

بسم الله الرحمن الرحيم



Sudan University of Science and Technology

College of Agricultural Studies

Plant Protection Department



Evaluation of the efficacy of Coriander Extracts against wilt disease caused by Fusarium solani in Potato plant

تقييم فعالية مستخلص الكسبره ضد مرض الزبول علي نبات البطاطس الذي يسببه فطر فيوزيريوم سولاني

By

Shima Omer Elhaj

Sudan University of Science and Technology College of Agricultural Studies – Shambat

B.Sc. Agric. (Honors), 2016

Supervisor

Supervisor

Dr .Ekhlash Hussien Mohammed

September 2016

بسم الله الرحمن الرحيم

الآية

قال تعالى:

(وَوَضَّلْنَا عَلَيْكَ الْغَمَامَ وَأَنْزَلْنَا عَلَيْكَ الْمَنَّاءَ وَالسَّلْوَىٰ كُلًّا مِنْ طَيِّبَاتِ
مَا رَزَقْنَاكَ وَمَا ظَلَمُونَا وَلَكِنْ كَانُوا أَنْفُسَهُمْ يَظْلِمُونَ (٥٧))

صدق الله العظيم

سورة البقرة (57)

Dedication

To my parents

*To my friends and to everyone who helped me in
this research*

With Love

ACKNOWLEDGEMENTS

I would like very much to render His Almighty Allah who gives me the power and health to complete this work.

I would also like to express my sincere gratitude to my supervisor. **Dr .Ekhlash Hussien Mohammed** for her keen interest, constant guidance, help and encouragement throughout the course of this study to bring this work to reality. . It has been a privilege and a pleasure to work with her.

I will also take the opportunity to express my sincere thanks to **Ustaza. Mawada Ibrahim** for her help throughout the study.

My Sinsere gratitude is also extended to **Ustaza Khansaa Alfa Hashim** who statistically analysed this research.

My thanks are also extended to all my friends and colleagues who stand before me to complete this study.

TABLE OF CONTENTS

	Page
الآية	I
Dedication	II
Acknowledgements	III
Table of contents	IV
Abstract.....	V
الملخص العربي.....	VI
Chapter I	1
INTRODUCTION	1
Chapter II	4
LITERATURE REVIEW	4
Chapter III	16
Materials & Methods	16
Chapter IV	20
Results & Discussion	20
References	37
Images	38

Abstract

This study was carried out in the laboratory of the Department of plant protection ,College of Agricultural Studies, Sudan University of Science and Technology, Shambat area during December and January 2015-2016. The objective of this study is to evaluate the efficacy of Coriander aqueous extracts and fungicides against Fusarium wilt disease in Potato.

The result showed that the treatment were effective in the inhibition of the fungi *Fusarium oxysporum*.

Coriander (50% - 25%) and showed inhibition growth the Fusarium wilt, all the treatments used inhibition zone of the growth fungi.

الملخص

أجريت هذه الدراسة في معمل قسم وقاية النبات, (معمل امراض النبات) كلية الدراسات الزراعية, جامعة السودان للعلوم والتكنولوجيا (شمبات) خلال ديسمبر 2015-2016م . تهدف هذه الدراسة الي تقييم فعالية المحلول الكحولى لبذور الكزبرة والمبيد الفطري (التلت) ضد فطر الذبول في البطاطس.

أوضحت النتائج انها توجد اختلافات بين المعاملات فيما بينها . أوضحت المعاملات فعالية في تثبيط نمو فطر الفيوزاريوم.

كل التراكيز المستخدمة فعالة في تثبيط نمو الفطر.

CHAPTER ONE

INTRODUCTION

Potato (*Solanumtuberosum*) is a starchy, tuberouscrop from the perennialnightshade*Solanumtuberosum* L. The word "potato" may refer either to the plant itself or the edible tuber. In the Andes, where the species is indigenous, there are some other closely related cultivated potato species. Potatoes were introduced outside the Andes region approximately four centuries ago, and have since become an integral part of much of the world's food supply. It is the world's fourth-largest food crop, following maize, wheat, and rice. Wild potato species occur throughout the Americas from the United States to southern Chile.

In terms of global production, potato (*Solanumtuberosum* L.) is the fourth most important food crop after corn, rice and wheat. This crop is grown throughout the world. Present world production is some 321 million tons fresh tubers from 19.5 million ha.

The potato plays a strong role in developing countries with its ability to provide nutritious food for the poor and hungry. The demand for potato is growing as both a fresh and processed food. The decreasing availability of land for area expansion means that yields will have to be improved. Critical to achieving improved tuber yields will be access to an adequate water supply, including more efficient use of scarce water and costly fertilizer inputs. Potato is grown in about 100 countries under temperate, subtropical and tropical conditions.

Fusarium solani sp. *euemartii* 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).

Pesticides are considered indispensable for sustainable agriculture production, in addition to their role in the protection of human health especially in the tropics. (Karan, *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 (Akimbo, and Carvel, 2004). 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 (Research Council Board of Agriculture, 1987). Accordingly, increasing efforts have been primarily directed towards minimizing pesticides risks in the environment through ecologically sound innovative measures of diseases control (Guideword, *et.al*, 1990).

Recently, the uses of natural products for crop protection were greatly emphasized by scientists in everywhere (Guideword, *et.al*, 1990).

Medicinal plants have become the focus of intense study in terms of validation of their traditional uses, and then it can be used as a natural pesticides. These pesticides are generally more selective in their action, economically feasible and less harmful to the environment than synthetic chemicals. (Songhua and Michailides, 2005).

