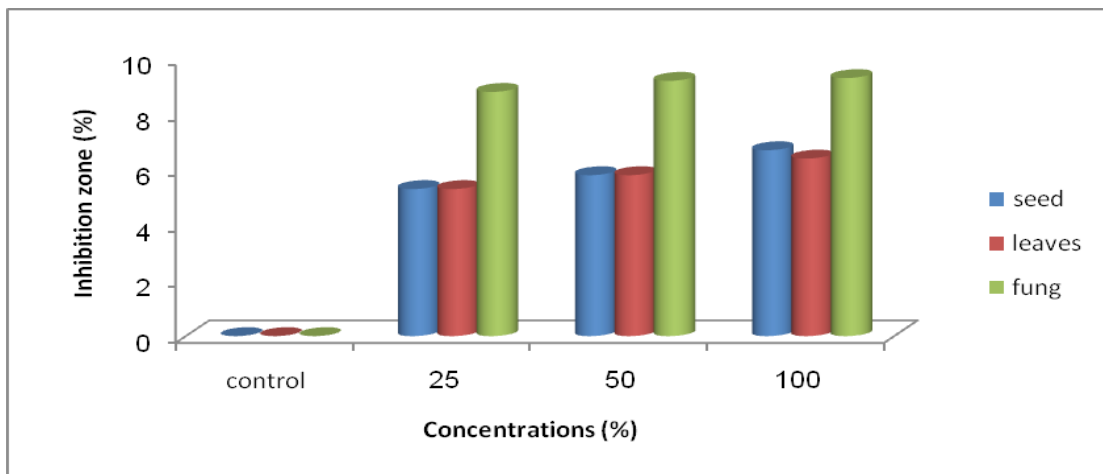
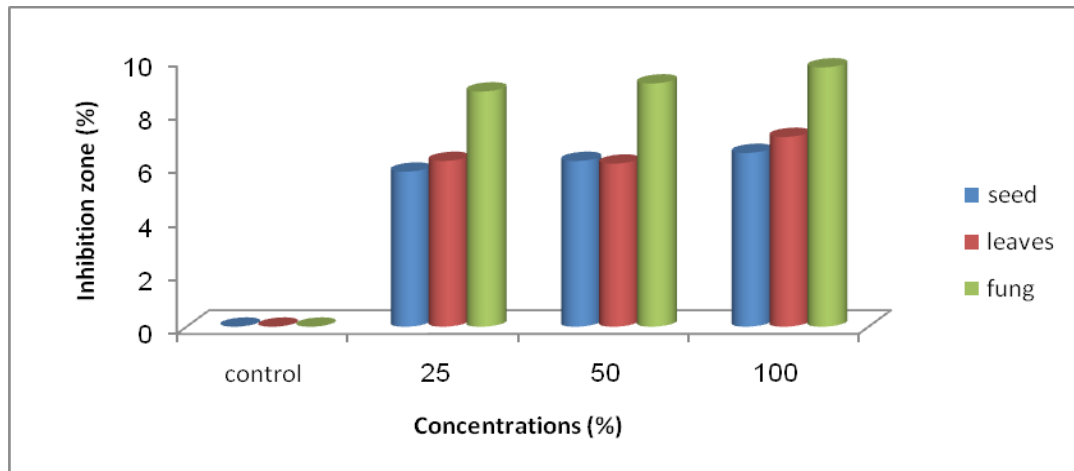


$$\sqrt{X + 0.5}\sqrt{X + 0.5}$$

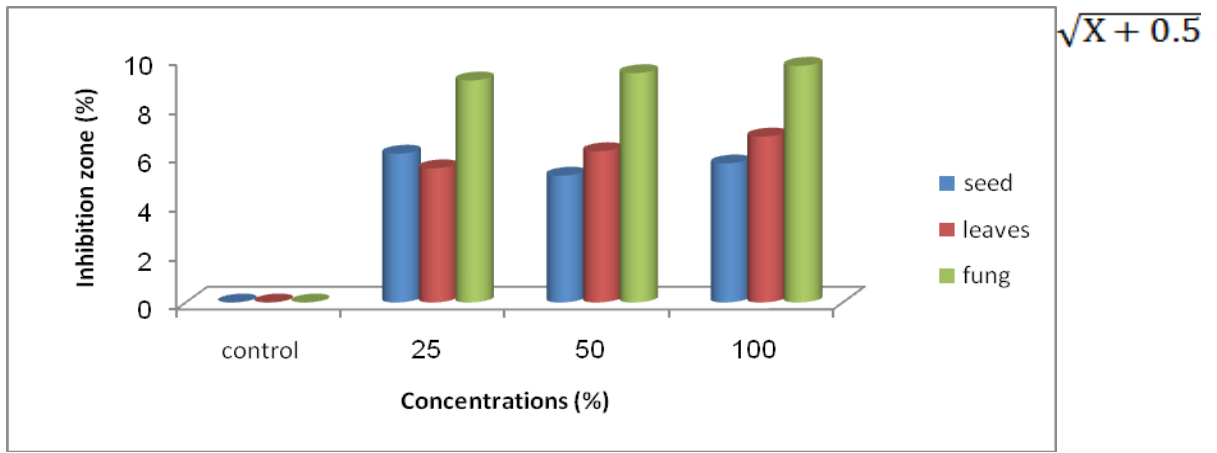


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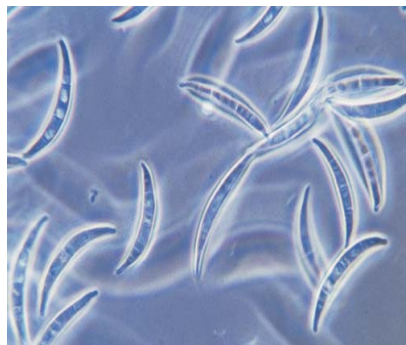
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$\sqrt{X + 0.5}$





بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

**Sudan University of Science and Technology  
College of Graduate Studies**

**Antifungal Activity of *Coffea senna* (*Cassia occidentalis*)  
and Tilt fungicides against (*Fusarium solani* ) in Potato**

تأثير التضاد الفطري للمستخلص الإيثانولي لنبات السوربب والمبيد الفطري في  
فطر الفيوزاريوم سولاني في البطاطس

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

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**2015**

قال تعالى:  
(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ ثَبَاتٍ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا  
تُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنَ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ  
وَالزَّيْتُونِ وَالرَّيْحَانِ مُنْتَبِهًا وَعَيْرَ مُتَشَابِهٍ انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي  
ذَلِكَ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ ((99))  
صدق الله العظيم  
سورة الأنعام 99 )

### Dedication

I would like to dedicate this work my father my mother my  
sisters my brothers, teachers my friends

With my love

And also to staff member of the department of plant protection  
Collage of Agricultural Studies, Sudan University of Science and  
Technology (SUST)

Abd Elrazeg Adam

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## Abstract

The present study was conducted on the Laboratory of plant pathology at the College of Agricultural Studies, Sudan University of Science and Technology (SUST) to control the dry rot of potato caused by *Fusarium solani*. Wilt is an important disease of potato crop causing significant reduction in yield. In present study, the pathogenic fungus was isolated from infected plant parts and identified based on morphological and cultural characters as *Fusarium solani f.sp.eumartii*. The *in vitro* studies were conducted to evaluate the efficacy of *Coffea senna* (*Cassia occidentalis* L) Leaves and Seeds ethanol extract and fungicide Tilt (250 EC) to control potato wilt pathogen. Different concentrations (25, 50 and 100 %) respectively, of plant extracts were used in the study. All the plants extracts showed significant reduction of pathogen. Among the different extracts leaves ethanol extracts it was most effective followed by seed ethanol extract in the growth. The results showed that all tested concentrations of both plant parts and fungicide caused inhibitory effect on the growth of the tested fungi compared to control. The highest concentration used in this study (100%) scored higher inhibition percentage (42.2, 50.7 and 94.9) respectively. Generally, the results showed that the antifungal activity increase with the increasing in concentration of each extract and Tilt. However, the inhibitory effect of Leaves

ethanolic extract of *Coffee senna* on fungi was more pronounced than that of Seeds ethanolic extract of *Coffee senna*. Application of plant extract which are easily available for controlling plant diseases are non-pollutive, cost effective nonhazardous and do not disturb ecological balance. Investigations are in progress to test the efficacy of these extracts in field applications.

In conclusion, this study showed that *Coffee senna* leaves and seeds extracts contain antifungal properties that could be investigated in further studies.

