Sudan University of Science and Technology

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Bioactivity of Ethanolic Extract of Some plants Compared to Bayleton® 50WP (Standed fungicide) against Aspergillius Flavus and Penicillium digitum

الفعالية الحيوية للمستخلص الإيثانولي لبعض النباتات والمبيد الفطري ضد فطري الأسبرجلس فلافس والبنسليم ديجتاتم تحت ظروف المعمل

A thesis submitted in partial fulfilment of the requirements for the M. Sc. degree in plant protection

BY

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بسم الله الرحمن الرحيم

قال تعالى في سورة البقرة:

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ) (32)

صدق الله العظيم

Dedication

To my mother

father

brothers and sisters

To my love

To all my teachers

Colleagues and friends

With love and respect.

SARA Mohammed

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is considered as one of the major oil crop widely grown in tropical and subtropical regions of the world, and is an important source of protein. Diseases are major constraints to groundnut production throughout the world. In fact, food contaminants especially spoilage fungi and its associated risks to humans, wild animals and livestock are considered as ones of the most important diseases of this crop worldwide. The present investigation was undertaken in the laboratory of Plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology, to evaluate the antifungal activity of Ethanolic extract of seeds and leaves of Mesquite (*Prosopis juliflora),* seeds of Datura (*Datura innnoxia*) and efficacy of fungicide (Byleton® 50 WP) against two fungi, *Aspergillus flavus* and *Penicillium digitatum*. Three concentrations of Ethanolic extract of leaves and seeds of Mesquite and seeds of Datura and Byleton each of 25, 50 and 100% were used in addition to the control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth inhibition zone percentage. The results showed that all Ethanolic extracts (leaves and seeds of Mesquite and seeds of Datura) as well as fungicide induced a significantly high inhibition zones percentage against the two test fungi compared to the control. Among all treatments the inhibition zones percentage against the two fungi ranges between 82.6 and 100% where no growth. Moreover, concentration of each extract as well as that of the fungicide reacted differently against the fungi. In this respect the effect of the fungicide was found to be pronounced than the extracts. The results also showed that the fungicide result in complete inhibition of the fungal growth (100%) at all tested concentrations. Among Ethanolic extracts, Mesquite leaves and seeds expressed at 100% concentration relatively high inhibition zone against the two fungi. Moreover, this relative increase in inhibition zone was also demonstrated by

leaves of Mesquite compared to it seeds extracts against the two fungi. Obviously, the growth inhibition increased with increasing concentration and the sensitivity of the fungus *P. digitatum* towards the plant extracts was more pronounced compared to *A. Flavus.* The current results were considered promising and encouraging to carry out a phytochemicals analysis of different parts of Mesquite plant using different solvents so as to determine the bioactive ingredient in each of these parts.

ملخص البحث

يعتبر الفول السودانى احد المحاصيل الزيتية الرئيسية التى تزرع على نحو واسع فى المناطق الإستوائية و شبه الإستوائية وايضأ مصدر مهم للبروتين. الامراض هى من المعوقات الرئسية لإنتاج الفول على نطاق العالم. من المعروف ان ملوثات الاطعمة وخاصة الفطريات وماتسببه من مخاطر على لانسان والحياة البرية والماشية تعتبر من اهم امراض هذا المحصول على نطاق العالم. أجريت هذه الدراسه تحت ظروف المختبر(معمل أمراض النبات) بقسم وقاية النبات، كلية الدراسات الزراعية، جامعه السودان للعلوم و التكنولوجيا لدراسه تاثير المستخلص الكحولي لاوراق وبذور نبات المسكيت ، وبذور نبات السيكران مقارنة مع المبيد الفطرى بايلتون 50% على نمو فطر الاسبيرجلس فلافس والبنسليم دجتاتم في الفول السوداني . استخدمت ثلاثه تراكيز من المستخلص الكحولي لاوراق وبذور نباتات المسكيت ،وبذور السيكران و المبيد الفطرى بايلتون 50%كل على حده (25 ،50، 100%) أضافة الى الشاهد. تم تقيم الاثر التثبيطى لهذه التراكيز بتسجيل نسبه تثبيط نمو الفطريات المستهدفه . أوضحت النتائج ان كل تراكيز المستخلص الكحولي لاوراق وبذور نبات المسكيت، بذور السيكران والمبيد الفطرى قد أظهرت تأثير معنوى هام ضد الفطرين المختبرين مقارنه بالشاهد. تراوحت نسبة التثبيط ما بين كل المعاملات ضد الفطرين ما بين 82.6 و100 حيث لا يوجد نمو. اضافة الى ذلك فان تراكيز ايا من المستخلصات الايثانولية النباتية والمبيد الفطرى قد تفاعلت كل على حده ضد الفطرين المختبرين لصفه عامه فان تاثير المبيد الفطرى ضد الفطرين المختبرين وجد بانه اكثر تثبيطا من المستخلصات النباتية والذى نتج عنه تثبيط كامل لنمو الفطرين (100%) لكل التراكيز وعلى طول زمن التجربة مابين المستخلصات الكحولية فان مستخلص الاوراق والبذور للمسكيت قد اظهر نسبة تثبيط عالية نسبيا ضد الفطرين عند التركيز الاعلى (100%) على طول زمن التجربة (جدول 1-4) (90.4،96.3،97.2،98.4%)، (89.3’96،93.4،93.3%)، (86.9،89.8،92.5،92.9%)، (85.7،86.9،88.7،89.9%) بالمقارنة مع السيكران الذى اعطى (94.9،94.6%)، (89.4،91.7%)، (88.5،91.7%)، (94.9،94.6،90.4%)، (89.4،91.7%)، (88.5،91.7%)، (86.8،90.4%) على التوالى . هذه الزياده النسبيه فى التثبيط ايضا اظهرها مستخلص الاوراق فى المسكيت مقارنة ببذوره ضد الفطرين من الواضح ان نسبة التثبيط ضد الفطر تزداد بزيادة تركيز المستخلصات كما ان حساسية فطر البنيسليم للمستخلصات اعلى مقارنة بفطر الاسبيرجلس. النتائج الحالية تعتبر مشجعه للقيام بتحاليل كيميائية لمختلف اجزاء نبات المسكيت باستعمال مستخلصات مختلفه لتحديد الماده الفعاله المكونه فى كل من هذه الاجزاء.

CHAPTER ONE

Introduction

Groundnut (*Arachis hypogaea* L.),belong to family (Fabaceae) is a major oil seed 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). 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%), Indonesia (4.1) and Sudan (30.6%) (Nwokoto, 1996).Worldwide, approximately 25.7 million tons of groundnuts are produced annually from about 21 million hectares of cropped land. Asia alone produces 17.9 million tons, 70% of global production. Africa produces another 20%. About 60% of Africa's production comes from Western Africa (FAO, 2006).

The crop 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). Groundnut seeds are nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. The kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil). It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (Nwokoto, 1996).

In Sudan Groundnut plays an important role in the diets of rural populations, particularly children, because of its high contents of protein (21-30%), fat (41-52%), and carbohydrate (11-27%). It is also rich in calcium, potassium,

phosphorus, magnesium and vitamin E. Protein meal, a by-product of oil extraction, is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock. This crop attacked by several diseases mainly fungal diseases, among these fungi, *Aspergillus spp*. which produced secondary metabolites called aflatoxin which a contaminant that result in acute and chronic poisoning in humans and animals through ingestion. The health impacts of ingestion in humans include stunted growth and development as well as an increased risk in liver cancer (IARC, 1993).

Obviously, the infection of groundnuts by various *Aspergillus spp*. not only results in reduction in crop yield and quality but also contamination of products 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, 1996)

The foregoing reflect the potential of risk of contamination of groundnuts and its by-products with *Aspergillus spp*. 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 plant pathogens and to protect the crop produce against them, chemical control methods are in practice. However, although the use of chemicals has helped increase of yields obtained (Ali, 1996), but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms in addition to contamination of the environment with very toxic substances (Okigbo, 2004; Carvalho, 2004). This has initiated the exploration of safe alternate methods of control.

Obviously, no single approach for control of *Aspergillius spp*. contaminants of groundnuts was proved to be effective and without drawback. Therefore, a

holistic approaching by combining different methods of control is the only solution to minimize the risk of contaminants.

This current study amide to investigate the effect of some plant derivative extracts as an alternative to toxic fungicides to be included as components of any integrated pest management approach allocated for controlling *Aspergillus spp.* in groundnut and *Penicillium* simultaneously increase the yield with following objectives:-

- To study the antifungal potential of some higher plants crude extract against *Aspergillus flavus and Penicillium digitatum*.
- To evaluate the efficacy of systemic fungicide on fungal growth

CHAPTER TWO

2. LITERATURE REVIEW

2.1. The groundnut

Classification:

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

Groundnut (*Arachis hypogaea L.*), a species in the family leguminasea, is an annual legume. It is known by many local names, including peanut, earthnut, monkey-nut and goobers. The groundnut originated in Latin America and was introduced to African continent from Brazil by the Portuguese in the 16th century (Abalu & Etuk, 1986; Adinya *et al*., 2010; Hamidu *et al*., 2007). The crop is mainly grown for oilseed, food, and animal feed (Pande *et al*., 2003; Upadhyaya *et al*., 2006). It is the world's 13th most important food crop, 4th most important source of edible oil and 3rd most important source of vegetable protein (Taru et *al*., 2010).

