. (2014): Evaluation of phytochemicals proximate, minerals and anti-nutritional compositions of yam peel, maize chaff and bean coat. International Journal of Applied Biological Research 6(2):21-37

MarksonA. A; Amadioha, A, C; Omosun, G; Madunagu, B. E; Udo, S. E and Umana , E. J. (2012): Control of Botryodiplodia theobromae causing Tissue Rot of WhiteYam (*Dioscorea rotundata Poir*) Scholarly Journal of AgriculturalScience; 2(1):1-7.

Mba, P. C, Ihejirika. G. O; Onyewuchi, O. P; Ofor, M. O; Ngwuta, A.A and Nwufo, M. I(2017): Evaluation of some plant extracts on the control Rot disease of yam (*Dioscorea rotundata poir*) Futo Journal Series Vol. 3, ISSUE-pp-54-64

Mbata, T, and Saikia. A (2017): Antibacterial Activity and Phytochemical Screening of Crude Ethanolic Extract Of Leaves Of *Ocimum Gratissimum* L On Listeria Monocytogenes. The Internet Journal of Microbiology.2007 Volume 4 Number 2.

Mathur, S. B. & Kongsdal, O. (2003). Common Laboratory Seed Health Testing Method for detecting Fungi. 1st Edition International Seed Testing Association, Bassersdorf, CH-Switzerland, pp: 425.

Nahanga, V. and Becvarora, V. (2014): Yam Production as a Pillar of Food Security in Logo Local Government Area of Benue State, Nigeria. *European Scientific Journal,* Nov. 2014 edition. Vol. 10 No. 31 ISSN 1857-7881.

National Academies Press U.S. (1992).*A tree for solving global problems. Washington D.C: The National Academic Press.* http://doi.org/109.17226/10924. *ISBN: 978-0-309-04686-2.*

National Plant Germplasm System (2017): Scientific classification of *Carica papaya*

Nesom, Guy L. (2006*):*"*Chromolaena odorata*". *In Flora of North America Editorial Committee (ed.).* Flora of North America North of Mexico (FNA)***.21***. *New York and Oxford – via* eFloras.org, Missouri Botanical Garden, *St. Louis, MO &*Harvard University Herbaria, *Cambridge, MA.*

Ngue-Bissa, T., Mbairanodji, A., Njualem, D. (2007): Technical Guide for the Production and Conservation of Yams (*Dioscorea spp).* National Programme for Roots and Tubers Development (NPRTD).Ministry of Agriculture and Rural Development, Republic of Cameroon. Pp 19 – 27.

Norman MJT, Pearson CJ, Searle PGE (1995): *Tropical Food Crops in their environment 2nd Edition. Cambridge Univ. press, U.K. pp. 305 – 316.*

Nweke, F. U., Ugwu, B.O. and Asadu, C.L. (1991), "Production cost in yam based cropping system in South western Nigeria", Research monograph No. 6, IITA Ibadan, Nigeria, pp. 4 – 12.

Nweke, F. U. (2013): The effect of Staking and Plant Spacing on the growth and yield of cucumber (Cucumis Sativus L.) .Journal of Environmental Science, Toxicology and Food Technology ER. 3(4):26-31 2013. Doi: 10.9790/2402-0342631.

Nweke, F. U. and Agbogidi, O. M. (2008): Fungi associated with the spoilage of pawpaw fruits sold in Asaba-Nigeria. J. Pest, Disease and Vector Management 8:449 – 451.

Nwufo, *et al.*, (2016): In their study reported on moisture, Ash, Crude fibre, crude protein, Fat, NFE(CHO), Nitrogen and Carbon contents of *Dioscorea rotundata* respectively Of *Vernonia amygdalina*. International Journal of Scientific World, 4 (1) (2016) 11-14

Obi, (2002): Statistical methods of detecting different between treatment means and research methodology issues in laboratory and field experiments AP Express Publishers Ltd Nsukka. Nigeria. PMCid:PMC2732465.

Ogaraku, A. O. and Usman,H. O. (2008): Storage Rot of some yams (*Dioscorea spp*) in Keffi and environs, Nasarawa State Nigeria. *Production and Agricultural Technology* 4: 22-27

Ogbo Frank C. and Agu Kingsley C., (2013): Open side shelves. www.sciencedomain.org.

