بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection and Identification of Some Seed Borne Fungi of Groundnut from Different locations with Emphasis on *Aspergilus flavus* and its Control in Sudan

فحص وتعريف بعض الفطريات المحمولة علي الفول السوداني من مناطق مختلفة بالتركيز علي فطر*Aspergillus flavus* ومكافحته في السودان

A thesis submitted in partial fulfillment of the requirements for the Master of Science Degree in Plant Protection

By

Mohamed Adam Osman Ibrahim

Agriculture of Science (B.Sc honor) October 2005

Department of plant protection

Sinnar University

Supervisor: Dr. Ekhlass Hussein Mohamed Ahmed

December 2015

Sudan University of Science & Technology

College of Graduate Studies

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية الدراسات العليا

Approval Page

Name of Candidate: Mohamed Adam Osman Abraium

Thesis title: Detection and Identification of seed borne fungi on groundnut with emphasis of A. Spergillus flavus and its control in Sudan.

Approved by:

1. External Examiner

Name: Mundasir Adam Mohamed Elamien

Signature: ..... Date: 3-1-2016

2. Internal Examiner

Name: Mawahib Ahmed Elsiddig

Signature: ..... Date: 3-1-2016

3. Supervisor

Name: Eknlass Hussein mohmed Ahmed

Signature: ..... Date: 3.1.2016

Sudan University of Science and Technology
College of Graduate Studies

Declaration

I, the signing here-under, declare that I'm the sole author of the (M.Sc.) thesis entitled Detection and Identification of Same seed Borne Fungi of Groundnut from Different Locations with Emphasis on Aspergillus flavus and its Contral in Sudan

which is an original intellectual work. Willingly, I assign the copy-right of this work to the College of Graduate Studies (CGS), Sudan University of Science & Technology (SUST). Accordingly, SUST has all the rights to publish this work for scientific purposes.

Candidate's name: Mohammed Adam Osman Ibrahim

Candidate's signature: ..... **Date:** .....

إقرار

أنا الموقع أدناه أقر بأننى المؤلف الوحيد لرسالة الماجستير المعنونة فحص وتعريف بعض الفطريات المحمولة على البذور السوداني من مناطق مختلفة بالتركيز على فطر Aspergillus flavus ومكافحته في السودان

وهى منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعه السودان للعلوم والتكنولوجيا، عليه يحق للجامعه نشر هذا العمل للأغراض العلمية .

اسم الدارس : محمد آدم عثمان ابراهيم

توقيع الدارس : ..... التاريخ : .....

بسم الله الرحمن الرحيم

قال تعالى :

{ هُوَ اللَّهُ الَّذِي لا إِلَهَ إِلاَّ هُوَ عَالِمُ الْغَيْبِ وَالشَّهَادَةِ هُوَ الرَّحْمَنُ الرَّحِيمُ*هُوَ اللَّهُ الَّذِي لا إِلَهَ إِلاَّ هُوَ الْمَلِكُ الْقُدُّوسُ السَّلامُ الْمُؤْمِنُ الْمُهَيْمِنُ الْعَزِيزُ الْجَبَّارُ الْمُتَكَبِّرُ سُبْحَانَ اللَّهِ عَمَّا يُشْرِكُونَ*هُوَ اللَّهُ الْخَالِقُ الْبَارِئُ الْمُصَوِّرُ لَهُ الأَسْمَاءُ الْحُسْنَى يُسَبِّحُ لَهُ مَا فِي السَّمَوَاتِ وَالأَرْضِ وَهُوَ الْعَزِيزُ الْحَكِيمُ*}

صدق الله العظيم

سورة الحشر الآيات من {22-24}

Dedication

To my wife,

To my family and

To my friends

With love and respect

Mohammed

ACKNOWLEDGEMENT

All thanks are due to Almighty Allah who gave me health and strength, and helped me to produce this work.

I would like to express my thanks to my supervisor Dr. Ekhlas Hussein Mohamed, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology, who devoted most of her time to teach me on various disciplines of scientific research. Her enthusiasm and integral view on research and her mission for providing only high-quality and not less, has made a deep impression on me. I owe her lots of gratitude for having me shown this way of research.

I would like to thank my wife Sakina who kept an eye on the progress of my research and always available when I needed her advices.

I also like to extend my thanking to all my colleagues and others that have assisted me, for their kind inspiration to publish this work and their encouragement and support during the work in laboratory and data base analysis, this is uncountable support. I have been accompanied and fostering from my family and friends. It is a pleasant aspect that I have now the opportunity to express my gratitude and thanks for all of them.

Also thanks to Ministry of Agriculture, Department of Plant Protection West Darfur.

Many thanks and deep gratitude is due to all members, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology.

Special thanks are also accorded to all those who have assisted in one way or another in the compilation and production of this report,

LIST OF CONTENTS

TITLE	Page
DEDICATION ……..	III
ACKNOWLADGEMENT …….....	III
LIST OF CONTENTS ……	IIIIII
LIST OF TABLES ……...	III
LIST OF PALTES ……	III
LIST OF FIGURES …….	IIII
ABSTRACT ……..	III
ARABIC ABSTRACT …….....	III
CHAPTER ONE ……	1
INTRODUCTION ……..	1
CHAPTER TWO ……..	5
2.1 The Groundnut ……	5
2.1.1 Scientific classification ……..	6
2.1.2 Importance ……	6
2.2 The diseases ……...	7
2.2.1 Seeds born fungi …….	8
2.2.1.1 *Aspergillus spp.* ……..	9
2.2.1.2 *Penicillium spp.* …….	10
2.2.1.3 *Alternaria spp*. ……..	10
2.2.1.4 *Rhizopus spp.* ……	11
2.2.1.5 *Aspergillus niger* ……...	11
2.3 Control management …….	11
2.3.1 Seed health testing ……...	11
2.3.2 Practices and biological control ……	12

2.3.3 Fungicides and extracts ……. 12
2.4 *Asperogillus flavus* ……... 13
2.4.1 Ecology and distribution ……. 14
2.4.2 Soil and air …… 14
2.4.3 Genome ……. 15
2.4.4 Taxonomy and classification …… 15
2.4.4.1 Scientific classification …… 15
2.4.5 Identification ……. 15
2.4.6 Molecular typing …… 16
2.4.7 Contamination levels …… 16
2.4.8 Control methods …… 20
2.5 The extract of botanicals …… 20
2.5.1 Tree of Neem ……. 20
2.5.1.1 Scientific classification …… 21
2.5.1.2 Uses of the Neem ……. 21
2.5.2 The Ginger ……... 22
2.5.2.1 Scientific classification …… 22
2.5.2.2 Uses of the Ginger ……... 22
2.6 Fungicides …… 23
2.6.1Chemical identification …….. 23
CHAPTER THREE …… 24
MATERIALS AND METHODS ……... 24
3.1 Study location ……... 24
3.2 The materials …….. 24
3.2.1 Tools and equipments used ……. 24

3.3 The methods …… 24
3.3.1 Samples collection …… 24
3.3.2 Dry seed inspection …… 25
3.3.3 Detection and isolation of seed borne fungi …… 25
3.3.3.1 Blotter method ……. 25
3.3.3.2 Agar bate method …… 26
3.3.4 Slide preparation …….. 26
3.3.5 Pure culture preparation of fungi ……. 26
3.3.6 Identification of fungi ……. 27
3.3.7 Plant extracts preparations ……. 27
3.3.7.1 Ethanol extracts preparations …….. 27
3.3.7.2 Ginger and Neem extracts …….. 28
3.3.8 Preparation of fungicide Topas Ec. 100 ……. 28
3.4 Effect of botanical extracts and fungicide on the linear growth of *Aspergillus flavus in vitro* …… 29
3.5 Fungicides Topas Ec. 100 …..…… 29
3.6 Experimental design …… 29
3.7 Statistical analysis …….. 29
CHAPTER FOUR …….. 30
RESULTS ……... 30
4.1 Detection and isolation of seed borne fungi from Groundnut……..... 30
4.1.1 Dry Seed inspection ……. 30
4.1.2 Blotter Method (filter papers) …….. 33
4.1.3 Agar Plate Method (PDA) ……... 33
4.2 Isolation and identification of *A. flavus strain* …… 41
4.2.1 Identifications of *A. flavus* in groundnut…….. 41

4.2.2 Isolated of *A. flavus* from groundnut…….. 44
4.3 Effect of ethanol extracts of botanicals and fungicide were used to inhibition growth of *A. flavus* in vitro…… 44
4.4 Effects of the fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro……. 48
4.4.1 Effect of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 3rd day……. 48
4.4.2 Effect of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 4th day…….. 51
4.4.3 Effect of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 5th day…….. 54
CHAPTER FIVE…… 57
DISCUSSION……..... 57
CONCOLUSSION…….. 61
RECOMMENDATIONS…… 62
REFERENCES…….. 63
APPINDIX…….. 79

LIST OF TABLS

No.	Title	Page
1	Dry inspection of seed groundnut showing different categories of 200 seeds tested, Seasons (2013-2014 and 2014-2015).	31
2	Table 2 fungi incidence on seed of groundnut by blotter method in the 3rd day from incubating (disease incidence %).	35
3	Fungi incidence on seed of groundnut by blotter method in the 4th day from incubating (disease incidence %).	36
4	Fungi incidence on seed of groundnut by blotter method in the 5th day from incubating (disease incidence %).	37
5	Table 5 fungi incidence on seed of groundnut by Aga plate-method in the 3rd day from incubating (disease incidence %).	38
6	Fungi incidence on seed of groundnut by Aga plate-method in the 4th day from incubating (disease incidence %).	39
7	Fungi incidence on seed of groundnut by Aga plate-method in the 5th day from incubating (disease incidence %).	40
8	The growth of *A. flavus* in the 3rd day from treatment on Blotter-method (growth/day).	41
9	The growth of *A. flavus* in the 3rd day from treatment on Agar plate-method (growth/day).	42
10	Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 3rd day from incubation.	49
11	Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 4th day from incubation.	52
12	Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 5th day from incubation.	55

LIST OF PLATES

No.	Title	Page
1	The composite samples and two hundred to process of dry inspection test inspection test.	32
2	Processes of dry inspection test for 200 seeds from each one of eight samples.	34
3	*A. flavus* strain isolated from seed samples and re-culturing on PDA in the 5th day from incubation.	45
4	Plate 4 slides *of A. flavus* isolated from groundnut seed samples.	46
5	Inhibition of *Aspergillus flavus* in the 5th day from incubation comparison between ethanol **A=**fungicide**, B=** extract of Neem and **C=** extract of Ginger with **D=**control.	47

LIST OF FIGURES

No.	Title	Page
1	Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 3rd day from incubation. ……..	50
2	Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 4th day from incubation. …….	53
3	Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 5th day from incubation. ……	56

ABSTRACT

The experiment was conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology (SUST), During March and April 2015. The objective of this research is to detect, identify seed borne fungi associated with seeds of groundnut, (*Arachis haypogaea L.*), and test the effect of ethanol extracts of (Ginger rhizomes *Zingiber officinale* Roscoe) and Neem (leaves *Azadirachta indica* A.Juss)، against fungi *Aspergillus flavus* strain. Seeds samples of groundnut was collected from four regions in Sudan (Algeneina, Nyla, New hafa and Alrahad-alfao), two samples from each region of seasons (2013-2014 and 2014-2015), the study covered Seed health Testing process, detection and identification of Seed borne fungi before-after cultured seeds of groundnut on PDA media and Filter papers methods. Five types of fungus were detected, identificated and isolated, these fungus are *Aspergillus flavus*, *Aspergillus niger*, *Penicillium digitatum*, *Alternaria alternata* and *Rhizopus.* The result showed that the degree of inhibition of fungi increase with the increasing of ethanol extract concentration which added with concentrations (25%, 50% and 100%) to *A. flavus* strain, moreover the ethanol extracts of Ginger and Neem at concentration of 100% gave the highest redaction of the growth on *A. flavus* in the last day from incubation, the inhibition growth was (89% and 86.4%), respectively.

Ethanol extracts contain antifungal compounds which could be used as alternatives for the fungicides for assurance the safety of human and plant from risk of fungicides.

ملخص الدراسة

أُجريت التجربة تحت ظروف معمل قسم وقاية النبات، كلية الدراسات الزراعية، جامعة السودان للعلوم والتكنولوجيا خلال شهري مارس وابريل 2015م. الهدف من البحث هو الكشف والتعرف على الفطريات المحمولة على بذور الفول السوداني (*Arachis haypogaea L.*) والمرتبطة به، واختبارتأثيرالمستخلصات الايثانولية لدرنات الزنجبيل (rhizomes of *Zingiber officinale* Roscoe) وأوراق النيم (leaves of *Azadirachta indica* A.Juss) على سلالة الفطر(أسبرجلس فلافس)*Aspergillus flavus* . ثمانية عينات من بذور الفول السوداني جُمعت من اربعة مناطق في السودان (الجنينة ، نيالا، حلفا الجديدة، الرهد-الفاو)، أخذت عينتان من كل منطقة للمواسم (2013-2014) و (2014-2015).غطت الدراسة عملية اختبار صحة البذور والكشف والتعرف على الفطريات المحمولة على البذور قبل وبعد زراعة البذور بطريقتي الأجار وورق الترشيح. تم الكشف والفحص والعزل لخمسة انواع من الفطريات وهي *Rhizopus, Aspergillus flavus, Aspergillus niger, Penicillium digitatum, Alternaria alternata*. أظهرت النتائج ان درجة تثبيط الفطر تزيد بزيادة تركيز المستخلص الايثانولي والذي أضيف بتراكيز (25%، 50%،100%) للفطر *Aspergillus flavus*. علاوة علي ذلك المستخلصات الايثانولية للزنجبيل والنيم بتراكيز 100%. أعطت أعلى درجة تخفيض نمؤ الفطر*Aspergillus flavus* في اليوم الأخير من التحضين وكانت نسبة التثبيط (89% و 86.4%)،على التوالي.

المستخلصات الايثانولية تحتوي على مركبات مضادة للفطريات والتي يمكن استخدامها كبدائل للمبيدات الفطرية لضمان سلامة الانسان والنبات من خطر المبيدات.

CHAPTER ONE

INTRODUCTION

Groundnut (*Arachis haypogaea L.*) plant belongs to the family Fabaceae (Gregory, 1973). It is the 3rd most important food crop and 4th most important oil seed crop of the world. This crop is known throughout the world by different names, Peanut, Earthnut, Monkey-nut and Goobers. It is grown in nearly 100 Countries. The origin of the groundnut South American, (Hommons, 1994 and weiss, 2000).

The main producing Countries are China, India, Nigeria, USA, Indonesia and Sudan (Mondal; *et al.,* 2006). In addition it is widely grown in many Countries such as Myanmar, Vietnam, Senegal, the Democratic Republic of Congo, Chad, Burkina Faso, Zimbabwe, Mali, Mozambique, Uganda, and Tanzania (Shiferaw *et al.,* 2004). China and India together are the world's leading groundnut producers accounting for nearly 60% of the production and 52% of the crop area. Especially in the developing countries, groundnut has to play an important role both as oil and food crop. Most of the groundnuts are used for extraction of oil for domestic consumption and export. For example, Sudan accounted for 17% of the world groundnut export trade (Abulu, 1978).

Groundnut is growing primary as an oilseed crops and most of the produces is used for oil production (Singh, 1982). It is consumed directly in processed food and snacks as valuable source of protein, energy, minerals, oil, meal and confectionery products (Abu Assar *et al.,* 2008). Groundnut is growing for food that including peanut butter, roasted in the shell, candy, and as shelled whole seed that are salted, or dry-roasted (Isleib and Wynne, 1992). The seed cake and hay are valuable source animal feed. Shells maybe used

as a source of heat and raw source of many products, (e.g.) activated carbon, organic chemicals and combustible gases.

In the Sudan groundnut is produced under two environmental conditions irrigated and rain fed conditions. Irrigated areas include Gazira, Rahad, New Halfa, Suki, Blue and White Nile schemes. Rain-fed areas include South, West, North and East Darfur, South and North Kordofan. Irrigation peanut is producing on heavy black cracking of central Sudan, where only late maturing (Virginia) types are grown (Khidir, 1997 and Ismail, 2000).

Groundnut production in Africa has suffered from fluctuation and downward trend low yield in the Eastern Africa, that have been attributed to the variable rains with recurrent drought, lack of high-yielding cultivars, insect pests and diseases, as well as low input used in groundnut cultivation (Mahmoud, 1995). Groundnuts that are used as raw materials for peanut butter processing are liable to colonization by fungal molds during harvest-handling, storage and transportation, exposing them to the risk of contamination with aflatoxin (Polixeni and Panagiota, 2008; Mutegi, 2012).

The main aflatoxin producing fungi in groundnuts are *Aspergillus flavus,* (CAST, 1998), which mostly infect groundnuts as a complex. (Varga, 2012; Mutegi, 2012) reported *A. flavus L*-strain, *A. flavus* S-strain, and *A. niger* as the major fungal pathogens infecting groundnuts and groundnut products from Busia and Homa Bay districts in Western Kenya. *Aspergillus flavus* and aflatoxin have been reported in groundnuts in Sudan (Omer, *et al.;* 2001) and Egypt (Abdelhamid, 1990). *Aspergillus flavus* is the main producer of aflatoxin, only grows in groundnut when the moisture content exceeds 9% and has optimum growth conditions of between 25 and 30^{o}C (Ribeiro, *et al.;* 2006). The warm and humid environmental conditions in Africa are ideal for growth of *A. flavus* making aflatoxin contamination of

food, including groundnuts, a widespread problem across the continent (Bankole, 2006; Wagacha and Muthomi, 2008). Acute exposure of humans to aflatoxins leads to outbreak of a disease condition known as aflatoxicosis contributing significantly to the disease burden in Africa, chronic effects of aflatoxins result from continuous exposure to relatively low levels of toxins over a prolonged period (Mehan, 1991).