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 to great economic losses.

Based on the foregoing this study was undertaken to focus on investigation of three components for management of Fusarium dry rot of potato caused by *Fusarium solani* f.sp. *eumartii* higher plant extracts /synthetic fungicides and biological control on green house condition in order to formulate promising disease management approach with following objective:-

The aim of this work is to find an alternative to chemical fungicides currently used in the control of plant pathogenic fungus *F. solani*.

In this work we intended to:

- 1- Isolate the fungus *F. solani* from infected Potato plant.
2. Investigate the efficacy of alternative control measures involving the Coriander ethanolextracts against the growth of *F. solani in vitro*.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Crop

Potato (*Solanumtuberosum*L.) is a member of the family Solanaceae that family includes also tomato (*Lycopersicumesculentum*), sweet pepper (*Capsicum annum*), eggplant (*S. Melongena var. esculentum*), tobacco (*Nicotianatabacum*), and petunia (*Petunia hybrida*) (Fernald, 1970).It is the world's fourth most important food.

2.1.1Scientific classification

Kingdom: Plantae (unranked):

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *S. tuberosum*

(Binomial name: *Solanumtuberosum* L.)

The genus *Solanum* is largely tropical and subtropical. 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 (Hoopes and Plaisted, 1987).

Potato is one of the major vegetable crops grown worldwide following wheat, maize, and rice, with a production estimates of 368million

tons(FAOSTAT, 2015).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 (Howard, *etal.*, 1970).

2.1.2 Potato in Sudan

Potato was apparently introduced by the British to Sudan in the early twentieth century as a home garden vegetable. Cultivation increased around army posts during the First and Second World Wars in an effort to augment British army rations (Kambora, 1978). This expansion was accompanied by agricultural research in both the northern and southern parts of the country, although these efforts were somewhat disjointed and many records have been lost (ARC, 1985). The first recorded importation of seed was in 1939 from India, and subsequent imports occurred from Kenya (El Hilo *el al.*, 1974). More systematic research dates from the early 196's (Taha, 1983), although research and farming activities have been periodically disrupted by civil and military strife in the south (Bayeh and Dirks, 1976). Although not presently a major food crop in Sudan, demand has increased steadily since independence (ARC, 1985; Geneif, 1986).

Currently, 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 Zalinge potato (Abdelgadir, 2003). The crop is an important cash crop for small-scale

growers, and has 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 (Ahmed, 1985).

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 (Elsir, 2005). This is one of the problems to further expansion of potato production (Elrasheed and Ballal, 2009). The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddans (Hind and Mohamed, 2010).

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.

The threat to potatoes from fungal infections has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977). One of the main fungal pathogens that attack potatoes is *Fusarium* spp which are a worldwide economic problem (Nielson, 1981).

2.2 Fusarium dry rot

Fusarium dry rot of potato is a devastating post-harvest losses disease affecting both seed potatoes and potatoes for human consumption. In fact, Fusarium dry rot of potatoes is a worldwide economic problem. There are many species of Fusarium reported to cause dry rot of potato Worldwide (Nielson, 1981). The disease may cause greater losses of potatoes than any other-post harvest disease. Crop losses attribute to dry rot have been estimated to an average of 6 to 25% (Powelson *et al.*, 1993). *Fusarium* species which cause dry rots are also important to the consumer because some, *Fusarium* which cause dry rots also produce mycotoxins, one of such toxins is trichothecene which is an inhibitor of eukaryotic protein synthesis and can pose serious health problem to man and animals (Beremaid *et al.*, 1991).

This fungus which prefers warmer climates causes a variety of colored rots in potatoes (Rowe *et al.*, 2013). There are many species of *Fusarium* reported to cause dry rot of potato Worldwide of which *Fusarium solani* has been reported as the most pathogenic *Fusarium* species causing potato dry rot (Sharifi *et al.*, 2009).

2.2.1 Classification

Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Sordariomycetes
Subclass:	Hypocreomycetidae
Order:	Hypocreales
Family:	Nectriaceae
Genus:	Fusarium

Species: *F. solani*

2.2.2 Host range and distribution

The predominant hosts for *Fusarium solani* are Potato, Pea, Bean, and members of the cucurbit family such as melon, cucumber, and pumpkin. Some strains may cause infections in humans. *Fusarium* damping-off, corn rot, fruit rot, root rot, and surface rot are caused by *Fusarium solani* Mart. Sacc and are found in most states in the United States (<http://www.bugwood.org/fungi.html>).

The fungus has a worldwide distribution, but its frequency as an important plant pathogen is well known and hence remains the most common disease-causing fungus in its genus (Aoki, *et al.*, 2003).

2.2.3 Description

F. solani is a filamentous fungus in the genus *Fusarium*, and the anamorph of *Haematonectria aematococca*. *F. solani* (Mart.) Sacc is a name that has been applied broadly to what is now known as the *F. solani* species complex (FSSC; O'Donnell 2000). Members of the FSSC, which includes several additional named species and currently corresponds to approximately 50 phylogenetic species (Zhang *et al.* 2006, O'Donnell *et al.* 2008), are ubiquitous in soil, plant debris and in other plant and animal substrata and can be serious plant and human pathogens (Booth 1971). The FSSC contains both heterothallic and homothallic strains and species, as well as strains that have no known sexual stage.

2.2.4 Symptoms

The fungus can be soil borne, airborne or carried in plant residue and can be recovered from any part of the plant from the deepest root to the highest flower (Summerville *et al.*, 2003).

