بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Department of Plant Protection

Bioactivity of Aqueous Extracts from some Parts of Mesquite (*Prosopis juliflora*) and Fungicide (Amistar Top®) on Growth of *Fusarium oxysporum f. sp. tuberosi* Under Laboratory Conditions

الفعالية الحيوية للمستخلصات المائية من بعض أجزاء المسكيت و المبيد الفطري أمستار توب علي نمو الفطر فيوزاريرم اوكسيسبورم تحت ظروف المعمل.

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الآيـــــــــــة

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قال تعالي:

(وَإِذْ قَالَ رَبُّكَ لِلْمَلائِكَةِ إِنِّي جَاعِلٌ فِي الأَرْضِ خَلِيفَةً قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ قَالَ إِنِّي أَعْلَمُ مَا لا تَعْلَمُونَ (30) وَعَلَّمَ آدَمَ الأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلاء إِنْ كُنتُمْ صَادِقِينَ (31) قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ (32)

صدق الله العظيم

DEDICATION

To my mother

To soul of my father Eltahir

To my brothers and sisters

To all my family

To all my teachers

To all my friends

With love and respect

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First of all I render my gratitude and praises to the Almighty Allah. I wish to express my sincerest gratitude to my supervisor **Dr. Ibrahim Saeed Mohammed** for his helpful guidance, encouragement and supervision of this work. Also special thanks to all staff members of the Department for their helps and valuable suggestions. Thanks are also extended to **Dr. Salah Eldein Sidahmed** the head of Research Lab of Agricultural Studies – Sudan University of Science and Laboratory, the technician **Ustaz Reem Hassan.** Thanks and gratitude is also due to the Laboratory technician **Ustaz Mawda Ibrahim** who helped me a lot during the experiment period. Great thanks to **Ustaz Nasreddin Idris** who gave me help and support during the study. Also especial thank to my colleagues at **M. Sc. group** for their help during the experiment period. I am Grateful to all my **friends** for unbounded help and interesting feeling.

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With respect

The researcher

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

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, Collage of Agricultural Studies, Sudan University of Science and Technology, January 2019; to investigate, under laboratory conditions, the bioactivity of aqueous extracts of some parts of mesquite plant namely, leaves and fruits, plus Fungicide Amistar top® against the growth of *Fusarium oxysporum f. sp. tuberosi* wilt fungus of potato. Three concentrations (25%, 50% and 100%) of each of the two 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 two extracts, the mesquite leaves extract, especially the high concentration, was more effective than fruits extract in inhibiting of fungus growth, which give higher inhibition in all concentration (64.3% 80% and 96.6%) while the fruits. When comparing all the treatments, it was found that the extracts of the leaves and fruits of the Mesquite in addition to Amistar top® fungicide were exhibited superior inhibitory on the growth of the fungus by 65%, 96.6% 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 environment.

ملخص البحث

إن الأخطارالمتزايدة علي الصحة العامة والبيئة نتيجة للاستعمال الغير مرشد للمبيدات المصنعة إلى جانب ظهورسلالات مقاومة من الفطريات الممرضة للنبات قد أدت إلى الشروع في إستكشاف منتجات بديلة آمنة .هنالك عدد جيد من التقاريرالتي 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 2019م ،لبحث الفعالية الحيوية للمستخلصات المائيةَ لبعض أجزاء نبات المسكيت تحديداً ,الثمار ،الأوراق ، والمبيد أميستار توب تحت ظروف المعمل علي نمو فطرالذبول الفيوزاريمي في البطاطس استخدمت ثلاث تراكيز (25% ، 50 % و100%) لكل من المستخلصات الثلاثة و100% من المبيد أميستار توب بالإضافة للشاهد. أوضحت الدراسة أن كل المستخلصات ذات أثرمعنوي في تثبيط نمو الفطر ومن بين المستخلصات الاثنان وجد أن مستخلص أوراق المسكيت وخاصة التركيز العالي كان الأمثل في تثبيط نمو الفطر حيث بلغت نسبته (64,3%, 80% , 96,6%) علي التوالي مقارنة بمستخلص الثمار ..عند مقارنة كل المعاملات وجد أن مستخلصي الأوراق والثمار المائيين ومبيد Amistar top® علي التوالي أظهروا نتائج ممتازة (65 % , 96.6 % و100 %) في تثبيط نموالفطر .كما أظهرت النتائج أن الفعالية ضد الفطر تزداد بزيادة تركيزالمستخلصات. 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 والإنتفاع بها كبدائل لإستخدام المبيدات الضارة علي صحة الانسان ، الحيوان والبيئة.