Groundnut seeds, known as kernels, contain 40-50% fats, 20-50% protein and 10-20 % carbohydrates (Sorrensen *et al*., 2004). They are a nutritional source of vitamin E and other minerals for human health including niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut is useful in the treatment of haemophilia, can cure

stomatitis and prevent diarrhoea, and is beneficial for pregnant women, nursing mothers and growing children (Akobundu, 1998). The kernels can be eaten raw, roasted or boiled and the groundnut vines are used as fodder for cattle (Pompeu, 1980; Hong *et al*., 1994). The crop can be used for producing industrial materials, such as oil-cakes and fertilizer. Extracted oil from the kernel is used as culinary oil and other crop extracts are used as animal feeds (Nigam & Lenné, 1996). Almost every part of the crop is used in some way. The multiple uses of the groundnut plant make it an important food and cash crop for domestic consumption and export in many developing and developed countries. Globally, 50% of total groundnut production is used for oil extraction, 37% for confectioner.y use and 12% for seed (Taru *et al*., 2010).

Groundnut is grown in nearly 100 countries. Globally, it is grown on almost 23.95 million hectares with total production of 36.45 million tons and an average yield of 1,520 kg/acre in 2009 (FAOSTAT, 2011). China, India, Indonesia, Nigeria, Senegal, Sudan, USA and Myanmar are the major groundnut growing countries (Taru *et al*., 2010; FAOSTAT, 2011).

2.2. Mesquite tree

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons

Subclass Rosidae

Order Fabales

Family Fabaceae/Leguminosae – Pea family

Genus *Prosopis* L. – mesquite

Mesquites (*Prosopis* spp.) are ever green leguminous trees or shrubs. The genus comprises 44 species which are natives to the Americans; Africa, Middle East and Pakistan (Broun and Massey, 1929 and Bukart, 1976). *Prosopis* species grow in arrays of environments and are not restricted by soil type, pH, salinity or fertility (Sidahmed, 2005).

Mesquite is an alien plant. It had been introduced to Sudan in 1917 by seeds imported from Egypt and South Africa to combat desertification and to lessen sand movement, under the name of *Prosopis juliflora*. In the first time the Mesquite plant was established at limited area at Khartoum and then extended to different parts of the country. Several species of Mesquite were introduced, in the period 1978-1986, with the objective of selecting suitable species for the different ecological zones. Mesquite seedlings failed to establish on sand dunes, but were well established within oases leading to lowering of water tables and suppression of native vegetation (Babiker, 2006).

The plant has spread into various ecological niches, where it is not desired and has become a noxious weed in Sudan (Abdel Magied, *et. al.*, 2010). It has invaded both natural and managed habitats, including watercourses, floodplains, and highways, degraded abandoned land and irrigated areas (Babiker, 2006 and Elkhalifa, 2010). The plant colonizes open niches and is suppressive and allelopathic (Abdel Magied, *et. al.*, 2010).

2.2.1 Benefit of Mesquites:

The tree has some benefits that include combating desertification, nitrogen fixation as a leguminous plant, increasing the global green coverage, using its timber for furniture, fencing and fuel, also as animal feed. However, recently it was realized that the problems caused by the plants far overweigh the benefits derived from them (Sidahmed, 2005 and Elkhalifa, 2010).

2.3. Datura

2.3.1 Scientific classification

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Solanales
Family:	Solanaceae
Genus:	*Datura*
Species:	*D. innoxia*

Binomial name: *Datura innoxia* L.

Datura innoxia (thorn-apple, downy thorn-apple, Indian-apple, lovache, moonflower, nacazcul, toloatzin, tolguache or toloache) is a species in the family Solanaceae. It is rarely called sacred datura, but this name in fact refers to the related *Datura wrightii*. It is native to Central and South America, and introduced in Africa, Asia, Australia and Europe. The scientific name is often cited as *D. innoxia* (Preissel *et al*., 2002). When English botanist Philip Miller

first described the species in 1768, he misspelled the Latin word *innoxia* (inoffensive) when naming it *D. inoxia*. The name *Datura meteloides* was for some time erroneously applied to some members of the species, but that name has now been abandoned.

2.3.2 Description

D. inoxia with ripe, split-open fruit, *Datura inoxia* is an annual shrubby plant that typically reaches a height of 0.6 to 1.5 metres (Annapoorani, 2013). Its stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. It has elliptic entire-edged leaves with pinnate venation. All parts of the plant emit a foul odor similar to rancid peanut butter when crushed or bruised, although most people find the fragrance of the flowers to be quite pleasant when they bloom at night. The flowers are white, trumpet-shaped, 12–19 cm (4.75-7.5 in) long. (Richard, 1970) (Retrieved, 2007) they first grow upright, and later incline downward. It flowers from early summer until late fall.

The fruit is an egg-shaped spiny capsule, about 5 cm in diameter. It splits open when ripe, dispersing the seeds. Another means of dispersal is by the fruit spines getting caught in the fur of animals, which then carry the fruit far from the mother plant. The seeds have hibernation capabilities, and can last for years in the soil. The seeds, as well as the entirety of this plant, act as deliriants, but have a high probability of overdose (Richard, 1970).

2.3.3 Toxicity

All parts of *Datura* plants contain dangerous levels of poison and may be fatal if ingested by humans and other animals, including livestock and pets. In some places it is prohibited to (Richard, 1970).

2.3.4 Cultivation

When cultivated, the plant is usually grown from seed, but its perennial rhizomes can be kept from freezing and planted in the spring of the following year. *Datura inoxia*, like other *Datura* species, contains the highly toxic alkaloids atropine, hyoscine (scopolamine), and hyoscyamine. The Aztecs called the plant *toloatzin* (Richard, 1970).

Datura inoxia is quite similar to *Datura metel*, to the point of being confused with it in early scientific literature. *D. metel* is a closely related Old World plant for which similar effects were described by Avicenna in eleventh century Persia. The closely related *Datura stramonium* differs in having smaller flowers and tooth-edged leaves, and *Datura wrightii* in having wider, 5-toothed (instead of 10-toothed) flowers. *Datura inoxia* differs from *D. stramonium*, *D. metel* & *D. fastuosa* in having about 7 to 10 secondary veins on either side of the midrib of the leaf which anastomose by arches at about 1 to 3 mm. from the margin. No anastomosis of the secondary veins is seen in the other 4 major species of Datura.

2.3.5 TRADITIONAL USES:

Datura innoxia, or toloache, is the most ethnopharmacologically important of all thorn apple species in the Americas. Excavations dating to 1200 C.E. have shown that the prehistoric Pueblo Indians of the Southwest used the seeds in rituals (Litzinger, 1981). The plant has also clearly been used in Mexico since the prehistoric period. It has been suggested that Aztec sacrificial victims were given Datura preparations in order to prepare them for death. At present, toloache is still used in Mexico for medicinal, ritual and aphrodisiac purposes (Ratsch, 1998).

In the Yucatan, *D. innoxia* is regularly cultivated as an ornamental and an entheogen. Shamans smoke cigars rolled from *D. innoxia* leaves or eat the seeds in order to do divinations with quartz crystals. Tarot cards are also sometimes used. The datura is said to allow the shaman to gain insight he would not have been able to discover otherwise. The flowers are used as offerings for the gods in ritual, as well (Ratsch, 1998).

In modern Mexican witchcraft, or *brujeria*, toloache has a connection to dark practices and a reputation for causing insanity and death. It is said to give the user dark power. The Huichol regard *D. innoxia* as a 'bad plant of the gods' and associate it with sorcery (Ratsch, 1998).

D. innoxia is sacred to the Navajo, who use it in healing ceremonies. During one ceremony known as the Beauty way, *D. innoxia* preparations are consumed to produce visions. The plant is also used as a medicine to treat hallucinations. The Navajo take small amounts of *D. innoxia* to protect themselves from the attacks of dark sorcerers, and utilize the plant in divination and love magic. The Navajo *Ajilee* ceremony is one in which the practitioner is transformed into the Datura spirit and is able to gain power over women he desires and game he wishes to hunt. The ritual is also used to heal individuals who are suffering from sexual excess, and women who have been forced into prostitution (Brugge, 1982). The Apache use powdered *D. innoxia* root in secret ceremonies as a plant medicine. Hopi medicine men chew the roots to induce visions that allow them to diagnose diseases (Ratsch, 1998).

D. innoxia was introduced to Pakistan from the Americas and now grows wild there. A few crushed seeds or a dried leaf mixed with tobacco (Nicotiana tabacum) is used as an aphrodisiac and inebriant (Goodman & Gharfoor, 1992 c Ratsch, 1998). In India, *D. innoxia* is used in the same way as *D. metel*.

