Sudan University of Science and Technology

College of Graduate Studies

Antifungal Evaluation of some plants extracts and fungicide against *(Fusarium oxysporum f.sp. Lycopersici*) causal agent wilt of Tomato.

تقييم التضاد الفطري لدي بعض المستخلصات النباتية والمبيد الفطري ضد الفطر المسبب لمرض الذبول الفيوزيرمي في الطماطم *(فيوزاريم أوكسي إسبوريم)*.

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in Plant Protection.

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الآية

قال تعالى:

{قُلْ لَوْ أَنَّ عِنْدِي ما تَسْتَعْجِلُونَ بِهِ لَقُضِيَ الْأَمْرُ بَيْنِي وَبَيْنَكُمْ وَاللَّهُ أَعْلَمُ بِالظَّالِمِينَ (58) وَعِنْدَهُ مَفَاتِحُ الْغَيْبِ لَا يَعْلَمُهَا إِلَّا هُوَ ۚ وَيَعْلَمُ مَا فِي الْبَرِّ وَالْبَحْرِ ۚ وَمَا تَسْقُطُ مِنْ وَرَقَةٍ إِلَّا يَعْلَمُهَا وَلَا حَبَّةٍ فِي ظُلُمَاتِ الْأَرْضِ وَلَا رَطْبٍ وَلَا يَابِسٍ إِلَّا فِي كِتَابٍ مُبِينٍ (59)}

صدق الله العظيم

[سورة الأنعام: الآيات 58-59]

Dedication

I dedicate this work to my dear parents,

To my brothers and sisters,

To my distinguished teachers,

To my colleagues and friends.

With love and respect.

Amani osman

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I would like to express my appreciation and gratitude to all who made it possible for me to accomplish this work. First of all my grateful to the Almighty of ALLAH who gave me the power and patience to complete this study. Really, I am greatly indebted to my supervisor Dr. Ibrahim Saeed Mohamed, College of Agricultural studies, Sudan University of science and technology, who devoted most of his time to teach us on various disciplines of scientific research. Deep gratitude to Ms. Mawada Hamid, Plant Pathology Laboratory. I would like to express my cordial and deep gratitude to my father, mother, brothers and sisters for their continual encouragement, unlimited support and strong moral support.

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ABSTRAC

Fusarium wilt of tomato is considered as one of the most important diseases of the worldwide. The present investigation was undertaken under laboratory of Plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology, to study the effect of aqueous leaves extracts of *Eucalyptus camaldulensis*, Mint, Sweet basil plants and fungicide Revus top on growth of the fungi *Fusarium oxysporum f. sp. Lycopersici* causal agent of wilt in tomato. Three concentration of aqueous leaves extract of *Eucalyptus camaldulensis*, Mint, Sweet basil and fungicide each of 25, 50 and 100% were used in addition to control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth inhibition percentage. The results showed that all concentration of the leaves aqueous extracts of all plants tested and fungicide were of significantly high inhibitory effect against the linear growth of test fungus compared to control. Moreover, concentration of each aqueous extract as well as that of fungicide reacted differently against test fungus. However, the effect of fungicide was more pronounced against test fungus than the plant extracts and which range from 83.2% to 100% where no growth was recorded. Among aqueous extracts, *E. camaldulensis* expressed relatively high inhibition zone throughout the course of the experiment (44.1, 53.1 and 53.1 %) than Basil (36.8, 51.5 and 54.4 %) and Mint aqueous extract as well (35.5, 39.6 and 39.6 %) respectively. Generally, by the end of the test the highest concentration of the plant extracts (Mint, Basil and *Eucalyptus* 100%) and fungicide (100%) gave significantly highest inhibition zones percent (41.9%, 48.5%, 39.3%, and 99.3%) respectively compared to the untreated control. the results showed that the antifungal activity increase with increase in extract concentration. Obviously, the test fungus differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration. The current results were considered promising and encouraging to carry out a phytochemicals analysis of different parts of *Eucalyptus camaldulensis* plant using different solvents so to determine the bioactive ingredient in each of these parts.

ملخص التجربة

يعتبر مرض الذبول الفيوزيريومى فى الطماطم من أهم أمراض هذا المحصول فى العالم.

أجريت هذه الدراسه تحت ظروف المختبر(معمل أمراض النبات) بقسم وقاية النبات، كلية الدراسات الزراعية، جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسه تاثير المستخلص المائى لأوراق نباتات النعناع ، الريحان، الكافور والمبيد الفطرى ريفص توب على نمو فطر الفيوزاريم اوكسيسبوريم المسبب لمرض الذبول الفيوزيرمي فى الطماطم. استخدمت ثلاثه تراكيز من المستخلص المائى لأوراق نباتات النعناع ، الريحان والكافور ، كل (25، 50 و 100%) وكذلك ثلاثه تراكيز من المبيد الفطرى ريفص توب (25، 50 و 100%) أضافة الى الشاهد. تم تقيم الأثر التثبيطى لهذه التراكيز بقياس نسبه تثبيط نمو الفطر. أوضحت النتائج ان كل تراكيز المستخلص المائى لأوراق النباتات المختبرة و المبيد الفطرى قد أظهرت تأثير معنوى ضد نمو فطر الفيوزاريم مقارنه بالشاهد. تراكيز المستخلص المائى للنباتات و المبيد الفطرى قد تفاعلت مع نمو فطر الفيوزاريم. علي أية حال فان تاثير المبيد الفطرى ضد نمو فطر الفيوزاريم اكثر وضوحاً من المستخلصات النباتية والذى تراوحت بين 38.2 و 100% حيث لا يوجد نمو للفطر . التركيز الأعلى (100%) فى كل من المستخلصات المائية و المبيد الفطرى أعطت أعلى نسبة تثبيط مقارنة بالشاهد (41.9، 48.5، 39.3و 99.3 %) على التوالى . فيما بين المستخلصات المائيه المختبرة كان مستخلص الريحان نسبيا الأكثر فعاليه فى تثبيط نمو الفطر مقارنة مع بقية المستحلصات النباتية. عموما أظهرت النتائج إن فعالية المستخلصات المائية ضد نمو فطر الفيوزاريم تزداد بزيادة تركيزالمستخلصات. النتائج الحاليه تعتبر مشجعه للقيام بتحاليل كيميائية لمختلف أجزاء نبات الريحان باستعمال مستخلصات مختلفة لتحديد الماده الفعاله في أجزاء النبات المختلفة

CHAPTER ONE

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a member of the family solanaceae that includes also other cultivated crops such as potato, pepper, eggplant, tobacco etc… The origin of tomato is believed to be central and south America from where the crop was transferred to Europe in the 16th century, then to old world continents (Rick , 1977). The crop plays an important role in human nutrition by providing essential amino acids, vitamins and minerals (Sainju *et al*., 2003). Its vitamin C content is particularly high (Kanyomeka and Shivute, 2005). It also contains lycopene, a very potent antioxidant that may be an important contributor to the prevention of cancers (Agarwal and Rao, 2000). In fact it is considered as one of the most important and popular vegetable in many countries (FAO, 2002) and this is because of its acceptable flavour, nutritive value and ability to fruit in a wide range of environments and the relative ease with which it can be cultivated. The production estimate worldwide was 95 million Mt annually (FAO, 2002).

In Sudan tomato is becoming increasingly important for local consumption and for export. It is cultivated throughout the year under irrigation in an area that exceeds 36540 hectares with an average yield of 17.57 tons per hectare (AOAD, 2007 and Dawelbeit *et al*., 2010).

The crop is affected by several diseases, reflecting negatively on plant growth and the produced yield worldwide. Out of these, pathogenic fungi especially the wilt caused by species of Fusarium; remain to be a challenging task in terms of management (Agrios, 2000; Rick, 1976;

Srinon *et al*., 2006). Wilt of tomato caused by *Fusarium oxysporum f.sp. lycopersici* (Sacc.) W., is one of the most economically important diseases world-wide (Rick, 1979; Cal *et al*., 2004 and Srinon *et al*., 2006).

Likewise, in Sudan, several diseases are known to limit production of tomato, of which Fusarium wilt caused by (*Fusarium oxysporum*f. sp. Lycopersici) is one of the most important (Bhatia *et al.*, 2004*)*. In fact, Fusarium wilt is one of the major yield limiting factors of tomato production in Sudan (Bhatia *et al.*, 2004*)*. It is reported that the disease is especially serious in the traditional production areas where tomato is grown on stored soil moisture after the flood waters of the Nile River subside.

Fusarium wilt of tomato has been managed primarily by the use of resistant varieties (Jalali and Chand, 1992) but breakdown in resistance of these varieties due to evolution of virulent races of the pathogen have undermined their importance in recent years (Haware and Nene, 1982 and Jiménez-Díaz, *et al*., 1993). In fact, numerous strategies have been proposed to control this fungal pathogen (Biondi *et al*., 2004; Ahmed, 2011). Methods like solarization, disinfection, seed treatment with synthetic fungicides, crop rotation and mixed cropping were also in use (Sullivan, 2004). However, management of seed-borne and soil-borne diseases such as wilt caused by Fusarium species has always been problematic (Haware and Kannaiyan, 1992; Rao and Balachadran, 2002).