Some of the common effects of chronic aflatoxicosis include impaired food conversion, slower rates of growth, and a decrease in absorption of various micronutrients (Jolly, 2007). Aflatoxin contamination of groundnuts therefore poses a risk to human health and has been identified as a major constraint to trade in Africa (Mutegi, 2009). Peanut butter is one of the main products of groundnut processing (Campos-Mondragón, 2009).

If raw groundnuts are contaminated with aflatoxins, there is a high risk of exposure to the consumer through consumption of peanut butter processed from such groundnuts practices such as poor storage and handling within the peanut butter cottage industry could contribute to further aflatoxin contamination of peanut butter. High aflatoxin levels up to 22 mg/kg in groundnut products such as roasted nuts and peanut butter have been reported in Nairobi (Mutegi, 2010).

The significant economic and health hazards caused by fungi and mycotoxin especially in developing countries that have poor food storages is of great concern so to ensure a healthy food supply thereby minimizing consequences to food security, international trade and animal and human health, there is a need to monitor fungal and mycotoxin contamination periodically so as to meet international and national mycotoxin regulatory standards (128 Afr. J. Food Sci.).

This study therefore conducted to determine the status of fungi contamination in groundnuts on four regions. Standard laboratory methods used for the detection of the fungi *Aspergillus flavus* from Seed of groundnut samples.

In this regard, the objectives of this study are to:

- Validate and determine the contamination of groundnut by the fungi associated with seeds of groundnut.
- Detection, identification and isolation the fungi *Aspergillus flavus* from groundnut Seed samples.
- Inhibit the *Aspergillus flavus* growth by using ethanol extracts of botanical, (Ginger rhizomes and Neem leaves), and use the fungicide Topas Ec.100 as a standard to control *Aspergillus flavus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 The Groundnut

Groundnut or peanut (*Arachis hypogea Linn*), is a plant which belongs to the family of Fabaceae (Eke-Ejiofor, *et al.;* 2012). Botanically, groundnut is a legume although it is widely identified as a nut and has similar nutrient profile with tree nuts (Ros, 2010). This annual plant is generally distributed in the tropical, sub-tropical and warm temperate areas and represents the second most important legume in the world based on total production after soybean (Pattee and Young, 1982; Redden, *et al.;* 2005).

Groundnut (*Arachis hypogea Linn*), consumed directly in processed food and snacks constitute valuable source of protein, energy and minerals, utilization of oil, meal and confectionery groundnuts is increasing concurrently with a gradual shift away from oil and meal into confectionery products and snacks. Groundnut is primarily used for oil extraction in Sudan. It is also consumed directly because of its high food value. The total area under groundnut production is approximately one million hectares with an average yield of 855 kg ha-1 and total production of 826,000t. High yielding Virginia types with different seed size categories have been released for the irrigated sector (El Ahmadi, 1993; Abu Assar, *et al.;* 2000).

2.1.1 Scientific classification

Kingdom	:	Plantae
Subkindom	:	Viridiplantae
Infrakindom	:	Streptophyta
Superdivision	:	Embryophyta
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Dlass	:	Magnoliopsida
Superorder	:	Rosanae
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Arachis*
Species	:	*Arachis hypogaea L*.

(www.itis.gov/servlet/singleRpt.)

2.1.2 Importance

Peanuts are among the most common nuts included in many diets worldwide and play an important role in the diets of several African populations, especially children, because of their high content of protein (approximately 25%), fat and carbohydrate. (Kamika and Takoy, 2011).

The average world production of groundnut pods amounted to about 35.88 million tons/year from 24.4 million hectare and the total production in sub-Saharan Africa was 8.2 million tons/year from 9.5 million hectare (USDA, 2012). The main producing Countries are China, India, Nigeria, United States, Indonesia and Sudan. Peanut constitutes a major annual oilseed crop and a good source of protein containing high lysine content, which makes it a good complement for cereal (Okaka, 2005). The proximate biochemical composition of mature groundnut Seeds /100gm edible portion, moisture

(6.5g), protein (25.8 g), lipids (49.2 g), carbohydrate(16.1g), dietary fibre (8.5 g), calcium (92 mg), magnesium(168 mg), phosphorus (376 mg) and iron (4.6 mg) (USDA, 2010). However, peanut contains some antinutritional factors as physic acid, condensed tannins, trypsin and amylase inhibitor, that may limit its usage and nutritional value (Njintang, *et al.;* 2001).

2.2 The diseases

Peanut (*Arachis hypogaea L*.) is one of most important and widespread oil crops. One of the major problems in peanut production worldwide is aflatoxin contamination, which is of great concern in peanut as this toxin can cause teratogenic and carcinogenic effects in animal and human. Infection of peanut by *A. flavus* occurs not only in post-harvest but also in pre-harvest conditions. Several biotic (soil-born and insects) and abiotic (drought and high temperature) factors are known to affect pre-harvest aflatoxin contamination, while the late season drought (20-40 days before harvest) which predispose peanut to aflatoxin contamination is more important in the semi-arid tropics. Irrigation in late season can reduce peanut pre-harvest aflatoxin contamination, but this cultural practice seems to be impractical in some areas, especially in semi-arid and arid areas, (Wang, 2010).

Moreover, groundnuts are liable to fungal contamination during handling, storage and transportation, exposing them to the risk of contamination with aflatoxin (Polixeni and Panagiota, 2008; Mutegi, 2012). Indeed, groundnuts can be contaminated with aflatoxin during pre-harvest and post-harvest processing and the risk of contamination increases along the marketing chain due to poor handling practices (Kladpan, 2004; Kaaya, 2006). The main aflatoxin producing fungi in groundnuts are *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*, which mostly infect groundnuts as a complex (Varga, 2012).

A. flavus grows in groundnuts when the moisture content exceeds 9% and has optimum growth conditions of between 25 and 30°c, and water activity of 0.99% with a minimum of 0.83%, while production of aflatoxin occurs optimally at 25°c and water activity of 0.99%. (Ribeiro, *et al.;* 2006). According to (IARC, 2002), aflatoxin produced by *Aspergillus spp*., has immune suppressive effects and epidemiological studies have shown a positive correlation between aflatoxin intake and the incidence of liver cancer. Peanuts and its derivatives are often classified as street food which satisfies essential need of the urban population by being affordable and available (Donkor, *et al.;* 2009). Peanut seeds are eaten raw, boiled or roasted, made into butter or paste and are used for thickening soups (Campos-Mondragón, *et al.;* 2009).

Peanut butter is made by grinding dry roasted groundnuts into a paste (Mutegi, *et al.;* 2009). Peanuts are also used as major ingredients in the formulation of weaning food with other cereals such as sorghum, corn, and millets because of their high protein and omega 6 fatty acid contents (Iro, *et al.;* 1995). All the fungal densities are based on the wet mass of final soil preparation used for inoculating seeds.

2.2.1 Seeds born fungi

Seeds play a vital role in the production of healthy crops. Healthy seed is the foundation of healthy plant; a necessary condition for good yields (Diaz, *et al.;* 1998). Seed is the most important input for crop production. Pathogen free healthy Seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Islam, 2009).

Fungi, or moulds in this context to differentiate them from single celled yeasts, are destructive agents causing losses of agricultural commodities in many zones of the world, ranking alongside insects and weeds for crop loss or yield reduction. They can occur on growing in-field crops as well as harvested commodities, leading to damage ranging from rancidity, odor, flavor changes, loss of nutrients, and germ layer destruction. This can result in a reduction in the quality of grains, as well as gross spoilage and possible mycotoxin production (Oerke and Dehne, 2004).

2.2.1.1 *Aspergillus spp.*

The role of *Aspergillus spp.* in food spoilage is well-established (KRN Reddy, *et al.;* 2010). Many *Aspergilli* are xerophilic and present particular problems during commodity harvest, and during subsequent drying and storage. About 30 species of *Aspergillus* or their teleomorphs are associated with food spoilage, these include: *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus ochraceus, Aspergillus candidus, Aspergillus restrictus, Aspergillus penicillioides, Aspergillus niger, Aspergillus carbonarius, Aspergillus fumigatus, Aspergillus clavatus, Aspergillus carbonarius, and Aspergillus versicolor*. (Haq Elamin, 1988). However, *(Haq Elamin NH, 1988; Yousif,et al,; 2010; and* Olusegun, *et al,;* 2013). Reported that *Aspergillus species* tend to be associated more with tropical and warm temperate crops, for example oilseeds and nuts, since they prefer to grow at relatively high temperatures. They concluded that, *Aspergillus flavus, Aspergillus parasiticus* and Aflatoxins typically affect oilseeds, including groundnuts, soya, tree nuts, maize and various oilseed-based animal feed stocks - cotton seed cake, copra, sunflower, but can also affect rice, wheat, sorghum, figs, coffee and sweet potatoes, for example. Aflatoxins are also noted in milk, via contaminated animal feed.

2.2.1.2 *Penicillium spp.*

Penicillium as well is a large genus containing 150 recognized species, of which 50 or more occur commonly. Many species of Penicillium are isolated from foods causing spoilage; in addition, some may produce bioactive compounds. Some of the most important toxigenic species in foods are *Penicillium expansum, Penicillium citrinum, Penicillium crustosum and Penicillium verrucosum.* A much larger number of *Penicillium species* are mainly associated with food spoilage.

Those include *Penicillium aurantiogriseum, Penicillium chrysogenum, Penicillium digitatum, Penicillium griseofulvum, Penicillium italicum, Penicillium oxalicum* and *Penicillium viridicatum*; some of these produce mycotoxins. However, *Penicillium species* are associated more with cool temperate and temperate crops, mainly cereals, since most species do not grow very well above 25-30°c (Pitt J.I., 2006). Obviously, *Fusarium species* causes a huge range of diseases on an extraordinary range of host plants. As mentioned earlier the fungus can be soil borne, airborne or carried in plant residue and can be recovered from any part of the plant from the deepest root to the highest flower (Booth 1971; Summeral, *et al,;* 2003).

2.2.1.3 *Alternaria spp.*

Alternaria species are plant pathogens that can produce toxins in both pre- and post-harvest commodities. They are characterized by very large brown conidia with a characteristic "beak" at the tip. The most common species is *Alternaria alternata*; others include *Alternaria tenuissima, Alternaria infectoria, Alternaria citri, Alternaria brassicicola and Alternaria brassicae.* The species *Aternaria alternata* and *Alternaria tenuissima* are pathogenic to a wide range of crops; the other species have more limited host ranges (Agrius 2005).

2.2.1.4 *Rhizopus spp.*

These species typically affect fruits and vegetables, since they can only grow at relatively high water activities (Agrius 2005).

2.2.1.5 *Aspergillus niger*

Aspergillus niger belonges to section *Asperogillus flavi*. These species typically affect grain in moisture conditions, *Aspergillus niger* is prevalent in warm and dry climatic zones and its incidence ranges from 2% to 14% (Pande and Narayana Rao 2000). The pathogen attacks groundnut plants at all the growth stages and causes pre-emergence rotting in seeds, soft rot in emerging seedlings, and crown rot in mature plants.

2.3 Control management

2.3.1 Seed health testing

Seed health testing for the presence of seed borne pathogens is an important step in the management of crop diseases. This is simply because, seed-borne diseases have been found to affect the quality and quantity of food crops. Accordingly, the importance of seed health testing cannot be under estimated. The pathogens can present externally or internally or associated with the seed as contaminant.

A number of laboratory seed health testing methods for detecting fungi sampling were in use. This include, examination of dry seeds, washing test, blotter method and its modification, agar plate method, embryo and seedling symptom test). However, Blotter test is the simplest and most widely used method especially in developing countries (Mathur and Kongsdal, 2003). In respect of the blotter test, seeds are typically surface sterilized with dilute hypochlorite solution and planted in 6×9 inches blotters. These are incubated and observed for 7–10 days. Fungal growth is recorded and confirmed with

microscopic examination (www.worldseed.org). It is possible that two methods may be required to detect a pathogen.

2.3.2 Practices and biological control

Fungal pathogens associated with food grains are major problem of many economically important food crops. Some are soil-borne pathogen, which can live in the soil for long periods of time, so rotational cropping is not a useful control method. It can also spread through infected dead plant material, so cleaning up at the end of the season is important (Jones, 1982).

The potential for using microorganisms to detoxify mycotoxins has been reported by (Murphy, *et al.;* 2006) to be promising. One of the management strategies that had been developed is biological control using the competitive exclusion mechanism, which has been successfully implemented in the USA; biological control has been used to reduce aflatoxin contamination in various crops such as cotton, maize and groundnut. The International Institute for Agricultural research (IITA) has pioneered this technique in Nigeria, by the development of its product called *Aflasafe. Aflasafe* has proven successful and is being tried on a number of crops. (Bandyopadhyay and Cardwell, 2003).

2.3.3 Fungicides and extracts

One of the control methods is to improve soil conditions because soil borne pathogens spreads faster through soils that have high moisture and bad drainage. Other control methods include removing infected plant tissue to prevent over win (Smith, *et al.;* 1988). Control of the disease using soil and botanical extracts.

(Thomas, 1998), reported that it is difficult to find a biological control method because research in a green house can have different effects than testing in the field. However, the best control method found for soil borne

fungi is planting resistant varieties, although not all have been bred for every forma specialist.

Group of studies were carried out to investigate the antifungal activity of plant extract. In fact the antifungal activities of some plants extracts in controlling different pathogens have been reported by several workers who pointed out that the active compounds present in plants were influenced by many factors which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials, (Tewarri and Nayak, 1991; Amadioha, 2000).

(Babu Joseph, *et al.;* 2008) reported that Antifungal activity of the in vitro efficacy of different plant extracts *Azadirachta indica*, were found to control wilt pathogens. Systemic fungicides to eradicate the pathogen from the soil, flood, fallowing, and using clean seeds each year are very common methods (Booth, 1971).

However, alternative methods of controlling the disease have been studied with emphasis on novel compounds derived from plant sources (Alabouvette, 1999). Plant extracts and plant essential oils have been reported to be effective antimicrobials against food and grain storage fungi, foliar pathogens and soil borne pathogens (Bowers and Locke, 2000). Many plants and their products have been reported to possess pest control properties. These are good alternatives to chemical pesticides, as they are readily biodegradable in nature (Singha, *et al.;* 2010).

2.4*Asperogillus flavus*

A. flavus fungi belong to *A. flvus*, section *flavi*, commonly invade oil seeds and grain, such as peanut and in which they produce the carcinogenic aflatoxin (payne, 1998). Aflatoxins show considerable toxicity in some animal and humans (Hussain and Barasel, 2001). And epidemiologically

studies of human populations that prolonged ingestion of aflatoxin result in an increase in heptacellular carcinoma when interacting with repetitive viruses (Turner, *et al.;* 2002). *A. flavus* unlike most fungi is favored by hot dry conditions. The optimum temperature for growth is 37°c (98.6F), but the fungus readily grows between the temperatures of 25-42c° (77-108F), and will grow at temperatures from 12-48°c (54-118F). Such a high temperature optimum contributes to its pathogenicity on humans. (Payne, 1998, Scheidegger, and Richard, and Payne, 2003).

2.4.1 Ecology and distribution

Like other *Aspergillus species, A. flavus* has a worldwide distribution. This probably results from the production of numerous airborne conidia, which easily disperse by air movements and possibly by insects. Atmosphere composition has a great impact on mould growth, with humidity being the most important variable (Gibson, *et al.;* 1994). *A. flavus* grows better with water activity between 0.86 and 0.96 (Vujanovic, *et al.;* 2001). The optimum temperature for *A. flavus* to grow is 37°c, but fungal growth can be observed at temperatures ranging from12 to 48°c. Such a high optimum temperature contributes to its pathogenicity in humans.

2.4.2 Soil and air

A. flavus appears to spend most of its life growing as a saprophyte in the soil, where it plays an important role as nutrient recycler, supported by plant and animal debris (Scheidegger and Payne, 2003). The ability of *A. flavus* to survive in harsh conditions allows it to easily out-compete other organisms for substrates in the soil or in the plant (Bhatnagar, *et al.;* 2000). *A. flavus* has been particularly prevalent in the air of some tropical countries (Gupta,

et al.; 1993; Adhikari, *et al.;* 2004). Climatic conditions markedly influence the prevalence of *A. flavus* in outdoor air (Calvo, 1980).

2.4.3 Genome

The sequencing of *A. flavus* is in progress, and will provide a rich source the primary assembly indicates that the *A. flavus* genome is 36.3 Mb in size and consists of eight chromosomes and 13 071 predicted genes. The mean gene length is 1384 bp (Yu, *et al.;* 2005).

2.4.4 Taxonomy and classificatio

2.4.4.1 Scientific classification

Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Eurotiomycete
Order	:	Eurotiales
Family	:	Trichocoaceae
Genus	:	*Aspergillus*
Species	:	*Aspergillus flavus*

Agrios, (2005)

2.4.4.2 Classification

Classically, the systematic of *Aspergillus* and its associated teleomorphs have been based primarily on differences in morphological and cultural characteristics (Raper and Fennel, 1965; Samson, 2000). Moreover, the taxonomy of the *A. flavus* complex group is further complicated by the existence of morphological divergence amongst isolates of the same species (Klich and Pitt, 1988).

2.4.5 Identification

Accurate species identification within *A. flavus* complex remains difficult due to overlapping morphological and biochemical characteristics. In

general, *A. flavus* is known as a velvety, yellow to green or brown mould with a goldish to red brown reverse. The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 mm in diameter. Based on the characteristics of the sclerotia produced, *A. flavus* isolates can be divided into two phenotypic types. The S strain produces numerous small sclerotia (average diameter 400 mm). The L strain produces fewer, larger sclerotia (Cotty, 1989).

