Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Occurrence and Identitication of Seed Borne Fungi Associated with Groundnuts in Kordofan States

وجود وتعريف الفطريات المحمولة على بذور الفول السوداني في ولايات كردفان

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

BY

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الآية

﴿ بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ ﴾

قال تعالى : -

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) الإِنسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَرَبُّكَ الأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الإِنسَانَ مَا لَمْ يَعْلَمْ (٥) كَلاَّ إِنَّ الإِنسَانَ لَيَطْغَى (٦) أَن رَّآهُ اسْتَغْنَى (٧) إِنَّ إِلَى رَبِّكَ الرُّجْعَى (٨) أَرَأَيْتَ الَّذِي يَنْهَى (٩) عَبْدًا إِذَا صَلَّى (١٠) أَرَأَيْتَ إِن كَانَ عَلَى الْهُدَى (١١) أَوْ أَمَرَ بِالتَّقْوَى (١٢) أَرَأَيْتَ إِن كَذَّبَ وَتَوَلَّى (١٣) أَلَمْ يَعْلَم بِأَنَّ اللَّهَ يَرَى (١٤) كَلاَّ لَئِن لَّمْ يَنتَهِ لَنَسْفَعًا بِالنَّاصِيَةِ (١٥) نَاصِيَةٍ كَاذِبَةٍ خَاطِئَةٍ (١٦) فَلْيَدْعُ نَادِيَهُ (١٧) سَنَدْعُ الزَّبَانِيَةَ (١٨) كَلاَّ لاَ تُطِعْهُ وَاسْجُدْ وَاقْتَرِب (١٩)

سورة العلق - سورة 96 - عدد آياتها 19

Dedication

To my mother

To my father

to my wife

to my sons

To my brothers and sisters

To my family

To my teachers

To my colleagues and friends

With love and respect

Osman

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First of all thanks to Allah who gave me strength to complete this work.

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Table of Contents

List of Tables

Title **Page No.**

List of Figures

Title **Page No.**

List of Plates

ABSTRACT

Groundnut (*Arachishypogaea L.*) is considered the 4th most important oilseed crop worldwide due to its multiple uses. The production of the crop is constrained by several factors, among which are the seed borne fungi. In Sudan, the impact of these fungi and their secondary metabolites as food contaminants is under continuous investigation. This study was conducted at laboratory of Plant Pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during February -April, 2016.(Stored since2015 season). The objectives to determine the occurrence and identify the seed borne fungi associated with groundnuts in samples collected from four locations in the Agricultural Bank of Sudan stores, namely, Alfulah , and Almoglad in Western Kordofan whereas Alobied and Alnehud in Northern Kordofan State,filter paper method and agar plate method were used as recommended by ISTA (1966). The laboratory examination revealed that five seed borne fungi were identified namely, *Aspergillus flavus, Aspergillus niger, Rhizopus nigricans, Penicillium digitatum and Alternaria solani.* Among these fungi the most predominantly occurred and consistently recorded across locations were *A. flavus* and*A.niger.* Their percentages incidences were significantly higher (42.5% and 30%) respectively followed by *Alternaria solani* (6.5%), *R. nigricans* (5.0%) *and P. digitatum* (1.25%). The results obtained by the two methods used are very close. The results obtained in this study highlighted the major human and animal health risk encountered due to high contamination of groundnut with mycotoxins producing fungi.

ملخص البحث

يعتبر الفول السودانى الرابع كاحد اهم المحاصيل الزيتية عالمياً لاستعمالاته المتعددة. الفطريات هى من بين العوامل العديدة التى تعيق انتاجية هذا المحصول. فى السودان تاثير هذه الفطريات وإيضها الثانوى كملوثات للاطعمة تحت الدراسة المستمرة. أجريت هذه التجربة بمعمل أمراض النبات ـ كلية الدراسات الزراعية ـ جامعة السودان للعلوم والتكنولوجيا خلال فبراير والى ابريل عام 2016 بهدف تحديد تواجد وتعريف الفطريات المحمولة على البذور المرتبطة بعينات من الفول السودانى جمعت من اربعة مناطق من مخازن البنك الزراعى السودانى بالتحديد , النهود المجلد من غرب كردفان, الابيض من ولاية شمال كردفان بالسودان باستعمال طريقتى ورق الترشيح والاقار كما هو موصى به من قبل المظمة العالمية لفحص البذور. اوضحت النتائج التى تم الحصول عليها انه تم الكشف عن خمسة فطريات تسمى, اسبرجلس فلافس, اسبرجلس نيغر, رايزوبس نيقريكانز, الترناريا سولانى و بنسليم ديجيتاتم. من بين هذه الفطريات سبرجلس فلافس, اسبرجلس نيغر سجلت انها الاكثر تواجدا وبالدرجة الاولى وبصورة ثابته بجميع المناطق. نسبة الإصابة بها كانت عالية معنوياً (42.5 و 30%) على التوالى يليهن الإلترناريا سولانى (6.5%) و بنسليم ديجيتاتم(1.25). النتائج التى تم الحصول عليهما بواسطة الطريقتين اللتين استخدمتا لكشف تلوث العينات كانتا متقاربتين جداً كما ان طريقة ورق الترشيح وضح انها عملية اكثر من طريقة الأقار. النتائج التى تم الحصول عليها فى هذه الدارسة سلطة الضوء على الخطر الماثل نتيجة تلوث الفول السودانى بالفطريات المنتجة للافلاتوكسن .

CHAPTER ONE

INTRODUCTION

Groundnut (*Arachishypogae*a L.) which believed to be originated from South America (Wiess, 2000) is an important food and cash crop in many countries worldwide and it contribute significantly to food security and alleviate poverty (Smart et al., 1990) in some developing countries. 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). In Sudan, the total area under groundnut production is approximately one million ha with an average yield of 855 kg/ha (Mahmoud *et al*., 1995; ARC, 2003-2010 and FAOSTAT, 2010).

Moreover, the crop which belongs to the family Fabaceae is the 13th most important food crop of the world and the world's 4th most important source of edible oil and 3 rd most important source of vegetable protein (Taru, *et* al., 2010). Sudan considered among the main producing countries beside China, India, Nigeria, USA, Indonesia (Mondal; *et al.*, 2006). The crop is also widely grown in countries like Myanmar, Vietnam, Senegal, the Democratic Republic of Congo, Chad, Burkina Faso, Zimbabwe, Mali, Mozambique, Uganda, and Tanzania (Shiferaw *et al.*, 2004).

Among groundnuts uses, the nut is consumed directly in processed food and snacks as valuable source of protein, energy, minerals, oil, meal and confectionery products (Abu Assar *et al.*, 2008). 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 and the by-product of oil extraction is an important ingredient in

livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock (Mahmoud *et al*., 1995). 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.

One of the major constraints facing the productivity and availability of healthy groundnut produce worldwide are the losses and spoilage caused by Fungi, bacteria, viruses, insects, nematodes and parasitic weeds. In fact, the threat to this crop from fungi species which produce secondary metabolites has now reached a level that outstrips that posed by other biotic and a biotic factor (Berger, 1977). These fungi continue 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).

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 *Aspergillusspp*. (Anon, 1989).

In Sudan, the impact of these fungi and their secondary metabolites as food contaminants is well-established (Ali, 1989; HaqElamin*et al*., 1988; Reddy KRN. 2010 and Yousif*et al*., 2010). Shami and Ahmed(2010) indicated that *Aspergillusflavus* 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.

Obviously, the infection of groundnuts by various fungi spp. not only results in reduction in crop yield and quality but also contamination of produce with poisonous fungal secondary metabolites called mycotoxins. These substances arise from the secondary metabolism of fungi in response to a wide range of genetic and environmental factors and are capable of causing diseases in man and animals (Ali, 1989 and Bhat and Vasanth, 2003).Based on the foregoing, this study was undertaken with the following objectives:-

- To determine the occurrence of mycoflora associated with groundnut in East and North Kordofan State in Sudan.
- To identify mycoflora species associated with groundnut in this States.
- To determine the frequency of seeds contamination in each location.

CHAPTER TWO

LITERATURE REVIEW

2.Groundnut

2.1.1. Origin and producing countries

Groundnut (*Arachis hypogaea* L.) which believed to be originated from South America (Wiess, 2000) is an important food and cash crop in many countries worldwide and it contribute significantly to food security and alleviate poverty (Smart et al., 1990) in some developing countries. According to FAO (2006), around 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). Ground cultivation is primarily cultivated in areas of the world bounded by latitudes 40º N to 40º S. and warm temperate zones countries. The major growing countries are; India (26%), China (19%) and Nigeria (11%),U.S.A (5.9%), Indonesia (4.1) and Sudan (3.6%) (Nwokoto, 1996).

2.1.2 Scientific classification of groundnut plant

Domain : Eukaryota

Kingdom : Plantae

Subkindom : Viridiplantae

Infrakindom : Streptophyta

Superdivision : Embryophyta

Division : Tracheophyta

Subdivision : Spermatophytina

Class	:	Magnoliopsida
Superorder	:	Rosanae
Order	:	Fabales
Family	:	Fabaceae
Genus	:	Arachis
Species	:	*Arachishypogaea L*.

2.1.3.Groundnut plant

Groundnut (*Arachis hypogaea*) is a self-pollinated, tropical annual legume which is fairly drought resistant and mainly cultivated in dry tropical areas. It has the advantage of generating residual nitrogen in the soil which benefits subsequent crops, especially when groundnut residues are incorporated into the soil during ploughing. Despite the high local demands for groundnuts, farmers' yields in South Nyanza (Province in Kenya) continue to be low, averaging 250kg/acre of dry shelled seeds.

