بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Agricultural Studies

Department of Plant Protection

Antimicrobial Effect of Mesquite (*Prosopis juliflora*) leaves alcoholic Extract and Fungicide (Amstar top) on fungus (*Fusarium oxysporum p.f mangiferae*) Causal Agent of Wilt in Mango

أثر التضاد الميكروبي للمستخلص الكحولى لاوراق المسكيت والمبيد الفطري (Amstar top) نمو الفطر فيوزاريم اوكسسبورم المسبب لمرض الذبول فى المانجو

A thesis submitted in Partial Fulfillment of Requirements for the B.Sc (honors) Degree in Plant Protection

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الآيـــــة

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قال تعالي:

(وَهو الذَّي أَنْزلَ مَن السَّماِء ماءاً فأَخْرْجنا بهِ نبَاتَ كُلِّ شَيٍء فأَخْرْجنا مِنهْ خَضرًا نُخْرُج منهْ حَبا مَتراكباً وِمَن النَّخْلِ مْن طلَعهِا قنِوْانٌ دانيةَ وَجنَّاتٍ مْن أَعْنابٍ والزَّيتوُنَ والرُّمانَ مشْتبَهِا وغْير متشَابهٍ انظْرُوا إلِى ثَمَرِه إ ذِاَ أثمر وينعه إ نِّ فِي ذلكِم لآيات لقِوٍم يؤِمنوُنَ)

صدق الله العظيم

سورة الأنعام الاية (٩٩)

Dedication

To my father

My mother

Sisters and brothers

To my Supervisor Dr: Ibrahim Saeed

I dedicate this work with sincerer love

Hafiz

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First of all I renderer my gratitude and praises to The Almighty Allah.

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With respectful

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ABSTRACT

This study was conducted at the Faculty of Agricultural Studies, Sudan University of Science and Technology, in October 2018, to study the effect of the alcoholic extracts of the leaves of mesquite (*Prosopis juliflora*) and the fungicide Amistar top on the growth of the Fusarium oxysporum. The Mesquite tree is a family of legumes. It is found throughout the year in South America. It spreads in large areas in Sudan and has swept most of the agricultural land, which is now threatened by agriculture and biological diversity. The pathogen was isolated from a pure environment obtained from the Plant Pathology laboratory. Three concentrations of 25%, 50% and 100% were used for each of the three extracts and 20% of the fungicide (Amstar top) in addition to the control

The results showed that all the extracts of the leaves of mesquite and especially the high concentration 100% were optimal in inhibiting the growth of fungi by 1.5. Of the three concentrations of leaves 100%, was found to be optimal in inhibiting the growth of fungi and the Amstar top® (1.0) with a concentration of 20% compared to the control. When comparing all the treatments, it was found that the extracts of the leaves (25, 50 and 100%), fungicide the Amstar top® (1.8, 1.7, 1.5 and 1.0) respectively showed results in inhibiting the growth of the fungus. The results obtained from the leaves extracts are promising and encouraging for chemical analyzes of various parts Mesquite tree using different extracts to identify the active substance in each of these parts and use them as alternatives to the use of harmful pesticides and harmful to human health, animal and environment.

ملخص البحث

اجريت هذه الدراسة بمعمل امراض النباتات بكلية الدراسات الزراعية ، جامعة السودان للعلوم والتكنولوجيا لدراسة تاثير المستخلص االكحولي لاوراق المسكيت (*Prosopis juliflora*) والمبيد امستار توب علي نمو فطر الفيوزاريم المسبب المرضي للذبول الفيوزيرمي فى المانجو. تتبع شجرة المسكيت للعائلة البقولية,وتوجد مخضرة طول العام موطنها امريكا الجنوبية ، وتنتشر في مساحات واسعة فى السودان واجتاحت معظم الاراضي الزراعية مما اصبحت مهددا للزراعة الان والتنوع الحيوى.ينمو المسكيت في صفائف البيئات ولا يقتصر على نوع التربة أو الرقم الهيدروجيني أو الملوحة أو الخصوبة. ويزهرعلى مدار السنة كما لوحظ عدم وجود نباتات تحت اشجار المسكيت مما يعزز فرضية وجود تضاد بيوكيميائى. تم عزل المسبب المرضي من بيئة نقية تم الحصول عليها من معمل أمراض النبات ، بعد إعادة تزريعها للحصول علي بيئة نقية مرة اخري واجريت الدراسة لتقييم نمو الفطر علي البيئة الغذائية وأوضحت النتائج أن النمو الأمثل في بيئة البطاطس PDA علي درجة حرارة 25م. استخدامت ثلاث تركيزات 25% ،50% و 100 % لكل من التراكيز الثلاثة و20% من المبيد امستار توب إضافة الي الشاهد. أوضحت الدراسة أن كل المستخلصات ذات فعالية معنوية في تثبيت الفطر ومن بين التراكيز االثلاثة وجد أن التراكيز الثلاثة وخاصة التركيز العالي 100% كان الأمثل في تثبيط نمو الفطر بنسبة 1.5.