Progressed achieved in recognizing antimicrobial compounds in higher plants gave more promises in combating plant pathogenic diseases. Such compounds, being biodegradable and selective in their toxicity, are

considered valuable for controlling some plant diseases (Schmutterer, 2002).

This study was undertaken to focus on investigation of two components for management of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici*, higher plant extracts and synthetic fungicides under laboratory conditions in order to formulate promising disease management approach with following objectives:-

- To explore the antifungal potential of some higher plants aqueous crude extract against *F. oxysporum* f.sp. *lycopersici.*
- To evaluate the effect of systemic fungicide on fungal growth.
- To develop promising disease control components against Fusarium wilt of tomato.

CHAPTER TWO

Literature review

2.1 Tomato

Tomato, (*Lycopersicon esculentum* Mill.), is one of the most important edible and nutritious vegetable crops in the world. It belongs to Solanaceae family. It ranks next to potato and sweet potato with respect to world vegetable production. It is widely cultivated in tropical, sub-tropical and temperate climates and thus ranks third terms of world vegetable production (FAO, 2006). The tomato crop (*Lycopersicon esculentum*) originated in tropical central South America it was domesticated in Mexico and later taken to Europe (Rick, 1978).

World production of tomato covers approximately 4 million hectares of arable land with production estimated at 100.5 million tons and valued at 5-6 billion US$ (Costa and Heuvelink, 2005).

2.1.1 Economic importance

The importance of tomato, both as vegetable food and cash crop cannot be over-emphasized. It is a vegetable crop of considerable economic importance in tropical and subtropical countries where high yields of tomato result in high incomes to farmers when it is cultivated on large scale (Thompson and Kelly, 1957).

Tomato is also a versatile crop that can be classified according to use into fresh market tomatoes and processing tomatoes which are cultivated for industrial canning and processed foods.

Tomato fruit is considered to be fairly high in vitamins A and C, of high cash value and with potential for value added processing. Tomato was

regarded as a top priority vegetable by scientists interviewed under the Technical Advisory Committee of the Consultative Group on International Agricultural Research (CGIAR) (FAO, 1990). Tomato grows best in fertile, well-drained soils, with pH 6 and ambient temperatures of about 25 ºC (Villareal, 1979 and Rice *et al*., 1987).

2.1.2 Scientific classification

Kingdom: planta
Sub kingdom: tracheobionia
Division: Magnoliopida
Sub class: Asterielae
Order: Solanacaea
Genus: Lycopesricon
Species: *Esculentum* (Mill)

2.1.3 Fungal diseases of tomatoes

In total, there are more than 200 pathogens that infect the tomato crop and diseases are often the limiting factor in tomato production (Jones *et al*., 1997). The epidemics of a disease depend on complex interactions between host, pathogen and environment. The disease development may also be influenced by cultural practices such as fertilization and irrigation (Aust and v. Hoyningen Huene, 1986). Plant pathogens have different strategies for surviving and spreading to new hosts. Most pathogens have a life cycle that includes both plants and soil, but they usually need to infect a specific host to increase the population (Agrios, 1997).

Tomatoes are parasitized by a number of pathogens, including *Fusarium oxysporum f. sp. lycopersici* (Sacc.) W.C. Snyder H.N. Hansen, the causal agent of fusarium wilt of tomato (Ivanović and Mijatović, 2003), which is

one of the most important species as tomato pathogen (Agrios, 1988; Smith *et al*., 1988). In an indoor environment due to high temperature and humidity, *F. oxysporum f. sp. lycopersici* can cause significant damage. However, pathogenic fungi of the genus Fusarium that is the causal agents of tomato wilt cause root and basal stem deterioration and result in the wilting of vegetable plants. On the other hand, fresh vegetable fruits are quite perishable because their high moisture content makes them vulnerable to microbial decay as well as physiological deterioration (Eckert and Ogawa, 1988). Fresh vegetable fruits can be contaminated with various fungi, including Alternaria, Aspergillus, Fusarium, Rhizopus, Penicillium and Trichoderma species (Brackett, 1988). These species can produce mycotoxins, contaminators of food, which is an important issue for human health. Fusarium rot is one of the most common diseases of fresh tomato fruits in storage and warehouses. The major role in the intensity of rots in tomato fruits in the warehouse is the harvest season. Fruits should be harvested when 70% of the fruits become red. Rot contamination rate increases if harvest cannot be carried out for any reason such as in appropriate weather (Yoltas, 1985).

In Sudan, cultivated tomatoes suffer from many fungal diseases such as are Fusarium wilt (*Fusarium oxysporum f. sp. lycopersici*), Verticillium wilts (*Verticillium dahliae*), powdery mildews (*Leveilula taurica*) and early and late blights, which are caused by *Alternaria solani*/ *alternata* and *Phytophthora infestans*, respectively. In fact, Fusarium wilt disease is considered one of the major agents of yield reduction of the crop (Awad, 1990).

2.2 The Genus Fusarium

Fusarium is a filamentous fungus widely distributed on plants and in the soil. It is found in the normal mycoflora of commodities, such as rice, bean, soybean, and other crops (Pitt *et al*., 1994).

Approximately 1000 Fusarium species had been described by 1900, based largely on examination of fruiting structures (sporodochia) on plant material. This large number of species was reduced by Wollenweber and Reinking, (1935) to 65 species, 55 varieties and 22 forms, in 16 sections, and all taxonomic systems proposed since then have been based on this system (Burgess *et al*., 1994).

The most disastrous disease caused by Fusarium species in agricultural history throughout the world was the infection of *F. oxysporum f. sp. cubense* on banana in Panama, thus known as Panama disease (Ploetz, 1994) affecting the whole Panama's economic sectors in the agricultural industry. Another major event caused by this genus was the disease called Fusarium head scab on wheat and barley in the United States (Windels, 2000).

2.2.1 Ecology of Fusarium

Fusarium species can be found in soil, water and on seeds, roots and leaves of most plants. Several selective media have been developed for the isolation, growth and sporulation of Fusarium species, including Selective Fusarium Agar (SFA), Dichloran Chloramphenicol Peptone Agar (DCPA), Spezieller Nahrstoffarmer Agar (SNA) and Modified Potato Dextrose Agar (MPDA). The isolation of Fusarium species from plants is affected by the nature of the source material, method of surface

sterilization, plating procedures, medium and incubation conditions (Burgess *et al*., 1994).

The choice of medium depends largely on the nature of the tissue involved in the isolation exercise. Selective media are normally used for the isolation of Fusarium species from diseased crown or root samples. There are several other techniques for recovering Fusarium species, directly or indirectly, from plant samples, which do not involve plating tissue segments on agar media. Some species produce sporodochia on the surface of the diseased tissue. Macroconidia can be taken from these sites and used to prepare a conidial suspension, which is plated on Water Agar containing antibiotics. Germinated single conidia are later taken to initiate pure cultures for identification of Fusarium species (Burgess *et al*., 1994).

2.2.2 Mycotoxins Produced by Fusarium species

Besides the diversity and distribution around the world, toxic substances produced by Fusarium species in post harvest products are what matters most. Fusarium species produced a range of mycotoxins that could pose a serious threat to plant, animal and human health (Marasas *et al*., 1984; Joffe, 1986). Mycotoxins are secondary metabolites produced by fungi that are associated with a variety of animal disorders and some human health problems. Mycotoxicoses are diseases or disorders caused by the ingestion of foods or feeds made toxic by these fungal metabolites. Trichothecenes, zearalenone, and fumonisins, for instance, are the major Fusarium mycotoxins produced in infected maize kernels (D'Mello *et al*., 1999; Logrieco *et al*., 2002). Many mycotoxins produced by Fusarium species were discovered in cereals especially maize. For that discovery, the infected maize kernels are of great concern worldwide.

2.2.3 Host range

These fungi attack a diverse group of plants including crops, ornamentals and trees (Nelson *et al*., 1981).

The most important Fusarium wilt pathogens have wide range of host and including numerous forma specials some of them contain two or several pathogenic races, causing devastating wilt diseases and many are seed borne as listed by Anderson (1974) for the following hosts Allium cannabis. *Beta vulgaris*, *Cucumis sativa*, *Phaseolus vulgaris* and *Psumi stativum.*

Fusarium oxysporum is one of the major causal agents of wilt disease (Nene *et al*; 1991). The disease is prevalent in most Tomato growing countries and is a major disease. It is seed and soil borne disease .The fungal pathogen *F*. *oxysporium* affects a wide variety host of different age Tomato, Tobacco, Legumes, Cucurbits. Sweet Potatoes, Chickpea and Banana are a few of the most susceptible plant, but it also affects other herbaceous plants (Pan Germany, 2010).