Fusarium dry rot of potatoes a devastating post-harvest losses (vegetables) disease affecting both seed potatoes and potatoes for human consumption (Loria, Rosemary 1993). Dry rot causes the skin of the tuber to wrinkle. The rotted areas of the potato may be brown, grey, or black and the rot creates depressions in the surface of the tuber. Seed pieces may not completely before they have the chance to be planted. The genus that causes dry rot of potato, *Fusarium*, is a fungus. Signs of a pathogenic *Fusarium species* can be seen on an infected potato, and include white or pink mycelia (masses of vegetative fungal tissue) and very colorful spores that can be blue, black, purple, grey, white, yellow, or pink (Loria, Rosemary. 1993).

2.2.5 Identification and Life Cycle

Fusarium dry rot is an important postharvest disease of potato worldwide. *Fusarium* dry rot can be caused by several different *Fusarium* spp. including *F. solani*, *F. sambucinum*, *F. avenaceum*, *F. culmorum*, and *F. oxysporum*, but *F. solani* appears to be the most aggressive and important. Dry rot *Fusarium* spp. originate from contaminated seed or infested soils, infecting tubers through wounds in the periderm that are common after potato cutting and handling practices. *Fusarium* spp. introduced into soils by contaminated seed can persist for years. Soil borne inoculum can infect tubers through wounds caused by other pathogens, insects, or during harvest and handling. (Howard, *et al* 2005).

2.2.6 Environment

Warmer climates are preferred. (Warton., *et al* 2013). However; different species of *Fusarium* may be more prevalent in different areas. Michigan is currently having trouble with *Fusariumsambucinum* (Rowe, *et al* , 2013). *Fusariumsambucinum* can be found in Ohio, Michigan, Wisconsin, North Dakota, and Montana. *Fusarium* requires higher moisture to grow and is not able to when conditions are only periodic. The temperatures preferred for growth and spread are above 50F. (Rowe., *et al*, 2013).

2.2.7 Importance

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, 2012). In Michigan, over 50% of seed lots have reported having variable levels of dry rot.

Fusarium spp., are among the most important plant pathogens in the world and are highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes (Nelson, 1983). Species of *Fusarium* are responsible for vascular wilt, e.g., *F. solani*, *sp. cucurbitae*, cause crown, foot rot and fruit rot and root rot caused by *Fusarium solani* has been considered among the most deleterious diseases, which cause great losses in many parts of the world (Celar, *et al.*, 2000). *Fusarium* dry rot of potatoes is a worldwide economic problem. There are many species of *Fusarium* reported to cause dry rot of potato worldwide. The dry rot disease may cause greater losses of potatoes than any other post-harvest disease. Crop losses attributed to dry rot have been estimated to an average of 6 to 25% (Powelson *et al*, 1993).

Mycotoxins are toxic of secondary metabolites of low molecular weight compounds that do not produce immediate symptoms and they are harmful to humans (Conner, 1993; Corrier, 1997; FDA, 2004).

Fusarium species that causes dry rots are also important to the consumer, because some *Fusarium* that causes dry rots also produce mycotoxins, one of such toxins is trichothecene which is an inhibitor of eukaryotic protein synthesis and can pose serious health problem to man and animals (Beremaid *et al*, 1991).

2.2.8 Management

There are many ways to manage dry rot. This includes application of fungicides, cultural practices, sanitation, biological control and botanical pesticides. However, most techniques for managing dry rot are aimed at preventing injury to the tubers, either seed or the harvested crop. Preventing bruises will greatly aid in avoiding infection (Wartonet *al.*, 2007).

2.2.8.1 Chemical control

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. 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, *et al*, 2005).

2.2.8.2 Cultural practices

Cultural practices can also limit the spread of dry rot. This could include planting of high quality seed free from *Fusarium* dry rot pathogens into soils without a history of *Fusarium* dry rot. Varieties vary in their reaction to dry rot, and highly susceptible varieties should be avoided. Harvest tubers at least 14 days after vine kill to promote good skin set and reduce skinning injury that can increase storage dry rot. Avoid harvesting cold tubers that are more susceptible to injury. Provide conditions that promote rapid wound healing early in storage, including high humidity, good aeration, and temperatures of 55 to 64°F for 14 to 21 days. Since *Fusarium* dry rot increases with length in storage, short-term storage is advisable for fields where severe infection is expected (Howard, *et al.*, 2005).

2.2.8.3 Biological control

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., 2013). Scientists in Tunisia have found that several bacterial species of the genus *Bacillus*, commonly found in the salty soils of Tunisia, can reduce the amount of rot seen due to *Fusarium sambucinum*. *Bacillus thuringiensis* can help control dry rot.

2.3 Coriander plant

There exist very different uses of coriander and these are based on different parts of the plant. The traditional uses of the plant, which are based on the primary products, i.e. the fruits and the green herb, are two-fold: medicinal and culinary. During industrialization, the specific chemical compounds of coriander were recognized and identified, and these became important as raw materials for industrial use and further processing. The essential and fatty oils of the fruits are both used in industry, either separately or combined. After extraction of the essential oil, the fatty oil is obtained from the extraction residues either by pressing or by extraction.

A further benefit of coriander derives from the reproductive biology of this plant. Coriander produces a considerable quantity of nectar and thereby attracts many different insects for pollination, an external effect which is of both ecological and economic value. Coriander is also a good melliferous plant, and Luk'janov and Reznikov (1976) state that one hectare of coriander allows honey bees to collect about 500 kg of honey.

