Sudan University of Science and Technology
College of Graduate Studies

Bioactivity of Alcohol Extracts from some Parts of Mesquite (*Prosopis juliflora*) and Fungicide (Amistar top) on Growth of *Fusarium oxysporum f. sp. ciceri* under laboratory conditions

الفعالية الحيوية للمستخلصات الكحولية من بعض اجزاء المسكيت والمبيد الفطري امستار توب علي نمو الفطر فيوزاريم اوكسيسبورم تحت ظروف المعمل

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By :

Hafiz Osman Ramadan Omer

Supervisor:
Dr. Ibrahim Saeed Mohammed

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الآيــــة

قال تعالي:

﴿فَلْيَنظُرِ الْإِنسَانُ إِلَى طَعَامِهِ (24) أَنَّا صَبَبْنَا الْمَاءَ صَبًّا (25) ثُمَّ شَقَقْنَا الْأَرْضَ شَقًّا (26) فَأَنبَتْنَا فِيهَا حَبًّا (27) وَعِنَبًا وَقَضْبًا (28) وَزَيْتُونًا وَنَخْلًا (29) وَحَدَائِقَ غُلْبًا (30) وَفَاكِهَةً وَأَبًّا (31) مَتَاعًا لَّكُمْ وَلِأَنْعَامِكُمْ (32)﴾

صدق الله العظيم

{من الآية (24) إلى الآية (32)}سورة عبس

DEDICATION

To Soul of my father

My mother

Sisters and brothers

To my wife

I dedicate this work with sincerer love

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Greatest thanks to **my family** especially my **mother** and my **wife** for their care, love and continuous encouragement in all my life.

With respect

The researcher

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Abstract

Increasing hazards to public health and environment due to indiscriminate use of synthetic pesticides coupled with development of resistant strains of phytopathogenic fungi has initiated the exploration of safe alternate product. A good number of reports outlined the antimicrobial effects of some plants extracts for plant disease control. This study was conducted at the Plant Pathology Laboratory, Faculty of Agricultural Studies, Sudan University of Science and Technology January 2018; to investigate, under laboratory conditions, the bioactivity of alcoholic extracts of some parts of mesquite plant namely, fruits, leaves and bark plus Fungicide Amistar top against the growth of Fusarium wilt fungus of chick pea. Three concentrations of 5%, 10% and 20% of each of the three extracts of mesquite tree parts and 100 % concentration of fungicide Amistar Top in addition to control were used in this study. The results showed that all extracts had a significant inhibitory effect on the growth of the fungus. Among the three extracts, those of leaves and bark, especially concentration of 20%, were found to be superior in inhibiting the growth of the fungus by 61.8 % and 71.8 % respectively compared to control. When comparing all the treatments, it was found that the extracts of the leaves and bark of the Mesquite tree in addition to Amistar top fungicide were exhibited superior inhibitory on the growth of the fungus by 61.8% ، 65% ، 71.8% and 100% respectively compared to control. The results also showed that effectiveness of the extract was directly proportional to its concentration. The results obtained from this study are promising and encouraging carrying out further chemical analyzes of various parts of the Mesquite tree using different extracts to identify the effective ingredient in each of these parts to

use it as alternatives to harmful pesticides that adversely affect human, animal and environmental.

ملخص البحث

إن الأخطار المتزايدة علي الصحة العامة والبيئة نتيجة للاستعمال الغير مرشد للمبيدات المصنعة إلى جانب ظهور سلالات مقاومة من الفطريات الممرضة للنبات قد أدت إلى الشروع في استكشاف منتجات بديلة آمنة. هنالك عدد جيد من التقارير التي أبرزت التأثير المضاد للميكروبات لدى بعض المستخلصات النباتية لمكافحة الأمراض النباتية. أجريت هذه الدراسة بمعمل أمراض النباتات بكلية الدراسات الزراعية ، جامعة السودان للعلوم والتكنولوجيا يناير 2018م ، لبحث الفعالية الحيوية للمستخلصات الكحولية لبعض أجزاء نبات المسكيت تحديداً, الثمار، الأوراق و اللحاء والمبيد اميستار توب تحت ظروف المعمل علي نمو فطر الذبول الفيوزاريمي في الحمص. استخدمت ثلاث تراكيز(5% ، 10% و 20 %) لكل من المستخلصات الثلاثة و100% من المبيد اميستار توب بالإضافة للشاهد. أوضحت الدراسة أن كل المستخلصات ذات آثر معنوي في تثبيط نمو الفطر ومن بين المستخلصات الثلاثة وجد أن مستخلصي أوراق ولحاء المسكيت و خاصة التركيز العالي 20% كانا الأمثل في تثبيط نمو الفطر بنسبة 61.8% و % 71.8 علي التوالي.. عند مقارنة كل المعاملات وجد أن مستخلصي الأوراق واللحاء الكحوليين و مبيد Amistar Top® (61.8% ، 65% ، 71.8% و 100%) علي التوالي أظهروا نتائج ممتازة في تثبيط نمو الفطر. كما أظهرت النتائج أن الفعالية ضد الفطر تزداد بزيادة تركيز المستخلصات. النتائج المأخوذة من مستخلصات شجرة المسكيت تعتبر واعدة ومشجعة للقيام بتحاليل كيميائية لمختلف أجزاء شجرة المسكيت باستعمال مستخلصات مختلفة لتحديد المادة الفعالة في كل من هذه الأجزاء والانتفاع بها كبدائل لاستخدام المبيدات الضارة علي صحة الإنسان, الحيوان والبيئة.