2.3 Fusarium wilt of tomatoes

Fusarium oxysporum f. sp. lycopersici (FOL) is a soil borne pathogen causing wilting occasionally accompanied with severe yield loss in tomato.

Fusarium species causes a huge range of diseases on an extraordinary range of host plants. 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 (Booth 1971; Summeral *et al*. 2003). Fusarium wilt of tomato (*Lycopersicon esculentum) c*aused by *Fusarium oxysporum f. sp. lycopersici* is a disease that causes serious economic loss

(Agrios 2005). The fungus causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele (Bowers and Locke 2002).

Fusarium wilt of tomato is most severe where the crop is grown at relatively high temperature or when seasons are hot and dry (Rick, 1983).

2.3.1 Scientific Classification of pathogen and identification

Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Sordariomycetes
Subclass:	Hypocreomycetidae
Order:	Hypocreales
Family:	Nectriaceae
Genus:	Fusarium
Species:	*F. oxysporum f.sp. lycopersici*

Binomial name: *Fusarium oxysporum f.sp. lycopersici*

W.C. Snyder & H.N. Hansen, (1940).

The Ascomycota fungus emended by (Snyder and Hansen 1940) comprises all the species , varieties and forms recognized by (Wollenweber and Reinking, 1935) with in an infra generic grouping called section Eleganns while the species, as defined by Snyder and Hansen, has been widely accepted for more than 50 years, (Booth, 1971 and Nelson *et al*.,1983). More recent work indicates this taxon is actually a genetically heterogeneous polytypic morpho species (O'Donnell, and Cigelnik 1997, Waalwijk *et al*, 1996) whose strains represent some of the most abundant and widespread microbes of the global soil micoflora

(Gordon and Martyn, 1997). Although this last statement has not been proven or supported by actual data. These remarkably diverse and adaptable fungi have been found in soils ranging from the Sonoran Desert, to tropical and temperate forests, grasslands and soils of the tundra. (Stoner, 1981).

Fusarium oxysporum strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes and degrade lignin (Rodriguez *et al.,* 1996, Sutherland, *et al.,* 1983) and complex carbohydrates (Christakopoulos *et al.,* 1995/1996), associated with soil debris. They are also pervasive plant endophytes that can colonize plant roots (Gordon *et al* 1989, Katan, 1971) and may even protect plants or be the basis of disease suppression (Larkin *et, al.,* 1993 and Lemanceau e*t al*., 1993). Although the predominant role of these fungi in native soils may be as harmless or even beneficial plant endophytes or soil saprophytes, many strains within the *F. oxysporum* complex are pathogenic to plants, especially in agricultural area.

2.3.2 The pathogen

Fusarium oxysporum f. sp. lycopersici is a soil borne pathogen with a high level of host specificity. There are more than 120 described formae speciales and races within the species. Together they cause diseases on a wide range of agricultural crops (Correll, 1991 and Agrios, 1997). The causal agent of Fusarium wilt on tomato is *Fusarium oxysporum f. sp. lycopersici* (Sacc.) Snyd. and Hans (Chambers, 1963).

Fusarium oxysporum produces three kinds of spores: microconidia, macroconidia and chlamydospores. Microconidia are small, 5-12 x 2-3 µm, and produced abundantly at the end of mycelium branches in all conditions. Macroconidia have three to five cells and pointed ends. The

macroconidia are produced on the surface of dead plant tissue (Agrios, 1997; Jones *et al*., 1997). Chlamydospores are round spores with thick walls that can survive in the soil for a long time. They are usually produced at the end of old mycelia in decaying plants (Nelson et al., 1981).

2.3.3 Description

The mycelium of *F. oxysporum f. sp. lycopersici* is colorless at first, but with age it becomes cream colored, pale yellow, pale pink, or purplish (Agrios, 2005).

2.3.4 Distributions

Worldwide, pathogenic races may have different distribution, defined by range common in temperature regions; North and South America, Europe, Africa, Australia and New Zealand. Those are Fusarium in linum spp and Gossypium spp as reported. Whose strains represent some of the most abundant and widespread microbes of the global soil micoflora, (Gordon, and Martyn, 1997). These remarkably diverse and adaptable fungi have been found in soils ranging from the Sonoran Desert, to tropical and temperate forest, grassland and soils of the tundra (Stoner, 1981).

2.3.5 Economic importance

Fusarium wilt is considered as one of the most destructive diseases to tomato in temperate regions (Agrios, 1997). The disease is described as a serious pathogen on the tomato crop in El Salvador (Pérez *et al*., 2003).

Fusarium oxysporum f. sp. lycopersici causes Fusarium wilt specifically in tomato for which it is one of the most prevalent and damaging

diseases. This disease, first described in England in 1895, it is of worldwide importance because at least 32 countries had reported the disease. Fusarium wilt is most destructive in warm climates and sandy soils of temperate regions. The disease causes great losses when soil and air temperatures are rather high during much of the season. Infected plants become stunted and soon wilt and finally die. Occasionally, entire fields of tomatoes are killed or damaged severely before a crop can be harvested (Agrios, 2005).

2.3.6 Symptoms

The first symptoms appear as slight vein clearing on the outer and younger leaflets. Subsequently, the oldest and lowest leaves show yellowing and epinasty caused by drooping of the petioles. Plants infected at the seedling stage usually wilt and die soon. As the disease progresses, growth is typically stunted, and little or no fruit develops. Older plants in the field may wilt and die suddenly, if the infection is severe and if the weather is favorable for the pathogen. However, in older plants, vein clearing and leaf epinasty are more commonly followed by stunting of the plants, yellowing of the lowest leaves, occasional formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of the remaining leaves, and finally death of the plant. After an initial period of stunting, the smallest side roots rot. Also fruit may occasionally become infected and then it rots and drops off (Agrios, 2005).

2.3.7 Development of disease and life cycle

Fusarium wilt occurs both in the field and in greenhouses. The disease develops quickly in warm weather and on sandy soils and the fungus is favored when the soil is low in nitrogen and phosphorous and high in

potassium (Jones *et al*., 1997). Growth and spreading of *Fusarium oxysporum f. sp. lycopersici* is reduced if the soil is low in micronutrients such as copper, iron, manganese, molybdenium and zinc. The micronutrients are more available at a low pH and this is a reason why a high pH (6.5-7.5) is preferable to reduce infection. The source of nitrogen also affects the pathogen, when the nitrogen source is ammonium the pathogen is more virulent (Nelson *et al.*, 1981).

The optimum temperature for growth of *F. oxysporum* is 28-29 °C. There are no symptoms of infection if the soil temperature is below 20 °C or above 30 °C (Holliday, 1980). Growth and survival of *F. oxysporum* is favoured by dry soil, but after infection the spread of the pathogen inside the plant is favoured by moist soil and a high transpiration (Nelson et al., 1981).

In the periods between tomato productions the fungus survives as mycelia, micro or macroconidia on plant debris or as dormant chlamydospores in the soil. When new plants grow in the contaminated soil the spores are stimulated to germinate. A germinating chlamydospore forms conidia or new hyphae (Mace *et al*., 1981). Germ tubes or mycelia penetrate the plant root directly or through wounds in the cuticle. Inside the plant the fungus moves towards the vascular tissue (Agrios, 1997).

The fungus excretes metabolites and auxin, which are transported upwards in the stem, ahead of the mycelia. When the diseased plant reacts to the high auxin levels, the cells surrounding the vascular tissue start to expand. The pressure on the vascular tissue increases and eventually the vessels collapse (Chambers *and corden*, 1963). F. oxysporum also excretes pectolytic and cellolytic enzymes which cause collapse of the vascular tissue (Holliday, 1980).

Mycelia grow and produce microconidia inside the vessels. Spores are transported upwards in the xylem and germinate when the upper wall in the vessel stops them. Mycelia penetrate the walls and spread upwards in the plant, but also to the adjacent vessels (Agrios, 1997). Damaged vessels are filled with mycelia and spores and they lose their capacity to transport water to the upper parts of the plant (Chambers and cordon, 1963). When the water potential decreases the stomata close and the leaves wilt and die. The vessels of diseased plants are smaller, fewer and deformed and the ability to transport water is reduced to 6 % of the capacity of a healthy plant (Mace *et al*., 1981). The fungus sporulates on the surface of dead plant tissue. The spores spread to new plants by wind or water (Agrios, 1997).

2.3.8 Control

2.3.8.1 Culture control

The culture control is the only practical measure for controlling the diseases in the field. The wilt fungus is so widespread and so persistent in soils that seed bed sterilization and crop rotation although always sound practices but are of limited value. Soil sterilization is too expensive for application but it should be always practiced for greenhouse grown tomato plant (Agrios, 2005).

