Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Occurrence and control of seed borne fungi of four food crops in Sudan

تواجد ومكافحة الفطريات المحمولة على البذرة في أربعة محاصيل غذائية في السودان

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in plant protection.

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

وَإِذْ قَالَ رَبُّكَ لِلْمَلائِكَةِ إِنِّي جَاعِلٌ فِي الأَرْضِ خَلِيفَةً قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ قَالَ إِنِّي أَعْلَمُ مَا لا تَعْلَمُونَ (30)وَعَلَّمَ آدَمَ الأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلاءِ إِنْكُنتُمْ صَادِقِينَ(31) قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ(32)

صدق الله العظيم

الآيات (30-32سورة البقرة)

Dedication

To my mother

To my father

To the soul of my sister Amna

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Mawda

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ABSTRACT

The present study was plant pathology laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology to investigate the occurrence of seed-borne fungi on sorghum, pearl millet, groundnut and sesame seeds collected from four different locations, each in one state of Sudan(Gazera , Gadarif, Elobid , and Niyalla) and their possible control using aqueous plant extracts (Garlic Neem, Argel, Damas and Tilt fungicide at (100%,50%,25%) respectively . Out of the 16 seed samples, 4 of each crop, tested for seed borne fungi, a total of eleven genera of twelve species of fungi were recorded. The seed borne fungi recorded were *Penicillium digitatum, , Aspergillus niger, Rhizopus nigricans, Drechslera spicifera, Alternaria solani, Fusarium solani, Colletotricum graminicola, Rhizoctonia solani, Phoma glomerata, Curvularia lunata , Macrophomina phaseolina* and *Aspergillus flavus* with mean percent incidence ranging from (23%-16% – 12%-8.0%-10%-4.0%-6.0%-2.0%-2.0%-2.3%-2.5%-2.3%and 53%) respectively . The higher percent incidence was recorded by *Aspergillus flavus* (53%) in sesame calculated by used rule (infected seeds –healthy seeds)100 . The four most prevailing seed borne fungi recorded across crops seeds were *Penicillium digitatum, Aspergillus flavus, Aspergillus Niger, and Rhizopus nigricans* with varying level of incidences. Likewise, all concentrations of the leaves aqueous extracts of all plants tested and fungicide (Tilt –Garlic- Argel- Neem , , and Damas) ranging from (100%,50%,25% respectively) exhibited significantly high inhibitory effect against the linear growth of test fungus(93.8-100-100,27.2-91.3-100,66.0-73.8-84.1,23.8-54.9-66.7,13.3-21.9-31.1) as well at all concentrations gave the highest inhibition zones percent compared to control. Moreover, concentrations of each aqueous extract as well as that of fungicide reacted differently against test fungus. However, among plant extracts the Garlic at all concentrations tested (25, 50 and 100%) exhibited consistently

the highest inhibitory effect throughout the days (three ,four and five respectively in Table 1,2,and,3 of experiments (100%,100%,46.1% , 100%,100%,90.4% and 100%,100%,93.8%) than the other equivalents. Similar effect was also demonstrated by the fungicide (100%, 100%, 94.5%; 100%, 100%, 94%; 100%, 100%, 100%) throughout that days respectively. However, the inhibitory effect of Damas Leave Aqueous extracts (100%, 50%, 25%) respectively gave lowers inhibitory effect that was (70%, 42.2%, 18.5%; 63.5%, 50.6% 48.3%; and 31.1%, 21.9%66%) reduced with time of recording. Generally, the results showed that the antifungal activity increase with extract concentration. However the Garlic Extract gave highest effect of fungus compared to Damas aqueous extract and similar effect of fungicide. 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 highlighted the need for adhering to effective measures that aimed at reducing seed-borne fungi incidence in stable food crops seeds in Sudan.

ملخص الدراسة

أجريت هذه الدراسة بمعمل أمراض النبات الخاص بقسم وقاية النباتات بكلية الدراسات الزراعية -جامعة السودان للعلوم والتكنولوجيا بغرض التحقق من تواجد الفطريات المحمولة علي البذور في اربعة محاصيل غذائية بالسودان هى (الذرة الرفيعة ،الدخن , السمسم ,والفول السوداني) لاربعة ولايات بالسودان وهى (الجزيرة ,القضارف ,الابيض ونيالا) ومعرفة النسبة المئوية للفطيات المحمولة عليها والفطريات الاكثر انتشارا وامكانية مكافحتها بإستخدام بعض المستخلصات النباتية المائية والمبيد الفطري (التلت) كإستاندر وهى:- (الثوم ,النيم ,الحرجل ,والدمس ومبيد التلت) بنسب 25%-50%-100%علي التوالى.. باستخدام طريقة رق الترشيح (Filter paper method) وطريقة الاجار(Potato Dextrose Agar) او PDA اي بيئة البطاطس والد كستروز والاجار للتجربة الاولية وللعينات النقية والتجربة النهائية للفطر المختبر (الاسبرجلس فلافس *Aspergillus flavus*). ومن بين الستة عشر عينة لكل منطقة ،أربعة لكل محصول والتي اختبرت الفطريات المحمولة عليها تم الكشف عن ثلاثة عشر جنسا لاثني عشر نوعا من هذه الفطريات والتى تم تدوينها وهي :- *Aspergillus ,Penicillium digitatum Alternaria ,Drechslera spicifera,*(Rhizopus nigricans,*Aspergillus niger,flavus Macrophomina phaseolina ،Curvularia lunata, Phoma glmerata , solani Colletotricum graminicola).* solani،*Rhizoctonia solani* الفطرياتات الاكثر تواجدا والتي تم تدوينها في هذة المحاصيل هي(Aspergillus flavus (Penicillium digitatum , Aspergillus niger, Rhizopus nigricansبمستويات اصابة مختلفة وعل نفس النمط فإن كل تركيزات المستخلصات النباتية (الثوم ,النيم ,الحرجل ,والدمس) (25‘50‘100) علي التوالي والمبيد الفطري قد اظهرت اثر تثبيطي عالي (وذو اثر معنوي هام علي النمو الميسليومي للفطر المختبر مقارنة بالشاهد وكان تاثيرها مختلف عن بعض اما فيما بين المستخلصات النباتية فإن كل تراكيز الثوم قد اظهرت اعلي تثبيط وبصورة مستمرة طيلة ايام التجربة. كما اظهر المبيد الفطري تاثيرا مشابها للثوم في حين اظهر الدمس تاثيرا متدنيا مع الوقت, وعامة كان التاثير عاليا مع زيادة التركيز. لهذا فان نتائج هذه الدراسة مهمة لإلغائها الضوء علي الحوجة للإلتزام لإجراءت الفعالة والتي تهدف إلي الحد من إصابة المحاصيل الغذائية بالسودان في الحقل والمخزن بالفطريات المحمولة علي البذور.

CHAPTER ONE

INTRODUCTION

Cereal grains and edible oil seeds are important human food resources and livestock feeds worldwide. In fact, cereal grains are food staples in many, if not most, countries and cultures and are the raw materials of many of our foods. The main cereal grains used for foods include sorghum, corn (maize), wheat, barley, rice, oats, rye, millet, and ground nuts, soybean and sesame are not a cereal product, but rather, are legumes or a pulse, but are often considered with cereals because of their importance as a food source.

The major constraints facing the productivity and availability of healthy food crops worldwide are the losses and spoilage caused by plant pathogens, insects, nematodes and parasitic weeds. Among these fungi that contaminate seeds of food crops and edible oil. The threat to food crops from fungal pathogens has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977).

Seed borne fungi associated with seeds of food crops and edible oil continues to represent a major human health risk throughout the world and particularly in the humid tropics being major spoilage agents of food crops (Olusegun, *et al.,* 2013). Moreover, 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. Other seed-borne fungi were also most frequently isolated from pear millet seeds such as *Alternaria alternata*, *Fusarium semitectum*, and *Curvularia lunata* (Azhar, *et al.,* 2009.)

The Food and Agriculture Organization (FAO) estimates that 25 % of the world’s food crops are affected by food contaminants, of which the most

notorious are those resulted from *Aspergillus spp* .Losses to livestock and poultry producers from contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Anon, 1989)

In Sudan, Shami and Ahmed who surveyed and determined the fungi associated with stored peanut in Sudan indicated that *Aspergillus flavus* was isolated from twenty six samples (43.33%) out of the total number of samples investigated. Younis and Malik (2003) who studied contamination in Sudanese groundnut and groundnut products found that the percentage of contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively.

Severalseed borne fungi associated with seeds of food grainsare known to limit utilization of these crops, of which *Aspergillusssp*. are the most important. In fact, these fungi and their secondary metabolites are one of the most important food crops spoilage agents in the Sudan (Haq Elamin et al., 1988; Yousif *et al*., 2010).

Obviously, the infection of plants by various fungi not only results in reduction in crop yield and quality with significant economic losses but also contamination of grains with poisonous fungal secondary metabolites called mycotoxin. These substances arise from the secondary metabolism of fungi in response to a wide range of genetic and environmental factors (Haq Elamin et al., (1988).

The ingestion of such contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases in man and animals (Bhat and Vasanth, 2003).

Seed borne fungi frequently occur in the field following infection of plants with specific pathogenic fungi or with symbiotic endophytes. In addition, contamination may occur during processing and storage of harvested seeds

and feed whenever environmental conditions are appropriate for spoilage fungi. However, the fungal seeds contaminants 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 (Azhar, et al., 2009).

These reflect the potential of risk of food crops contamination with spoilage pathogens a situation that necessitate more scientific studies to be carried out in order to help overcoming the risk involved.

In most cases in order to control 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 , 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 (Carvalho, 2004). This has initiated the exploration of safe alternate products.

Obviously, no single approach for control of fungal contaminants of seeds was proved to be effective and without drawback. Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen population, optimization of cultural practices and safe alternate antimicrobial compounds of higher plants (Azhar, et al., 2009).

Historically, the presence of antimicrobial compounds, in higher plants, has been recognized as important products in combating plant pathogenic diseases. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Siva, *et.al.,* 2008). Thus, the development of new and different antimicrobial agents more safe has been a very important step (Agrafotis, 2002). However, the step of

validation of traditional uses of antimicrobial compounds in higher plants was studied by a number of researchers. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrafotis, 2002).

Natural products play an important role in the treatment of different diseases. The history of use of plants for different conditions is very old. The earliest records found show that plants have been used in Egypt thousands of years ago. Numerous photochemical have been isolated from different plants which are now being prescribed by medical practitioners all around the world (Newman, *et al.*, 2000). Moreover, the presence of antimicrobial compounds in Neem 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). The antifungal activity of the in vitro efficacy of different plant extracts viz., *Azadirachta indica, Artemisia annua, Eucalyptus globulus; Ocimum sanctum* and *Rheumemodi* were also reported by Babu Joseph (2008) who that found them to control wilt pathogens (Azhar, *et al.*, 2009).