2.1.4. Importance of Groundnut in Sudan

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 and the by-product of oil extraction is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock.

2.1.5. Production constraints

In Sudan, this crop attacked by several constraints mainly fungal diseases and contaminants, among these is*Aspergillus spp.* which produces secondary metabolites called Aflatoxin. The health impacts of ingestion in humans include stunted growth and development as well as an increased risk in liver cancer(HCC) (IARC and ICRISA, 2002).

2.2. 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 (Ali, 1989; El-Naghy, *et al*., 1998; HaqElamin,*et al*., 1988; Holbrook, *et al*., 2000; 1998; Osman,*et al*., 1999; Saber, *et al*., Thompson and Henke, 2000; Yousif, *et al*., 2010).

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 on 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 mycotoxins, for example *Penicilliumspp*, although many penicillia associated with grains are pathogenic. The seed borne fungi of most concern are produced by species within the genera of *Aspergillussp*p, *Fusariumspp*, and *Penicilliumspp* 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).

However, *Aspergillusspp, Penicilliumspp, Alternariaspp* and *Fusariumspp* 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.

2.2.1. *Aspergillus* spp :

The impact of *Aspergillusspp* in as food contaminants is well-established (Ali, 1989; HaqElamin NH *et al*., 1988; KRN Reddy, 2010 and Yousif.M.A.*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: *A. flavus, A.parasiticus, A. nomius, A.ochraceus, A.candidus, A. restrictus, A.penicillioides, A. niger, A.carbonarius, A. fumigatus, A. clavatus, and A. carbonarius, and A. versicolor* (Peter, 2010.) However, Ali, (1989); HaqElamin NH et al., (1988); Olusegun, (2013), and Yousif M. A.*et al*., (2010), and reported that *Aspergillusspp* 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, *A.flavus, A. parasiticus* and aflatoxins typically affect oilseeds, including groundnuts, soya, tree nuts, maize and various oilseed-based animal feed stocks - cotton seed cake, copra, sunflower, but can also affect rice, wheat, sorghum, figs, coffee and sweet potatoes, for example Aflatoxins are also noted in milk, via contaminated animal feed.

2.2.2. *Penicillium* spp:

Penicillium spp as well is a large genus containing 150 recognized species, of which 50 or more occur commonly. Many species of *Penicilliumspp are* isolated from foods causing spoilage; in addition, some may produce bioactive compounds.

Important mycotoxins produced by *Penicilliumspp* include ochratoxin A, patulin, citrinin and penitremA. Some of the most important toxigenic species in foods are *P. expansum, P. citrinum, P. crustosum and P. verrucosum*(Pitt J.I., 2006).

Amuch larger number of *Penicilliumspp*are mainly associated with food spoilage. Those include *P. aurantiogriseum, P.chrysogenum, P.digitatum, P. griseofulvum, P. italicum, P. oxalicum and P. viridicatum*; some of these produce mycotoxins. However, Penicillium*spp* are associated more with cool temperate and temperate crops, mainly cereals, since most species do not grow very well above 25-30°C (Pitit, 2006).

2.2.3. *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 *Fusariumspp*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 *Fusariumchlamydosporum, F.culmorum, F.solani, F. equiseti, F.graminearum, F. oxysporum, F. proliferatum, F. poae, F. semitectum, F.subglutinans, F. sporotrichioides and F.verticillioides* (alternative name (synonym) *F. moniliforme*).

Obviously, *Fusariumspp* causes a range of diseases on an extraordinary range of host plants. As mentioned earlier the fungus can be soil borne, airborne or carried in plant residues and can be recovered from any part of the plant from the deepest root to the highest flower (Booth 1971; Summeral *et al*. 2003).

2.2.4. *Alternaria* spp:

Alternaria spp 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 *A. alternata; others include A. tenuissima, A. infectoria, A. citri A. brassicicola and A. brassicae*. The species *A.alternata and A. tenuissima* are pathogenic of wide range of crops; the other species have more limited host ranges.

2.2.5. *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 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 arepresent externally or internally or associated with the seed as contaminants . A number of laboratory seed health testing methods for detecting fungi sampling were in use. This include, examination of dry seeds, washing test, blotter method and its modification, agar plate method, embryo and seedling symptom test). However, blotter test is the simplest and most widely used method especially in developing countries (Mathur and Kongsdal, 2003).

In respect of the blotter test, seeds are typically surface sterilized with dilute hypochlorite solution and planted in 6 × 9 inches blotters. These are incubated and observed for 7 – 10 days. Fungal growth is recorded and confirmed with microscopic examination (www.worldseed.org). It is possible that two methods may be required to detect a pathogen (Mathur, 1975).

2.4 . Management of fungal contaminants associated withcrops seeds

Several effective ways for prevention and control of fungal contaminants associated with seed crops and their dangerous mycotoxins 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 promoteand support efforts aiming the benefits for the economics and health of people.

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 winter (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 Antifungalactivity ofthe in vitro efficacy of different plant extracts viz.,*Azadirachtaindica*, *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, *Allium sativum, Capsicum annuum, Artimesia vulgaris, Eupatorium adenophorum, Gaultheria fragrantissima* and *Phyllanthusemblica* were found to have activity against the fungus *F. solani* (Asha, *et. al*., 2009).

Igbinosa (2009) investigated the ability of the crude stem extracts of *Jatrophacurcas* 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 *Jatrophacurcas* and *Ricinuscumunis*seed extracts in the control of mycelia growth and rot development of yam caused by *F. verticilliodes*and*A. flavus*reported that these plants possess antimicrobial activity.

In Sudan, ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results (Mustafa,*et al*., 1982). Crude extracts solution obtained from the plant *Gordenialutea*, showed antibacterial activity against *Bacillus subtilis, Staphylocousaureus, Escherichia coli* and *Pseudomonas aeruginoza* (Ahmed,*et al*., 1984). Badreledin (2006) reported that ginger oil showed antimicrobial activity against *Staphylococcus aureus*, while, ELboshra (2005) reported that *Staphylococcusaureus* was sensitive to clove oil. 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.

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 February -April, 2016. The aim of this study was to detect and identify seed borne fungi associated with seeds samples of groundnuts collected from four different locationsin the stores of Agricultural Bank of Sudan,(stored since 2015 season) namely, Alfulah andAlmoglad in Western Kordofan whereas Alobied and Alnehudin Northern KordofanState of Sudan using filter paper method and agar plate method as recommended by ISTA(1996).

3.2. Materials, tools and equipments used in the study

- Gloves
- camera
- marker pen
- electric blender
- Petri-dishes
- sensitive balance
- incubator
- needle
- 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. Clorox (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).

3.2.1. Collection of samples

One random and homogeneous sample of five kilo grams was secured from each of the four locations in the stores ofAgricultural Bank of Sudan. Thepeanut sample was obtained fromOne random and homogeneous sample of five kilo grams was secured from each of the four locations in the storesof Agricultural Bank of Sudan. The peanut samples seeds on shells were obtained from grains market seed stocks in each location. The 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 transformed to the laboratory where they were stored at 5^0C refrigerator for further investigations.

3.3. Detection and isolation of seed borne fungi :

3.3.1. Dry Seed Inspection:

A sample of 400seeds 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.

3.3.2.Methods for the detection of seed borne fungal pathogens:

The seed samples were tested by the standard Filter paper and Agar methods for detection of seed borne fungi as described by ISTA. Normal and discolored seeds were tested separately for seed borne fungi.

3.3.3 .Filter Paper Method

For the detection of seed borne fungi, standard Filter paper 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 were then platted on moistened filter papers (dia. 9.0 cm) in 9.0 cm sterilized plastic Petri-dishes. Five seeds were plated from each sample, 3 arranged at the periphery of the plate and 2 at the centre. Each sample was replicated four times and then kept in dark place for seed germination.

After seven days of incubation, seeds were then examined for fungal growth under stereo microscope. Fungi identification by habit character was supplemented by microscopic examination of spores and fruiting bodies using a compound microscope. Other identification aids wereAgarwal*et al.*,(1989); Burgess *et al.*, (1994); Mathur SK, SB Mathur, P Neergaard (1975);and Mathur and Kongsdal (2003). Incidence levels were recorded as the percentage of infected seeds in a sample

3.3.4. Agar Method:

All seed samples 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, five each, were then plated in sterilized glass Petri-dishes on Agar medium.

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.

3.4. 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; Barnet and Hunter, 1999; and Rifai, 1969).The binocular compound microscope was also used to determine the type of fungus in each plate. Fungi identified and their percentage frequency of occurrence was calculated by applying the following formula:

PF = (No. of seeds on which fungus appear / Total number of seeds) X 100

CHAPTER FOUR

RESULTS

This study which was conducted under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology during the period, February to April 2016aimed to investigate the occurrence of seed borne mycoflora associated with groundnuts samples collected from four locations in the Stores of Agricultural Bank of Sudan inNorth and West Kordofan Estates using Filter Paper and Agar Methods as recommended by ISAT (1966).

4.1. Seed Borne mycoflora of groundnut seeds using Filter Paper Method

All the seed samples were found significantly infected by *Aspergillusflavus* and *Aspergillusniger* whereas the occurrence of *Rhizopus. nigricans and Alternariasolani* is sporadically.In fact, *A. flavus* and *A. niger*were isolated at higher frequencies from samples collected from all locations while *Rhizopusnigricans and Alternariasolani*was not consistently isolated and their occurrence is low.