Moreover, use of healthy seed and transplants is of course mandatory, and hot water treatment of seed suspected of being infected should precede planting (Agrios, 2005).

As mentioned above, Fusarium wilts affect and cause severe losses on most vegetable and flowers, several field crops such as cotton, Tobacco, banana, plantain, coffee, sugarcane and a few shade trees. Fusarium wilts

are most severe under warm soil conditions and green house (Agrios, 2005).

Prevent spreading of the pathogen to disease free areas by using clean tools and equipment (Agrios, 1997; Jones *et al*., 1997).

2.3.8.2 Botanical controls

The antifungal effect of certain medicinal and aromatic plants extracts have been investigated by many workers (Singh and Dwivedi, 1987; Handique and Singh 1990). Thus, the development of new and different antimicrobial agents more safe has been a very important step (Agrafotis *et al*., 2002). However, the step of validation of traditional uses of antimicrobial compounds in higher plants was studied by a number of researchers. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrafotis *et al*., 2002).

The use of plant extracts for controlling Fusarium wilt, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of 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*. *oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2000). If natural plant products can reduce populations of soil borne pathogens and control diseases development, than these plant extracts have potential as environmentally safe alternatives and as component in integrated pest management programs. Chand and Singh (2005) reported that the plant extracts, VIZ *Calotropis procera*, *Eucalyyptus globulens*, *Jatropha*

multifida, *Azadirachta indicia*, *Allium sativum* were significantly pronounced in reducing wilt incidence in *Cicer arietinum* L. Mycelial growth of various Fusarium species were inhibited by the plant extracts of Adhatoda vasica, *Azadirachta indica*, *Cinnamomum camphora* and *Ocimum sanctum* (Prasad and Ojha, 1986; Reddy and Reddy, 1987*)*. Bansal and Rajesh, 2000; Nwachukku and Umechuruba (2001) reported the antifungal effect of *Eucalyptus amgdalline, Ailanthus exclsa and Lantana camera*.

2.3.8.3 Chemical control

Presently, Anon (1994) and Ristaino *et al.*,(1997) reported that methyl bromide fumigation is used extensively for tomato production in some geographical areas in addition to reducing or eliminating soil borne diseases like Fusarium wilt. Fumigation allows more rapid transplant growth allowing for earlier harvesting and optimizes fresh markets. The use of methyl promide may be curtailed in near future and alternative chemicals are being examined.

2.2.8.4 Biological control

Recently, biological control of Fusarium wilt seems to be successful. Such control methods include prior inoculation of plants with nonpathogenic strains of *F. oxysporum* or the use of antagonistic fungi, such as Trichoderma and Gliocladium, *Pseudomonas fluorescens* and *Burkholderia cepacia* bacteria. It was shown that spraying tomato plants with a suspension of zoospores of the oomycete Phytophthora cryptogea induces systemic acquired resistance. Although promising, none of these methods have been used for control of Fusarium wilt in practice so far (Agrios, 2005).

2.4 Botanical product used in control of *Fusarium oxysporum f. sp. lycopersici*

2.4.1 Basil (Sweet basil)

2.4.1.1 Scientific Classification

Kingdom: Plantae

(Unranked): Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Ocium

Species: basilicum

Binomial name: *Ocium basilicum* L.

2.4.1.2 Uses of Basil

2.4.1.2.1 Culinary

Basil can be used in soup, stew, stuffing and rice as well as with fish, chicken, vegetables and meats. They can also be a key ingredient in cheeses, vinegars, oils, jellies, teas, drinks and liqueurs, and seeds can be used in beverages (Bown and Deni. 2001, Makinen *et al*,. 1999, Simon, James E. 1995, Simon *et al*,. 1984, Small and Ernest, 1997 and Tucker *et al*,. 2000). Small leaves can be added whole to salads, vegetable dishes, pasta and rice.

2.4.1.2.2 Craft Uses

Basil can also be used in crafts and homemade cosmetics. Leaves can be added to bath bags, facials and hair rinses (Hampstead and Marily, 1984) as well as potpourris, nosegays/tissue mussies and wreaths (Webster 2000). Looking for a functional craft? Try making an herbal moth repellent bag with equal parts camphor basil, lavender and rosemary (Buchanan and Rita, 1987).

2.4.1.2.3 Economic Uses

The essential oil of basil is used in a variety of common products including soaps, cosmetics, dental products, colognes/perfumes, prepared foods and beverages (Bown and Deni. 2001, Simon and James E. 1995, Simon *et al*., 1984, and Tucker *et al*., 2000). Ocium *basilicum* are classified as economic materials in their own right, and European and Egyptian varieties are primary sources of *O. basilicum* oils (Simon and James E, 1995). The essential oil of some types of *O. basilicum* is used in aromatherapy and in soaps, cosmetics and foods (Lawless and Julia,. 1992).

As mentioned in the Pests and Diseases section, basil does have insect repellent properties. The essential oils of *O. gratissimum* and *O. basilicum* are used in commercial insect repellents (Bown and Deni, 2001), the seeds and leaves of *O. kilimandscharicum* have repellent properties (Bown and Deni, 2001), and *O. gratissimum* is used as a mosquito repellent as a live plant (Bown and Deni, 2001, and Tucker *et al*., 2000).

2.4.1.2.4 Medicinal & Ethnobotanical Uses

Most people don't generally think of basil as a medicinal plant, but it has been used in traditional medicine in countries around the world and is showing promise for a variety of medical conditions. *Ocium americanum* has been used in Brazil for kidney problems and rheumatism and in Sudan and India for skin parasites (Small and Ernest, 1997). *Ocium basilicum* has been used in traditional Chinese medicine for kidney problems, gum ulcers and as a hemostyptic in childbirth (PDR for Herbal Medicines, 2000) and for problems as diverse as earache, rheumatoid arthritis, anorexia, skin conditions, menstrual irregularities, and malaria in India (PDR for Herbal Medicines, 2000).

Several basil species have antimicrobial and antifungal properties. *Ocium basilicum* (Blumenthal and Mark, 1998, Opalchenova, G. and D. Obreshkova, 2003) and *O. gratissimum* (Ngassoum, M.B. *et al*., 2003) are reported to be antimicrobial/antibacterial and antifungal (Holm and Yvonne, 1999).

Despite its ethnobotanical history and potential, it's important to keep in mind that basil has not been approved for medicinal use by the German Commission E. Due to a

2.4.1.2.5 Garden Uses

Basils can also be used in the landscape as edgings and annual borders (Tucker *et al*, 2000) and in knot gardens and mazes (Hampstead and Marilyn, 1984, Ross and Marty. 1996).

2.4.2 Peppermint

2.4.2.1 Scientific Classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Mentha

Binomial name: *Mentha piperita* L.

Peppermint (*Mentha piperita* L.) belongs to Lamiaceae family and originated from Mediterranean Regions. It is widely cultivated in the world and is a hybrid mint, a cross between water mint and spearmint (Frampton, 2009).

2.4.2.2 Uses

Peppermint can also be found in some shampoos, soaps and skin care products. Menthol activates cold-sensitive TRPM8 receptors in the skin and mucosal tissues, and is the primary source of the cooling sensation that follows the topical application of peppermint oil (Eccles, 1994).

Peppermint oil has a high concentration of natural pesticides, mainly polygone and menthone (Krieger, 2001). Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches,

and migraines (Hendriks, 1998). The well-known and widely used peppermint is a cultivated natural hybrid of *M. aquatica* L. (water mint) and *M. spicata* L. (spearmint). Although a native genus of the Mediterranean Regions, it is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Peppermint oil is one of the most widely produced and consumed essential oils (Foster, 1990). Menthol is an organic compound made synthetically or obtained from peppermint or other mint oils. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts slightly above. *Mentha piperita* has been shown to possess strong antifungal activity, even when compared to synthetic fungicides. Peppermint oil showed antifungal activity against *Aspergilus niger*, *Alternaria alternata* and Fusarium sp. by agar well diffusion method (Aqil *et al*, 2000). The chemical responsible for this action was menthone (Soković et al., 2009).

2.4.3 *Eucalyptus camaldulensis* (River Red Gum)

Eucalyptus camaldulensis is a common and widespread tree along watercourses over much of mainland Australia. It is frequently a dominant component of riparian communities, and is an iconic and important species of the Murray-Darling catchment, both ecologically and economically (Chippendale, 1988).

2.4.3.1 Scientific Classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Eudicots

(Unranked): Rosids

Order: Myrtales

Family: Myrtaceae

Genus: Eucalyptus

Species: Camaldulensis

Binomial name: Eucalyptus camaldulensis Dehnh.

2.4.3.2 Uses and Economic importance

Eucalyptus camaldulensis fibers are particularly appreciated for manufacturing high-quality grades of tissue paper, writing and printing papers (Foelkel 2009). Improved plant raw materials for pulp and paper purposes have been obtained by modifying lignin biosynthetic pathway; these changes can affect lignin content, composition, or both (Baucher *et al* 2003).