CHAPTER ONE

INTRODUCTION

Potato plant (*Solanum tuberosum* L.) is a member of the family Solanaceae that includes: eggplant, tobacco and tomato. The crop which was originally believed to be domesticated independently in multiple locations is an important crop worldwide and ranks fourth in production among food crops after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aesitvum* L.) (FAOSTAT, 2006).The importance of potatoes is increasing due to the rising world population, the capability of potatoes to grow well in adverse conditions, and its high nutritional value with an annual production of 3.6 x108 tones (Hamilton, 2005 and Anonymous, 2012).

In Sudan, although potato cultivation depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato. The crop is cultivated in wide area around large Cities along the Nile and on seasonally flooded plains (FAO, 1999). However, the area around Khartoum accounts for over 70 percent of the country's potato production (Geneif, 1986).

The losses caused by diseases and insects constitute the major constraints that facing the production of potato worldwide and among these, the most wide spread and important are fungi, affecting tubers and vegetative parts. One of the main fungal pathogens that attack potato is *Fusarium* dry rot which is a worldwide economic problem. There are many species of *Fusarium* reported to cause dry rot of potato Worldwide (Nielson, 1981) of which *Fusarium oxysporum f. sp. tuberosi* has been reported as the most pathogenic Fusarium species causing potato dry rot (Sharifi *et al*., 2009).

The disease affects tubers in storage and seed potato pieces after planting. (Hanson *et al*, 1996) reported that *Fusarium* dry rot of feed tubers can cause crop losses up to 25%, while more than 60% of tuber can be infected

in storage. Indiscriminate use of chemical pesticides to control various pests and pathogenic microorganisms of crop plants is causing health hazard both in terrestrial and aquatic lives through their residual toxicity (Viana et al., 1996), much attention is being focused on the alternative methods of pest control (Ali, 1996).

Natural plant extracts have been recommended as suitable alternative choices to synthetic chemicals to control diseases and pests of crops (Suhr & Nielsen, 1981). In Sudan: the bioactivity of many cultivated and wild Plants are demonstrated by many researchers such as (Al-Doghairi *et al*., (2004); and Sidahmed *et al.*, (2005).

This study was undertaken to find out an alternative and nontoxic biological control agents to control the dry rot of Fusarium in potatoes. The aim of this study was: to explore the antifungal activity of extracts of different parts of Mesquite plant (leaves and fruits), and the efficacy of systemic fungicide in suppressing the growth of the Fusarium dry rot of potato under laboratory conditions with following objectives:-

- To explore the inhibitory effect of the aqueous extracts of different part of mesquite (leaves, and fruits) against *F. oxysporum f. sp. tuberosi*
- To evaluate the efficacy against systemic fungicide (Amistar Top®) in suppressing the fungus *F. oxysporum f. sp. tuberosi* in vitro.
- To develop promising disease management component against Fusarium wilt.

CHAPTER TWO

LITEREATURE REVIEW

2.1 Potato (*Solanum tuberosum L.*)

The potato plant which belongs to the family Solanaceae includes, among 2000 other species, tomato *(Lycopersicum esculentum L.*), sweet pepper (*Capsicum annuum L.*), eggplant (*S. Melongena var. esculentum L.*), tobacco *(Nicotiana tabacum L.*), and petunia (*Petunia hybrid L.)*, (Fernald, 1970).

2.1.1 Scientific classification

Kingdom: Plantae (unranked)

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *tuberosum*

(Binomial name: *Solanum tuberosum L.*)

The genus Solanum is a polymorphous and largely tropical and subtropical genus containing more than 1000 species. The origin agreed to be the high elevation of South America and the area of first domestication was reasoned to be the area where wild diploids are still found and where the greatest diversity of cultivated forms can still be found, and is identified as the high plateau of Bolivia and Peru, in the general region of Lake Titicaca (Hoopes and Plaisted, 1980).

Potato is one of the major vegetable crops grown worldwide following wheat, maize, and rice, with a production estimates of 368 million tons (FAOSTAT, 2015). It is the staple food of many cultures and civilizations past and present.

In Sudan, the potato is grown mainly as winter crop and the main area of production are along the Nile bank in both Khartoum and Northern States. Although potato cultivation in Sudan depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato (Geneif, 1986).