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.sp lini), 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 & Armstrong 1981, MacHardy & 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.(George N. 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)

Fusarium oxysporum f. sp. lycopersici (Sacc.) W.C. Snyder and H.N. 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. For the fungus to be successful in infecting the plant, it must mobilize different sets of genes for early plant–host signaling, attachment to root surface, enzymatic breakdown of physical barriers, defense against antifungal compounds of the host, and inactivation and death of host cells by fungal toxins.(George N. Agrios,2005)

soil borne plant pathogen in the class Hyphomycetes, causes Fusarium wilt specifically on tomato. There are more than 100 Fusarium vascular wilt diseases worldwide. Apart from causing diseases, they colonize outer cells of roots as harmless endophytes after the pathogen has killed the root tissues and others live as saprophytes in soil (Burgess et al., 2008). This disease was first described by G.E. Massee in England in 1895. The pathogen has three physiological races (1, 2, and 3, hereafter r1, r2 and r3) and are distinguished by their specific pathogenicity on tester plants carrying dominant race-specific resistance genes (Cai et al., 2003).

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) and produces three types of asexual spores; microconidia, macroconidia and chlamydospores (Arios, 1988). Some strains of Fusarium oxysporum are not pathogenic and may even antagonize the growth of pathogenic strains and can be used as biological agents (Fravel et al., 2003).

The fungus colony of oxysporum Fusarium appears in different manifestations on the potato culture and dextrose agar (PDA), according to its specific form. In most cases, however, the mycelium appears white first and then changes to Different colors ranging from dark to purple violet (Smith, et al ,1988)

It is a major wilt pathogen of many economically important crop plants it can also spread through infected dead plant material, so cleaning up at the end of the season is important. A recent example of this is the spread F. oxysporum f.sp. Cubense which may have originated in Asia and just recently has appeared in banana producing areas in the South Pacific(David and Richard,2004).

Obviously, Fusarium oxysporum is one of the most hazardous diseases that widely spread. 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 leavs of Mesquite (Fruit, Bark and Leaves) against fungal.
- To evaluate in vitro the efficacy against systemic fungicide (Amstar top) in suppressing the fungus.
- 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 wilts 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:

Fusarium 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. Vasin fectum; carnation, F. oxysporum f. sp. dianthii; and so on. (Agrios, 2005).

2.1.3. Description:

Fusarium 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 & 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 & 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 microconidio phores, 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 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 e.g: banana industry the threat of more virulent strains or mutants that dmage previously resistant crops in of major concern(Drestadt and Clark, 2004) Other commercially important plants are affected include basil, beans, carnation, chrysanthemum ,peas, and watermelon. (Ekhlass, 2013)

Fusarium 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.

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. 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 & 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 a,b, Beckman & 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 & 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 of 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; Jones *et al*., 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 bioagents 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 greenhouse 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), arbuscular mycorrhizal fungi (AMF) G. intraradices and some Gram-negative and fluorescent rhizobacteria (RB), P. fluorescens, P. putida and Enterobacter cloaceae, 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 Peudomonas 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, gibberellins, cytokinin, ethylene, dissolved minerals, and also indirectly prevents the development of pathogenic microorganisms through siderofore, and antibiotic production (McMilan, 2007; Sharma 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. In 2007, Nel et al. reported that benomyl was partly effective against F. oxysporum f.sp cubense 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. 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).

2.3.1. Classification of Mesquite (Prosopis):

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).

2.3.2. characterized of mesquite:

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).

2.3.3.damage of mesquite:

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 neighbouring 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).

2.3.4. The Benefit Uses of Mesquites:

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).

All parts of P. juliflora and P. pallida are used in the preparation of medicinal products to treat human ailments. There are many records of the use of these products from historical literature where Prosopis species are native and from recent descriptions where they have been introduced. In India, for example, an astringent decoction is made from boiling wood chips, a bark extract is used as an antiseptic on wounds, and gum is used to treat eye infections (Vimal and Tyagi 1986). In Brazil, P. juliflora flour is used as an aphrodisiac, syrup as an expectorant and tea infusion against digestive disturbances and skin lesions (Rocha 1990).

2.4.1. The Fungicide Amstar top ®

Amstar 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. Amstar top ® provides excellent disease control of many Leaf spots, Powdery mildews, and Downy mildews. Amstar top ® is applied as a foliar spray and can be used in block, alternating spray, or tank mix programs with other crop protection products. All applications must be made according to the use directions that follow.