It is in view of this crop that the current study aimed at exploring and investigating on (i) Presence of pathogenic fungi associated with seeds in samples of four food crops namely sorghum, millet, groundnut and sesame collected from Gadarif, Gezira, Kordofan and Khartoum States in Sudan (ii) The efficacy of some higher plant extracts and fungicide for management of fungi associated with seeds of these crops in order to formulate promising. method of control strategies with following objectives:-

- To investigate the occurrence of seed borne mycoflora associated with seeds of four food crops

- To explore the antifungal potentials of some higher plants crude extract against most commonly occurring fungus
- To evaluate the efficacy of systemic fungicide on fungal growth
- To develop Integrated Management Approach for pathogens associated with seeds of food crops.

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CHAPTER TWO

2: LITERATURE REVIEW

2.1. Food grains

In Sudan the main food grains used for foods include sorghum, wheat, maize, rice, millet, plus ground nuts and sesame which are importance crop in 1991 according to FAO statistic (Mahmud et al.,1995).

2.2.1 *Sorghum bicolor L.* (Moench)

2.2.1.1 Scientific classification

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Liliopsida
Order:	Poales
Family:	Poaceae
Genus:	Sorghum
Species:	bicolor

Sorghum [*Sorghumbicolor*L. (Moench)] is one of the major cereal crop and staple food as well for millions of the poorest and most food insecure people in the Semi-Arid Tropics of Africa and Asia. The greatest diversity in both cultivated and wild types of Sorghum is found in north-eastern tropical Africa. The crop may have been domesticated in that region, possibly Ethiopia (ICRISAT, 1993) .

The total area cultivated by sorghum in the entire world is 106 million feddans and the fives top countries area wise are India, Sudan, USA, Nigeria and China. The areas under cultivation in these countries represent 66% of the total world areas cultivated by sorghum. In the Sudan sorghum is produced

mainly in rain-fed agriculture. The cereal harvest for the 15 northern states of the Republic of the Sudan is estimated at 5.707 million MT, comprising 4.606 million MT of sorghum Vast acreage are cultivated in mechanized crop production Schemes in Gadarif, Damazin, Blue Nile state and both Kordofan and Darfur states .the crop is also grown in irriga ted Schemes of Gazera and Rahad as important crop in rotation. In the traditional rain fed, sorghum is cultivated in Kordofan , Darfur, and White Nile. But in Butana and Blue Nile it is produced mechanically by rains. (ISTA 1985).

Sorghum is affected by a range of fungal seed borne diseases including ergot (Claviceps africana), seed rot (Fusarium moniliforme), zonate leaf spot (Gloeocercospora sorghi), downy mildew (Sclerospora sorghi), loose smut (Sphacelotheca cruenta), covered smut (Sphacelotheca sorghi), leaf spots (Phoma sorghina), Bipolaris bicolor, anthracnose (Colletotrichum graminicola) and grey leaf spot (Cercospora sp.) (Almekinders and Louwaars, 1999; Kaula and Chisi, 2002 and Neergaard, 1979). Of all these

diseases, smuts are the most destructive. Therefore, seed health testing is a prerequisite to minimize losses by assessing the quality of seed before it is sown (International Seed Testing Association (ISTA), 1985).

The common name of sorghum in Sudan is “Aish” which means life. In Sudan, sorghum is used as food for human beings,food for the animals. Industrial uses include extraction of many products such as starch, oil, alcohol, sugar, and sugary juices (Khatab and Hassan 2000).

Common seed and seedling rot diseases in sorghum are caused by soil- and seed-borne Aspergillus, Fusarium, Pythium, Rhizoctonia and Rhizopus spp. They are controlled by treatment of the seed with fungicides.(Taylor,2003).

2.2.2 Pearl mille(Pennisetum glaucum)

2.2.2.1Classification:

Kingdom : Plantae

Unranked: Angiosperms

Unranked : Monocots

Unranked : Commelinids

Order : Poales

Family : Poaceae

Subfamily : Panicoideae

Genus : *Pennisetum*

Species : *glaucum*

Pearl millet is an important food for millions of people inhabiting the semi-arid tropics and is a major source of calories and vital component of food security in the semiarid areas in the developing world (FAO and ICRISAT, 1996.) The plant is a cereal crop that belongs to family Poaceae. While millet is indigenous to many parts of the world, millet most likely had an evolutionary origin in tropical Western Africa, as that is where the greatest number of both wild and cultivated forms exists .It is an 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(Manning et al., 2010). The most widely grown millet is pearl millet, which is an important crop in India and parts of Africa (Fuller, 2003). The millet is nutritionally equivalent or superior to most cereals, containing high levels of methionine, cystine, and other vital amino acids for human health, they are also unique sources of pro-vitamin A (yellow pearl millet) and micoronutrients(Zn,Fe and Cu) (Food security 2003).

A series of research findings showed the contamination of pearl millet with fungal pathogens. (Syed Danish , *et al.*, 2013) swho studied soilborne fungi

in millet and other field crop indicated that the fungi of Aspergillus, Fusarium, Helminthosporium, Rhizopus and Penicillium were frequently isolated from sample of millet grains collected from farmers saved crops. Also at the maturity stage the crop suffers from many seed borne pathogens that include *Alternaria alternata*, *Aspergillus flavus, A niger* and *Fusarium semitectum* deteriorate the quality and quantity of developing floral parts that reduces grain yield at maturity . Pearl millet pathologists working at International Crop Research Institute for the Semi Arid-Tropics estimated global yield losses of 45%, 32%, 9%, 3%, and 1% due to Downey mildew, Striga, smuts, rusts, 56 and viruses, respectively. (FAO and ICRISAT, 1996).

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

In Sudan, Pearl millet, locally known as "Dukhun", is one of the important cereal crops, coming as the second most-important cereal crop, after sorghum, in both area and total production. It is the preferred staple food crop for the majority of the inhabitants of western Sudan (Kordofan and Darfur States). The average total area annually planted in the country is about 6 million feddans (2.5 million ha). About 95% of this area is found in Western Sudan.The crop is favored due to its productivity and short growing season under dry, high temperature conditions (Abulgasim, E.H. (1997).

Millet is nutritionally equivalent or superior to most cereals; containing high levels of methionine, cystine, and other vital amino acids for human health. - They are also unique sources of pro-vitamin A (yellow pearl millets) and micronutrients (Zn, Fe and Cu) (Food security 2003).

Downy mildew is one of the major diseases that receive more attention in breeding programmes to develop resistant varieties to minimize yield losses associated with this problem (Abulgasim, 1997).

2.2.3 Groundnut (*Arachis hypogaea L.*)

2.2.3.1Classification:

Domain:	Eukarya
Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Fabales
Family:	Leguminosae
Sub-family:	Papilionaceae
Genus:	*Arachis*
Species:	*hypogaea*

Groundnut 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 40º N to 40º S. Major groundnut producing countries are: China (40.1%), India (16.4%), Nigeria (8.2%), U.S.A (5.9%) and Indonesia (4.1) Sudan (30.6%) (Nwokoto,1996).

It is an annual legume which is also known aspeanut, earthnut, monkey-nut and goobers .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).

2.2.4 Sesame (*Sesamum indicum* L.)

2.2.4.1 Classification

Kingdom : Plantae

Unranked : Angiosperms

Unranked : Eudicots

Unranked : Asterids

Order : Lamiales

Family : Pedaliaceae

Genus : *Sesamum*

Species : *indicum*

Sesame which is a flowering plant in the genusSesamumconsidered the oldest oilseed crop known to humanity (Raghav Ram, *et al.*, 1990). Numerous wild relatives occur in Africa and a smaller number in India. The crop has many species, and most are wild. Most wild species of the genus *Sesamum* are native to sub-Saharan Africa.Sesame is widely naturalized in tropical regions around the world and is cultivated for its edible seeds, which grow in pods. 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, as attested to by the discovery of many ancient presses for sesame oil in the region. Sesame is drought-tolerant and is able to grow where other crops fail (Raghav Ram, *et al.*, 1990).

The world harvested about 3.84 million metric tonnes of sesame seeds in 2010. The largest producer of sesame seeds in 2010 was Burma. (Food and

Agriculture Organization , 2012). The world's largest exporter of sesame seeds was India, and Japan the largest importer. developed economies (Ray Langham , 2008)**.**

Sesame has one of the highest oil contents of any seed with a rich nutty flavor. It is a common ingredient in cuisines across the world(Ray Hansen, 2011). Like other nuts and foods, it can trigger allergic reactions in some people.

The crop comes 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 Penicillium spp. The crop is also contaminated with secondary metabolites like Aflatoxin which caucused by *Aspergillus spp*. (FAO, 2012).

2.2.5 Argel (*Solenostemma argel* Del. Hayenne)

2.2.5.1 Classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Asterids

Order: Gentianales

Family: Apocynaceae

Subfamily: Asclepiadoideae

Genus: *Solenostemma*

Species: *S. argel*

The plant harjal (*Solenostemma argel*) is a member of the family Apocynaceae, which comprises numerous medicinal plants, like Calotropis

procera, Marsdenia obyssinicna and Huernia mecrocarpa, known for their cardiac activity. Harjal grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria) and the Saudi Arabia (Ahmed, 2004). Harjal leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps and stomach colic.

Argel is an herb of wide use in Sudanese traditional medicine that grows wild in the Northern and Nile States (Elkamali and Khalid, 1996). Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Kamel et al., 2000; Hamed, 2001). Antimicrobial properties of argel were reported by Roos *et al.*, (1980), Elhady et al. (1994) and Sulieman et al. (2009). According to Idris et al., (2011), soil application of argel's dry leaves under the conditions of the Northern State enhanced flowering and yield of a dry date cultivar and the influence was attributed to either pesticide or growth promoting ingredients

2.2.6Garlic (*Allium sativum*)

2.2.6.1Scientificclassification

Kingdom: *Plantae*

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Amaryllidaceae

Subfamily: Allioideae

Genus: *Allium*

Species: *A. sativum*

Allium sativum((NGRP,2006).

The crop is commonly known as garlic, is a species in the onion genus, Allium of the family Alliaceae.Its close relatives include the onion, shallot, leek, chive (Block, (2010) With a history of human use of over 7,000 years, garlic is native to central Asia, and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. It was known to Ancient Egyptians, and has been used for both culinary and medicinal purposes.