Out of the four groundnuts samples collected from the four locations tested for incidence of seed borne fungi, a total of three genera of four species of fungi were recorded (Table 1 and Fig. 1). The mean percentage incidences of seed borne fungi of groundnut revealed by the filter papermethod are given in (Table 1 and Fig. 1).

The seed borne fungi identified were, *Aspergillusflavus, Aspergillusniger, Rhizopusnigricans andAlternariasolani.* Among these fungi the most predominantlyoccurred and recorded across locations were the storage fungi (saprophytes), *Aspergillusflavus*and*Aspergillusniger.*Their percentages

incidences were significantly higher (42.5% and 30% respectively followed by *Alternariasolani (6.5%) and Rhizopusnigricans (5.0%)*.

Among all locations, the mean percentage incidence of fungi detected on groundnut were occurred with varying level of incidences; *Aspergillusflavus* was significantly high at Alnehud (65%) compared to other locations. However, there are no significant differences in the incidences of *A. niger* across all locations, it was 25%, 30%, 35% and 30% in Alobied, Alfulah, Almuglad and Alnehud, respectively. The percentage incidences ofR. *nigricans* and *A. solani* were either 0.0% as in Almuglad and Alnehud or the highest was *A. solani* (15%) in Alobied

Table 1: Mean frequency percentage of seed borne fungi on various seed samples of groundnut collected from four different locations, each from one location using Filter paper method

Fungi	Locations				Overall mean
	Alobied	Alfulah	Almuglad	Alnehud	
Aspergillus flavus	30.00^{bc}	35.00^{b}	40.00^{b}	65.00^{a}	42.50^{A}
Aspergillus niger	25.00^{bcd}	30.00^{bc}	35.00^{b}	30.00^{bc}	30.00^{B}
Alternaria solani	15.00^{cde}	05.00^{e}	05.00^{e}	00.00^{e}	06.25^{C}
Rhizopus nigricans	05.00^{e}	10.00^{de}	00.00^{e}	05.00^{e}	05.00^{C}
Overall mean	15.00^{A}	16.00^{A}	16.00^{A}	20.00^{A}	
C.V%	13.52%				
P-value	0.0507*				
$Lsd_{0.05}$	17.42				

Mean(s) sharing same superscript(s) are not significantly different (P>0.05) according to DMRT.

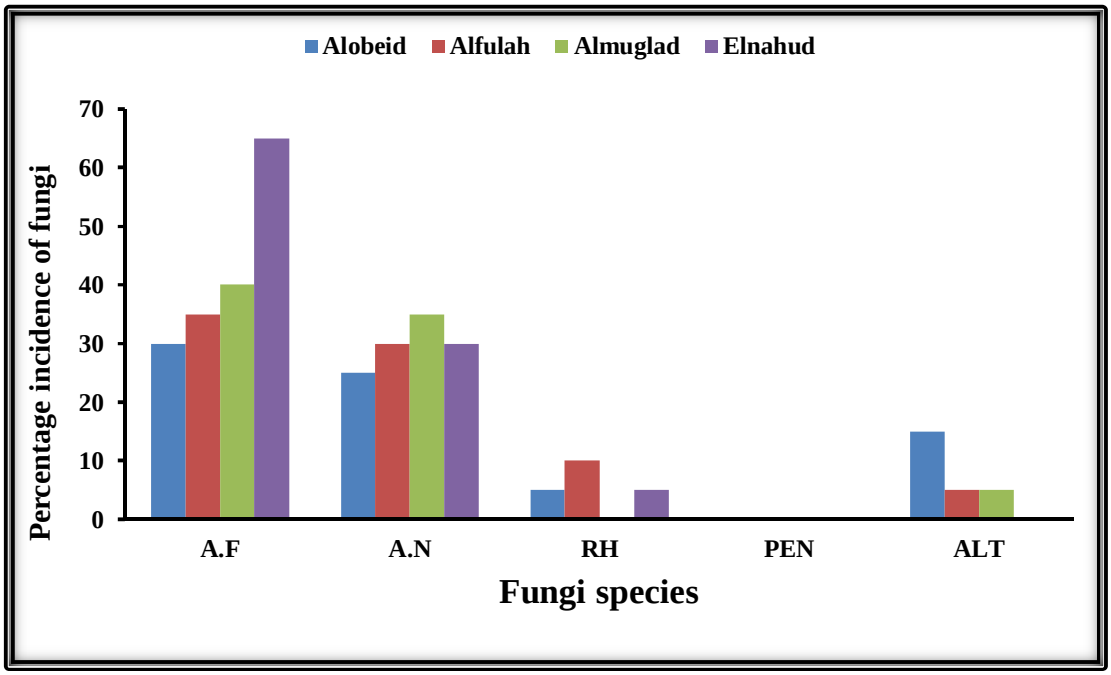

Fig 1: Mean frequency percentage of seed borne fungi on various seed samples of groundnut collected from four different locations, each from one location using Filter paper method

4.2. Seed borne mycofloraof groundnut Seeds using Agar Method

Table, 2 and Figure, 2 show the results of detection of mycoflora associated with groundnut seed samples from the four locations. The species *A. flavus was* found significantly occurredall across locations followed by *A. niger,*R. *nigricans, A. solani* and *Penicilliumspp*. In fact, A. *flavus*was the most prevalentmycotoxigenic fungi across the locations in Northern and WesternKordofanStates. The species of fungi isolated were(*A. flavus*)at the rate of *52.5*% , (*A. niger*)16.25%, (R. *nigricans)*11.25%,(A. *solani)* 6.25% and (*Penicilliumspp*.) 1.25%.

Among the locations, the proportion of nuts contamination by *Aspergillus spp*. varied from 65% at Muglad to 55% at Alfulla. The seeds contamination by other contaminants was found to be far less behind. It varies between 0.0% and 25%.

Table 2: Mean frequency percentages of seed borne fungi on various seed samples of groundnut collected from four different locations, each from one location using Agar method

Fungi	Location				Overall mean
	Alobeid	Alfulah	Almuglad	Elnehud	
Aspergillusflavus	45.00^{ab}	55.00^{a}	65.00^{a}	45.00^{ab}	**52.50^{A}**
Aspergillusniger	15.00^{c}	15.00^{c}	10.00^{c}	25.00^{bc}	**16.25^{B}**
Rhizopusnigricans	0.00^{c}	10.00^{c}	20.00^{bc}	15.00^{c}	**11.25^{BC}**
Alternariasolani	0.00^{c}	20.00^{bc}	5.00^{c}	0.00^{c}	**06.25^{BC}**
Penicilliumdigitatum	0.00^{c}	0.00^{ac}	0.00^{c}	5.00^{c}	**01.25^{C}**
Overall mean	**12.00^{A}**	**20.00^{A}**	**20.00^{A}**	**18.00^{A}**	
C.V%	**15.05%**				
P-value	**0.041***				
$Lsd_{0.05}$	**23.53**				

Mean(s) sharing same superscript(s) are not significantly different (P>0.05) according to DMRT.

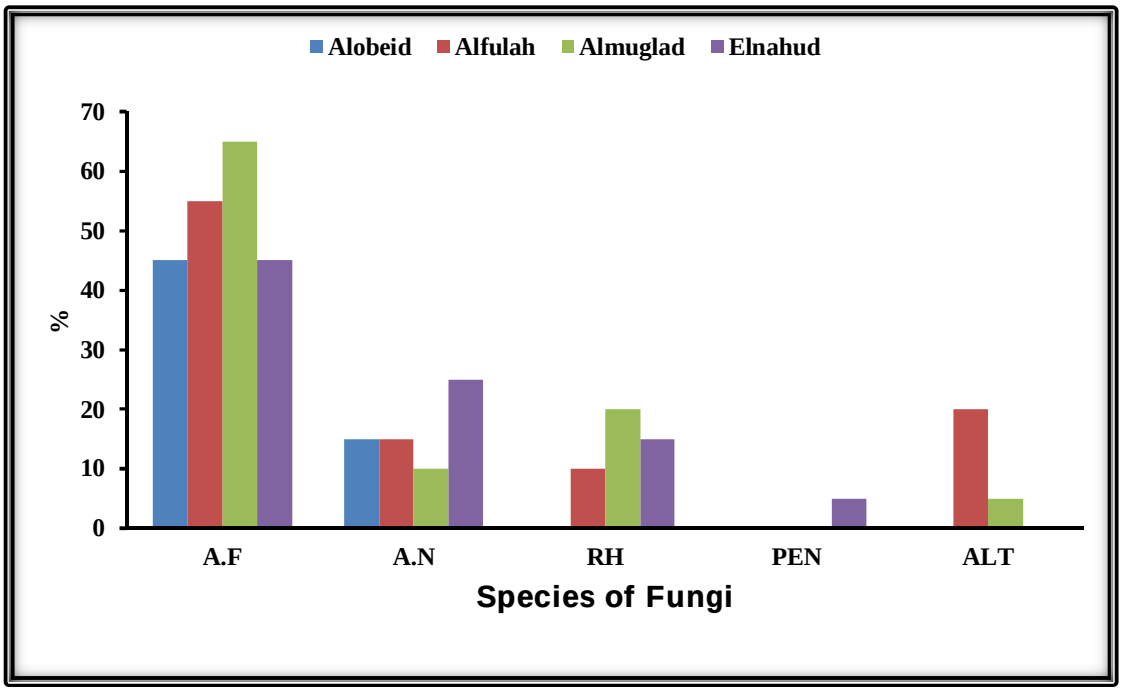

Fig 2. Mean frequency percentages of seed borne fungi on various seed samples of groundnut collected from four different locations, each from one location using Agar method

CHAPTER FIVE

DISCUSSION

Groundnut (*Arachis hypogaea* L.) is an important food and feed crop, which also serve as significant source of cash in developing countries that contribute significantly to food security and alleviate poverty (Pande *et al.*, 2003; Upadhyaya *et al.*, 2006). However, groundnut production and commercialization in developing countries and particularly in Africa is constrained by several factors, among which is mycoflora associated with seeds especially *Aspergillus spp*., (Caliskan *et al*., 2008; Pande *et al*., 2003; Upadhyaya *et al*., 2006). These facultative fungi, in addition to causing quantitative losses, produce highly toxic and carcinogenic chemical substances known as Aflatoxin (Yousif M.A.*et al*., 2010).