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 October to November to evaluate the antifungal effect of alcoholic extract of mesquite leaves ethanol extracts and efficacy of fungicide Amstar top® against the growth of the fungus *Fusarium oxysporum p.f mangiferae in vitro*

3.2. Equipments, Tools and Materials used in the Study:

- Incubator Laminarflowcabinet
- Autoclave Compound microscope
- Needle Injection
- Slide Marker pen
- Petri-dishes Conical flask
- Sensitive balance Aluminum foul
- Gloves Face mask
- Regestration form Camera
- Potato Dextrose Agar (PDA).
- Mesqiute root Mesqiute leave
- Mesqiute park Soap
- Ethanol 95% Medical cotton
- Filter paper
- Fungicide Amstar Stop®

- All Tools, which used in the experiments, were sterilized**.**

3.3 . Source of materials:

Leaves part of mesquite was collected from trees growing in the premises of the College of Agriculture Studies, Shambat and efficacy of fungicide (Amistar top) against the fungus plant pathogens (fungi) was obtained from the laboratory of Plant Protection Department College of Agri. Studies.

3.4. Preparations

3.4.1. Preparation of extract:

Extraction was carried out according to method described by Sukhdev *et*. *al*. (2008): 50 g of leaves sample was extracted by soaking in 750 ml 80 % ethanol 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.

3.4 .2. Preparation of inoculums:

The pure culture of *Fusarium oxysporum* was prepared using 7 days old mycelia. The fungi was cultured on PDA then transferred aseptically to the center of Petri dishes containing PDA medium and incubated at 25^0c the linear growth of the fungus was assessed in cm after 72 hrs

3.4.3. Preparation of fungicide:

The chemical tested was Amstar Top fungicide. Two ml was dissolved in 100 ml of sterilized distilled water.

3.5. 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 leaves, Peppermint and fungicide Amstar 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 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.

3.6 Calculation:

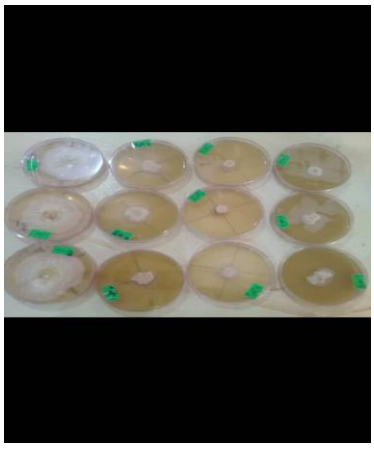
Every 48 hours, the diameter of growth was measured by taking the average of two crossed dimensions for each disc in the Petri dish. The radial growth was then calculated as a percentage from the diameter (9.0 cm) of the glass Petri dish. The effect of each extract concentration on linear fungal growth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



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CHAPTER FOURE
RESULT

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 august to October 2018 was to confirm that Fusarium oxysporum and to explore the antifungal potentials of different parts of mesquite plant and efficacy of fungicide Amstar stop® against the fungus. The results cover effect of plant extracts on growth of Fusarium oxysporum mangiferae and confirmation of the causal agent.

4. 2. Effect of Mesquite leaves Extracts and Fungicide Amstar top on radial growth of Fusarium oxysporum in vitro three days after inoculation:

The results (Table 1 and 2) showed that the leaves alcohol extracts of all concentration tested and fungicide (Amstar 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.

Moreover the highest concentration of leaves extract (100%) gave significantly higher inhibition compared to the untreated control which gave (1.5). However, fungicide exhibited the most effective inhibitory effect (Table, 1 and 2).

The results showed that the antifungal activity increase with increasing of extract concentration.

Table, 1: MEAN, LOWER, UPPER Results.

Tratments	Mean	Lower	Upper
Control	2.081	2.013	2.150
Fungicide	1.000	0.931	1.069
Mousket 100%	1.494	1.425	1.563
Mousket 25	1.788	1.719	1.856
Mousket 50%	1.653	1.584	1.722

Table: 2. Duncan's multiple range test

Tratments	Mean	
Fungcide	1.000	a
Mousket 100%	1.494	b
Mousket 50%	1.653	c
Mousket 25	1.788	d
conrol	2.081	e

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 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 and Mawda, 2015) 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 alcoholic extracts and fungicide, Amstar 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 Shimaa , Huda (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.

CONCLUSIONS

In conclusion, the findings presented in this study indicate promising potentials of Mesquite, (*Prosopis juliflora*). Leaves 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 (Amstar top) could be applied as part of an integrated approach to control Fusarium wilt .

- The screened concentrations of Mesquite, (*Prosopis juliflora*) leaves alcohol 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.

RECOMMENDATIONS:

1. Further studies and research on mesquite trees according to the current results aimed at knowledge of the chemical resistance of the mesquite tree using different solvents

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Appendixes

Individual 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)
EAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
Treatment			
control	2.081	2.013	2.150
M			
Fungicide	1.000	0.931	1.069
Mousket 100%	1.494	1.425	1.563
Mousket 25	1.788	1.719	1.856
Mousket 50%	1.653	1.584	1.722

38 AMCOMPARISON [METHOD=duncan; DIRECTION=ascending; PROB=0.05] Tratment

Duncan's multiple range test

Treatment

	Mean	
Fungicide	1.000	a
Mousket 100%	1.494	b
Mousket 50%	1.653	c
Mousket 25	1.788	d
control	2.081	e