2.2.6.2 Uses:

Garlic is central to the cuisines of Mexico, the Caribbean, South America, the Middle East, India and China and can impart flavor to many different type of dishes. Economically, garlic is used in commercial food flavoring (Wiersema et al., 1999). Garlic is also used in folk medicine including treatment of bronchitis and respiratory problems, gastrointestinal problems, flatulence, leprosy, menstrual cramps, high blood pressure, diabetes and externally for warts, corns, arthritis, muscle pain, neuralgia and sciatica (Grieve and Mrs.M., 1971; Simon *et al*., 1984; Heinerman and John, 1995 and PFAF, 2002). Sangoyomi (2004) reported that aqueous extract of garlic effectively inhibited mycelia growth, conidia, pycnidia and sclerotial production of *Butryodiplodia theobromae, Aspergillus niger, Sclerotium rolfsii , Rhizoctonia solani and Neofusicoccum mangiferae* fungal pathogen in yam storage.

2.2.7 Neem Tree

2.2.7.1 Classification

Kingdom: Plantae

Division: Magnoliophyta

Order: Rutales

Suborder: Rutinease

Family: Meliaceae

Genus: Azadirachta

Species: Azadirachta indica

S.N: *Azadirachta indica* A.Juss

Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management, environment protection and medicine. Also it has showing great promise as potential fertilizer (Ramose, et al., (2007). Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management, environment protection and medicine. Also it has showing great promise as potential fertilizer (Abdalla, 2010).

2.2.7.2 Uses of Neem in pest and disease control

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) reported from Sudan that Neem seed water extracts at1Kg/1Liter of water repelled foliage pest of potato including B. tabaci, Aphis gossypii and J. lybica and yield increased to 5 ton/ ha. Mohammed (2002) reported that Neem seed showed good performance against A. gossypii, B. tabaci, andJ. Lybica on Okra.

2.2.8 Damas

2.2.8.1 Classification

Kingdom: Plantae

Phylum: Tracheophyta

Class : Magnoliopida

Order : Myrtales

Family : Combretaceae

S. N. : *Conocarpus lancifolius* Engl

Family Combretaceae comprises about 20 genera and about 600 species found in tropical and subtropical regions of the world. The family has few genera with great economic value, an useful timber is obtained from some species belong to it and other species has medicinal importance. Damas *Conocarpus lancifolius* Engl is one of the most important species in this family (Pandey and Misra, 2008).

2.2.8.2 Uses of Damas in disease control

C. lancifolius 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 and Wickens, 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. A drought-resistant species, *C. lancijolius* 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 (NAS, 1983).

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 tribes owing the Damas (Tugs) dry river valleys (wades) containing *C. lancifolius* have restricted cutting because of the threat of overexploitation (Booth and Wickens, 1993).

2.2.9 Seeds borne fungi

The importance of seed borne pathogens to crop quality and quantity cannot be ignored. Results by Bipen *et al.*, (1999) showed that there was a significant decrease in oil content of sunflower seeds infected with Rhizopus oryzae. Wanyera (1998) analyzed wheat seed and concluded that fungal infection led to abnormal seedlings and dead seeds. Aflatoxin contamination and its associated risks to humans, wild animals and livestock and reduced grain quality have been reported by several authors (Haq Elamin NH et al., 1988; Thompson 2000; and Yousif .et al., 2010).

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 (Williams and Mc Donald, 1983; Dawson and Bateman, 2001; Islam et al., 2009).The most common fungi were *Aspergillus flavus, Aspergillus niger, Alternaria alternate, Fusarium moniliforme ,Rhizopus nigricans and Trichoderma viridae* were common in all selected seed samples. *Incidence* of *Aspergillus sniger Fusarium moniliforme, Rhizopus nigricans* and *Trichoderma virida* was found when compared to other. Many fungal species of *Alternaria alternata, Aspergillus terrus, A. flavus, A. fumigatus, A. niger ,Botrytis sp, Cladosporium, Curvularia lunata, Fusarium solani, F. moniliforme, F. oxysporum, Macrophomina phaseolina, Penicillium notatum, Rhizoctonia sp.,* and *Rhizopus nigricans* etc has been reported from *Cicer arietinum* L.. Similar observation recorded by (Ahmad et al., 1993).

The most common seed borne fungi were *Aspergillus niger, A. flavus, Fusarium oxysporum, Botrytis sp, Chaetomium sp, Penicillium notatum. Rhizopus spp., Cladosporium sp*. and *Trichothecium*.The fungi isolated from Lens *culinaris Medik.* Treated seeds were *Fusarium moniliforme, Alternaria alternata*, Mucor hiemalis, Chaetomium sp, *Penicillium citrinum, Aspergillus niger,* A. *flavus, A .terreus and Nigrospora sp. F. Moniliforme ,A. alternata, M. hiemalis, Chaetomium spp., and A. Niger* were common in all samples while *P. citrinum ,A. flavus, A. terreus* and *Nigrospora spp*., were only isolated from untreated seed. (Muhammad et al(2007).

Richardson, (1979) gave a list of seed -borne diseases of lentil, according to which Botrytis spp., and F. oxysporum were isolated from lentil seed from Czechoslovakia and Uromyces fabae from debris mixed with seeds .

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).

Spoilage fungi may not be able to attack crops in the field, but cause problems once the crop is harvested, if conditions allow. Some spoilage fungi can also produce mycotoxin, for example Penicillium, although many penicillia associated with grains are pathogenic. Agrios (2005).

Commercially, discolored sorghum seeds caused by fungi are of poor quality reducing their acceptability and thus, low market value of the produce. Grain mold causes crop loss by reducing seed size and weight, the food value and keeping quality of grains (Bandyopadhyay, 1986). 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 mycotoxin, to some of which legislative restrictions may apply (patulin from *Byssochlamys*, for example), others do not.

2.2.*10 Aspergillus spp*.

The role of Aspergillus species in food spoilage is well-established Haq Elamin NH *et al.*, 1988; Ali, 1989; Yousif M.A.et al., 2010and KRN Reddy, 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*. (Haq Elamin, 1988).However, Haq Elamin NH et al., (1988); Ali, (1989); Yousif M. A.et al., (2010), and Olusegun,(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.

The role of *Aspergillus* species in food spoilage is well-established. Mycotoxins produced by *Aspergillus flavus* include aflatoxins and cyclopiazonic acd. Other important mycotoxins from aspergilli include ochratoxin A and patulin. Some Aspergilli have an ascomycete teleomorphic (sexual) stage; for example, *Eurotium*, *Neosartorya*, and *Emericella*; Many Aspergilli are xerophilic and present particular problems during commodity harvest, and during subsequent drying and storage.(Cutler.1991).

2.2.10.1*Aspergillus flavus*

Aspergillus flavus is a fungus. It grows by producing thread like branching filaments known as hyphae. Filamentous fungi such as *A. flavus* are sometimes called molds. A network of hyphae known as the mycelium secretes enzymes that break down complex food sources. The resulting small molecules are absorbed by the myceilium to fuel additional fungal growth. The unaided eye cannot see individual hyphae, but dense mats of mycelium with conidia (asexual spores) often can be seen. The ear of maize below shows the growth of the fungus covering four maize kernels. When young, the conidia of *A. flavus* appear yellow green in color. As the fungus ages the spores turn a darker green (Scheidegger, and Payne. 2003). Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound. *Aspergillus flavus* is also the second leading cause of aspergillosis in humans. Patients infected with *A. flavus* often have reduced or compromised immune systems (Richard, and Payne. 2003).

2.2.11 *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. Important mycotoxin 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*. 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).

2.2.12 *Fusarium spp*.

Fusarium spp. is 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. In Sudan, several diseases are known to limit production of tomato, one of which Fusarium wilt caused by (*Fusarium oxysporumf.sp. Lycopersici*)is one of the most important (Bhatia et al., 2004).

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).

Use of fungicide to control *Fusarium* diseases is indispensable (Minton 1986 and DeVay***e****tal*., 1988). Several fungicides have been used for control of different plant pathogens including fusaria (Liggit et al. 1997; Diehl and Fehrmann 1999) 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).

2.2. *13 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. *Alternaria solani* is the fungal pathogen than produce the disease in tomato plants called early blight .The pathogen produces distinctive (bulls eye) patterned leaf spots and can also cause stem lesion and fruit rot on Tomato and tuber blight in Potato .Despite the name (early),foliar symptoms usually occur on older leaves .If uncontrolled, early blight can cause significant yield reduction in Sudan Cultivar Tomatoes suffer from many fungal disease such as , Verticillium wilts (*Verticillium dahlia*) are *Fusarium spp*. and early blight caused by *Alternaria solani* and *Phytophthora infestans,* respectively in fact the Fusarium wilt disease is considered one of the major agents of yield redaction of the crop(Awad , 1990**).**

2.2.14 *Phoma sorghina*

Fungi are responsible for agricultural product losses, both while crops are growing and when they are later stored. In fact, during storage, fungi can make food crops unfit for consumption, by changing the nutritional value of the seeds or producing mycotoxin that are harmful for human and animal health. At global . level, over 25% of cereals are contaminated by known mycotoxin and more than 300 of the metabolites produced by fungi are toxic for human beings and animals (Satish et al., 2007). Phoma sorghina is a fungus frequently found on sorghum seeds. Studies were conducted in 2006, with the aim of evaluating fungal populations, on 50 sorghum samples from different regions of Burkina Faso and again in 2008 on 67 sorghum samples collected from different regions of the same country. These studies revealed that this fungus was present in all the samples collected. Somda et al., (Somda et al.,2007). Show that, out of 37 samples of the different species that were cultivated and evaluated, 33 were infected with Phoma sorghina. Studies by Boiron (Boiron 2009). have shown that Phoma sorghina asecretes a toxin called tenuazonic acid, which is dangerous to human health. Phoma sorghinaalso contributes to the pre-emergent and post-emergent mortality of cultivated plants (Punithalingam ., 1985,).

2.2.15 *Colletotricum graminicola*

Colletotrichum graminicola is known to be one of the most damaging fungal agents affecting sorghum in Burkina Faso. It is responsible for stunted growth, seed rot, leaf necrosis, red rot on the stems and seed discoloration. Losses caused by C. graminicola can range from 30-70%, depending on which organ is infected (Thomas et al., 1996).

2.2.16 Mucor and Rhizopus

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

2.3 Seed Health Testing:

Seed health testing for the presence of seed borne pathogens is an important step in management of crop diseases. This is simply because. seed-borne diseases have been fund to affect the quality and quantity of food crops. According , The important of seed health testing cannon be under estimated. Seed-borne diseases have been found to affect the growth and productivity of crop plants. A seed-borne pathogen present externally associated with the seed as contaminant may cause seed abortion , seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection. Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Besides, the mold fungi which grow on the seed substratum produce mycotoxin which are hazardous to humans and animals (Halt, 1994).