CHAPTER ONE

INTRODUCTION

Fusarium oxysporum. Causes vascular wilt diseases in a wide variety of economically important crops (Beckman 1987). Vascular wilt has been a major limiting factor in the production of many agricultural and horticultural crops, including banana (*Musa spp*.) (*F. oxysporum* f. sp. cubense), cabbage (*Brassica spp*.) *(F. oxysporum* f. sp. conglutinans), cotton (*Gossypium spp*.) (*F. oxysporum f.* sp. vasinfectum), flax (*Linum spp*.) *(F. oxysporum* f.splini), muskmelon (*Cucumis spp*.) (*F. oxysporum* f. sp. melonis), onion (*Allium spp.)* (*F. oxysporum* f. sp. cepae), pea (*Pisum spp*.) (*F. oxysporum f. sp. pisi*), tomato (*Lycopersicon spp*.) (*F. oxysporum f. sp. lycopersici*), watermelon (*Citrullus spp*.) (*F. oxysporum f. sp. niveum*), china aster (*Calistephus spp*.) (F. *oxysporum f. sp.* callistephi), carnation (*Dianthus spp*.) (*F. oxysporum f. sp*. dianthi), chrysanthemum (*Chrysanthemum spp*.) (*F. oxysporum f. sp.* chrysanthemi), gladioli (*Gladiolus spp.*) (*F. oxysporum f. sp*. gladioli) and tulip (*Tulipa spp*.) (*F. oxysporum f. sp. Tulipae*) (Armstrong and Armstrong 1981, Mac Hardy and Beckman 1981)

The genus Fusarium is a soil borne, necrotrophic, plant pathogenic fungus with many species that cause serious plant diseases around the world.

F. oxysporum causes primarily vascular wilts on many crops, whereas numerous species, especially *F. solani*, cause root and stem rots and rots of seeds that are accompanied by the production of mycotoxins. A *Fusarium* species causing disease in immune compromised human patients has been reported.(Agrios,2005).

Fusarium wilt is a common vascular wilt fungal pathogen .exhibiting symptoms similar to Verticillium wilt. The pathogen that causes Fusarium wilt is *Fusarium oxysporum* (Snyder and Hansen,1940) The fungus can survive in

the soil as mycelium or as spores in the absence of its hosts. If a host is present, mycelium from germinating spores penetrates the host roots, enters the vascular system (xylem) in which it moves and multiplies, and causes the host to develop wilting symptoms.

It is of worldwide importance where at least 32 countries had reported the disease, which is particularly severe in countries with warm climate (Mui-Yun, 2003). The Fusarium fungus is a known pathogen of tomato plant (Suarez *et al*., 2007) which is present in all important tomato growing regions of the world (Mohammed, 1990).

There is a limited information or lack of effective control measures of the disease. Accordingly, an effective control measures should be developed to control this devastating disease that represent real threat to fruit, forest and ornamental trees. The aim of this study was to explore the antifungal activity of extracts of different parts of Mesquite plants and the efficacy of systemic fungicide in suppressing the growth of this fungus in vitro with the following objectives**:**

- To explore the inhibitory effect on alcohol extracts of different part of Mesquite (Fruit, Bark and Leaves) against F. oxysporum .
- To evaluate the efficacy against systemic fungicide (Amistar Top) in suppressing the fungus F. oxysporum. in vitro
- To develop promising disease management component against Fusarium wilt.

CHAPTER TOW

LITEREATURE REVIEW

2.1. Fusarium Wilt :

As mentioned earlier, Fusarium wilts affect and cause severe losses on most vegetables and flowers; several field crops, such as cotton and tobacco; plantation crops, such as banana plantain, coffee, and sugarcane; and a few shade trees. Fusarial wilts are most severe under warm soil conditions and in greenhouses. Most Fusarial w0ilts have disease cycles and develop similar to those of the Fusarium wilt of Tomato (Agrios, 2005).

2.1 .1. Classification :

Kingdom : Fungi

Division : Ascomycota

Class : Sordariomycetes

Order : Hypocreales

Family : Nectriaceae

Genus : Fusarium

Species : *Fusarium oxysporum*

(Snyder and hansen, 1940).

2.1.2.The Pathogen:

F. oxysporum the mycelium is colorless at first, but with age it becomes cream-colored, pale yellow, pale pink, or somewhat purplish. The fungus produces three kinds of asexual spores. microconidia, which have one or two cells, are the most frequently and abundantly produced spores under all conditions, even inside the vessels of infected host plants. macroconidia are the typical "Fusarium" spores; they are three to five celled, have gradually pointed and curved ends, and appear in sporodochia-like groups on the surface of plants killed by the pathogen. Chlamydospores are one- or two celled, thick-walled,

round spores produced within or terminally on older mycelium or in macroconidia. All three types of spores are produced in cultures of the fungus and probably in the soil, although only chlamydospores can survive in the soil for long. (Agrios, 2005).

There are four genera of fungi that cause vascular wilts: Ceratocystis, Ophiostoma, Fusarium, and Verticillium. Each of them causes disease on several important crop, forest, and ornamental plants. Ceratocystis causes the vascular wilt of oak trees (*C. fagacearum*), of cacao, and of eucalyptus. Ophiostoma causes the vascular wilt of elm trees, known as Dutch elm disease (O. novo-ulmi). Fusarium causes vascular wilts of vegetables and flowers, herbaceous perennial ornamentals, plantation crops, and the mimosa tree (silk tree). Most of the wilt causing Fusarium fungi belong to the species *Fusarium oxysporum*. Different host plants are attacked by special forms or races of the fungus. The fungus that attacks tomato is designated *F. oxysporum f. sp.* lycopersici; cucurbits, *F. oxysporum* f. sp. conglutinans; banana, *F. oxysporum* f.sp. cubense; cotton, *F. oxysporum* f. sp. Vasinfectum; carnation, *F. oxysporum* f. sp. dianthii; and so on. (Agrios, 2005).