The importance of seed borne fungi to food and oil crops quality and quantity cannot be ignored. The risk encountered have been reported by several authors (Ali, 1989; El-Naghy *et al*., 1998; Haq Elamin NH *et al.*, 1988; Holbrook *et al*., 2000; Osman *et al*., 1999; Saber *et al.*, 1998; Thompson and Henke 2000 and Younis and Malik (2003).Azhar *et al., (*2011)reported that the seed mycoflora of most concern are produced by species within the genera of *Aspergillusspp*, *Fusarium spp*, and *Penicillium spp*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 study was carried out to investigate the occurrence of seed borne fungi and to identify them on four groundnut samples collected from different locations in North and west Kordofan State. The results revealed that irrespective of load of mycoflora, their association with groundnut crop seeds in different locations of North and west Kordofan appears to be a prevalent situation. Apart from *R.nigricans, A. solani* and*P. Digitatum*where their

occurrence is low and inconsistent on seeds samples tested with standard filter paper method as described by the International Seed Testing Association (ISTA 1976),*A. flavus, and A.niger, were consistently and significantly isolated from all location.*

These results are in line with Younis and Malik (2003) in Sudan and Eshetu (2010) in Ethiopia who reported in their study the most frequent occurrence of *Aspergillusspp*. (*A. flavus, A. niger* and other fungi) in wet shelled one year stored peanut sample.The results is also in agreement with those of Kamal and Mughal (1968) and Khan *et al*., (1974) ; Syed Danis, *et al*., (2013) who reported the presence of *Aspergillusspp*, *Penecilliumspp, Alternariaspp, Fusariumspp*, and *Rhizopusspp*in seeds of food crops. This common occurrence of seed borne fungi like *Aspergillus.spp*had been widely reported by HaqElamin NH *et al.,* 1988 and Martin *et al.,* (1984).The results also corroborate those ofBhutta and Hussain (1999; Khan and Bhutta (1994; and Singh (1983) who reported the occurrence of *Aspergillus, Penicillium* and *Fusarium spp*. were common associates of seeds crops.

The high load of seed borne fungi in seed samples from some locations compared to others demonstrated by this study could be attributed to favorable weather or storage 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 werepredominant fungi of groundnut. In Sudan Abdela (2009) also reported contamination of groundnut samples by *A. niger*and *A. flavus*, which were isolated at frequencies of 29-60% for *A. niger*and 4-52% for *A. flavus*. Furthermore, Tefera and Tana, (2002) attributed the predominant species of fungi associated with diseased plants to the involvement of these fungi in pre- and post- emergence death of groundnut plants.

The data also demonstrated that the results obtained by the two methods were found to be very close and the filter paper method was found to be more practical than Agar method. These findings confirm that of Jovicevic (1980) who reported that the filter paper method was most practical method for routine analysis of seed health. Such similar results were also observed by Khan *et al.*, (1988) on rice seed and Dawar & Ghaffar (1991) on sunflower seed.

Conclusion

- Groundnut seeds besides being of high quality and purity should also be free from mycotoxigenic fungi. In this study four genera were encountered in wide range of incidence percentage in all samples collected from four locations, each in one location in North Kordofan of Sudan.

- Of the fungi occurred in seed samples, the two most consistently prevailing seed borne fungi across locations were the storage ones; *Aspergillusflavus* and *Aspergillusniger* with varying level of incidences.

- The other fungi identified from samples and which were not consistent in their incidence are *R.nigricans, A. solani* and *P. digitatum.*

- The results obtained by the two methods used to detect contamination of samples are very close whereasthe filter paper method was found to be more practical than agar method

Recommendations

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

- Establishment of field research to demonstrate to farmers the ideal management practices that minimize the level of seed borne fungi in groundnut.
- The importance of conduction of reconnaissance survey to determine the consistency of the seed borne fungi isolated across the country to determine percentage incidences and severity and favorable conditions for contamination.
- Use the clean healthy seeds and selective seed for producing food.

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Appendices

APPENDIX (1)

Experiment Model: Two Factor Completely Randomized Design; where Factor A = Location and Factor B = Fungi

Filter paper

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

S. of Var.	df	SS	MS	F-cal	P-value
Factor A	3	295.000	98.333	0.6484	>0.05
Factor B	4	21880.000	5470.000	36.0659	0.0000
AB	12	3480.000	290.000	1.9121	0.057
Error	60	9100.000	151.667		
Total	79	34755.000			

Coefficient of Variation: 13.52%

1- Locations

Duncan's Multiple Range Test

LSD value = 7.790 SE = 2.754 at alpha = 0.050

Mean 1 = 15.00 A

Mean 2 = 16.00 A

Mean 3 = 16.00 A

Mean 4 = 20.00 A

2- Fungi

Duncan's Multiple Range Test

LSD value = 8.710

SE = 3.079 at alpha = 0.050

Mean 1 = 42.50 A

Mean 2 = 30.00 B

Mean 3 = 5.000 C

Mean 4 = 0.0000 C

Mean 5 = 6.250 C

Interaction

Duncan's Multiple Range Test

LSD value = 17.42 SE = 6.158 at alpha = 0.050

Mean 1 = 30.00 BC

Mean 2 = 25.00 BCD

Mean 3 = 5.000 E

Mean 4 = 0.0000 E

Mean 5 = 15.00 CDE

Mean 6 = 35.00 B

Mean 7 = 30.00 BC

Mean 8 = 10.00 DE

Mean 9 = 0.0000 E

Mean 10 = 5.000 E

Mean 11 = 40.00 B

Mean 12 = 35.00 B

Mean 13 = 0.0000 E

Mean 14 = 0.0000 E

Mean 15 = 5.000 E

Mean 16 = 65.00 A

Mean 17 = 30.00 BC

Mean 18 = 5.000 E

Mean 19 = 0.0000 E

Mean 20 = 0.0000 E

APPENDIX(2)

POTATO DIXTROSE AGAR

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

S. of Var.	df	SS	MS	F-cal	P-value
Factor A	3	860.000	286.667	1.0361	0.3831
Factor B	4	26500.000	6625.000	23.9458	0.0000
AB	12	2740.000	228.333	0.8253	0.0411
Error	60	16600.000	276.667		
Total	79	46700.000			