Several reports about seed-borne mycoflora on sorghum, pearl millet and groundnut [Soetan et al.(2006), Grish et al. (2004)] have been published. Post-harvest fungal infection, according to farmers, has been one of the constraints for mass production of these grains and less seed germination and viability. Mathur et al. (1975) observed reduction in germination rate of sorghum and pearl millet due to Alternaria alternata, Aspergillus spp., Rhizopus spp., Curvularia lunata and Fusarium equiseti present in or on seed surface . Seed health testing constitutes part of the seed certification and plant quarantine practices aimed at reducing the distribution of seed borne pathogens by both national and international trade of seeds. Mathur and Kongsdal (2003) also reported that percent frequency of seed-borne fungal pathogens were more in pearl millet as compared to sorghum .Groundnut seed mycoflora: Results of fungal identification in showed that all the seed samples were contaminated with various fungal pathogens , Fungal pathogens identified included Alternaria, Aspergillus, Fusarium, Helminthosporium

and Rhizopus ,All the seed samples were found to be infected by Aspergillus whereas five samples with Fusarium. The test also shows loss in seed germination and symptom development in seedlings and can be used to evaluate seed treatments.

2.4 Management of fungal contaminants associated with crops seeds

Several effective ways for prevention and control of fungal contaminants associated with seed crops and their dangerous mycotoxin have been discussed by many researchers(FAO, 1979; Sanders et al., 1981 and WHO, 1988). In their recommendations they concentrate on optimization of cultural practices, development of resistant varieties, biological control and physical treatments. Farmers should be aware of pre-harvesting preparation of the field and environments ,drying of commodities after post harvest is the most economical and effective means for farmers. Chemical treatments such as alkalinization and ammonization are well-recognized and industrially used. They call for International cooperation through authorized organizations to promoted and support efforts aiming the benefits for the economics and health of people of all the nations. 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 et.al., 1982). 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 systemic fungicides to eradicate the pathogen from the soil, flood, fallowing, and using clean seeds each year are very common methods (Booth, 1971). 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. 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). Babu Joseph (2008) reported that Antifungal activity of the in vitro efficacy of different plant extracts viz., *Azadirachta indica, Artemisia annua, Eucalyptus globulus; Ocimum sanctum* and *Rheumemodi* were found to control wilt pathogens. Varma et al., 2002 also reported that extracts of tulsi (20%) was found to be least effective in inhibition of growth of *Fusarium.* The crude extracts of six plants viz, *Alliumsativum*, *Capsicumannuum*, *Artimesiavulgaris, Eupatorium adenophorum, Gaultheria fragrantissima* and *Phyllanthus emblica* were found to have activity against the fungus*Fusariumsolani* (Asha, *et. al.*,2009). Igbinosa (2009) investigated the ability of the crude stem extracts of *J. curcas* to inhibit the growth of fungi and bacteria is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. Aiyelaagbe and Ekunday (2000) investigated in-vitro and in-vivo the antifungal properties of *Jatroph acurcas* and *Ricinus cumunis* seed extracts in the control of mycelia growth and rot development of yam caused by *Fusarium verticilliodes* and *Aspergillus flavus r*eported that these plants possess antimicrobial activity. In Sudan, ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results , The fenugreek oilwas also found to inhibit *Salmonella typhimurium* (Sulieman, 2009). 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.

2.5 Fungicide(Tilt ® 250EC)

Tilt fungicide 250ec is systemic fungicide is recommended for the control of many important plant diseases , multiple purpose in fungi control for controlled the Powdery mildew in different vegetables (tomato 15mililiter\100liter) and used with wilt fungal disease. SYNGENTA encourages responsible resistance management to ensure effective short term control of the fungal diseases on this label Tilt ® 250ec. The number reported in Sudan is 524.

2.5.1 Storage:- in temperature little from (10-25).

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

3.2.Materials, tools and equipments used in the study

- Gloves
- Camera
- Marker pen
- Electric blender
- Petri-dishes glass
- Needle
- Autoclave
- Corcopuran
- Sensitive balance
- Incubator
- Flame
- Laminar flow cabinet
- Microscope
- Autoclave
- Slide
- Aluminum foul
- Water path
- Potato dextrose agar(PDA)
- Filter papers

- Medical cotton

All materials 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 (Aneja, 2004).

3.3 Collection of samples

A total of 16 seed samples, 4 of each crop, of four major food grinsnamely, *Sorghum bicolor L.,Pennisetum glaucum L., Arachishypogaea L. and Sesame indicum L.,*were collected from grains market' seed stocksof four different locations, Wadmadani, El-Gadarif, Niyalla and Elobied, one in each Estate. One random and homogeneous sample of one kilo gram was secured from each of the four crops in each location. 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 Detection and isolation of seed borne fungi

3.4.1 Dry Seed Inspection

A sample of four hundred (400) seeds of each seed sample were randomly selected and examined under stereoscopic binocular microscope (25-4x) and 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.

3.4.2 Incubation procedures

The seed samples were tested by the standard blotter method and (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.

3.5 Methods for the detection of seed borne fungal pathogens

3.5.1 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. Twenty five seeds were plated from each sample, 15 arranged at the periphery of the plate and10at the centre in case of sorghum, pearl millet and sesame while in case of groundnut, 3 seeds were arranged at the periphery of the plate and 2 at the centre. A total of four seed samples per crop, with three replications, were used and then kept in dark place for seed germination.

After seven days of 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. Other identification aids were Burgess*et al.,* (1994); Mathur SK, SB Mathur, P Neergaard (1975);Agarwal *et al.,* (1989) and Mathur and Kongsdal (2003). Infection levels were recorded as the percentage of infected seeds in a sample .

3.5.2. Agar Method

All seed samples (Sorghums, Millets, Groundnuts, sesame) was pre-treated with sodium hypochlorite 1% solution for 5 minutes then washed three times with sterilized distilled water (SDW) and dried between tow filter papers. The seed samples were then plated in the sterilized glass Petri-dishes on potato dextrose agar medium (PDA). The plates were incubated for seven

days at 25^0C. On the 8 th days the seeds were examined under light microscopes using slides preparation.(Lioyd B 2011).

3.5.3 Slide preparation and identification

The samples of fungus were taken randomly from each crop 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) X 100

3.6 Pure culture

The amount of the mycelium of *(Aspergillus flavus, A niger Penicillium digitatum, Rhizopus nigricans,Macrophomina phaseolina, Fusarium oxysporum, Fusarium solani, Alternaria solani,Colletotricum graminicola, Drechslera spicifera, Curvularia lunata,Cladosporium spp., Phoma spp.,* isolated from crops samples(sorghum, millet, sesame and 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; Barnett & Hunter, 1972; Ellis, 1980). Fungal growth continued for 7-10 days and then kept in the refrigerator as a stock for further investigation.

3.7 Identification of the pathogen

The identification of the fungi was based on visual culture characteristics mentation .furthermore, microscopic examinations were carried out for cMycelial and conidia structure based on the method of (Booth1977).

3.8 Preparation of plant extract

Neem and Damas leaves were collected from Shambat area and brought to the laboratory where they were shade dried. After complete dryness plant samples were crushed separately to obtain fine powder for extraction but the Garlic collected from market.

3.9Aqueous extract preparation:

The obtained fine powder form each plant was weighted (25, 50 and 100 gm.) and placed in 100,50,75 ml distill water respectively in plastic page each and completed to 100 ml distilled water at 24hrs to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 100 % 50% and 25% concentrations.

3.10 Test procedures

Inhibition zone technique was used in this study (Rao and Srivastava, 1994). The fungus spores suspension was prepared from previously prepared pure culture by allowing the spores to grow on PDA media (Ramprasad, 2005) treated with a desired concentration of neem damas leaves aqueous" extract.

The PDA media was amended with the required concentration (5ml, 10 and 15) 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 center of the plate after cut by used Corcopuran. In case of the control the disc was treated with sterilized distilled water and placed at the centre of Petri-dishesand inoculated Petri dishes inIncubation at 25 C^0 for 3 days. The growth of the fungus was calculated every day. 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 and dt= diameter of treated growth

3.11 Experimental design:

These experiments were arranged in a Complete Randomized Design

3.12 Statistical analyses:

The obtained data was statistically analyzed by Mstatc software computer program according to analysis of variance (ANOVA); -Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

This study which conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of science and Technology during august and September2013, was to detect and identify seed borne mycoflora associated with seeds samples of four food crops collected from four Estates in Sudan and to explore the antifungal potentials of some higher plants crude extract and fungicide against most commonly occurring fungus. The results cover Seed Health Testing and effect of plant extracts on growth of *Aspergillus flavus in vitro*.

4.1. Incidence of fungal species on crops seeds from different locations

Out of the sixteen seed samples, 4 of each crop, tested for occurrence of seed borne fungi, a total of 11 genera of 12 species of fungi were recorded (Table 1-4 and fig 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, and 3 respectively. The seed borne fungi identified were *Penicillium digitatum, Aspergillus flavus, Aspergillus niger, Rhizopus nigricans, Drechslera specifer, Alternaria solani, Fusarium solani, Colletotricum graminicola, Rhizoctonia solani, Phoma glomerata, Curvularia lunata and Macrophomina phaseolina*.

The most predominant seed borne fungi recorded across crops seeds were the storage fungi (saprophytes),*Penicillium digitatum*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus nigricans* with varying level of incidences (Table 1-4 and fig. 1-4).

Most samples tested for seed borne fungi gave a wide and large number of fungi with varying incidences (Table, 1-4). However, among all seed borne fungi mean percent incidence of the storage fungi, *Penicillium digitatum*,

Aspergillus flavus, Aspergillus niger and *Rhizopus nigricans,* was higher with varying level of incidences ; *Penicillium digitatum was* 36.3% in groundnut, *Aspergillus flavus*was38.8% in sesame, *Aspergillus niger* was32% insorghumand *Rhizopus nigricans*was 13.8 in groundnut (Table, 1-4). Among all crops, fungi detected on groundnut occurred in relatively higher incidence 22.5% as compared to other crops (Table, 4).

4.1.1. Mean percent incidence of seed borne fungi on millet seeds

The results obtained (Table, 1) and showed that out of the eleven genera of seed borne fungi detected in all samples of seeds, 12 species of fungi were recorded in millet. The highest percent frequency of occurrence of the seed borne fungi in millet recorded was 30% by *Penicillium digitatum* in Niyalla, *Aspergillus flavus* in Wad madani and *Drechslera specifer* in Elobied. However, *Penicillium digitatum* was the most prevailing fungus with 23% frequency of occurrence in all locations compared to other fungi.

