Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Detection and Identification of Seed Borne Fungi in Sudan

كشف وتعريف الفطريات المحمولة على البذرة فى السودان

A thesis submitted in partial fulfillment of the requirements for the Degree of M.Sc. (Agric) in Plant Protection

By

Alawia sharaf Eldeen Beshir Abdalla

University of Zalingy, Agriculture Faculty

Department of Plant Protection

B.Sc. Agric. (Honours) 2005

Supervisor: Dr. Ibrahim Saeed Mohamed

Department of Plant Protection

College of Agricultural Studies -Shambat

Sudan University of Science and Technology

2015

Sudan University of Science & Technology

College of Graduate Studies

بسم الله الرحمن الرحيم

كلية الدراسات العليا

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

Approval Page

Name of Candidate: Alawia Sharaf Eldeen Beshir

Thesis title: Detection and Identification of Seed borne fungi on some food crops and their control in Sudan

Approved by:

1. External Examiner

Name: Muntasir Adam Mohamed Elameen

Signature: ..... Date: 10-5-2015

2. Internal Examiner

Name: Ekhlass Hussein mohamed Ahmed

Signature: ..... Date: 10-5-2015

3. Supervisor

Name: Ibrahim Saeed Mohamed

Signature: ..... Date: 10-5-2015

الآية

قال تعالى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّماءِ ماءً فَأَخْرَجْنا بِهِ نَباتَ كُلِّ شَيْءٍ فَأَخْرَجْنا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَراكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِها قِنْوانٌ دانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشابِهٍ انْظُرُوا إِلى ثَمَرِهِ إِذا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذلِكُمْ لَآياتٍ لِقَوْمٍ يُؤْمِنُونَ (99))

صدق الله العظيم

سورة الأنعام الآية (99)

Dedication

To my mother

To the soul of my father

To my husband and my daughters

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

ACKNOWLEDGEMENTS

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

I would like to express my thanks to my supervisor Dr. Ibrahim Saeed Mohamed College of Agricultural studies, Sudan University of science and technology, who devoted most of his time to teach us on various disciplines of scientific research.
For his helpful assistance, guidance, patience and keen supervision during this work.

Thanks are also extended to my dear teachers Ustaz Mode Ibrahim Ustaz Mohammed Alziber, and Wlaid Elamin for their continuous and unlimited helps during this study.

Also Special Thanks are due to my colleagues at the M.Sc. who helped me during the experiments. Grateful thanks are due to all the staff of the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology.

CONTENTS

List of tables

Abstract

Seed borne fungi are one of the major food limiting factors contaminate of food grains in many countries. The present study was undertaken under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology to detect and identify the seed-borne fungi on sorghum, pearl millet, groundnut and sesame seeds collected from four different locations, each in one state of Sudan and their possible control using aqueous plant extracts chemical fungicides. Out of the 16 seed samples, 4 of each crop, tested for seed borne fungi, a total of 7 genera of 8 species of fungi were deced. The fungal pathogens recorded were *Aspergillus flavus, Aspergillus niger, Fusarium spp. Penicillium spp. and Rhizopus spp. Alternaria spp., Macrophomina spp. and Drechslera spp.* with mean percent incidence ranging from 1.0 – 70%. The higher percent incidence was recorded by *Aspergillus flavus* (70%) in groundnut. The four most prevailing seed borne fungi recorded across crops seeds were *Aspergillus flavus, Aspergillus Niger, Penicillium spp. and Rhizopus* spp. with varying level of incidences. Likewise, all concentrations (25, 50 and 100%) of the leaves aqueous extracts of all plants tested neem, damas and fungicide exhibited significantly high inhibitory effect against the linear growth of fungus compared to control. Moreover, concentrations of each aqueous extract as well as that of fungicide reacted differently against test fungus. However, the Neem plant extracts (50 and 100%) and tilt

as well at all concentrations gave the highest inhibition zones percent (78.9%, 88.8%, 90.4%, 100% and 100%) respectively after five days from inoculation. Generally, the results showed that the antifungal activity increase with increase in extract concentration. Obviously, the test fungus differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration. The findings of this study are therefore, important as they highlight the need for effective measures aimed at reducing seed-borne fungi incidence in stable food crops seeds in Sudan.

ملخص البحث

الفطريات المحمولة على البذور هى احدى اهم العوامل الملوثة والمحددة للحبوب كغذاء فى عدة دول . اجريت هذه الدراسة تحت ظروف المعمل بقسم وقاية النبات بكلية الدراسات الزراعية بجامعة السودان للعلوم و التكلنولوجيا بغرض كشف وتعريف الفطريات المحمولة علي البذور في الذرة الرفيعة , الدخن , الفول السوداني و السمسم و التي اخذت من اربعة مناطق مختلفة , من ولايات المختلفة من السودان, تحت طريقة الكشف بستعمال المستخلصات النباتيه وبعض المبيدات الكميائية . للكشف عن الفطريات المحمولة علي البذور تم استعمال طريقة ورق النشافه القياسيه , من بين الستة عشرة عينه (اربعه من كل محصول) التي اختبرت تم كشف و تعريف سبعه انواع لثمانيه اجناس من الفطريات المحمولة علي البذور . الفطريات التي تم تدوينها هي اسبيراجلس فلافس *Aspergillus flavus*,اسبيرجلس نيغر *Aspergillus niger*, فيوزاريم *Fusarium*, بنسليم Penicillium, رايزوبس *Rhizopus* spp., الترناريا *Alternaria*, ماكروفومينا *Mucor*,و دريشلارا *Drechslera* و بنسبه اصابه تراوحت ما بين %(01.70)بالمائه و اعلي نسبه اصابه سجلت بواسطه اسبيرجلس فلافس %(70) في الفول السوداني . اكثر الاجناس تواجدا هي اسبيرجلس فلافس *Aspergillus flavus* ,, اسبيرجلس نيغر *A. niger,* , بنبسليم Penicillium, ورايزويص Rhizopusو نسب اصابه مختلفة . كما ان جميع تراكيز المستخلصات كل من النيم والدمس النباتيه (25 ,50, 100%) والمبيد الفطري تلت قد اظهرت نسبه تثبيط عاليه ضد نمو الفطر مقارنه بالشاهد كما كان تاثير كل منها مختلفا عن الاخر و اعلي نسبه تثبيط كانت لمستخلص النيم (50, 100%) و المبيد الفطري النسب بعد خمسه ايام من بدايه التجربه . اظهرت هذه الدراسه ان الاثر التثبيطي لكل المحاليل يزداد مع زيادة التركيز للدراسه اهمية لانها القت الضوء علي اهميه اتخاذ اجراءات فعاله للحد من اصابه .

CHAPTER ONE

INTRODUCTION

Food crops are ones of the most important food crops in many part of the world. Among these food grains, sorghum, sesame, millet and groundnuts play an important role in food security. According to FAO (2006), food grains are cultivated on about 9.89 million hectares in the world, with an average annual production of 7.80 million Mt in the Year 2002 (FAO, 2002). Its average productivity is 789 kg/ha.

Currently, these crops are being cultivated throughout the semi-arid region of the world (Agrios,. (1997).). Out of the 33 countries growing food crop, 18 cultivate in access of 20 thousands hectares which represent 92 of the production area, 89 percent of this is concentrated in semi-arid tropical countries (Agrios,. (1997)). In fact, these crops provide major source of low-cost carbohydrates for masses, of low-income groups. Apart from being an important source of dietary protein for human consumption, this crop is also important for the management of soil fertility (Agrios, . (1988).).

In Sudan, where agriculture continues to dominate the economy of the country, more than 80% of the population is engaged in crops production. Food crops, which occupy more than 7,000 ha, are considered one of the principal cultivated crops in the Sudan. These crops have a significant role in the diets of the Sudanese people and

contribute substantially to their income. It is also gaining further importance as a source of protein (FAO Stat, 2006).

The major constraints facing the productivity of these food crops worldwide are the losses caused by diseases, insects, nematodes fungus diseases and parasitic weeds. Among these, the most important are fungi, affecting roots, stems, leaves, flowers, and seeds. Unfortunately, the threat to these food crops from fungal contamination of seeds has now reached a level that outstrips that posed by bacterial and viral diseases (Aiyelaagbe,.(2001).). The seed borne fungi of most concern are produced by species within the genera of Aspergillus, Fusarium, and Penicillium that frequently occur in seeds of major food crops in the field and continue to contaminate them during storage, including cereals and oil seeds (Aiyelaagbe.(2001).

In fact, *Aspergillus spp.* are ones of the major food limiting factors contaminate in many countries (Singh and Dahiya, , and Mohamed, 2002).

In Sudan, several seed borne fungi are known to limit utilization of food grains, of which *Aspergillus sp.* is one of the most important. (Yousif *et al.,* (2010) indicated that *Aspergillus spp.* are the most important spoilage of food grains in the Sudan. Moreover, and in most cases, in order to prevent the plant pathogens and to protect the crop plants against them, chemical control methods are in practice. However, although the use of chemicals has helped increase of yields obtained (

Yousif *et al.,* (2010).but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms in addition to contamination of the environment with very toxic substances (Okigbo, 2004; Carvalho, 2004). This has initiated the exploration of safe alternate products.

Historically, the presence of antimicrobial compounds in higher plants has been recognized as important products in combating food contaminants. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some of these fungal contaminants (Schmutterer,. 2002).

Based on the foregoing, this study was an exploratory investigation to focus on (i) detection and identification of seed borne fungi associated with seeds of four food crops in Sudanese grains markets, (ii) and to evaluate the antifungal potentials of some plants extract and fungicide (Tilt) efficacy against most frequently occurring fungus in order to formulate promising method of control with following objectives:-

- To detect and identify seed borne fungi associated with seeds of four food crops
- To explore the antifungal potentials of some higher plants crude extract against most common fungus seed born fugi
- To evaluate the efficacy of systemic fungicide on fungal growth
- To develop Integrated Management Approach for pathogenic fungi associated with seeds of food crops.