2.3.1 Scientific classification

Kingdom: Plantae

Order: Apiales

Family: Apiaceae

Genus: *Coriandrum*

Species: *C. sativum*

Binomial name: *Coriandrum sativum*

2.3.2 Distribution

A native of the Mediterranean region there for several thousand years, now cultivated in tropical Asia (India, Malaysia, Thailand, and china), the Middle East and Brazil. Coriander was brought to the British colonies in North America in 1670, and was one of the first spices cultivated by early settlers. (Aggarwal, and Kunnumakkara, 2009) and (Platte, 1962)

2.3.3 Chemical composition of the fruits

The essential oil content is of greatest importance, and Table 8 and Figure 6 show that there are accessions that have almost no essential oil and others with up to 2.60% of essential oil in the air-dried fruits. The extremely leafy types from Syria have very low essential oil content in the fruits. Despite this, the essential oil content is positively correlated with the foliation of the plant ($r=0.348$). High essential oil content of was found in all accessions from the Caucasus.

The taste of the green leaves of the plant was more aromatic in the accessions that had high essential oil content in the fruits. The Georgian types had leaves with a very spicy taste. The Syrian types must have been subject to a selection towards plants with a mild taste more suited to use in salads than as a spice. The Ethiopian accessions show the same tendency as the Syrian, but their flavor is more aromatic.

Usually, the plants with low foliation and large fruits have allowed essential oil content. The Indian group with the lengthened fruits also belongs in this category.

This fact has been known and described in literature for a long time (e.g. Luzina and Michelsson 1937). But the chemical composition of the essential oil of the plant is also important, as it affects its flavour.

The organoleptic differences between coriander plants of different origin have been described by Purseglove.,*et al.*1981.

CHAPTER THREE

MATERIALS AND METHODS

This study which conducted under laboratory condition of Plant Pathology, College of Agricultural Studies, Sudan University of science and Technology during the period November to December 2015 to investigate the inhibitory effect of ethanolic extracts of coriander and standard fungicide, **score**^{EC} efficacy against the fungus *F. solani*.

3.1. Isolation and Identification of the causal pathogen:

Fungal isolate was isolated from naturally diseased tomato leaves showing blight symptoms and Identification of the causal pathogen. Pathogenicity tests of *Fusarium* sp. Isolate was conducted under laboratory conditions at Plant pathology.

3.2. Plant Material

Freshly collected plant parts (Coriander) were shade-dried at room temperature for 10–15 days. Dried bark and leaf samples were separately crushed and ground into fine powder with mortar and pestle.

3.4. Preparation of extracts:

Extracts from seeds of Coriander (*Coriandrum sativum*) were obtained or collected from local market in Khartoum “Shambat”, Sudan and tested for their efficacy in reducing the mycelial growth of *A. solani* *in vitro* using the poisoned food technique (Schmitz, 1930).

Powdered plant materials were sequentially extracted with different solvents in a Soxhlet apparatus for 8 h according to the method described elsewhere (Pandey, A.K. 2007). The solvents used for

extraction included petroleum ether (PE), ethanol (ET). The respective extracts were filtered and dried under reduced pressure using rotary evaporator to yield solid/semi-solid residues. The residues were lyophilized to get dry solid mass.

The PDA media amended with five milliliters of ethanol extract, 1 and 5%, of each plant extracts individually were inoculated with mycelial discs (9 mm diameter) taken from the advancing edges of 7 day-old pure cultures of *A. solani*. The control experiments had distilled water instead of plant extracts. The inoculated media were incubated at temperature $27 \pm 1^\circ\text{C}$. Four plates were each treatment was used as a replicates. The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate 7 days after inoculation. Each treatment was replicated three times with four plates per replication (Pandey, A.K. 2007).

3.5. Preparation of fungicide concentrations

One mL of the score 250 EC (Difenoconazole 25%), fungicide was dissolved in 90 ml of sterilized distilled water of 2500 ppm was prepared.

Five mL of recommended fungicide doses (score ^{250EC}) and nine mL of molten PDA medium was poured into sterilized glass Petri dishes 2500 ppm fungicide concentrations.

3.6. Test procedure:

The antifungal in vitro assays were carried out following the modified method of (Okigbo *et al.* 2005) and (Chohan, S. and R. Perveen, R. (

2006). Inhibition zone technique was used in this study to evaluate the effect of each concentration on mycelia linear growth of the fungus. Initially, fresh fungal growth was prepared from previously maintained culture of *Alternaria solani*. Prepared PDA media was amended with the required concentration from Coriander and fungicide before being solidified in a conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 25 ml of PDA, were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

One mycelia disc of the fungus was placed in the centre of PDA plates where opposite poles were marked at the back of the plate and incubated at $27 \pm 1^\circ\text{C}$ in incubator and radial growth of pathogen was measured at 24 h intervals.

The Petri dishes of each concentration were arranged in a complete block design in incubator and incubated at $27 \pm 1^\circ\text{C}$ for 3 days. The growth of the fungus was measured and calculated successively after 3, days after inoculation. The effect of each extract concentration on linear fungal growth was calculated as percentage of reduction in diameter of fungal growth. The formula suggested by Vincent (1947). The formula Where: -

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

Where: 2

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

3.7. Experimental design:

The experiment was arranged in a Complete Randomized block Design.