Essential oils from Eucalyptus leaves have been widely used as antiseptic and for the relief of cough symptoms, colds, sore throat and other infections (Kumar *et al*; 2007). Mulyaningsih *et al.*, (2010) have suggested that aromadendrene may significantly contribute to antimicrobial activity of its fruit oil. Combinations of aromadendrene and 1,8-cineole showed additive effects in most cases, but also synergistic behavior.

Eucalypts extracts contain an array of defensive allelochemicals exhibiting various biological effects, antibacterial, antioxidant, and antihyperglycemic, among them (Takahashi et al 2004), with essential oils playing a central role in many of these biological functions. Most essential oils, including those obtained from eucalypts, have shown to

display some degree of antimicrobial activity that is in general related to the presence of terpenoid and phenylpropanoid compounds that have proved to exhibit individual antimicrobial effects (Einhellig 1995).

Different assays were also performed by our research group to determine Eucalyptus essential oil biological activities. Antifungal activity of four Eucalyptus species (*E. camaldulensis, E. globulus, E. sideroxylon, E. tereticornis*) was tested against common pathogens affecting crops production (*Aspergillus flavus, Aspergillus niger, Cladosporium cucumerinum*). Antifungal activity was tested by means of bioautographic assay (Homans and Fuchs 1970), *E. camaldulensis* being more active than *E. globulus, E. sideroxylon* and *E. tereticornis.*

Plant extracts and isolated natural compounds represent a wide range of possibilities to replace or at least diminish the use of synthetic products to control pests and diseases affecting plants, animals and/or human beings. Bioactive natural products should also be seriously considered as they have proved to be more specific in most of their biological activities.

Forestry-derived industries have been focused during last decades on development of breeding techniques to produce trees with higher timber yields and enhanced wood quality (Wallis et al 2010).

Chinese folk medicine has used Eucalyptus species for centuries, hot water extracts of dried leaves from E. citriodora have been, and are still used to prepare anti-inflammatory, analgesic and antipyretic formulas for respiratory infections, such as sinus congestion and flu. Essential oils are easily biodegraded and have proved to exhibit low toxicity against vertebrates also playing an important role as bioherbicide for weed management (Barton 1999, Batish *et al;* 2007, Batish *et al*; 2008).

E. camandulensis essential oil has also proved to be useful for pharmaceutical purposes. It has been used to treat lung diseases and cough in medicines like expectorants, also taking advantage of its antituberculosis, antibacterial, and antifungal properties.

The significant negative effects of *E. camaldulensis* and *E. urophylla* essential oils on *S. aureus* and *E. coli* development contribute to point out the potential of both Eucalyptus species for antiseptic, microbiostatic, or as disinfectant activities (Bachir Raho and Benali 2008).

Eucalyptus camaldulensis essential oils have been preferred over those obtained from other forestry exploited species because they have proved to be useful in perfumery, pharmaceutical and other industries playing multipurpose roles (FAO 1995). They have also proved to negatively affect virus development, Schnitzler et al (2001) reported in vitro activity against antiherpes virus. *E. globulus* essential oil components, alone or in combination with other antibacterial agents, may provide a promising new scheme in phytotherapy.

Some Eucalyptus essentials oils containing high 1,8-cineole amounts have also proved to be effective to control mites. They could be used as a natural acaricides, as they have shown to be effective against varroa mite, *Varroa jacobsoni*, an important parasite of honeybee, *Tetranychus urticae* and *Phytoseiulus persimilis* (Choi *et al*; 2004) and *Dermatophagoides pteronyssinus* (Saad *et al*; 2006).

Essential oils and their major constituents have shown toxicity against a wide range of microbes including bacteria and fungi, both soil-borne and post-harvest pathogens. Su *et al;* (2006) demonstrated the antifungal activity of essential oils from *E. grandis, E. camaldulensis*, and *E. citriodora* against the mildew and wood rot fungi viz, *Aspergillus*

clavatus, A. niger, Chaetomium globosum, Cladosporium cladosporioides, Myrothecium verrucaria, Penicillium citrinum, Trichoderma viride, Trametes versicolor, Phanerochaete chrysosporium, Phaeolus schweinitzii, and *Lenzites sulphureus*.

Eucalypts cause reduction in crop growth and yield when agricultural crops are grown close to eucalypts (Jagger and Pender, 2003; Jiregna Gindaba, 2003; Selamyihun Kidanu *et al.*, 2004, 2005; Tilashwork Chanie *et al*., 2013).

Eucalypts have been reported to cause crop loss by outcompeting crops for water and soil nutrients (Michelsen *et al*., 1993; Jiregna Gindaba, 2003; Tilashwork Chanie *et al.*, 2013), through shading (Tilashwork Chanie *et al*., 2013) and producing allelochemicals (Lisanework and Michelsen, 1993; Ahmed *et al*., 2008). Studies conducted so far mainly focused on reduction in crop growth and yield when eucalypt trees are grown on or close to farmlands (e.g. Jagger and Pender, 2003; Tilashwork *et al*., 2013). There has been also fear of crop loss owing to the perceived long-term site deterioration allegedly caused by eucalyptus (Jiregna, 2006)

CHAPTER THREE

Material and Method

3.1. Experimental site

This study was conducted under laboratory conditions at Plant pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology (SUST) within the period January to March 2015, to evaluate **the antifungal activity of Sweet basil, peppermint and** River red gum **leaves aqueous extracts and efficacy of fungicide,** Revus top®**, against *Fusarium oxysporum f. sp. lycopersici*.**

3.2. Fungal inoculum

Random samples were collected from roots and stems of infected tomatoes plants (*Lycopersicon esculentum* Mill.) in fields at Wad Ramli area. Secured samples were put in paper bags and brought to laboratory where they kept in refrigerator for further investigations.

3.3 Isolation of the pathogen from tomato

Isolation was done from diseased roots and stem of diseased tomato plant showing typical symptoms of Fusarium wilts. They were then cut into pieces of 0.5 to 1 cm, washed under tap water for about 5 minutes to remove soil particles. The washed pieces were dipped in 70% ethyl alcohol (5% concentration) for 2 minutes and rinsed three times in changes of sterilized distilled water and dried on sterilized filter paper. The sterilized sections were then after plated at the rate of 6 sections per plate on potato dextrose agar (PDA) medium.

3.4. Identification of pathogen

The Petri dishes were incubated at 25^0C. After incubation for 7days, growing fungus was sub-cultured on PDA medium for further

purification of the fungus. Furthermore, Compound microscopic examinations were carried out for Mycelia and conidia structure based on the method of (Booth key, 1977) to confirm that the fungus is ***Fusarium oxysporum* f.sp. *lycopersici***. Standard books and research papers were also consulted during the examination of this fungus (Aneja, 2004; Rifai, 1969; Barnet and Hunter, 1999). The purified Isolates were maintained on PDA medium for further studies.

3.5. Preparations

3.5.1 Preparation of *Fusarium oxysporium* inoculum

Using a cork-borer (1cm), agar plugs were taken from the actively growing region of the mycelial growth for sub-culturing in other sterilized Petri dishes containing PDA medium and left for 7 days under fluorescent light at the room temperature. From these plates pure cultures of *Fusarium oxysporum f. sp. lycopersici* isolates were used for the experiment (Ramprasad, 2005).

3.5.2 List of plant species tested for antifungal activity

Name of plant	Family
Basil (*Ocium basilicum L.*)	Lamiaceae
Peppermint (*Mentha piperita L.)*	Lamiaceae
River red gum *(Eucalyptus camaldulensis*)	Myrataceae

3.5.3 Aqueous extract preparation

Sweet basil and River red gum leaves were collected from Shambat area where **peppermint was obtained from Omdurman Market. All samples were** brought to the laboratory where they were shade dried. The

samples were freed from foreign materials like stones, sand and dust, before being kept in the laboratory for further investigation. The leaves were then washed with water, dried, and milled using laboratory mill into fine powder. The powdered samples were then weighted separately (25, 50 and 100 g) and placed in 75, 50 and 100 ml of sterilized distilled water respectively and placed in a shaker for 24 hrs. The extracts were then filtered overnight to obtain the concentrations 100%, 50%, and 25%.

3.5.4 Preparation of Revus top fungicides

The chemical tested was Revus Top fungicide. Two ml was dissolved in 100 ml of sterilized distilled water and the concentration 25, 50 and 100 ppm was obtained by serial dilution test.

3.6 Inhibition of Fusarium growth

Inhibition zone technique was used in this study (Rao and Srivastava, 1994). The PDA media was amended with the required concentration from Sweet basil, River red gum, Peppermint and fungicide Revus top® before being solidified in a conical flask of 250 ml containing 100ml of PDA medium, agitated and poured 25 ml into each sterilized Petri dish. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

Each solidified medium was then inoculated centrally by a fungal growth disc cut by a sterile cork-borer (5 mm) from an edge of an actively growing culture of the fungus *Fusarium oxysporum f. sp. Lycopersici* grown on PDA as described above. The inoculated Petri dishes were then incubated at room temperature and the radial growth was measured every two days. All treatments were done in triplicates and were arranged in a Complete Randomized Design.