2.1.3. The Description:

F.oxysporum produces three types of asexual spores: microconidia, macroconidia and chlamydospores (Nelson *et al*. 1983). Conidia are produced on monophialides and in sporodochia, and are scattered loosely over the surface of a mycelium (Griffin, 1994). Microconidia are predominantly uninucleate and germinate poorly and variably, with germination efficiency ranging from 1 - 20% (Ebbole and Sachs, 1990). The macroconidia are produced abundantly, are multinucleate, and germinate rapidly, thereby reproducing the fungus efficiently. Chlamydospores are viable, asexually produced accessory spores resulting from the structural modification of a

vegetative hyphal segment(s) or conidial cell possessing a thick wall, mainly consisting of newly synthesized cell wall material (Schippers and van Eck 1981). Its function is primarily survival in soil. morphological characterization of *F. oxysporum* is based on the shape of macroconidia, the structure of microconidiophores, and the formation and disposition of chlamydospores (Beckman , 1987). Asexual reproduction in *F. oxysporum* is accomplished by macroconidia and microconidia, while a sexual state of the fungus has never been observed (Booth ,1971).

2.1.4. The 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).

2.1.5 . Economic Important:

Fusarium oxysporum is significal problem in many crops. it is economically damaging too many industrial crops , the threat of more virulent strains or mutants that damage previously resistant crops in of major concern (Drestadt and Clark, 2004) *F. oxysporum* is a seed and soil borne fungal pathogen that causes Fusarium wilt (Haware, 1990, Nene and Reddy, 1987).The wilt disease was found to be more serious in low rain fall areas, were the weather condition are favorable for disease development (Khan, 1980).

2.1.6. 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.

F. 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. oxysporum* 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).

2.1.7. Symptoms:

The first symptoms appear as slight vein clearing on the outer, younger leaflets. Subsequently, the older leaves show epinasty caused by drooping of the petioles. Plants infected at the seedling stage usually wilt and die soon after appearance of the first symptoms. Older plants in the field may wilt and die suddenly if the infection is severe and if the weather is favorable for the pathogen. More commonly, however, in older plants, vein clearing and leaf epinasty are followed by stunting of the plants, yellowing of the lower 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. Often these symptoms appear on only one side of the stem and progress upward until the foliage is killed and the stem dies. Fruit may occasionally

become infected and then it rots and drops off without becoming spotted. Roots also become infected; after an initial period of stunting, the smaller side roots rot . (Agrios, 2005).

2.1.8. Life Cycle :

The life cycle of *F. oxysporum* commences with a saprophytic phase when the fungus survives in soil as chlamydospores (Beckman and Roberts, 1995). Chlamydospores remain dormant and immobile in the remains of decayed plant tissue until stimulated to germinate by utilising nutrients that are released from extending roots of a variety of plants (Stover 1962, Beckman and Roberts, 1995). Following germination, a thallus is produced from which conidia form in 6-8 hours, and chlamydospores in 2-3 days if conditions are favorable. Invasion of the roots is followed by the penetration of the epidermal cells of a host or a non-host (Beckman and Roberts,1995) and the development of a systemic vascular disease in host plants (Stover,1970). In the advanced stages of the disease, the fungus grows out of the vascular system into adjacent parenchyma cells, producing vast quantities of conidia and chlamydospores. The pathogen survives in infected plant debris in the soil as mycelium and in all its spore forms, but most commonly as chlamydospores in the cooler temperate regions (Agrios ,1997).due to the growth of the fungus within the plants vascular tissue, the plant water supply is greatly affected , this lack of water induces the leaves stomata to close, the leaves with and the plant eventually disease. At this point the fungus invades the plants part until it finally reaches the surface of the dead tissue where it sporulates abundantly (Agrios, 2005).The resulting spores can be used as new inoculation for further spread of fungus.

2.2. Control :

2.2.1. Cultural Control :

Cultural practices can also limit the spread of Fusarium and also Plant high quality seed free from Fusarium (Howard *et al*., 2005).use of varieties resistant to the fungus is the only practical measure for controlling the disease in the field. Several such varieties are available today. The fungus is so widespread and so persistent in soils that seedbed sterilization and crop rotation, although always sound practices, are of limited value. Soil sterilization is too expensive for field application, but it should be always practiced for green house grown tomato plants. Use of healthy seed and transplants is of course mandatory, and hot-water treatment not seed suspected of being infected should precede planting. (Agrios, 2005).Prevent spreading of the pathogen to disease free areas by using clean tools and equipment (Agrios, 1997).

2.2.2. Biological Control:

To provide an environmentally friendly Fusarium disease control system, the use of antagonistic microorganisms represents an alternative disease management strategy (Lugtenberg and Kamilova, 2009). The mechanisms adopted by biological control agents could be direct, indirect or mixed (Pal and Gardener, 2006). The use of bio agents was reported quite effective to control Fusarium wilt disease on tomato (Freeman *et al*., 2002). According to (Momol *et al*. 2003), several isolates of non pathogenic *Fusarium spp* (*F. oxysporum* and *F. solani*) that effectively controlled Fusarium wilt in green house test have been identified. The isolates include CS-20, CS-1, CS-24 and Fo 47 of which was consistently effective when applied at high rate. (Attitala *et al*. 2001) showed that after spraying with zoospores of *Phytophthora cryptogea* followed by *Fusarium oxysporum f.* sp. lycopersici inoculation, tomato plants show no wilt disease. also, in another studies conducted by (Akkopru and

Demir 2005) , ar buscularmycorrhizal fungi (AMF) G. intraradices and some Gram-negative and fluorescent rhizobacteria (RB), *P. fluorescens*, *P. putida* and Enterobactercloaceae, isolated from the rhizoplane of solanaceous plants were effective against *Fusarium* oxysporum f. sp. lycopersici. (Monda, 2002) reported that bacterial biocontrol agents with promising biocontrol activities against *Fusarium oxysporum f. sp*. *lycopersici* include *Pseudomonas flourescens*, *P. putida*, *P. chlororaphis*, *Bacillus subtilis*, *Streptomyces pulcher*, *S. corchorusii* and *S. mutabilis*. Rhizobacteria which may act directly as biofertilizer ,and biostimulants through production of plant growth hormones such as indole acetic acid, gibberelin, cytokinin, ethylene, dissolved minerals ,and also indirectly prevents the development of pathogenic microorganisms through siderofore, and antibiotic production (McMilan, 2007) (Sarma *et al*., 2009). (Widnyana *et al*. 2013) reported that three isolates of rhizobacteria isolated from the rhizosphere plants of the families Solanaceae and Leguminoseae namely KtS1, TrN2 and TmA1 and identified as Pseudomonas alcaligenes exhibited antagonistic activity against *Fusarium oxysporum f. sp*. *lycopersici* by effectively reducing the incidence of wilt disease on tomato under greenhouse experiment.