CHAPTER TWO

LITERATURE REVIEW

2.1. Food Crop

Grains, as they are sometimes called, provide more food energy worldwide than any other crop and thus are staples. They are staple for two third of the earth's population, providing 85% of the world's food energy and protein intake (FAO, 2006).

In fact, consumption of food grains is moderate in developed countries whereas in Africa and Asia, it is a daily sustenance. Moreover, in Africa, grains contribute by 46% of the total energy intake; however, this figure could be as high as 78% in some African countries (FAO, 2010). Because of their rich nutrient composition, grains support fungal growth and mycotoxin production excellently on the farm, during storage and after processing into foods and feeds.

2.1.1. Millets (*Pennisetum glaucum*)

Millet is the common term for members of family Poaceae comprising of a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for both human food and fodder. While millets are indigenous to many parts of the world, millets most likely had an evolutionary origin in tropical western Africa, as that is where the greatest number of both wild and cultivated forms exists.

Millets have been important staples food in human history, particularly in Asia and Africa, and they have been in cultivation in East Asia for the last 10,000 years (Fuller-, 2003).

Pearl millet is an important staple food for millions of people inhabiting the semi-arid tropics because it is a major source of calories and a vital component of food in the developing world (Fuller- 2003).This crop is best suited to harsh climate of seasonally hot drought prone semi-arid region of Africa and Indian sub-continent. Millets are important crops in the semi-arid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries (Ibrahim, 1990).

In Sudan Pearl millet is an important coarse grain summer cereal crop. It is favored due to its productivity and short growing season under dry, high temperature conditions. The most widely grown millet is pearl millet, which is an important crop in India and parts of Africa (Fuller-, 2003)

In the Sudan pearl millet is mainly grown in the western states in Darfur and kordofan, in upper Nile, and soon it is also cultivated in small patches in Damazin, Gedarif and Gezira states. The straw is used as animal feeding, fuel f or making fences and the stalks are used for and building. The crop is consumed as staple food (78%), drinks and other

uses (20%). Feed use is still very small (2%). As food, they are nutritionally equivalent or superior to most cereals; containing high levels of methionine, cystine, and other vital amino acids for human health. It is used as flour for making kisra, Asida, Nasha, madeedah, Damergah (Ibrahim, 1990).

It is also unique sources of pro-vitamin A (yellow pearl millets) and micronutrients (Zn, Fe and Cu) which are especially high in finger millet (Munyaradzi and Makoni, 2013).

2.1.2. Groundnut (*Arachis hypogaea* L.)

Groundnut (Leguminosae) is a major oilseed crop widely grown in tropical and subtropical regions of the world, and is an important source of protein which believed to be originated from South America (Wiess, 2000). Major groundnut growing countries are India (26%), China (19%) and Nigeria (11%). Its cultivation is mostly confined to the tropical countries ranging from 40° N to 40° S. Major groundnut producing countries are: China (40.1%), India (16.4%), Nigeria (8.2%), U.S.A (5.9%), Indonesia (4.1) and Sudan (30.6%) (Nwokoto, 1996).

Worldwide, approximately 25.7 million tons of groundnuts are produced annually from about 21 million hectares of cropped land. Asia alone produces 17.9 million tons, 70% of global production. Africa produces another 20%. About 60% of Africa's production comes from Western Africa (FAO, 2006).

It is an annual legume which is also known as peanut, earthnut, monkey-nut and goobers (Nwokoto, 1996). It is the most important food crop and oil seed crop of the world. Groundnut seeds (kernels) contain 40-50% fat, 20-50 % protein and 10-20 % carbohydrate. Groundnut seeds are nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil). It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (Nwokoto, 1996).

In Sudan Groundnut plays an important role in the diets of rural populations, particularly children, because of its high contents of protein (21-30%), fat (41-52%), and carbohydrate (11-27%). It is also rich in calcium, potassium, phosphorus, magnesium and vitamin E. Protein meal, a by-product of oil extraction, is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock. This crop attacked by several diseases mainly fungal diseases, among these fungi, *Aspergillus spp*. which produced secondary metabolites called aflatoxin is the major is the contaminant that result in

acute and chronic poisoning in humans and animals on ingestion. The health impacts of ingestion in humans include stunted growth and development as well as an increased risk in liver cancer (Nwokoto, 1996).

2.1.3. Sesame (*Sesamum indicum* L)

Sesame is a flowering plant in the family *Pedaliaceae*. Numerous wild relatives occur in Africa and smaller number in India. Sesame seed is considered to be the oldest oilseed crop known to humanity (Raghav Ram, *et al.,* 1990). The crop has many species, and most are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa. The cultivated type is *Sesame indicum* which is widely naturalized in tropical regions around the world and is cultivated for its edible seeds. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. It was a major summer crop in the Middle East for thousands of years (Raghav Ram, *et al.,* 1990)

Sesame has one of the highest oil contents of any seed. With a rich nutty flavor and is a common ingredient in cuisines across the world (Ray Hansen, 2011). Although this crop is very important crop but in Sudan it became second class in oil crops after Groundnut in Sudan. The crop is attacked by several diseases and pest of which the most important are diseases caused by fungi. Among these are of the genus *Fusarium*, *Alternaria*, and *leveillula*. The crop is also used to be contaminated with

secondary metabolites like Aflatoxin which produced by *Aspergillus spp*. (Ray Hansen, 2011).

2.1.4. Sorghum (*Sorghum bicolor* (L.) Moench)

Sorghum bicolor (L.) Moench, sorghum of the family Poaceae, is one of the world's major grain crops. It is extensively cultivated in marginal rainfall areas of the tropics and subtropics, and selected varieties are widely grown in temperate climates. It is generally, considered to have first been domesticated in North Africa, possibly in the Nile or Ethiopian regions as recently as 1000 BC(Agrios 2005). Today, sorghum is cultivated across the world. It is quantitatively the world's fifth largest most important cereal grain, after wheat, maize, rice and barley. In Africa, sorghum is still largely a subsistence food crop that increasingly forming the foundation of successful food and beverage industries .(Agrios 2005). Sorghum in Africa is processed into a very wide variety of attractive and nutritious as traditional foods, such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. It is the grain of choice for brewing traditional African beers. Sorghum is also the grain of 21st century Africa. New products such as instant soft porridge and malt extracts are great successes. In the competitive environment of multinational enterprises, sorghum has been proven to be the best alternative to barley for lager beer brewing. The potential for sorghum to be the driver of economic development in Africa is

enormous. Continuing focused fundamental and applied research is essential to unleash sorghum's capacity to be the cornerstone of food security in Africa (Agrios 2005)

Sorghum grain is one of the major ingredients in swine, poultry and cattle feed in the western hemisphere, China and Australia. Sorghum is also grown for forage; in northern India it is very common and fed to animals fresh or as silage or hay. Sweet sorghum is used to a limited extent in producing sorghum syrup and 'jaggery' (raw sugar) in India and has recently gained importance in ethanol production (Agrios 1997).

In Sudan the crop is either from the semi-mechanized rain-fed sub-sector or irrigated sub-sector (Agrios 1997). semi mechanized farming is the main producer of sorghum, producing around 65 % of the 4.23 Million MT, which is the total sorghum production in Sudan (FAO/WFP, 2006) with an area of about 6 million hectares located in the region of Gadaref, Blue Nile, Sennar, Kosti, Renk, Dilling, and Kordofan, Darfur states (FAO/WFP, 2006)

In the Sudan the crop is subject to contamination by several contaminants, among those is *Drechslera* spp., *Fusarium* spp., *Aspergillus* spp., where *Aspergillus niger* is one of the most common species of the genus *Aspergillus*. (Samson, 2001).

2.2.hughar plant extract:

2.2. 1. Neem (Azadirachta Indica Juss.)

The Neem (family Meliaceae) which is tropical evergreen tree is thought to have originated in Asia and Burma. However, the exact origin is uncertain, some at authors said Neem is native to the whole Indian, other attribute it to dry forest areas, throughout all of south and south Asia (Ruskin, 1991).

In India, Neem is known as "the village pharmacy" because of its healing versatility, and it has been used in medicine for more than 4,000 years due to its medicinal properties. Neem is also called '*arista*' a word that means 'perfect, complete and imperishable'. The seeds bark and leaves contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses (Ruskin. 1991)

All parts of the tree have been examined by chemists which contains number of chemical compound called "triterpeness" or limonoids .there are nearly 100 proto limonoids, limonoids or triterpenoid, pentanor, hexane or triterpenoid and some none terpenioid (Janes *et al.,* 1985).

The Neem oil contains several terpenioid, steroids alkaloids, flavonoids, glycoside and other (Anonymous 2001).Biological activities of Neem products Neem has been used as an effective postharvest protestant for

many crops .Neem is especially against the cow pea weevil (David et al 2003).

In Sudan, Neem is introduced in the 20 century. The first one were planted at Shambat in 1916, today trees are spread in town and villages along the Blue and White Nile, irrigated areas of Central Sudan, Kordofan and Darfur (Schmutterer, 1969).

The Neem tree produce a compounds of many active ingredients called Azadirachtin and it is tetramer titer penoid compound which influences the hormonam, feeding activity reproduction and fling ability of insect. Azadirachtin hl systeas low mammalian toxicity. It degrades rapidly in the environment and has low side effects on non-target species and beneficial insects. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Salanin inhabits the feeding of wider any of insect pests, Nimbin and Nimbidin showed antiviral effects (Ganguli, 2002). Extract of various parts of the tree studied by many chemicals that isolated many different compounds. Most of the known active compounds belong to the group of titer penoids (Schmutterer, 1990). Azadirachtin and Solanin are the most important constituents of Neem seed kernel composition, other active compounds in the seed kernel are Salanin, Salanol, Acetate, Nimbin and Deactly nimbidin .