إجريت هذه الدراسة بمعمل امراض النبات , بكلية الدراسات الزراعية, جامعة السودان للعلوم و التكنولوجيا لمكافحة مرض الزبول في محصول البطاطس الذي يسببه الفطر فيوزارييم سولاني, الذي يسبب انخفاض كبير في انتاحية محصول البطاطس. في الدراسة الحالية تم عزل الفطر المسبب للمرض من أجزاء النباتات المصابة . تم تعريف الفطر علي اساس الصفات المورفولوجية والمزرعية باذة فطر فيوزارييم سولاني . اثر التضاد الفطري للمستخلصات الإيثانولية لأوراق و بذور نبات السوريب *Cassia occidentalis* L (ومبيد التلت 250 Tilt إي س) لمكافحة فطر فيوزارييم سولاني المسبب لمرض للزبول في البطاطس. إستخدمت تراكيز مختلفه ( 50,25 و 100% ) من المستخلصات النباتيه في هذه الدراسة واطهرت جميع المستخلصات النباتيه انخفاض معنوي كبير في نمو الفطر. النتائج التي تم الحصول عليها وضحت ان تأثير المستخلص الإيثانولي لأوراق و بذور نبات السوريب والمبيد في كل التركيزات اثرت على نمو الفطر المختبر مقارنة بالشاهد. التركيز الأعلى المستخدم في هذه الدراسة (100%) لكل من المستخلص الإيثانولي للاوراق والبذور والمبيد سجلت أعلى تثبيط لنمو الفطر. على أية حال، التأثير التثبيطي للمستخلص الإيثانولي للأوراق علي الفطر أكثر وضوحا من المستخلص الإيثانولي للبذور. عموما تزيد منطقة التثبيط بزيادة تركيز المستخلصات .نتيجة لذلك، توضح هذه الدراسة بأن أوراق و بذور نبات السوريب تحتوي على مواد ذات تأثير مضاد لنمو الفطريات ، يمكن أن تتحرى في دراسات أخرى.

عمل المستخلصات النباتيه سهل ومتوفر ويمكن تحضيره بسهولة للسيطرة على أمراض النبات ، كما انه غير مكلف ، وليس له خطوره على الانسان و فعال ولا يؤثر على البئيه.



## **CHAPTER ONE**

### **INTRODUCTION**

Potato is one of the most important high nutritive value crop grown in the world (Singh *et. al.*, 2004). It comes in the forefront of tuber crops and occupies the fourth position after wheat, sorghum and rice, as an edible and consumed crop in the world. The majority of potato production comes from industrial countries; China, Russia, India, and United States of America with production 72, 63, 23 and 20 million tons/annum, respectively (FAO, 2007). Egypt is the leading Arab countries in terms of potato production producing about 3.16 million tons/annum followed by Algeria (2.18 million tons) and Morocco (1.6 million tons). Sudan occupies the seventh position with annual production of 0.4 million tons (AOAD, 2006).

In Sudan the area around Khartoum, the capital of the Sudan, benefits from rich water resources (including the Nile and tributaries) and the fertile cultivable land along the River banks is available natural resource. The land suitable for cultivation accounts for about 750,000ha. Of which 11 percent is allocated to urban and periurban agriculture. In Jebel Marra, in the western part of the country, is reported to be the second most important potato production area of Sudan. The Gash Delta area in Kassala Province is often mentioned as a zone of high potential for potato production, though figures on actual production in the area are lacking (Elsir M. Elamin, 2005).

Viral diseases occur throughout the potato production areas of the Sudan, fostered by local seed multiplication practices and the apparent absence of rotation. This is especially true in Jebel Marra where little or no certified seed is used. PVY and leaf roll virus have been noted as have such bacterial diseases as soft rot and fungal diseases such as early blight, late blight, and powdery mildew, dry rot. Major insect pests include termites, cutworms, potato leaf beetle, aphids, white flies and tuber moth.

Dry rot of potato caused by *Fusarium solani* f.sp.*eumartii* is an internationally important disease of potato resulting in about 25 to 60% loss in yield in different countries and attempts have been made to manage the disease by treating with chemical compounds, biological agents as reported (Wharton and Kirk, 2007).

Pesticides were considered indispensable for sustainable agriculture production, in addition to their role in the protection of human health especially in the tropics (Kiran *et al.*, 2006). Meanwhile, the increasing and irrational use of synthetic pesticides has become a source of great concern because of their possible effect on human health and non-target components of the environment. This concern is heightened by the non-specificity and high toxicity of some pesticides and development of resistant strains of microorganisms against other ones. The foregoing has initiated the exploration of safe alternate antimicrobial agents (Okigbo, 2004).

Likewise, Mint (*Mentha spicata*), Ryhan (*Ocimum basilicum*), and Maharab (*Cymbopogon schoenanthus* Poximus) were tested to control sooty canker pathogen. Extract from garlic followed by Henna (*Lawsonia nermis*) leaf extract was reported to control minimum mycelia growth of *Pythium aphanidermatum* (Shenoi *et al.*, 1998). ( ElKorashy 1997) reported that the plant extract of *Mentha spicata* (Mint) at concentration of 50% and 100 % inhibited the growth of *Rhizoctonia solani*, *Fusarium solani*, and *Sclerotium rolfsii* , which cause damping - off disease of peanut. Currently, control of plant pathogens requires employment of alternative techniques because traditional handling with synthetic chemicals has caused various problems such as toxicity to users and impairment of beneficial organisms (Anderson *et al.*, 2003). Another important aspect is that pathogenic organisms have generated resistance to the active ingredient of some synthetic fungicides in response to selection pressure due to high dose and continuous applications, causing great economic losses. However, natural products proved to be economical and efficient alternative for disease control since it does not affect environment and their residues are easy to degrade .(Wilson *et al.*, 1999).

Based on the foregoing, this study was undertaken to focus on investigation of two components for management of *Fusarium* dry rot of Potato caused by *Fusarium solani* f.sp.*eumartii*, higher plant extracts and synthetic fungicides under laboratory conditions in order to formulate promising disease management approach with following objectives:-

- Isolation and Identification of the causal agent.
- To explore the antifungal activity of *coffee senna* (leaves and seeds) extract against *F. solani* f.sp.*eumartii*.
- To evaluate the effect of systemic fungicide on fungal growth (Tilt)

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Potato (*Solanum tuberosum* L.)

The potato plant which belongs to the family *Solanaceae* includes, among 2000 other species, tomato (*Lycopersicon esculentum*), sweet pepper (*Capsicum annum*), eggplant (*S. Melongena var. esculentum*), tobacco (*Nicotiana tabacum*), and petunia (*Petunia hybrida*). The potato was first domesticated in the region of modern-day southern Peru and extreme northwestern Bolivia<sup>[6]</sup> between 8000 and 5000 BC. It has since spread around the world and become a staple crop in many countries. ( Spooner, et al,. 2005).

##### 2.1.1 Scientific classification

Kingdom: Plantae (unranked):

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *S. tuberosum*

(Binomial name: *Solanum tuberosum* L.)

The genus Solanum is a polymorphous and largely tropical and subtropical genus containing more than 1000 species. The origin

agreed to be the high elevation of South America and the area of first domestication was reasoned to be the area where wild diploids are still found and where the greatest diversity of cultivated forms can still be found, and is identified as the high plateau of Bolivia and Peru, in the general region of Lake Titicaca (Spooner, *et al.*, 2005).

Potato is one of the major vegetable crops grown worldwide following wheat, maize, and rice, with a production estimates of 368 million tons. It is the staple food of many cultures and civilizations past and present. The term Potato is used to refer both to the plant, and the vegetable itself (AOSTAT, 2015).

In Sudan, the potato is grown mainly as winter crop and the main area of production are along the Nile bank in both Khartoum and Northern Estates. Although potato cultivation in Sudan depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato (Abdelgadir, 2003).

Potatoes in Sudan are an important cash crop for small-scale growers, and have the potential to increase incomes in per urban areas, improve living standards and create employment opportunities. Potato production is steadily increasing in Khartoum; the acreage devoted to this crop has more than tripled in the last ten years.

The total acreage under potato cultivation in the Khartoum region amounts to about 6,500 hectares, with yields of 17 to 25 ton/ha.



However, production costs of potatoes are high in comparison with those of other crops; seed potatoes have to be imported and account for more than half of the total production cost of potatoes. This is a major constraint to further expansion of potato production. The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddans.

One of the major constraints facing the quantity, quality and availability of healthy crop worldwide are the losses and contamination caused by post harvest diseases. The major groups of postharvest diseases are those which arise from infections initiated during and after harvest. (Elsir, 2005).