Potatoes in Sudan are an important cash crop for small-scale growers, and have the potential to increase incomes in periurban areas, improve living standards and create employment opportunities. Potato production is steadily increasing in Khartoum; the acreage devoted to this crop has more than tripled in the last ten years (Ahmed, 1985).

The total acreage under potato cultivation in the Khartoum region amounts to about 6,500 hectares, with yields of 17 to 25 ton/ha. However, production costs of potatoes are high in comparison with those of other crops. Seed potatoes have to be imported and account for more than half of the total production cost of potatoes (Elsir, 2005). The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddans (Hind and Mohamed, 2010).

One of the major constraints facing the quantity, quality and availability of healthy crop worldwide are the losses and contamination caused by post-harvest diseases. The major groups of post-harvest diseases are those which arise from infections initiated during and after harvest.

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

2.1.2 Economic importance

- The potato is a starchy, tuberous crop from the perennial *Solanum tuberosum* of the Solanaceae family (also known as the nightshades).
- The word potato may refer to the plant itself as well as the edible tuber.
- In the region of the Andes, there are some other closely related cultivated potato species.
- Potatoes are the world's fourth largest food crop, following rice, wheat, and maize.
- Long-term storage of potatoes requires specialized care in cold warehouses and such warehouses are among the oldest and largest storage facilities for perishable goods in the world.
- Once established in Europe, the potato soon became an important food staple and field crop.
- The annual diet on of an average global citizen in the first decade of the twenty-first century included about 33 kg (or 73 Ib) of potato.
- However, the local importance of potato is extremely variable and rapidly changing.
- It remains an essential crop in Europe, where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in southern and eastern Asia.
- China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India (Thompson and Morgan, 1855).

2.2 Fusarium dry rot

Fusarium dry rot of potato is a devastating post-harvest disease affecting both potato tubers and potatoes for human consumption. In fact, *Fusarium* dry rot of potatoes is a worldwide economic problem. There are many species of *Fusarium* reported to cause dry rot of potato worldwide (Nielson, 1981). The disease may cause greater losses of potatoes than any other-post harvest disease. Crop losses attributed to dry rot have been estimated to an average of 6 to 25% (Powelson *et al*., 1993).

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

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

2.2.1 Classification

Kingdom: Fungi

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: *Fusarium*

Species: *oxysporum*

Fusarium oxysporum f. sp. tuberosi

2.2.2 The Pathogen

Fusarium oxysporum f. sp. tuberosi 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.

Microconidia 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 belongs 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).

2.2.3 The Description

The fungus 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 micro-conidiophores, 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).

2.2.4 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 *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).

2.2.5 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,).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).

2.2.6 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) *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 potato, 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).

2.2.7 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).

2.2.8 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 utilizing 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.

2.3 Control

2.3.1 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).

2.3.2 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 nonpathogenic *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 were 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), arbuscularmycorrhizal fungi (AMF) G. intra radices 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) 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. tuberosi* by effectively reducing the incidence of wilt disease on tomato under greenhouse experiment.

2.3.3 Chemical Control

Agricultural chemicals are commonly used for management of pests and diseases. Tuber 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*

the root dip treatment method. This method was applied to using carbendazimal on potato 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.

2.4 Allelopathic activity of mesquite plant

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.

Actually, allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Allelochemicals are a subset of secondary metabolites which are not required for metabolism (i.e. growth, development and reproduction) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory (Stamp and Nancy, 2003).

2.4.1 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*. grows in arrays of environments and is not restricted by soil type, pH, salinity or fertility (Sidahmed, 2005 and Babiker, 2006).

2.4.2 Classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Mangoliophyta
Class: Mangoliopsida
Subclass: Rosidae
Order: Fabales
Family: Leguminosae
Sub-family: Mimosoideae
Genus: *Prosopis*
Species: *juliflora*

(Felker *et al*, 2001).

2.4.3 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).

2.4.4 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 primitive secondary infections on prickling (Takur and Sharma, 1985).

2.4.5 The Benefits

Mesquite, at its center 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).

2.5 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 under laboratory conditions at Plant pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology (SUST) within the period March to evaluate the antifungal activity of mesquite parts (leaves , and fruits) aqueous extracts and efficacy of fungicide, (Amistar top®) 250 EC ، against *Fusarium oxysporum f. sp. tuberosi*.