2.2.2. Damas (Conocarpus lancifolius Engl.)

Damas is one of the most important species in this family Combretaceae (Pandey and Misra, 2008). It is an evergreen tree that grows up to 20 m in height and 60 - 250 cm or more in diameter. It is usually a multi-branched tree in its natural habitat, trees planted in the Sudan formed a single, straight stem.

The tree is multipurpose; wood which is the main product is used domestically for house construction, firewood and excellent charcoal. Commercially timber was more useful formerly; it was cut and exported from Somalia to Arabia for dhow construction. Other potential uses include wood based board. Bark may be a useful source of tannins (Booth, 1993).

The tree is evergreen and its foliage makes a good fodder, also it is a good shade and roadside tree. It is used as wind breaks around irrigated agricultural areas and for avenue planting. As drought-resistant species, *C. lancijoliusis* is one of the more promising trees for trials in arid areas. It is recommended for a variety of soil types including saline soils, and yields excellent charcoal and valuable wood(Booth, 1993) .

Information on the importance of *C. lancifolius* in its native distribution areas relative to other species with similar wood, fuel and forage uses is lacking hence it is difficult to assess its importance. However, Somali tribe owing the Damas at the dry river valleys (wadis) containing *C.*

lancifolius have restricted cutting because of the threat of overexploitation (Booth, 1993).

Damas grows best in areas where the mean annual temperature ranges from 20°c -30°c, but where the maximum summer temperature has reached 50°c. The tree grows from sea level up to about 1000 m. The rainfall in its natural habitat is generally between 50 mm and 400 mm, but the tree grows mainly along seasonal watercourses. It can be grown in plantations in areas with less than about 400 mm but grows well only if irrigated or within reach of groundwater. It withstands drought conditions for several months when irrigation fails. Damas does well on deep soils ranging from pure sand to clays and loams, but has difficulty on shallow soils. It will tolerate moderately saline soils (Pandey and Misra, 2008).

2.3. Seeds borne fungi

The negative impact of seed borne pathogens on crop quality and quantity was demonstrated by many investigators (Agrios ,2005). showed that there was a significant decrease in oil content of sunflower seeds infected with Rhizopus oryzae(Agrios,2005).) who analyzed wheat seeds concluded that fungal infection led to abnormal seedlings and dead seeds. Foods contamination and its associated risks to humans, wild animals and livestock and reduced grain quality have been reported by several authors (Haq Elamin ,*et al.*, 1988 Yousif.et al., 2010).

Generally, fungi are one of the 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).

Spoilage fungi under inductive condition may cause problems once the crop is harvested if not able to attack crops in the field. Some spoilage fungi can also produce mycotoxins although they are not pathogenic. The seed borne fungi of most concern are produced by species within the genera of *Aspergillus*, *Fusarium*, and *Penicillium* that frequently occur in major food crops in the field and continue to contaminate them during storage, including cereals, oil seeds, and various fruits (*Azhar, et al.*, 2009).

Seed-borne mycoflora of sorghum reported from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* spp., *Fusarium moniliforme, F. oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora* spp., *Phoma* spp., and *Rhizopus* spp. (Haq Elamin, 1988). In his study he reported that there are a large number of other moulds that have been isolated from food and

feeds, particularly cereals, oilseeds, herbs and spices. These include *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus*, *Moniliella*, *Paecilomyces*, *Wallemia*, *Byssochlamys*, *Talaromyces*, *Eupenicillium*, *Claviceps*, *Phoma*, *Phomopsis*, *Curvularia*, *Chaetomium*, *Xeromyces* and *Chrysosporium*. Some of these produce mycotoxins, to some of which legislative restrictions may apply (patulin from *Byssochlamys*, for example), others do not.

However, Fusarium, Aspergillus, Penicillium and Alternaria are amongst the most common fungal species associated with growth in and damage to food crops in the field, and in store, if poor storage conditions prevail after harvest, especially in case of previously dried commodities (Bandyopadhyay, 1986).

It is well known that *Fusarium* spp. are major wilt pathogen of many economically important crop plants. It is a soil-borne pathogen, which can live in the soil for long periods of time. Jones *et al.,* (1982) reported that Fusarium species are mainly plant pathogens and normally occur in association with plants and cultivated soils. Infection may occur in developing seeds, and in maturing fruits and vegetables. Damage is usually confined to pre-harvest, for cereals, or immediately post-harvest until drying is well under way. Vegetables can continue to be spoiled in store, due to their higher water activity.

Examples of species are Fusarium chlamydosporum, Fusarium culmorum, Fusarium solani, Fusarium equiseti, Fusarium graminearum, Fusarium oxysporum, Fusarium proliferatum, Fusarium poae, Fusarium semitectum, Fusarium subglutinans, Fusarium sporotrichioides and Fusarium verticillioides (alternative name (synonym) F. moniliforme).

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).

The role of Aspergillus species in food spoilage as well is well-established Haq Elamin et al., 1988; Yousif,.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,* and *Aspergillus carbonarius, and Aspergillus versicolor (Peter, 2010.)* However, *Haq Elamin NH et al., (1988); Yousif ,et al., (2010).* 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 feedstocks - 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.

Moreover, 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. Important mycotoxins produced by Penicillium include ochratoxin A, patulin, citrinin and penitrem A. Some of the most important toxigenic species in foods are *Penicillium expansum, Penicillium citrinum, Penicillium crustosum and Penicillium verrucosum* (Pitt., 2006).

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., 2006).

One of the plant pathogens that can produce toxins in both pre- and post-harvest commodities are Alternaria species. 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.

2.4. Control of Seed Borne Fungi

The control of seed borne fungi can be considered broadly in terms of exclusion and elimination of inoculum (*(Peter, 2010.)* However, use of chemical to control diseases is indispensable). Several fungicides have been used for control of different plant pathogens including fusaria (Liggit et al. 1997).and the number of effective fungicides with negligible effect on the environment is rare. Fungicides are expensive, can cause environmental pollution and may cause the selection of pathogen resistance (Lumsden and Locke 1989).

However, alternative methods of controlling the disease have been studied with emphasis on novel compounds derived from plant sources

(Garibaldi et al. 1990; Alabouvette 1999). Plant extracts and plant essential oils have been reported to be effective antimicrobials against food and grain storage fungi, foliar pathogens and soilborne 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).

A 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; Okigbo, 2005)

In Sudan, ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results. Crude extracts solution obtained from the plant Gordenia lutea, showed antibacterial activity against Bacillus subtilis, Staphylocous aureus, Escherichia coli and Pseudomonas aeruginoza (Ahmed et al., 1984). reported that ginger oil showed antimicrobial activity against Staphylococcus aureus, while,(Singha *et al.,* 2010).

reported that Staphylococcus aureus was sensitive to clove oil. The fenugreek oilwas also found to inhibit Salmonella typhimurium (Ahmed et al., 1984). Despite of the growth of global market for herbal products, following complementary and/or alternative medicines, homeopathy, health foods and natural-pharmaceuticals therapy, yet the majority of these herbs were not assessed for quality, safety and efficacy.

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 (Mathur and Kongsdal, 2003. 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 şimplest 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 (Mathur, 2003).

CHAPTER THREE

MATERISLS AND METHODS

3.1. Experimental site

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 May-July, 2013. The aim of this study was to detect and identify seed borne mycoflora associated with seeds samples from four food crops collected from four different locations, each in one estates of Sudan, and to explore the methods of control under laboratory conditions where temperature 28^0C.

3.2. Materials, tools and equipments used in the study

- Gloves
- camera
- marker pen
- electric blender
- Petri-dishes
- sensitive balance
- incubator
- needle
- flame
- fomalin
- laminar

- microscope
- slide
- aluminum foul
- water path
- potato dextrose agar(PDA)
- filter papers
- medical cotton

All materials except seeds, which used in this experiment, were sterilized either using 1% sodium hypochlorite or 70% ethyl alcohol. Formalin (10%) was used for Petri plate sterilization. staining of fungal cytoplasm was used according to (Aneja,2004) where cotton blue and lacto phenol were used for providing a light blue background, against which the walls of hyphae can easily be seen

3.3. Collection of seed samples

Sixteen seed samples, 4 of each crop, of sorghum, pearl millet, sesame and groundnut were collected for detection and identification of seed-borne fungi from four Estates of Sudan, during may 2013. The seeds were randomly sampled from grains market' seed stocks of four different zones, El-Gazera, El-Gadarif, Niyalla and Elobied, one in each Estate. Four seed samples, one of each crop, were obtained from each of the four zones. From each seed sample, an amount of 1000 gm of homogeneous seeds were taken. 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 stored at 5^0C refrigerator for further analysis.

3.4. Methods for detection and identification of seed borne fungi

3.4.1. Dry seed inspection

All seed samples under test were examined under the stereoscopic binocular microscope (using low magnification) for impurities such as plant debris, weed seeds and for disease symptoms such as discoloration, wrinkling or malformation.

3.4.2. Seed Health Testing

For the detection of the seed-borne fungi associated with each seed samples standard blotter method as described by the International Seed Testing Association (ISTA ,1993), was used. The seed samples in their various forms according to their crops were then plated on moistened filter papers (dia. 9.0 cm) in 9.0 cm sterilized Petri-dishes. Hundred untreated seeds from each sample were used and plated at equalized distances then incubated at 28°c for 7 days under alternating cycles to enhance sporulation of fungi. A total of four seed samples per crop, with three replications, were used.