2.4.6 Molecular typing

Phenotypic methods to discriminate *A. flavus* showed only a moderate discriminatory power for distinguishing isolates (Rath, 2001). Genotypic methods that have been used for typing *A. flavus* isolates (Moody and Tyler, 1990; James, 2000; Rath, 2001; Heinemann, *et al.;* 2004). And microsatellite polymorphism analysis (Guarro, 2005). Restriction endonuclease analysis of total cellular DNA has not proven.

2.4.7 Contamination levels

Aflatoxin is the name for a group of toxins known as B1, B2, G1, G2, M1 and M2 (carcinogenic compounds) that are produced mainly by two fungi called (*A. flavus* and *A. parasiticus*). These toxins occur naturally and have been found in a wide range of commodities (including peanuts) used for animal and human consumption. Depending on their levels, toxins can severely affect the liver and induce a human carcinogen, i.e., causes cancer. In many developing countries, aflatoxin is a major health risk to both humans and animals due to the high levels of the contaminated products consumed (Wright, 2002).

The causative agents grow on food and feed grains at moisture level of 15% or greater in the presence of warm temperatures (21°c- 37°c). The toxin can be found in a variety of grains but most often occurs in peanut and corn. Contamination can occur while the grain is standing in the field, and soon after harvesting and during storage before or after the grain is processed into food or feed (Allen, 2003). Considered as the most important mycotoxins aflatoxin is a natural potent carcinogen known to affect both humans and animals. Mycotoxins, secondary metabolites produced by fungi, are toxic to both animals and humans and their occurrence in the food chain may have public health effects (Wu, 2011). The International Agency for Research on Cancer (IARC, 1993), has reported that Aflatoxins, especially aflatoxin B1 (AFB1), are the most potent natural carcinogenic substances and are being linked to severe illnesses and also increase the risk of liver cancer in humans. Several food crops such as peanut *(Arachis hypogaea L.*), are susceptible to contamination by aflatoxigenic fungi (*Aspergillus spp.*) (Kamika and Takoy, 2011).

Aflatoxins are more prevalent in tropical and sub-tropical areas where environmental conditions such as high temperature and humidity prevail, which favors the growth of fungi and production of mycotoxins on the crops (Klich, 2007). There was variation in aflatoxin levels among groundnut and peanut butter. The wide variation in aflatoxin contamination in peanut butter could be attributed to contamination during processing in the cottage industry and using groundnuts that were already infected by aflatoxin-producing fungi. For instance, the processor whose peanut butter recorded the highest aflatoxin contamination did not grind roasted nuts immediately after roasting but stored them to grind later based on customer purchase orders. It was also observed that the processor had not cleaned the grinder

after the previous peanut butter production. This practice could predispose the peanut butter to increased aflatoxin levels, on the other hand, the processor from whose peanut butter Aflatoxins were not detected observed hygiene in processing and was also a trained food technologist. Defective nuts are more likely to be infected with aflatoxin–producing fungi than sound nuts. (Omer, 2001). Reported that aflatoxin B1 causes liver cancer in Sudan. High aflatoxin content (25–600 ppb) was reported in groundnut in Sudan. The occurrence of liver cancer in Sudan could be substantially reduced by lessening contamination of food with aflatoxins to internationally accepted levels. (Younis and Malik 2003). Studied aflatoxin contamination in Sudanese groundnut and groundnut products and found that percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively. They confirmed that aflatoxin B1 was predominant in all samples followed by G1, B2, and G2.

The economic impact of aflatoxins was derived directly from crop, livestock losses, and indirectly, from the cost of regulatory programs designed to reduce risks to animal and human health. The Food and Agriculture Organization (FAO) estimates that 25 % of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins. Aflatoxins losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency.

Other adverse economic effects of aflatoxins include lower yield for food and fiber crops (Anon, 1989). Aflatoxin contamination of groundnut is a major problem in most of the groundnut production regions across the world. It is mostly influenced by the occurrence of drought during the late seed filling duration. It is caused by the growth of the moulds *Aspergillus*

flavus and toxicity of groundnut from aflatoxin endangers the health of humans and animals and lowers market value, (Abdalla, 2005). Hence, it is a problem to groundnut producers as well as consumers. The moulds are common saprophytic fungi found in soils throughout the major groundnut producing areas of the world (Griffin and Garren 1974) reviewed the influence of changing environmental conditions on the activity of the moulds on groundnuts; Aflatoxin is more serious during and following alternating dry and wet periods. (Pettit and, 1973; 1971) observed that peanuts grown under dry land conditions and subjected to drought stress accumulated much more aflatoxin before digging than peanuts grown under irrigation. (Wilson and Stansell, 1983) reported that water stress during the last 40-75 days of the crop contributed to higher aflatoxin 21 levels in mature kernels.

In a field study in *A. niger*, (Craufurd, 2006) confirmed that infection and aflatoxin concentration in peanut can be related to the occurrence of soil moisture stress during pod filling when soil temperatures are near optimal for *A. flavus*, (Cole, 1985; Dorner, *et al*.; 1989) have shown that preharvest contamination of aflatoxin requires drought period of 30-50 days and a mean soil temperature of 29 - 31ºC in the podding zone. In Sudan, the irrigated region (Central Sudan) used to be free from aflatoxins while the rainfed region (Western Sudan) showed high levels of aflatoxin contamination, (Hag Elamin, 1988). In the same study, temperature of 30o C and relative humidity of 86% were identified as optimum conditions for aflatoxin production. (Rachaputi, *et al.;* 2002) observed aflatoxin contamination to be widespread in the Queensland region of Australia during the 1997-98 seasons with severe and prolonged end of season drought and associated

elevated soil temperature and lower aflatoxin risk during 1999-2000 seasons with well-distributed rainfall and lower soil temperatures.

2.4.8 Control methods

Although drought stress is known to predispose peanut to aflatoxin contamination limited researches were reported on the mechanism of late season drought stress aggravating the *A. flavus* infection. (Dorner, *et al.;* 1989). Observed that drought stress could decrease the capacity of peanut seeds to produce phytoalexins, and thus resulted in higher aflatoxin contamination. The active water of seeds is the most important factor controlling the capacity of seeds to produce phytoalexins. Application of fungicides at the beginning of windrow or storage periods may reduce this problem (JACKSON, 1965). The chemical treatment efficiency depends on the ability of the sprayed substances to cross the shell barrier.

2.5 The extract of botanicals

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (Wolfe and Liu, 2003; Valko, *et al.;* 2007). The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods (Sun and Ho, 2005; Suhaj, 2006).

2.5.1 Tree of Neem

Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. research in Sudan started in the 60 concentrating on it is use as pesticide. Currently three is extensive research, reported and projects published by the National Centre for Research (Khartoum, Sudan) NCR and many other universities (El-abjar, 1992).

2.1.2 Scientific classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Order	:	Rutales
Suborder	:	Rutinease
Family	:	Meliaceae
Genus	:	*Azadirachta*
Species	:	*Azadirachta indica*
Binominal name	:	*Azadirachta indica* A.Juss

https://en.m.wikipedia.org/wiki/Neem

2.5.1.2 Uses of the Neem

Neem as biohebicides or control to plant diseases and Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated and is recommended for those who are sensitive to Permethrin. A known insecticide which might be irritants and also the scabies mite has yet to become resistant to Neem, so in persistent cases Neem has been shown to be very effective, there is also anecdotal evidence of its effectiveness. In treating infestations of head lice in humans, it is also very good for treating worms (soak the branches and leaves in lukewarm water and drink it). In the traditional medicine Neem trees originated on the Indian subcontinent.

The Neem twig is nature's tooth brush to over 500 million people daily in India alone. Herbal medicine is the oldest form of therapy practiced to be mankind and much of the oldest medicinal use of plants seems to have been based on highly developed 'dowsing instinct' (Grigs, 1981,. Siddig, 1993).

2.5.2 The Ginger

Ginger is commonly use in food as spice. (Kim, *et al,;* 2007, and Schwertner and Rios 2007), reported that the main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10-gingerol and these constituents had exhibited strong antioxidative activity. Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (Wolfe and Liu, 2003; Valko et al., 2007).

2.5.2.1 Scientific classification

Kingdom	:	Plantae
Clade	:	Angiosperms
Clade	:	Monocots
Clade	:	Commelinids
Order	:	Zingiberales
Family	:	Zingiberlaceae
Genus	:	*Zingiber*
Species	:	*Z. officinale*
Binominal name	:	*Zingiber officinale* Roscoe

(https://en.m.wikipedia.org/wiki/Ginger)

2.5.2.2 Uses of the Ginger

Many scientists have reported antimicrobial properties of several plants. The antimicrobial, (Khalil, *et al,;* 2005; Akroum, *et al,;* 2009; Omoya and Akharaiyi, 2012), anti-inflammatory and anti-necrotic (Lin and Huang, 2002; Omoya and Akharaiyi, 2012) activities have been reported from the use of plants extracts. In many parts of the world, Zingiber officinale has medicinal and culinary values (Omoya and Akharaiyi, 2012).

The volatile oil gingerol and other pungent principles not only give ginger its pungent aroma, but are the most medically powerful because they inhibit

prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation (Longe, 2005; Omoya and Akharaiyi, 2012). Ginger has been found to be more effective than placebo in multiple studies for treating nausea caused by seasickness, morning sickness and chemotherapy (Ernst and Pittler, 2000), though ginger was not found superior to placebo for presumptively treating postoperative nausea (Omoya and Akharaiyi, 2012). These studies also show superiority of odansetron over ginger in the treatment of chemotherapy related nausea. Ginger compounds are active against a form of diarrhea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active constituent against enterotoxigenic Escherichia coli heat-labile enterotoxin-induced diarrhea (Ernst and Pittler, 2000; Chen, *et al,;* 2007).

2.6 The fungicide

The chemical Topas 100 Ec. Is Fungicide used as standard control their active ingredient contended 100 of liter penconazol, made of Suasra, signature in Sudan under No. 673. The Active ingredient (a.i) of Topas 100 Ec. is dosage 100gm/litter Binchonazol.(R.S)-1-(2-(4,2-Dichlorophynile)-N-Phynile)-1-ASH-1,2,4 Trapzole. Mode of action systemic Fungicide with penetrated of plant and effect on fungi hypha growth stage.

2.6.1Chemical identification

Penconozole, Calcium alkyl benzene, Sulphonate, Aromaticpetroleum hydrocarbon liquid Poisoning symptoms in laboratory animals were non-specific. Stable under standard conditions.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study location

This study was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology, during, March and April 2015. The aim of this Study was to detect and identify fungi *Aspergillus flavus* , associated with seeds samples collected from four location , each one from different Estate of Sudan, to explore the methods of control under laboratory conditions where temperature around 25-28°c.

3.2 The materials

3.2.1 Tools and equipments used

All materials and tools except seeds, which used in the experiments, were sterilized using 70% ethyl alcohol. Formalin (10%) was used for Petri plate sterilization. Cotton blue and lacto phenol were used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen (Aneja2004).

3.3 The methods

3.3.1 Samples collection

Eight groundnut seeds samples were obtained from four different regions in Sudan (Nyala , Algeneina , Alrahad and New halfa), two samples from each region , one from storage (post harvest in year of 2013) and the other from field (harvested in year 2014). Randomizes and homogeneous as 1 kg. was secured from each samples. Seed samples were drawn according to international standards for seed testing association (ISTA, 1966). Collected samples were labeled and kept separately in sealed paper bags and transported to the laboratory where they were ready to test.

3.3.2 Dry seed inspection

Two hundred (200) seeds of each seed sample were randomly selected and examined under stereoscopic , binocular , microscope (25-4x), by magnified lens and naked eye according to the international seed testing association (ISTA Rules,1966). The samples were examined for impurities, plant debris, weed seeds, discoloration and malformation.

3.3.3 Detection and isolation of seed borne fungi

The seed samples were tested by the standard blotter method and media (PDA) potato dextrose agar method for detection of seed borne fungi as described by IST. Normal and discolored seeds were tested separately for seed borne fungi.

3.3.3.1 Blotter method

For the detection of seed borne fungi, standard blotter method as described by the International Seed Testing Association (ISTA 1996), was used for the detection of the seed-borne fungi associated with each seed sample. The seed samples in their various forms according to their crops were then platted on moistened filter papers (dia. 9.0 cm) in 9.0 cm sterilized plastic Petri-dishes. in case of groundnut, 3 seeds were arranged at the periphery of the plate and 2 at the centre with three replications, were used and then kept in dark place for seed germination.

After seven days from incubation, seeds were then examined for fungal growth under a stereo microscope. Fungi identification by habit character was supplemented by microscopic examination of spores and fruiting bodies using a compound microscope. (Mathur, P Neergaard, 1975; Mathur and Kongsdal; 2003). Infection levels were recorded as the percentage of infected seeds in each sample.

3.3.3.2 Agar bate method

All seed samples was pre-treated with sodium hypochlorite 1% solution for 5m, then washed three times with sterilized distilled water and dried between tow filter papers. The seed samples were plated in the sterilized glass Petri-dishes on potato dextrose agar medium (PDA). The plates were incubated for 7 days at 25^0C. In the 8^{th} days the Seeds were examined under light Microscopes using slides preparation. (Lioyd, *et al,;* 2011).

3.3.4 Slide preparation

The samples of fungus were taken randomly from any each seed samples. These samples were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi (Aneja, 2004).The binocular compound microscope was also used to determine the type of fungus in each plate. Fungi identified and their percentage frequency (PF) of occurrence of fungal was calculated by applying the following formula:

PF = (No. of seeds on which fungus appear / Total number of seeds) x100

3.3.5 Pure culture preparation of fungi

The amount of the mycelium of *(A. flavus, A. niger Penicillium digitatum, Rhizopus spp., Alternaria spp., Colletotricum Phoma spp.,* isolated from seed samples of groundnut were picked and cultured into sterilized glass Petri-dishes(9.0 cm in diameter) containing PDA media for further identification with the help of various keys (Raper and Fennel, 1965; Booth, 1971). Fungal growth continued for 5-7 days and then kept in the refrigerator as a stock for further investigation.

3.3.6 Identification of fungi

The identification of the fungi was based on visual culture characteristics. Furthermore, Microscopic examinations were carried out for Mycelial and conidia structure based on the method of (Booth1977).

3.3.7 Plant extracts preparations

Neem leaves were collected from Shambat area and brought to the laboratory was dried in shade. Ginger rhizomes' of parched collected from local market of bahri. Ginger and Neem leaves were crushed separately to obtain fine powder.

3.3.7.1 Ethanol extracts preparations

The powder prepared from each plant material mentioned above was used for preparing the different botanical treatments for the study. Preparation of the methanol extracts started 3 days before the experiment time. Exactly 60gm powder was extracted in ethanol for 6 hours using a soxhlet. The extracts were concentrated using rotary flash evaporator and preserved at 58°C in airtight brown bottles for further use.

An additional methanol extract from each Neem leaves and ginger were prepared separately. The fresh Neem leaves and Ginger rhizomes were washed. After drying, Neem and Ginger slices were ground to fine powder separately using electric blender. 10 g powder of each was soaked in 100 ml of methanol separately. The flasks were incubated at room temperature for 72 hours with shaking at 120 rpm. The crude extracts were centrifuged at 3000 rpm for 10 minutes at 25°C. The extracts were evaporated at 50°C. All dried extract samples were dissolved in distilled water separately to the final concentration of 100 mg/ml and centrifuged again at 10,000 rpm to remove the undissolved residues. The extract solutions were stored at 4°c.

3.3.7.2 Ginger and Neem extracts

Fresh rhizomes of Ginger and Neem leaves plants their names (rhizomes of *Zingiber officinale),* (leaves of *Azadirachta indica*), respectively was powdered with grinding and obtain 60gm from each one for ethanol extracts. After that we must be have three concentrations from each extract, as well as 25, 50 and 100%.

3.3.8 Preparation of fungicide Topas Ec. 100

The chemical tested were Topas Ec.100 fungicide 5 ml dissolved in 100 ml methanol to give 25 , 50, 100 ppm respectively .For this solution 25, 50, 100 were completed to 100 ml by adding sterilized potato dextrose agar medium to give final concentration.

The PDA media was amended with the required concentration from Neem, Ginger and fungicide Topas Ec.100, (25, 50 and 100ml from each) before being solidified in a conical flask of 250 ml, agitated and poured it into sterilized Petri dishes. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

The Petri dishes of each concentration were incubated at 25 ºC for 5 days. The growth of the fungus was measured and calculated successfully after 3 to 5 days after inoculation.

The effect of each extracts was evaluated as percentage of reduction in diameter of fungal growth (R) where:-

$$R = \frac{\mathrm{dc} - \mathrm{dt}}{dc} \mathrm{X}100\%$$

Where R = Percentage reduction of the growth, dc=diameter of controlled growth and dt= diameter of treated growth

3.4 Effect of botanical extracts and fungicide on the linear growth of *A. flavus in vitro*

The PDA media was amended with the required concentration from Neem leaves ;(25ml, 50 and 100ml from each) before being solidified in a conical flask of 250 ml, agitated and poured it into sterilized Petri dishes. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

The Petri dishes of each concentration were incubated at 25ºC for 5 days. The growth of the fungus was measured and calculated successfully after 3 to 5 days after inoculation.

3.5 Fungicides Topas Ec. 100 Ec.

The chemical Topas 100 Ec. Is Fungicide used as standard control their active ingredient contended 100 of liter penconazol, made of Suasra, signature in Sudan under No. 673.