2.2.3. Chemical Control:

Agricultural chemicals are commonly used for management of pests and diseases. Seed treatment with synthetic fungicides considerably reduce wilt incidence in tomato. However, their use is costly as well as environmentally undesirable (Song and Goodman, 2001). Some of these chemicals include prochloraz, propiconazole, thiabendazole,carbendazim, benomyl, thiophante, fuberidazole and all of the benzimidazoles, (Nel et al., 2007) reported that benomyl was partly effective against *F. oxysporum f. spcubense* using the root dip treatment method. This method was applied to using carbendazimal on

tomato seedlings infected with Fusarium wilt and it led to about 24 % increase in yield (Khan and Khan, 2002).

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.

2.3. Plant Extract Botanical:

The phenomenon of plants influencing neighboring plants through the release of chemicals in the environment has been known as early as c. 370 BC. Greeks and Romans have used this knowledge in agriculture since c. 64 AD (Fraenkel, 1959). From this, he cautiously speculated that chemicals of plant origin (allelochemicals) have potential for bringing about population level change by affecting the growth of neighboring plants.

2.3.1 Mesquite :

The tree of Mesquite (*Prosopis juliflora*) is an ever green and multi-purpose leguminous tree or shrub that adapted to arrays of environments (Pasiecznik, 2001).The plant which was native to semi-arid areas of the West Indies, Mexico, Central America and Northern South America has been introduced to Sudan Since 1917's. (Broun and Massey, 1929 and Felker *et al*, 2003). *Prosopis spp* grow in arrays of environments and are not restricted by soil type, pH, salinity or fertility (Sidahmed, 2005 and Babiker, 2006).

2.3.2. Classification :

Kingdom : Plantae

Subkingdom : Tracheobionta

Superdivision : Spermatophyta

Division : Mangoliophyta

Class: Mangoliopsida

Subclass : Rosidae

Order : Fabales

Family : Leguminosae

Sub-family : Mimosoideae

Tribe : Mimoseae

Group: Prosopis

Genus: Prosopis

(Felker, *et. al*., 2001).

2.3.**3. Characteristics** :

The seeds, characterized by coat imposed dormancy, germinate in flushes and establish a huge persistent seed bank. Goats, sheep, cows and feral animals, attracted by the green foliage, eat ripened pods and liberate the seeds. The seeds encapsulated in animal droppings, are spread into new sites over long distances. The pods are also transported by flood waters and run-off (Babiker, 2006).

2.3.4.Damage :

The trees have many competitive advantages over other plants however, the seedlings are somewhat sensitive (Pasiecznik, 1999) They colonize disturbed, eroded, overgrazed or drought-ridden land associated with unsustainable agronomic practices (Pasiecznik, 1999). The trees are believed to deplete groundwater reserves and to smother and suppress, through both allelopathic and competitive effects, growth of neighboring plants (Ahmed, 2009). *Prosopis* pollens are said to be a major cause of allergic reactions and the thorns are poisonous and/or promotive secondary infections on prickling (Takur and Sharma, 1985).

2.3.5. The Benefits:

Mesquite, at its centre of origin, the arid areas in South America, has played an important social role. In addition to its role in combating desertification and supply of high-value mechanical wood products, firewood and charcoal mesquite provides shelters, animal feed and food for humans in areas where protein intake is very low and under adverse conditions of drought and famines (Ibrahim, 1989). The plant is important for fencing stalks, and as bee forage for honey production. Mesquite pods are a source of good quality flour and syrup (Felker *et al.*, 2003). Flour and syrup from mesquite are used in making foodstuffs at household levels (Pasiecznik, 2001, Felker *et al*., 2003). Mesquite species exude a water soluble gum that has been used as a substitute for gum Arabic during periods of restricted trading or international market shortages (Vilela and Ravtta, 2005). Mesquite species have ameliorating effects on soil under canopy. The tree fixes nitrogen and the leaf litter, when incorporated, improves soil physical and chemical properties. In Peru, leaves of mesquite are valued as compost (Pasiencznik, 2001). Foliage of mesquite contains several chemicals which are effective against several weeds; insects, fungi and some are of medical and/or industrial value (Pasiecznik, 1999). Moreover, mesquite, when properly managed, is a suitable tree for agroforestry in low-input low-rainfall areas (Luukkanen et al., 1983).

2.4.1. The Fungicide Amistar Top ®

Amistar Top ® is a broad spectrum product containing two fungicides. It has preventative, systemic and curative properties and is recommended for the control of many important plant diseases such as (Powdery mildews (*Leviliulla turika*), and Alternaria early leave blight (*Alternaria solani*) and Alternaria late blight (*Phytophthora infistance*) and rust stem of wheat (*puccinia graminis* vr

tirtici) . Amistar top ® is applied as a foliar spray and can be used in block, alternating spray, or tank mix programs with other crop protection products.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Location :

This study was conducted in the Laboratory of Plant Pathology Department of Plant Protection, College of Agricultural Studies, Shambat, Sudan University of Science and Technology during the period January, to February 2018 to evaluate the antifungal effect of different parts of Mesquite (fruit, Bark and Leaves). ethanol extracts and efficacy of fungicide Amistar Top® against the fungus *Fusarium oxysporum f. sp. Ciceris*. *In vitro.*

3.2 . Collection of Plant Materials:

Different parts of mesquite (Fruits, Leaves and Barks) will be collected from trees growing in the premises of the College of Agriculture Studies, Shambat. to study the efficacy of alcohol extract against *F. oxysporum* . All the plant parts were cleaned from dust and foreign material by hand and washed with water, and dried under shade. After complete dryness, plant samples were crushed separately to obtain fine powder for extraction.