3.2 Isolation, characterization and identification of the fungus

Infected potato plant (tuber) showing typical symptom of the disease was obtained from Shambat research field. The tubers were cut into small part (0.5, 1.0 cm) washed thoroughly with the tap water, surface sterilized with Clorox (NaOCl) (1% concentration) for 1 minute, rinsed three times in sterilized distilled water and dried on sterilized filter paper. The sterilized fruits sections were then plated at the rate of 6 section spar plate on potato dextrose agar medium (PDA).

The inculcated Petri dishes were incubated at 25° C for 7days. After incubation, isolated fungi were 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. tuberosi* Standard books and research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified Isolates were maintained on PDA for further studies.

3.3 Collection and preparation of extracts

Different parts of Mesquite (Leaves and fruits), were obtained from (Shambat area), All the plant parts were cleaned from dust and foreign material by hand and washed with distilled water and Clorox, and dried under shade. After complete dryness, plant samples were crushed separately to obtain fine powder for extraction.

The obtained fine powder form different parts of mesquite was weighted (25, 50 and 100 gm.), and placed in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 25 % 50% and 100% concentrations. Aqueous extracts of each of the plant materials were prepared as they recommended by (Okigbo, 2006), figure (1 and 2).

3.3.1 Preparation of Medium

The medium was prepared by dissolving 39 mgs of media in 1000 ml distilled water, Heat to dissolve the medium completely, and sterilize by autoclaving at 121 C°. 15 Ibs pressure for 15 minutes.

3.3.2 Preparation of Inoculums

The pure culture of *Fusarium oxysporum f. sp. tuberosi* 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.

3.3.3 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, figure (3).

3.3.4 Inhibition of plant extract

Effect of Mesquite aqueous extract on the radial growth of *F. oxysporum f. sp. tuberosi in vitro* (Leaves and Fruits) (25%, 50 % and 100%). and then measure the radial growth of *F. oxysporum* after 3-4-5 days

3.3.5 Inhibition of *F. oxysporum f. sp. tuberosi* 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.

3.3.6 Measurement of the inhibition effect of plant extracts growth on *F. oxysporum f. sp. tuberosi*

The diameter of growth was measured every 24 hours by taking the average of two crossed diameters 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:

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

Where:-

dc = diameter growth of the fungal in control.

dt = growth of treatment.