Coefficient of Variation: 15.05%

Duncan's Multiple Range Test

LSD value = 10.52 SE = 3.719 at alpha = 0.050

Mean 1 = 12.00 A

Mean 2 = 20.00 A

Mean 3 = 20.00 A

Mean 4 = 18.00 A

Duncan's Multiple Range Test

LSD value = 11.76 SE = 4.158 at alpha = 0.050

Mean 1 = 52.50 A

Mean 2 = 16.25 B

Mean 3 = 11.25 BC

Mean 4 = 1.250 C

Mean 5 = 6.250 BC

Duncan's Multiple Range Test

LSD value = 23.53

SE = 8.317 at alpha = 0.050

Mean 1 = 45.00 AB

Mean 2 = 15.00 C

Mean 3 = 0.0000 C

Mean 4 = 0.0000 C

Mean 5 = 0.0000 C

Mean 6 = 55.00 A

Mean 7 = 15.00 C

Mean 8 = 10.00 C

Mean 9 = 0.0000 C

Mean 10 = 20.00 BC

Mean 11 = 65.00 A

Mean 12 = 10.00 C

Mean 13 = 20.00 BC

Mean 14 = 0.0000 C

Mean 15 = 5.000 C

Mean 16 = 45.00 AB

Mean 17 = 25.00 BC

Mean 18 = 15.00 C

Mean 19 = 5.000 C

Mean 20 = 0.0000 C

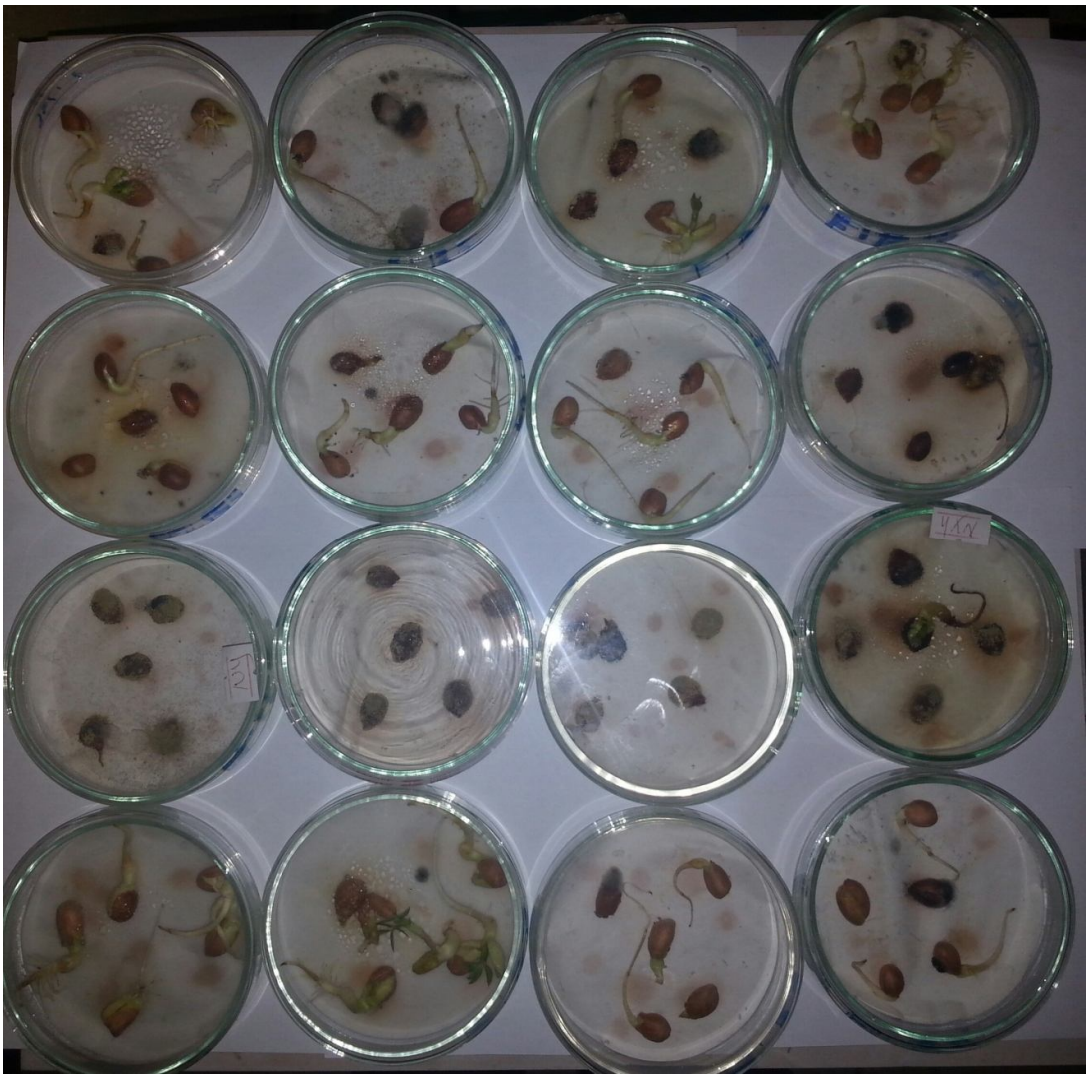
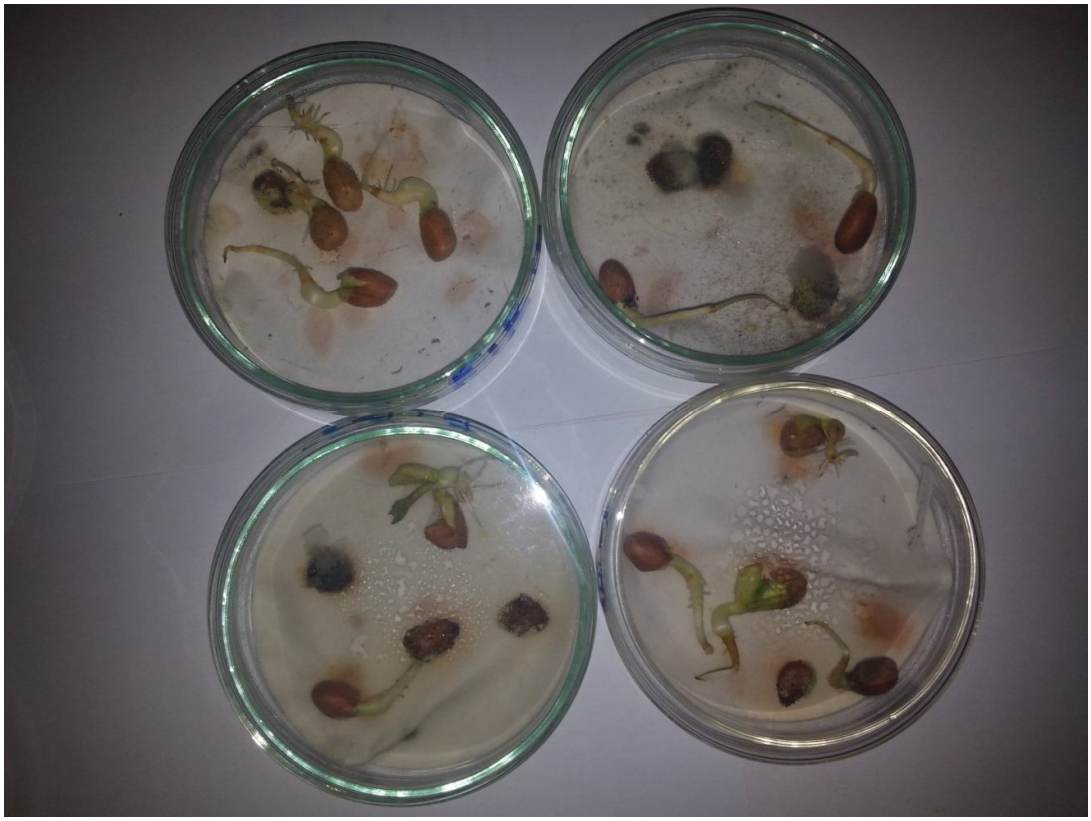


Plate 1: Detection of major seed borne fungi by Filter paper method

(A)