3.8. Statistical analyses:

The obtained data was statistically analyzed according to analysis of variance (ANOVA) Duncan's Multiple Range Test (DMRT) was used for means separation using Mstat-C statistical package..

CHAPTER FOUR

RESULTS

This study was conducted under laboratory condition of plant protection department, College of Agricultural Studies, Sudan university of science and Technology (During November and December 2015-2016) the aim of this study was to investigate the antifungal activities of Coriander (*Coriander sativum*) Ethanol extracts, and fungicides score against *Fusariumsolani in vitro*.

4.1 Identifications of *Fusariumsolani*

Identification of *Fusariumsolani* was performed depending on the cultural characteristic and conidia, hyphae, mycelium and spores shapes as described by Booth (1977).

4.2 Effect coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* three days after inoculation *in vitro*.

The results (Table 1 and Figure 1) showed that the coriander ethanolic extracts tested and fungicide had effects on the fungal growth after three days from inoculation. Additionally, the percentages of fungal growth inhibition were significantly high compared to the control. The effect of coriander ethanolic extract at concentrations (12.5, 50, and 100 %) and fungicide at recommended dose gives the full Reduction of fungal.

Otherwise, concentrations of the coriander extracts and fungicide gave the same result and inhibition zones percent are (100%) compared to the untreated control.

Table 1 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusarium solani* three days after inoculation *in vitro*.

Treatments	Inhibition zone %				Mean
	Con. %	R1	R2	R3	
Coriander	12.5	100(10)	100(10)	100(10)	100 (10) a
	25	100(10)	100(10)	100(10)	100 (10)a
	50	100(10)	100(10)	100(10)	100(10)a
Fungicide		100(10)	100(10)	100(10)	100 (10)a
Control		0(.7)	0(.7)	0(.7)	0 (.7)b
CV%					0
SE±					0
LSD					0

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

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

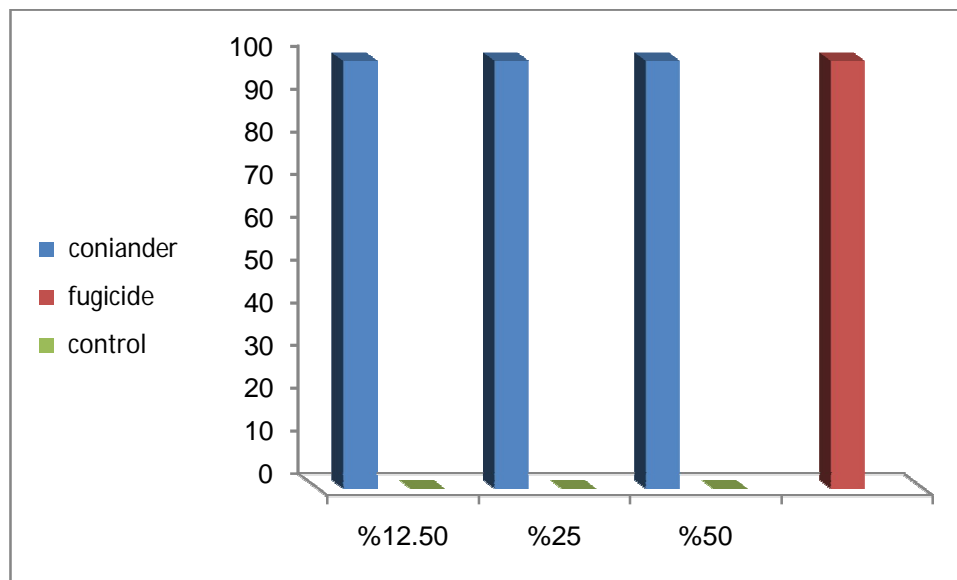


Fig. 1 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* three days after inoculation *in vitro*.

.2.1 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* five days after inoculation *in vitro*.

Five days after inoculation, coriander extract concentrations as well as that of the fungicide were positively continued exhibiting inhibitory effects against the fungal growth. However, the highest concentration of the coriander extract at concentration (50%) and fungicide gave the highest inhibition zones percent (100%) equality. This inhibitory effect from all concentrations tested was significantly different from control (Table, 2 and Fig. 2). Followed by coriander extract in concentrations in 25 and 12.5% were at inhibition zone percentage (97.7 and 74.4) respectively.

Table 2 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* five days after inoculation *in vitro*.

Treatments	Inhibition zone %				Mean
	Con. %	R1	R2	R3	
Coriander	12.5	73.3(8.8)	50(7.1)	100(10)	74.4 (8.6)b
	25	93.3(9.6)	100(10)	100(10)	97.7(9.8) a
	50	100(10)	100(10)	100(10)	100 (10)a
Fungicide		100(10)	100(10)	100(10)	100 (10)a
Control		0(.7)	0(.7)	0(.7)	0 (.7)d
CV%					8.42
SE±					.3808
LSD					1.2