Okigbo, R.N. & Ikediegwu, FEO (2000).Studies on biological control of postharvest rot of yams *(Dioscorea Spp)* with *Trichodermaviride. Journal of Phytopathology*, 148:351-355. http://dx.doi.org/10.1111/j.1439-0434.2000.tb04786.x

Okigbo, R.N. & Ikediegwu, FEO (2001). Biological control of tuber surface mycoflora of yam (*Dioscorea rotundata)* Trop. Sci. 41: 85 – 89.

Okigbo, R. N. (2004): A Review of Biological Control Methods for Post-Harvest Yams (*Dioscorea spp*) in Storage in South Easter Nigeria. *KMITL Sci J*. 4(1): 207-215.

Okigbo, R. N. (2005): Biological Control of Post-Harvest Fungal Rot of Yams (*Dioscorea spp*) with *Bacillus subtilis. Mycopathologia*, 159: 307-314

Okigbo, R. N. and Ogbonnaya, U. O. (2006): Antifungal Effects of Two Tropical Plant Leaf Extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on Post- Harvest Yam (*Dioscorea spp*) Rot. *Afr. J. Biotechnol,* Vol. 5 (9). Pp727-731

Okigbo, R. N. and Igwe, D. I (2007): The antimicrobial effects of piper guineense "Uziza" and *Phyllanantus* "ebebenizo" on *Candida albica* and *Streptococus faecalis*. Acta Michrobiological etImmunologica Hungaric 54: 353-366

Okigbo, R. N; Putheti, R; and Achusi, C. T. (2009): Post-harvest deterioration of cassavaand its control using extracts of *Azadirachta indica* and Afromonium meleguata.E-J Chem 6: 1274-1280.

Okigbo RN, Agbata CA, Echezona CE (2010): Effect of leaf extract of*Azadirachta india* and *Chromolaena odorata* on post harvest spoilage fungi of yams in storage. Current Res J of boil Sci 2(1): 9-12

Okigbo, R. N. and Emeka, A. N. (2010): Biological Control of Rot Inducing Fungiof Water Yam (*Dioscorea alata*) with Trichodermahar zianum,Pseudomonas syringae and Pseudomonas chlororaphis. Journal ofStored Products and Postharvest Research; 1(2):18-23..

Okigbo, R. N., Anukwuorji, C. A. and Ezeabara, C. A. (2013): Effects of leaf extracts of *Chrmolaena odorata* (L.) and *Terminaliacatappa* (L.) on mycelia growth of yam tuber pathogens. Nigerian Journal Plant Protection 27(1): 74 – 84

Okigbo RN and Ezebo RO (2017): Effect of leaf extracts of *Cymbopogon citratus, Chromolaena odorata and Newbouldialaevis on the Dioscore aalata* rot. DOI:10.15761/IFNM.1000205

Okigbo, R. N and Ezebo, R. O (2017): Effect of leaf etractsof *Cymbopogoncitratus, Chromolaena odorata* and *new bouldialaevis* on the *Dioscorea alata* Integr. *Food Nutr.Metab.*Vol. 5(1): 1-7

Okigbo, R. N., Enweremadu, E. C., Agu, K. C., Irondi, C. R., Okeke, C. B., Awah, N. S., Anaukwu, G. C., Okafor, O. I., Ezenwa, U. C. and Iloanusi, C. A. (2015): Control of White Yam (*Dioscorea rotundata*) Rot Pathogen Using Peel Extract of Water Yam (*Dioscorea alata*). *Advances in Applied Science Research*. 6(10): 7-13.

Oladeebo, J. O. and Okanlawon, O. M. (2010): Profitability level of yam (*Dioscorea spp.) production in Oyo State of Nigeria*. *In: Proceedings of the 44th Annual Conference of* Agricultural Society of Nigeria, held at Ladoke Akintola

Omodamiro, R.M., Ojimelukwe, P.C, Asiedu, R and Etudaiye, H.A. (2015): Effect *of Fusarium nygamai* infection on the chemical composition of flour from two yam varieties. J. of Adv.In Chemistry, Vol.12 No1 pp 3881-3887.

Onuh J. O., Dooshima S., Simon T. U., Tseaa S. (2015). Isolation of Six Microorganisms from rotten *Dioscorea alata* (Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts. Food and Nutrition Sciences, 2015, 6, 1381-1394. http://www.scrip.org/journal/fns, http://dx.doi.org/10.4236/fns.2015.615144

Onuegbu, B. A. (1999): Composition of Four Cocoyam Cultivars and their tolerance to Corm Rot. *Journal of Tropical Science.*39: 136-139.