2.3.6 TRADITIONAL PREPARATION:

The dried leaves and flowers of *D. innoxia* may be smoked alone or with other herbs in a smoking blend. Yucatec Maya shamans combine the leaves with tobacco to make cigars that they call chamal. One leaf of each plant is used to make one chamal. The shaman smokes until he reaches the state of consciousness he desires. The amount needed varies considerably from person to person. The seeds and leaves of *D. innoxia* may be crushed and fermented to make an alcoholic beverage. The roots are sometimes added to pulque, beer, or chicha (Ratsch, 1998).

The Yaqui tribe add crushed seeds and leaves of *D. innoxia* to lard and rub this ointment on to the abdomen in order to induce visions. Fresh roots may be crushed and applied externally, chewed, or dried and powdered. However, dosage information regarding the roots is not available (Ratsch, 1998).

Four leaves is an appropriate dose for smoking if one wants to receive the aphrodisiac effects of the plant. Working with the plant in this way prevents overdose, as well. Tea made from the leaves should be consumed carefully – just one small leaf can cause very intense hallucinations. Alkaloid concentration will vary widely from plant to plant, and individuals can react very differently to tropane_alkaloids, so detailed dose information is difficult to provide. 30-40 seeds are considered a strong visionary dosage, but as few as 10 seeds can result in significant perceptual changes. In Pakistan, 150 grams of leaves, fruits, or flowers is considered to be a lethal dose, but even significantly less than this can cause death in some individuals (Goodman & Ghafoor, 1992 Ratsch, 1998).

2.3.7 MEDICINAL USES:

In Mexico, toloache is used as a remedy for many disorders and symptoms, particularly fevers. The Apache use the juice of the flowers and roots to disinfect wounds. Dew drops that have collected in the flowers are used as an eye wash (Ratsch, 1998).

The Aztecs used thorn apple leaves to treat broken bones and swollen joints. Leaves that had been warmed in a steam bath were placed directly on to the affected areas. Toloache is one of the most important aphrodisiacs and sedatives in Mexican folk medicine. It is given during childbirth to help with pain. In Israel, a decoction of the leaves is consumed to treat diarrhea, and a paste of the leaves is applied externally to treat pain (Dafni & Yaniz, 1994). In many parts of the world, the leaves of *D. innoxia* have been smoked, alone or in blends, as a most effective treatment for asthma (Ratsch, 1998).

2.3.8 TRADITIONAL EFFECTS:

The entire *D. innoxia* plant is rich in tropane_alkaloids, particularly scopolamine and hyoscyamine. Some plants produce significantly more scopolamine than others. The effects of *D. innoxia* are dependent on dosage and method of preparation. The American Indians say that a mild dosage produces medicinal, healing effects, a moderate dosage produces aphrodisiac effects, and high doses produce shamanic visions (Ratsch, 1998).

Shamanic doses of *D. innoxia* cause profound visions and hallucination and delirium. Overdose may begin with excitation, an urge to dance and fits of laughter, and end in acute hallucinosis and death through respiratory paralysis. In Mexico, peyote is used as an antidote for toloache overdose (Ratsch, 1998).

2.4 Seeds-borne fungi

The term seed-borne describes the state of any micro-organisms being carried with, on, or in the seed (Agarwal and Sinclair, 1997). Seed may either be infected or contaminated.

2.4.1 History of fungi in seeds

Knowledge concerning seed-borne fungi, which cause diseases in plants, is almost two and half centuries old. It was in France when Tillet (1755) showed that he could increase the number of smut-infected wheat plants by adding smut dust to wheat seeds before planting and reduce them if the seeds were treated with copper sulphate before planting (Agrios, 1997). However, it was only during the first half of the last century when seed-borne diseases received greater attention, especially in Europe and North America (Mathur *et al.*, 2003).

2.4.2 Economic significance of seed-borne diseases in crop plants

Seed damage due to microorganisms is responsible for some of the most severe yield losses in the world today. Seed-borne diseases cause crop losses which may be of economic importance to many nations all over the world (Neergaard, 1977). *Altenaria solani* can cause extensive defoliation leading to a reduction of economic fruit yield of tomato (Spletzer and Enyedi, 1999). The brown blotch disease of cowpea (Vigna unguiculata Walp), causes damping-off arising from infected seeds and brown spots on infected cotyledons, stems, pods and seeds, all resulting in considerable yield losses (Emechebe, 1981). In Ghana, *Phytophthora palmivora* accounts for an average annual loss of 19% of the crop (Blencowe and Wharton 1961). Darkwa (1987) reported that the less virulent *Phytophthora palmivora* causes yield losses in cocoa of 4.0%-19%, but with the more virulent *Phytophthora megarkarya*, losses of 60% - 80% are possible.

2.5. *Aspergillus spp.*

Kingdom: Fungi

phylum: Ascomycota

Class: Deuteromycetes

Order: Eurotiales

Family: Trichocomaceae

Genus: *Aspergillus*
Micheli (1729)

Aspergillus flavus Link is the major producer of aflatoxins worldwide in corn, peanuts, tree nuts, cottonseed, spices and other crops. These polyketide-derived mycotoxins are among the most carcinogenic compounds known from nature and are also acutely hepatotoxic as well as immunosuppressive (Eaton and Groopman 1994, Turner *et al*., 2003). Aflatoxigenic strains of *A. flavus* generally produce aflatoxin B_1, the most toxic of the naturally occurring aflatoxins (Cullen and Newberne 1994), and lesser amounts of B_2 (Horn *et al*., 1996). Aflatoxins are highly regulated in human and animal food in more than 100 countries (van Edmond and Jonker, 2005), and commodities with aflatoxin concentrations that exceed established limits either must be reprocessed or destroyed. In regions of the world where aflatoxins are not regulated, outbreaks of aflatoxicosis and associated deaths in human populations occur periodically (Krishnamachari *et al* 1975, Lye *et al*., 1995, Azziz-Baumgartner *et al*., 2005). In addition to aflatoxins *A. flavus* produces another unrelated mycotoxin, cyclopiazonic acid (CPA), an indol-tetramic acid that targets the liver, kidneys and gastrointestinal tract in animals (Burdock and Flamm 2000). Aflatoxins and CPA often cocontaminate agricultural products, and several of the symptoms associated with turkey "X" disease in poults that led to the discovery of aflatoxins in the early 1960s can be attributed to CPA (Cole 1986). *Aspergillus flavus* is also an important opportunistic human pathogen in aspergillosis. The

species is the most common cause of aspergillosis involving skin, oral mucosa and subcutaneous tissue and is second only to *A. fumigatus* Fresen. In invasive aspergillosis that includes the systemic infection of immunocompromised patients (Hedayati *et al.*, 2007).

Aspergillus flavus belongs to section *Flavi*, which contains an assemblage of phylogenetically related aflatoxin-and nonaflatoxin-producing species (Peterson 2008). One of the hallmarks of *A. flavus* populations is the extreme genetic diversity, as reflected by differences in morphology and mycotoxin production (Bayman and Cotty 1993, Horn et al 1996)

2.6. Penicillium spp.

Scientific classification

Kingdom: Fungi

Order: Eurotiales

Class: Deuteromycetes

Family: Ascomycota

Genus: *Penicillium*
Link (1809)

Penicillium as well is a large genus containing 150 recognized species, of which 50 or more occur commonly. Many species of Penicillium are isolated from foods causing spoilage; in addition, some may produce bioactive compounds. Important mycotoxins produced by Penicillium include ochratoxin A, patulin, citrinin and penitrem A. Some of the most important toxigenic species in foods are *Penicillium expansum, Penicillium citrinum, Penicillium crustosum and Penicillium verrucosum* (Pitt, 2006).

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

2.7 Management of seed borne fungal diseases/pathogens

The control of seed-borne fungal pathogens can be considered broadly in terms of exclusion and elimination of inoculum (Maude, 1996). Exclusion strategies include; use of legislation, the isolation of seed production areas, the setting of minimum inoculum tolerance levels for seeds and breeding for resistance, while direct eliminatory measu res include, control of organisms by seed treatments and crop treatments. Disease control may be achieved by single or by combined strategies contained within the concepts of exclusion and elimination (Maude, 1996).

2.7.1 Seed treatment

Seed treatment is a generic term (Scott, 1989) which does not specify the application method but indicates that seeds are subjected to a compound of chemical, nutrient, hormone, etc treatments; a process (such as wetting or drying) or to various energy forms (e.g. radiation, heat, magnetism, electricity). Research has shown that seed borne pathogens can be controlled substantially using various physical, mechanical, chemical, botanical, and other methods (Messiaen, 1992).

2.7.2 Physical control measures

Cowpea seeds naturally infected with *Macrophomina phaseolina* and *Fusarium equiseti* given hot water treatment at eight different temperatures showed reduced infection frequency (Sinha and Khare, 1977). Duration of each treatment was 5, 10, 15 and 20 minutes. The most effective treatment to check both pathogens was dipping of seeds in water at 46 degree Celsius for 20 minutes.). Baker (1972) reported that dry heat has been used to eliminate or reduce artificial or natural bacterial contamination of seeds often with little impairment of seed germination. According to Megahed and Moore (1969) exposure of infected Prunus seeds to radiation at doses of (20, 000 rad) reduced the transmission of Prumus Necroti Ringspot Virus (PNRSV) and Prunus Dwarf Virus (PDV).