3.4 Experimental Design and Statistical Analysis

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

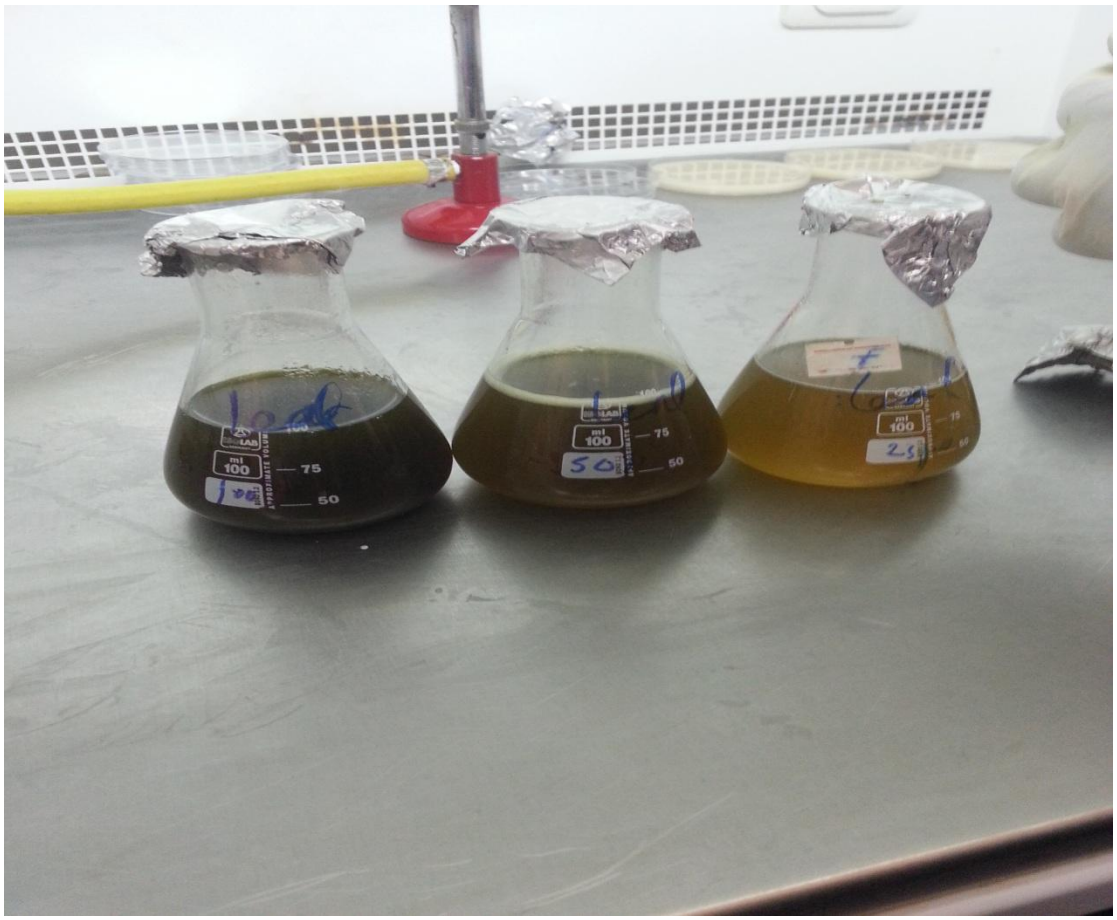

Figure (1) leaves extract.

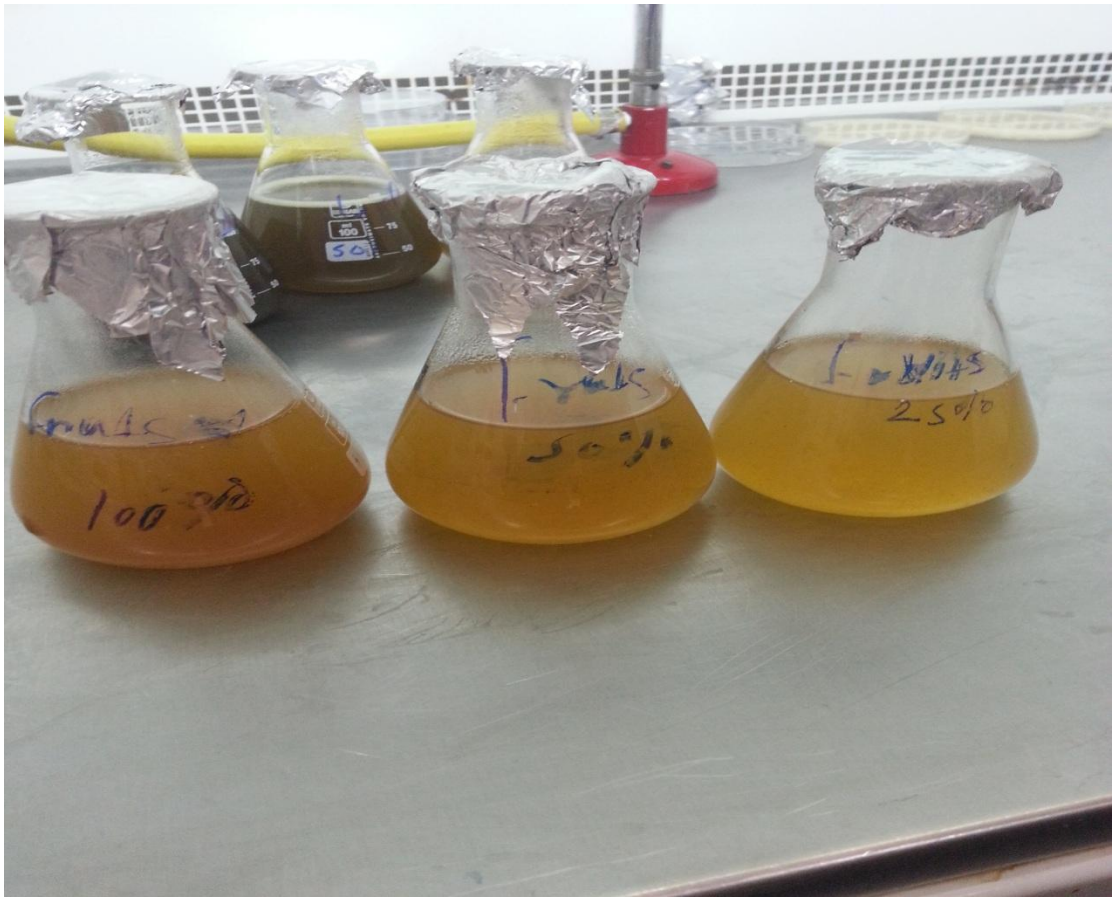

Figure (2) Fruits extract.