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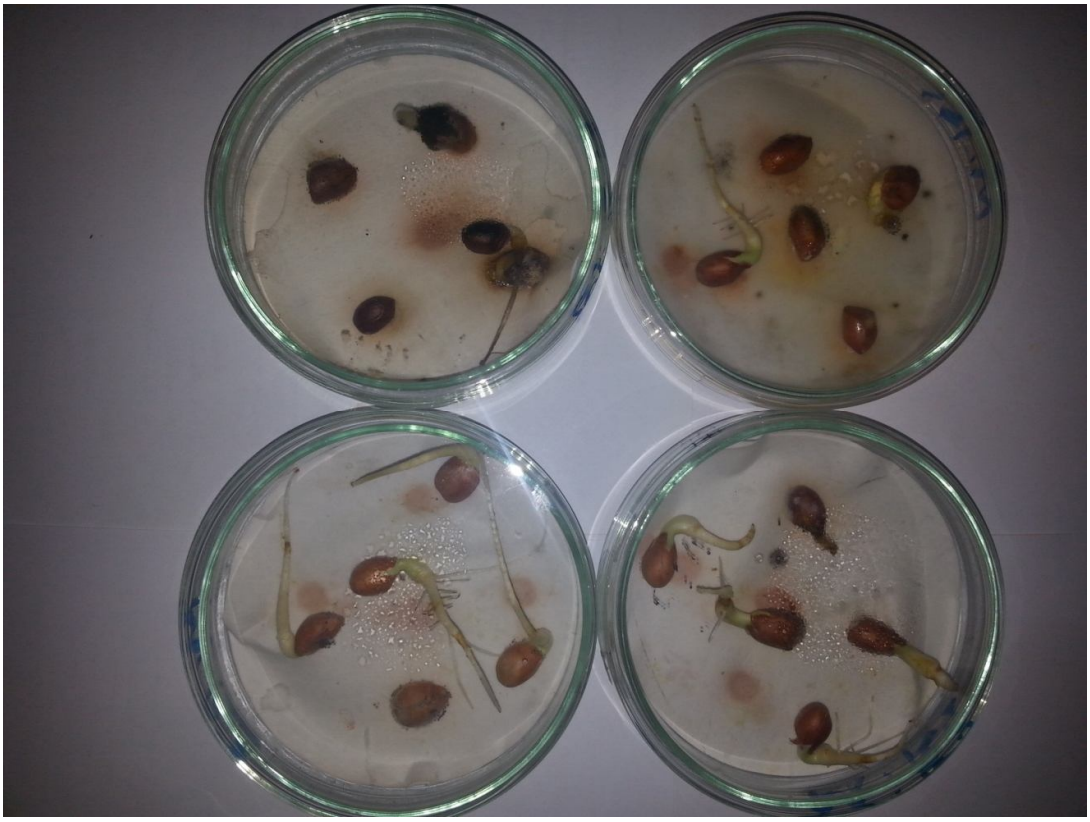
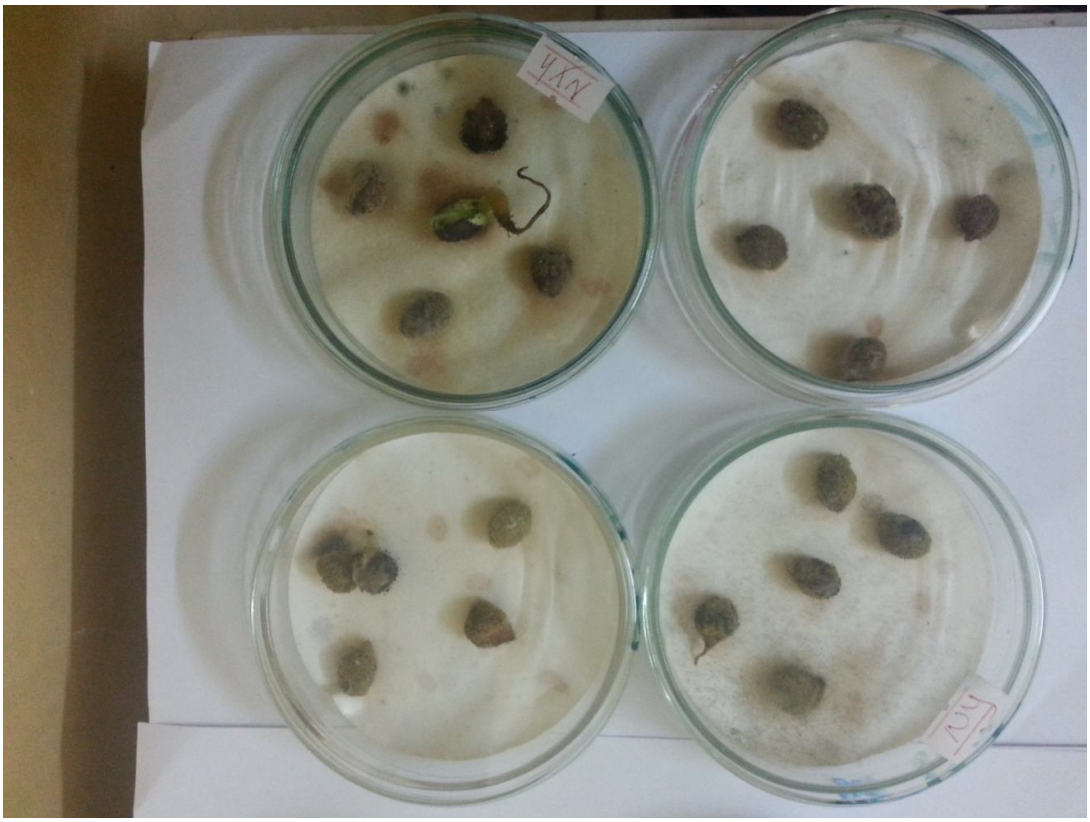
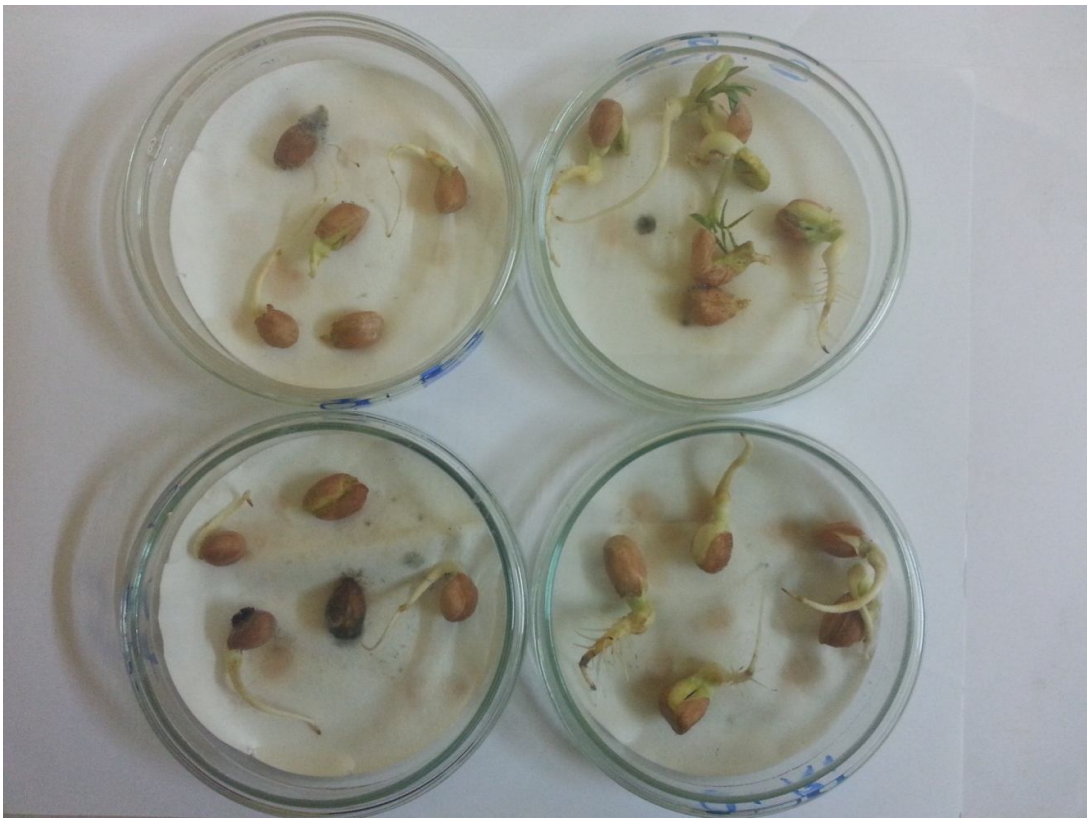


(C)



(D)



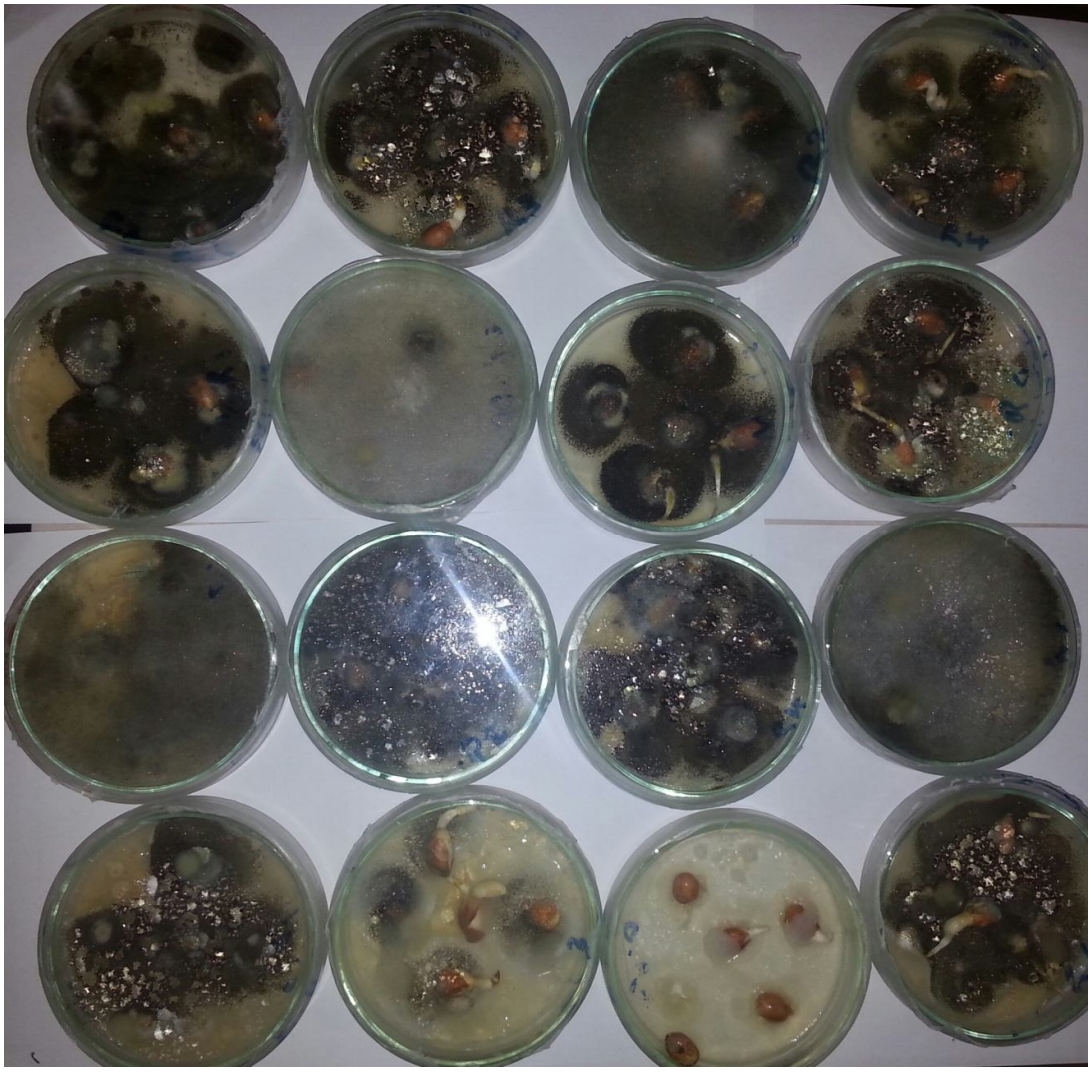
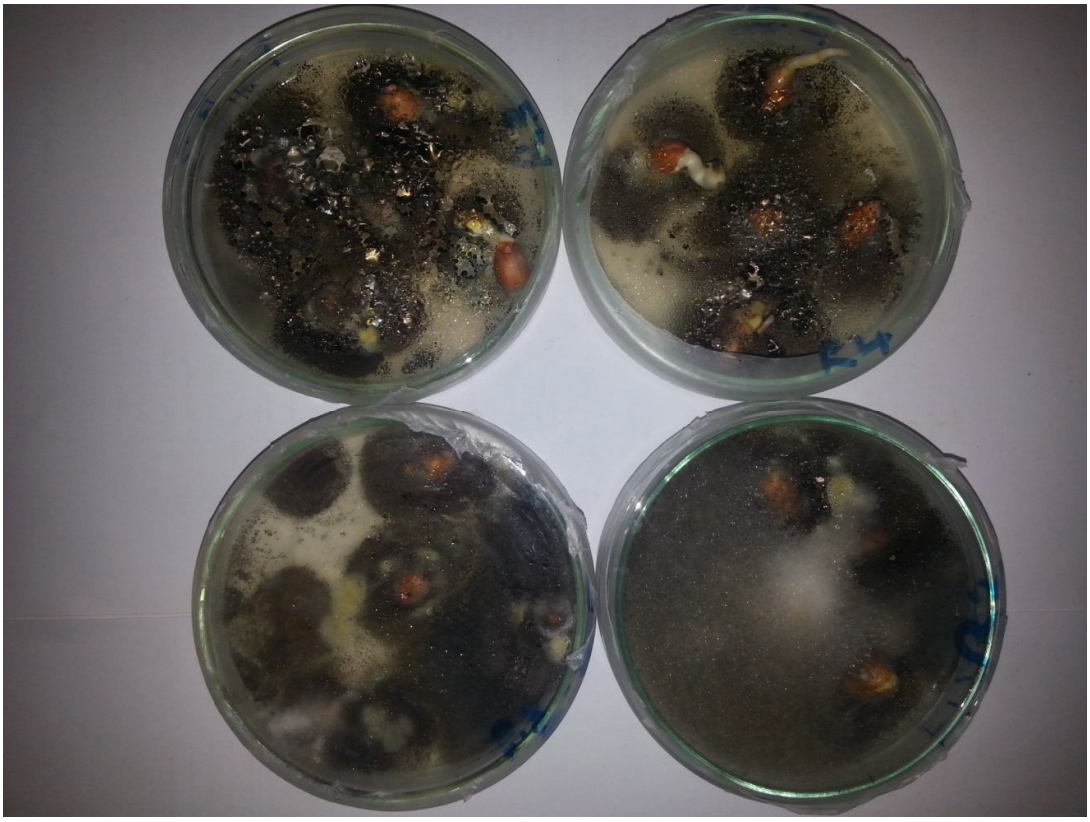