Each seed sample at the end of the incubation was examined thoroughly under compound microscope for the growth of fungi. Fungi found

associated with seeds were carefully examined and identified based on 'habit characters' (Mathur, 2003). Slide preparation of fruiting structures, such as conidia born in conidiophores, spores held together in spore masses, sporodochia, and acervuli, pycnidiospore in pycnidia, ascospores in perenthecia were each examined using compound microscope to confirm their identity using reference publication (Mathur, 2003). Records were then taken on incidence and infection percent of the seed borne fungal pathogens identified on seeds.

3.4. Agar test

Seed samples were first treated with 1% sodium hypochlorite solution for 3 minutes, washed 5 times with sterilized distilled water and dried between 2 sterilized filter papers. Seeds were then planted in glass petri-dishes containing Potato Dextrose Agar medium (PDA). The plates were incubated for 7 days at 25°c. On the 8 day the seeds were examined under compound microscope.

3.5 Source of fungus

Aspergillus niger was used in this test being the most frequently identified fungus in seed samples. The fungus was obtained from fresh culture previously isolated from seeds.

3.6 Collection of leaves samples

Neem and Damas leaves were collected from trees growing in the premises of the college of Agricultural study, Shambat.

and brought to the laboratory where they were shade dried under ambient The sample were then left to dry under room temperature for two weeks, and then washed with distilled water before grinding using an electric blender (Moulinex). The collected powder was used directly for making aqueous extracts.

Temperature ground and powdered separately to obtain fine powder for extraction.

3.6.1. Preparation aqueous extracts

The aqueous extract of Neem and Dams leaves powder were prepared by adding 10 grams of the -leaves powder to 90 ml sterilized distilled waters in a conical flask 250ml. The mixtures were shaken every 8 hours till 24 hours at room temperature. The mixture was then strained using a light cloth, and then filtered through filter paper what man No. 1 and stored till the experiment time.

3.6.2. Preparation of concentrations of aqueous extracts

Three concentrations of.25- 50 and 100 ml of leaves and water extract were prepared through series of dilution using distilled sterilized water. The control was treated with sterilized distilled water.

The obtained fine powder form each plant leaves was weighted in to 25, 50 and 100gm and placed in a separate conical flask each containing 100 ml distilled water and was placed in a shaker for 4 hours. The solutions

were later filtered through layers of muslin cloth. Concentrations of 25%, 50% and 100% concentrated of the Neem and damas leaves extracts were prepared.

3.7. Test procedure

Inhibition zone technique was used in this study as described by(Rao and Srivastava (1994). The fungus spores suspension was prepared from previously prepared pure culture by allowing the spores to grow on PDA media treated with the desired concentration of Neem and Damas leaves water extract.

The PDA media was amended with the required concentration (25, 50 and 100%) before being solidified in a conical flask of 250 ml, agitated before pouring 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 inoculated using sterilized filter paper disc dipped in a fresh culture suspension of corresponding fungus and placed at the centre of the plate. In case of the control the disc was treated with sterilized distilled water and placed at the centre of Petri-dishes. Inoculated Petri dishes were then incubated at 28 C^0 for 3, 4 and 5 days. The growth of the fungus was calculated every day. Treated plates were arranged in a randomized complete block design.

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

$$R = \frac{dc - dt}{Dc} \times 100$$

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

3.8. Statistical analysis

The data obtained was statistically analyzed according to analysis of variance (ANOVA), L.S.D test was used for means separation.

CHAPTER FOUR

RESULTS

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 May-July, 2013. The aim of this study was to detect and identify seed borne mycoflora associated with seeds samples from four food crops collected from four different zone, each in one Estates of Sudan, and to explore the effect of aqueous plant leaves extract of Neem and Damas and fungicide (Tilt) on the linear growth *Aspergillus niger* in culture media under laboratory conditions where temperature around 28 ^{0}C.

The results are presented in Tables 1 to 7 respectively under the different parameters investigated. The results cover detection and identification of seed borne mycoflora associated with seeds samples of four food crops collected from four different locations, each in one Estate of Sudan and effect of aqueous plant leaves extract of Neem and Damas and fungicide (Tilt) on the linear growth of *Aspergillus niger* in culture media under laboratory conditions where temperature around (+28 ^{0}C).

4.1. Detection and Identification of seed borne fungi

Aspergillus clavatus. *A. niger* . *Cladosporium* spp., *Fusarium moniliforme* .

Drechslera tetramera, Nigrospora spp., *Phoma* spp., and *Rhizopus* spp. *Penicillium spp. Macrophomina spp.*

4.1.1 Incidence of fungal species on the four crops seeds from different locations.

Out of the sixteen seed samples, 4 of each crop, tested for seed borne fungi, a total of 7 genera of 8 species of fungi were recorded (Table 1-4). The mean percentage incidence of seed borne fungi of sorghum, millet, groundnut and sesame revealed by the Blotter Method are given in Tables 1, 2, 3 and respectively.

The fungal pathogens Detected were *Aspergillus flavus, Aspergillus niger, Fusarium spp. Penicillium spp. and Rhizopus spp. Alternaria spp., Macrophomina spp. and Drechslera spp.*

The four most prevailing seed borne fungi Detected across crops seeds were the non– pathogenic ones; *Aspergillus flavus, Aspergillus Niger, Penicillium spp. and Rhizopus* spp. with varying level of incidences (Table 1-4).

Irrespective of the source of seed, same fungi were detected, with variation in fungus percentage infection e.g. *Aspergillus niger* was 86 % in Nyalla as compared to 8.0 % in Gadarif (Table 1). *Aspergillus flavus* on groundnut was higher in Elobied 70% than Wad madani 25% (table, 3). Most samples tested for seed borne fungi gave a wide and large number of fungi with varying incidences (Table, 1-4). However, among all four crops average percent frequency of seed borne fungi in

groundnut was the highest 34% (table, 4) and fungi detected on groundnut occurred in relatively higher incidence as compared to other crops.

4.1.2. incidence of seed borne fungi on sorghum seeds

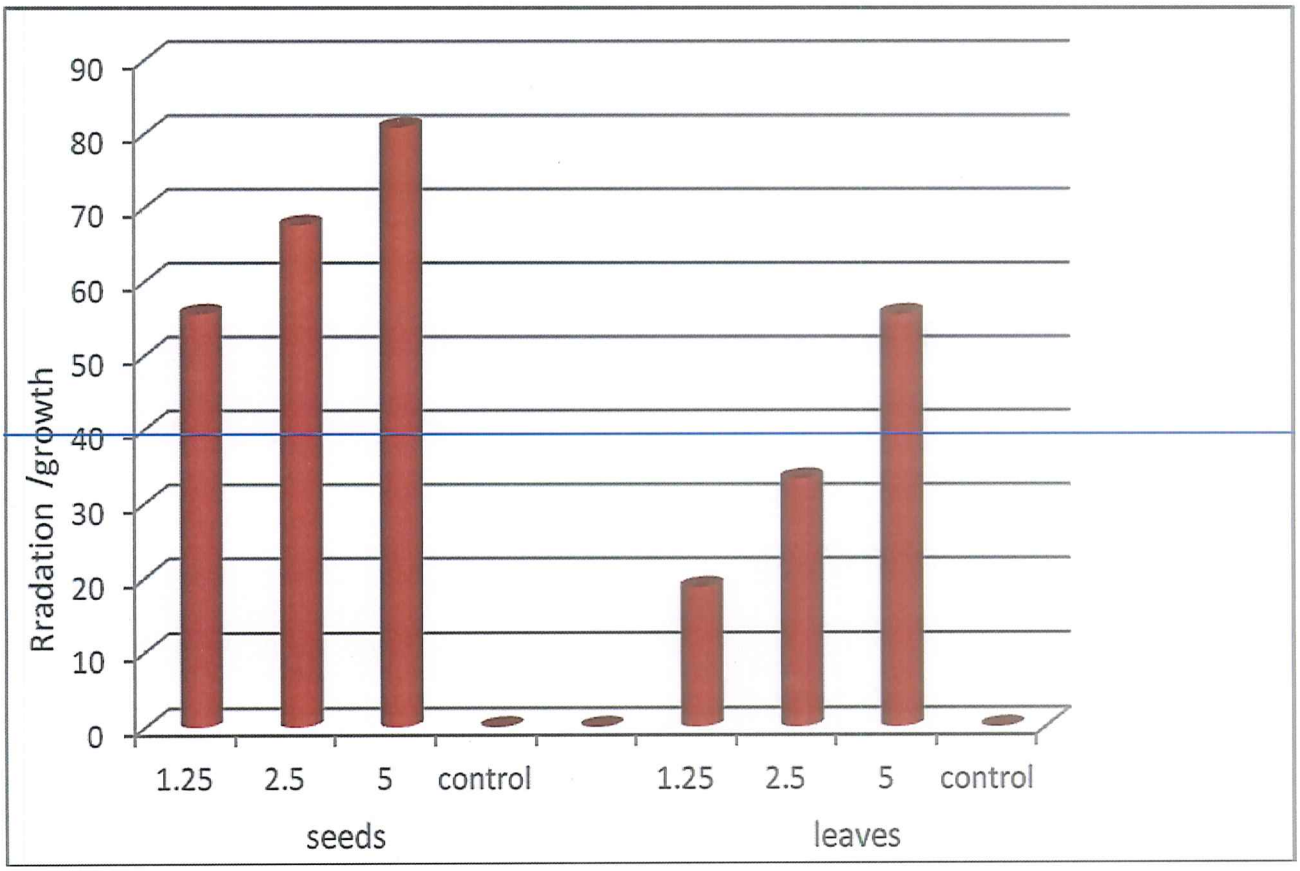
The results obtained (Table, 1) showed that out of the seven genera of seed borne fungi detected in all samples of seeds, a total of species of fungi were recorded I sorghum. The percent frequency of occurrence of the seed borne fungi in seeds is higher in Niyalla 15% and Gadarif 14% followed by Wad madani 13% and Elobied 11%. However, *Aspergillus Niger* in Niyalla was the most prevailing fungus with 86 % followed byWodmadni 54% and lower in Gadarif 8% .while Drechslera was high in Gadarif recorded 43% and lower in Niyalla recorded 1% also high incidence of Penicillium in Gadarif 21% followed by Elobied 9% bat lower in Wodmadni recorded 2%.