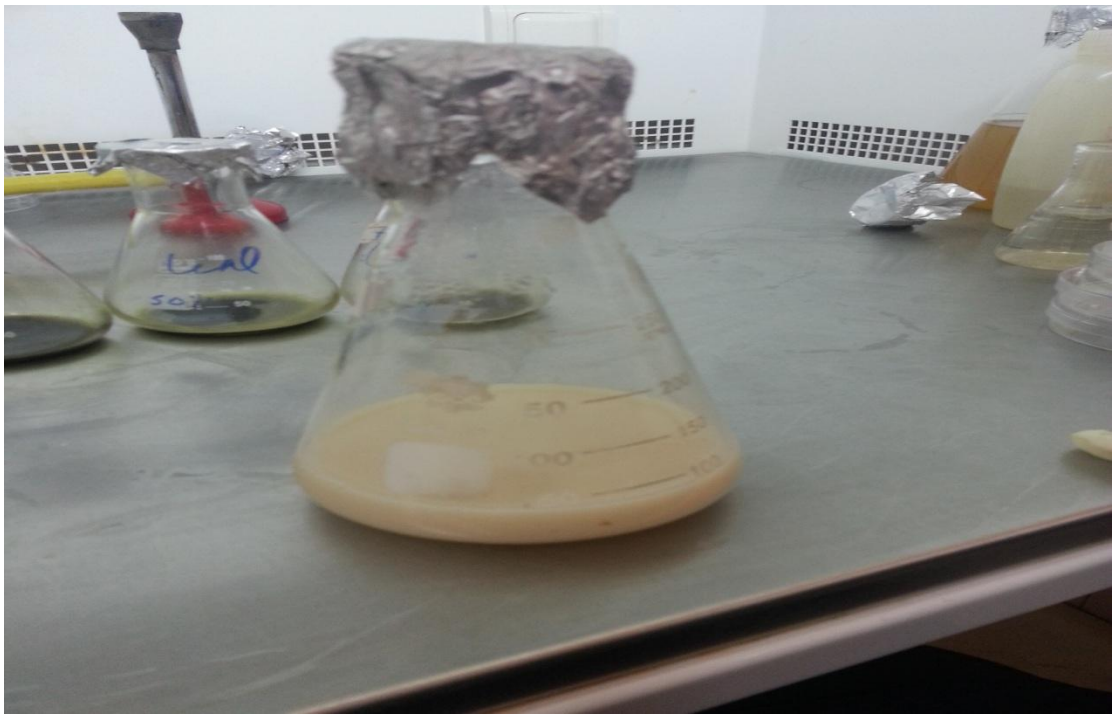

Figure (3) Fungicide (Amistar top®)

CHAPTER FOUR

RESULTS

4. 1 Location

This study which conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of Science and Technology in January, 2019 to investigate the inhibitory effect of different parts of mesquite (leaves and fruits) aqueous extract and fungicide, Amistar top® against the fungus *Fusarium oxysporum.f.sp.tuberosi.*

4.2 Isolation and Identification of the Fungus

The Isolation and Identification of *Fusarium oxysporum f. sp. tuberosi* was performed according to the shape of spores and conidia.

Fusarium oxysporum f. sp. tuberosi 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.

Microconidia 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).