3.3. Preparations:

3.3.1. Preparation of Plant Extract:

Extraction was carried out according to method described by (Sukhdev *et. al.* 2008). 50 g of each sample was extracted by soaking in 750 ml 80 % ethanol (National Distillation Company Sudan)for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness and different concentration were prepare (5% , 10% and 20)

3.3.2. Preparation of the Fungus:

The fungus *F. oxysporum* were obtained from the Hediba Research Station lab, Eddamer .and the pure culture for further studies.

3.3.3. Preparation of Media:

Prepare by dissolving 39 gms of media in 1000 ml distilled water . Heat to dissolve the medium completely. And sterilize by autoclaving at 121 Deg C . 15 Ibs pressure for 15 minutes.

3.3.4. Preparation of Inoculums:

The pure culture of *Fusarium oxysporum* were prepared using 7 days old mycelia. The fungi was cultured on PDA then transferred as, aseptically to the center of Petri dishes containing PDA medium and incubated at 25 °C the linear growth of the fungus was assessed in cm after 72 hrs.

3.3.5. Preparation of Fungicide:

The chemical tested was Amistar Top fungicide. 0.2 ml was dissolved in 100 ml of sterilized distilled water to obtain 100% of the fungicide .

3.4. Inhibition of plant extract:

Effect of Mesquite alcoholic extract on the radial growth of *F. oxysporum in vitro*.(Leaves . Bark and Fruits) (5% . 10 % and 20%). and then measure the radial growth of *F. oxysporum* after 3-4-5 days

3.4.1. 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 Mesquite, and fungicide Amistar Top® before being solidified in a conical flask of 250 ml containing 100 ml 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 grown on PDA as described above. The inoculated Petri dishes were then incubated at 25 °C the linear growth of the fungus was assessed in cm after 72 hrs. All treatments were done in triplicates and were arranged in a Complete Randomized Design.

3.5. Inhibition of Fungicide:

The effect of fungicide Amistar Top on the liner growth of *F. oxysporum in vitro.*

3.6 Measurement of the inhibition effect of growth of *F. oxysporum* :

The diameter of growth was measured every 24 hours 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 = diameter growth of the fungal in control.

dt = growth of treatment.

3**.7. Experimental Design and Statistical Analysis:**

The experiment was arranged in a Complete Randomized Design, the obtained data was statistically analyzed by MSTATc software computer program according to analysis of variance (ANOVA). Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOURE

RESULTS

4.1. Laboratory Experiment

This study which conducted under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology, during January to February 2018 was to confirm and to explore the antifungal potentials of different parts of Mesquite plant alcoholic extract and efficacy of fungicide Amistar Top® against the fungus *F. oxysporum*. The results cover the effect of plant alcoholic extracts on growth of *F. oxysporum f. sp. Ciceris.* and confirmation of the causal agent.

4 . 2 . Effect of Mesquite different Parts alcohol Extracts and Fungicide Amistar top on radial growth of *Fusarium oxysporum f. sp. Ciceris* three days after inoculation *in vitro(%)*:

The results (Table 1 and Figure 1) showed that the leaves alcohol extracts of all part extracted plants tested and fungicide Amistar Top had negative effects on the fungal growth after three days from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.(which gave (71.8) and 100% respectivly

Moreover the highest concentration of plant extract (20%) gave significantly higher inhibition compared to the untreated control which gave (71.8% , 61.8% and 41%) among the parts of Mesquite extract tested .

Leaves extract was the most effective in suppressing of the fungus growth than the other part of Mesquite respectively which gave (71.8 %) and the Bark inhibit the growth by (61.8%)followed the Fruit gave 41% in (table 1) The results showed that the antifungal activity increase with increasing of extract concentration.

Table, 1: Effect of Mesquite different Parts (Bark , Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* three days after inoculation *in vitro* (%):

Treatments	Inhibition Zone (%)			
Concentration	Replicates			
	R1	R2	R3	Means%
Bark 20%	61.5 (7.9)	61.5 (7.9)	61.5 (7.9)	61.8(7.9) bc
Bark 10 %	46.2(6.8)	46.2(6.8)	53.8(7.4)	48.7(7) cd
Bark 5%	53.8(7.4)	61.53(8)	38.5(6.2)	51.27(7.2) cd
Leaves 20%	69.23(8.4)	69.23(8.4)	76.9(8.8)	71.8(8.5) b
Leaves 10 %	61.5 (7.9)	53.8(7.4)	53.8(7.4)	56.4(7.56) c
Leaves 5 %	53.8(7.4)	46.2(6.83)	46.2(6.83)	48.7(7.0) cd
Fruits 20 %	30.8(5.6)	38.5(6.24)	53.8(7.4)	41.0(6.4) d
Fruits 10 %	23.1(4.9)	15.4(4)	23.1(4.9)	20.5(4.6)e
Fruits 5%	15.4(4)	7.7(3.3)	0(5)	7.7(4.1)e
Amistar Top 100%	100(10)	100(10)	100(10)	100(10)a
Control	0(0.7)	0(0.7)	0(0.7)	0(0.7)f
SE±		0.30		
C.V. (%)		8.17%		