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

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

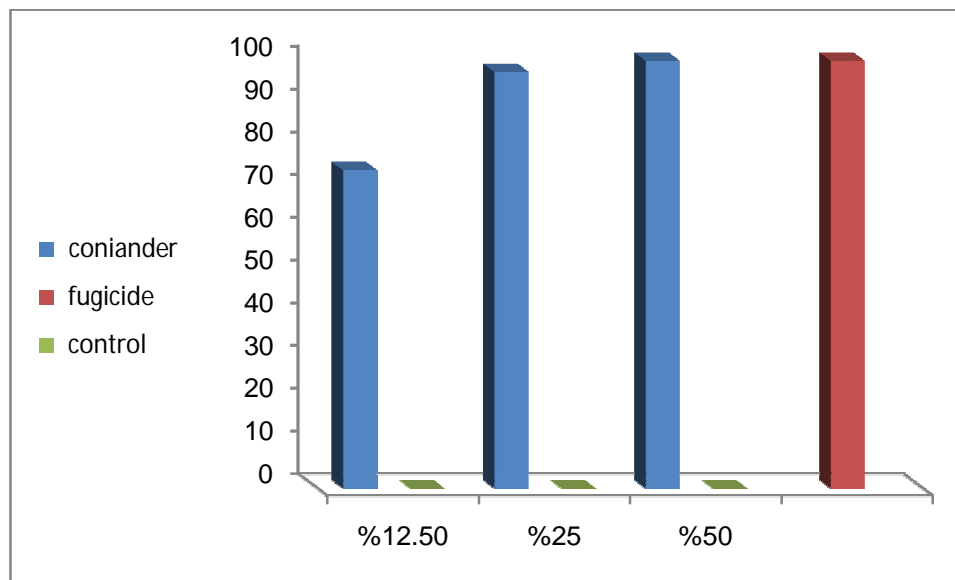


Figure 2 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* five days after inoculation *in vitro*.

4.2.2 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* seven days after inoculation *in vitro*.

seven days after inoculation the results (Table, 3 and Figure, 3) showed that the fungicide and coriander extract in 50 were gave the highest inhibition zone percentage against the fungal growth (100 %) and followed by rest of coriander extract at concentration (25 and 12.5%) were gave inhibition zone percentage (85.3 and 56.8%) .

Clearly, the coriander extracts and fungicide 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 3 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* seven days after inoculation *in vitro*.

Treatments	Con. %	Inhibition zone %			Mean
		R1	R2	R3	
Coriander	12.5	78.9(8.9)	41.6(6.4)	50(7.1)	56.8(7.4) b
	25	89.4(9.4)	83.3(9.1)	83.3(9.1)	85.3(9.2)a
	50	100(10)	100(10)	100(10)	100 (10)a
Fungicide		100(10)	100(10)	100(10)	100 (10)a
Control		0(.7)	0(.7)	0(.7)	0(.7) c
CV%					7.79
SE±					.3362
LSD					1.059

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

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

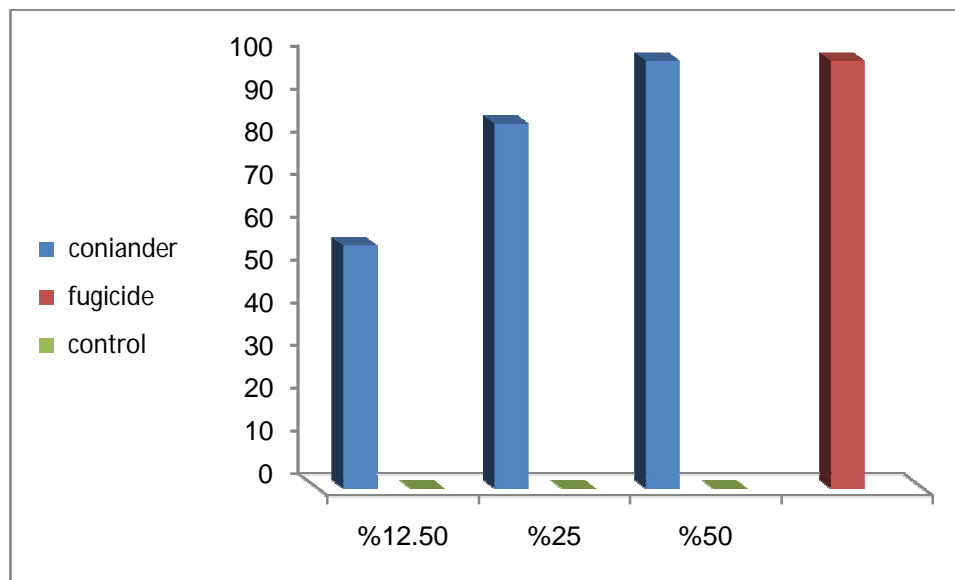


Figure 3 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* seven days after inoculation *in vitro*.

4.2.3 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* ten days after inoculation *in vitro*.

The treatments of fungicide and coriander extracts (12.5, 25, and 50%) in ten days post inoculation were gave significant inhibitory effects against the fungal growth, the fungicide and coriander extract at concentration were give full inhibition percentage 100%. followed by coriander in 25% was gave 89.3% and the lowest inhibition percentage was happen in coriander at 12.5 concentration.

Table 4 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* ten days after inoculation *in vitro*.

Treatments	Con. %	Inhibition zone %			Mean
		R1	R2	R3	
Coriander	12.5	79.1(8.9)	66.6(5.8)	50(7.1)	71.9(8.4) c
	25	87.5(9.3)	90.4(9.5)	90(9.5)	89.3 (9.4)b
	50	100(10)	100(10)	100(10)	100 (10)a
Fungicide		100(10)	100(10)	100(10)	100(10) a
Control		0(.7)	0(.7)	0(.7)	0 (.7) d
CV%					2.50
SE±					0.11
LSD					0.35