3.7 Calculation

Every 48 hours, the diameter of growth was measured by taking the average of two crossed dimensions for each disc in the Petri dish. The radial growth was then calculated as a percentage from the diameter (9.0 cm) of the glass Petri dish. The effect of each extract concentration on linear fungal growth was calculated as percentage of inhibition in diameter of fungal growth: -

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times 100$$

Where:-

dc = Average increase in mycelial growth in control,

dt = Average increase in mycelial growth in treatment,

3.8 Statistical analyses

The obtained data was statistically analyzed according to analysis of variance (ANOVA); -Duncan's Multiple Range Test was used for mean separation using SAS software for windows version 9.

CHAPTER FOUR

RESULTS

This study was conducted under laboratory conditions at Plant Protection Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology within the period January to March 2015, to evaluate **the antifungal activity of Sweet basil, peppermint and** River red gum **leaves aqueous extracts and efficacy of fungicide,** Revus top®**, against *Fusarium oxysporum f. sp. lycopersici*.**

4.1. Effect of different concentrations of plants leaves aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum f. sp. lycopersici in vitro* two days after inoculation

The results (Table, 1) showed that the leaves aqueous extracts of all plants tested and fungicide exhibited an inhibitory effect on the fungal growth after 2 days from inoculation. The percentage inhibition ranged from 5.9 % at 25 % concentration of River Red Gum to 100 % inhibition achieved by 50 and 100 % concentrations of fungicide. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

Among plant extracts, Basil aqueous extract at all concentrations (25, 50, and 100 %) gave the highest inhibition of mycelial growth (31.9, 39.9 and 51.7 %) followed in descending order by River red gum which gave reduction in linear growth of the fungus as (5.9, 41.3 and 43.5 %) at the three concentrations (25, 50, and 100 %) respectively and the lowest reduction (15.5, 24.6 and 27.4 %) was obtained by Mint at the three concentrations (Table 1). Moreover, the fungicide especially at 50 and 100 % concentration demonstrated 100% inhibition. However, the

suppressing effect of fungicide was more pronounced (83.2, 100 and 100 %) at all concentrations tested than other treatments.

Table 1: Effect of different concentrations of plants leaves aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum f. sp. lycopersici in vitro* two days after inoculation

Products		Inhibition zone (%)			
Plant extract	Concentrations	R1	R2	R3	Mean
Mint	25%	28.5 (5.4)	10.3 (3.3)	7.6 (2.8)	15.5(3.8)ef
	50%	14.2 (3.8)	17.2 (4.2)	42.3 (6.5)	24.6(4.8)de
	100%	46 (6.8)	24.1 (5)	11.5 (3.5)	27.4(5.1)de
Basil	25%	28.5 (5.4)	17.2 (4.2)	50 (7.1)	31.9(5.6)de
	50%	57.1 (7.6)	24.1 (5)	38.4 (6.2)	39.9(6.3)cd
	100%	39.2 (7.6)	62 (7.9)	53.8 (7.4)	51.7(7.6)bc
River red gum	25%	0 (0.07)	13.7 (3.8)	3.8 (2.1)	5.9 (2.2)fg
	50%	39.2(6.3)	34.4 (5.9)	50 (7.1)	41.3(6.4)cd
	100%	42.8 (6.6)	41.3 (6.5)	46.1 (6.8)	43.5(6.7)cd
Fungicide	25%	92.8 (9.7)	75.8 (8.7)	80.7 (9)	83.2(9.1)ab
	50%	100 (10)	100 (10)	100 (10)	100 (10.0)a
	100%	100 (10)	100 (10)	100 (10)	100 (10.0)a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0.0 (0.7)g
C.V. (%)					17.03
SE±					0.46
LSD					1.725

Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

4.2. Effect of different concentrations of plants leaves aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum f. sp. lycopersici in vitro* four days after inoculation

In day four after inoculation, all plant extracts concentrations as well as that of the fungicide were invariably continued exhibiting suppressing effects against the fungal growth. However, all concentrations of the fungicide (25, 50, and 100%) demonstrated the significantly highest inhibition zones percent (86.1, 92.5 and 99.3 %) respectively followed by Basil which gave 22.7, 42.8 and 48.5 and the lowest inhibition zone percent was given by Eucalyptus at 25 and 50 % concentrations (22.4 and

39.3) . Moreover the inhibitory effect from all concentrations tested was significantly different from control (Table, 2).

Table, 2: Effect of leaves aqueous extracts of Sweet basil, peppermint, River red gum and fungicide Revus top on the linear growth of *Fusarium oxysporum f. sp. Lycopersici in vitro* four days after inoculation

Treatment		Inhibition zone (%)			
Plant extract	Concentration	R1	R2	R3	Mean
Mint	25%	38.3 (6.2)	24.4 (5)	61.5 (7.9)	41.5(6.4)bc
	50%	45 (6.7)	42.8 (6.6)	36.5 (6.1)	41.5(6.5)bc
	100%	53.3 (7.3)	20.4 (4.6)	51.9 (7.2)	41.9(6.4)bc
Basil	25%	33.3 (5.3)	2 (1.6)	32.6 (5.8)	22.7(4.2)c
	50%	53.3 (7.3)	32.6 (5.8)	42.3 (6.5)	42.8(6.5)bc
	100%	55 (7.4)	34.6 (5.9)	55.7 (7.5)	48.5(6.9)b
Eucalyptus camaldulensis	25%	38.3 (6.2)	2 (1.6)	26.9 (5.2)	22.4(4.3)c
	50%	40 (6.4)	20.4 (4.6)	50 (7.1)	36.8(6)bc
	100%	56.6 (7.6)	28.5 (5.4)	32.6 (5.8)	39.3(6.2)bc
Fungicide	25%	90 (9.5)	83.6 (9.2)	84.6 (9.2)	86.1(a
	50%	93.3 (9.7)	91.8 (9.6)	92.3 (9.6)	92.5 a
	100%	100 (10)	97.9 (9.9)	100 (10)	99.3 a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7) d
C.V. (%)					19.71
SE±					0.42
LSD					2.120

Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

4.3. Effect of leaves aqueous extracts of Sweet basil, peppermint, River red gum and fungicide Revuse top on the linear growth of *Fusarium oxysporum f. sp. lycopersici in vitro* six days after inoculation

After six days from inoculation the results (Table, 3) showed that extracts of all the plants tested as well as the fungicide proved to be effective in suppressing the fungal growth.

In fact, all tested concentrations of Mint, **Sweet basil,** River red gum and fungicide continued inducing a significant inhibition zones percentage against test fungus compared to control (Table, 3). Meanwhile, the River red gum aqueous extract at all concentrations tested (25, 50, and 100 %)

gave relatively more inhibitory effect (44.1, 53.1 and 53.1 %) than Basil (36.8, 51.5 and 54.4 %) and Mint aqueous extract as well (35.5, 39.6 and 39.6 %) respectively.

Obviously, the test organism differs in its response to the different concentrations but on the whole, growth inhibition increased with increasing concentration. This inhibitory effect from all concentrations was significantly different from control.

Table, 3: Effect of leaves aqueous extracts of Sweet basil, peppermint, River red gum and fungicide Revuse top on the linear growth of *Fusarium oxysporum f. sp. Lycopersici in vitro* six days after inoculation.

Treatments		Inhibition zone (%)			
Plant extract	Concentrations	R1	R2	R3	Mean
Mint	25%	40.3 (6.4)	16.6 (4.1)	49.5 (7.1)	35.5(5.8) d
	50%	53.8 (7.4)	34.3 (5.9)	30.6 (5.6)	39.6(6.3) bcd
	100%	25.9 (5.1)	35.2 (6)	58.4 (7.7)	39.9(6.2) bcd
Basil	25%	41.3 (6.5)	35.2 (6)	33.6 (5.8)	36.8(6.1) cd
	50%	51.9 (7.2)	48 (7)	54.4 (7.4)	51.5(7.2) bc
	100%	55.7 (7.5)	54.9 (7.4)	53.4 (7.3)	54.7(7.4) b
River red gum	25%	49 (7)	43.1 (6.6)	40.5 (6.4)	44.3(6.6) bcd
	50%	55.7 (7.5)	50.9 (7.2)	52.4 (7.3)	53.1(7.3) bc
	100%	55.7 (7.5)	51.9 (7.2)	51.4 (7.2)	53.1(7.3) bc
Fungicide	25%	94.2 (9.7)	92.1 (9.6)	92 (9.6)	92.8(9.6) a
	50%	92.3 (9.6)	96 (9.8)	92 (9.6)	93.5(9.6) a
	100%	99 (10)	100 (10)	100 (10)	99.7(10) a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)e
C.V. (%)					13.04
SE±					0.40
LSD					1.090