Plate 2: Detection of major seed borne fungi by Agar method

(A)



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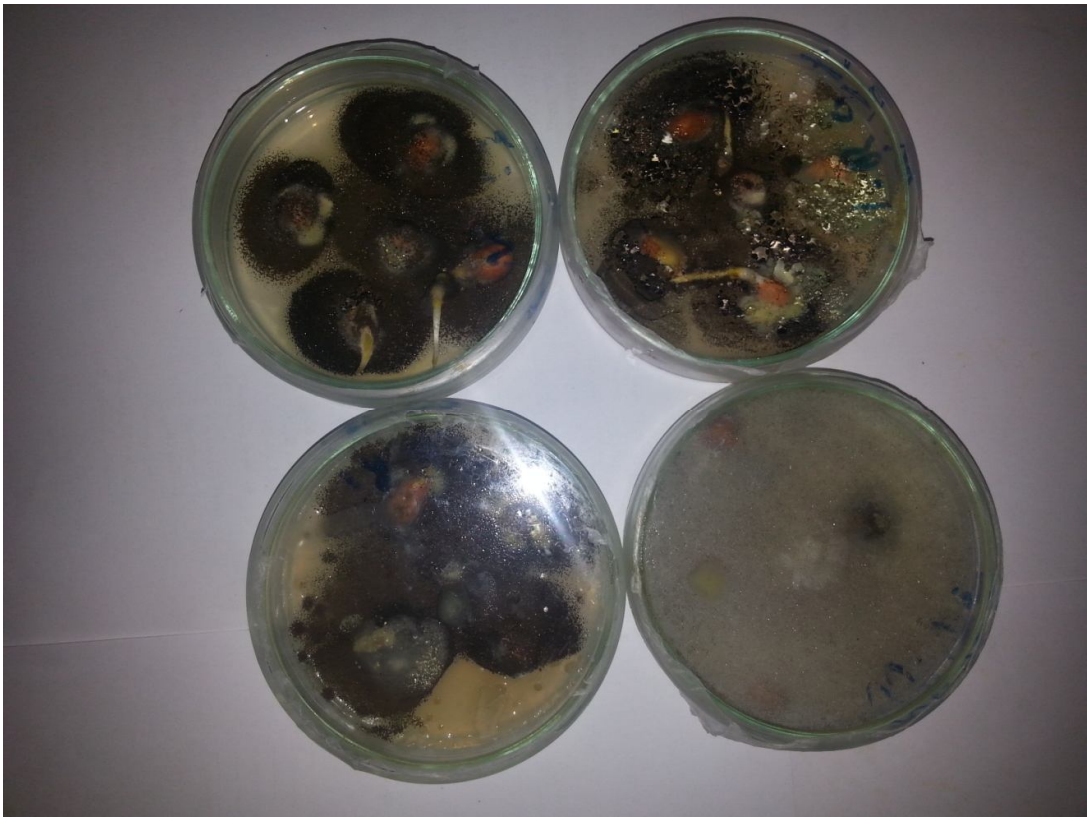
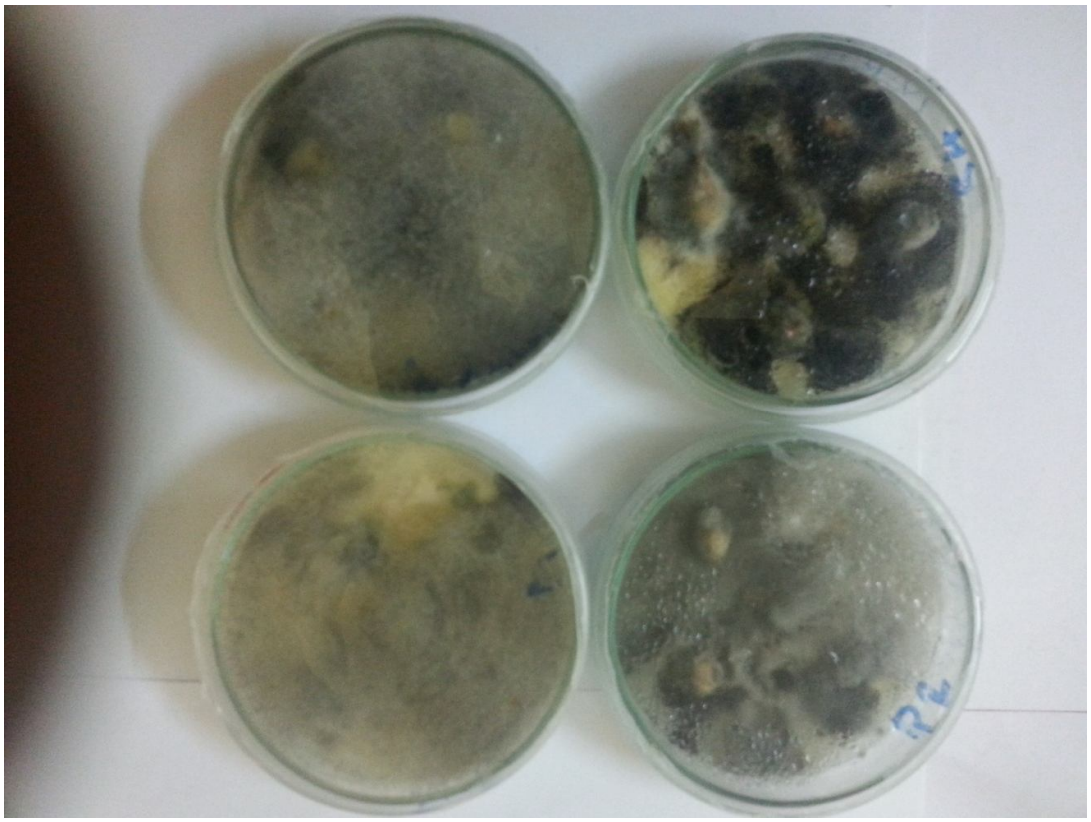
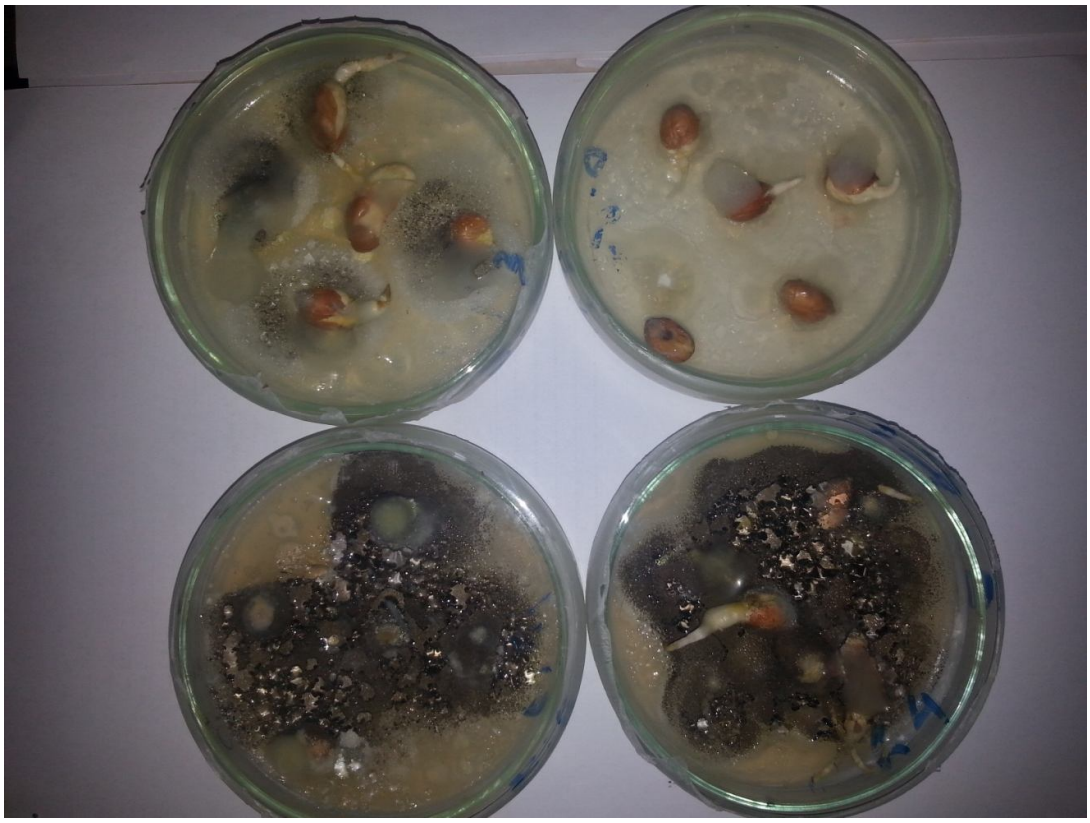