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

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

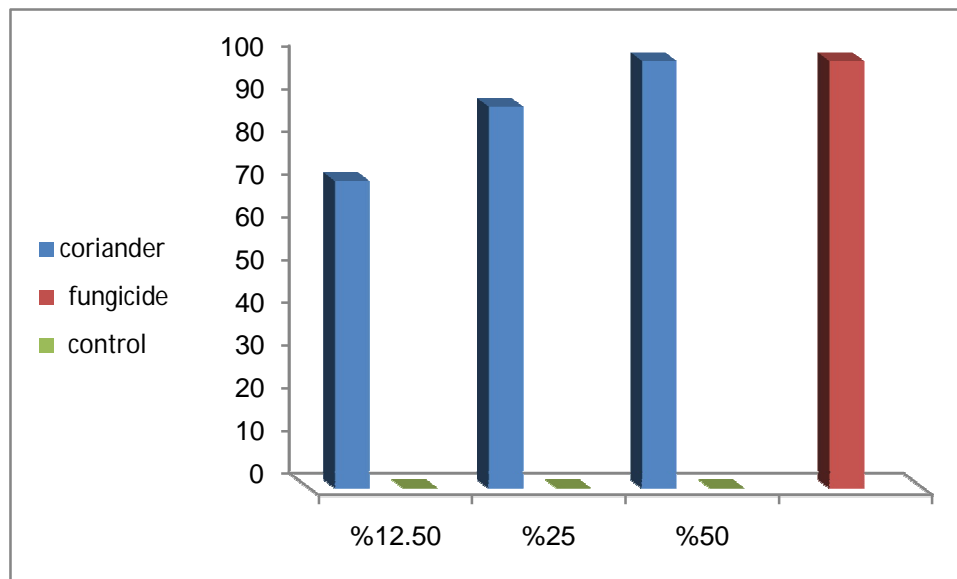


Figure 4 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* ten days after inoculation *in vitro*.

4.2.4 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* eleven days after inoculation *in vitro*.

The results (Table, 5 and fig 5) showed that the ethanolic extracts of plants and fungicide exhibited inhibitory effects against fungal growth after eleven days from inoculation. The percentages fungal growth inhibition was significantly high compared to the control.

Moreover, the highest concentration of the coriander extracts (50%) and that of fungicide gave significantly higher inhibition zones percent against test fungus (100%). Among the coriander extracts at concentration (25 and 12.5%) were affected in inhibition zones percentage against tested fungus at (89 and 74.3%) respectively, compared to the untreated control.

Generally, the results showed that the antifungal activity increase with concentration.

Table 5 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* eleven days after inoculation *in vitro*.

Treatments	Inhibition zone %				
	Con. %	R1	R2	R3	Mean
Coriander	12.5	75.8(8.7)	70.3(8.4)	76.9(8.7)	74.3(8.9)c
	25	86.2(9.3)	92.5(9.6)	88.4(9.4)	89(9.4)b
	50	100(10)	100(10)	100(10)	100(10) a
Fungicide		100(10)	100(10)	100(10)	100 (10)a
Control		0(.7)	0(.7)	0(.7)	0(.7) d
CV%					1.33
SE±					0.06
LSD					0.19

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

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

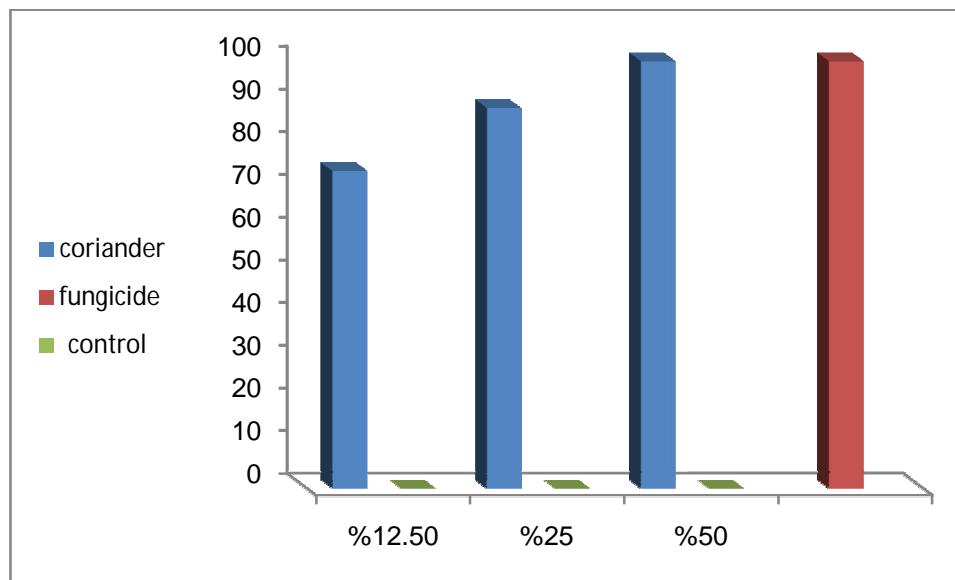


Figure 5 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* eleven days after inoculation *in vitro*.

4.2.5 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* thirteen days after inoculation *in vitro*.

Generally, it could be seen from the results (Table, 6 and fig 6) that after thirteen days from inoculation, extracts of coriander tested as well as the fungicide at proved effective in suppressing the fungal growth.

In fact, tested fungicide and coriander extract 50% induced significantly high inhibition zones percentage (100%) in all days post inoculation against test fungus compared to control , followed by coriander extract at 25% by inhibition percentage 88.6

However, the inhibitory effect of coriander extract at 12.5% increase with time of recording.

Table 6 Effect of different concentrations of coriander ethanolic extracts and fungicide on leaner growth of *Fusariumsolani* thirteen days after inoculation *in vitro*.