3.6 Experimental design

These experiments were arranged in a Complete Randomized Design (CRD)

3.7 Statistical analysis

The data was collected statistically analyzed according to analysis of Mstat variance (ANOVA); - Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

This study was conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of science and Technology (During March and April 2015).The aim of this study was to detect and identify seed borne fungi associated with seeds samples of groundnut, (*Arachis haypogaea L*.). Eight samples collected from four regions in Sudan, seasons (2013-2014 and 2014-2015) from each region, to evaluate the antifungal activity of ethanol extracts of Ginger and Neem with fungicide Topas Ec. 100 (as standard), to inhibit the growth of the Fungus *Aspergillus flavus in vitro*. The study also covers Seed Health Testing, detection and identification of seed borne fungi.

4.1 Detection and isolation of seed borne fungi from Groundnut samples

4.1.1 Dry Seed inspection

Two hundred (200) seeds from each sample of the four Ground nut regions (Algeneina, New Halfa, Nyala and Alrahad) were tested. The result revealed the presences of impurities, plant debris, broken, discoloration, and disease symptom as shown in table (1) and plate (1). The results showed that Algeneina scored a higher percentage of healthy seed (83.5%) but New Halfa scored a higher percentage of unhealthy seed (22.5%) and Nyala scored a higher percentage of broken seed (2%) season (2013-2014).

Table 1 Dry inspection of seed groundnut showing different categories of 200 seeds tested, Seasons (2013-2014 and 2014-2015).

Regions		Algeneina		Alrahad		New halfa		Nyala	
Categories	Seasons	2013 2014	2013 2014	2013 2014	2013 2014	2013 2014	2013 2014	2013 2014	2013 2014
Healthy	Number	160	168	149	161	158	175	156	160
	percentage%	80	83.5	79.5	80.5	66.5	82.5	78	80
Unhealthy	Number	38	32	39	38	45	23	40	37
	percentage%	19	16	19.5.	19	22.5	11.5	20	18.5
Broken	Number	2	1	2	1	2	2	4	3
	percentage%	1	0.5	1	0.5	1	1	2	1.5

Plate 1 the composite samples and two hundred seeds to process of dry inspection test.

4.1.2 Blotter Method (filter papers)

One hundred (100) groundnut seeds from each region were treated with Sodium hypochlorite 5%; the treatment by this chemical disinfectant has excluded all saprophytic fungi carried on the surface of seeds. Fungi detected in groundnut seeds are, *Alteenaria solani , Aspergillus flavus, Aspergillus niger, Pencillium digitatum, Rhyzopus nigrican* in the two season but in season (2013-2014) the incidence was higher and, *A. flavus* was scored the higher incidence percentage in the 3rd, 4th and 5th day from incubation (4.5 , 5.5 and 8%) respectively, as shown in Tables (2,3 and 4).

4.1.3 Agar Plate Method (PDA)

One hundred seeds of groundnut (*Arachis haybogaea)* from four regions were tested using agar plate method. The fungi were detected from groundnut Seeds were, *Alternaria solani, Aspergillus flavus, Aspergillus niger, Pencillium digitatum, Rhyzopus nigricans. nigrican* in the two season but in season (2013-2014) the incidence was higher and, *Aspergillus flavus* was scored the higher incidence percentage in the 3rd, 4th and 5th day from incubation (4.25, 5.5 and 8%) respectively, as shown in Tables (5, 6 and 7).

Plate 2 processes of dry inspection test for 200 seeds from each one of eight samples

Table 2 Fungi incidence on seed of groundnut by blotter method in the 3rd day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	0	0	1	0	0	0	0
Aspergillus flavus	10	2	6	0	1	0	1	2
Aspergillus niger	2	0	0	2	0	1	0	0
Penicillium digitatum	1	1	0	0	6	2	6	1
Rhizopus nigricans	0	0	0	1	1	4	1	1
Mean	26	06	12	08	16	14	16	08

Table 3 Fungi incidence on seed of groundnut by blotter method in the 4th day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	0	0	1	0	0	0	0
Aspergillus flavus	12	8	8	4	3	4	9	4
Aspergillus niger	2	3	3	2	0	1	1	1
Penicillium digitatum	0	4	1	0	7	3	1	4
Rhizopus nigricans	1	0	3	1	3	4	3	1
Mean	3	3	3	1.8	2.6	2.4	2.8	2

Table 4 Fungi incidence on seed of groundnut by blotter method in the 5th day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	0	0	1	0	0	0	4
Aspergillus flavus	12	8	8	4	3	4	9	1
Aspergillus niger	2	3	3	3	0	1	1	4
Penicillium digitatum	0	4	1	0	7	3	1	1
Rhizopus nigricans	1	0	3	2	2	4	3	0
Mean	3	3	3	2	2.4	2.4	2.8	2

Table 5 Fungi incidence on seed of groundnut by Aga plate-method in the 3rd day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	2	3	6	3	9	0	4
Aspergillus flavus	7	7	4	3	4	0	2	0
Aspergillus niger	0	0	0	1	0	2	0	0
Penicillium digitatum	3	5	0	0	0	0	0	1
Rhizopus nigricans	5	1	0	1	0	0	0	1
Mean	3	3	1.4	2.2	1.4	2.2	0.4	1.2

Table 6 Fungi incidence on seed of groundnut by Aga plate-method in the 4th day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	0	7	6	3	10	0	4
Aspergillus flavus	9	7	7	6	1	2	5	4
Aspergillus niger	0	5	1	0	0	1	0	0
Penicillium digitatum	0	0	0	0	1	0	3	1
Rhizopus nigricans	5	1	0	3	10	0	0	1
Mean	2.8	2.6	3	3	3	2.6	1.6	2

Table 7 Fungi incidence on seed of groundnut by Aga plate-method in the 5th day from incubating (disease incidence %)

Regions	Algeneina		Alrahad		New halfa		Nyala	
Seasons	2013	2014	2013	2014	2013	2014	2013	2014
Species	2014	2015	2014	2015	2014	2015	2014	2015
Alternaria alternata	0	0	0	1	0	0	0	4
Aspergillus flavus	12	8	8	4	3	4	9	1
Aspergillus niger	2	3	3	2	0	1	1	4
Penicillium digitatum	0	4	1	0	7	3	1	1
Rhizopus nigricans	1	0	3	1	3	4	3	0
Mean	3	3	3	1.6	2.6	2.4	2.8	2

4.2 Isolation and identification of *A. flavus strain*

4.2.1 Identifications of *A. flavus* in groundnut

Tables 8 and 9 showed that the growth of the *A. flavus* in groundnut seed, after culturing in Agar plate (PDA media) and Blotter methods (Blotter-filter papers), after the 3rd, 4th and 5th day from incubation.

Identification was performed depending on the cultural characteristics and conidia, hyphae, mycelium, sclerotia and spores shapes as described by (Booth 1977; and Agrios 2005)

Table 8 The growth of *A. flavus* in the 3rd day from treatment on Blotter-method (growth/day)

Days after	Algeneina		Alrahad		New halfa		Nyala	
incubation	2013	2014	2013	2014	2013	2014	2013	2014
Seasons	2014	2015	2014	2015	2014	2015	2014	2015
after 3rd day	10	2	6	0	1	0	1	2
after4th day	12	8	8	4	3	4	9	4
after 5th day	12	8	8	4	3	4	9	1
Mean	34	18	22	8	7	8	19	7

Table 9 The growth of *A. flavus* in the 3rd day from treatment on Agar plate-method(growth/day)

Days after	Algeneina		Alrahad		New halfa		Nyala	
incubation	2013	2014	2013	2014	2013	2014	2013	2014
Seasons	2014	2015	2014	2015	2014	2015	2014	2015
after 3rd day	7	7	4	3	4	0	2	0
After 4th day	9	7	7	6	1	2	5	4
after 5th day	12	8	8	4	3	4	9	1
Mean	28	23	19	13	8	6	16	5

4.2.2 Isolated of *A. flavus* from groundnut

Plate 3 showed the *A. flavus strain* isolated from groundnut seed in the 5th day after incubation on PDA media and re-culturing on pure culture of PDA media, and then obtained pure strain of *A. flvus*. Also Plate 3 showed the slide of *A. flavus* isolated from groundnut samples.

4.3 Effect of ethanol extracts of botanicals and fungicide were used to inhibition growth of *A. flavus* in vitro

plate 5 showed the comparison between effect of ethanol extract of botanicals (Ginger and Neem), Fungicide Topas 100 Ec. and control in the 5th day from the test of experimental were shown as the results of three concentrations 25%, 50% and 100% for each one of inhibitory materials (fungicide and ethanol extract of Ginger rhizomes and Neem leaves), showing as flowing:

A=Fungicide, B= Neem, C=Ginger and D= control. (Plate 5)

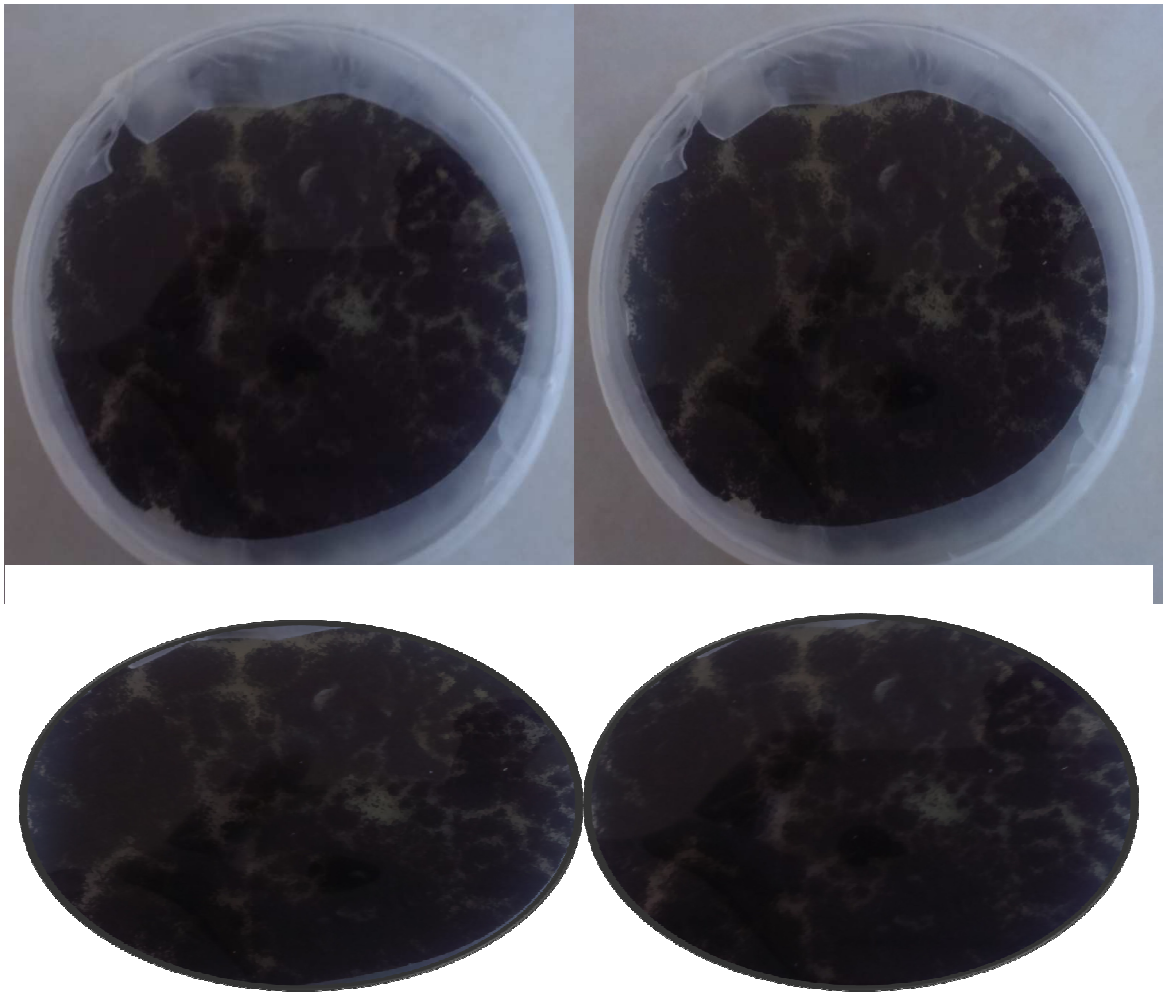
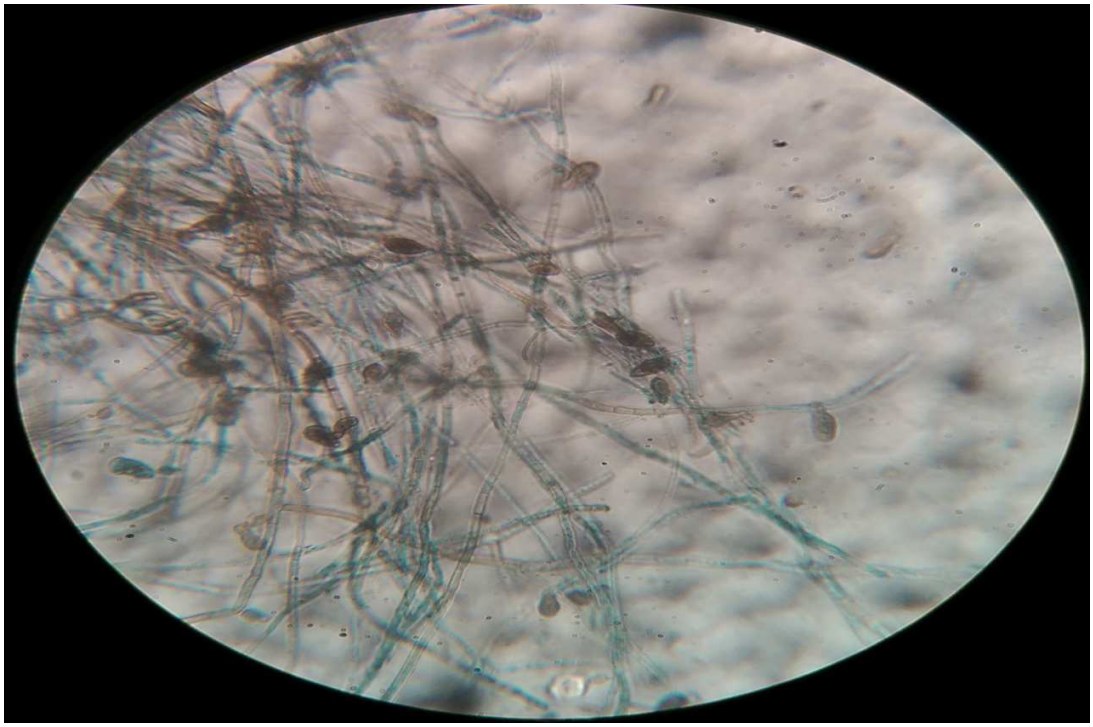


Plate 3 *A. flavus* strain isolated from seed samples and re-culturing on PDA in the 5th day from incubation