Table 4. 1:Mean percentage incidence of seed borne fungi on various seed sample of millet collected from four different locations, each from one estate of Sudan

Location/species	Wad madani	Gadarif	Elobied	Niyalla	Total	mean %
Penicillium digitatum	20	22	20	30	92	23
Aspergillus flavus	30	19	6.0	10	65	16
Aspergillus niger	17	16	5.0	11	49	12
Rhizopus nigricans	8.0	10	10	5.0	33	8.0
Drechslera specifer	3	5	30	4	42	10
Alternaria solani	2	3	7	5	17	4.0
Fusarium solani	5	5	8	7	25	6.0
Colletotricum graminicola	2	2	3	1	8	2.0
Rhizoctonia solani	2	2	2	3	9	2.0
Phoma glomerata	1	3	3	2	9	2.3
Curvularia lunata	5	2	0.0	3	10	2.5
Macrophomina Phaseolina	3	1	2	3	9	2.3
Mean %	8.0	7.5	8.0	7.0	30.5	7.6

4.1.2. Mean percent incidence of seed borne fungi on sorghum seed

Frequency of occurrence of seed borne fungi in sorghum was recorded in Table (2). Apart from *Drechslera specifer* all species of fungi recorded on millet was recorded on sorghum. Among all seed borne fungi detected, average percent frequency of *Aspergillus niger in* sorghum was the highest 32% followed by *Penicillium digitatum* and *Aspergillus flavus,* 13.3 and 13.5 respectively.

Table 4. 2:Mean percentage incidence of seed borne fungi on various seed sample of sorghum collected from four different locations, each from one estate of Sudan

Location/species	Wad madani	Gadarif	Elobied	Niyalla	Total	mean %
Penicillium digitatum	6	15	22	10	53	13.3
Aspergillus flavus	10	10	12	13	54	13.5
Aspergillus niger	52	24	21	31	128	32.0
Rhizopus nigricans	5	13	17	10	45	11.3
Drechslera specifer	4	5	4	6	19	04.8
Alternaria solani	3	7	5	5	20	05.0
Fusarium solani	4	12	5	5	26	06.5
Colletotricum graminicola	3	2	3	2	10	02.5
Rhizoctonia solani	2	2	1	0	5	01.3
Phoma glomerata	2	3	2	2	9	02.3
Curvularia lunata	3	1	3	1	8	02.0
Macrophomina Phaseolina	2	2	1	1	6	01.5
Mean %	8.0	8.0	8.0%	8.0	383	8.0

4.1.3. Mean percent incidence of seed borne fungi on sesame seeds

Results of fungi detected sesame were reported in table (3). Apart from *Drechslera specifer* and *Macrophomina Phaseolina* all species of fungi recorded on millet and sorghum was recorded on sesame (table, 1, 2 and 3).Among all seed borne fungi detected, average percent frequency of *Aspergillus flavus* in groundnut was the highest 38.8% followed by Penicillium digitatum 24.8%. The highest percent frequency of occurrence of the seed borne fungi in sesame recorded was 53%, 42% and 38% by *Aspergillus flavus* in wad madani, Elobied and Gadarif.

Table 4. 3:Mean percentage incidence of seed borne fungi on various seed sample of sesame collected from four different locations, each from one estate of Sudan.

Location/species	Wad madani	Gadarif	Elobied	Niyalla	Total	mean %
Penicillium digitatum	10	25	28	35	99	24.8%
Aspergillus flavus	53	38	42	22	155	38.8%
Aspergillus niger	16	10	6	10	42	10.5%
Rhizopus nigricans	8	8	6	13	35	8.8%
Drechslera specifer	2	3	5	4	14	3.5%
Alternaria solani	2	2	2	2	8	2.0%
Fusarium solani	3	4	1	3	11	2.8%
Rhizoctonia solani	2	0	3	1	6	1.5%
Phomaglomerata	0	1	2	1	4	1.0%
Curvularia lunata	1	2	2	4	8	2.0%
Mean %	9.7	9.4	9.7	9.5	382	9.5

4.1.4. Mean percent incidence of seed borne fungi on groundnut seeds.

The seed borne mycoflora of groundnut was presented in table 4. A total of four species of fungi were detected in samples collected from the four locations, namely, *Penicillium digitatum Aspergillus flavus*, *Aspergillus niger* and *Rhizopus nigricans.* However, among all fungi occurred, *Penicillium digitatum* groundnut showed the highest frequency of occurrence 36.3% in seed samples from the four locations followed by *A. flavus* 27.5 and the lowest *Rhizopus nigricans* 12.5%. Moreover, the mean percentage incidence of *Penicillium digitatum* was higher in Niyalla 45% followed by Gadarif 4o%.

Table 4. 4:Mean percentage incidence of seed borne fungi on various seed sample of groundnut collected from four different locations, each from one estate of Sudan

Location / Species	Wad madani	Gadarif	Elobied	Niyalla	Total	mean %
Penicillium digitatum	30	40	30	45	145	36.3%
Aspergillus flavus	25	20	35	30	110	27.5%
Aspergillus niger	10	15	15	10	50	12.5%
Rhizopus nigricans	20	20	10	5.0	55	13.8%
Mean %	21.25	23.75	22.5	22.5	360	22.5

4.2. Effects of plants extracts and fungicide on radial growth of fungus

4.2.1. Effect on radial growth of fungus on day three

After three days from inoculation the results indicated that plants extracts at all concentrations reduced the fungal growth significantly compared to control (Table, 1and fig 5). Moreover, the garlic extract and fungicide at 50 and 100% concentration completely inhibited the growth of the fungus. In fact, among plant extracts, garlic at the three concentrations (25, 50, and 100%) demonstrated the highest inhibition of fungal growth (63, 100 and 100%) followed in descending order by Neem (53.7, 68.1 and 85.3%), Harjal (39.2, 60.8 and 81.7) and Damas (18.5, 42.2 and 70%) respectively (Table, 1). However, the suppressing effect of fungicide was more pronounced at all concentrations tested.

Table 4. 5: Effect of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillus flavus* after three days from inoculation *invitro*.

Treatment concentrations (%)		Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	25	44.4(6.7)	66.7(8.2)	50.0(7.1)	53.7(7.3)DEF
	50	66.6(8.2)	77.7(8.8)	60.0(7.8)	68.1(8.3)BCD
	100	77.8(8.8)	88.9(9.5)	90.0(9.5)	85.3(9.3)AB
Harjal	25	37.5(6.2)	40.0(6.4)	40.0(6.4)	39.2(6.3)F
	50	62.5(7.9)	50.0(7.1)	70.0(8.4)	60.8(7.8)CDE
	100	75.0(8.7)	90.0(9.5)	80.0(9.0)	81.7(9.1)ABC
Damas	25	25.0(5.0)	21.4(4.7)	9.1(3.1)	18.5(4.3)B
	50	58.3(7.7)	50.0(7.1)	27.3(5.3)	42.2(6.7)EF
	100	75.0(8.7)	71.4(8.5)	63.6(8.0)	70.0(8.4)BCD
Garlic	25	42.1 (6.5)	33.3(5.8)	63.0(9.4)	46.1(7.3)DEF
	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
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(0.70)H
CV					9.05%
SE					0.36
LSD					1.176

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

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

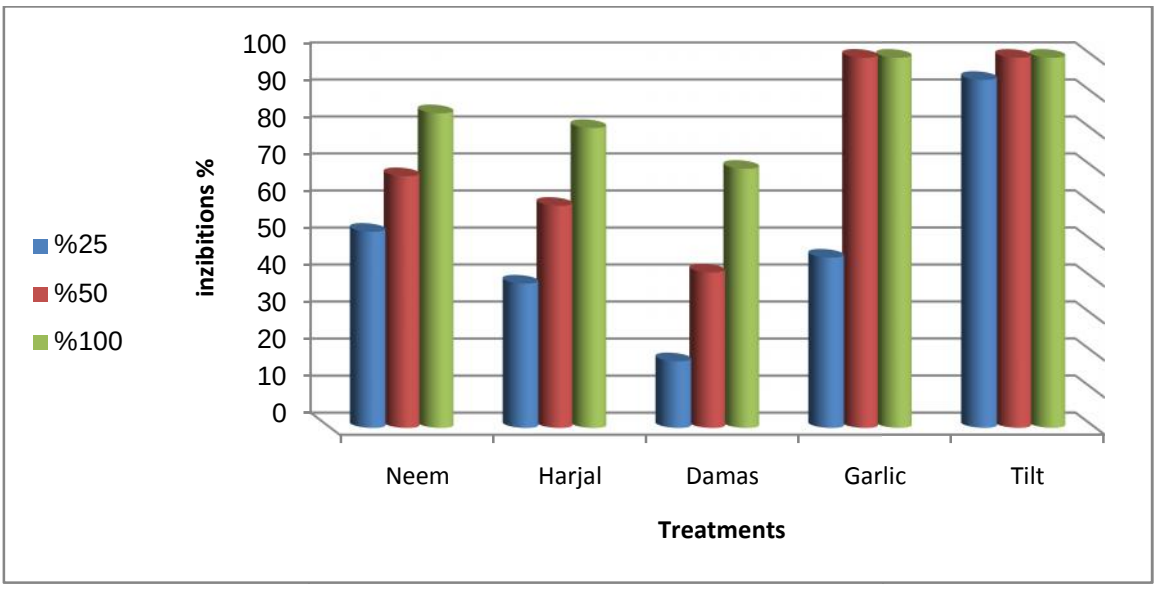


Figure 1: Effect of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillus flavus* after three days from inoculation *invitro*.

4.2.2. Effect on radial growth of fungus on day four

The results (Table, 2 and fig 6) showed that the aqueous extracts of all plants screened and fungicide tilt exhibited inhibitory effects against fungal growth after three days from inoculation. The percentages fungal growth inhibition was significantly high compared to the control.

Moreover, the highest concentration of the plant extracts (100%) and that of fungicide (Tilt) gave significantly higher inhibition zones percent against test fungus (85.3, 81.7, 70, 100% and 100%) respectively compared to the untreated control. Among the plant extracts screened that of garlic was invariably the most effective in suppressing the fungus growth at all concentrations screened than its equivalent Neem, Damas and Harjal (Table, 2). Generally, the results showed that the antifungal activity increase with concentration.

Table 4. 6: Effect of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after four days from inoculation.