Means followed by the same letter are not significantly different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

4.4. Effect of leaves aqueous extracts of Sweet basil, peppermint, River red gum and fungicide Revus top on the linear growth *of Fusarium oxysporum f. sp. lycopersici in vitro* eight days after inoculation

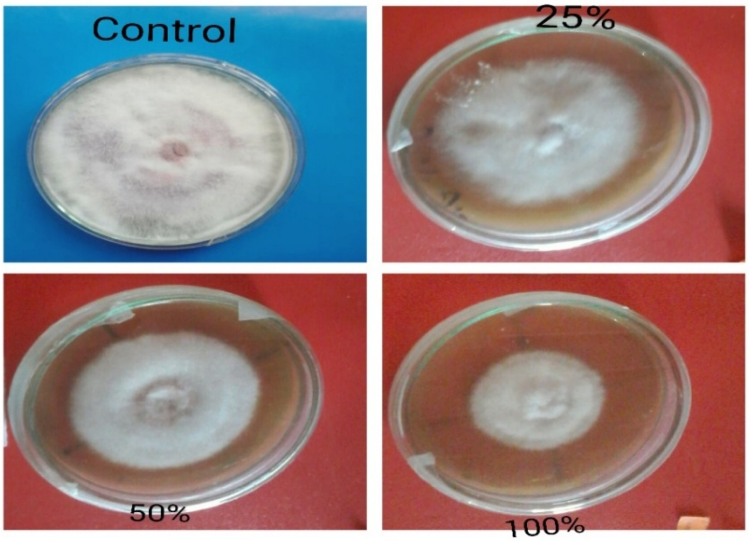
After eight days from inoculation the results (Table, 4) showed that extracts of all the plants tested as well as the fungicide maintained their suppressing effect on the fungal growth. This suppressing effect of all tested concentrations of Mint, **Sweet basil,** River red gum and fungicide was significantly higher than the control (Table, 4). However, among all treatments, the inhibitory effect of the fungicide at all concentrations was more pronouncing than others. Moreover, the assessment of the fungicide effect on fungal growth after eight days from inoculation showed a concentration dependant differential inhibition (Table 4) where the percentage inhibition increased with increasing concentration.

Table, 4: Effect of different concentrations of plants leaves aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum f. sp. lycopersici in vitro* eight days after inoculation

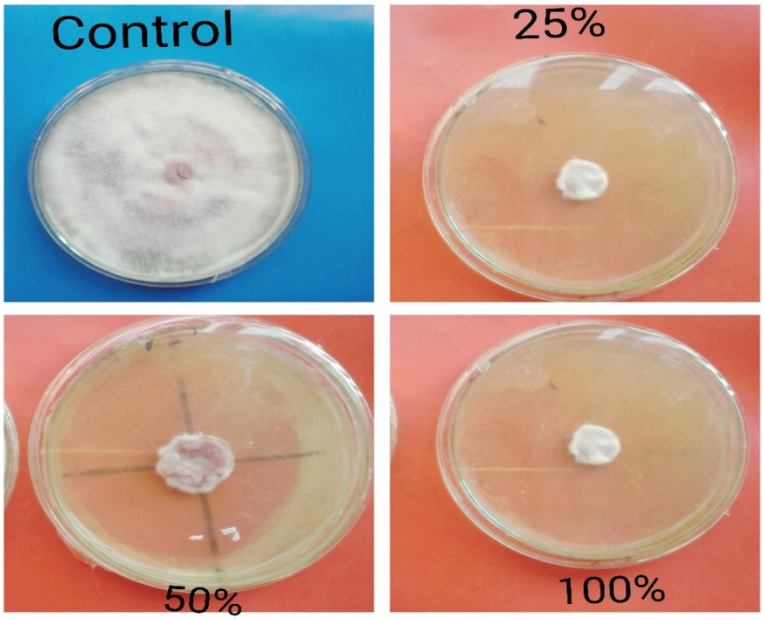
Treatments		Inhibition zone (%)			
Plant extract	Concentrations	R1	R2	R3	Mean
Mint	25%	38.2 (6.2)	19.4 (4.5)	53.3 (7.3)	37.0(6) c
	50%	21.9 (4.7)	38.8 (6.3)	51.8 (7.2)	37.5(6) c
	100%	58.5 (7.7)	49.2 (7.1)	40.7 (6.4)	49.5(7) bc
Basil	25%	39.8 (6.4)	40.2 (6.4)	41.4 (6.5)	40.5(6.4) c
	50%	49.5 (7.1)	46.2 (6.8)	46.6 (6.9)	47.5(6.9) bc
	100%	54.4 (7.4)	58.2 (7.7)	57 (7.6)	56.6(7.5) b
River red gum	25%	38.2 (6.2)	41 (6.4)	40.7 (6.4)	40.0(6.2) c
	50%	36.5 (6.1)	42.5 (6.6)	41.4 (6.5)	40.2(6.4) c
	100%	43 (6.6)	41 (6.4)	45.1 (6.8)	43.1(6.6) bc
Fungicide	25%	95.1 (9.8)	94 (9.7)	94 (9.7)	94.4(9.7) a
	50%	93.4 (9.7)	97 (9.9)	94 (9.7)	94.9(9.7) a
	100%	99.1 (10)	100 (10)	100 (10)	99.7(10) a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)d
C.V. (%)					8.25
SE±					0.38
LSD					0.9553

Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

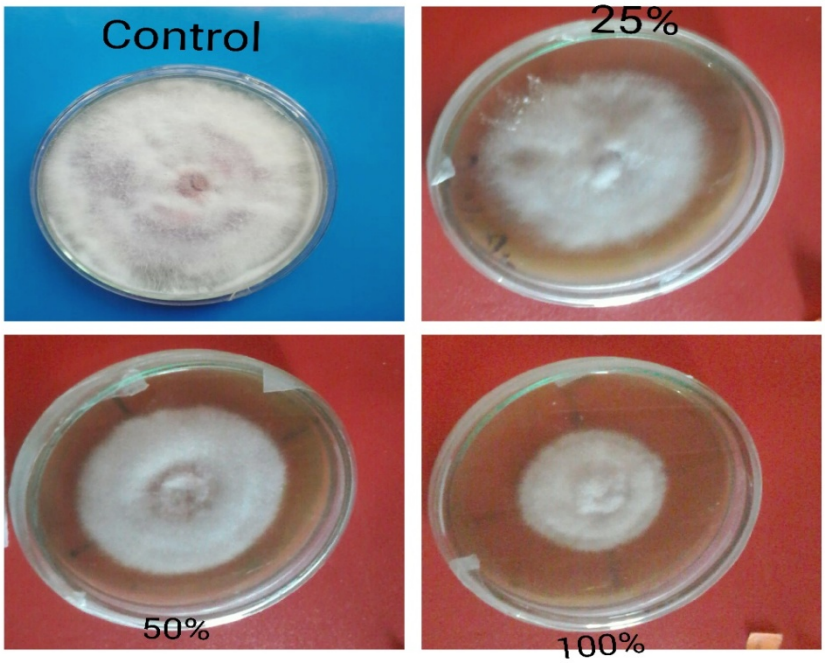
Experiments plates which explain effect of aqueous extract (Mint, Basil and Eucalyptus) and fungicide on growth of *Fusarium oxysporum f. sp. lycopersici in vitro* eight days after incubation.



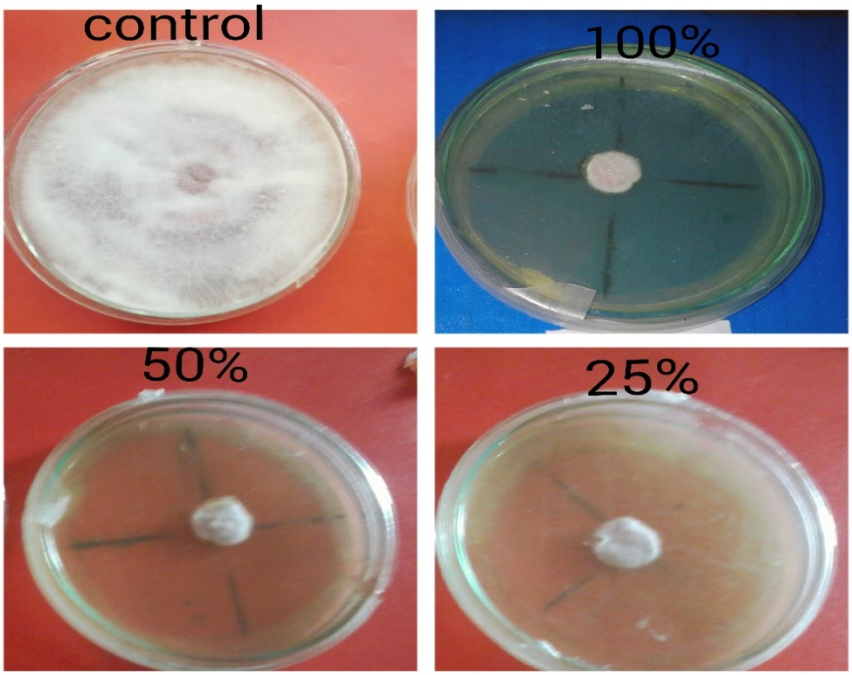
Plate, 1: Effect of leaves aqueous extracts of Basil on growth of *Fusarium oxysporum f. sp. lycopersici in vitro*



Plate, 2: Effect of leaves aqueous extracts of Basil on growth of *Fusarium oxysporum f. sp. lycopersici in vitro*.