Frequency of occurrence compared to other fungi.

Table. 1 ;seed borne Fungi and their frequency (%) isolated form sample of sorghum from deferent location in Sudan

Species/location	Niyalla	Elobied	Gadarif	wad	Total	mean
Aspergillus flavus	0.0	25	13	10	48	12.0a
Aspergillus niger	86	21	8.0	54	169	42.3ac

Alternaria spp.	11	1.0	0.0	0.0	12	03.0b
Macrophomina spp.	0.0	3.0	10.0	8.0	13	03.3ab
Rhizopus spp.	6.0	13	3.0	18	40	10.0c
Drechslera spp.	1.0	4.0	43	1.0	49	12.3ab
Penicillium spp.	4.0	9.0	21	2.0	36	09.0d
Mean %	15	11	14	13	53	13

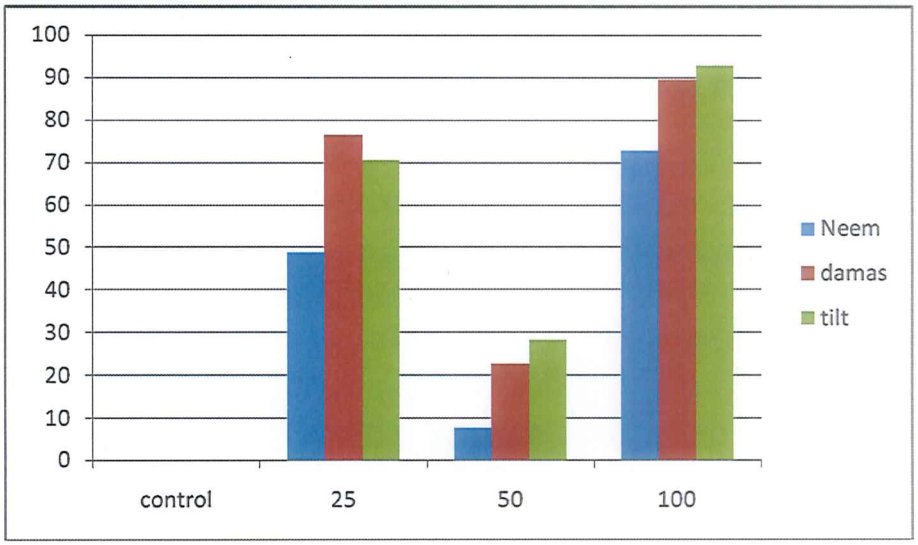


4.1.3 incidence of seed borne fungi on millet seeds

Frequency of occurrence of seed borne fungi in pearl millet (Table 2) *Penicillium* spp was higher in Niyalla 57% followed by Elobied 61% Gadarif 20% and Wad medani.5%. Among all species of seed borne fungi detected in all locations, average percent frequency of *Penicillium* spp. in pearl millet was the higher 36% followed by *Aspergillus flavus* 29% and *Aspergillus Niger* 18%.

Table. 2 : seed borne Fungi and their frequency (%) isolated form sample of millet from deferent location in Sudan

Species/location	Niyalla	Elobied	Gadarif	Wad	Total	mean
Aspergillus flavus	27	18	33	37	115	29.0a
Aspergillus niger	27	32	4.0	0.8	71.0	18.0ab
Alternaria spp.	0.0	12.0	0.0	0.0	12.0	03.0d
Fusarium spp.	0.0	7.0	0.0	3.0	10.0	03.0cd
Rhizopus spp.	18	11	9	14	52.0	13.0b
Drechslera spp.	3.0	4.0	11	11	29.0	07.0d
Penicillium spp.	57	61	20	5.0	143	36.0a
Mean %	18	21	11	11	62	15.5


100
90
80
70
60
50
40
30
20
10
0
control
25
50
100
Neem
damas
tilt

4.1.4. Incidence of seed borne fungi on groundnut seeds

Results of fungal identification in Table 3 showed groundnut seed mycoflora. Among all seed borne fungi dtected, average percent frequency of *Aspergillus flavus* in groundnut was the highest 70% followed by Penicillium spp. 38%. Fungal pathogens identified included Rhizopus, *Penicillium and Aspergillus.* Frequency of occurrence of seed borne fungi in groundnut (Table 3) was higher in Elobied 60% followed by Gadarif 39% then Wad medani 20% and Niyalla 11%.

Table. 3 seed borne Fungi and their frequency (%) isolated form sample of groundnut from deferent location in Sudan

Location/species	Niyall	Elobie	Gadarif	Wad	Total	Mean %
Aspergillus flavus	18	70	60	25	287	20a
Aspergillus Niger	0.0	15	35	0.0	50.0	13d
Penicillium spp.	26	35	40	50	157	38cd
Rhizopus spp.	0.0	15	20	15	50.0	13b
Mean %	11	60	39	23	133	34

4.1.5. Incidence of seed borne fungi on sesame seeds

In case of sesame, higher frequency of occurrence of the seed borne fungi was recorded in Wad madani 24% whereas minimum in Elobied 19%. A total of four species of fungi were detected in samples collected from the four locations, namely, *Aspergillus flavus, Aspergillus Niger, Penicillium spp. and Rhizopus spp. However, A. flavus* in sesame showed the highest frequency of occurrence 83% in seed samples from Wad madani followed by *Penicillium* spp. 82% detected in seed samples from Niyalla. Moreover, among all fungi detected in seeds from the four locations, the mean percentage incidence of *A. flavus* was the higher 47 % followed by Penicillum spp. 30%.

Table. 4 incidence(%) of seed borne fungi on various seed sample of sesame collected from four different locations.

Localalies. Fungi	Niyalla	Elobied	Gadarif	Wad	Total	Mean
Aspergillus flavus	17	50	38	83	188.0	47.0
Aspergillus Niger	0.0	15	35	0.0	050.0	13.0
Penicillium spp.	82	9.0	20	10	121.0	30.0
Rhizopus spp.	1.0	3.0	4.0	2.0	010.0	03.0
Mean %	25	19	24	24	92.00	23

4.2. Effect of leaves aqueous plant extracts of Neem and damas,fungicides on the fungal growth

4.2.1. Effect of plant extracts on fungal growth invitor after three days from inoculation

The results (Table, 5) showed that the leaves aqueous extracts of all plants tested and fungicide had demonstrated an inhibitory effect on the fungal growth after three days from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

Moreover, the 100% concentration of the plant extracts (Neem and Damas) and 50% and 100% of fungicide Tilt gave significantly higher inhibition zones percent (85.6%, 70%, 100% and 100%) respectively compared to the untreated control. Among the plant extracts tested that of Neem was better than Damas in suppressing the fungus growth whir inhibit the fungal growth 85%whil Damas 70%. (Table, 5). The results also showed that the antifungal activity increase with increase in extract concentration.

Table. 5: Effect of leaves aqueous crude extracts of Neem, Damas and fungicide Tilt on the linear growth (invitor%) of the fungus *Aspergillus niger* after three days from inoculation.

Treatments		Inhibition zone (%)			
Concs. (%)		R1	R2	R3	Mean
Neem	25	*44.4(6.7)*	*66.7(8.2)*	*50.0(7.1)*	*53.7(7.3)d*
	50	*66.6(8.2)*	*77.7(8.8)*	*60.0(7.8)*	*68.1(8.3)c*
	100	*77.8(8.8)*	*88.9(9.5)*	*90.0(9.5)*	*85.6(9.3)ab*
Damas	25	*25.0(5.0)*	*21.4(4.7)*	*27.1(5.2)*	*24.5(5.0)e*
	50	*58.3(7.7)*	*50.0(7.1)*	*27.3(5.3)*	*45.2(6.7)d*
	100	*75.0(8.7)*	*71.4(8.5)*	*63.6(8.0)*	*70.0(8.4)bc*
Tilt	25	*95.1(9.8)*	*93.3(9.7)*	*95.2(9.8)*	*94.5(9.8)a*
	50	*100.0(10.0)*	*100.0(10.0)*	*100.0(10.0)*	*100(10.0)a*
	100	*100.0(10.0)*	*100.0(10.0)*	*100.0(10.0)*	*100(10.0)a*
Control		*0.0(0.7)*	*0.0(0.7)*	*0.0(0.7)*	*0.0(0.7)f*
C V					*6.99%*
SE					*0.52*
LSD					*0.898*

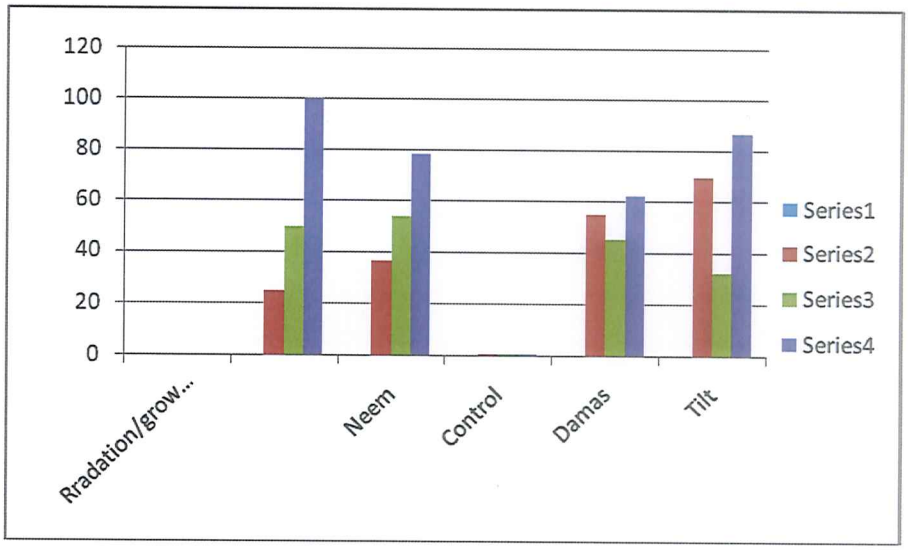
Data in parentheses transformed using square root transformation$\sqrt{X+0.5}$) before analysis

R = A-B\A %

R =percent reduced of liner growth .