(C)



(D)



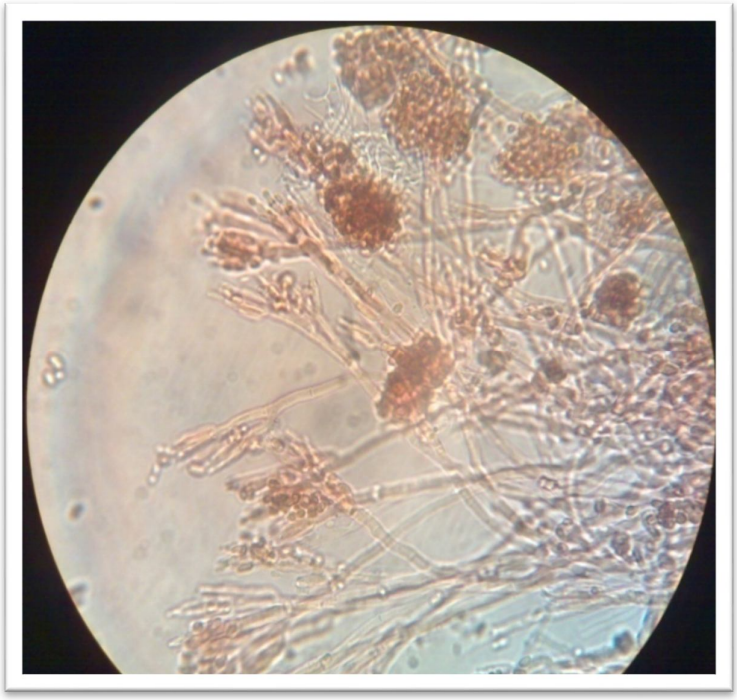


Plate 3. ***Penicilliumspp***

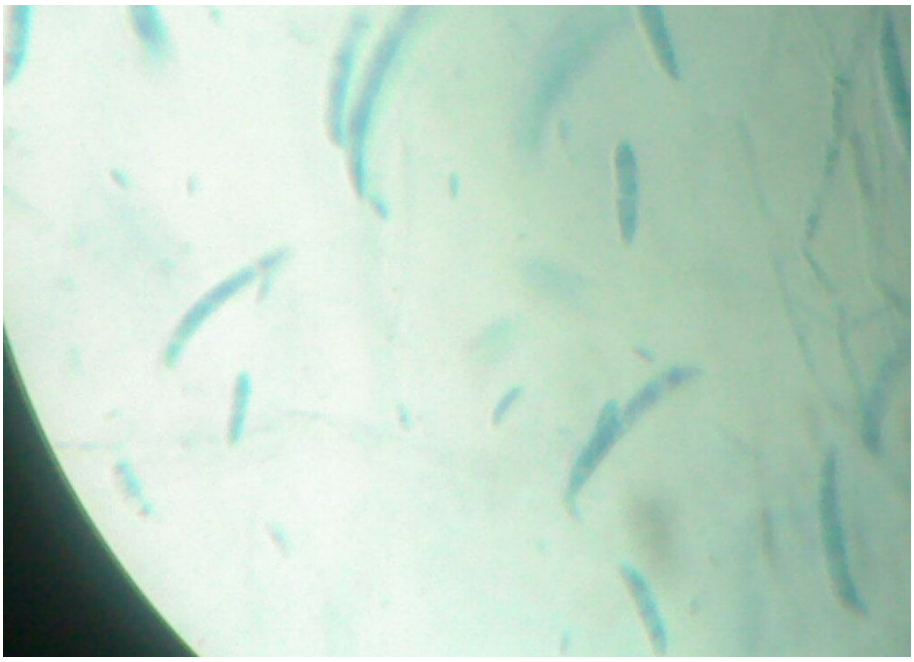


Plate 4. ***Fusarium spp.***

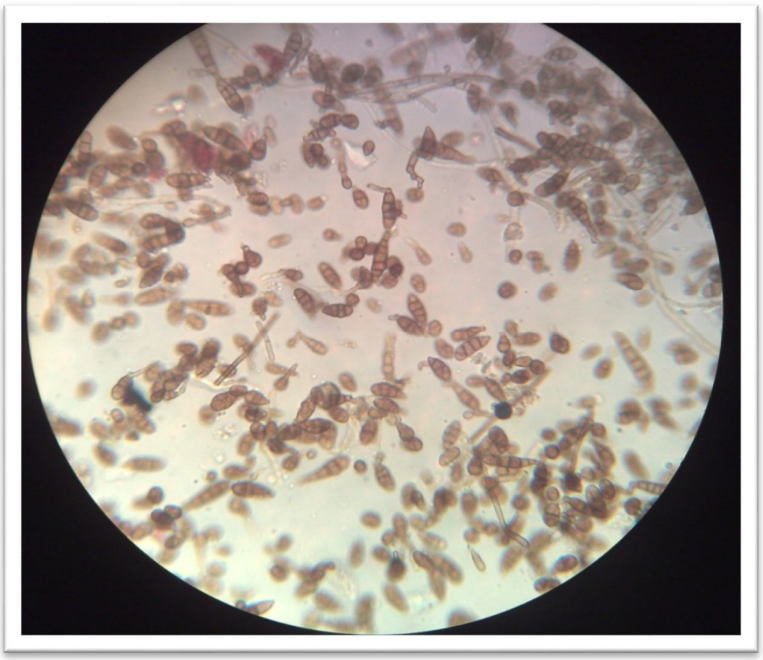


Plate 5. ***Alternariaspp***

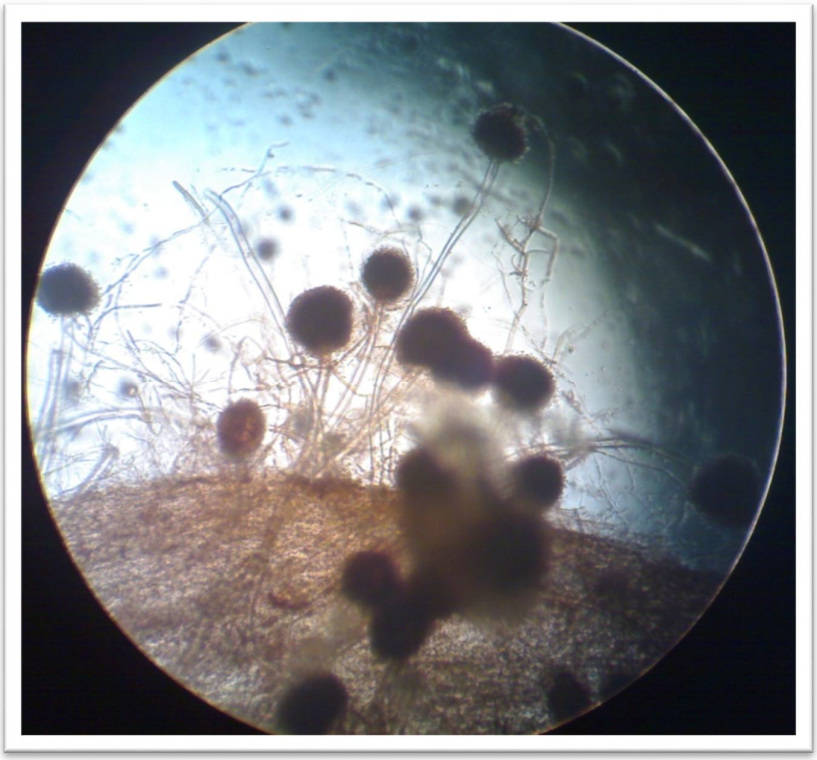


Plate 6. ***Aspergillus spp*****.**