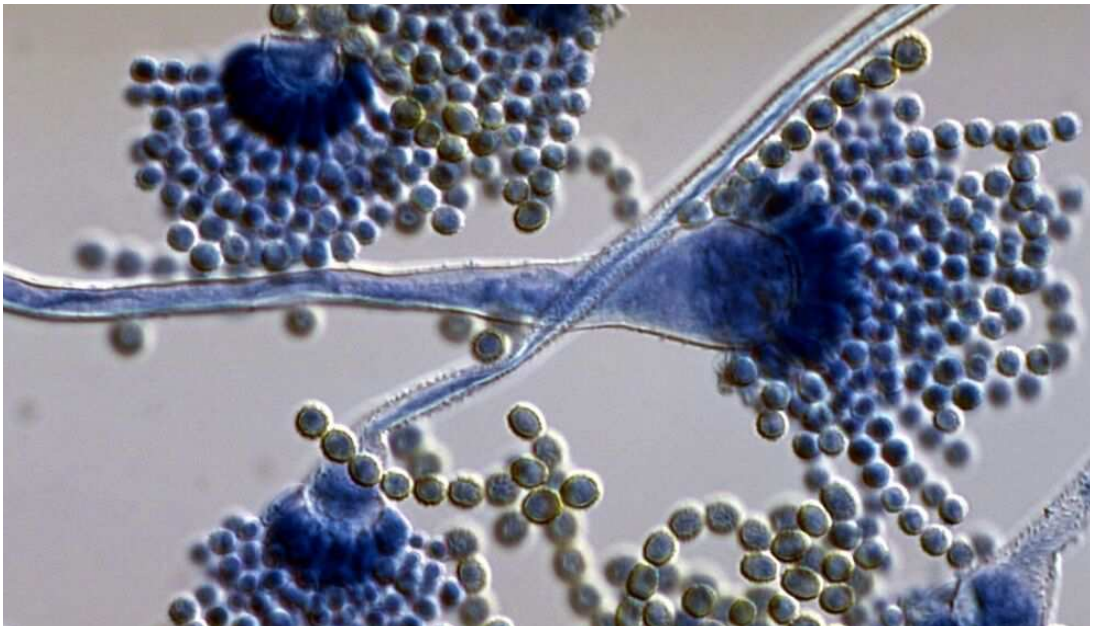


Plate 4 slides *of A. flavus* isolated from groundnut seed samples

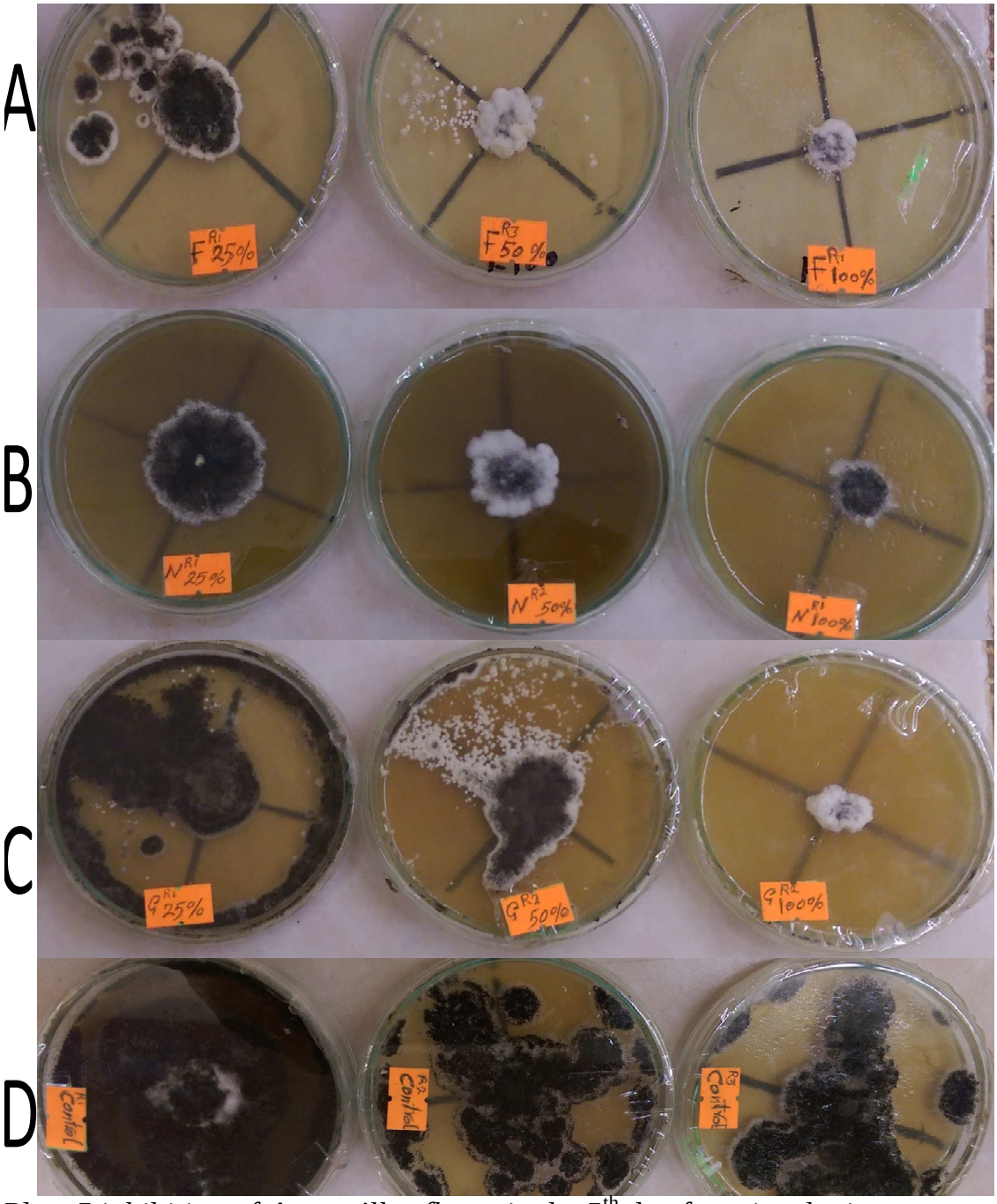


Plate 5 inhibition of *Aspergillus flavus* in the 5th day from incubation comparison between ethanol **A=**fungicide**, B=** extract of Neem and **C=** extract of Ginger with **D=**control.

4.4 Effects of the fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro

The antifungal activity of Ginger rhizomes and Neem leaves extracts to study the effects of plant extracts on the growth of *A. flavus in vitro* after 48houre from incubation. The results showed that fungicide and the ethanol extracts of Ginger and Neem were effective in reducing the mycelia growth of *A. flavus.* All concentrations of fungicide and extracts of botanicals (Ginger and Neem) were given different significantly of inhibition growth percentage against fungus *A. flavus.* That there was a significant decrease in the mycelia growth of the *A. flavus* with an increase in fungicidal concentration. Figure (1, 2 and 3)

4.4.1 Effect of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 3rd day.

Table 10 and figure 1 shown result in the 3rd day from incubation the fact, all concentrations of fungicide and extracts Ginger and Neem were given different significantly of inhibition growth percentage against fungus of *A. flavus* in vitro. Ginger were given (12.4, 49.6 and 70.2 %), Neem were given (25, 49.8 and 60.7 %), fungicide were given (41.3, 73.2 and 89.3 %) respectively. The sensitivity of mycelial growth of *A. flavus* was checking against fungicide. That there was a significant decrease in the mycelia growth of the fungus with an increase in fungicidal concentration.

Table 10 Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 3rd day from incubation

Treatments		Inhibition growth %
Ginger	25%	12.4(20.2) e
	50%	49.6(44.8)c
	100%	70.2(57.3)e
Neem	25%	25(29.8)de
	50%	49.8(44.9)c
	100%	60.7(51.3)bc
Fungicide	25%	41.3(40)cd
	50%	73.2(59)b
	100%	89.3(74.5)a
Control		0 (0.7) c
LSD		11.58
SE±		3.89
C.V %		16.12%

Statistical analysis was performed transformations by using Table X Angular. (Appendix, 8 and 9)

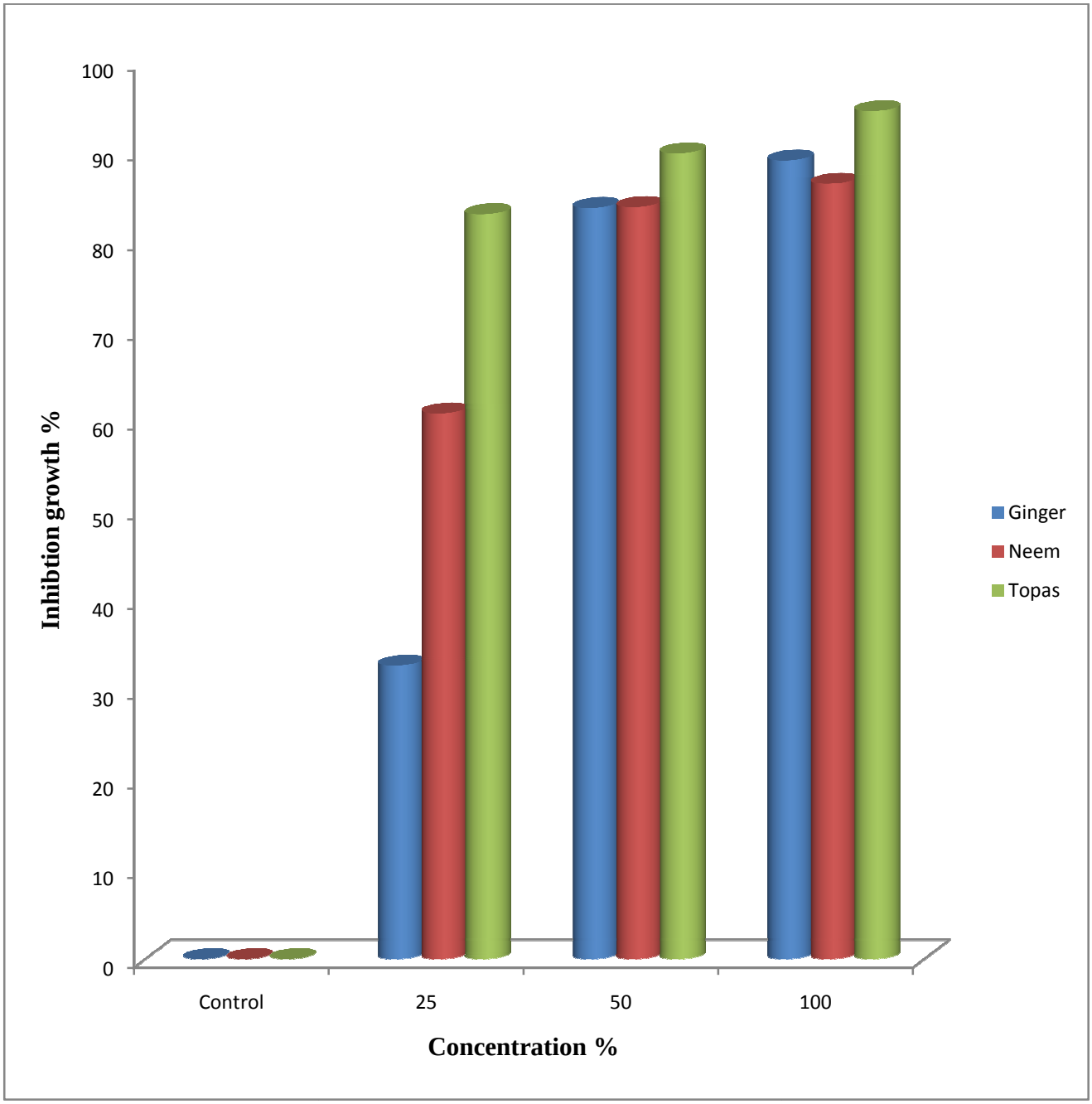


Figure 1 Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 3rd day from incubation.

4.4.2. Effect of the fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 4th day.

Table 11 and figure 2 shown results in the 4th day from incubation. The fact, all concentrations of fungicide and extracts Ginger and Neem were given different significantly of inhibition growth percentage against fungus of *A. flavus* in vitro. Ginger concentrations (25, 50, and 100%) were given (28.7, 82.5 and 85.1 %) respectively. Neem concentrations (25, 50 and 100 %) were given (35.9, 79.3 and 84.3 %) and fungicide concentrations (25, 50, and 100%) were given (60.3, 84.5 and 89.2 %) respectively. The Ginger gives the highest reduction than the Neem.

Table 11 Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 4th day from incubation

Treatments		Inhibition growth %
Ginger	25%	28.7(32.2)e
	50%	82.5(62.7)bc
	100%	85.1(70.7)ab
Neem	25%	53.9(47.3)d
	50%	79.3(60.7)c
	100%	84.3(66.7)abc
Fungicide	25%	60.3(51)d
	50%	84.5(66.9)abc
	100%	89.2(70.9)a
Control		0 (0.7) c
LSD		11.58
SE±		4.08
C.V %		14.55%

Statistical analysis was performed transformations by using Table X Angular.

(Appendix, 8 and 9)

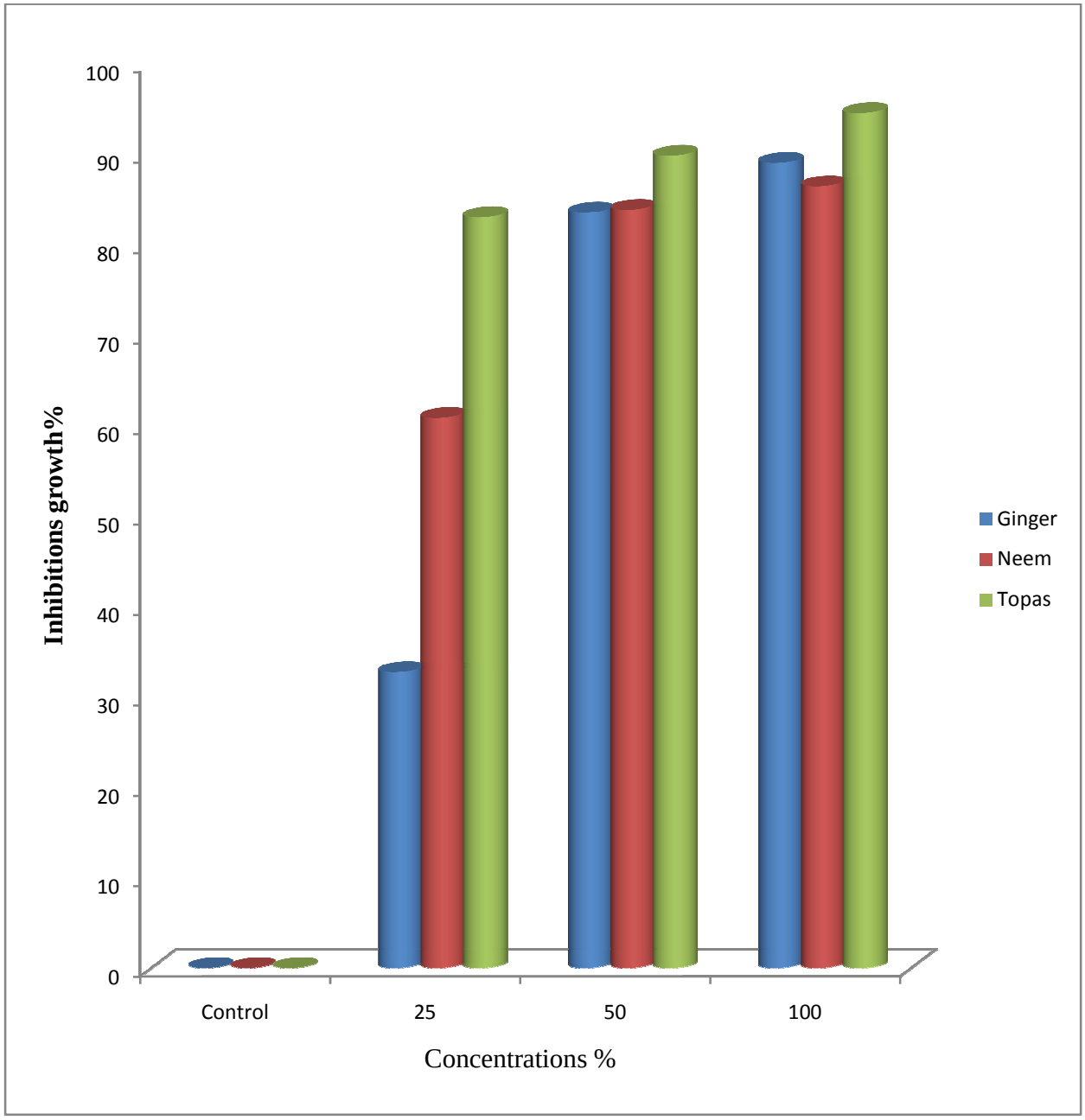


Figure 2 Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 4th day from incubation.

4.4.3. Effect of the Fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 5th day.

Table 12 and Figure 3 shown results in the 5th day from incubation. The fact all concentrations of Ginger, Neem and fungicide were gives different significantly of inhibition percentage against fungus. In fungicide concentrations (25, 50 and 100%) were gives (60.3, 84. and 89.2 %) respectively. Ginger concentrations (25, 50 and 100 %) were gives (32.7 83.5 and 89%) respectively, Neem concentrations (25, 50, and 100%) were gives (60.8, 83.8 and 86.4 %) and fungicide r concentrations (25, 50 and 100 %) were gives (83, 89.8 and 94.5 %) respectively.

Table 12 Effects of fungicide and ethanol extracts of Ginger and Neem on the liner growth of *A. flavus in vitro* in the 5th day from incubation

Treatments		Inhibition growth %
Ginger	25%	32.7 (34.8)b
	50%	83.5 (66)a
	100%	89 (70.8)a
Neem	25%	60.8 (54.3)b
	50%	83.8 (66.3)a
	100%	86.4(68.4)a
Fungicide	25%	83 (65.2)a
	50%	89.8 (67.9)a
	100%	94.5(76.6)a
Control		0 (0.7) c
LSD		11.91
SE±		4.07
C.V %		12.10%

Statistical analysis was performed transformations by using Table X Angular.

(Appendix, 8 and 9)

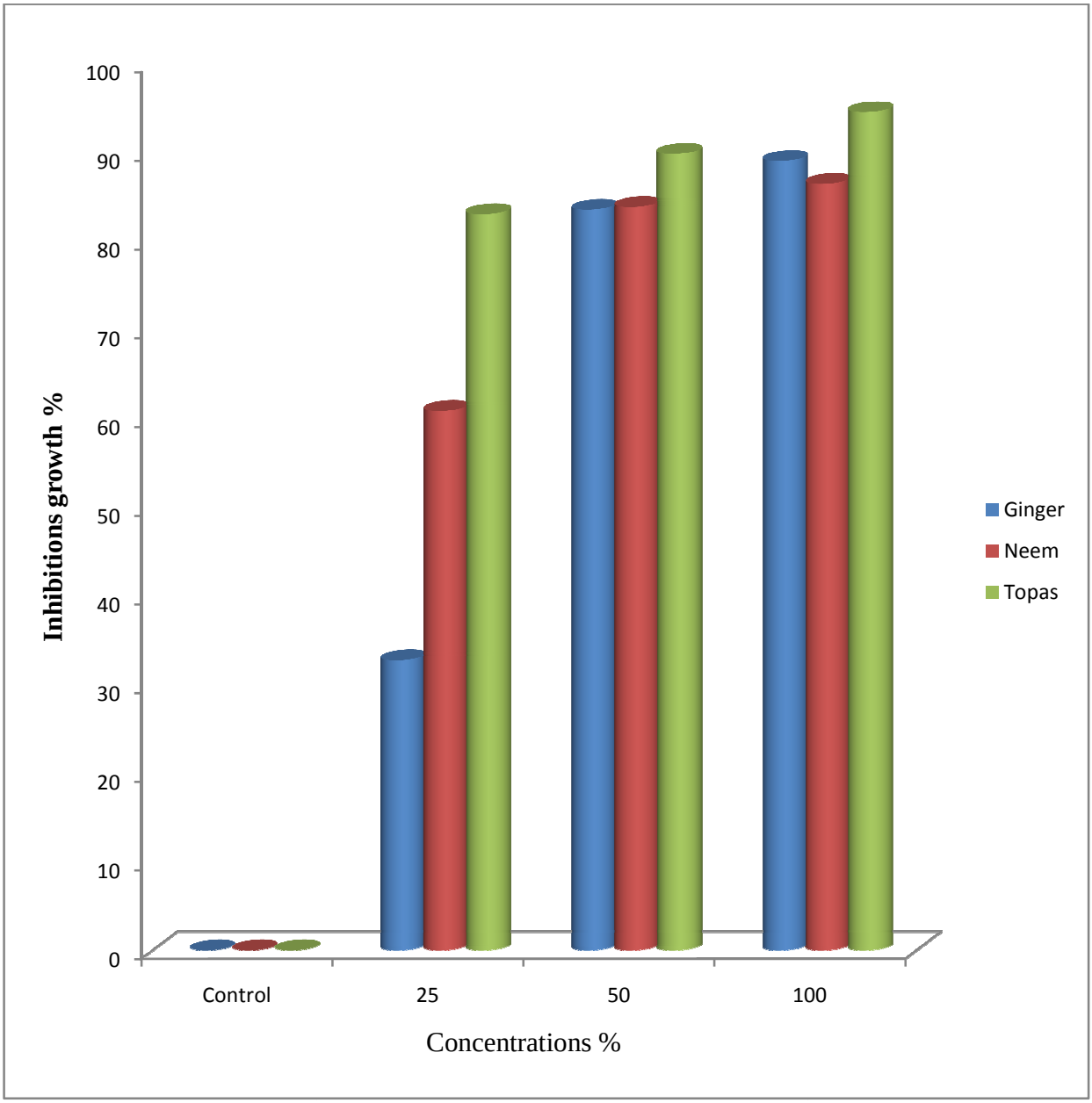


Figure 3 Effects the Ginger and Neem ethanol extracts and fungicide on the liner growth of *A. flavus in vitro* in the 5th day from incubation

CHAPTER FIVE

DISCUSSION

Mycotoxin-producing by fungi *Aspergillus spp.* is significant contaminant and destroyers of agricultural products and seeds in the field, during storage and processing, and in the markets reduces their nutritive value (Jimoh and Kolapo, 2008). Mycotoxin as Aflatoxin contamination indeed increases under drought stress (Girdthai *et al.*, 2010) because of decrease in the water activity, that creates cracks in pod wall that allow the penetration of the *Aspergillus flavus*. Damaged pods are likely to contain more aflatoxin than pods with undamaged shells (Sudhakar *et al.*, 2007).