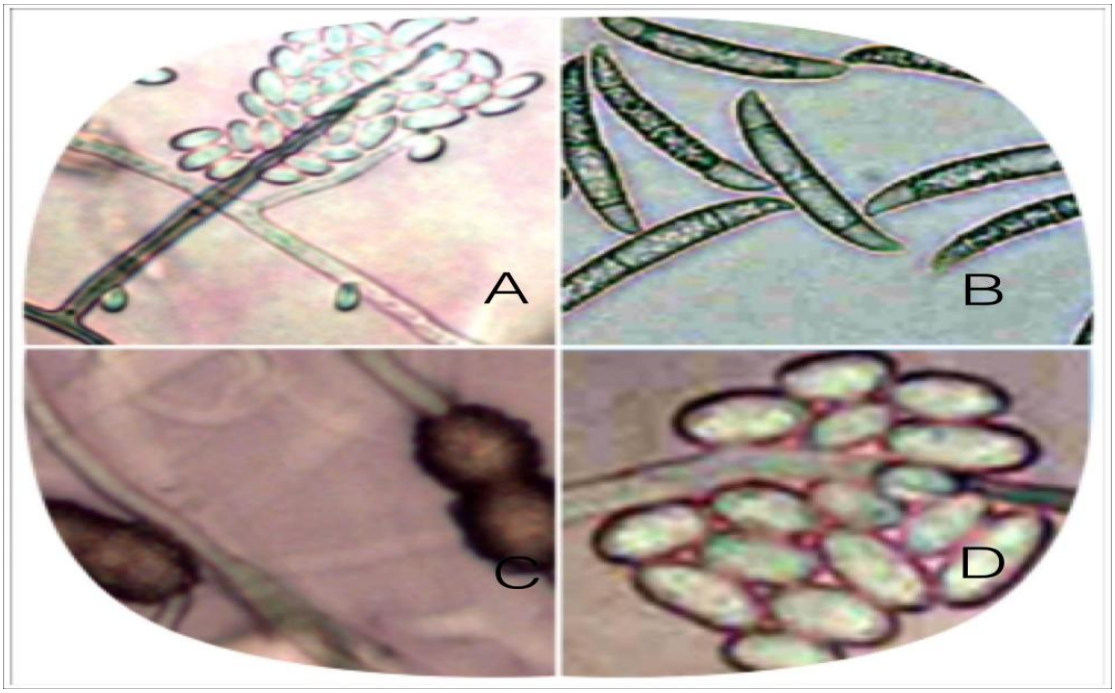

Figure: (4) Morphological characters of *F.oxysprum* (Morphotype II).A= Conidiophores, B = Macroconidia, C = Chlamydospores, D = Microconidia (scale bar = 25 um).

4.3 Effect of Mesquite different Parts aqueous Extracts and Fungicide Amistar top on radial growth of *Fusarium oxysporum f. sp. tuberosi* three days after inoculation *in vitro*.

The aqueous extract of mesquite ware prepared of concentration (25%, 50% and 100%), it effect was studied mesquite leaves extract inhibited the growth of the fungus in all concentrations within three days after inoculation (tables 1).The extract at 50% concentration ware effective in radial growth. The results (Table 1 and figure 1) showed that after three days from inoculation all plant extracts (leaves and Fruits) of mesquite and Fungicide Amistar top® as will induced significantly high inhibition zones percentage against the test fungi compared to control.

Moreover the highest concentration of the two parts plant extracts (100%) gave (100 %, 46 %) significantly higher inhibition compared to the untreated control. among the parts of Mesquite extract tested. Leaves extract was the most effective in suppressing of the fungus growth than Fruits in (Table 1) which gave (100%, 89.5% and 67%)in all concentration which fruits extract gave (46%, 43.6% and 40.6%) which Amistar top® gave 100% to the *Fusarium oxysporum f. sp. tuberosi* The results showed that the antifungal activity increase with increasing of extract concentration.

Table: (1):- Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide (Amistar Top®) on radial growth of *Fusarium oxysporum f. sp. tuberosi* three days after inoculation *in vitro*.

Treatment / Concentration %		Percentage of mycelium inhibition %			
Mesquite Leaves	100	100(10)	100(10)	100(10)	100(10 a)
	50	97(9.7)	95(9.5)	77(7.7)	89.6(8.9 a)
	25	69(6.9)	69(6.9)	63(6.3)	67(6.7 b)
Mesquite Fruits	100	53(5.3)	36(6.6)	49(6.9)	46(4.6 b)
	50	36(5.3)	49(3.6)	46(4.9)	43.6(4.3 c)
	25	38(4.2)	36(4.2)	48(4.4)	40.6(4.0 c)
Fungicide		100(10)	100(10)	100(10)	100(10 a)
Control		0(0.7)	0(0.7)	0(0.7)	0(0.7 c)
LSD		1.1			
C.V (%)		9.92			
SE±		0.4			

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.