### **2.1.2 Nutrition**

---

#### **Nutritional value per 100 g (3.5 oz)**

Energy	321 kJ (77 kcal)
Carbohydrates	17.47 g
Starch	15.44 g
Dietary fiber	2.2 g
Fat	0.1 g

Protein	2 g
Vitamins	
Thiamine (B1)	(7%) 0.08 mg
Riboflavin (B2)	(3%) 0.03 mg
Niacin (B3)	(7%) 1.05 mg
Pantothenic acid (B5)	(6%) 0.296 mg
Vitamin B6	(23%) 0.295 mg
Folate (B9)	(4%) 16 µg
Vitamin C	(24%) 19.7 mg
Vitamin E	(0%) 0.01 mg

Vitamin K	(2%)
	1.9 µg
Trace metals	
Calcium	(1%)
	12 mg
Iron	(6%)
	0.78 mg
Magnesium	(6%)
	23 mg
Manganese	(7%)
	0.153 mg
Phosphorus	(8%)
	57 mg
Potassium	(9%)
	421 mg
Sodium	(0%)
	6 mg
Zinc	(3%)
	0.29 mg

## **Other constituents**

Water	75 g
-------	------

(Picard Andre, 2002).

### **2.1.3Uses**

---

Potatoes are used to brew alcoholic beverages such as vodka, potcheen, or akvavit.

They are also used as food for domestic animals.

Potato starch is used in the food industry as, for example, thickeners and binders of soups and sauces, in the textile industry, as adhesives, and for the manufacturing of papers and boards.

Maine companies are exploring the possibilities of using waste potatoes to obtain polylactic acid for use in plastic products; other research projects seek ways to use the starch as a base for biodegradable packaging.

Potato skins, along with honey, are a folk remedy for burns in India. Burn centers in India have experimented with the use of the thin outer skin layer to protect burns while healing.

Potatoes (mainly Russets) are commonly used in plant research. The consistent parenchyma tissue, the clonal nature of the plant and the low metabolic activity provide a very nice "model tissue" for experimentation. Wound-response studies are often done on potato tuber tissue, as are electron transport experiments. In this respect, potato tuber tissue is similar to *Drosophila melanogaster*, *Caenorhabditis elegans* and *Escherichia coli*: they are all "standard" research organisms. (Jai Gopal *et al*, 2006).

### **2.1.3.1 Culinary uses**

- Potatoes are prepared in many ways: skin-on or peeled, whole or cut up, with seasonings or without. The only requirement involves cooking to swell the starch granules. Most potato dishes are served hot, but some are first cooked, then served cold, notably potato salad and potato chips/crisps.
- Common dishes are: mashed potatoesyogurt and butter; whole baked potatoes; boiled or steamed potatoes; French-fried potatoes or chips; cut into cubes and roasted; scalloped, diced, or sliced and fried (home fries); grated into small thin strips and fried (hash browns); grated and formed into dumplings, Rösti or potato pancakes. Unlike many foods, potatoes can also be easily cooked in a microwave oven and still retain nearly all of their nutritional value, provided they are covered in ventilated plastic wrap to prevent moisture from escaping; this method, which are first boiled (usually peeled), and then mashed with milk or produces a meal very similar to a steamed potato, while retaining the appearance of a conventionally baked potato. Potato chunks also commonly appear as a stew ingredient.



- Potatoes are boiled between 10 and 25 minutes, depending on size and type, to become soft (Cookbook:Potato.2011).

#### **2.1.4 Diseases:-**

##### **2.1.4.1 Bacterial diseases**

Bacterial wilt, Common scab, Erwinia black leg, Erwinia wilt

##### **2.1.4.2 Fungal disease**

Black dot, Botrytis, Botrytis, Early blight, Fusarium dry rot, Fusarium wilt, Gangreen, Late blight, Powdery scab, Rhizoctonia, Sclerotinia rot, Sclerotinia wilt, Silver scurf, Verticillium.

##### **2.1.4.3 Viral diseases**

Potato virus Y (PVY), Potato virus X (PVY).

Orzolek *et al.*, (2010).

#### **2.2 Fusarium dry rot in potato:**

*Fusarium solani* f.sp.*eumartii* is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these *Fusarium* species are fumonisins and trichothecenes. (Howard, 2003)

##### **2.2.1 Classification by (Desjardins, 2006):**

Kingdom	: Fungi
Subkingdom	: dikarya
Phylum	: Ascomycota
Subphylum	: Pezizomycotin
Class	: Sordaiomycetes

Order : Hypocrites  
Family : Nectraceae  
Genus : *Fusarium*  
Species : *Solani*  
S.N : *F.solani f.sp.eumartii*

### **2.2.2 Morphology:-**

On potato dextrose agar medium, *F. solani* produces sparse to abundant, white cream mycelium. Macroconidia have three to four septa on average, are slightly curved, are rather wide and thick walled, and may have a slightly blunted apical end. Microconidia are abundant, oval to kidney shaped, and formed in false heads on very long monophialides. Chlamyospores are abundant.

microconidia and macroconidia

### **2.2.3 Biology:**

In solid media culture, such as Potato Dextrose Agar (PDA), the different special forms of *F. solani* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple according to the strain (or special form) of *F. solani*. If sporodochia are abundant, the culture may appear cream or orange in color (Zaccardelli, *et al*,.2008).

#### **2.2.4 Symptoms:**

The diseased plant generally, produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. The most important of these is vascular wilt (Ramsamy *et al.*, 1996).

*Fusarium* dry rot starts out looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. On older plants, symptoms are more distinct between the blossoming and fruit maturation stages. *Fusarium* dry rot is characterized by an internal light to dark brown or black rot of the potato tuber-and it is usually dry. The rot may develop at an injury such as a bruise or cut. The pathogen penetrates the tuber, often rotting out the center. Extensive rotting causes the tissue to shrink and collapse, usually leaving a dark sunken area on the outside of the tuber and internal cavities. Yellow, white, or pink mold may be present (Zaccardelli *et al.*, 2008).

#### **2.2.5 Disease cycle:**

*Fusarium solani f.sp.eumartii* produces asexual spores (micro conidia and macro conidia). Its sexual state is *Nectria haematococca* (Ascomycete). It produces chlamydospores and overwinters as mycelium or spores in infected or dead tissues or seed. It can be spread by air, equipment, and water .

The fungus can persist in the soil for several years. The spores and the mycelium are carried into the soil on tools and in bean

straw manure. They may also be splashed by rain or carried by floods. The chlamydospores are the survival structure in the absence of a host plant (Cho *et al.* , 2001).

#### **2.2.6 Hosts Range:**

The fungal pathogen *F. Solani f.sp.eumartii* affects a wide variety of hosts at any age. Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana are a few of the most susceptible plants (Koenning, 2001).

#### **2.2.7 Disease:**

Fusarium wilts affect and cause severe losses on most vegetable and flowers, several field crops such as cotton vegetable and flowers, and tobacco, plantation crops such as banana, plantain, coffee and sugar cane, and a few shade trees, Fusarium wilts are most severe under warm soil conditions and in green houses. Most Fusarium wilts have disease cycles and develop symptoms. (Koenning, 2001).

#### **2.2.8 Environment:**

As previously stated *F.solani f.sp.eumartii* is a common soil saprophyte that infects a wide host range of plant species around the world. It has the ability to survive in most soil- arctic, tropical, desert, cultivated and non-cultivated. Though *Fusarium* spp. may be found in many places and environments .Development of the disease is favored by high temperatures and warm moist soils. The optimum temperature for growth on artificial media is between 25-30°C, and the optimum soil temperature for root infection is 30°C or above (Koenning, 2001).

### **2.2.9 Importance of dry rot:**

Dry rot is not just a cosmetic problem like many other pathogens. It destroys tubers and leaves them completely inedible or unusable as seed in the future. Long-term storage losses have been reported to be as high as 60% while annual dry rot losses can range from 6to25%(Gachango *et al*,.2012).

### **2.2.10 Management:**

There are many ways to manage dry rot. Application of thiabendazole, also known as Mertect, was a common and efficacious method used from 1970-1985. Eventually, however, the pathogen developed resistance to the chemical treatment, and while some people still use thiabendazole, it is no longer an effective treatment. Effective chemical control of dry rot can be achieved with chemicals like Tops MZ, Maxim MZ, and Moncoat MZ. These chemicals protect not only against dry rot, but also against other potato diseases like rhizoctonia, silver scurf, and black dot. These chemical treatments can delay emergence of the young plants, but this doesn't mean these chemicals shouldn't be used. Many fungicides, including thiabendazole, work best when they are applied to tubers before they are cut into seed pieces (Schwartz,2005).

Cultural practices can also limit the spread of dry rot. Farmers are advised to only use certified, disease-free seed, and to inspect seed pieces personally to ensure that they are symptom-free. Seed should be stored at 40-42 degrees Fahrenheit, but gently warmed to 50 degrees prior to cutting. The cooler temperatures antagonize growth of *Fusarium*, and the warmer temperature encourages potato tubers to heal any post-harvest wounds, minimizing the chance that *Fusarium* will get inside the tuber. Sanitation is very important in controlling dry rot. Storage facilities and cutting equipment should be disinfected frequently. The blades used for cutting

should be sharp to ensure clean cuts. Farmers also should not keep “cull piles” of potato tubers. Stored tubers should be checked regularly for signs and symptoms of dry rot.