2.7.3 Mechanical control measures

Sheppard (1983) during his investigation, revealed that the use of furrow rather than overhead irrigation system in low rainfall areas further restricts the spread of splash- dispersal pathogens including *Xanthomonas campestris pv. Phaseoli, Phaseoli suyringae pv. Phaseolicola* and *Colletotrichum lindemuthianum* on phaseolus bean, *Septoria apiicola* on celery *Xanthomonas campestris pv. Campestris* and *Phoma lingam* on cabbage and *Ascohyta spp.* on peas.

2.7.4 Biological control measures

Biological control had attained importance in modern agriculture, due to attempts to reduce hazards of intensive use of chemicals for pests and disease control (Tuber and Baker, 1988). Trichoderma spp have shown to inhibit *Macrophomena phaseolina* growth on PDA. (Mahaber *et al.*, 1995). Okigbo and Ikediugwu (2000) showed that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of yam tubers. Single application of *Trichoderma viride* effectively controlled the normal tuber surface mycoflora

throughout six months storage, greatly reducing rotting. Okigbo (2002) also used *Bacillus subtilis* to control pathogens that affect white yam (*Dioscorea rotundata*) and it was reported that *Bacillus subtilis* displaced the naturally occurring mycoflora on the surface of yam tubers as was observed in yams with *Trichoderma viride*.

2.7.5 Chemical control measures

Attempts have been made to reduce seed-borne infection by chemical treatment of the seeds and some successes have been reported. Seed dressings are used to eliminate most surface infestation of seeds but have relatively little effect on internally borne organisms (Jackson, 1963). Several seed dressing chemicals, including soaking of seeds in dilute nitric acid have been used to eradicate seed-borne inoculum of rice. However, some of these chemicals in organic solvents have also been used successfully (Singh and Monga, 1985). According to Ramadoss and Srivaprakasam (1987), three chemical fungicides, carbendazim, quionotozene and TMPTD (Thiram) and four insecticides; carbosulfar, Chloropyriphos, phosalone and monocrotophos and combinations of these were evaluated for control of *Macrophomena phaseolina* in-vitro. Of the fungicides, carbendazim produced the largest inhibition zone. None of the insecticides alone inhibited *Macrophomina phaseolina* but acted synergistically in combination with fungicides. The efficacy of seven fungicides to control *Macrophomina phaseolina* causing blight on cowpea (*vigua unguiculata*) seeds was determined. Agrosan Gn (Phengl mercury acetate), vita vase (carbozin), Bavistin (Carbendazim), Thiram and Topsin-(thiophamate-methyl) inhibited growth of the fungus completely (Gautam and Udit, 1996). Thiophamate methyl (as Topsinum) and benonyl (as Benlate) were shown to be effective in controlling *Macrophomina phaseolina* (Ali *et al.*, 1995). Complete control of *Fusarium oxysporum* was found in 60 day old tomato plants where captan was used at 2 g a.i/kg naturally infected and artificially inoculated seeds with

Fusarium oxysporum. Similar results have been reported by Dwivedi and Pathak (1981) who found that the use of captan completely checked the population of *Fusarium oxysporum* on tomato. In another study where *Aspergillus niger* and *Aspergillus flavus* caused spoilage of groundnuts in storage and reduced quantity and quality of oil during pathogenesis. Propionic acid and sodium metabisulphide sprays and dips were effective against the organisms (Vaidya and Ivr, 1989).

2.7.6 Control with Botanical Products

The search for plants with anti-microbial activity started in 1940s (Mathews, 1995). Through this research work, a number of discoveries have been made about the use of plant products to either kill or inhibit the spread of plant pathogens. Plant extracts have been used to control diseases in cowpea (Amadioha and Obi, 1998) and banana (Okigbo and Emoghene, 2004). Amadioha and Obi (1998) demonstrated the fungitoxic activity of seed extract of *Azadirachta indica* (neem) and *Xylopia aethiopica* against the anthracnose fungus (*Colletotrichum lindemuthianum*) of cowpea. Both hot and cold water extracts inhibited spore germination and significantly reduced the growth of *Colletotrichum lindemuthianum* on PDA medium. Onifade (2002) also reported the control of *Colletotrichum lindemuthianum* using neem seed; fruit ,leaf, bark and root extracts, recording a 100% inhibition of spore germination and mycelia growth. Extracts of *Alliums sativum* (L) have been used to inhibit spore germination in *Sclerotium spp*. Pesticides of plant origin are biodegradable, cheap, readily available and environmentally safe than synthetic chemicals. The antifungal activity of essential oil from *Caesulai axillararis* (L) against *Helminthosporium oryzae* (Petch) was demonstrated by Pandey *et al.*, (1981).

The ginger root extract has microbial action at levels equivalent to 200mg/ml of the spice. Ginger inhibits *Aspergillu spp*, a fungus known for the production of

Aflatoxin, a carcinogen (Nanir and Kadu, 1987). In-vitro studies have shown that active constituents of ginger rhizome inhibit multiplication of colon bacteria. It inhibits the growth of *Eschericha coli* and *Salonella* (Gugnani *et al.*, 1985). Fresh ginger juice showed inhibitory action against *Aspergillus niger, S. cerevisiae, Mycoderma spp* and L. acidophilus at 4, 10, 12 and 14% respectively at ambient temperatures (Meena, 1992).

Eugena aromatica and *Allium sativum* have been found to possess anti-microbial properties. These properties have been ascribed to the presence of essential oils, acrolein and crotonaldehyde in garlic and eugenol (Ali, 1994; Godwin and Mercer, 1973). An in-vitro study of Eugenia aromatica extracts showed that they posses antifungal properties against spoilage fungi such as *Apsergillus niger, Apsergillus fumigatus, Mucor spp and Cephalosporium spp* (Adekalu *et al*., 2007). Garlic (*Allium sativum*) has been found to posses' anti-bacterial activity on ground camel meat (Al-Delamy *et al*., 1971). Also reported fungicidal properties of garlic in poultry feed substrate. Experiments conducted by Khan and Fakir (1995) revealed that seed treatment with garlic extracts at different concentration significantly, reduced seed-borne infection by *Macrophomina phaseolina* in jute. Amadioha (1998) reported that leaf extracts of Carica papaya was shown to effectively inhibit the growth of powdery mildew fungi (*Erysiphe cichoracerarum)* in-vitro, with the greatest inhibition level recorded with 100% cold water extract.

2.8 Seed Health Testing

Sampling, examination of dry seeds, washing test, blotter method and its modification, agar plate method, embryo and seedling symptom test are the common laboratory seed health testing methods for detecting fungi (Mathur and Kongsdal, 2003). Blotter test however, 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 hypochrorite 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, 1995). The importance of seed health testing cannot be under estimated. Laboratory screening of seed for sowing is a cheap and effective means of the spread of many seed borne diseases if correctly carried out. Sowing healthy seed of high quality is our concern to improve crop yield thus increasing food production (www.bpi.da.gov.ph).

CHAPTER THREE

3. MATERISLS AND METHODS

3.1. Experimental site

This study was conducted at the laboratory of the plant Pathology Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) "Shambat" during the period January-March, 2015. The aim was to evaluate the antifungal activity of seeds and leaves Ethanolic extract of *Prosopis juliflora and seeds of Datura innoxia* in comparison to fungicide (Byleton 50 WP) against the two fungi, *Aspergillus flavus and Penicillium digitatum*

3.2. Collection of plant samples

Random samples of infected groundnut (pods) showing typical sign of the disease were obtained from sick groundnut field of the college farm in January 2015 and brought to laboratory to be used as source of inoculum for fungi.

3.2.1. Isolation of *Aspergillus flavus* and *Penicillium digitatum*

The infected of groundnut pods cut into small sections (0.5-1.0cm), washed thoroughly with tap water, and surface sterilized by immersing 1:4 Clorox (NaOcl) for 5 minutes, rinsed three times in changes of sterilized distilled water to remove the adhering Clorox and dried on sterilized filter papers ready for culturing. A culture medium Potato Dextrose Agar, (PDA) was used. The medium was supplemented with Chloramphenical (0.05g/l) as Bacteriostatic agent (Anon., 1981). The medium was poured in 9cm Petri dishes. Five sections of the dried parts were aseptically placed in a Petri dish and incubated at 28°C. The growing fungi were further sub-cultured to obtain pure cultures of the inoculum from each of the two fungi. Slides were prepared from these pure cultures, and examined under compound (x: 100) based on the method of (Booth key, 1977) to confirm that the fungi are *Aspergillus flavus* and

Penicillium digitatum. Fungus identification by growth habit character and spores using microscopic examination to confirm the two fungi was supplemented by other identification aids such as Burgess *et al.*, (1994)

3.2.2. Collection of botanical plants

Mesquite seeds and leaves in addition to Datura seeds were collected from Shambat area and brought to the laboratory where they were shade dried. After complete drying, plant samples of each sample {mesquite (leaves and seeds) and Datura seeds} were crushed separately to obtain fine powder for extraction.