Treatments	Inhibition zone %				
	Con. %	R1	R2	R3	Mean
Coriander	12.5	79.4(8.9)	77.1(8.8)	80(8.9)	78.8(8.8) c
	25	85.2(9.2)	94.2(9.7)	86.6(9.3)	88.6 (9.4)b
	50	100(10)	100(10)	100(10)	100 (10)a
Fungicide		100(10)	100(10)	100(10)	100 (10)a
Control		0(.7)	0(.7)	0(.7)	0 (.7) d
CV%					1.55
SE±					0.07
LSD					0.22

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

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

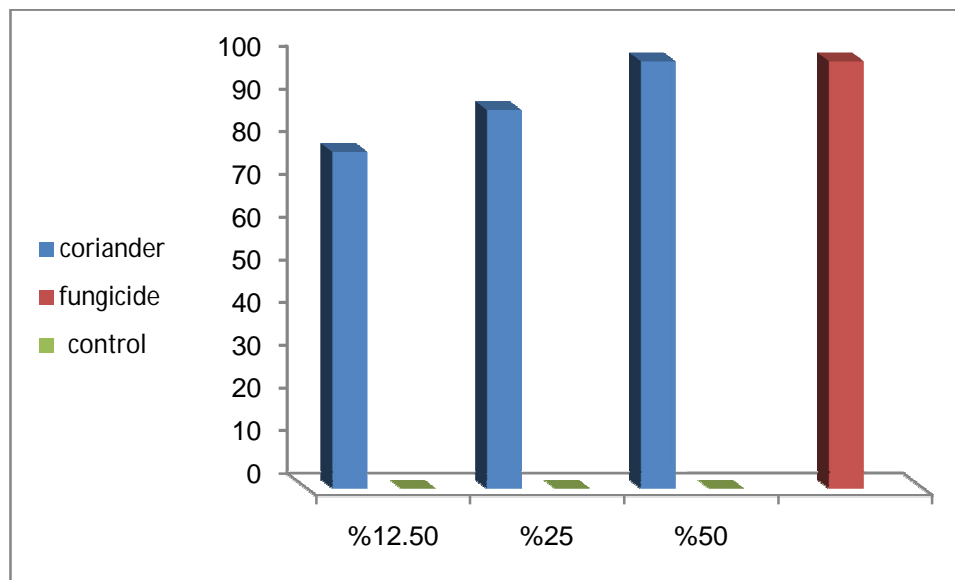


Figure 6 Effect of different concentrations of coriander ethanolic extracts and fungicide on learner growth of *Fusarium solani* thirteen days after inoculation *in vitro*.

DISCUSSION

Higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenoid, Phenols and other secondary metabolite were produced by higher plants (Siddig., 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical fungicides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Kelang, 2001).

The use of chemical control, cultural practices and the use of other methods they are not available or effective. The use natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Plant extracts or plant essential oils have been tested against *F. oxysporium* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2000).

In the study, the highest concentration of the coriander fruit aqueous extracts (50%) give a significantly higher inhibition zones percent (100%) after three days. In fact this finding is in agreement with Hanaa *et al* (2011). Who tested the effects of coriander aqueous extracts on the leaner growth of *Fusariumsolani* in potato .

The results of this study that the concentration of Tilt fungicides generated asignifictly higher inhibition zone percentage against *Fusariumsolani* it induced (100%) inhibition after four days, this agrees by Abdelgader, (2005) who found that Tilt induced 100% inhibition against *F. solani* when applied

100ppm after 7 days of exposure similar finding were also revealed with Mohammed (2005) who found that Tilt when applied at 10ppm against *Drechslerahawaiiensis* induced 100% inhibition after 4 days.

RECOMMENDATIONS

It was recommending using natural antifungal such as coriander which inhibits the fungal growth especially at concentration, 12.5%, 25% and 50%.

- It was recommended to use natural antifungal such as Tilt which inhibits the fungal growth especially at concentration (100%).

REFERENCES

- Agrafiotis D.K.; Bone, R. and Salemmme, F.R (2002), soil R .method of geerating chemical compounds having desired properties .US patent 6:434,490 August 13.
- Agricultural Research Corporation (ARC), Republic of Sudan (1985).Improving Potato Production in the Sudan.
- Agrios, G. N. (1969). Plant Pathology, 1st Ed.New York Academic Press Inc. 49- 629pp.
- Agrios, G.N. (1988). Plant Pathology, 3rd. ed. Academic Press, Inc.: New York. 803 pp.
- Agrios, G.N. (2005) Environmental effect is on development of the infectious disease. (In)plant pathology.5th end, Elsevier Acad .press Burlington, mass, USA pp251-262.
- Ahmed Abdelmageed (2013). Potential Degradation of Certain Alkanes by *Pseudomonas frederiksbergensis*. Journal of Pure and Applied Microbiology. Vol. 7(Spl. End.), p. 13-2
- Ahmed Abdelmageed (2014). In-vitro AntibacClinical Pathogens. Journal of Pure and Applied Microbiology. 8 (Spl. End. 1) 221-226.
- Ahmed, E.H (2013). Management of Chickpea wilts Disease Caused by *Fusarium f.sp.* Ciceri PhDThesis, Faculty of Agriculture, Department of Plant Production, Sudan University of Science and Technology.
- Ali, M.E.K. (1996) .A review of wilt and root –rot diseases of food legumes. In production and important of cool-season food legumes.In the Sudan