Before planting, cut seed pieces should be treated with fungicide, such as Tops MZ, Moncoat MZ, or Maxim MZ. The seed pieces should be planted in warm, well-drained soil within 24 hours of cutting; this environment is conducive to sprout growth and emergence. The seed pieces should be shielded from wind and sunlight before they are planted, to prevent dehydration (Loria, 2013).

Tubers shouldn't be harvested until their skins have set and their internal temperature is greater than 50 degrees Fahrenheit. These measures minimize the risk of harvest injury, which could give the *Fusarium* pathogen entrance into the tuber. Biological control of dry rot is an intriguing concept, but currently nothing is available commercially. Researchers at Michigan State University are investigating the efficacy of *Bacillus subtilis* and *Bacillus pumilis* (both bacteria) and *Trichoderma harzianum* (a fungus) in controlling *Fusarium* dry rot (Warton *et al.*, 2013).

### **2.3 Coffee senna (Soreib):**

*Cassia* species (Caesalpinaceae) are annual under shrub grows all over the tropical countries. Traditionally, the leaves of *Cassia* species are popular as pot herb. It is used as natural pesticide in the organic farming. Also *Cassia* species contain chrysophanic acid-9-anthrone which is an important fungicide (Singh *et al.*, 2013).

The genus *Cassia* comprises more than 40 species amongst which some are economically important in the production of timber,

gum, tanning, dying materials and fish poisons. In the Sudan, this genus is represented by at least 13 species (Omer *et al.*, 2012).

### **2.3.1 Taxonomy:**

Family: Caesalpinaceae

Genus: Cassia

Species: occidentalis

S. N: *Cassia occidentalis* L.

C. N: Coffee senna

### **2.3.2 Botanical description:**

Coffee senna (*C. occidentalis*) is an erect somewhat branched, smooth, half woody herb or shrubby plant, about 0.8 -1.5 meters in height. The flowers are yellow about 2 cm in length and borne on auxiliary and terminal racemes. Seed pods are narrow and semi-flattened about 10 cm long, thickened and containing about 40 or more brown to dark-olive, ovoid seeds about 4 mm long. The species is distinguished by a *fitted* odour, absence of spines. Leaves with 3 - 7 leaflets about 2–10cm long and 0.6–4cm wide. Flowers with 10 fertile and sterile stamens, 6 or 7 fertile anthers and cylindrical seeds (Podsilva, 2003).

### **2.3.3 Geographical distribution:**

Coffee senna grows throughout the tropics and subtropics including the United States from Texas to Iowa eastward, Hawaii, the Pacific Island territories, Puerto Rico, and the U.S. Virgin Islands. It appears to be of South American or New World origin (Singh *et al.*, 2013). *C occidentalis* are most commonly found in

savannah areas of Africa and are utilized for various purposes. This plant is found in many parts of the Sudan and commonly known as Soreib (Mariod and Matthäus, 2008).

#### **2.3.4 Cultivation:**

*C. occidentalis* can flower and fruit throughout the year or only periodically, depending on rainfall and temperature conditions and seasons. In cold or dry climates, the life cycle of *C. occidentalis* is completed in 6 to 9 months. In warm, continually moist areas, however, plants may last a full year. Well-dried seed stored in airtight containers remain viable for more than three years. Seed should be treated to enhance germination. The distal end of each seed should be nipped, or the seed can be immersed in concentrated sulphuric acid for 10 minutes and then rinsed with plenty of water. Seed should germinate between 5 and 36 days after sowing. *C. occidentalis* is planted in hedges and as an ornamental, but has the potential to become a weed in farmland, and is often found in disturbed areas. It should therefore be managed carefully. The species can be controlled with broadleaf herbicides (Dharani *et al.*, 2010).

(a)

(b)

***C. occidentalis* plant (a) and *C. occidentalis* seeds (b).**



### **2.3.5 Chemistry:**

Chemical constituents isolated from *C. occidentalis* including sennoside, anthraquinone glycoside, fatty oils, flavonoid glycosides, galactomannan, polysaccharides, and tannins (Yadav *et al.*, 2010). The stem, bark and leaf extract of *C. occidentalis* have been found to contain important phytochemicals such as anthraquinones, carbohydrates, glycosides, cardiac glycosides, steroids, flavanoids, saponins, phytosterols, gum and mucilage (Colle *et al.*, 2003). This plant also include wide range of chemical compounds such as achrosin, aloe-emodin, emodin, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol and chrysoeriol (Dave and Ledwani, 2012). Terpenoids, flavonoids and anthraquinone derivatives have been detected in active fractions obtained from the leaf extract. Also in a separate investigation, new C-glycosidic flavonoids (cassia occidentalis A, B and C) were isolated from this plant (Dharani *et al.*, 2010).

### **2.3.6 Medicinal uses:**

*C. occidentalis* leaves are used for the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of southwestern Nigeria. In addition to this, the leaves are also known to be effective against jaundice, headache and toothache. Infusion of *C. occidentalis* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria (Taiwo *et al.*, 2013). Also *C. occidentalis* leaves have ethno medical importance like wound healing, treatment of sores, cutaneous diseases, bone

fracture, fever, and throat infection . It also used as a diuretic and in the treatment of snake-bite (Yadava and Satnami, 2011). Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities (Tona *et al.*, 2004). *C. occidentalis* has long been used as natural medicine in rainforests and other tropical regions for the treatment of inflammation, liver disorders, constipation, worms, fungal infections, ulcers, and respiratory infections (Dave and Ledwani, 2012). This plant is also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma, and disorder of hemoglobin and is also reported to cure leprosy. An infusion of the plant bark is given by the folklore in diabetes (Sini *et al.*, 2011). Aqueous extract of stem and leaves of this plant showed a suppressive effect on *Trypanosoma cruzi* infected rats (Ibrahim *et al.*, 2010). The ethanol extract of this plant has also been found to show significant antihepatotoxic activity against carbon tetrachloride and thioacetamide as hepatotoxins and antidiabetic activity in normal and alloxan induced diabetic rats (Mustapha *et al.*, 2013).

### **2.3.7Uses in pest control:**

The leaves of this plant are used for various disease treatments as well as in the control of some stored product insects especially in many parts of Africa (Abdullahi, 2011). In Senegal the leaves of *C. occidentalis* are used to protect cowpea seeds against *Callosobruchus maculatus* . Both fresh and dry leaves as well as whole and ground seeds had no contact toxicity on the cowpea beetle, in contrast, seeds oil induced an increase in mortality of *C.*

*maculata* eggs and first larval instars at the concentration of 10 ml/kg cowpea (Lienard *et al.*, 1993). In addition, coffee senna has been used to reduce the number of mosquitoes indoors at night (Paisson and Jaenson, 1999) and for the control of a large variety of insects (Dweivedi and Kumar, 1998).

### **2.3.8 Other uses:**

Coffee senna is used as a flowering shrub for landscape purposes. It is also used as a coffee substitute, where it has some medicinal uses as seeds are brewed into the coffee-like beverage which is used for asthma (Nassar *et al.*, 2011). The leaves are widely used as a leaf vegetable and are eaten either raw or mixed with coconut, chilli, and onion (Nassar *et al.*, 2013). The gum derived from seed endosperm can be potentially utilized in a number of industries to replace the conventional gum (Gupta *et al.*, 2005)

### **2.3.9 Animal toxicity:**

Several animal studies have demonstrated the toxicity of the fresh and / or dried / roasted beans (seeds). Ingestion of large amounts of the seed pods by grazing animals has caused serious illness and death. Cattle, sheep, goats, horses, pigs, rabbits, and chickens have been shown to be susceptible to poisoning by *Cassia* spp. (Rowe *et al.*, 1987). Also all parts of the plant are toxic, most poisoning occurs when animals eat the pods and beans, or fed green chop containing *Cassia* plants. The toxic effects are seen on skeletal muscles, liver, kidney and heart in animals. One interesting attribute of *C. occidentalis* poisoning in

animals is its propensity to cause different manifestations of toxicity in different animal species. However, the physiologic systems involved in toxicity depend also upon the dose of the beans consumed. When the dose is low the animal develops features of mild liver damage and myodegeneration and at higher doses hepatic degeneration may be rapidly fatal before myodegeneration has time to develop (Vashishta *et al.*, 2009).