3.3. Preparation

3.3.1. Preparation of Ethanolic extracts

The plant powder prepared from each plant material as mentioned above was used for preparing the different botanical treatments for the study. Preparation of the methanolic extracts started 3 days before the experiment time.

Exactly 60g of each plant material were extracted in ethanol for 6 hours using a soxhlet. The extracts were concentrated using rotary flash evaporator and preserved at 58°C in airtight brown bottles until further use.

3.3.2. Preparation of the fungicide concentrations

The concentrations of the fungicide tested were prepared by dissolving ,3 g in 1000ml of sterilized distilled water to obtain 25, 50 and 100ppm using serial dilution.

3.4. Testing of the antifungal activity of the extracts and the fungicide

The plant extracts and the fungicide were then subjected to antifungal activity against the two fungi where Potato Dextrose Agar (PDA) medium was prepared in conical flasks (1000ml). Ethanolic extract bioassays were carried out in the

prepared PDA. To avoid bacterial contamination, antibacterial Chloromyctin capsules (125 PPM) were used.

Three concentrations of each plant extract and fungicide (100%, 50% and 25%) were prepared. Prepared PDA media was amended with the required concentration from [Mesquite (seeds and leaves) and Datura seeds] and fungicide before being solidified in the conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 25 ml of PDA, were assigned for each concentration and left to solidify. In case of control Ethanol was added instead of plant extract and three plates with PDA medium were assigned to it.

One mycelial disc (Cork borer) of *Aspergillus flavus* and *Penicillium digitatum* colony (7days old) was plugged and inoculated aseptically in the centre of each Petri dish where opposite poles were marked at the back of the plate.

The inoculated Petri dishes were arranged in a complete block design in incubator and incubated at 25 C^0 for 5 days. The growth of the fungus was measured and calculated successively after 3, 4 and 5 days after inoculation. The effect of each extract concentration on linear fungal growth was calculated as percentage of reduction in diameter of fungal growth using the Formula according to Awuah (1989):-

$$MP = \frac{M1 - M2}{M1} \times 100$$

Where MP = Percentage inhibition of myceliam growth, M1= myceliam growth in control petri-dish without extract/fungicide, M2= myceliam growth extract/fungicide petri-dish.

3.5. Statistical analyses.

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

CHAPTER FOUR

RESULTS

The results of this study, which was conducted at the laboratory of the plant Pathology Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) "Shambat" during the period January-March, 2015, aimed to evaluate the antifungal activity of Ethanolic extract of seeds and leaves of *Prosopis juliflora* and seeds of *Datura innoxia*. and efficacy of fungicide (Byleton® 50 WP) against the two fungi, *Aspergillus flavus and Penicillium digitatum in vitro,* is presented in table 1-4.

4.1: Effect of different concentrations of Ethanolic extracts (leaves and seeds of mesquite, seeds of Datura,) and Byleton on linear growth *of Aspergillus flavus,* and *Penicillium digitatum* three days after inoculation

The results after three days from inoculation all Ethanolic extracts (leaves and seeds of mesquite and seeds of Datura) as well as fungicide induced a significantly high inhibition zones percentage against the two test fungi compared to control.

The inhibition zone percentage against the two fungi range from 81.0% at 25% concentration of mesquite leaves extract to 100% at all concentrations of fungicide. Moreover, the Fungicide at all treatments, Mesquite and Datura extracts at 100% concentration expressed the highest inhibitory effect against the growth of the two fungi that range between 100% and 90.4%. However, the fungicide at all concentrations completely inhibited the growth of the two fungi (100% inhibition). Among fungi, the fungus *P.*

digitatum showed slightly higher sensitivity to all extracts compared to *A. Flavus*.

Table, 1: Effect of different concentrations of Ethanolic extracts (leaves and seeds of mesquite, seeds of Datura,) and fungicide on linear growth *of Aspergillus flavus* and *Penicillium digitatum,* three days after inoculation

Treatments Product	Concs.	Fungus[1]	Inhibition zone (%) R1	R2	R3	Means
		Asp.	80.0 (8.9)	84.4 (9.2)	83.6(9.1)	82.6(9.0)ef
	25%	Pen.	83.6(9.1)	89.3(9.4)	83.0(9.1)	85.3(9.2)f
Mesquite		Asp.	81.6(9.0)	87.9(9.4)	83.6(9.1)	84.4(9.1)ef
Seeds	50%	Pen.	93.4(9.6)	93.9(9.7)	90.7(9.5)	92.7(9.6)d
		Asp.	90.0(9.5)	89.6(9.4)	91.8(9.6)	90.4(9.5)cd
	100%	Pen.	98.3(9.9)	96.9(9.8)	93.8(9.7)	96.3(9.8)bc
		Asp.	83.3(9.1)	81.0(9.0)	78.6(8.8)	81.0(8.9)f
	25%	Pen.	83.6(9.1)	87.8(9.4)	89.2(9.4)	86.9(9.3)ef
Mesquite		Asp.	91.6(9.6)	89.6(9.4)	91.8(9.6)	91.0(9.5)cd
leaves	50%	Pen.	91.8(9.6)	93.9(9.7)	90.7(9.5)	92.1(9.6)d
		Asp.	100.0(10)	98.2(9.9)	93.4(9.6)	97.2(9.8)ab
	100%	Pen.	96.7(9.8)	98.4(9.9)	100(10)	98.4(9.9)ab
		Asp.	86.6(9.3)	77.5(8.8)	80.3(8.9)	81.5(9.0)f
	25%	Pen.	90.1(9.5)	90.9(9.5)	87.6(9.3)	89.5(9.4)e
Datura		Asp.	88.3(9.4)	84.4(9.2)	86.8(9.3)	86.5(9.3)de
Seeds	50%	Pen.	93.4(9.6)	93.9(9.7)	93.8(9.7)	93.7(9.6)cd
		Asp.	95.0(9.7)	96.5(9.8)	93.4(9.6)	94.9(9.7)bc
	100%	Pen.	95.0(9.7)	95.4(9.7)	93.8(9.7)	94.7(9.7)cd
		Asp.	100(10)	100(10)	100(10)	100(10)a
	25%	Pen.	100(10)	100(10)	100(10)	100(10)a
		Asp.	10010)	100(10)	100(10)	100(10)a
Fungicide	50%	Pen.	100(10)	100(10)	100(10)	100(10)a
		Asp.	100(10)	100(10)	100(10)	100(10)a
	100%	Pen.	100(10)	100(10)	100(10)	100(10)a
Control		Asp.	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7) g
		Pen.	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)g
SE±		Asp.				0.07958
		Pen.				0.05164
C.V. (%)		Asp.				1.56%
		Pen.				1.02%
LS.D		Asp.				0.2313
		Pen				0.1501

Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis. Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5

1. **Asp**. = ***Aspergillus flavus*** ***Pen. = Penicillium digitatum***

4.2: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* four days after inoculation

In day four after inoculation, all plant extracts concentrations as well as that of the fungicide were invariably continued exhibiting suppressing effects against the growth of the two fungi. The inhibitory effect from all concentrations tested was significantly different from control (Table, 2). Moreover, the fungicide at all concentrations (25, 50, and 100%) continued to inhibit completely the growth of the two fungi (100%).

Among plant extracts, the mesquite (seeds and leaves extract) at 100 concentration gave the highest inhibition zones percent (89.3, 93.4, 93.3 and 96.0) against the two fungi, *A. flavus*, and *P. digitatum* respectively. Moreover, the leaves extract of Mesquite demonstrated more effect (93.3 and 96.0) than that of seeds (89.3, 93.4). However, the sensitivity of the fungus *P. digitatum* towards the plant extracts was more pronounced compared to A. *Flavus.*

Table, 2: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* four days after inoculation