Aflatoxin, a toxin produced by fungi *A.flavus*,is acutely toxic to some animals but also carcinogenic to humans (Thirumala-Devi *et al.*, 2002). High level of aflatoxin contains groundnut-derived products for consumption is one of the main problems related to groundnut commercialization. Breeding groundnut for aflatoxin contamination resistance would havea broad impact on groundnut kernel quality, thereby enhancing the economic return and wellbeing of smallholder farmers, and health of consumers. However, contamination by aflatoxin is a multi-stage process and it is not clear what among these is the most critical to curb the final aflatoxin content (Liang *et al.*, 2006;Cotty *et al.*, 2007).

Fungal contamination of edible greasy seeds, mostly pistachio and almond were reported in different countries. Previous study revealed that the presence of Aspergillus in soil may be the main causes of the contaminations in groundnuts. Regarding to direct contact of the soil with the groundnuts in growth phases, fungi can penetrate through the groundnut's shell and grow there (Pitt, *et al.*; 1991).

This study was investigated occurrences of the seed borne fungi on the

groundnut in two seasons (2013-2014 and 2014- 2015), the study found that the high incidence of fungal contamination of groundnuts, it seems in the late season compared to the early season, this contamination could be due to long-term storage, marketing under non-hygienic conditions of the food products in the poor environmental conditions including high moisture and temperature. Also the traditional methods of handling grains during harvesting in the field, drying process and transferring lead to mechanical damages of grains that due to the high incidence of fungal contamination of groundnuts. In this condition, broken and ground grains are more vulnerable to fungal attack than whole grains. These results are similar to those (Lund, 2000) who reported that the 27 samples of groundnut and their products showed positive to Aflatoxins This similar to the eight samples of groundnut studied in this study. Also (Suliman, 2007) reported that the stored peanut kernels showed positive to Aflatoxins this resembles result in our study the shown *A. flavus* that response present of Aflatoxins. . Identification was performed depending on laboratory investigation (cultural characteristics and conidia, hyphae, mycelium, sclerotia and spores shapes as described by (Booth 1977).

Eight seed samples of groundnut were obtained from four regions in Sudan (Algeneina, Alrahad, New Halfa and Nyala), for two seasons (2013-2014 and 2014-2015). The samples were tested for seed-borne fungi by the internationally accepted techniques. International Seed Testing Association according to (ISTA 1966). The study cleared presence of species of fungi after incubation by blotter and Agar plate methods. The fungi were presences are: - *Aspergillus flavus, Aspergillus niger, Alternaria alternate, Penicillium digitatum, Rhizopus nigricans.*

Present investigations revealed that the in vitro growth of *Aspergillus flavus* was significantly checked by ethanol extracts of Ginger rhizomes and Neem leaves at all concentration, results showed that, Ginger have the highest antifungal activity against *A. flavus* which gave 25% inhibition reduction at concentration 25% in the 3rd day from incubation. In concentration 50% the effects shown as (49.6 and 49.8), inhibition of the Ginger and Neem are similar. In concentration 100% the inhibition of Ginger highest than the inhibition of Neem (70.2 and 60.7%) respectively.

In the 4th day after incubation the experimental it was noticed that the effect were shown when the concentrations 25% (32.7 and 60.8) the low inhibition in Ginger and medium in Neem, respectively. In concentration 50% (83.5 and 83.8), inhibition of the Ginger and Neem are similar. In concentration 100 % the effect showed as (89 and 86.4) the inhibition of Ginger better than the inhibitory of Neem.

However the higher inhibition of reduction result recorded in Ginger followed by Neem leaves at all dose compared with control .who is the reported less inhibition in the experimental.

In the 5th day after the incubation the experiment it was noticed that the low effect were shown when the concentrations 25% the effect shown as (28.7, 53.9 and 60.3) Ginger, Neem and fungicide respectively. In concentration 50% the effects shown as (82.5, 79.3 and 84.5) Ginger, Neem and fungicide respectively, inhibition reduction of the Ginger and Neem are closely, and best inhibition in fungicide. At concentration 100 % the effect shown as (85.1, 84.3 and 89.2) the Ginger better than the Neem, and the high inhibition was in fungicide. The result of this all treatment were comparisons with control who is the reported less inhibition in the experimental.

In this experimental the three test of inhibition reduction growth of *A. flavus* Tables (10, 11 and 12) and Figures (1, 2 and 3) showed the different significant in reduction growth to the fungus of *A. flavus* and the some different significant was explained in concentrations 25% and 50% , that the means the inhibition growth increase with increasing of concentration.

The results also demonstrated that the Ginger extract exhibited more inhibitory effect than the Neem extracts in concentrations 50% and 100%, this could be attributed to the high concentration of the bioactive inhibiting compound in the Ginger extracts than the Neem extracts. Moreover, the data on concentrations from each plants ethanol extract exhibited different inhibitory abilities on fungal growth.

The highest concentrations of the botanical extracts (100%) were the most suppressive followed in a descended order by 50% and 25%. Likewise the test organism responded differently to the different concentrations of extracts. This variability in response which expressed by test organism to different plants extracts was also reported by (Aiyelaagbe 2001). In his investigation, the explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree. Our results are in agreement with the study of (Reem, Alhadi and Faiza, *et al.;* 2012).

Comparison of the inhibitory activity of the both ethanol extracts, the botanical ethanol extracts revealed the highest zone of inhibition against the fungi *A.flavus* strain, and they possess antifungal compounds which could be used as substitutes for the antibiotics.

Also present study signs the interesting assurance of designing potentially antifungal active real agents from the both (*Zingiber officinale* and *Azadirachta indica)*.

CONCOLUSSION

Aspergillus flavus is the second most important causing infections. The importance of this fungus increases in regions with a dry and hot climate.

Commercial formulation based on extracts of Neem leave and Ginger rhizomes, have been successfully used in past as fungicide and control of the plants diseases (with respect to pathogenic and mycotoxins producing fungi), based on experimental investigations carried out to the present study. All the treatment had three replicates and the experiment was not repeated. This study has examined the relationship between growth of *A. flavus* and the impact of ethanol extracts of botanicals and fungicide. Thus optimum conditions for growth of this of *A. flavus strain* were $(30\text{-}35)^{\circ c}$ with marginal conditions at $(15\text{-}40)^{\circ c}$ production optimum conditions were $(25\text{-}30)^{\circ c}$. The results showed that the 100 % best concentration for the inhibitory of the largest amount of the *A. flavus.* The Tables (10, 11 and 12) showed significant differences between the three treatments Ginger, Neem and fungicide at three concentrations (25%, 50% and 100%).

This result was generalized by pairing isolate of *A. flavus* with fungicide and ethanol extracts of botanicals. Inhibitor growth of *A. flavus* accept Fungicide, the Ginger that the best than the Neem in concentrations 50% and 100%, however all test means the botanical extracts of ethanol has good in vitro activity against a range growth of *A. flavus*. Observed that severe drought promoted growth and persistence of *A. flavus* population leading to high aflatoxin contamination.

Hopefully, recently published information about the *Aspergillus* genomes will help us to better understand the pathogenesis of these infections, as well as providing insights into toxin production and allergens

RECOMMENDATIONS

Although, in this study the Ginger rhizomes and the Neem leafs ethanol extracts have been proven to inhibit fungi *Aspergillus falvus*, there are still many aspects that need more research.

The storage of groundnut in optimum conditions, the moisture content must be less than the 9%, and temperature is above 35^{o}c.

- Further researches should be carried out for finding other botanical extracts to inhibit the fungi.
- Further experiments should be done to investigate the influence of botanical extracts on fungi inhibition.
- It is better to cooperate with economic professionals for evaluating and comparing between botanical extracts from economic perspective.
- Use the clean healthy seeds and selective seed for producing food.
- Add powder of Neem in storages and container of groundnut.

The abstract article focuses on experimental advances of the extracts of Ginger and Neem as antifungal

REFRENCES

Abdalla, A.T., Stigter, C.J., Mohamed, H.A., Mohammed, A.E. and Gough, M.C., 2005. Identification of micro-organisms and mycotoxin contamination in underground pit stored sorghum in central Sudan. Paper 5 in: Mycotoxin contamination in stored sorghum grains, health hazard implications and possible solutions. Ministry of Council of Ministers, Sudanese Standardsand Metrology Organization & Standards Administration, in collaboration with Wageningen University (The Netherlands), Khartoum, Sudan, 10 pp.

Abdelhamid AM, 1990. Occurrence of some mycotoxins (*aflatoxin, ochratoxin A, citrinin, zearalenone* and *vomitoxin*) in various Egyptian feeds. Arch Tierernahr 40: 647–64.

Abu Assar, A.H., El Ahmadi, A.B., Abdalla, E.G.H., El Mustafa, E.S.A., Eleasir, M.B.A and Hussein, A.A. (2008). Bunting a new large-seeded Virginia groundnut variety for the irrigated clays in Sudan. *Journal of SAT Agricuitural research*, 6.

Abu Assar AH, El Ahmadi AB, EL Gailani AA, Allaa Eldeen I, Abdel Rahman E and Saeed M. 2000. Two groundnut genotypes C69A-25A-25-1-1-1-B and C109A-4-7-B-2 for the irrigated clays. Variety Release Committee, First Meeting, March 2000. Khartoum, Sudan: Ministry of Agriculture and Forestry.

Abulu, G.O., 1978. An economic analysis of groundnut production in Northen Nigeria. In Proceedings of the National Seminar on Groundnut Production. pp. 83-90, Kano, Nigeria.

Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S. & Chanda, S. (2004). Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Sci Total Environ 326, 123–141.

Aiyelaagbe, O, O. Adesogan ,E. K. and Ekunday,O, O. (2000). The antimicrobial activity of roots of Jatrophapodagrica (Hook). *Journal* Article, Research Support .14(1):60-2.

Agrios GN (2005). Plant Pathology *5th Ed. Academic. Press London.pp.* 410-413.

Akroum, S., Satta, D., and Lalauoui, K. (2009) Antibacterial antioxidant, cytotoxic activities and phytochemical screening of some Algerian plants. Euro. J Sci. Res., 31(2), pp. 289-295.

Alabouvette C (1999) *Fusarium* wilts suppressive soils: an example of disease suppressive soils. Australas Plant Pathology 28:47–64.

Alhadi .M.(2012) Antifungal Activity of jatropha (*Jatropha curcas L.*) Seeds and Leavs Aqueous Extract under Laboratory Conditions.

Allen, L.H., Prasad, P.V.V., Boote, K.J. and Thomas, J.M.G. (2003). *Super-Optimal Temperatures are Detrimental to Peanut (Arachis haypogaea L.) Reproductive Processes and Yield at Both Ambient and Elevated Carbon Dioxide.* Global Change Biological, 9:1775-1787. [Provides seasonal responses of groundnut to elevated temperature and elevated carbon dioxide].

Amadioha A.C. 2000. Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch phytopathology Pflan. 4: 1-9.

Aneja KR,(2004). Experiments in Microbiology, Plant Pathology and Biotechnology. Fourth edition, New International (P) limited publishers, India.121-128.

Anon A.1989. Mycotoxin, Economic and Health Risks.Council for Agricultural Science and Technology; Report No. 116.Pp. 91.

Babu Joseph ,Muzafar Ahmad ; Dar and Vinod Kumar. (2008). Department of Microbiology and Microbial Technology, College of Biotechnology and Allied Sciences, Allahabad Agricultural Institute, Deemed University, Allahabad 211 007, Uttar Pradesh, India.

Bandyopadhyay, R., and Cardwell, K.F. (2003) Species of *Trichoderma* and *Aspergillus* as biological control agents against plant diseases in Africa. In Biological Control in Integrated Pest Management Systems in Africa. Neuenschwander, P., Borgemeister, C., and Langewald, J. (eds). Wallingford, UK: CABI Publishing, pp. 193–206.

Bankole SA, Schollenberger M, Drochner W, 2006. Mycotoxin contamination in food systems in sub-Saharan Africa:A review. Mycotoxin Research 22:163–169.

Bhatnagar, D., Cleveland, T. E. & Payne, G. A. (2000). Encyclopedia of Food Microbiology, pp. 72–79. London: Academic Press.

Booth C (1971) the genus *Fusarium*. Commonwealth Mycological Institute, Kew.

Booth, C. (1977) *Fusarium lahoratory* guide to the identification of the major species commonwealth mycological institutes, Kew, surrey PP 325.

Bowers JH, Locke JC (2000) Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of.

Calvo, A., Guarro, J., Suarez, G. & Ramirez, C. (1980). Air-borne fungi in the air of Barcelona (Spain). III. The genus *Aspergillus* Link. Mycopathologia 71, 41–43.

Campos-Mondragón MG, Calderón AM, Durán-Prado A, Campos-Reyes LC, Oliart-Ros RM, Ortega-García J, Medina-Juárez LA, Angulo O, 2009. Nutritional composition of new peanut (*Arachis hypogaea L.*) cultivars. Grasas Y Aceites 60: 161 – 167.

CAST, 1998. Mycotoxins: Economic and health risks. Task force report 116. Council of Agricultural Science and Technology, Ames, IA.

Chen, Jaw-Chyun; Li-Jiau Huang, Shih-Lu Wu, Sheng-Chu Kuo, Tin Yun Ho, Chien-Yun Hsiang (2007). "Ginger and Its Bioactive Component Inhibit Enterotoxigenic Escherichia coli Heat-Labile Enterotoxin-Induced Diarrhoea in Mice". Journal of Agricultural and Food Chemistry 55 (21): 8390– 8397

Cole,R.J.,Sanders,T.J.,Hill,R.A.,Blankenship,P.D.,1985.Mean geocarposphere temperatures that induce preharvest aflatoxin concentration of peanuts under drought stress. Micopathologia 91:41-46.

Cotty P.J 1989. Virulence and cultural characteristicss of two *A. flavus* strains Pathogenic on cotton phytpathology, 79: 808-814.

Cotty, P., Jaime-Garcia, R., 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol. 119, 109–115.

Craufurd, P.Q., Prasad, P.V.V., Waliyar, F., Taheri, A., 2006. Drought, pod yield, pre-harvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger.Field Crops Res. 98, 20–29.

Dawson W.A.J.M. and Bateman G.L. (2001).Bateman. Fungal communities on roots of wheat and barley and effects of seed treatments containing fluquinconazole applied to control take- all. Plant Pathology, 50: 5-82**.**

Diaz C., Hossain M., Bose M. L., MerceaS and Mew T.W. (1998). Seed quality and effect on rice yield: findingsfrom farmer sparticipatory experiment in Central Luzon, Philippines. J. Crop. Sci. 23(2):111-119.

Donkor ES, Kayang BB, Quaye J, Akyeh ML, 2009. Application of the WHO keys of safer food to improve food-handling practices of food vendors in a poor resource community in Ghana. International Journal of Environmental Research and Public Health 6: 2833-2842.

Dorner,J.W.,Cole,R.J.,Saders,T.H.,Blankenship,P.D.,1989.Inter-relationship of kernel water activity, soil temperature, maturity and phytoalexin production in pre-harvest aflatoxin concentration of drought-stressed peanuts. Micopathologia 105:117-128.

Eke-Ejiofor J, Kiin –Kabari DB, Chukwu EC, 2012. Effect of processing method on the proximate, mineral and fungi properties of groundnut (*Arachis hypogea*). Seed *Journal of Agricultural* and Biological Science 3:257–261.

Elabjar,Z,E,Bashir,N.H.andNasor,O.E.(1992)Use of Neem Biocide to protect .

El Ahmadi AB, Ahmed M Ghorashi and Abdel Rahman E. 1993. Proposal for the release of a new groundnut cultivar (C50-231-1-B) for the irrigated clays. Khartoum, Sudan: Sudan Variety Release Committee, Ministry of Agriculture.