Treatment concentrations. (%)		Inhibition zone (%) R1	R2	R3	Mean %
Neem	25	36.8(6.1)	47.6(6.9)	56.5(7.6)	47.0(6.9)DE
	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)	88.8(9.5)AB
Damas	25	40.0(6.4)	31.8(5.7)	41.7(6.5)	37.8(6.2)E
	50	55.0(7.4)	54.5(7.4)	50.0(7.1)	53.2(7.3)CD
	100	60.0(7.8)	59.1(7.7)	52.5(7.9)	57.2(7.8)C
Harjal	25	41.7(6.5)	33.3(5.8)	42.9(6.5)	39.3(6.3)E
	50	58.3(7.6)	50.0(7.10)	64.3(8.1)	57.5(7.6)CD
	100	75.0(8.7)	83.3(9.2)	71.4(8.5)	76.6(8.8)B
Garlic	25	43.5(6.6)	21.1(4.6)	21.1(4.6)	28.6(5.3)F
	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
Tilt	25	95.5(9.8)	94.7(9.8)	91.7(9.6)	94.0(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(0.70)G
CV					5.43%
SE					0.35
LSD					0.7056

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

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

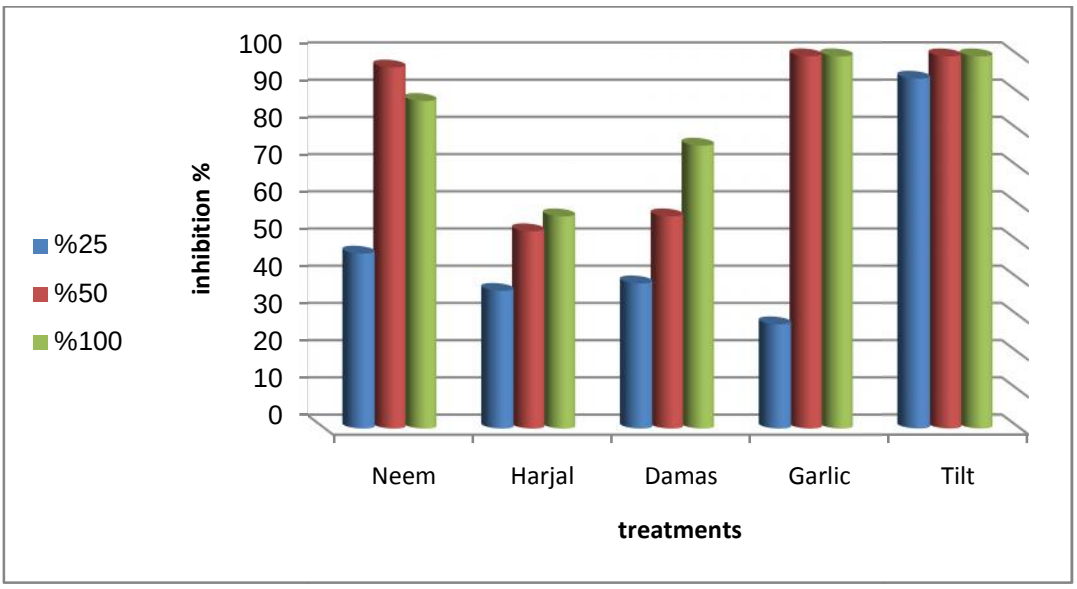


Figure 2: Effect of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after four days from inoculation

4.2.3. Effect on radial growth of fungus on day five

In day five after inoculation (Table, 3 and fig 7), treatments of fungicide, Garlic, Neem, Harjal and Damas at all concentrations (25, 50, and 100%) were invariably continued exhibiting significant inhibitory effects against the fungal growth (100%, 100%, 90.4; 100%, 95.9% ,44.0%; 74.8% 67.1%, 43.2%; 63.5%, 50.6%, 48.3% and 43.3%, 27.1% 20.7%) respectively. However, the inhibitory effects of Neem, Harjal and Garlic were more pronounced than that of Damas which showed decreasing inhibitory effect against test fungus compared to day three and four (Table, 1 and 2). Furthermore, the fungicide irrespective of concentration, (25, 50 and 100%) effected significant reduction of fungal growth (94.0, 100, and 100%) respectively compared to control.

Table 4. 7:Effects of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after five days from inoculation.

Treatment concentrations (%)		Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	25	42.9(6.6)	40.9(6.4)	45.8(6.8)	43.2(6.6)E
	50	61.9(7.9)	72.7(8.6)	66.7(8.2)	67.1(8.2)CD
	100	76.2(8.8)	77.3(8.8)	70.8(8.4)	74.8(8.7)BC
Damas	25	16.7(4.1)	21.9(4.7)	23.5(4.5)	20.7(4.4)F
	50	26.7(5.2)	31.2(5.6)	23.5(4.5)	27.1(5.1)F
	100	33.3(5.8)	40.6(6.4)	55.9(7.5)	43.3(6.6)E
Harjal	25	61.5(7.9)	41.7(6.5)	41.7(6.5)	48.3(6.5)E
	50	65.4(8.1)	36.4(6.2)	50.0(7.1)	50.6(7.1)E
	100	64.6(8.1)	54.5(7.4)	71.4(6.5)	63.5(7.3)DE
Garlic	25	44.0(6.7)	22.7(4.8)	65.4(8.1)	44.0(6.5)E
	50	100.0(10.0)	95.5(9.8)	92.3(9.6)	95.9(9.8)A
	100	100.0(10.0)	100.0(10.0)	100.0(10.0)	100(10.0)A
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)G
CV					8.16%
SE					0.36
LSD					0.9937

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

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

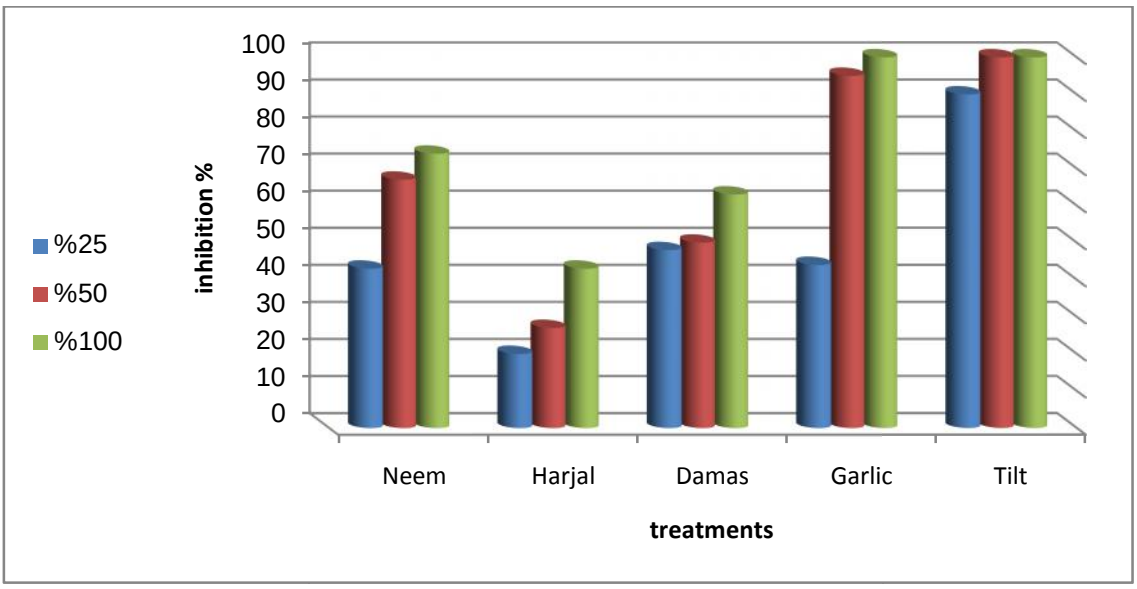


Figure 3: Effects of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after five days from inoculation

4.2.4 Effect on radial growth of fungus on day six

Generally, it could be seen from the results (Table, 4 and fig 8) that after six days from inoculation, extracts of all plants tested as well as the fungicide at proved effective in suppressing the fungal growth.

In fact, all tested concentrations of fungicide, Garlic, Harjal, Neem, and Damas (100, 50 and 25%) induced significantly high inhibition zones percentage (100%, 100%, 93.8; 100%, 91.3% ,27.2%; 84.1%, 73.8%, 66.0%; 66.7% 54.9%, 23.8% and 31.1%, 21.9% 13.3%) respectively against test fungus compared to control (Table, 3).

Meanwhile, among plant extracts the Garlic at all concentrations tested (25, 50 and 100%) exhibited consistently the highest inhibitory effect throughout the days of recording (Table, 1, 2 and 3) than the other equivalents. Similar effect was also demonstrated by the fungicide. However, the inhibitory effect of Damas plant extracts reduced with time of recording.

Obviously, in all tested products, growth inhibition increased with the concentration.

Table 4. 8:Effects of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after six days from inoculation

Treatment	concentrations. (%)	Inhibition zone (%) R1	R2	R3	Mean %
	25	23.3(4.9)	22.6(4.7)	25.5(5.0)	23.8(4.9)E
Neem	50	60.0(7.8)	51.6(7.2)	53.1(7.3)	54.9(7.4)D
	100	70.0(8.4)	67.7(8.3)	62.5(7.9)	66.7(8.2)CD
	25	17.1(4.2)	17.1(4.2)	5.6(2.5)	13.3(3.6)F
Damas	50	12.1(3.5)	34.1(5.9)	19.4(4.5)	21.9(4.6)EF
	100	21.2(4.7)	41.5(6.5)	30.6(5.6)	31.1(5.6)E
	25	65.8(8.1)	64.7(8.1)	67.6(8.3)	66.0(8.2)CD
Harjal	50	73.6(8.6)	73.5(8.6)	74.3(8.6)	73.8(8.6)BC
	100	84.2(9.2)	85.3(9.3)	82.9(9.1)	84.1(9.2)ABC
	25	23.1(4.9)	13.5(3.7)	45.0(6.7)	27.2(5.1)E
Garlic	50	100.0(10.0)	86.5(9.3)	87.5(9.4)	91.3(9.6)AB
	100	100.0(10.0)	100.0(10.0)	100.0(10.0)	100(10.0)A
	25	94.6(9.8)	93.9(9.7)	92.9(9.7)	93.8(9.7)A
Tilt	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(0.700)G
CV					8.40%
SE					0.40
LSD					1.008