Treatments Product	Conc.	Fungus	Inhibition zone (%) R1	R2	R3	Means
Mesquite Seeds	25%	Asp.	78.5(8.8)	83.1(9.1)	79.5(8.9)	80.4(8.9)^{d}
		Pen.	78.7(8.9)	84.4(9.2)	78.0(8.8)	80.4(8.9)^{g}
	50%	Asp.	82.1(9.0)	89.2(9.4)	85.2(9.2)	85.5(9.3)^{c}
		Pen.	88.7(9.4)	90.9(9.5)	84.9(9.2)	88.1(9.3)^{de}
	100%	Asp.	86.9(9.3)	90.3(9.5)	90.9(9.5)	89.3(9.4)^{b}
		Pen.	93.7(9.7)	94.8(9.7)	91.7(9.6)	93.4(9.6)^{bc}
Mesquite leaves	25%	Asp.	82.1(9.0)	80.7(9)	81.8(9)	81.5(9.0)^{cd}
		Pen.	81.2(9.0)	87(9.3)	83.5(9.1)	83.9(9.1)^{fg}
	50%	Asp.	90.4(9.5)	90.3(9.5)	93.1(9.5)	91.3(9.5)^{b}
		Pen.	88.7(9.4)	89.6(9.4)	89.0(9.4)	89.1(9.4)^{de}
	100%	Asp.	94.0(9.6)	92.7(9.7)	93.1(9.6)	93.3(9.6)^{b}
		Pen.	97.5(9.8)	96.1(9.8)	94.5(9.7)	96.0(9.7)^{b}
Datura Seeds	25%	Asp.	84.5(9.2)	74.6(8.6)	81.8(9)	80.3(8.9)^{d}
		Pen.	87.5(9.3)	84.4(9.2)	84.9(9.2)	85.6(9.2)^{ef}
	50%	Asp.	88.0(9.4)	83.1(9.1)	84(9.1)	85.1(9.2)^{c}
		Pen.	90.0(9.5)	87(9.3)	86.3(9.3)	87.7(9.3)^{de}
	100%	Asp.	88.0(9.4)	90.3(9.5)	89.7(9.5)	89.4(9.4)^{b}
		Pen.	92.5(9.6)	89.6(9.4)	93.1(9.6)	91.7(9.5)^{cd}
Fungicide	25%	Asp.	100(10)	100(10)	100(10)	100(10.0)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10.0)^{a}
	50%	Asp.	100(10)	100(10)	100(10)	100(10.0)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10.0)^{a}
	100%	Asp.	100(10)	100(10)	100(10)	100(10.0)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10.0)^{a}
Control		Asp.	0.0 (0.7)	0 .0(0.7)	0 (0.7)	0 .0(0.7)^{e}
		Pen.	0.0 (0.7)	0.0 (0.7)	0 (0.7)	0.0 (0.7)^{h}
SE±		Asp.				0.07303
		Pen.				0.05774
C.V. (%)		Asp.				1.45%
		Pen.				1.11%
LS.D		Asp.				0.2123
		Pen				0.1678

Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis. Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5

Asp. = ***Aspergillus flavus*** ***Pen. =* penicillum *digtatum* .**

4.3: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* five days after inoculation

After five days from inoculation the results in Table, 3 showed that extracts of all plants tested as well as the fungicide proved to be significantly effective in suppressing the fungal growth at all concentrations compared to control.

In fact, this significant effect was demonstrated by all the concentrations of Mesquite, Datura and fungicide tested against the two fungi compared to control (Table, 3). Meanwhile, at day five, the inhibitory effect of Mesquite leaves Ethanolic extract at all concentrations tested (25, 50, and 100%) was more pronounced than other plant extracts against the two fungi. It gave relatively more inhibitory effect (81.0, 81.7), (88.1, 88.4), (92.5, 92.9) than mesquite seeds (78.5, 82.9), (86.9 76.9) and (78.7, 89.8) and Datura Ethanolic extract as well (76.7, 84.7), (82.6, 85.8) and (88.5, 91.7) respectively.

Moreover, the fungicide at all concentrations (25, 50, and 100%) continued to demonstrate its superiority in completely inhibiting the growth of the two fungi (100%) at day five.

Table, 3: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* five days after inoculation

Treatments			Inhibition zone (%)			
Product	**Conc.**	**Fungus**	**R1**	**R2**	**R3**	**Means**
Mesquite Seeds	**25%**	**Asp.**	75.4(8.7)	82.2(9)	77.9(8.8)	78.5(8.8)^{ef}
		Pen.	72.7(8.5)	81.8(9)	76.3(8.7)	76.9(8.7)^{g}
	50%	**Asp.**	79.2(8.9)	86.9(9.3)	82.5(9.1)	82.9(9.1)^{be}
		Pen.	77.2(8.8)	79.5(8.9)	79.5(8.9)	78.7(8.8)^{fg}
	100%	**Asp.**	84.9(9.2)	87.8(9.3)	88(9.4)	86.9(9.3)^{cd}
		Pen.	86.3(9.3)	87.5(9.3)	95.6(9.8)	89.8(9.6)^{bc}
Mesquite leaves	**25%**	**Asp.**	81.1(9)	80.3(8.9)	81.6(9)	81(8.9)^{ef}
		Pen.	73.8(8.6)	85.2(9.2)	86(9.3)	81.7(9)^{ef}
	50%	**Asp.**	86.7(9.3)	88.7(9.4)	88.9(9.4)	88.1(9.3)^{bc}
		Pen.	88.6(9.4)	87.5(9.3)	89.2(9.4)	88.4(9.3)^{bcd}
	100%	**Asp.**	91.5(9.5)	93.4(9.6)	92.6(9.6)	92.5(9.5)^{b}
		Pen.	93.1(9.6)	92(9.6)	93.5(9.6)	92.9(9.6)^{b}
Datura Seeds	**25%**	**Asp.**	80.1(8.9)	71(8.4)	78.8(8.9)	76.7(8.7)^{f}
		Pen.	85.2(9.2)	84(9.1)	84.9(9.2)	84.7(9.1)^{de}
	50%	**Asp.**	84.9(9.2)	80.3(8.9)	82.5(9.1)	82.6(9)^{de}
		Pen.	88.6(9.4)	84(9.1)	84.9(9.2)	85.8(9.2)^{cde}
	100%	**Asp.**	87.7(9.3)	89.7(9.4)	88(9.4)	88.5(9.3)^{bc}
		Pen.	92(9.6)	89.7(9.5)	93.5(9.6)	91.7(9.5)^{b}
Fungicide	**25%**	**Asp.**	100(10)	100(10)	100(10)	100(10)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10)^{a}
	50%	**Asp.**	100(10)	100(10)	100(10)	100(10)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10)^{a}
	100%	**Asp.**	100(10)	100(10)	100(10)	100(10)^{a}
		Pen.	100(10)	100(10)	100(10)	100(10)^{a}
Control		**Asp.**	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)^{g}
		Pen.	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)^{h}
SE±		**Asp.**				**0.07746**
		Pen.				**0.09129**
C.V. (%)		**Asp.**				**1.56%**
		Pen.				**1.81%**
LS.D		**Asp.**				**0.2252**
		Pen				**0.2654**

Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis. Any two mean value (s) bearing different superscripts (s) are differing significantly (P < 0-0.5)

Asp. = ***Aspergillus flavus*** ***Pen.* = Penicillium *digitatum***

4.4: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* six days after inoculation

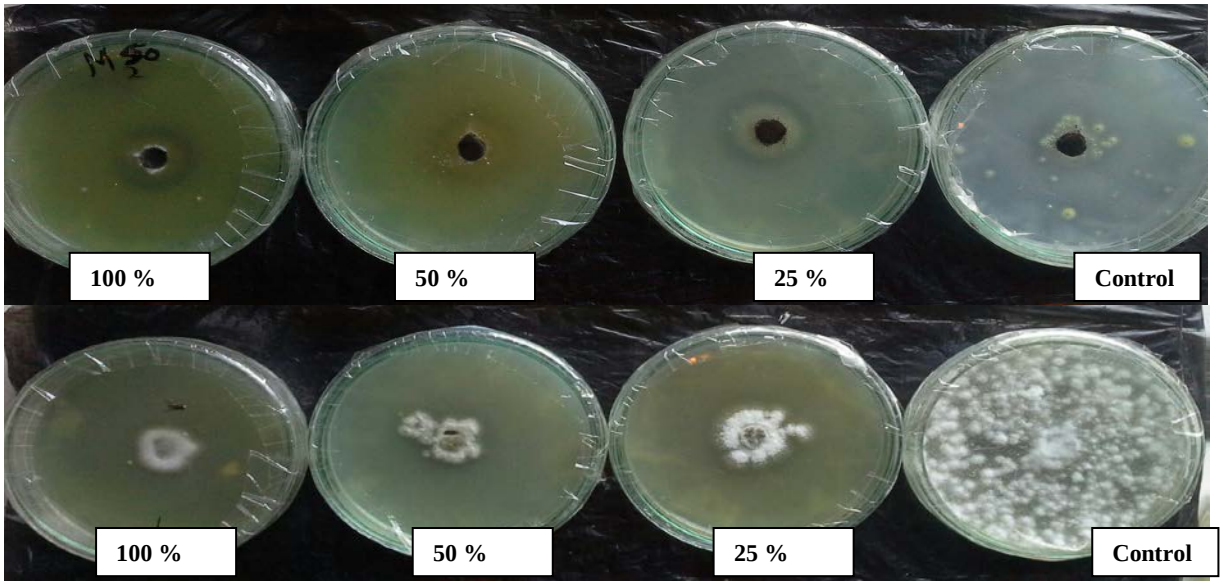
After six days from inoculation the results (Table, 4) showed that extracts of all the plants tested as well as the fungicide maintained their suppressing effect on the fungal growth compared to control. This suppressing effect of all tested concentrations of Mesquite (seeds and leaves), **Datura** and fungicide was significantly higher than the control (Table, 4). However, among all treatments, the inhibitory effect of the fungicide at all concentrations was more pronouncing than others. Moreover, the assessment of the plant extracts effect on fungal growth after six days from inoculation showed a concentration dependant differential inhibition (Table 4). Obviously, the test organism differs in its response to the different concentrations but on the whole, growth inhibition increased with increasing concentration. This inhibitory effect from all concentrations was significantly different from control.

