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

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

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2015
Dedication

I would like to dedicate this work to 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
ACKNOWLEDGEMENTS

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A lot of thanks are due to my dearest mother for her help, support and encouragement throughout my life.

I express my thanks and sincere gratitude to my supervisor Dr: Ekhlass Hussein and Ustaz: Mohammed Alziber Hassan Alziber for his help during this study.

For this guidance and support, finally thanks are due to all my friends especially to Ahmad Abd Elm geed –and Waleed Elamain and I wish them a happy life and good luck in the future.

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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*. Wlti 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 *Coffee 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.
إجربت هذه الدراسه بمعامل أمراض النباتات بكلية الدراسات الزراعية, جامعة السودان للعلوم والتكنولوجيا لمكافحة مرض الزبول في محصول البطاطس الذي يسببه الفطر فوزاريم سولاني, الذي يسبب انخفاض كبير في انتاجية محصول البطاطس. في الدراسة الحاليه تم عزل الفطر السبب للمرض من أجزاء النباتات المصابة . تم تعريف الفطر على أساس الصفات المورفولوجية والمزرعية ينتمي فيوزاريم سولاني. اثر انتشار الفطري المسؤول عن المحصول للعديد من الخصائص الإيثانولية لأوراق وبذور نبات السوريب (Cassia occidentalis L) . ومبيد التلت (250 تlt) لمكافحة الفطر السبب لمرض الزبول في البطاطس. استخدمت تراكيز مختلفة (25,50, و100%) من المستخلصات النباتيه في هذه الدراسة واظهرت جميع المستخلصات النباتيه انخفاض معنوي كبير في نمو الفطر. النتائج التي تم الحصول عليها وضح ان تأثير المستخلص العذرلي لأوراق وبذور نبات السوريب والمبيد في كل التكبيرات أثرت على نمو الفطر المختبر مقارنه بالشاهد.التركيز الأعلي المستخدم في هذه الدراسة (100%) للاوراق والبذور. في كل من المستخلص العذرلي لأوراق وبذور السوريب والمبيد سجلت أعملا تثبيط لنمو الفطر. على أية حال، التأثير التثبيطي للمستخلص العذرلي لأوراق السوريب على الفطر أكثر وضوحا من المستخلص العذرلي للمبيد. عموما تزيد منطقه التثبيط بزيادة تركيز المستخلصات. نتيجة لذلك، توضح هذه الدراسة بأن أوراق وبذور نبات السوريب تحتوي على مواد ذات تأثير مضاد لنمو الفطريات، يمكن أن تتحري في دراسات أخرى.

عمل المستخلصات النباتيهسهل ومتوفر ويمكن تحضيره بسهولة للسيطرة على أمراض النبات. كما انه غير مكلف، وليس له خطوره على الإنسان وفعالة ولا يؤثر على البيئة.
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 Jebal 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 Jebal 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 (*Lowsonia inermis*) 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 (*Lycopersicum esculentum*), sweet pepper (*Capsicum annuum*), 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 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

<table>
<thead>
<tr>
<th>Nutritional value per 100 g (3.5 oz)</th>
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<tr>
<td>Energy</td>
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<tr>
<td>Carbohydrates</td>
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<td>Starch</td>
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<tr>
<td>Dietary fiber</td>
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<td>Fat</td>
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<td>Nutrient</td>
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<td>--------------------------</td>
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<tr>
<td>Protein</td>
</tr>
<tr>
<td>Thiamine (B1)</td>
</tr>
<tr>
<td>Riboflavin (B2)</td>
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<tr>
<td>Niacin (B3)</td>
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<tr>
<td>Pantothenic acid (B5)</td>
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<tr>
<td>Vitamin B6</td>
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<tr>
<td>Folate (B9)</td>
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<td>Vitamin C</td>
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<td>Nutrient</td>
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<td>Vitamin K</td>
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<td>Potassium</td>
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<td>Sodium</td>
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<tr>
<td>Zinc</td>
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</tbody>
</table>
### Other constituents

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>75 g</td>
</tr>
</tbody>
</table>

(Picard Andre, 2002).