Ernst, E., and Pittler, M.H. (2000) Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *British Journal of Anesthesia* 84 (3): 367–371.

Gibson, A. M., Baranyi, J., Pitt, M. J., Eyles, M. J. & Roberts, T. A. (1994). Predicting fungal growth: the effect of water activity on *A. flavus* and related species. Int J Food Microbiol 23, 419–431.

Girdthai, T., Jogloy, S., Vorasoot, N., Akkasaeng, C., Wongkaew, S., Holbrook, C.C.,Patanothai, A., 2010 b. Heritability of, and genotypic correlations between, afla-toxin traits and physiological traits for drought tolerance under end of seasondrought in peanut (*Arachis hypogaea L.)*. Field Crops Res. 118, 169–176.

Gregory, W.C., Gregory, M.P., Karpovickas, A., Smith, B.W. and yarbrought, J.A. (1973). Structure and genetic Resources of Peanut. In: Peanut culture and uses. Pp.47-134.American Peanut Research and Education Association, Inc.Oklahoma State University. Stillwater, Oklahoma 74074. USA.

Griffin, G.J.and Garren, K.H.1974.Population levels of *Aspergillus flavus* and the *A. niger* group in Virginia peanut field soils. Phytopathology 64:322-325.

Grigs,(1981).The Neem tree *Azadirachita indica*A.Juss. and other meliaceous plant.

Guarro J, Sole M, Castany R, Cano J, Teixido A, Pujol I, Gene J, Castro A, Sarda P (2005). Use of random amplified microsatellites to type isolates from an outbreak of nosocomial aspergillosis in a general medical ward. Med. Mycol. 43:365-371.

Gupta, S. K., Pereira, B. M. & Singh, A. B. (1993). Survey of airborne culturable and non-culturable fungi at different sites in Delhi metropolis. Asian Pac J Allergy Immunol 11, 19–28.

Haq Elamin NH1, Abdel-Rahim AM, Khalid AE. (1988). Aflatoxin contamination of groundnuts in Sudan.Mycopathologia. 1988 Oct; 104(1):25-31.detection and enumeration of molds in cereal products. Food Quality & Safety magazine, February/March 2011.

Heinemann, S., Symoens, F., Gordts, B., Jannes, H. & Nolard, N. (2004). Environmental investigations and molecular typing of *A. flavus* during an outbreak of postoperative infections. J Hosp Infect 57, 149–155.

Home page site, http://www. *Aspergillus flavus.* org/*aflavus*/. 5/8/2015.

Hommons, R.O. (1994). The origin and history of the groundnut, in: J. Smartt (Ed.), the Groundnut Crop. A Scientific Basis for Improvement. New York. Chapman and Hall.

Hussain H.S., and Barasel, J. M. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals,. Toxicology, 167: 101-134.

https://en.m.wikipedia.org/wiki/Neem.

https://en.m.wikipedia.org/wiki/Ginger.

IARC (1993): IARC monographs on the evaluation of carcinogenic risks to humans: Some naturally occurring substances food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monographs 56:245.

IARC, 2002. Some traditional herbal medicines some mycotoxins, naphthalene and styrene. monographs on the evaluation of carcinogenic risks to humans, Volume 82. Lyon: IARC Press. pp. 171-274.

Iro N, Iliyas A, Tato A, 1995. Studies on the preparation and nutrient composition of Kunu Gyado. A traditional Nigerian groundnut- cereal based weaning food. Food Nutrition Bulletin 16: 238-240.

Islam S.M.M., Masum M. M. I. and Fakir M. G. A. (2009). Prevalence of seed-borne fungi in sorghum of different.

Isleib, T.G. and Wynne, J.C. (1992). Groundnut and Research in North America. In: S.N. Nigam, (Ed.). Groundnut-a global perspective: proceeding of an International workshop, 25-29 Nov. 1991, ICRISAT Centre, India, patancheru, A.P. India.

Ismail, M.A. (2000). Evaluation of some peanut (*Arachis hypogaea L.*) genotype for yield and its components. M.Sc. thesis, U of k.

ISTA (1966). International Rules of Seed Testing; Pros. Int. Seed. Test. Assoc. 32:565-589.

JACKSON, C.R. Evaluation of fungicides for control of some fungi found on peanuts. Plant Disease Reporter, Washington, n.49, n.ll, p.928-931, 1965.

James MJ, Lasker BA, McNei MM, Shelton M, Warnock W, Reiss E (2000). Use of a repetitive DNA probe to type clinical and environmental isolates of *Aspergillus flavus* from a cluster of cutaneous infections in a neonatal intensive care unit. J. Clin. Microbiol. 38:3612-3618.

Jimoh KO, Kolapo AL. 2008. Mycoflora and Aflatoxin production in Market Samples of Some Selected Nigerian Foodstuffs. Res J Microbiol 3: 169-179.

Jones, J.P. Jones.; J.B. And Miller, W. (1982). *Fusarium* wilts on tomato. Fla, Dept. Agric. & Consumer Serv., Div. of Plant Industry Plant Pathology Circular No. 237.

Jolly PE, Jiang Y, Ellis MO, Awuah RT, Appawu J, Nnedu O, Stilles JK, Wang JS, Adjei O, Jolly CM, Williams JH, 2007. Association between aflatoxin exposure and health characteristics, liver function, hepatitis and malaria in Ghanaians. Journal of Nutritional and Environmental Medicine 16: 242–257.

Kamika I. and Takoy, L.L. 2011. Natural occurrence of aflatoxin B1 in peanut collected from Kinshasa, Democratic Republic of Congo. Food Control 22(11): 1760-1764.

Kaaya AN, Harris C, Eigel W, 2006. Peanut aflatoxin levels on farms and in markets of Uganda. Peanut Science, 33: 68 – 75.

Khalil, A., Hassawi, D.S., and Kharma, A. (2005) Genetic relationship among salvia species and antimicrobial activity of their crude extracts against pathogenic bacteria. Asian J. Plant Sci., 4(5), pp. 544-549.

Khidir, M.O.(1997). The origin, variability and spread of groundnut (*Arachis hypogaea L.*) (English translation). In the domestication and exploitation of plant and animals (Ucko, P.J., and falk, I.S., eds). London, O.K.

Kim, J.K., Kim, Y., Na, K.M., Surh, Y.J. and Kim, T.Y. 2007. [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression in vitro and in vivo. Free Radical Research 41: 603-614.

Kladpan S. Mahakachanaka W, Yonmanitchai V,Boonyaratanakornkit M, Chinbuti A, 2004. Situationof aflatoxin contamination in groundnut products in Thailand.

Klich, M. A. & Pitt, J. L. (1988). Differentiation of *Aspergillus flavus* from A. parasiticus and other closely related species. Trans Br Mycol Soc 91, 99–108.

Klich MA (2007). Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. Mycoscience, 48: 71-80. Young HV, YL Luo, HY Cheng, WC Hsieh, JC Liao and WC Peng, 2005. Analgesic and anti-inflammatory activities of [6]-gingerol. J Ethnopharmacol, 96: 207-210.

KRN Reddy, B Salleh, B Saad, HK Abbes, CA Abel, and WT Shier (2010). An overview of mycotoxin contamination in foods and its implications for human health. Inform, Toxin Reviews, 2010; 29(1): 3–26.

Kubiak K. and Korbas M. (1999).Occurrence of fungal diseases on selected winter wheat cultivars. PostepyOchronie Roslin, 39 (2): 801-804.

Longe, J.L., Crawford, S., and Odle, T.G. (2005). The Gale Encyclopedia of Alternative Medicine. Detroit, Thomson Gale, pp. 163-167.

Liang, X.Q., Luo, M., Guo, B.Z., 2006. Resistance mechanisms to *A. flavus* infection andaflatoxin contamination in peanut (*Arachis hypogaea*). Plant Pathol. J. 5, 115–124.

Lin, Y. C, Huang, C. C, Huang, S. F, Lee, T. J (2002). Isolated Sphenoid Sinvs *Aspergillsis:* Report of Two cases, Chang Gung Jvol. 25 No 7.

Lioyd B. Bullerman and Andreia Bianchini(2011).Variety of media and methods available for detection and enumeration of molds in cereal products.Food Quality & Safety *magazine*, February/March 2011.

Lund B Parker T and Gould G. 2000. The Microbiological and Safety and Quality of Food:" Toxigenic Fungi and Mycotoxins", Aspen. Inc. Pub. Hong Kong. 53: 1490- 1517.

Mahmoud, M.A., Khidir, M.O., Khlifa, M.A., Musnad, H.A., Mohamed, El.I. (1995). Country report to the FAO international technical conference on plant genetic resources (Leipzig).

Mathur SK, SB Mathur, P Neergaard (1975).Detection of seed borne fungi in sorghum. Pear millet and groundnut. Seed Science Technology, 3: 683-690.

Mathur SB, O Kongsdal (2003). Common laboratory seed health testing method for detecting fungi.Ist Edition International Seed Testing Association, P.O. Box 308, 8303 Bassersdorf, CH- Switzerland, pp: 425.

Mehan, V.K., D. Mc Donald, L. J. Haravu, and S. Jayanthi, 1991. The groundnut aflatoxin problem: Review and literature data-bas, ICRISAT, Patancheru, Andhra Predesh, India.

Mondal, S., Badigannavar, A.M., Kale, D.m. and Murty, G.S.S. (2006). Induction of genetic variability in disease-resistant groundnut breeding line. Tamil Nadu Agriculture University Cambridge. (Brac newsletter founders day special issue No. 285. October, 2007).

Moody, S. F. & Tyler, B. M. (1990). Restriction enzyme analysis of mitochondrial DNA of the *Aspergillus flavus* group: *A. flavus, A. parasiticus,* and *A. nomius.* Appl Environ Microbiol 56, 2441–2452.

Murphy, P. A. S Hendrich, C Landgren, and C. M Bryant, 2006. Food Mycotoxins: an Update. A scientific status summary. *Journal of Food science* 7(5) R51R65 doi:10.1111/j.1750-3841.2006.00052.x

Mutegi CK, Ngugi HK, Hendriks SL, Jones RB, 2009. Prevalence and factors associated with aflatoxin contamination of peanuts from Kenya. International Journal of Food Microbiology 130: 27 – 34.

Mutegi C, Kimani J, Otieno G, Wanyama R, Christie ME, Mallikarjunan K, Kaaya A, 2010. Peanut aflatoxin levels under different market attributes. East African Agricultural and Forestry Journal 77: 95 – 103.

Mutegi CK, Ngugi HK, Hendriks SL, Jones RB, 2012. Factors associated with the incidence of *Aspergillus* section *Flavi* and aflatoxin contamination of peanuts in the Busia and Homa bay districts of western Kenya. Plant Pathology, 61: 1143 – 1153.

Njintang YN, Mbofung CMF, Waldron KW, 2001. In vitro protein digestibility and physicochemical properties of dry red bean flour (Phaseolus vulgaris) flour: Effect of processing and incorporation of soybean and cowpea flour. Food Chemistry 49: 2465- 2471.

Oerke, E.C. and Dehne, H.W. 2004.Safeguarding production-losses in major crops and the role of crop protection. Crop Protection 23, 275-285.

Okaka JC, 2005. Handling, storage and processing of plant foods. Academic publisher, Nigeria, pp. 250-270.

Omer, R.E., Verhoef, L., Veer, P.V., Idris, M.O., Kadaru, A.M.Y., Kampman, E., Bunschoten, A. and Kok, F.J. 2001. Peanut butter intake, GSTM1 genotype and hepatocellular carcinoma: a case-control study in Sudan. Cancer Causes and Control 12: 23-32.

Omoya F.O. and F.C. Akharaiyi. 2012. Mixture of Honey and Ginger Extract for Antibacterial Assessment on Some Clinical Isolates. *Int. Res. J. of Pharmaceuticals, 2(5):127-132.*

Olusegun Atanda, Hussaini Anthony Makun, Isaac M. Ogara, Mojisola Edema, Kingsley O. Idahor, Margaret E. Eshiett and Bosede F. Oluwabamiwo (2013). Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds: in Mycotoxin and Food Safety in Developing Countries, edited by Hussaini Anthony Makun, ISBN 978-953-51-1096 5, Published: April 10, 2013 under CC BY 3.0 license.

Pande, S., and Narayana Rao, J. 2000. Changing scenario of groundnut diseases in Andhr a Pradesh, Karnataka, and Tami l Nadu states of India. Internat ional *Arachis* Newslet ter 20:42-44.

Pattee HE, Young CY, 1982. Peanut Science and Technology, American Peanut Research and Education Society, Inc. Yoakum, Taxas, USA.

Payne, G. A. 1998. Process of contamination by aflatoxin producing fungi and their impacts on crops. In, Mycotoxins in Agriculture and Food Safety. K.K. Sinha and D. Bhatnagar. Marcel Dekker, Inc. New York.

Pettit, R.E., R.A. Taber, and N.K.Person, 1971. Microbial infestation of peanuts as related to windrow-curing conditions. Proceedings of the American Peanut Research and Education Association, 3: 127-136.

Pitt, J.I., S.K. Dyer and M. Olsen, 1991. Invasion of developing peanut plant by *Aspergillus flavus*. Let. Appl. Microbiol., 13: 16-20.

Pitt J.I. (2006). Penicillium and related genera: In Food Spoilage Microorganisms, Blackburn C. de W. Woodhead Publishing, Cambridge, 2006, 437-50.

Polixeni V, Panagiota M, 2008. Aflatoxin B1 and ochratoxin A in breakfast cereals from Athens market: Occurrence and risk assessment. Department of chemistry, University of Athens, Panepistimiopolis, 15784 Athens, Greece.

Rachaputi, N.,Wright, G.C. and Krosch, S.2002. Management practices to minimize pre-harvest aflatoxin contamination in Australian peanuts, Australian Journal of Experimental Agriculture 42(5):595-605.

Raper, K. B. & Fennel, D. I. (1965). The Genus *Aspergillus*. Baltimore: Williams & Wilkins.

Rath, P.M. (2001). Phenotypic and genotypic characterization of reference strains of the genus *Aspergillus*. Mycosis 44:65-72.

Reem .*H.* (2012).*Bioactivity of (Jatropha* curcas L.*)* seeds kernel and shell cold Methanol Extract Against for Bacteria Species.

Redden RJ, Chen W, Sharma B, 2005. Chickpea Breeding and Management. United Kingdom: CABI.

Ros E (2010). Health benefits of nut consumption. Nutrients, 2: 652 - 682.

Richard, J. L. and G. A. Payne. 2003. Mycotoxins in plant, animal, and human systems. Task Force Report No. 139. Council for Agricultural Science and Technology.

Ribeiro J, Cavaglieri L, Fraga M, Direito G, Dalcero A, Rosa C,2006. Influence of water activity, temperature and time on mycotoxins production on barley rootlets. Letters in Applied Microbiology, 42: 179 – 184.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C. & Filtenborg, O. (2000). Identification of the common food and airborne fungi, Aspergillus. In

Introduction to Food and Airborne Fungi, pp. 64–97. Utrecht: Centraalbureau voor Schimmekultures.

Scheidegger, K. A. and G. A. Payne. 2003. Unlocking the secrets behind secondary metabolism: A review of *Aspergillus flavus* from pathogenicity to functional genomics. Journal of Toxicology-Toxin Reviews. 22(2-3): 423-459.

Schwertner, H.A. and Rios, D.C. 2007. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in ginger–containing dietary supplements, spices, teas, and beverages. Journal of Chromatography B 856: 41-47.

Shiferaw, B., Pantilan, M.C., Gupta, S.C. and Shetly, S.VR. (2004). Research Spillover Benefits and experiences in Inter-Regional Technology Transfer. An Assessment and Synthesis ICRISAT.

Siddig, S.A. (1993). Evaluation of Neem seed and leaf water extracts and powder from the control of insect pest insect in the Sudan / Agric. Res. Cro Tech .Bull. Bull, NO. 6.

Singha IM, Unni BG, Kakoty Y, Das J, Wann SB, Singh L, Kalita MC (2010) Evaluation of in vitro antifungal activity of medicinal plants against phytopathogenic fungi. Arch Phytopathol Plant Prot. doi: 10.1080/03235401003672913.

Singh, B. (1982). And Interdisciplinary Approach to Optimum Food Utility of Peanut is SAT Africa. In the Annual Report of the Peanut Collaborative Support Program (CRSP). Pp. 35-41. Peanut CRSP.

Smith, I.M. Dunez, J. Phillips, D.H. Lelliott, R.A., and Archer, S.A. eds. (1988).European handbook of plant diseases. Blackwell Scientific Publications: Oxford 583pp.

Sudhakar, P., Lathat, P., Babitha, M., Reddy, P.V., Naidu, P.H., 2007. Relationship of drought tolerance traits with aflatoxin contamination in groundnut. Indian J.Plant Physiol. 12, 261–265.

Suhaj, M. 2006. Spice antioxidants isolation and their antiradical activity: review. Journal of Food Composition and Analysis 19: 531-537.

Suliman M Yee C Hamid A and Yatim A. 2007. The Occurrence of Aflatoxin in Raw Shelled Peanut Samples from Three District of Peark, Malaysia, Elec. J. of Envio. Agri. and Food Chemi. 6(5): 2045 – 2052.

Summeral BA, Salleh B, Leslie JF (2003). A utilitarian approach to *Fusarium* identification. Plant Dis 87:117–128.

Sun, T. and Ho, C.T. 2005. Antioxidant activities of buckwheat extracts. Food Chemistry 90:743-749.

Tewarri S.N., Nayak N. 1991. Activity of four plants leaf extracts against three fungal pathogens of rice. Tropical Agriculture. (Trinidad). 68: 373-375.