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

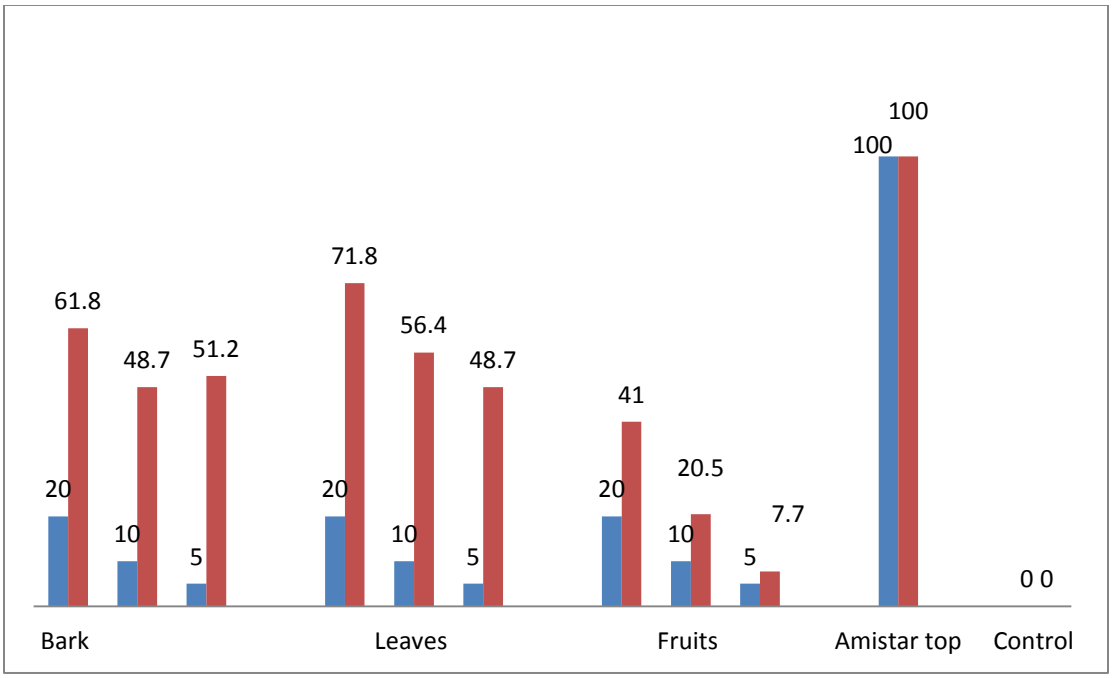


Fig 2: Effect of Mesquite different Parts (Bark , Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* three days after inoculation *in vitro* (%).

4.3. Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* four days after inoculation *in vitro* (%):

The results in Table, 2 and Fig. 2 showed that the alcohol extracts of different parts of Mesquite and fungicide (Amistar Top®) at all concentrations **(5, 10 and 20)** continued exhibiting an inhibitory effect against of *F. oxysporum* after four days from inoculations. The percentages of the fungal growth inhibition was significantly high compared to the control. Moreover, the highest inhibitory effect was demonstrated by concentration of leaves and Bark extracts at 20% concentration and that of fungicide Amistar Top® at all concentrations (58 %) and Amistar Top® 100 % significantly high against test fungus. Among the plant extracts screened that of leaves and Bark were the most effective in suppressing the fungus growth at all concentration. However the fruit extract has lower inhibition of the fungal growth . which gave (33%)

The results showed that the inhibitory effect increase with increased concentration

Table, 2: Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* four days after inoculation *in vitro* (%):

Treatments	Inhibition Zone (%)			
Concentration%	Replicates			
	R1	R2	R3	Means%
Bark 20%	65(8.1)	65(8.1)	65(8.1)	65(8.1) b
Bark 10 %	55(7.4)	45(6.7)	55(7.4)	51.7(7.16) bc
Bark 5%	45(6.7)	45(6.7)	40(6.4)	43.3(6.6) cd
Leaves 20%	65(8)	65(8)	65(8)	65(8.26) b
Leaves 10 %	60(7.8)	60(7.8)	55(7.4)	58.3(7.66) b
Leaves 5 %	50(7.1)	25(5)	50(7.1)	41.7(6.4)cd
Fruits 20 %	25(5)	40(6.4)	35(6)	33.3(5.8)de
Fruits 10 %	20(4.5)	24(4.9)	30(5.5)	24.7(4.96)ef
Fruits 5%	20(4.5)	20(4.5)	10(3.2)	16.7(4.06)f
Amistar top 100 %	75(8.7)	100(10)	100(10)	91.7(9.56)a
Control	0(0.7)	0(0.7)	0(0.7)	0(0.7)g
SE±	0.33			
C.V. (%)	9.14%			