Onumadu, F. N and Eze, C. I. (2008): Private Sector Extension Service on Crop Production. *Journal of Economic and Sustainable Development.*ISSN: 2222-2700.

Onwueme, I. C., (1982): The Tropical Tuber Crops: Yam, Cassava, Sweet potato, Cocoyam. The pitman press, Bath, Great Britain., pp: 234.

Onwueme, I. C., and Sinha, T. D. (1991): Field Crop Production in Tropical Africa. Onuh, J. O., Shiriki D., Ubwa1, S. T., and Shambe T. (2015): Isolation of Six Microorganisms from Rotten *Dioscorea alata*(Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts. Food and Nutrition Sciences, 20151, 6, 1381-1394.

Opara, L.U. (1999): Yam Storage. In Bakker-Arkema. F.W., Ed., CIGR Handbook of Agricultural Engineering 171 volume IV Agro processing. The American Society of Agricultural Engineers, St Joseph, 182-214.

Opara Linus U, (2003): Yams: Post-harvest operation. Inpho-postharvest compendium. In *Food and Agriculture Organization of the United Nations* (p. 23). Rome: FAO. Retrieved May 2, 2016, from https://www.fao.org/3/a-ax449e.pdf.

Orkwor, G.C. (1998): The importance of yams. In: Food yams. Advances in Research, Orkwor, G.C., Asiedu, R. & Ekanayake, I.J., eds. IITA and NRCRI, Nigeria, Page, 249.

Oyelana, O.A., Durugbo, E.U.., Olukanni, O.D., Ayodele, E.A., Aikulola, Z.O. and Adewole, A.I. (2011): Antimicrobial Activity of *Ficus* Leaf Extracts on Some Fungal and Bacterial Pathogens of *Dioscorea rotundata* from Southwest Nigeria, Journal of biological science, 2011.

Plants data base, (2003): Plants that feed the world: Database for in-dept research. University of Florida, Gainesville, FL, 32611 (352) 392 – 4357.http://guides.uflib.edu/PLS2003.

Prince Odeh Jones Isaiah, (2017): Igede Traditional Yam barn. Ed. By Cletus AdoleAruta.fb.me/igedeculture.

Princewill-Ogbonna, I. and Ibeji, C. (2015): Comparative study on nutritional and anti-nutritional composition of three cultivars (red, green and yellow) of aerial yam (*Dioscorea bulbifera*). IOSR Journal of Environmental Science and Toxicological Food Technology 9:79-86.

Rajan, (2014): Showed that tannin, phlobatannis, saponin, flavonoids, steroids, terpenoids, triterpenoids, carbohydrate, polyphenol and glycoside were present in acqueous extract of *Psidium guajava*.

Rasal V.P. (2005): Screening Shorearo besta Gaertn F. For Burnealing and Antioxidant activities. M. Pharm. Thesis. K.L.E.S. College of Pharmacy.Belgaum, Karnataka, India.(2005) p 33.

Ravi, A., Rao, D.S, Yedukondalu, R., (2005): Growth response and carcass characteristics of crossbred finisher pigs fed rations containing green gram chuni. Indian Vet. J., 82 (1): 48 - 51.

Ravi, K; Bharavi, K; Ravi Kumar P; and Vamsi Krishna B (2015): Phytochemical Screening and Antibacterial Activity of Ethanol Extract of Leaves and Twigs of *Azadirachta indica* A. Juss. Pharmaceutical and Biosciences Journal || ISSN: 2582-0540 [Formerly known as UK Journal of Pharmaceutical and Biosciences || ISSN: 2347-9442 (2013-2018)]

Sadiku, N. A; and Sadiku, I. B. S. (2011): Indigenous Efforts by African Farmers in Ensuring Sustainability in Agricultural Productivity in the face Changing Climate: A Proceeding of the Environmental Management Conference, FUNAAB, Nigeria, Pp: 64-72.

Sahore, D.A., Nemhi, G.J and Kamena, A, (2007).Changes in nutritional properties of yam (*Discorea species*), green plant (*Musa spp*) and Cassava (*Manihotesulenta*) during storage.Food Sci and Tech, 47, pp81-88.