Toxicity studies on the aerial parts, leaves and roots of *C. occidentalis* reported that various leaf and root extracts given to mice (administered orally and injected at up to 500mg/kg) cause mortality (Sadiq *et al.*, 2012). In another recent study, the leaf extract was observed to be potentially toxic to mice with an intraperitoneal LD<sub>50</sub> of 1000mg/kg body weight (Mustapha *et al.*, 2013). Roasting of the beans partially reduces their toxicity such that goats fed on 2.5 g/kg per body weight of roasted beans were unaffected, whereas unroasted beans at this dosage were fatal (Suliman and Shommein, 1986). Apparently all toxic effects are acute and it is believed that the toxins do not accumulate in body tissues. However, when consumed repeatedly over time the ill effects would be seen as chronic, but in fact it is the result of repeated acute poisoning due to the inclusion of *Cassia* vegetation in fresh green feed installed fed animals. *C. occidentalis* was proved to be toxic to heifers with more prominent clinical symptoms depressed muscular tone, weakness, and slow march (Marrero *et al.*, 1998). There are several compounds that bind strongly to cell membranes occur in *Cassia* spp., but the specific toxin(s) responsible for muscle degeneration have not been identified while the exact toxic principles are yet to be defined, various anthraquinones and their derivatives like emodin glycosides, toxalbumins, and other alkaloids are usually blamed for *C. occidentalis* toxicity (Vashishtha *et al.*, 2009)

#### **2.4 Fungicide:-**

#### **2.4.1 Tilt 250EC:-**

Common name:	Propiconazole
Company :	Syngenta Agro
The Recommended Dose:	0.15 - 0.20ml/L
The Pest :	Powdery mildew disease
The Crop :	Cucumber
The Year of Recommendation:	1987

### **CHAPTER THREE**

#### **MATERIALS AND METHODS**

These experiments were conducted at the laboratory of the plant Pathology Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) "Shambat" in February 2015. To evaluate the inhibitory effect of seeds and leaves ethanolic extract of *cassia occidentals* and Fungicide (Tilt) against the *Fusarium solani f.sp.eumartii*.

The Materials and Methods used in this study are mentioned below:

### **3.1 Equipments:**

- 1-Sensitive balance
- 2-Soxhlet extractor apparatus
- 3-Rotary evaporator
- 4-Petri-dishes
- 5-Marker pen
- 6-Pencil
- 7-Gloves
- 8-Masks
- 9-Camera
- 10-Electronic blender
- 11-Filter paper
- 12-Registration form
- 13-Scalpel
- 14-Conical flask
- 15-Laminar
- 16-Water bath
- 17-Outoclave
- 18-Incubator

### **3.2 Materials:**

- 1-Cassia seeds and leaves
- 2-Ethanol 95%
- 3-Distilled water

- 4-Soap
- 5-Praphen
- 6-Tubers of potato diseased
- 7-fungicide Tilt

### **3.3 Collection of plant samples:**

Infected potato (tuber) showing symptoms of the disease were obtained from sick blots from Shambat Research Station in February 2015. Random samples from infected potato field were collected to the laboratory for isolation and identification.

### **3.4 Isolation method:**

#### **3.4.1 Isolation of *Fusarium solani* f.sp.eumartii:**

#### **3.4.2 Isolation from plant materials:**

Infected potato (tubers) showing symptoms of the disease were obtained from sick blots from Shambat Research Station in February 2015 parts showing disease symptoms were cut into small sections (0.5-1.0), washed thoroughly with tap water, and surface sterilized by immersing 1:4 Clorox (NaOCl) for 5 miles, 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 antibacterial agent (Anon., 1981). The medium was poured in 9cm Petri dishes. Five sections of the dried parts were as optically placed in a Petri dish and incubated at 28°C. Sub-

cultures were later prepared to get pure cultures. Slides were prepared from these pure cultures, and examined microscopically(x: 40).

### **3.5 Effect of plant extract:**

The aim of this experiment to study the antifungal activities of plant extract on the growth of *Fusarium solani f.sp.eumartii* in vitro. Coffee senna (*Cassia occidentalis*) seeds and leaves were tested for their effect on the fungus.

#### **3.5.1 Collection of plant material and preparation of plant extract:**

Exactly 60g of the each plant material were extracts in ethanol for 6hure using a soxhlet. The extracts were concentrated using rotary flash evaporator and preserved at 58°C in air light brown bottles until further use. The extracts were subjected to antifungal activity against fungi. Potato Dextrose Agar (PDA) medium was prepared in conical flasks (250ml). Ethanolic extract bioassays were carried out in the prepared PDA. To avoid bacterial contamination, antibacterial Chloromycetin capsules (parts/million) were used. Three concentrations of each plant were used (100%, 50%, 25%).The plant extract (Coffee senna seeds and leaves ethanolic extract) were mixed with a PDA media in a conical flask of 250ml. Ethanol was added instead of plant extract as control. Three plates after the solidification of the medium, one disc (corborer) of *Fusarium solani* colony (7days old) was plugged and inoculated aseptically in the center of each petridishes and then they were incubated at 28°C.



The fungal growth was stimulated daily by measuring the growth of the fungus along the two diameters drawn on the back of each Petri dish.

The effect of each extracts was evaluated percentage of Reduction of the growth. Percent growth reduction was calculated by Formula (1) given by Jab Tap *et al.*,.2007).

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

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

### **3.6 Preparation of Tilt fungicides:**

Tilt fungicides were tested 10ml dissolved in 1000ml of sterilized distilled water to give 1, 0.5, 0.25 ppm respectively. Potato Dextrose Agar (PDA) medium was prepared in conical flasks (250ml). Three concentrations were used (100%, 50%, 25%). The Fungicide was mixed with a PDA media in a conical flask of 250ml. Three plates after the solidification of the medium, inoculated with one disc (corborer) of *Fusarium solani f.sp.eumartii*. Colony (7days old) was plugged and inoculated aseptically in the center of each petridishes and then they were incubated at 28°C.

The fungal growth was stimulated daily by measuring the growth of the fungus along the two diameters drawn on the back of each Petri dish.

. The effect of the fungicide was evaluated percentage of Reduction growth by the Formula (1)

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

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

### **3.7 Statistical analysis:**

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

Plate. : Soxhlet Extractor Apparatus

Plate. : Soxhlet and Rotary Evaporator

## CHAPTER FOUR

### RESULTS

#### 4.1 Laboratory Experiments:

This study was conducted at the laboratory of Plant Pathology, Department of Plant Protection, college of Agricultural studies, Sudan University of Science and Technology during February 2015. The aim of this study was to investigate the antifungal activities of seeds and leaves ethanolic extract of *cassia occidentals* on *Fusarium solani f.sp.eumartii*.

#### 4.2 Isolation and Identification of the Pathogens

The causal agents of the potato dry rot disease were identified as isolate of *Fusarium spp.* Identification was performed depending on the cultural characteristics and conidial shapes as described by Booth (1977).

#### 4.3 Effect of plant extracts (*coffee senna*) on linear growth of *Fusarium solani* *in vitro*:

The antifungal activity of *Coffe senna* seed and leaves extracts to study the effects of plant extracts on the growth of the *F. solani* *in vitro* after three days. The results showed that the plant extracts were effective in reducing the mycelia growth of *F. solani* *in vitro*.

Table (1) figure (1) results indicated that, all concentrations of *Coffe senna* extracts was gave different significantly of inhibition percentage against fungus, *Coffe senna* seed extracts concentrations (25, 50, and 100%) were gave (12, 25 and 33.3 %)

respectively. *Coffe senna* leaves extracts concentrations (25, 50 and 100 %) were gives (37.5, 49.8 and 58.5 %) respectively. The results indicated that, leaves extracts have the highest antifungal activity against the growth of *F. solani*. Concentrations of Tilt fungicide (25, 50 and 00%1) was gave (79.1, 93.7 and 100 %) two days after of incubation.

Table (2) and figure (2) results indicated that, all concentrations of seed and leaves was gave different significantly of inhibition percentage against fungus, seed extracts concentrations (25, 50, and 100%) were gave (16.6, 27.7 and 33.3 %) respectively. Leaves extracts concentrations (25, 50 and 100 %) were gives (30.5, 38.8 and 47.2 %) respectively. The results indicated that, leaves extracts have the highest antifungal activity against the growth of *F. solani*. Concentrations of tested fungicide (25, 50 and 00%1) was gave (83.2, 90.2 and 97.1 %) three days after of incubation.

#### **4.4 The Effects of Fungicides Tilt on the liner growth of *F. solani in vitro***

The sensitivity of mycelial growth of *F. solani* was checking against fungicide Tilt. Table (2) showed that there was a significant decrease in the mycelia growth of the fungus with an increase in fungicidal concentration (Fig 2) 97.1% three days after incubation.