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

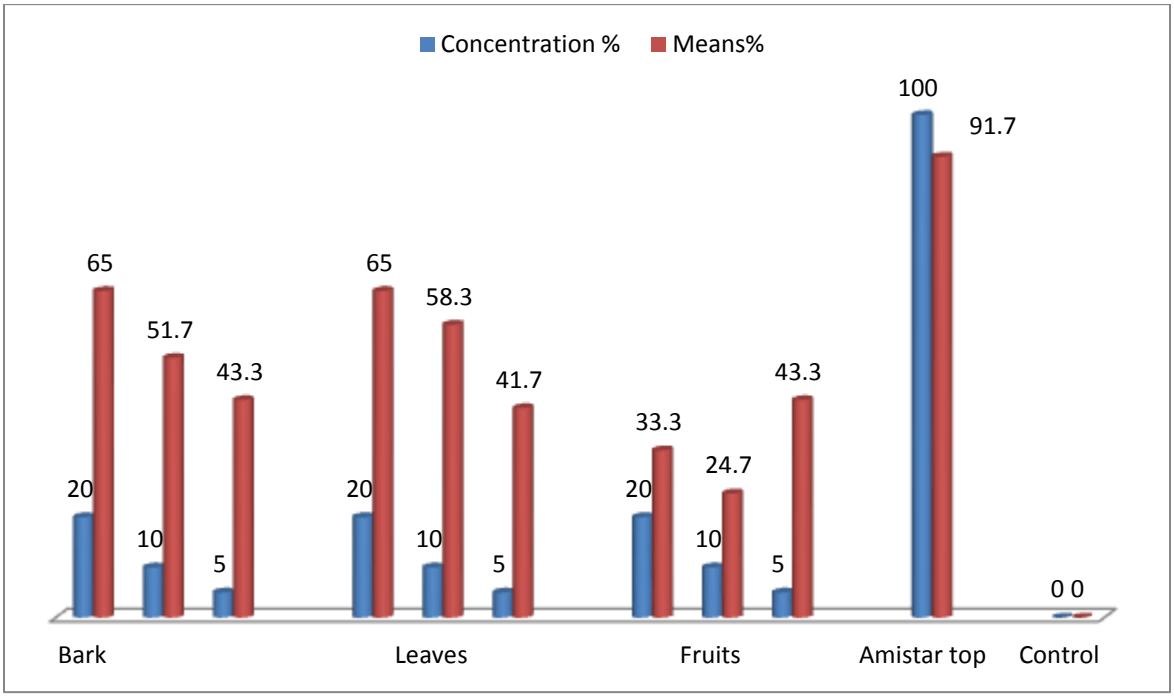


Fig. 2: Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* four days after inoculation *in vitro* (%).

4.4. Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* five days after inoculation *in vitro* (%):

In five day from inoculation , the result Table 3 and Fig. 3 showed that extracts of all parts of Mesquite plants tested proved to be effective in suppressing the fungal growth.

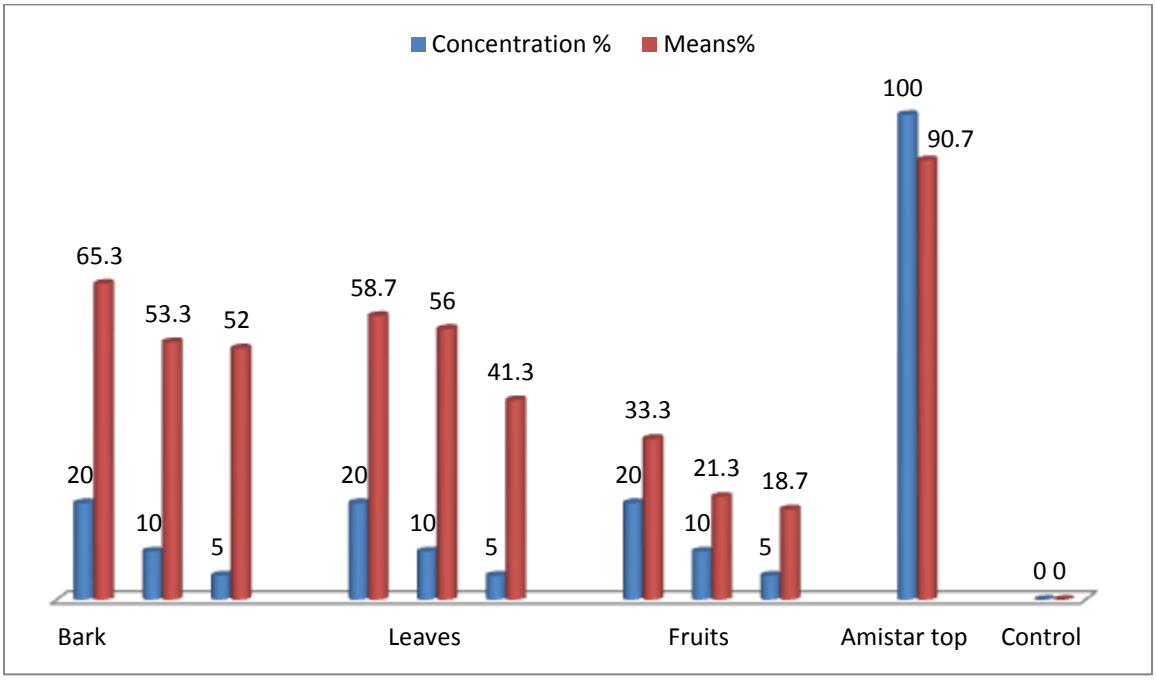
In fact, all tested concentration of all parts of Mesquite (5% , 10 % and 20%) induced significantly higher inhibition against *F. oxysporum* compared to control (65.5 %, 58.7 %, 33.3 %,) . meanwhile , the bark and leaves alcohol extract at high concentration tested exhibited consistently more inhibitory effect than the other parts of Mesquite plant alcohol extracts which give (65.5 %,and 58.7 %).respectively .

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

Table, 3: Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* five days after inoculation *in vitro* (%):

Treatments	Inhibition Zone (%)			
Concentration%	Replicates			
	R1	R2	R3	Means%
Bark 20%	60(7.8)	68(8.3)	68(8.3)	65.3(8.13) b
Bark 10 %	52(7.24)	52(7.2)	56(7.5)	53.3(7.3) c
Bark 5%	48(6.9)	60(7.8)	48(6.9)	52(7.2) c
Leaves 20%	56(7.5)	60(7.8)	60(7.8)	58.7(7.7) bc
Leaves 10 %	64(8)	52(7.2)	52(7.2)	56(7.4) c
Leaves 5 %	44(6.7)	40(6.4)	40(6.4)	41.3(6.5) d
Fruits 20 %	28(5.3)	36(6)	36(6)	33.3(5.7) e
Fruits 10 %	24(5)	16(4)	24(5)	21.3(4.66) f
Fruits 5%	20(4.5)	16(4)	20(4.5)	18.7(4.33) f
Amstar top 100 %	80(9)	96(9.8)	96(9.8)	90.7(9.5) a
Control	0(0.7)	0(0.7)	0(0.7)	0(0.70) g
SE±	0.21			
C.V. (%)	5.76%			

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



Fig, 3: Effect of Mesquite different Parts (Bark, Leaves and Fruits) alcohol Extracts and Fungicide Amistar Top on radial growth of *Fusarium oxysporum* five days after inoculation *in vitro* (%).