#### 2.1.3 Uses

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 potatoes, yogurt 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 : Sordariomycetes
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. Chlamydospores 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 chlamydomspores 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 filed 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 6 to 25% (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) C. occidentalis plant (a) and (b) 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, flavonoids, 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, cassinollin, 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).

2.3.7 Uses 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.*
maculates 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$_{50}$ 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 install 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 (Vashishta 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. Sensetive balance
2. Soxthlet extractor apparatus
3. Rotary evaporator
4. Petri-dishes
5. Marker pen
6. Pencle
7. Gloves
8. Masks
9. Camera
10. Electronic blender
11. Filter paper
12. Regestration form
13. Scalpel
14. Conical flask
15. Laminar
16. Water bath
17. Outoclave
18. Incubater

3.2 Materials:

1. Cassia seeds and leaves
2. Ethanol 95%
3. Distilled water
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 (7 days 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).
$$R = \frac{dc - dt \times 100}{dc}$$

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.

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<td>25(4.9)</td>
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<td>Leaves</td>
<td>49.8(6.9)</td>
<td>62.1(7.5)</td>
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<tr>
<td>Fungicide Tilt</td>
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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.

<table>
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<tr>
<th></th>
<th>Mean</th>
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<th>Con .%</th>
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<tr>
<td></td>
<td>R3</td>
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<td>Seeds</td>
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<td>16.6(4.0) e</td>
<td>8.3(29)</td>
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<td>27.7(5.2) d</td>
<td>(25 (5.0)</td>
<td>33.3(5.8)</td>
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<td>33.3(5.8) c</td>
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<tr>
<td>38.8(6.2) d</td>
<td>33.3(5.8)</td>
<td>41.6(6.4)</td>
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<tr>
<td>47.2(6.8) c</td>
<td>50(7.1)</td>
<td>50(7.1)</td>
<td>41.6(6.4)</td>
<td>100</td>
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Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

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>
<thead>
<tr>
<th>Table</th>
<th>The inhibitory effect of the coffee senna seeds and leaves ethanolic extracts and Fungicide on <em>Fusarium solani f.sp.eumartii</em> after 96 hour.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition zone (%)</td>
</tr>
<tr>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Plant parts</td>
<td>Mean</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>Seeds</td>
<td>33.8(5.8)</td>
</tr>
<tr>
<td></td>
<td>35.4(6.2)</td>
</tr>
<tr>
<td></td>
<td>42.2(6.5)</td>
</tr>
<tr>
<td></td>
<td>38.8(6.2)</td>
</tr>
<tr>
<td></td>
<td>37.2(6.1)</td>
</tr>
<tr>
<td></td>
<td>50.7(7.1)</td>
</tr>
<tr>
<td>Leaves</td>
<td>78.7(8.8)</td>
</tr>
<tr>
<td></td>
<td>84.6(9.1)</td>
</tr>
<tr>
<td></td>
<td>94.9(9.7)</td>
</tr>
<tr>
<td>Fungicide</td>
<td>0(0.7)</td>
</tr>
</tbody>
</table>

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  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.

<table>
<thead>
<tr>
<th>Reduction growth</th>
<th>Con. % Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>R3</td>
</tr>
<tr>
<td>26.5(5.1)c</td>
<td>20.0(4.5)</td>
</tr>
<tr>
<td>35.8(5.9)c</td>
<td>40(6.3)</td>
</tr>
<tr>
<td>43.9(6.6)b</td>
<td>40(6.3)</td>
</tr>
<tr>
<td>29.0(5.4)c</td>
<td>32(5.7)</td>
</tr>
<tr>
<td>34.5(5.8)c</td>
<td>36(6.0)</td>
</tr>
<tr>
<td>39.8(6.3)b</td>
<td>36(6.0)</td>
</tr>
<tr>
<td>78.8(8.8)a</td>
<td>77(8.8)</td>
</tr>
<tr>
<td>83.9(9.1)</td>
<td>80(8.9)</td>
</tr>
<tr>
<td>93.4(9.4)</td>
<td>95(9.7)</td>
</tr>
<tr>
<td>0 (0.7)d</td>
<td>0 (0.7)</td>
</tr>
</tbody>
</table>

.1732  
6.91  
.5110  
SE±  
C.V. (%)  
L.S.D
Any two mean value(s) bearing different superscripts (s) are differing significantly (p<0-0.5).
Data in parentheses transformed using square root transformation () 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 significant 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 glycosidea, 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 a 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.