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

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

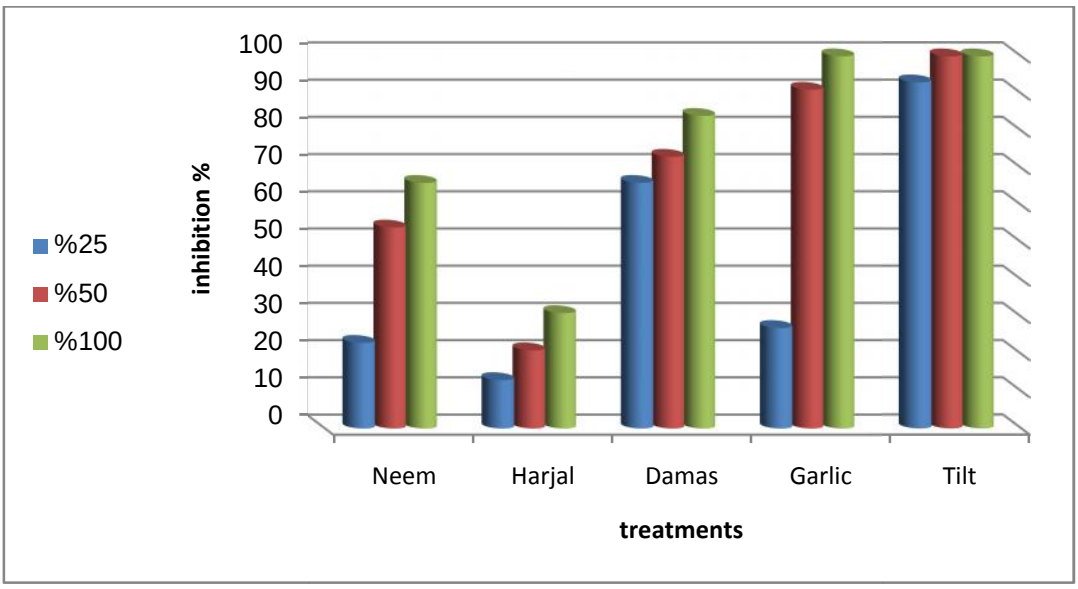


Figure 4: Effects of aqueous extracts of Neem, Damas, Harjal, garlic and fungicide on the linear growth (inhibition zone %) of *Aspergillusflavus* after six days from inoculation

CHAPTER FIVE

DISCUSSION

The study was carried out to investigate the occurrence of seed borne fungi 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 flavus in vitro*.

The importance of seed borne fungi to crop quality and quantity cannot be ignored. The risk encountered have been reported by several authors (Haq Elamin NH *et al.,* 1988; El-Naghy *et al*., 1998 ;and ;Yousif M.A.*et al*., 2010).

Azhar *et al., (*2011) reported that 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.

The results of this study revealed that irrespective of load of seed borne fungi, their association with food crops seeds in different locations of Sudan appears to be a prevalent situation. Apart from groundnut where only four storage fungi were occurred on seeds samples tested with standard blotter method as described by the International Seed Testing Association (ISTA 1976) all other crops were associated with at least ten known spoilage species of fungi (*Penicillium digitatum, Aspergillus flavus, Aspergillus niger, Rhizopusnigricans, Drechsleraspecifer, Alternariasolani, Fusarium solani, Rhizoctoniasolani, Phomalongum and Curvularialunata.* These results are in agreement with those of Syed Danis, *et al*., (2013); Kamal and Mughal (1968) and Khan e*t al.,* (1974) who reported the presence of Aspergillus, Penecillium, Alternaria, Fusarium, and Rhizopus, species in seeds of food

crops. The results also corroborate those of Khan and Bhutta (1994); Bhutta and Hussain (1999) and Singh (1983) 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, *Penicillium digitatum, Aspergillus flavus, Aspergillus Niger, and Rhizopus* nigricans with varying level of incidences. The common occurrence of seed borne fungi like Aspergillus and Penicillium had been widely reported by Haq Elamin NH *et al.,* 1988 and Martin *et al.,* (1984).

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) and Mukherjee *et al.,* (1992) also found that *Aspergillus* were the predominant storage fungi of groundnut seeds.

The results also revealed that the Neem,Harjal, Damas and Garlic 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 and Shariff et. al., 2006).

Garlic 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 garlic aqueous extracts expressed the highest fungal growth suppression with

significantly high inhibition zones percent compared to control. These results incorporate that of Karunyal (2000) who studied the antifungal effect of aqueous extract of *Allium sativum* bulbs against the fungus *Trichophytonrubrum* and that of AbdelMoneim E. *et al.*, (2009) who demonstrated the antimicrobial activity of Harjalaqueous extracts against two fungi (*Aspergillus niger* and *Pennicilium italicum*) and two Gram negative bacteria (*Escerichia coli* and *Salmonella typhi*).The Harjal which wasfound to inhibit mycelial radial growth of both fungi.Antimicrobial properties of Garlic, Harjal and Neem were also reported by *Roos et al.,* (1980), Elhady*et al.*, (1994), Abdel Moneim E. *et al.*, (2009) and Suliman*et al.*, (2009). 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.

The results also demonstrated that the Garlic, Harjal and Neem 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 highest concentrationsof the plants 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, he explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree. However, these results confirmed that obtained by (Reem, Alhadi and Faiza,(2012).

Conclusion

- Food crop seeds besides being of high quality and purity should also be free from spoilage fungi. In this study eleven fungal genera were encountered in wide range of incidence percentage in 16 samples of sorghum, pearl millet and groundnut and sesame collected from four locations, each in one state of Sudan.

- Of the fungi occurred in seed samples, the four most prevailing seed borne fungi recorded across crops seeds were the storage ones; *Aspergillus flavus, Aspergillus Niger, Penicillium digitatum and Rhizopus nigricans* with varying level of incidences.

- Among all crops, fungi detected on groundnut occurred in relatively higher incidence as compared to other crops

- 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.

- Garlic and Harjal aqueous extract exhibited the highest inhibitory effect compared to other plant extracts.

Recommendations

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

- It is vital to establish seed borne fungi mapping through continuous seed health analysis for sorghum, pearl millet, groundnut and sesame crops across Sudan and to be updated regularly so that research will target potentially important ones.
- 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.
- Introduction of testing seed health of major crops should in the national seed quality system is required.
- Further investigationof the antimicrobial properties of higher plants but in a group of medicinal plants against targets organism is needed to determine their potentials as botanical pesticides



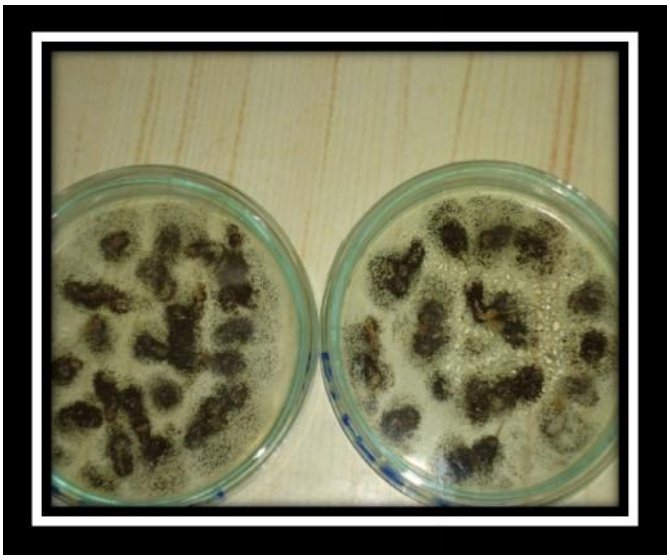
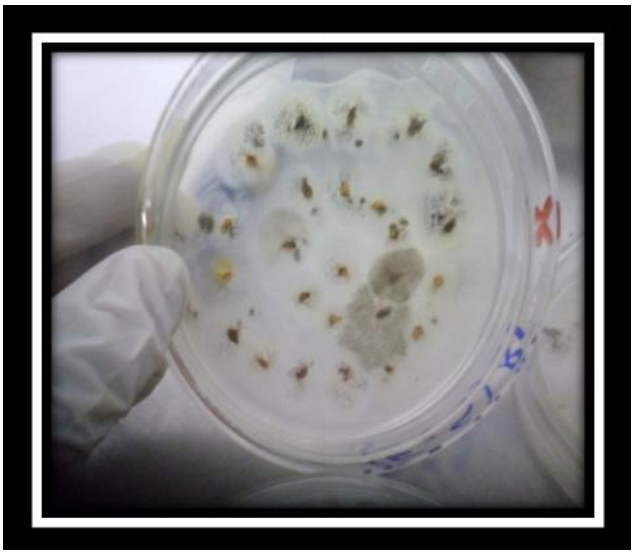



Plate 1: **Detectionof major seeds fungi by agar method in**

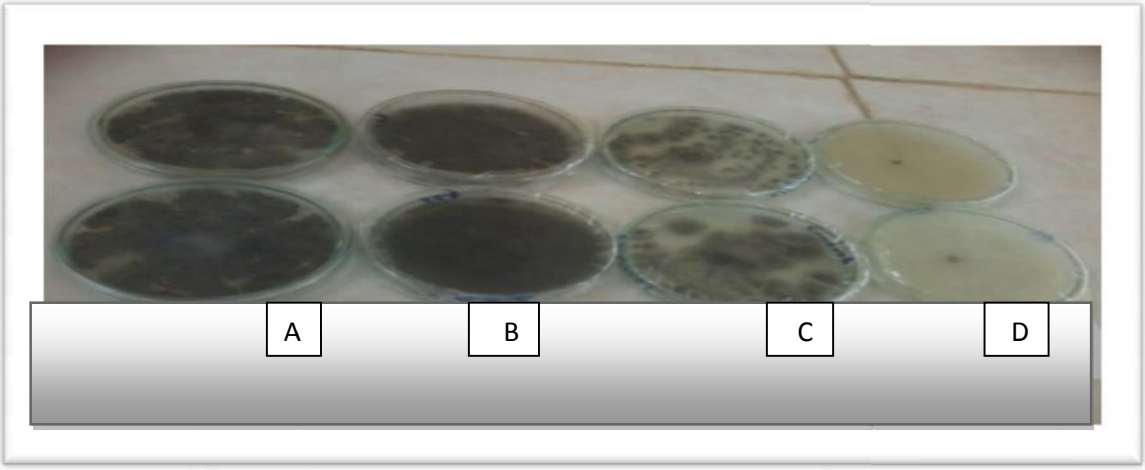


Plate 2: Effect of Damas aqueous extract of Aspergillus flavus after6days invitro. A/B/C/D/= control, 25%, 50%, 100% /respectively.