F. oxysporum control

F. oxysporum + Fungicide 20%

Plate 5: Effect of fungicide Amistar Top® on the growth of *Fusarium oxysporum* compare with untreated Control *in vitro.*

F. oxysporum Control

F. oxysporum+Mesquite20%

F.oxysporum+Mesquite 5%

F. oxysporum+Mesquite10%

Plate 6: Effect of Leaves alcohol extract on the growth of *Fusarium oxysporum* compare with untreated Control *in vitro*.

F. oxysporum Control

F. oxysporum+Mesquite20%

F.oxysporum+Mesquite 5%

F. oxysporum+Mesquite10%

Plate 7: Effect of Bark alcohol extract on the growth of *Fusarium oxysporum* compare with untreated Control *in vitro.*

F. oxysporum Control

F. oxysporum+Mesquite20%

F.oxysporum+Mesquite 5%

F. oxysporum+Mesquite10%

Plate 8: Effect of Fruits alcohol extract on the growth of *Fusarium oxysporum* compare with untreated Control *in vitro* .

CHAPTER FIVE

DISCUSSION

Generally, use of synthetic fungicides considerably reduce wilt incidence 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 which offers an alternative to fungicides is highly demanding.

The disease of wilt crops may cause greater losses of some crops ,Crop losses attributed to Fusarium has been estimated to an average of 25% (Powelson *et al*., 1993).Fusarium species are also important to the consumer because some, *Fusarium spp* 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).

Numerous research findings have presented a number of strategies to control this fungal pathogen ((Haware and Nene, 1982; Jiménez-Díaz, *et al.*, 1993; Biondi et al., 2004 and Ahmed, 2011).

Generally, management of seed-borne and soil-borne diseases such as *Fusarium spp*. always had been problematic (Haware, 1992) and (Rao and Balachadran, 2002). Based on the fact that botanical insecticides possess great advantages over synthetic pesticides (Karunyal, 2000; Abdel Moneim, *et al.*, 2009) in being more environmentally friend and accepted by the majority of the farmers, governmental organizations and decision makers.

The results (Tables 1 to 3) revealed that the Mesquite part (leaves, Barks and Fruits) alcohol extracts and fungicide, Amistar 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 (Abdelrahaman, 2016 and Harown,2016) who tested the bioactivity of Mesquite extract against fungi and demonstrated its suppressing effect on the fungal growth in vitro. also Similar results were obtained by (Fadl Elmola *et al.*, 2010). who reported that the extracts of Mesquite different plant parts were highly effective in suppressing bacterial growth. Also (Zainal *et al*., 1988). reported that *P . juliflora* contain antimicrobial compounds. (Abdelrahaman, 2016).

CONCLUSIONS

In conclusion, the findings presented in this study indicate promising potentials of Mesquite, (*Prosopis juliflora*). Leaves and Bark as sources of new antifungal in future that help in management of plant fungal diseases.

The leaves alcohol extracts of all plants tested exhibited an inhibitory effect on fungal growth. Thus the two components plus fungicide (Amistar Top) could be applied as part of an integrated approach to control Fusarium wilt .

- The alcohol extracts of different parts of Mesquite plant and fungicide Amistar Top® at all concentrations exhibited inhibitory effects against the radial mycelia growth of the test F. oxsysporum. The percentages zone of inhibition was significantly high compared to the Control.
- Among different parts of Mesquite, leaves and Bark at all concentrations tested (5 %, 10 % and 20%) and fungicide 100%. exhibited consistently the highest inhibitory effect throughout the test period than the other equivalents.
- The screened concentrations of Mesquite, (*Prosopis juliflora*). leaves alcohol extracts differ in their reactions to test F. oxsysporum. 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.
- The study revealed that the inhibitory effect of the fruits extracts were lower effect.

RECOMMENDATIONS:

1 - The Extracts of Leaves , Bark and Fruits of Mesquite tree can be used against other plant diseases.

2 - Further studies and research on Mesquite trees should be carried out to investigate more extracts from different Mesquite tree parts to be used as a biological control .

3- Increase concentration of extract increase inhibition .

4- check up the soil around Mesquite tree to studies of material Available Which cause biochemical antagonism and their characteristics.

5- Some research needed for the active ingredient of the all parts of mesquite.

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Appendix

Appendix 1: ANOVA

A) Variable 3 (inhibition in third day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	**10**	**190.408**	**19.041**	**68.522**	**0.0000**
Within	**22**	**6.113**	**0.278**		
Total	**32**	**196.522**			

Coefficient of Variation = 8.17%

B) Variable 4 (inhibition in four day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	**10**	**173.667**	**17.367**	**52.820**	**0.0000**
Within	**22**	**7.233**	**0.329**		
Total	**32**	**180.901**			

Coefficient of Variation = 9.14%

C) Variable 5 (inhibition in fifth day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	10	171.500	17.150	130.103	0.0000
Within	22	2.900	0.132		
Total	32	174.400			

Coefficient of Variation = 5.76%

Appendix 2: Equipments, Tools and Materials Used in the Study:

- Incubator Laminar flow cabinet
- Autoclave Compound microscope
- Needle Injection
- Slide Marker pen
- Petri-dishes Conical flask
- Sensitive balance Aluminum foul
- Gloves Face mask
- Registration form Camera
- Potato Dextrose Agar (PDA).
- Mesquite root Mesquite leave
- Mesquite park Soap
- Ethanol 95% Medical cotton
- Filter paper
- Fungicide Amistar Top®
- All Tools, which used in the experiments, were sterilized**.**

Appendix 3: FUNGICIDE

Name : Amistar Top®

Active ingredients : Azoxystrobin + Difenoconazole

Manufactured for : Syngenta Crop Protection, North Carolina

Fungicide Amistar Top®