**Table Effect of the *coffee senna* seeds and leaves ethanolic extracts and Fungicide Tilt on the growth of *Fusarium solani f.sp.eumartii*. 48 hour after incubation.**

Mean	Inhibition zone (%)			Con	Plant part
	R3	R2	R1		
12.5(3.1) <sup>e</sup>	12.5 (3.6)	25 (5.0)	0 (0.7)	25	Seeds
25(4.9) <sup>d</sup>	12.5 (3.6)	37.5 (6.1)	25 (5.0)	50	
33.3(5.7) <sup>c</sup>	25 (5.0)	37.5 (6.1)	37.5	100	
<sup>cd</sup>	37.5(6.1)	37.5 (6.1)	37.5	25	Leaves
49.8(6.9) <sup>c</sup>	62.1(7.5)	50(7.1)	37.5	50	
<sup>bc</sup>	62.1(7.5)	62.5(7.9)	50(7.1)	100	
79.1(8.8) <sup>a</sup>	81.2(9.0 )	75(8.6)	81.2(9.0	25	Fungicide Tilt
93.7(9.6) <sup>a</sup>	93.7(9.7)	96.8(9.8)	90.6(9.5	50	
100(10.0)	100(10.0)	100(10.0)	100	100	
0(0.7) <sup>f</sup>	0 (0.7)	0 (0.7)	0 (0.7)	Control	
	.5592			SE±	
	17.54			C.V. (%)	
	1.650			LS.D	

Any two mean value (s) bearing different superscripts (s) are differing significantly ( $p < 0.05$ ).

□ Data in parentheses transformed using square root transformation ( ) before analysis.

**Figure Effect of the *coffee senna* seeds and leaves ethanolic extracts and Fungicide Tilt on the growth of *Fusarium solani f.sp.eumartii* 48 hour after incubation.**

**Table Effect of the *coffee senna* seeds and leaves ethanolic extracts and Fungicide Tilt on the growth of *Fusarium solani f.sp.eumartii*. 72 hour after incubation.**

Inhibition zone (%)				Con .%	Plant part
Mean	R3	R2	R1		
16.6(4.0) <sup>e</sup>	8.3(29)	16.6(4.1)	25 (5.0)	25	Seeds
27.7(5.2) <sup>d</sup>	(25 (5.0	33.3(5.8)	25 (5.0)	50	
33.3(5.8) <sup>c</sup>	33.3(5.8)	41.6(6.4)	25 (5.0)	100	
<sup>de</sup>	33.3(5.8)	33.3(5.8)	25 (5.0)	25	Leaves
38.8(6.2) <sup>d</sup>	33.3(5.8)	41.6(6.4)	41.6(6.4)	50	
47.2(6.8) <sup>c</sup>	50(7.1)	50(7.1)	41.6(6.4)	100	

83.2(9.1) <sup>b</sup>	84.3(9.2)	84.3(9.2)	81.2(9.0)	25	Fungicide
90.2(9.4) <sup>a</sup>	91.6(9.5)	91.6(9.5)	87.5(9.3)	50	
97.1(9.8) <sup>a</sup>	97.9(9.9)	97.9(9.9)	95.5(9.8)	100	
0(0.7) <sup>f</sup>	0 (0.7)	0 (0.7)	0 (0.7)	Control	
2106.				SE±	
17.67				C.V. (%)	
.6211				L.S.D	

Any two mean value (s) bearing different superscripts (s) are differing significantly ( $p < 0.05$ ).

□ Data in parentheses transformed using square root transformation ( ) before analysis.

**Figure** Effect of *coffee senna* Leaves and Seeds ethanol extract and Fungicide on *Fusarium solani f.sp.eumartii* after 72hour.

No significantly different from all concentrations of Seeds and Leaves ethanolic extract of *C. occidentalis*.

The obtained (Table 3 and Figure 3) showed that all plant extracts of *C. occidentalis* concentrations as well as that of the fungicide were invariably continued exhibiting inhibitory effects against the fungal growth. However, all concentrations of the Leaves and Seeds ethanolic extract of *C. occidentalis* (25%, 50% and 100%) (6.2, 6.1, 7.1)(5.2, 6.2 and 6.1) respectively gave significantly

inhibition zones percent. Similarly the all concentrations of fungicide Tilt gave not significantly reduction in growth after 96 hrs of application. Furthermore, the Fungicide Tilt at all concentrations tested (25%, 50% and 100%) (8.8, 9.1 and 9.7) respectively continued to be the most suppressive, followed in descending order by the Seeds ethanolic extract and Leaves ethanolic extract of *C.occidentalis*.

This inhibitory effect from all concentrations tested was significantly different from control (Table, 4 and Fig. 4) all concentrations of the seeds and leaves ethanolic extract of coffee senna concentration gave significantly higher reduction in growth than the control after 120 hrs of application. The highest concentration of leaves ethanolic extract of *Cassia occidentalis* (100%)(6.3) generate inhibitory effect which were comparable and not significantly different than the inhibition caused by the highest concentration of seeds ethanolic extract of *C. occidentalis* (100%)(6.6). In fact, all tested concentrations of Leaves, Seeds and fungicide induced a significantly higher inhibition zones percentage against test fungus compared to control (Table, 4). Obviously, the test organism differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration. This inhibitory effect from all concentrations was significantly different from control.

**Table The inhibitory effect of the coffee senna seeds and leaves ethanolic extracts and Fungicide on *Fusarium solani f.sp.eumartii* after 96 hour.**

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Inhibition zone (%)

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Mean	R3	R2	R1	Con.	Plant parts
33.8(5.8)	31.5(5.6)	30(5.5)	40(6.3)	25	Seeds
35.4(6.2)	26.3(6.0)	45(6.7)	35(5.9)	50	
42.2(6.5) <sup>b</sup>	36.8(6.1)	45(6.7)	45(6.7)	100	
38.8(6.2)	31.5(5.6)	45(6.7)	40(6.3)	25	Leaves
37.2(6.1)	36.8(6.1)	35(5.9)	40(6.3)	50	
50.7(7.1) <sup>b</sup>	42.1(6.5)	50(7.1)	60(7.7)	100	
78.7(8.8) <sup>a</sup>	76.3(8.7)	75(8.6)	85 (9.2)	25	Fungicide
84.6(9.1) <sup>a</sup>	82.8(9.1)	87.5(9.3)	83.7(9.1)	50	
94.9(9.7) <sup>a</sup>	96.0(9.8)	95(9.7)	93.7(9.7)	100	
0(0.7) <sup>c</sup>	0 (0.7)	0 (0.7)	0 (0.7)		Control
	.5379				SE±
	6.42				C.V. (%)
	1.587				L.S.D

Any two mean value (s) bearing different superscripts (s) are differing significantly ( $p < 0.05$ ).

Data in parentheses transformed using square root transformation (s) before analysis.

**Figure** The inhibitory effects of *coffee senna* Leaves and Seeds ethanol extract and Fungicide on *Fusarium solani f.sp.eumartii* after 96hour incubation.

**Table** The inhibitory effect of the *coffee senna* seeds and leaves ethanolic extracts and Fungicide on *Fusarium solani f.sp.eumartii* after 120 hour incubation.

Mean	Reduction growth			Con. %	Plant part
	R3	R2	R1		
26.5(5.1) <sup>c</sup>	20.0(4.5)	25(5.0)	34.6(5.9)	25	Seeds
35.8(5.9) <sup>c</sup>	40(6.3)	29.1(5.4)	38.4(6.2)	50	
43.9(6.6) <sup>b</sup>	40(6.3)	45.8(6.8)	46.1(6.8)	100	
29.0(5.4) <sup>c</sup>	32(5.7)	29.1(5.4)	26.1(5.2)	25	Leaves
34.5(5.8) <sup>c</sup>	36(6.0)	29.1(5.4)	38.4(6.2)	50	
39.8(6.3) <sup>b</sup>	36(6.0)	37.5(6.1)	46.1(6.8)	100	
78.8(8.8) <sup>a</sup>	77(8.8)	76(8.7)	83.6(9.1)	25	Fungi cide
83.9(9.1)	80(8.9)	86.4(9.3)	85.5(9.2)	50	
93.4(9.4)	95(9.7)	93.7(9.7)	91.7(9.0)	100	
0 (0.7) <sup>d</sup>	0 (0.7)	0 (0.7)	0 (0.7)	Control	
		.1732			SE±
		6.91			C.V. (%)
		.5110			L.S.D

Any two mean value (s) bearing different superscripts (s) are differing significantly ( $p < 0.05$ ).  
Data in parentheses transformed using square root transformation ( $\sqrt{\quad}$ ) before analysis.

**Figure The inhibitory effect of coffee senna Leaves and Seeds ethanol extracts and Fungicide on *Fusarium solani f.sp.eumartii* after 120 hour incubation.**

## CHAPTER FIVE

### DISCUSSION

Several species of the genus *Fusarium* are ubiquitous fungal pathogens in a wide variety of crops. Dry rot is caused by several species of *Fusarium spp.* in potato tubers. *F. eumartii* infects tubers at wounded sites causing lesions on the surface that extend deeply in the tuber tissue producing a visible rot.