Table, 4: Effect of different concentrations of Ethanolic extracts of leaves and seeds of Mesquite, seeds of Datura and fungicide on the linear growth *of A. flavus and P. Digitatum* six days after inoculation

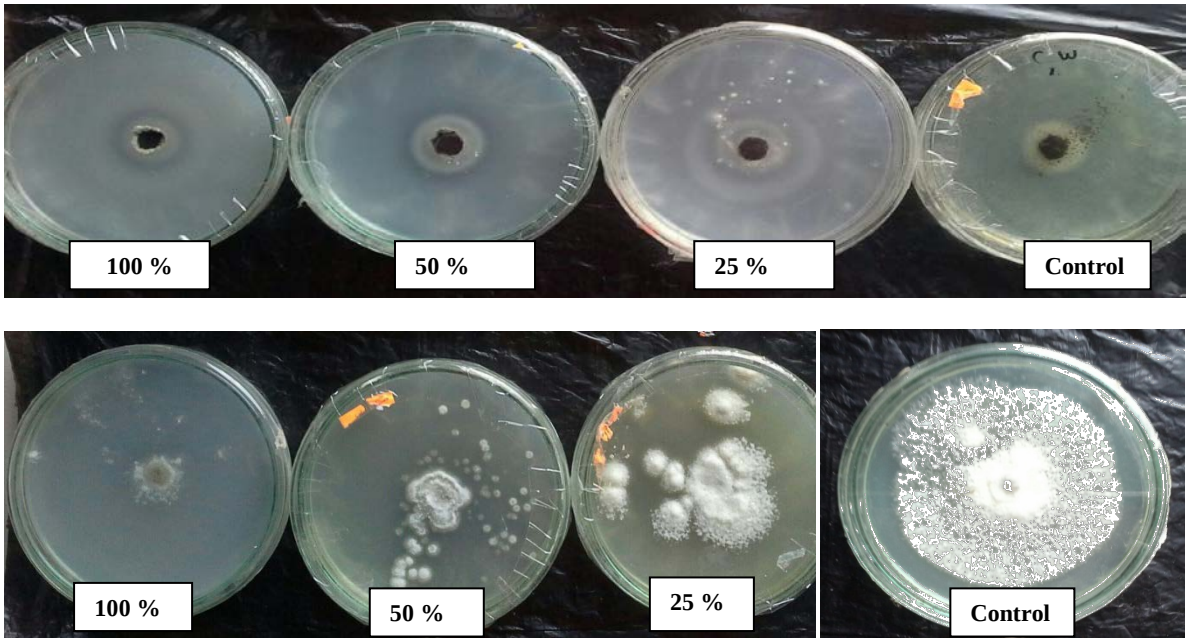
Treatments Product	Conc.	Fungus	Inhibition zone (%) R1	R2	R3	Means
Mesquite Seeds	25%	Asp.	74.3(8.6)	80(8.9)	75.6(8.7)	76.6(8.7)de
		Pen.	74.5(8.6)	79.8(8.9)	74.2(8.6)	76.2(8.7)g
	50%	Asp.	77.6(8.8)	85.6(9.2)	78.1(8.8)	80.4(8.9)cd
		Pen.	77.3(8.8)	78.8(8.9)	76.1(8.7)	77.4(8.8)fg
	100%	Asp.	85.1(9.2)	86.4(9.3)	85.7(9.2)	85.7(9.2)b
		Pen.	83.9(9.1)	84.6(9.2)	92.3(9.6)	86.9(9.3)bc
Mesquite leaves	25%	Asp.	80.1(8.9)	79.2(8.9)	79.8(8.9)	79.7(8.9)cd
		Pen.	80.1(8.9)	80.7(9)	81.9(9)	80.9(8.9)ef
	50%	Asp.	84.2(9.2)	86.4(9.3)	85.7(9.2)	85.4(9.2)b
		Pen.	85.8(9.2)	84.6(9.2)	87.6(9.3)	86(9.2)cd
	100%	Asp.	87.6(9.3)	89.6(9.4)	89(9.4)	88.7(9.3)b
		Pen.	90.5(9.5)	89.4(9.4)	89.5(9.4)	89.8(9.4)b
Datura Seeds	25%	Asp.	77.6(8.8)	72(8.5)	70.5(8.4)	73.4(8.5)e
		Pen.	80.1(8.9)	78.8(8.9)	80(8.9)	79.6(8.9)ef
	50%	Asp.	83.4(9.1)	78.4(8.8)	81.5(9)	81.1(8.9)c
		Pen.	83(9.1)	81.7(9)	83.8(9.1)	82.8(9)de
	100%	Asp.	86.7(9.3)	88(9.4)	85.7(9.2)	86.8(9.3)b
		Pen.	90.5(9.5)	89.4(9.4)	91.4(9.5)	90.4(9.4)b
Fungicide	25%	Asp.	100(10)	100(10)	100(10)	100(10)a
		Pen.	100(10)	100(10)	100(10)	100(10)a
	50%	Asp.	100(10)	100(10)	100(10)	100(10)a
		Pen.	100(10)	100(10)	100(10)	100(10)a
	100%	Asp.	100(10)	100(10)	100(10)	100(10)a
		Pen.	100(10)	100(10)	100(10)	100(10)a
Control		Asp.	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)f
		Pen.	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)h
SE±		Asp.				0.06583
		Pen.				0.05774
C.V. (%)		Asp.				1.30%
		Pen.				1.14%
LS.D		Asp.				0.1914
		Pen				0.1678

Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis. Any two mean value (s) bearing different superscripts (s) are differing significantly (P < 0-0.5)

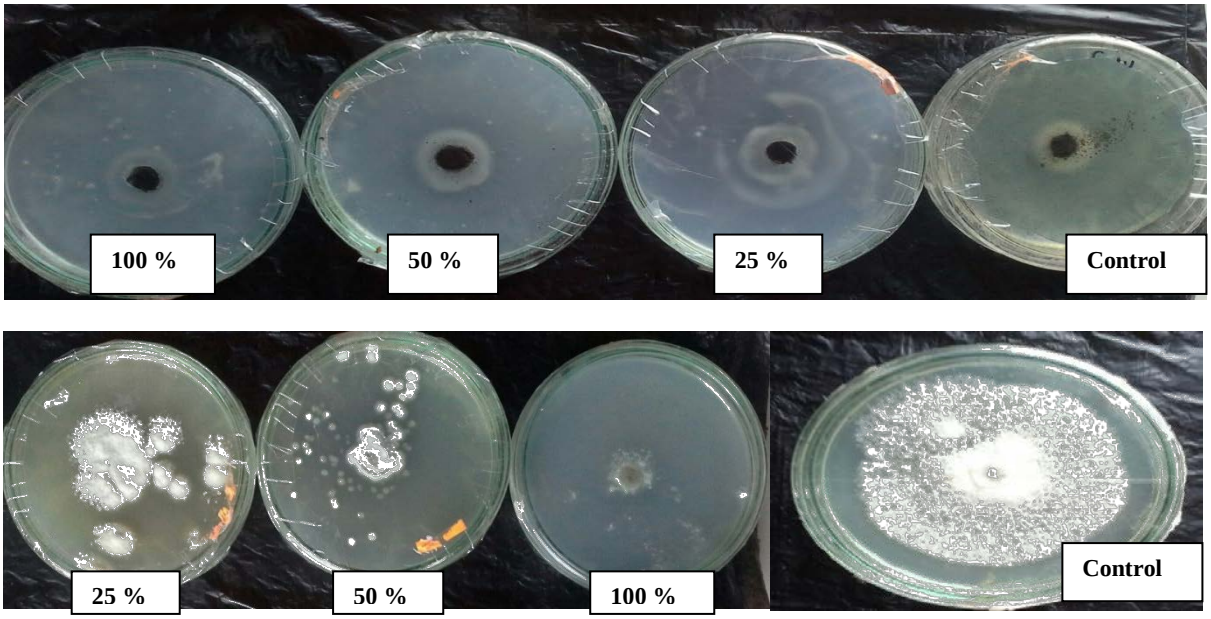
Asp. = ***Aspergillus flavus*** **Pen.** ***= Penicillium digitatum***