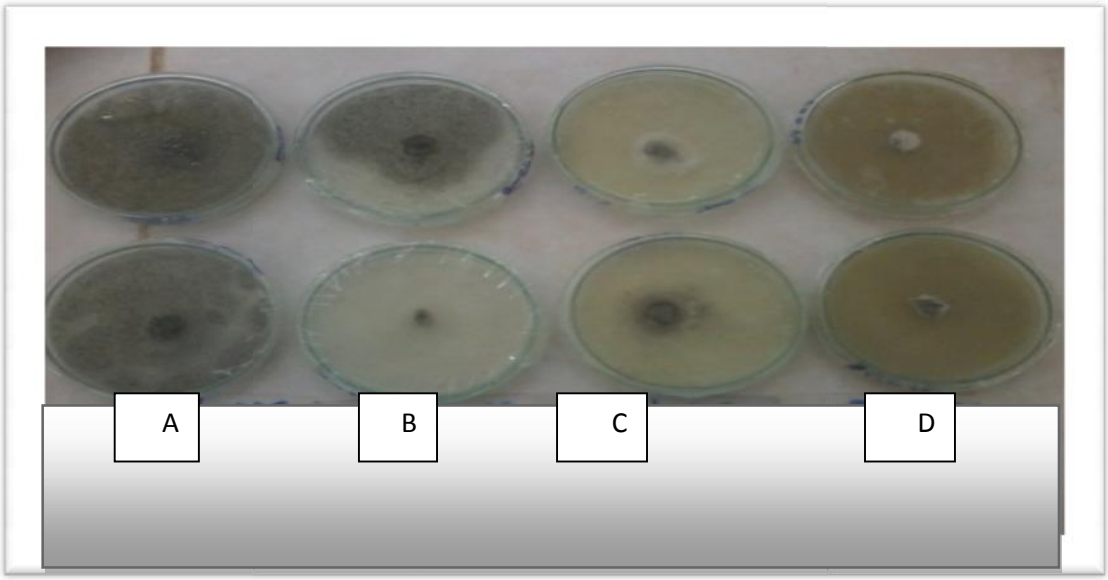


Plate 3: Effect of Neem aqueous extract of *Aspergillus flavus* after 6days invitro.A/B/C/D/control, 25%, 100% Respectively.

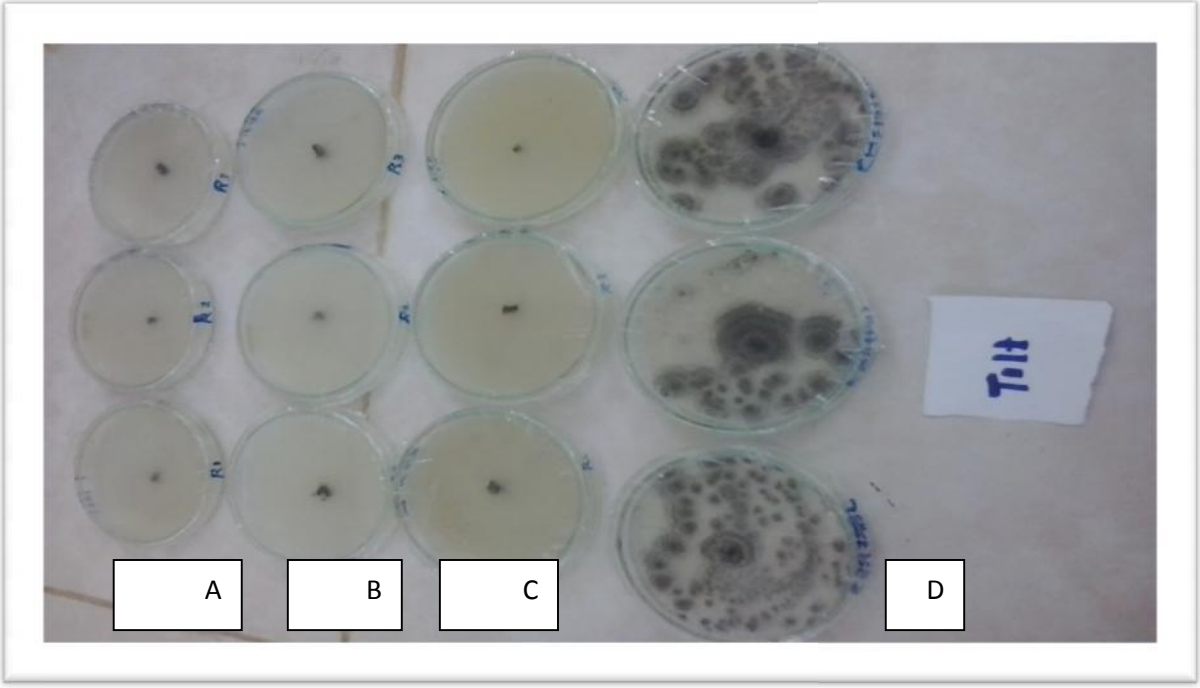


Plate 4: Effect of Tilt fungicide of *Aspergillus flavus* after 6daysinvitro. A/B/C/D=100%50%25% control respectively.

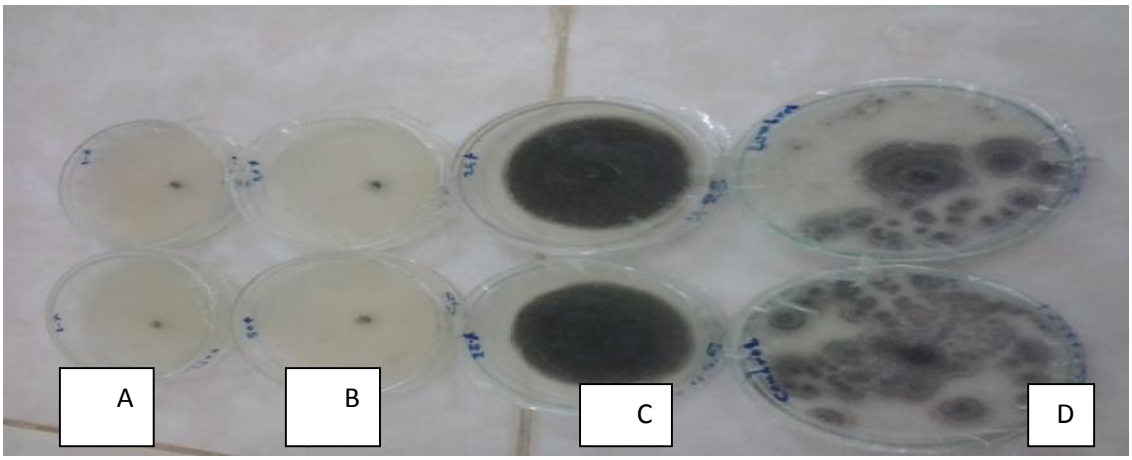
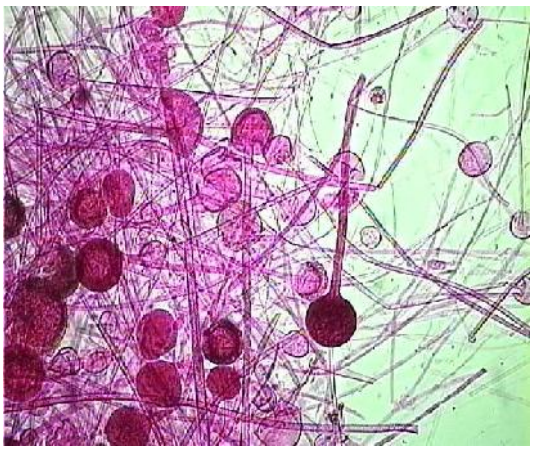
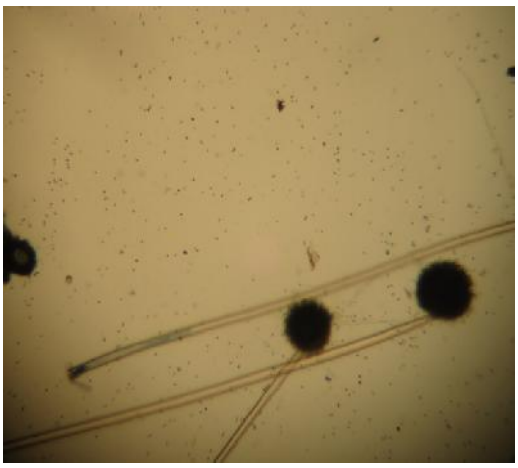


Plate 5: Effect of Garlic aqueous extract of *Aspergillus flavus* after 6days invitro/B/C/D=100%,50%,25%,control respectively.

Plate 6: Peroration of Neem aqueous extract

Page (7) Preparation of Tilt fungicide

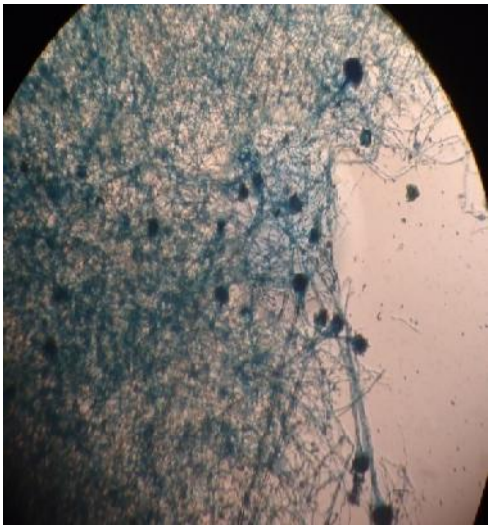


Plate (8)Rhizopus nigricans, Aspergillius flavus , Penicillium digitatum and Aspergillius niger respectively

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APPENDICES

Appendix1.2:

Table 1.2:A N A L Y S I S O F V A R I A N C E T A B L E(On way ANOVA table): (Effect of Damas Neem Argel Garlic aqueous extract and tilt fungicide after3days)

Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	15	283.046	18.870	37.708	0.0000
Within	32	16.013	0.500		
Total	47	299.059			

Coefficient of Variation = 9.05%

Appendix 2.2:-

Table 2.2: Analysis of variance table (One way ANOVA table):

(Effect of Damas Neem Argel Garlic aqueous extract and tilt fungicide after4days).

Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	15	273.177	18.212	101.177	0.0000
Within	32	5.760	0.180		
Total	47	278.937			

Coefficient of Variation = 5.43%

Appendix3.2:

Table 3.2: Analysis of variance table (One way ANOVA table):

Effect of Damas Neem Argel Garlic aqueous extract and tilt fungi after5days

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	15	284.226	18.948	53.126	0.0000
Within	32	11.413	0.357		
Total	47	295.639			

Coefficient of Variation = 8.16%

Appendix 4.2:

Table 4.2: Analysis of variance table (One way ANOVA table):

Effect of Damas Neem Argel Garlic aqueous extract and tilt fungi after 6days

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	15	352.373	23.492	63.995	0.0000
Within	32	11.747	0.367		
Total	47	364.120			

Coefficient of Variation = 8.40%