A = liner growth of control.

B = liner growth of treatment



4.2.2. Effect of plant extracts after four days from inoculation

In day four after inoculation, all treatments, plant extracts concentrations as well as that of the fungicide, were invariably continued exhibiting inhibitory effects against the fungal growth. However, the Neem plant extracts (100%) and tilt as well gave the highest inhibition zones percent (88.5, and 100%) respectively. This inhibitory effect from all concentrations tested was significantly different from control (Table, 6). Furthermore, the fungicide irrespective of concentration, (25, 50 and 100%) effected significant reduction of fungal growth 93.7%, 100%, and 100% compared to control.

Furthermore, the fungicide at all concentrations tested continued to be the most suppressive, followed in descending order by the Neem and Damas plant extract.

Table. 6 : Effect of leaves aqueous crude extracts of Neem, Damas and fungicide on the linear growth (inhibition zone %) of the fungus *Aspergillus niger* after four days from inoculation.

Concs. (%)		Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	25	36.8(6.1)	47.	56.5(7.6)	47.0(6.9)d
	50	73.7(8.6)	76.2(8.8)	87.0(9.4)	79.0(8.9)b
	100	89.5(9.5)	85.7(9.3)	91.3(9.6)	88.5(9.5)a
Damas	25	40.0(6.0)	31.8(5.7)	41.7(6.5)	37.8(6.1)e
	50	55.0(7.4)	54.5(7.4)	50.0(7.1)	53.2(7.3)d
	100	60.0(7.8)	59.1(7.7)	52.5(9.7)	57.2(8.4)c
Tilt	25	95.5(9.8)	94.7(9.8)	91.(9.6)	93.7(9.7)a
	50	100.0(10.0)	100.0(10.0)	100.0(10.0)	100(10.0)a
	100	100.0(10.0)	100.0(10.0)	100.0(10.0)	100(10.0)a
Control		0.0(0.7)	0.0(0.7)	0.0(0.7)	0.0(0.7)f
CV					6.11
SE					0.50
LSD					0.8061

Means followed by the same letter are not significant different at ($P< 0.05$)

❖ Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis.

4.2.3. Effect of plant extracts after five days from inoculation

After five days from inoculation (Table, 7) all treatments, plant extracts concentrations as well as that of the fungicide, were invariably continued exhibiting inhibitory effects against the fungal growth. However, the Neem plant extracts (50 and 100%) and Tilt as well at all concentrations gave the highest inhibition zones percent (78.9%, 88.8%, 90.4% 100% and 100%) respectively. This inhibitory effect from all concentrations tested was significantly different from control (Table, 7).

Furthermore, the fungicide irrespective of concentration, (25, 50 and 100%) continued to give consistent reduction effect against fungal growth throughout the test period. This consistent inhibitory effect against fungal growth was also exhibited by the Neem extract at 50 and 100% concentrations. However, the inhibitory effect of Damas plant extract reduced with time of recording of experiment.

Table. 7: Effect of leaves aqueous extracts of Neem, Damas and fungicide on the growth of the fungus (*Aspergillus niger*) after five days from inoculation

Treatment		*Inhibition zone (%)*			
		R1	R2	R3	Mean
Neem	*25*	*36.8(6.1)*	*47.6(6.9)*	*56.5(7.6)*	*46.9(6.8)a*
	50	*73.7(8.6)*	*76.2(8.8)*	*87.0(9.4)*	*78.9(8.9)b*
	100	*89.5(9.5)*	*85.7(9.3)*	*91.3(9.6)*	*88.8(9.5)a*
Damas	*25*	*16.7(4.1)*	*21.9(4.7)*	*11.8(3.5)*	*16.8(4.1)f*
	50	*26.7(5.2)*	*31.2(5.6)*	*23.5(4.5)*	*27.1(5.1)d*
	100	*33.3(5.8)*	*40.6(6.4)*	*55.9(7.5)*	*43.3(6.6)c*
Tilt	*25*	*91.7(9.6)*	*90.9(9.6)*	*88.5(9.4)*	*90.4(9.5)ab*
	50	*100.0(10.0)*	*100.0(10.0)*	*100.0(10.0)*	*100(10.0)a*
	100	*100.0(10.0)*	*100.0(10.0)*	*100.0(10.0)*	*100(10.0)a*
Control		*0.0(0.7)*	*0.0(0.7)*	*0.0(0.7)*	*0.0(0.70)f*
CV					*6.56*
SE					*0.55*
LSD					*0.79*

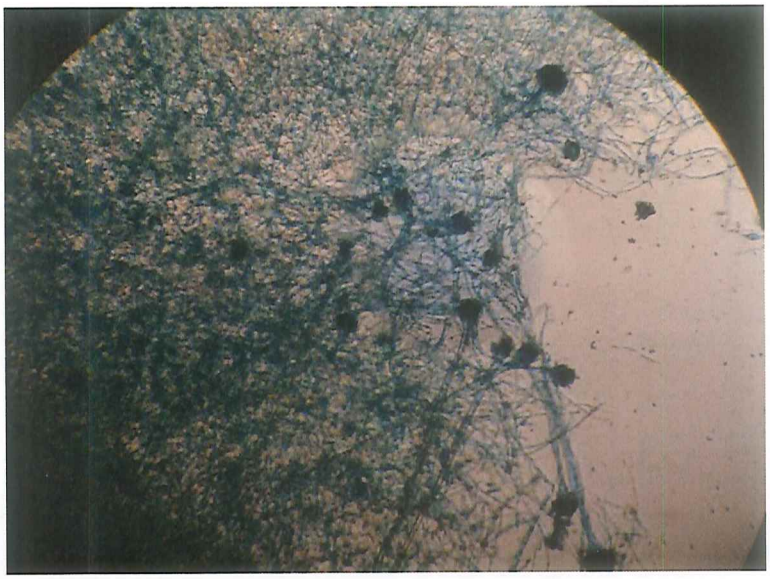
Means followed by the same letter are not significant different at ($P< 0.05$)

❖ Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis.

(1)*Penicillium sp.*

(2)*Aspergillus Niger*

(3)*Aspergillus Niger*



CHAPER FIVE

DISCUSSION

The study was carried out to detect and identify the seed borne fungi occurred on four food crops seeds collected from different estate of Sudan and to explore the potential of botanical extracts in suppressing the radial growth of fungus *Aspergillus niger in vitro*.

Foods contamination and its associated risks to humans, wild animals and livestock have been reported by several authors (Haq Elamin *et al.*, 1988 and Yousif, *et al.*, 2010).

In fact, the seed mycoflora of most concern are produced by species within the genera *of Aspergillus, Fusarium, and Penicillium that* frequently occur in major food crops in the field and continue to contaminate them during storage, including cereals, oil seeds, and various fruits (*Azhar, et al.*, 2009).

The results of this study revealed that the association of food crops seeds with seed borne fungi in different locations of Sudan appears to be a prevalent situation. All the seeds samples tested with standard blotter method as described by the International Seed Testing Association (ISTA, 1993) were associated with at least three known spoilage species of fungi (*Aspergillus, Rhizopus and Penicillium*). These results are in agreement with those of (Syed Danis, *et al., (2013))* who reported the

presence of *Aspergillus, Penecillium, Alternaria, Fusarium*, and *Rhizopus,* species in seeds of food crops who reported the occurrence of *Aspergillus, Penicillium and Fusarium* spp. were common associates of seeds crops.

The results showed four most prevailing seed borne fungi recorded across tested crops seeds, *Aspergillus flavus, Aspergillus Niger, Penicillium spp. and Rhizopus* spp. with varying level of incidences. The common occurrence of seed borne fungi like Aspergillus and Penicillium had been widely reported by (Bandyopadhyay (1986)The high load of seed borne fungi in some crop seeds or in some location compared to others demonstrated by this study could be attributed to favourable weather conditions for the different fungi in different environments. The implications of this variation was highlighted in the report of(Bandyopadhyay (1986) who determined that prevailing conditions at harvest and storage were responsible for incidence of spoilage fungi. Moreover, the present result showed that all the samples tested were associated with *Aspergillus which were* predominant fungi of groundnut.(Mathur *et al.,* (1975).also found that *Aspergillus* were the predominant storage fungi of groundnut seeds.

The study also investigated the potential of plant extracts against growth of fungi. Generally, use of synthetic fungicides considerably reduce the negative impact of fungal diseases incidence in crops but their use is

costly as well as environmentally undesirable (Patil, *et al.,* 2001). Moreover, the use of resistant varieties is faced with breakdown of resistance due to high pathogenic variability in the pathogen population (Patil, *et al.,* 2001).In this context, the searches for an eco-friendly way of managing seed borne fungi in crop seeds which offers an alternative to fungicides is highly demanding.

In fact, higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenioid, Phenols and other secondary metabolite were produced by higher plants (Siddig, 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Patil, *et al.,* 2001).