Dry rot is an important post-harvest disease that affects tubers in storage and seed pieces after planting causing important crop losses. Nowadays, dry rot is combated in the fields with chemical fungicides. This therapeutic approach for killing organisms with toxic chemicals has been the prevailing pest control strategy for over

50 years. However the Botanical control is likely to be an integrated part of the disease management strategy for many crops in the near future. Botanical control is becoming an accessory complement for safe and effective plant disease management with increasing knowledge about effect of chemical pesticides on the environment, as many chemicals may no longer be available.

Present investigations indicated that the *in vitro* growth of *F. solani* was significantly checked by Ethanol extracts of *Coffee senna* (*Cassia occidentalis* L) Leaves and Seeds at all concentration. Results showed that, the highest inhibitory effect of Leaves ethanol extract of *Coffee senna* was inhibitory more than Seeds ethanol extract. This could be due to the stem, bark and leaf extract of *C.occidentalis* have been found to contain important phytochemicals such as anthraquinones, carbohydrates, glycosides, cardiac glycoside, steroids, flavanoids, saponins, phytosterols, gum and mucilage (Colle et al.,2003). The inhibitory effect against the growth of tested fungi increases with increase in concentration and the bioefficacy of plant extracts has been shown to be affected by the extracting,

concentration and test organism. This plant also includes wide range of chemical compounds such as achrosin, aloe-emodin, emodin, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol and chrysoeriol (Dave and Ledwani, 2012). Terpenoids, flavonoids and anthraquinone derivatives have been detected in active fractions obtained from the leaf extract. Also in a separate investigation, new C-glycosidic flavonoids (cassia occidentalis A, B and C) were isolated from this plant (Dharani *et al.*, 2010).The obtained results were in line with that of Tona (1999).The data presented in this study in agreement with the result of work Tona (1999). As demonstrated by many researchers there are a considerable interest in the use of *Cassia occidentalis* ,for controlling various fungal diseases in plants ( Even (2002), Sharma (2007)and Yadav (2010).

## **CONCLUSIONS:**

The study also confirmed that the plant *Coffee senna* is potential sources of antimicrobial agents and indicated as well the

promising potentials of Coffee senna seeds and leaves in management of plant fungal diseases.

Overall, the present study indicates the antimicrobial properties of leaves extract of *C. occidentalis* and provides some idea about photochemical evaluation on *C. occidentalis*. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

#### **RECOMMENDATIONS:**

Based on the foregoing results the following studies were recommended;

- Screen a group of local medicinal plants against economically important plant diseases.
- Carry out a phytochemical analysis of different medicinal plants extract using different solvents so as to determine the bioactive ingredient in each of the test plants.
- Investigate the antimicrobial potentials of all parts of *Coffee senna* plant.

#### **REFERENCES**

Abdelgadir, K. E. (2003) Survey of city experiences with credit and investment for urban agriculture intervention, Sudan Case: Wadramli Cooperative Society (WACS)

- Abdullahi, N. (2011). Evaluation of the efficacy of different concentrations of mixed leaf powder of *Vittallaria paradoxa* and *Cassia occidentalis* against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) on stored cowpea seeds. *Bajopas*, 4 (1) : 94 - 97.
- Anderson, B.S., Hunt, J.W., Phillips, B.M., Nicely, P.A., Vlaming, V. de, Connor, V., Richard, N., and Tjeerdema, R. S. (2003) AOAD (2006) Yearly statistical book. Arab Organization for Agricultural Development. Khartoum, Sudan. AOSTAT". [faostat.fao.org](http://faostat.fao.org). Retrieved 25 January 2015.
- Cho, J. H., Rupe, J. C., Cummings, M. S., and Gbur, E. E. J. (2001) Isolation and identification of *Fusarium solani* f. sp. *glycines* from soil on modified Nash and Snyder's medium. *Plant Dis.* 85:256-260.
- "Cookbook:Potato - Wikibooks, open books for an open world". [En.wikibooks.org](http://En.wikibooks.org). 17 September 2011. Retrieved 16 October 2012.
- Colle, F., Kaminsky, R., Nkunya, M. H., Ogwal, E. N. and Brun, R. (2003). Trypanocidal activity of African plants. *Journal of Ethnopharmacology*, 5: 1-11.
- Dave, H. and Ledwani, L. (2012). A review on anthraquinones isolated from *Cassia* species and their applications. *Ind. J. Nat. Prod. Resour.*, 3 (3) : 291- 319.
- Dharani, N., Rukunga, G., Yenesew, A., Mbor, A., Mwaura, L., Dawson, I. and Jamnadass, R. (2010). Common antimalarial trees and shrubs of East Africa: a description of species and a guide to cultivation and conservation through use.

- Dawson 1 ed. The World Agroforestry Centre (ICRAF), Nairobi, Kenya, pp 40 - 42.
- Dweivedi, S. C. and Kumar, R. (1998). Evaluation of *Cassia occidentalis* leaf extract on development and damage caused by *Trogoderma granarium*, Khapra beetle. *Journal of Ecotoxicology and Environmental Monitoring*, 8: 55 -5 8.
- El.korashy, M (1997) Effect of some plant extracts against damping-off disease of peanut plant. *J. Agric. Sci. Mansoura Univ.* 22: 1921-1929.
- Elsir M. Elamin, A. (2005) Profitability analysis of potato production in the Sudan, *ARC Journal*, Volume 5, pp.97-114
- FAO (2007and 2008) The annual statistical report. Food and Agriculture Organization. Italy, Rome
- Gachango, E, L E. Hanson, A Rojas, J J. Hao, and W W. Kirk. "Fusarium spp. Causing Dry Rot of Seed Potato Tubers in Michigan and Their Sensitivity to Fungicides." *Plant Disease* 96.12 (2012): 1767-74. Print.
- Gupta, S., Sharma, P. and Soni, P. L. (2005). Chemical modification of *Cassia occidentalis* seed gum: carbamoylethylation, *Carbohydr Polym*, 59(4) : 501 - 506.
- Howard, D.H. (2003). Pathogenic fungi in human, animal, .Via ,.Google Books ISB, No. 8247. 683.8.
- Ibrahim, H. C., Ajagbonna, O. P., Mohammed, B. Y. and Onyeyili, P. A. (2010). *Cassia occidentalis* as a natural agent for the treatment of trypanosomiasis: A Preliminary report. In: *Proceedings of the 39th NVMA conference, Sokoto Nigeria.* pp 93 - 94.



- Kiran, K., Linguraju, S. and Adiver, S. (2006). Effect of plant extract on *Sclerotium rolfsii*, the incitant of, stem rot of ground nut, *J. Mycol. Pl. Pathol.*, Pathol.36: 77-79.
- Koenning, S, .(2001) Soybean Sudden Death Syndrome, Soybean Disease Information Note 7 Plant Pathology Extension, North Carolina
- Lienard, V., Seck, D., Lognay, G., Gaspar, C. and Severin, M. (1993). Biological activity of *Cassia occidentalis* L. against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *J. Stored Prod. Res.*, 29 (4) : 311 - 318.
- Loria, Rosemary. "Vegetable Crops: Fusarium Dry Rot of Potato." *Vegetable MD Online*. Department of Plant Pathology, Cornell University, Aug. 1993. Web. 22 Oct. 2013.  
[https://www.vegetablemdonline.ppath.cornell.edu/factsheets/Potato\\_Fusarium.htm](https://www.vegetablemdonline.ppath.cornell.edu/factsheets/Potato_Fusarium.htm)
- Mamatha, M.G. (2004). Studies on, foliar, diseases of, Turmeric crop M.Sc. (Agri).Thesis, Univ. of Agric. Sci. Dharwad (India).
- Mariod, A. and Matthäus, B. (2008). Physico-chemical properties, fatty acid and tocopherol composition of oils from some Sudanese oil bearing sources. *GRASAS Y ACEITES*, 59 (4): 321- 326.
- Mustapha, L., Angela, O. and David, M. Y. (2013). Anti-trypanosoma activity of the ethanolic leaf extract of *Senna occidentalis* (Fabaceae) on *Trypanosoma brucei brucei* infected mice. *International J. of Basic and Applied Sciences*, 2 ( 1) : 32 - 37.
- Nassar, M. A. A., Ramadan, H. R. H. and Ibrahim, H.M. S. (2011). Morphological characteristics of vegetative and