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http://www.potatodiseases.org/dryrot.html


APPENDIXES

Appendix (1)

Reduction percentage after 3th days of inoculums

<table>
<thead>
<tr>
<th>Reduction</th>
<th>Con.</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>0.6</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
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</table>
### Appendix (2)

Reduction percentage after 4th days of inoculums

<table>
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<th>Reduction</th>
<th>Con.</th>
<th>Plant part</th>
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</thead>
<tbody>
<tr>
<td>R3</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>1.1</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>0.8</td>
<td>0.7</td>
<td>0.9</td>
</tr>
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<td>0.7</td>
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</tr>
<tr>
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</tr>
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## Appendix (3)

Reduction percentage after 5th days of inoculums

<table>
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<tr>
<th>Reduction</th>
<th>Con. %</th>
<th>Plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>1.3</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>1.4</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
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<td>1.3</td>
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<td>1.1</td>
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<td>0.45</td>
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<td>1.9</td>
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## Appendix (4)
Reduction percentage after 6th days of inoculums

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</tr>
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<tbody>
<tr>
<td>R3</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>R2</td>
<td>1.8</td>
<td>50</td>
</tr>
<tr>
<td>R1</td>
<td>1.7</td>
<td>100</td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>1.5</td>
<td>25</td>
</tr>
<tr>
<td>R2</td>
<td>1.7</td>
<td>50</td>
</tr>
<tr>
<td>R1</td>
<td>1.6</td>
<td>100</td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>1.5</td>
<td>25</td>
</tr>
<tr>
<td>R2</td>
<td>1.5</td>
<td>50</td>
</tr>
<tr>
<td>R1</td>
<td>1.4</td>
<td>100</td>
</tr>
<tr>
<td>Fungicide</td>
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<td></td>
</tr>
<tr>
<td>R3</td>
<td>0.575</td>
<td>25</td>
</tr>
<tr>
<td>R2</td>
<td>0.575</td>
<td>50</td>
</tr>
<tr>
<td>R1</td>
<td>0.425</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
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Appendix (5)

Inhibition after 3th days of inoculums

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
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<td>166.440</td>
<td>23.777</td>
<td>24.779</td>
</tr>
<tr>
<td>Within</td>
<td>16</td>
<td>15.353</td>
<td>0.960</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>181.793</td>
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Coefficient of Variation = 17.54%
Appendix (6)

**ANALYSIS OF VARIANCE TABLE(2)**

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
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<td>148.293</td>
<td>21.185</td>
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<td>Within</td>
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<td>Total</td>
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</table>

Coefficient of Variation = 17.67%

Appendix (7)

**ANALYSIS OF VARIANCE TABLE(3)**

<table>
<thead>
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<th>Degrees of Freedom</th>
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<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
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<td>135.347</td>
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Coefficient of Variation = 6.42%

Appendix (8)

**ANALYSIS OF VARIANCE TABLE(4)**

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<th>Prob.</th>
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</thead>
<tbody>
<tr>
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<td>Within</td>
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<td>Total</td>
<td>23</td>
<td>136.400</td>
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</tr>
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</table>

Coefficient of Variation = 6.91%

Appendix (9) Seed of coffee senna

APPENDIX (10) Powder of coffee senna
APPENDIX (11) Infected potatoes by Dry rot disease
Reduction in fungal growth after 5\textsuperscript{th} days of incubation

\begin{tabular}{|c|c|}
\hline
L50\% & L100\% \\
\hline
L25\% &  \\
\hline
Control &  \\
\hline
S100\% & S50\% \\
\hline
S25\% & Control \\
\hline
\end{tabular}