In this study, the data revealed that the Neem and Damas leaves aqueous extracts consistently exhibited an inhibitory effect on fungal growth with significantly higher inhibition zones percent. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish *et. al.*, 1999; Okigbo and Ogbonnaya, 2006; Neem tree (*Azadirachta indica*) as well is one of the most known plants

for its multiuse in controlling insect pests and diseases. In this study the results revealed that the Neem leaves aqueous extracts gave the highest fungal growth suppression with significantly high inhibition zones percent compared to control. In field of plant protection Neem used to control: Grey leaf spots, powdery mildew and viruses. Similar results were obtained by Hanaa *et al.,* (2011) who found that treatment of tomato plants with Neem aqueous extracts reduced the percentage of Fusarium wilt disease incidence to the level of 25.5% and 27.8% after 6 weeks of infection respectively.

The results on effect of the Tilt on the fungus showed that the fungicide at all concentrations expressed consistently suppressive ability on the growth of the test fungus with significantly high inhibition zones percent compared to control throughout the experiment period. This finding is in line with the observations reported by(Abdelgader (2005). on efficacy of Tilt against *Fusarium oxysporum* where he found that tilt induced 100% inhibition against *Fusarium oxysporum* when applied at 100ppm after 7days of exposure. Similar finding were also revealed with (Mohammed (2002) who found that tilt when applied at 10ppm against *Drechslera hawaiiensis* induced 100% inhibition after 4 days.

The current study also demonstrated that the Neem leaves extract exhibited more inhibitory effect than that of the Damas. This could be attributed to the high concentration of the bioactive inhibiting compound

in the Damas plant leaves than in the Neem. Moreover, the data on concentrations from each plant leaves aqueous extract exhibited different inhibitory abilities on fungal growth.

The 100 % leaves aqueous extract concentration from the two plants was 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 Damas and Neem extracts was also reported by (Aiyelaagbe (2001). In his investigation, he explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree.

Aspergillus sp. is an important mycotoxin producer and produces four major metabolites of Aflatoxins B1, B2, G1 and G2 which are heptacarcinogenic (Abdelgader (2005). There is, therefore, need for reducing the mold growth and mycotoxin production in sorghum, pearl millet, groundnut and sesame seeds by improving the storage condition. The presence of so many pathogenic fungi at high level in stable food crop seeds from various geographical area indicates a clear need for field surveys for these and other pathogens. There also a real need to increase public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of seeds.

CONCLUSION

- One of the important aspects of food crop seeds besides high quality and purity is the absence of seed-borne fungi. In this study seven fungal genera were encountered in high percent frequencies of seed-borne fungi incidence percentage in 16 samples of sorghum, pearl millet and groundnut and sesame collected from four locations, each in one state of Sudan.

- *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Alternaria*, and *Drechslera* spp. were the main fungi occurring frequently in sorghum, pearl millet and groundnut and sesame seeds. Of the fungi isolated, the four most prevailing seed borne fungi recorded across crops seeds were the non– pathogenic ones; *Aspergillus flavus, Aspergillus Niger, Penicillium spp. and Rhizopus* spp. with varying level of incidences.

- *Aspergillus spp.* were the predominant seed borne fungi of all crop seed samples tested in all locations and notably the highest groundnut seeds

- The leaves aqueous extracts of all plants tested exhibited an inhibitory effect on fungal growth. Thus the two components plus fungicide (tilt) could be applied as part of an integrated approach to control seed borne fungi.

- The Neem plant leaves aqueous extract exhibited more inhibitory effect than that of the Damas.

RECOMMENDATIONS

Based on the foregoing result the following studies were recommended:-

- Further seed health analysis needs to be done for sorghum, pearl millet, groundnut and sesame crops across Sudan so that seed borne fungi mapping is done and updated regularly for research to target potentially important ones.
- Also more investigation needs to be done to determine consistency of the seed borne fungi isolated across locations to determine percentage incidences and severity under favourable conditions.
- Testing seed health of major crops should be introduced in the national seed quality system.
- To further investigate the antimicrobial properties of higher plants but in a group of medicinal plants against targets organism to determine their potentials as botanical pesticides.

REFERENCES

Abdelgader,H.S.M.(2005)Pathogenicity of two seed borne fungi isolated from seed of *Cicer arietinum* I.Msc.thesis Colege of Agricultural studies, Sudan unversites.Scual.

Agrios , G.N. (2005)Environmental effect is on development of the infectious disease. (in)plant pathology. 5th end, ElesvierAcad .press Burlington , mass ,USA pp251-262

Agrios, G. N. (1997). Plant diseases caused by *Mollicutes: phytoplasmas* and spiroplasmas, In Plant Pathology, Edited by G. N. Agrios. New York: Academic Press. pp. 457-470.

Agrios, G.N. (1988). Plant Pathology, 3rd. ed. Academic Press, Inc.: New York. 803 pp.

Ahmed, M. M. (2002). Molluscicidal and antimicrobial activity of certain Sudanese cucurbitacea plants. (Cited from Ahmed S.A., 2007).

Ahmed, O. M. (1984).Anti-hyperglycemic effects of water extract of Ulva lactucaand polysaccharides in nicotinamide-streptozotocin diabetic rats. Egypt. J. Zool., 54: 273-297.

Aiyelaagbe, O. O. (2001). Antibacterial activity of Jatropha multifida roots. Fitoterapia, 72:544–546.

Anon A. (1989). Mycotoxins, Economic and Health Risks. Council for Agricultural Science and Technology; Report No. 116. pp. 91.

Anon A. (2001). Mycotoxins, Economic and Health Risks. Council for Agricultural Science and Technology; Report No. 116. pp. 91.

Azhar H.; Safdar A.; Anwar1, G. M. and Sahi1, Q. A. (2009). Seed Borne Fungal Pathogens Associated with Pearl Millet (*Pennisetum typhoides*) and their impact on seed germination. Pak. J. Phytopathol., Vol. 21(1): 55-60, 2009.

Bandyopadhyay R (1986). Grain mold. In: Fredariksen, RA (ed). Compendium of sorghum diseases, Annual phytopathol. Soc., St. Paul Minnesota, USA, pp. 36-38.

Berger, R. D. (1977). Application of epidemiological principles to achieve plant disease control. Annual reviewof phytopathology 15, 165-183.

Booth, C. (1993) Fusarium lahoratory guide to the identification of the major species commonwealth mycological institutes , kew ,surrey PP 325 .

Booth, C. 1971. The Genus *Fusarium.Commonwealth* Mycological Institute, Kew, Surrey, UK, 237 pp.

Bowersand, J. C. (2000). Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil control of

David, H.; Akesson, M. and Nielsen, J. (2003). "Reconstruction of the central carbon metabolism of *Aspergillus niger*". European Journal of Biochemistry. 2003. Volume 270. p. 4243-4253

FAO, database, (2005/2006). FAO Repots, 2005 photopathological, 29(3): 225_233 bhp: //faostat. fao. org

FAOSTAT, (2010) Crops primary equivalent. Retrieved on 16th May, 2011 from www.faostat.fao.org

Fuller, D. Q. (2003). African crops in prehistoric South Asia: a critical review. inNeumann,K., Butler,A., Kahlheber,S. (ed.) Food, Fuel and Fields. Progress in Africa Archaeobotany. Africa Praehistorica 15 series. Cologne: H Fusarium wilt in the greenhouse, plant Disease, 84:300_305.

Ganguli, S. (2002). "Neem "A Therapeutic for all seasons "current science". 82 (11), June.Pp. 1304 (AL JAZEERA report on neem treatment in Senegal).

Garibaldi A, Guglielmone L, Gullino ML (1990) Rhizosphere competence of antagonistic Fusaria isolated from suppressive soils. Symbiosis 9:401–404

Hanaa, R.M.; Zeinab, A. A.; Dawlat, A. S; Meervat, A. R. and Ibrahim, A. M. (2011). Effect of Neem and Willon aqueous extracts on Fusarium wilt diseases in Tomato seedling: Induction enzymes. Annual Agricultural Sciences. Volume 56, pp.1-7.

Haq Elamin N. H.; Abdel-Rahim A. M. and Khalid A. E. (1988). Aflatoxin contamination of groundnuts in Sudan. Mycopathologia. 1988 Oct; 104(1):25-31.

Ibrahim, A. M. (1990). Survey of seed borne fungi of pearl millet in Sudan. M.Sc. thesis University of Khartoum.

ISTA (International Seed Testing Association) (1966). International Rules for Seed Testing. Rules Amendments. Seed Sci. Technol. 29:1-127.

ISTA (International Seed Testing Association) (1993). International Rules for Seed Testing. Rules Amendments. Seed Sci. Technol. 29:1-127.

Jones, J.B. and Jones, (1985).The effect of bactericides tank mixing time and spray schedule in bacterial leaf spot of tomato. Proc. Fla. State Hort. Soc., 98: 244-247.

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.

Khan, M. A.; Rashid, A. and Riaz, AC. (2000). Biological control of bacterial blight of cotton using some plant extracts. Pakistan Journal of Agricultural Sciences. 2000; 37:3-4.

Liggit J, Jenkinson P, Parry DW (1997) The role of saprophytic microflora in the development of Fusarium ear blight of winter wheat caused by *Fusarium culmorum*. Crop Prot 16:679–685.

Lumsden RD, Locke JC (1989) Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with Gliocladium virens in soilless

Manning, Katie, Ruth Pelling, Tom Higham, Jean-Luc Schwenniger and Dorian Q Fuller (2010). 4500-year old domesticated pearl millet (*Pennisetum glaucum*) from the Tilemsi Valley, Mali: new insights into an alternative cereal domestication pathway. Journal of Archaeological Science 38 (2): 312-322

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

Mathur, S.B. and N rearguard , Paul (2003) .Seed health testing of rice .1 Seed borne -borne fungi of rice in Philippine, India,Portugal, andEgypt.

Mohamed E.S. (2002). Towards an integrated pest management (IPM) PROGRAMME ON okra, *Ablemoschus esculentus* L. (Malvaceae) Ph.D. Degree Thesis, Faculty of Agriculture, University of Khartoum, Department of Plant Protection, Sudan.