Thirumala-Devi, K., Mayo, M.A., Hall, A.J., Craufurd, P.Q., Wheller, T.R., Waliyar, F.,Subrahmanyam, A., Reddy, D.V.R., 2002. Development and application of anindirect competitive enzyme linked immunoassay for aflatoxin M1 in milk and milk-based confectionery. J. Agric. Food Chem. 50, 933–937.

Thomas, A. Zitter, *Fusarium* Diseases of Cucurbits. Fact Sheet Page: 733.00 Date:1. (1998).Department of Plant Pathology, Cornell University.

Turner, P.C., Sylla, A., Diallo, M.S., Castegnaro, J.-J., Hall, A.J., and Wild, C.P. 2002. The role of aflatoxins and hepatitis viruses in the etiopathogenesis of hepatocellular carcinomas: a basis for primary prevention in Guinea-Conakry, West Africa. J. Gastro-entrol. Hepatol. 17: S441-S448.

USDA, 2010. Nutrient database. Retrieved from www.nal.usda.gov/fnic/ foodcomp.

USDA, 2012. World Agricultural Production. Retrieved from, http://www.fas.usda.gov/wap/current/

Valko, M., Leibfritz, D., Moncola, J., Cronin, M.T.D., Mazura, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. International Journal Biochemistry Cell Biology 39: 44–84.

Varga J, Frisvad JC, Samson RA, 2012. Two new aflatoxin producing species and an overview of *A*spergillus section *flavi*. Studies in Mycology, 69:57–**80.**

Vujanovic,V.,Smoragiewicz, W. & Krzysztyniak, K.(2001). Airborne fungal ecological niche determination as one of the possibilities for indirect mycotoxin risk assessment in indoor air. Environ Toxicol 16,1–8.

Wagacha JM, Muthomi JW(2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible.

Wang *et al*. BMC Plant Biology 2010, 10:267, http://www.biomedcentral.com/1471-2229/10/267.

Weiss, E.A. (2000). Oilseed crops. London, Blackwell Sciences.

Wilson, M.D. and Stansell,R.J.1983.effect of irrigation regimes on aflatoxin contamination of Peanut pods. Peanut Science 10:54 56.Witzenberger, A., Williams, J.H. and Lenz, F.1988.Influence of day length on yield-determining processes in six groundnut cultivars (*Arachis hypogaea L.*),.Field Crops Research 18: 89-100.

Wolfe, K. W. X. and Liu, R. H. 2003. Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry 51(3): 609–614

Wright GC Lamb DW and Medway J. 2002. Application of aerial remote sensing technology for the identification of aflatoxin affected areas in peanut fields. Brisbane, Australia.

www.itis.gov/servlet/singleRpt/singleRpt?search_topic=TSN&search_value=264633/ 13/1/2016 .

Younis, Y.M.H., Malik, M.K., 2003. TLC and HPLC assay of aflatoxin contamination in Sudanese peanuts and peanut products. Kuwait J. Sci. Eng. 30 (1), 79–93.

Wu. F. 2011. and Khlangwiset, P., G.S. Shepard. “Aflatoxins and growth impairment: A review.” Critical Reviews in Toxicology. 41(9): 740-755.

Yousif M.A. Idris, Abdalbasit A. Mariod, Ibrahim Alfaig Elnour, Adam Ali Mohamed (2010) Determination of aflatoxin levels in Sudanese edible oils. Food and Chemical Toxicology 48 (2010) 2539–2541.

Yu, J., Cleveland, T. E., Nierman, W. C. & Bennett, J. W. (2005). *A. flavus* genomics: gateway to human and animal health, food safety, and crop resistance to diseases. Rev Iberoam Micol 22, 194–202.

APPENDIXS

Appendix 1

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	9	12222.565	1358.063	29.367	0.0000
Within	20	924.893	46.245		
Total	29	13147.459			

Coefficient of Variation = 16.12%

Appendix 2

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	9	13462.323	1495.814	30.610	0.0000
Within	20	977.347	48.867		
Total	29	14439.670			

Coefficient of Variation = 12.10%

Appendix 3

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	9	13008.668	1445.408	120.914	0.0000
Within	20	239.080	11.954		
Total	29	13247.748			

Coefficient of Variation = 6.58%

Appendix 4

Table 11 Effects of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 4th day from incubation				
Treatment		Inhibition zone		
Product	Concentration	R1	R2	R3
Ginger	25%	15(22.8)	16.7(24.1)	5.6(13.7)
	50%	60(50.8)	44.4(41.8)	44.4(41.8)
	100%	55(47.9)	72.2(58.2)	83.3(65.9)
Neem	25%	25(30)	16.7(24.1)	33.3(35.2)
	50%	55(47.9)	50(45)	44.4(41.8)
	100%	60(50.8)	72.2(58.2)	50(45)
Fungicide	25%	35(36.3)	50(45)	38.9(38.6)
	50%	75(60)	66.7(54.8)	77.8(62)
	100%	90(71.6)	77.8(61.9)	100(90)
Control		0 (0.7)	0 (0.7)	0 (0.7)
LSD				11.58
SE±				3.89
C.V %				16.12%

Statistical analyses were performed transformations by using Table X Angular.

Appendix 5

Table 11 Effects of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 4th day from incubation				
Treatment		Inhibition zone		
Products	Concentration	R1	R2	R3
Genger	25%	28.1(32)	37.2(37.6)	20.7(27.1)
	50%	80.4(63.7)	80.4(63.7)	75.9(60.6)
	100%	88.2(69.9)	85.1(77.3)	82.1(65)
Neem	25%	62.1(52)	53.4(47)	46.2(42.8)
	50%	73.7(59.3)	78.4(62.3)	85.9(60.6)
	100%	83.7(66.2)	83.8(66.3)	85.5(67.6)
Fungicide	25%	71.2(57.5)	62.8(52.4)	46.9(43.2)
	50%	86.3(68.3)	82.4(65.2)	84.8(67.1)
	100%	91.5(73.1)	87.8(69.6)	88.3(70)
Control		0(0.7)	0 (0.7)	0 (0.7)
LSD				5.89
SE±				4.08
C.V %				4.55%

Statistical analyses were performed transformations by using Table X Angular.

Appendix 6

Table 11 Effects of fungicide and ethanol extract of Ginger and Neem on the liner growth of *A. flavus* in vitro in the 5th day from incubation				
Treatment		Inhibition zone		
Product	Concentration	R1	R2	R3
Genger	25%	36.4(37.1)	35.7(36.7)	25.9(30.6)
	50%	85(67)	85(67.2)	80.6(63.9)
	100%	91.4(73)	89.3(71)	86.3(68.3)
Neem	25%	70(65.8)	60(50.8)	52.5(46.4)
	50%	84.3(66.7)	85(67.2)	82(64.9)
	100%	87.1(69)	86.4(68.4)	85.6(67.7)
Fungicide	25%	85.7(67.8)	82.1(65)	79.1(62.8)
	50%	93.6(74.4)	88.6(70.3)	87.1(59)
	100%	92.9(74.6)	95.7(78)	95(77.1)
Control		0(0.7)	0 (0.7)	0 (0.7)
LSD SE± C.V %				11.91 4.07 12.10%

Statistical analyses were performed transformations by using Table X Angular.

Appendix 7

Informations of fungicide Tops 100 Ec

Product Name = Topas=100 Ec systemic fungicide

Synonym = C GA71818

Other Name = Product code: A6209Q

Company Details= Syngenta Crop Protection Pty Limited ABN 33 002 933 717

® Registered trademark of a Syngenta Group Company

Appendix 8

TABLE X. ANGULAR TRANSFORMATION

p%	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
0	0·00	1·81	2·56	3·14	3·63	4·05	4·44	4·80	5·13	5·44
1	5·74	6·02	6·29	6·55	6·80	7·03	7·27	7·49	7·71	7·92
2	8·13	8·33	8·53	8·72	8·91	9·10	9·28	9·46	9·63	9·80
3	9·97	10·14	10·30	10·47	10·63	10·78	10·94	11·09	11·24	11·39
4	11·54	11·68	11·83	11·97	12·11	12·25	12·38	12·52	12·66	12·79
5	12·92	13·05	13·18	13·31	13·44	13·56	13·69	13·81	13·94	14·06
6	14·18	14·30	14·42	14·54	14·65	14·77	14·89	15·00	15·12	15·23
7	15·34	15·45	15·56	15·68	15·79	15·89	16·00	16·11	16·22	16·32
8	16·43	16·54	16·64	16·74	16·85	16·95	17·05	17·15	17·26	17·36
9	17·46	17·56	17·66	17·76	17·85	17·95	18·05	18·15	18·24	18·34
10	18·43	18·53	18·63	18·72	18·81	18·91	19·00	19·09	19·19	19·28
11	19·37	19·46	19·55	19·64	19·73	19·82	19·91	20·00	20·09	20·18
12	20·27	20·36	20·44	20·53	20·62	20·70	20·79	20·88	20·96	21·05
13	21·13	21·22	21·30	21·39	21·47	21·56	21·64	21·72	21·81	21·89
14	21·97	22·06	22·14	22·22	22·30	22·38	22·46	22·54	22·63	22·71
15	22·79	22·87	22·95	23·03	23·11	23·18	23·26	23·34	23·42	23·50
16	23·58	23·66	23·73	23·81	23·89	23·97	24·04	24·12	24·20	24·27
17	24·35	24·43	24·50	24·58	24·65	24·73	24·80	24·88	24·95	25·03
18	25·10	25·18	25·25	25·33	25·40	25·47	25·55	25·62	25·70	25·77
19	25·84	25·91	25·99	26·06	26·13	26·21	26·28	26·35	26·42	26·49
20	26·57	26·64	26·71	26·78	26·85	26·92	26·99	27·06	27·13	27·20
21	27·27	27·35	27·42	27·49	27·56	27·62	27·69	27·76	27·83	27·90
22	27·97	28·04	28·11	28·18	28·25	28·32	28·39	28·45	28·52	28·59
23	28·66	28·73	28·79	28·86	28·93	29·00	29·06	29·13	29·20	29·27
24	29·33	29·40	29·47	29·53	29·60	29·67	29·73	29·80	29·87	29·93
25	30·00	30·07	30·13	30·20	30·26	30·33	30·40	30·46	30·53	30·59
26	30·66	30·72	30·79	30·85	30·92	30·98	31·05	31·11	31·18	31·24
27	31·31	31·37	31·44	31·50	31·56	31·63	31·69	31·76	31·82	31·88
28	31·95	32·01	32·08	32·14	32·20	32·27	32·33	32·39	32·46	32·52
29	32·58	32·65	32·71	32·77	32·83	32·90	32·96	33·02	33·09	33·15
30	33·21	33·27	33·34	33·40	33·46	33·52	33·58	33·65	33·71	33·77
31	33·83	33·90	33·96	34·02	34·08	34·14	34·20	34·27	34·33	34·39
32	34·45	34·51	34·57	34·63	34·70	34·76	34·82	34·88	34·94	35·00
33	35·06	35·12	35·18	35·24	35·30	35·37	35·43	35·49	35·55	35·61
34	35·67	35·73	35·79	35·85	35·91	35·97	36·03	36·09	36·15	36·21
35	36·27	36·33	36·39	36·45	36·51	36·57	36·63	36·69	36·75	36·81
36	36·87	36·93	36·99	37·05	37·11	37·17	37·23	37·29	37·35	37·41
37	37·46	37·52	37·58	37·64	37·70	37·76	37·82	37·88	37·94	38·00
38	38·06	38·12	38·17	38·23	38·29	38·35	38·41	38·47	38·53	38·59
39	38·65	38·70	38·76	38·82	38·88	38·94	39·00	39·06	39·11	39·17
40	39·23	39·29	39·35	39·41	39·47	39·52	39·58	39·64	39·70	39·76
41	39·82	39·87	39·93	39·99	40·05	40·11	40·16	40·22	40·28	40·34
42	40·40	40·45	40·51	40·57	40·63	40·69	40·74	40·80	40·86	40·92
43	40·98	41·03	41·09	41·15	41·21	41·27	41·32	41·38	41·44	41·50
44	41·55	41·61	41·67	41·73	41·78	41·84	41·90	41·96	42·02	42·07
45	42·13	42·19	42·25	42·30	42·36	42·42	42·48	42·53	42·59	42·65
46	42·71	42·76	42·82	42·88	42·94	42·99	43·05	43·11	43·17	43·22
47	43·28	43·34	43·39	43·45	43·51	43·57	43·62	43·68	43·74	43·80
48	43·85	43·91	43·97	44·03	44·08	44·14	44·20	44·26	44·31	44·37
49	44·43	44·48	44·54	44·60	44·66	44·71	44·77	44·83	44·89	44·94

Appendix 9

TABLE X. ANGULAR TRANSFORMATION—*continued*

p%	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
50	45·00	45·06	45·11	45·17	45·23	45·29	45·34	45·40	45·46	45·52
51	45·57	45·63	45·69	45·74	45·80	45·86	45·92	45·97	46·03	46·09
52	46·15	46·20	46·26	46·32	46·38	46·43	46·49	46·55	46·61	46·66
53	46·72	46·78	46·83	46·89	46·95	47·01	47·06	47·12	47·18	47·24
54	47·29	47·35	47·41	47·47	47·52	47·58	47·64	47·70	47·75	47·81
55	47·87	47·93	47·98	48·04	48·10	48·16	48·22	48·27	48·33	48·39
56	48·45	48·50	48·56	48·62	48·68	48·73	48·79	48·85	48·91	48·97
57	49·02	49·08	49·14	49·20	49·26	49·31	49·37	49·43	49·49	49·55
58	49·60	49·66	49·72	49·78	49·84	49·89	49·95	50·01	50·07	50·13
59	50·18	50·24	50·30	50·36	50·42	50·48	50·53	50·59	50·65	50·71
60	50·77	50·83	50·89	50·94	51·00	51·06	51·12	51·18	51·24	51·30
61	51·35	51·41	51·47	51·53	51·59	51·65	51·71	51·77	51·83	51·88
62	51·94	52·00	52·06	52·12	52·18	52·24	52·30	52·36	52·42	52·48
63	52·54	52·59	52·65	52·71	52·77	52·83	52·89	52·95	53·01	53·07
64	53·13	53·19	53·25	53·31	53·37	53·43	53·49	53·55	53·61	53·67
65	53·73	53·79	53·85	53·91	53·97	54·03	54·09	54·15	54·21	54·27
66	54·33	54·39	54·45	54·51	54·57	54·63	54·70	54·76	54·82	54·88
67	54·94	55·00	55·06	55·12	55·18	55·24	55·30	55·37	55·43	55·49
68	55·55	55·61	55·67	55·73	55·80	55·86	55·92	55·98	56·07	56·10
69	56·17	56·23	56·29	56·35	56·42	56·48	56·54	56·60	56·66	56·37
70	56·79	56·85	56·91	56·98	57·04	57·10	57·17	57·23	57·29	57·35
71	57·42	57·48	57·54	57·61	57·67	57·73	57·80	57·86	57·92	57·99
72	58·05	58·12	58·18	58·24	58·31	58·37	58·44	58·50	58·56	58·63
73	58·69	58·76	58·82	58·89	58·95	59·02	59·08	59·15	59·21	59·28
74	59·34	59·41	59·47	59·54	59·60	59·67	59·74	59·80	59·87	59·93
75	60·00	60·07	60·13	60·20	60·27	60·33	60·40	60·47	60·53	60·60
76	60·67	60·73	60·80	60·87	60·94	61·00	61·07	61·14	61·21	61·27
77	61·34	61·41	61·48	61·55	61·61	61·68	61·75	61·82	61·89	61·96
78	62·03	62·10	62·17	62·24	62·31	62·38	62·44	62·51	62·58	62·65
79	62·73	62·80	62·87	62·94	63·01	63·08	63·15	63·22	63·29	63·36
80	63·43	63·51	63·58	63·65	63·72	63·79	63·87	63·94	64·01	64·09
81	64·16	64·23	64·30	64·38	64·45	64·53	64·60	64·67	64·75	64·82
82	64·90	64·97	65·05	65·12	65·20	65·27	65·35	65·42	65·50	65·57
83	65·65	65·73	65·80	65·88	65·96	66·03	66·11	66·19	66·27	66·34
84	66·42	66·50	66·58	66·66	66·74	66·82	66·89	66·97	67·05	67·13
85	67·21	67·29	67·37	67·46	67·54	67·62	67·70	67·78	67·86	67·94
86	68·03	68·11	68·19	68·28	68·36	68·44	68·53	68·61	68·70	68·78
87	68·87	68·95	69·04	69·12	69·21	69·30	69·38	69·47	69·56	69·64
88	69·73	69·82	69·91	70·00	70·09	70·18	70·27	70·36	70·45	70·54
89	70·63	70·72	70·81	70·91	71·00	71·09	71·19	71·28	71·37	71·47
90	71·57	71·66	71·76	71·85	71·95	72·05	72·15	72·24	72·34	72·44
91	72·54	72·64	72·74	72·85	72·95	73·05	73·15	73·26	73·36	73·46
92	73·57	73·68	73·78	73·89	74·00	74·11	74·21	74·32	74·44	74·55
93	74·66	74·77	74·88	75·00	75·11	75·23	75·35	75·46	75·58	75·70
94	75·82	75·94	76·06	76·19	76·31	76·44	76·56	76·69	76·82	76·95
95	77·08	77·21	77·34	77·48	77·62	77·75	77·89	78·03	78·17	78·32
96	78·46	78·61	78·76	78·91	79·06	79·22	79·37	79·53	79·70	79·86
97	80·03	80·20	80·37	80·54	80·72	80·90	81·09	81·28	81·47	81·67
98	81·87	82·08	82·29	82·51	82·73	82·97	83·20	83·45	83·71	83·98
99	84·26	84·56	84·87	85·20	85·56	85·95	86·37	86·86	87·44	88·19