Sangoyomi, T. E. (2004): Post-Harvest Fungal Deterioration of Yam (*Dioscorea spp*) and its Control. Ph. D. Thesis, University of Ibadan, Nigeria. Pp179

Sanusi, W. A. and Salmon, K. K. (2006): Food Security among Households: Evidence from Yam Economics in Oyo State, Nigeria. *Agricultural Journal* 1(4): 249-254.

Second Life Market Place (2016): Thacthed roof barn building. http://secondlife.///app/5d8628a-13d42e8a3c53.-a7091bd.

Sharn Gagan; Richa, Shri; Pancha, l., Vivek Shamar, Narender, Singh, Bharpurand Mann, A. S. (2011): Scientific basis for the therapeutic use of *Cymbopogon citratus, stapf* (Lemon grass). J Adv Pharm Technol Res. 2011 Jan-Mar; 2(1): 3–8. doi: 10.4103/2231-4040.79796

Sofowara L.A. (1993): Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, 1993; pp 55 – 71.

Taiga, A. (2011): Comparative studies of the efficacy of some selected fungicidal aqueous plant extracts on yam tuber dry rot disease. Ann Biol Res 2: 332-336. *Theobromae* and *Penicillium spp* cause dry rot of yam.

Timothy, G. E. and Bassey, E. E. (2009): Preliminary study on the effects of tuber sett weight derived from regions of seed yam on growth and yield of yam in South Eastern Nigeria. Nigeria Journal of Agricultural Technology, 2: pp 32-40

Timothy, S.Y.1, Goji, S.Y.1, Abdussalam.B1, Mava, Y.2, and Galadima, I.H.1 (2011): Antibacterial and phytochemical screening of the ethanolic leaf extract of *Azadirachta indica* (neem) (meliaceae). International Journal of Applied Biology and Pharmaceutical Technology.*Volume: 2: Issue-3: July-Sept -2011. ISSN 0976-4550*

Titteh, J. P., and Saakwa, R. (1994): Prospects and constraints of yam production in Ghana. In: Ofori, C. G., Halm, P. C. (eds) Proceedings of the 9th symposium of the International Society for Tropical root Crops, 3. Accra, Ghana, pp 20-2.

Udoh, E.J. (2005), "Technical Inefficiency in Vegetable Farms of Humid Regions; An analysis of Dry season by Urban Women in South – South Zone, Nigeria", *Journal of Agriculture and Social Science,* Vol.1,pp.80-85.

Udochukwu, U, Omeje, F.I, Uloma, I. S and Oseiwe, F.D, (2016): Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. American journal of research communication, 2016, 3(5): www.usajournals.com. ISSN:2325-4076.

USDA Plants (2009): Scientific classification of *Ocimum gratissimum. Retrieved Jan 7, 2009.*

USDA Nutrient Database, (2018): Nutrient lists from standard reference legacy (2018). National Agriculture, Food and Nutrition Information Center (FNIC).USDA.gov.ng.

Usunomena U., Okolie P.N. (2016): Phytochemical analysis and proximate composition of *Vernonia amygdalina*. Int. J. Scienti. World 4(1), 11 – 14 (2016).

Weiss EA. (1997): Essential oil crops. Wallingford, UK: CAB International; 1997. pp. 59–137. World Development report (2011) *"A Breakthrough in Yam Breeding"www.openknowledge.worldbank.org. Retrieved 17 June, 2019.*

Wikipedia.org.ng: Three sided vertical yam barn with tubers tied with local materials.

Yusha, M., Onuorah F.C, Murtala, Y. (2009): In vitro sensitivity pattern of some urinary tract isolates to *Carica papaya* extracts. Bayero Journal of Pure and Applied Sciences Vol. 2 No. 2 (2009). Doi: 10.4314/bajopas.v2i2.63786.

Yusuf, C. and Okusanya, B. A. O. (2008): Fungi associated with the storage rot of *yam Dioscorea rotundata Poir* in Yola, Adamawa State. J. of Sustainable Development in Agriculture and Environment. 3(2): 99 – 103

Zakari, D. M. Mohammed, B., Madugu, N. I. and Sandra, I. (2014): Impact of climate change on yam production in Abuja, Nigeria. *International Journal of Science, Environment and Technology/* Vol. 3, No. 2: 458-474

Zaknayiba, D. B. and Tanko, L. (2013): Costs and Returns Analysis of Yam Production among Small-Scale Farmers in Karu Local Government Area, Nasarawa State, Nigeria. *PAT*. 9(1), 73-80.