- reproductive growth of *Senna occidentalis* L. Link (Caesalpiniaceae). Res. J. Agric. Biol. Sci., 7(2): 260-270.
- Nassar, M. A. A., Ramadan, H. R. H. and Ibrahim, H.M. S. (2013). Anatomical structures of vegetative and reproductive organs of *Senna occidentalis* (Caesalpiniaceae). Turk. J. Bot., 37 : 542-552.
- Okigbo, R.N. (2004). A review of biological control methods for post harvest yams (*Dioscorea* spp.) in storage in South Eastern Nigeria. KMITL Sci J. 4(1): 207 - 215.
- Omer, S. A., Al-Olayan, E. M., El-Amin, M. H., Hassan, Z. K., Daghestani, M.H. and Mohammed, O. B. (2012). Experimental *Cassia senna* intoxication in Lohmann broiler chicks. J. Med. Plants Res., 6 (17) : 3306 - 3310.
- Orzolek, M.D., Greaser, G.L., & Harper, J.K. 2010. *Commercial Vegetable Production Guide*. Penn State Cooperative Extension  
Agricultura  
Alternatives: The Pennsylvania State University.
- Paisson, K. and Jaenson, T. G. T. (1999). Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Tropica, 72: 39- 52.
- Picard, Andre (July 6, 2002). "Today's fruits, vegetables lack yesterday's nutrition". Globe and Mail. Retrieved February 16, 2015.
- Podsilva, V. (2003). Gastric cytoprotective antiulcer effects of the leaf of methanol extract of *Senna occidentalis* (Fabaceae) in rats. Journal of Ethnopharmacology, 82: 69-74.

- Ramasamy, P., Rajan, P.R. Jay Kumar, R. Rani, S. and Brenner, G. (1996). Infection and its control in cultured larval Indian tiger prawn, *Penaeus* New York.
- Rowe, L. D., Corrier, D. E., Reagor, J. C. and Jones, L. P. (1987). Experimentally induced *Cassia roemeriana* poisoning in cattle and goats. *Am. J. Vet. Res.*, 48 : 92-97.
- Sadiq, I. S., Shuaibu, M., Bello, A. B., Tureta, S. G., Isah, A., Izuagie, T., Nasiru, S. and Kamaru, B. (2012). Phytochemistry and antimicrobial activities of *Cassia occidentalis* used for herbal remedies. *J. of chem. Engineering*, 1 ( 1) : 38.
- Shenoi, M.M., Murthy, K.K, Sreen, Vas, S.S., and Wajid, S.M.A. (1998)., In vitro. Evaluation, of botanicals, for mycotoxic properties against *Alternaria alternate* causing ,brown spot disease, of tobacco, *Tobacco Research*, (1998).24:77-81.
- Singh, S., Singh, S.K. and Yadav, A. (2013). A review on *Cassia* species: pharmacological, traditional and medicinal aspects in various countries. *AJPCT*, 1 (3) : 291- 312.
- Singh NP, Bhardway AK, Kumar A, Singh KM (2004) *Modern Technology on Vegetable production*. International book distributing co. India.
- Sini, K. R., Sinha, B. N., Karpakavalli, M. and Sangeetha, P. T. (2011). Analgesic and antipyretic activity of *Cassia occidentalis* Linn. *Annals of Biological Research*, 2 (1): 195 - 200.

- Spooner, David M.; McLean, Karen; Ramsay, Gavin; Waugh, Robbie; Bryan, Glenn J. (2005). "A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping". PNAS 102 (41): 1469499.doi:10.1073/pnas.0507400102. PMC 1253605. PMID 16203994.
- Suliman, H. B. and Shommein, A. M. (1986). Toxic effect of the roasted and unroasted beans of *Cassia occidentalis* in goats. Vet. Hum. Toxicol., 28 : 6 -11.
- Taiwo F. O., Akinpelu D. A., Aiyegoro O. A., Olabiyi S. and Adegboye M. F. (2013) The biocidal and phytochemical properties of leaf extract of *Cassia occidentalis* linn. Afr. J. Microbiol. Res., 7 (27) : 3435 – 3441.
- Tona, L., Cimanga, R. K., Mesia ,K., Musuamba, C. T., De Bruyne, T., Apers, S., Hernans, N., Miert, S. V., Pieters, L., Totte, J. and Vlietinck, A. J. (2004).
- Vashishtha, V.M., John, T.J. and Amod, K. (2009). Clinical and pathological features of acute toxicity due to *Cassia occidentalis* in vertebrates. Ind. J. Med. Res., 130: 23-30.
- <http://www.potatodiseases.org/dryrot.html>
- Warton, Phillip, Ray Hammerschmidt, and William Kirk. "Potato Diseases: Fusarium Dry Rot." Potato Diseases. Michigan State University, May 2007.
- Web. 22 Oct. 2013. <http://www.potatodiseases.org/dryrot.html>
- Wilson. C.L., Ghaouth.A.E., and, Wisniewski., M.E. (1999). Prospecting in nature's storehouse for biopesticides,

Mexican Journal of physiopathology, Vol. 17, No. 1, (June 1999), pp. (49-53), ISSN 0185-3309

Yadav, J.P., Arya, V., Yadav, S. , Panghal, M., Kumar, S. and Dhankhar, S. (2010) *Cassia occidentalis* L. : A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, 81: 223 - 230.

Yadava, R. N. and Satnami, D. K. (2011). Chemical constituents from *Cassia*

*occidentalis* Linn. *Ind. J. Chem.*, 50B:1112-1118.

Zaccardelli, M., Vitale, S., Luongo, L., Merighi, M., Corazza, L. 2008. Morphological and Molecular Characterization of *Fusarium solani* Isolates *J. Phytopathology*: 156, 534-541.

## APPENDIXES

### Appendix (1)

Reduction percentage after 3th days of inoculums

Reduction			Con. %	Plant part
R3	R2	R1		
0.7	0.6	0.8	25	Seeds
7	0.5	0.6	50	
0.6	0.5	0.5	100	
0.5	0.5	0.5	25	Leaves
0.3	0.4	0.5	50	
0.3	0.3	0.4	100	

0.125	0.125	0.15	25	Fungicide
0.05	0.025	0.075	50	
0	0	0	100	
0.8	0.8	0.8		Control

## Appendix (2)

Reduction percentage after 4th days of inoculums

Reduction			Con. %	Plant part
R3	R2	R1		
1.1	1	0.9	25	Seeds
0.9	0.8	0.9	50	
0.8	0.7	0.9	100	
0.8	0.8	0.9	25	Leaves
0.8	0.7	0.7	50	
0.6	0.6	0.7	100	
0.225	0.3	0.225	25	Fungicide
0.1	0.1	0.15	50	
0.025	0.025	0.05	100	
1.2	1.2	1.2	control	

### Appendix (3)

Reduction percentage after 5th days of inoculums

Reduction			Con. %	Plant part
R3	R2	R1		
1.3	1.4	1.2	25	Seeds
1.4	1.1	1.3	50	
1.2	1.1	1.1	100	
1.3	1.1	1.2	25	Leaves
1.2	1.3	1.2	50	
1.1	1	0.8	100	
0.45	0.5	0.3	25	Fungicide
0.325	0.25	0.325	50	
0.075	0.1	0.125	100	
1.9	2	2	control	

### Appendix (4)

Reduction percentage after 6th days of inoculums

Reduction			Con. %	Plant part
R3	R2	R1		
2	1.8	1.7	25	Seeds
1.5	1.7	1.6	50	
1.5	1.3	1.4	100	
1.7	1.7	1.9	25	Leaves
1.6	1.7	1.6	50	
1.6	1.5	1.4	100	
0.575	0.575	0.425	25	Fungicide
0.35	0.325	0.375	50	
0.125	0.15	0.475	100	
2.5	2.4	2.6		Control

**Appendix (5)**

Inhibition after 3th days of inoculums

ANALYSIS OF VARIANCE TABLE(1)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	7	166.440	23.777	24.779	0.0000
Within	16	15.353	0.960		
Total	23	181.793			

Coefficient of Variation = 17.54%



### Appendix (6)

#### ANALYSIS OF VARIANCE TABLE(2)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	7	148.293	21.185	24.005	0.0000
Within	16	14.120	0.883		

Total 23 162.413  
Coefficient of Variation = 17.67%

### Appendix (7)

#### ANALYSIS OF VARIANCE TABLE(3)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	7	135.347	19.335	127.485	0.0000
Within	16	2.427	0.152		

Total 23 137.773  
Coefficient of Variation = 6.42%

### Appendix (8)

#### ANALYSIS OF VARIANCE TABLE(4)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	7	133.880	19.126	121.433	0.0000
Within	16	2.520	0.158		

Total 23 136.400  
Coefficient of Variation = 6.91%

**APPENDIX (9) Seed of coffee senna  
Powder of coffee senna**

**APPENDIX (10)**

**APPENDIX (11) Infected potatoes by Dry rot disease  
Reduction in fungal growth after 5<sup>th</sup> days of incubation**