Munyaradzi, M. (2013). "Biofortified pearl millet 'can combat iron deficiency'". SciDev Net. Retrieved 29 August 2013.

Nwokolo, E. (1996). Peanut (*Arachis hypogaea* L.). In: Food and Fee from Legumes and Oilseeds.

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

Okigbo RN, Nmeka IA,. (2005) . Control of yam tuber with leaf extracts of Xylopia aethiopica and Zingiber officinale. Afr. J. Biotechnol. 4(8): 804 – 807.

Okigbo, R.N. (2004). A review of biological control methods for post harvest yams(*Dioscorea* spp). In storage in South Eastern NigeriaKMITL Sci J. 4(1): 207 - 215.

Okigbo, R.N. and Ogbonna, U.O. (2006). Antifungal effects of two tropical plants leaf extracts (*Ocimum gratisimum* and *Afromonum melegatuata* and post Harvest yam (*Dioscoreaceae* spp). Afr. J. Biotech. 5: 717-731.

Patil, M.J; Ukey, S. P. and Raut, B. T. (2001).Evaluation of fungicides and botanicals for the management of early blight (*Alternaria solani*) of tomato. PKV-Research Journal, 25(1): 49-51.

Payne, G. A. (2008). Process of contamination by aflatoxin producing fungi and their impacts on crops. In, Mycotoxins in Agriculture and Food Safey. K.K. Sinha and D. Bhatnagar. Marcel Dekker, Inc. New York.

Peter Wareing (2010). The Fungal Infection of Agricultural Produce and the Production of Mycotoxins. European Mycotoxins Awareness Network.

Peter Wareing (2014). The Fungal Infection of Agricultural Produce and the Production of Mycotoxins. European Mycotoxins Awareness Network.

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

Rao, G.P. and A.K. Srivastava,.(1994). Toxicity of essential oils of higher plants against fungal pathogens of sugarcane. Current, Trend in Sugarcane Pathology,(eds). Rao,G.P.A.G. Gillasple, P.P. Upadhaya, A. Bergamin, V.P. Agnihotri and C.T. Chen.

International Books and Perodicals Supply Service, Pitampura, Delhi, pp. 347-365.

Ray Hansen (August 2011). "Sesame profile". Agricultural Marketing Resource Center

Samson, R.A.; Houbraken, J.; Summer bell, R.C.; Flannigan, B. and Miller, J.D. (2001). Common and important species of fungi and Actinomycetes in indoor environments. In: Microorganisms in Home and Indoor Work Environments. New York: Taylor & Francis. pp. 287–292. ,ISBN.

Satish, S.; Raveesha, K. A. and Janardhana, G.R. (1999). Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris. Letter in Applied Microbiology, 28:145-147

Schmutterer, H. (1969). Pests of Crops in North east and Central Africa. Gustav Fischer, Verlage, Stuttgart, Portland – USA. Pp.296.

Schmutterer, H. (1990). Properties and potential Natural pesticide from neem tree, Azodirachta indica. Annual reviewing Entromlo.35: 2797.

Siddig, S. A. (1993). An integrated Pest Management Proegramme including Neem treatment for combating potato pest in Sudan. proc. 2nd Int. Neem conf., Nairobi, Kenya, 1988.

Singh , A. N .Sharma and H.B. Sing (1973). Effect of UV light on the biocontrolpotenical of Trichodermaharzianum . Indian Jour .mycol. And pl. 24:97_107.

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

Stoll, G. (2000). Natural crop protection in the Tropics.Pp 117-199.

Syed Danish, Y. N.; Shiden T. M. and Mehret, S. (2013). Identification of seed borne fungi on farmer saved sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.) and groundnut (*Arachis hypogaea* L.) seeds. Agricultural Science Research Journals Vol. 3(4), pp. 107-114, April 2013.

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

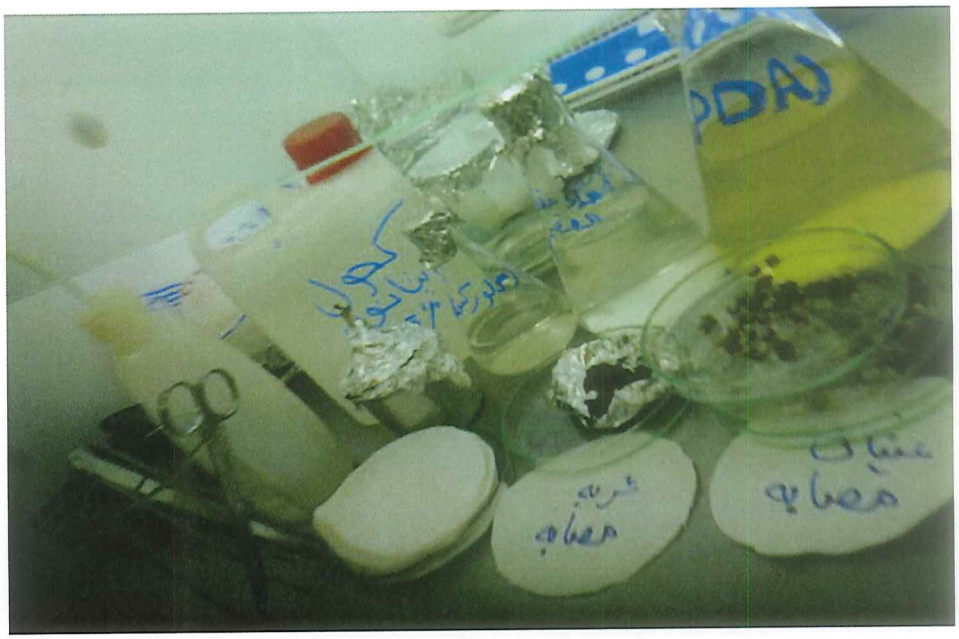
Weiss, E.A. (2000) Oilseed Crops. London: Blackwell Science.

Yousif, M. A. Idris; Abdalbasit, A. Mariod; Ibrahim, A. E. and Adam A. M. (2010). Determination of aflatoxins levels in Sudanese edible oils. Food and Chemical Toxicology 48 (2010) 2539–2541.

APPENDICES

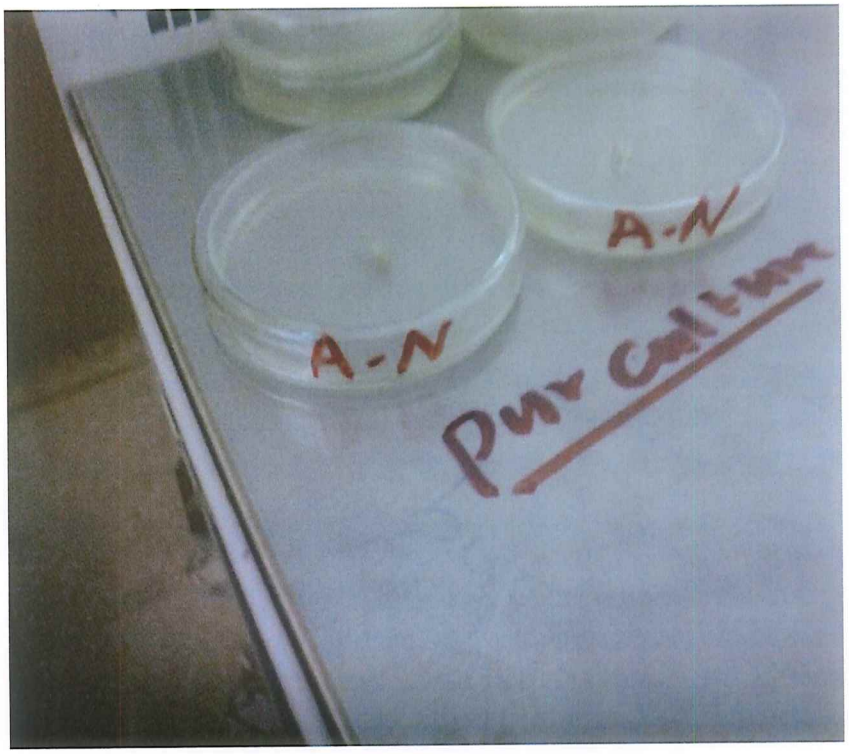
APPENDIX (1) Culthcer media PDA

Potato Dextrose Ager PDA




Samples of ELobyiD

Pur culihar



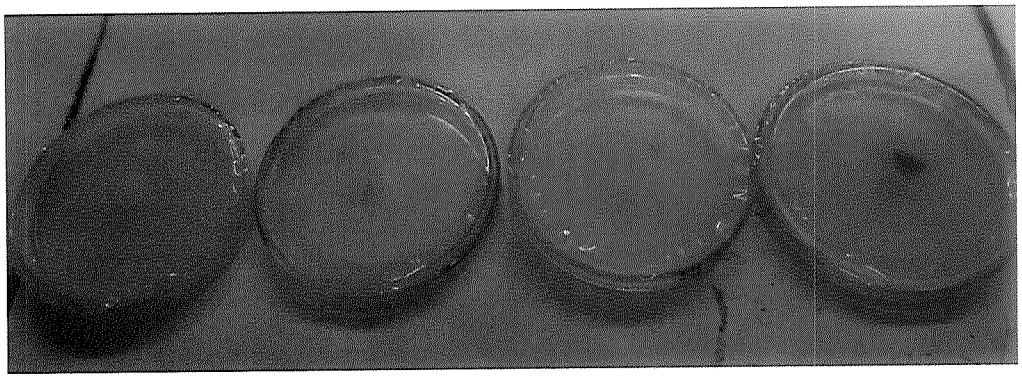
APPENDIX (4) Effect of leaves aqueous extracts of Neem

Control 25% 50% 100%

APPENDIX (5) Effect of leaves aqueous extracts of Damas

Control 25% 50% 100%

APPENDIX (6) Effect of Tilt fungicides



Control **25%** **50%** **100**