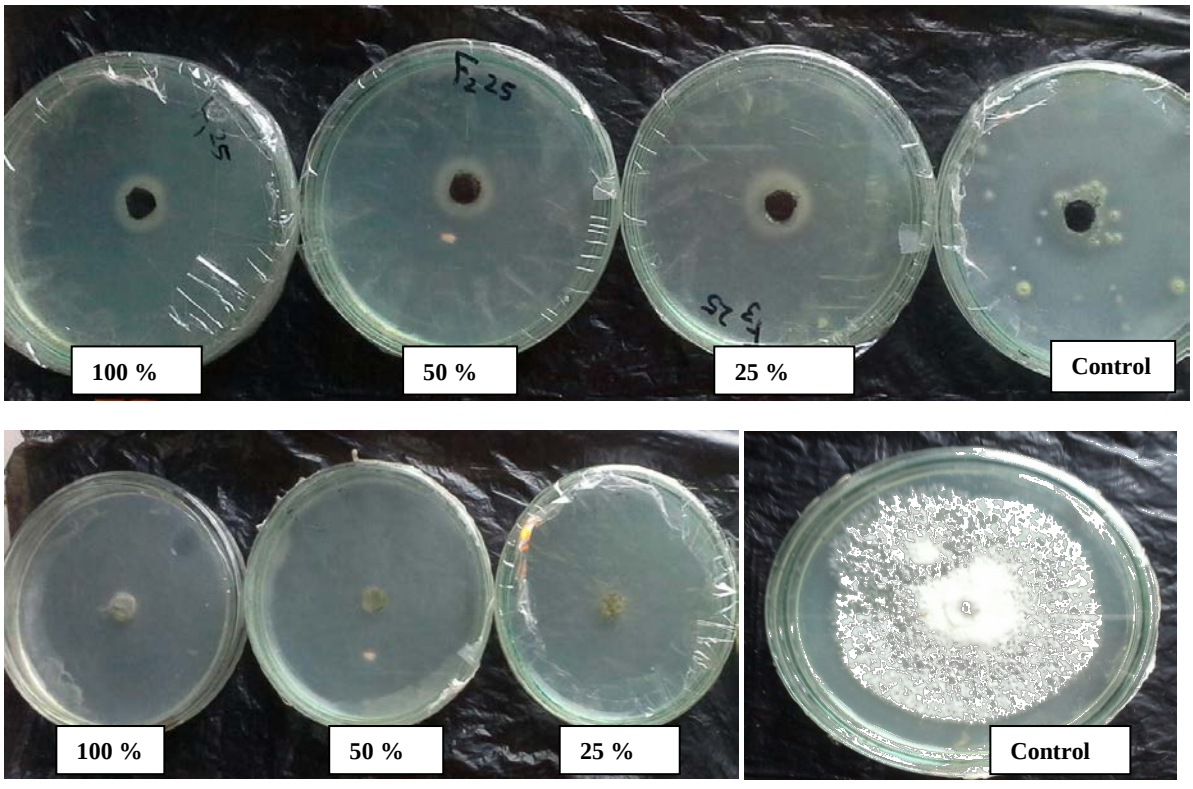
Plat (1) Effect of leaves Ethanolic extracts of mesquite on the growth of *Aspergillus flavus* and *Penicillium digitatum invitro.*



Plat (2) Effect of seeds Ethanolic extracts of mesquite on the growth of *Aspergillus flavus* and *Penicillium digitatum invitro*



Plat (3) Effect of seeds Ethanolic extracts of Datura on the growth of *Aspergillus flavus* and *Penicillium digitatum invitro*



Plat (4) Effect of fungicide Bayleton 50WP on the growth of *Aspergillus flavus* and *Penicillium digitatum invitro*

CHAPTER FIVE

DISCUSSION

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). 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%) and other macro and micro elements (Nwokoto, 1996).

The crop attacked by several food contaminants of which the most notorious are *Aspergillus spp.* And *Penicillium spp*. that produces secondary metabolites called mycotoxin. The health impacts of ingestion in humans include stunted growth and development as well as an increased risk in liver cancer (IARC and ICRISA, 2002). 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 risk encountered have been reported by several authors (Haq Elamin *et al.,* 1988; Yousif .*et al*., 2010).

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. This study was conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during the period January-March, 2015. The aim was to evaluate the

antifungal activity of seeds and leaves Ethanolic extract of *Prosopis juliflora* and seeds of *Datura innoxia* and efficacy of fungicide (Byleton® 50 WP) against the two fungi, *Aspergillus flavus and Penicillium digitatum.*

The results of this study (Tables 1-4) revealed that all Ethanolic extracts (leaves and seeds of mesquite and seeds of Datura) as well as fungicide, consistently throughout the course of the experiment exhibited an inhibitory effect on mycelial radial growth of the two fungi with significantly higher inhibition zones percent compared to control. Generally, botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Kelang, 2001). Moreover, these phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants of pesticidal significance and could be applied inform of extract, powders and cakes or as plant exudates (Owino and Wando, 1992; Anjorin and Salako, 2009). The obtained results are in line with similar studies which explored plant extracts and plant essential oils and reported to be effective antimicrobials against food storage fungi, foliar pathogens and soil borne pathogens (Garibaldi *et al.,* 1990; Alabouvette, 1999; Bowers and Locke 2000).

As demonstrated by many researchers there are a considerable interest in the use of mesquite extract to control pathogens. In this study the antimicrobial effect of Mesquite leaves and seeds Ethanolic extract at all concentrations tested was demonstrated against the two fungi tested. Similar results were obtained by Fadl Elmola *et al*., (2010) who reported that the extracts of mesquite different plant parts were highly

effective in suppressing bacterial growth. Also Zainal et al., (1988) reported that *Prosopis juliflora* contain antimicrobial compounds.

The data also demonstrated that the Ethanolic extract of Datura seeds have significantly high inhibitory effect on the growth of the two fungi tested. It is worth mentioning that various species of Datura are known and widely employed for their medicinal and toxic properties that are based upon more than 30 alkaloids (Neeraj *et al*., 2013). In a similar study Hadia *et al*., (2012) who investigated the antibacterial and antifungal of Datura reported all the extracts of *D. stramonium* tested have shown significant antifungal activity against *Saccharomyces cerevisiae, Aspergillus fumigatus and Aspergillus niger*. Similarly, *Vijai* (2015) reported the inhibitory effect of Datura on Aspergillus niger.

Generally, uses of synthetic fungicides considerably reduce the impact of plant diseases. In this study the fungicide Bayleton 50 WP consistently and throughout the course of the experiments, inhibited the radial mycelial growth of the two fungi tested and its suppressing effect was more pronounced at all concentrations tested compared to control. These results confirm that which reported by Rumkhsana *et al*., (2010) who indicated the effectiveness of systemic fungicide Bayleton against *Aspergillus sp. and Penicillium sp*.

Likewise in this study, the two fungi tested responded differently to the different concentrations of extracts. This variability in response which expressed by test fungi to different Mesquite and Datura 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, 2012; Alhadi 2013 and Faiza, 2013).

CONCLUSION

- The Ethanolic extracts of leaves and seeds of mesquite and seeds of Datura as well as fungicide induced a significantly high inhibition zones percentage against the two test fungi compared to control.
- Among all treatments the systemic fungicide Bayleton 50WP at all concentrations tested (25, 50 and 100%) exhibited consistently the highest inhibitory effect throughout the test period that results in complete inhibition of the tested fungi.
- The screened concentrations of all plants extracts treatments differ in their reactions to test fungi. Likewise the test fungi responded differently to the different concentrations of extracts.

RECOMMENDATIONS

Based on the foregoing results the following studies were recommended;

- To further investigate the antimicrobial properties in a group of medicinal plants against targets organism to determine their potentials as pesticides,
- To study different parts of mesquite plant using different solvents so as to determine the efficacy of these components in controlling plant diseases.
- Further research may be needed to look into on-field trial of the mesquite effective plant part (s) before embarking on further biochemical analysis.
- The variability in response which expressed by test organism towards the different concentrations of treatments could be investigated to adjust an optimum dose for controlling specific group of pathogens.

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APPENDIXS

Appendix 1: ANOVA table of *Aspergillus flavus*

Table 1: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	220.046	18.337	966.417	0.0000
Within	26	0.493	0.019		
Total	38	220.539			

Coefficient of Variation = 1.56%

Table 2: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	217.179	18.098	1120.370	0.0000
Within	26	0.420	0.016		
Total	38	217.599			

Coefficient of Variation = 1.45%

Table 3: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	214.430	17.869	967.912	0.0000
Within	26	0.480	0.018		
Total	38	214.910			

Coefficient of Variation = 1.56%

Table 4: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	211.569	17.631	1403.266	0.0000
Within	26	0.327	0.013		
Total	38	211.896			

Coefficient of Variation = 1.30%

Appendix 1: ANOVA table of *Pensililum digatatum*

Table 1: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	226.059	18.838	2226.348	0.0000
Within	26	0.220	0.008		
Total	38	226.279			

Coefficient of Variation = 1.02%

Table 2: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	220.323	18.360	1884.337	0.0000
Within	26	0.253	0.010		
Total	38	220.576			

Coefficient of Variation = 1.11%

Table 3: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	216.824	18.069	719.060	0.0000
Within	26	0.653	0.025		
Total	38	217.477			

Coefficient of Variation = 1.81%

Table 4: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	213.163	17.764	1823.101	0.0000
Within	26	0.253	0.010		
Total	38	213.416			

Coefficient of Variation = 1.14%