APPENDIX 1

ANOVA for depth of penetration

		Sum of Squares	Df	Mean Square	F	Sig.
A.N R	Between Groups	700.911	6	116.819	116.819	.000
	Within Groups	14.000	14	1.000		
	Total	714.911	20			
R.S .R	Between Groups	1019.111	6	169.852	187.731	.000
	Within Groups	12.667	14	.905		
	Total	1031.778	20			
Y .R	Between Groups	1112.880	6	185.480	185.480	.000
	Within Groups	14.000	14	1.000		
	Total	1126.880	20			
B.T R	Between Groups	1051.483	6	175.247	175.247	.000
	Within Groups	14.000	14	1.000		

	Total	1065.483	20			
A.N A	Between Groups	856.886	6	142.814	142.814	.000
	Within Groups	14.000	14	1.000		
	Total	870.886	20			
R.S.A	Between Groups	2184.626	6	364.104	364.104	.000
	Within Groups	14.000	14	1.000		
	Total	2198.626	20			
Y.A	Between Groups	699.951	6	116.659	116.659	.000
	Within Groups	14.000	14	1.000		
	Total	713.951	20			
F.S.A	Between Groups	836.066	6	139.344	133.071	.000
	Within Groups	14.660	14	1.047		
	Total	850.726	20			

APPENDIX 2

ANOVA for percentage inhibition

		Sum of Squares	Df	Mean Square	F	Sig.
yeast/ R	Between Groups	6.840	4	1.710	171.000	.000
	Within Groups	.100	10	.010		
	Total	6.940	14			
Aspergillus /R	Between Groups	13.260	4	3.315	331.500	.000
	Within Groups	.100	10	.010		
	Total	13.360	14			
Rhizopus/R	Between Groups	10.476	4	2.619	261.900	.000
	Within Groups	.100	10	.010		
	Total	10.576	14			
Boty/R	Between Groups	2.304	4	.576	57.600	.000
	Within Groups	.100	10	.010		

	Total	2.404	14			
yeast/ A	Between Groups	6.216	4	1.554	155.400	.000
	Within Groups	.100	10	.010		
	Total	6.316	14			
Aspergillus /A	Between Groups	5.640	4	1.410	141.000	.000
	Within Groups	.100	10	.010		
	Total	5.740	14			
Rhizopus/A	Between Groups	7.944	4	1.986	198.600	.000
	Within Groups	.100	10	.010		
	Total	8.044	14			
Boty/A	Between Groups	4.404	4	1.101	110.100	.000
	Within Groups	.100	10	.010		
	Total	4.504	14			

APPENDIX 3

ANOVA nutritional composition of diseased and healthy yam tubers

		Sum of Squares	Df	Mean Square	F	Sig.
mc/r	Between Groups	773.472	5	154.694	134.973	.000
	Within Groups	13.753	12	1.146		
	Total	787.225	17			
ash/r	Between Groups	3.342	5	.668	53.417	.000
	Within Groups	.150	12	.013		
	Total	3.492	17			
cf/r	Between Groups	4.725	5	.945	.614	.692
	Within Groups	18.480	12	1.540		
	Total	23.205	17			
cp/r	Between Groups	8.411	5	1.682	39.842	.000
	Within Groups	.507	12	.042		

	Total	8.918	17			
fat/r	Between Groups	.284	5	.057	6.400	.004
	Within Groups	.107	12	.009		
	Total	.391	17			
carbohy/r	Between Groups	858.123	5	171.625	10.896	.000
	Within Groups	189.013	12	15.751		
	Total	1047.136	17			
N/R	Between Groups	.404	5	.081	20.183	.000
	Within Groups	.048	12	.004		
	Total	.452	17			
C/R	Between Groups	11.845	5	2.369	13.537	.000
	Within Groups	2.100	12	.175		
	Total	13.945	17			
mc/A	Between Groups	1494.224	5	298.845	209.471	.000

	Within Groups	17.120	12	1.427		
	Total	1511.344	17			
ash/A	Between Groups	.396	5	.079	23.282	.000
	Within Groups	.041	12	.003		
	Total	.437	17			
cf/A	Between Groups	2.460	5	.492	49.200	.000
	Within Groups	.120	12	.010		
	Total	2.580	17			
cp/A	Between Groups	2.800	5	.560	56.000	.000
	Within Groups	.120	12	.010		