Plate, 3: Effect of leaves aqueous extracts Eucalyptus on growth of *Fusarium oxysporum f. Sp. Lycopersici* in vitro.



Plate, 4: Effect of leaves aqueous extractsfungicide (Revus Top) on growth of *Fusarium oxysporum f. Sp. Lycopersici* in vitro.

CHAPTER FIVE

DISCUSSION

Tomato (*Lycopersicon esculentum Mill.*) is considered as one of the most important and popular vegetable in many countries. The global production of tomatoes doubled three times in the last 4 decades (FAO, 2006). This is because of its acceptable flavor, nutritive value and ability to fruit in a wide range of environments and the relative ease with which it can be cultivated (Suarez *et al.*, 2007). Many diseases affect tomatoes during the growing season, both in greenhouse and field. Among these are Fusarium wilt disease, caused by pathogenic formae speciales of the soil-inhabiting fungus; *Fusarium oxysporum* f. sp *lycopersici*. In fact, wilt of tomato is one of the most economically important diseases world-wide (Rick, 1979; Cal *et al*., 2004 and Srinon *et al*., 2006).This pathogenic fungus remains to be a challenging task in terms of management (Rick, 1976; Agrios, 2005 and Srinon *et al*., 2006).

In Sudan tomato is becoming increasingly important for local consumption and for export. It is cultivated throughout the year under irrigation in an area that exceeds 36540 hectares with an average yield of 17.57 tons per hectare *(*AOAD, 2007 and Dawelbeit *et al*., 2010*)*. Likewise, in Sudan, several diseases are known to limit production of tomato, of which Fusarium wilt caused by (*Fusarium oxysporum*f. sp. *lycopersici*) is one of the most important (Bhatia *et al.*, 2004*)*.

A number of research findings have presented strategies to control this fungal pathogen ((Haware and Nene, 1982; Jiménez-Díaz, *et al*., 1993; Biondi *et al*., 2004 and Ahmed, 2011). However, management of seed-borne and soil-borne diseases such as tomato wilt caused by *Fusarium*

oxysporum f. sp. Lycopersici has always been problematic (Rao and Balachadran, 2002). Generally, use of synthetic fungicides considerably reduce wilt incidence in tomato but their use is costly as well as environmentally undesirable (Song and Goodman, 2001). Moreover, the use of resistant varieties is faced with breakdown of resistance due to high pathogenic variability in the pathogen population (Kutama *et al*., 2011; 2013). In this context, the searches for an eco-friendly way of managing Fusarium wilt in tomato which offers an alternative to fungicides is highly demanding.

Fortunately, progress achieved in recognizing antimicrobial compounds in higher plants gave more promises in combating plant pathogenic diseases. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer, 2002). In fact, higher plants with biologically active secondary metabolites are extremely abundant where over 80% of all known Alkaloids, Terpenioid, Phenols and other secondary metabolite were produced from them (Siddig, 1993 and Newman, *et al.*, 2000).

The results (Tables 1 to 4) revealed that the Sweet basil, peppermint and River red gum leaves aqueous extracts and fungicide, Revus top®, solution consistently and throughout the course of the experiments exhibited an inhibitory effect on mycelial radial growth of the fungus with significantly higher inhibition reduction growth percent compared to control. Similar studies which explored the effect of extracts of many higher plants and their essential oils have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Agrafotis, 2002; Okigbo and Ogbonnaya, 2006; Shariff et. al., 2006; Ergene et. al., 2006; Kiran and Raveesha, 2006). In fact, this finding is in agreement with Muntasir (2014) who tested the bioactivity of basil

extract against fungi and demonstrated its suppressing effect on the fungal growth in vitro. Bansal and Rajesh, 2000; Nwachukku and Umechuruba (2001) were also reported the antifungal effect of River red gum.

As demonstrated by many researchers there are a considerable interest in the use of Mint for controlling various fungal diseases in plants (Kalemba 2003 and Soković *et al*., 2009). Similar results were Moghtader (2013) who tests the effect of essential oil of its *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*. As well known Bicarbonates are widely used in the food industry and were found to suppress several fungal diseases of cucumber plants.

The data presented in this study showed that the use of Basil in vitro expressed an inhibitory effect against the mycelial growth of *Fusarium oxysporium* and the percentage zone of inhibition was significantly higher than the control. The obtained results were in line with that of Nafiseh Katooli et al., (2012) who tests the Antifungal activity of Eucalyptus (*Eucalyptus camaldulensis*, L.) essential oil evaluated on suppressed the mycelial growth of postharvest pathogenic fungi, *Penicillium digitatum, Aspergillus flavus, Colletotrichum gloeosporioides* and soilborne pathogenic fungi, *Pythium ultimum, Rhizoctonia solani, Bipolaris sorokiniana* pathogenic fungi.

Generally, uses of synthetic fungicides considerably reduce the impact of this disease. In this study the fungicide revus top consistently inhibited the radial mycelial growth of *N. mangiferae* and its suppressing effect was more pronounced at all concentrations tested throughout the time of the investigation. These results confirm that which reported by Themis *et al*., (2005) who indicated the effectiveness of fungicides against other fungi that infect limb dieback of figs in California.

CONCLUSIONS

- The leaves aqueous extracts of all plants tested exhibited an inhibitory effect on fungal growth. Thus the two components plus fungicide (Revus top) could be applied as part of an integrated approach to control Fusarium wilt in Tomato.

- The Sweet basil plant leaves aqueous extract exhibited more inhibitory effect than that of the *Eucalyptus camaldulensis* and Mint. This finding is the first one of its kind in Sudan in the invitro of Fusarium wilt control in Tomato which suggests more investigation to be carried out in this area.

- The screened concentrations of *Eucalyptus camaldulensis*, Sweet basil and Mint leaves aqueous extracts differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of extracts. This variability in response which expressed by test organism may be used to adjust an optimum dose for controlling Fusarium wilt in Tomato.

RECOMMENDATIONS:

Based on the foregoing results the following studies were recommended;

- To further investigate the antimicrobial properties in a group of medicinal plants against targets organism to determine their potentials as botanical pesticides,

- To carry out a phytochemicals analysis of different parts of sweet basil plant using different solvents so as to determine the bioactive ingredient in each of these parts.

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APPENDIXS

Appendix 1: ANOVA

a) Variable 1 (inhibition in two day after inoculum)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	285.153	23.763	22.505	0.0000
Within	26	27.453	1.056		
Total	38	312.607			

Coefficient of Variation = 17.03%

b) Variable 2 (inhibition after four day after inoculum)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	218.421	18.202	11.407	0.0000
Within	26	41.487	1.596		
Total	38	259.908			

Coefficient of Variation = 19.71%

b) Variable 3 (inhibition after six day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	221.116	18.426	24.303	0.0000
Within	26	19.713	0.758		
Total	38	240.829			

Coefficient of Variation = 13.04%

D) Variable 6 (inhibition after eight day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	201.454	16.788	51.880	0.0000
Within	26	8.413	0.324		
Total	38	209.868			

Coefficient of Variation = 8.25%

Appendix 2: (PDA)

Potato Dextrose Agar 93.5g/litter

Potato infusion 4.0g, Dextrose 20g, Agar 15g, Distilled water 1000ml

Appendix 3: (Fungicide)

Name: Revus top®

Active ingredients: Mandipropamid + Difenoconazole

Manufactured for: Syngenta Crop Protection, North Carolina.

Mode of action: inhibition the germination of fungal spores (zoo spore and sporangia spores), inhibit germination of hyphae and inhibit form of spores.

Appendix 4:

Tomato plant was isolated form it fungus *Fusarium oxysporum f. sp. Lycopersici.*

Appendix 5:

Materials, tools and equipments used in the study

- Gloves
- Camera
- Marker pen
- Electric blender
- Petri-dishes
- Sensitive balance
- Incubator
- Needle
- Flame
- Laminar flow cabinet
- Microscope
- Autoclave
- Slide
- Aluminum foul
- Water path
- Potato dextrose agar (PDA)
- Antibiotic
- Filter papers
- Medical cotton
- Thermometer
- 70% ethyl alcohol
- Cork poorer