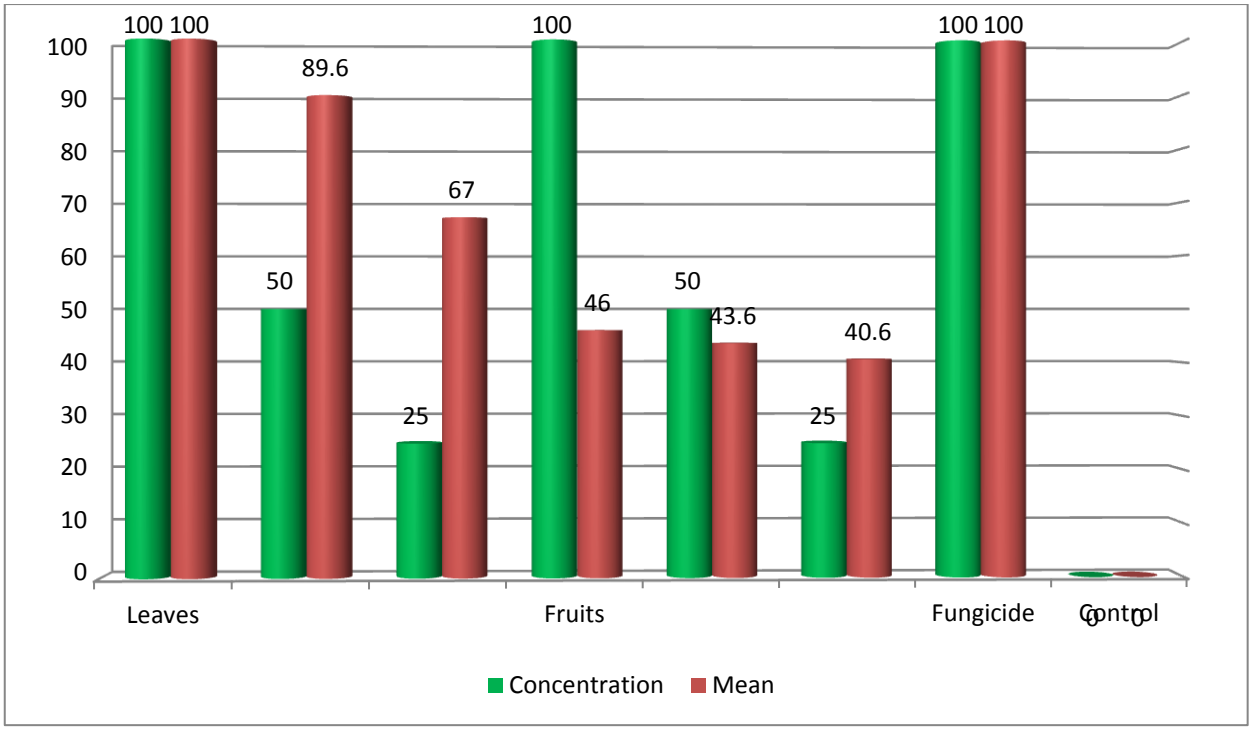

Figure: (5) Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum .f.sp.tuberosi* three days after inoculation *in vitro*.

4.4 Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum* four days after inoculation *in vitro.*

The results in (Table, 2 and Figure. 2 and Plates) showed that the aqueous extracts of different parts of Mesquite tested at all concentrations (25%, 50% and 100%) and fungicide (Amistar Top®)**)** continued exhibiting an inhibitory effect against *F. oxysporum.f.sp.tuberosi* after four days from inoculations. The percentage of the fungal growth inhibition was significantly high compared to the control. Moreover, the highest inhibitory effect (99.3 %) was demonstrated by concentration of leaves extracts at 100% concentration and that of fungicide Amistar Top® (100) were significantly high against fungus. Among the plant extracts screened those of leaves were the most effective in suppressing the fungus growth at all concentration which gave (99.3%, 86.3% and 74.3%). However, the fruit extract has lower inhibition of the fungal growth, which gave (51.6%, 41.3 and 37.3) the results showed that the inhibitory effect increase with increased concentration.

Table: (2) Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum.f.sp.tuberosi* four days after inoculation *in vitro.*

Treatment / Concentration %		Percentage of mycelium inhibition %			
Mesquite Leaves	100	100(10)	100(10)	98(9.8)	99.3(9.9 a)
	50	91(9.1)	84(8.4)	84(8.4)	86.3(8.6 ab)
	25	76(7.6)	72(7.2)	75(7.5)	74.3(7.4 b)
Mesquite Fruits	100	40(4)	60(6)	55(5.5)	51.6(5.1 c)
	50	23(2.3)	56(5.6)	45(4.5)	41.3(4.1 c)
	25	19(1.9)	41(4.1)	52(5.2)	37.3(3.7 c)
Fungicide		100(10)	100(10)	100(10)	100(10 a)
Control		0(0.7)	0(0.7)	0(0.7)	0(0.7 d)
LSD		1.6			
C.V (%)		14.8			
SE±		0.85			

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

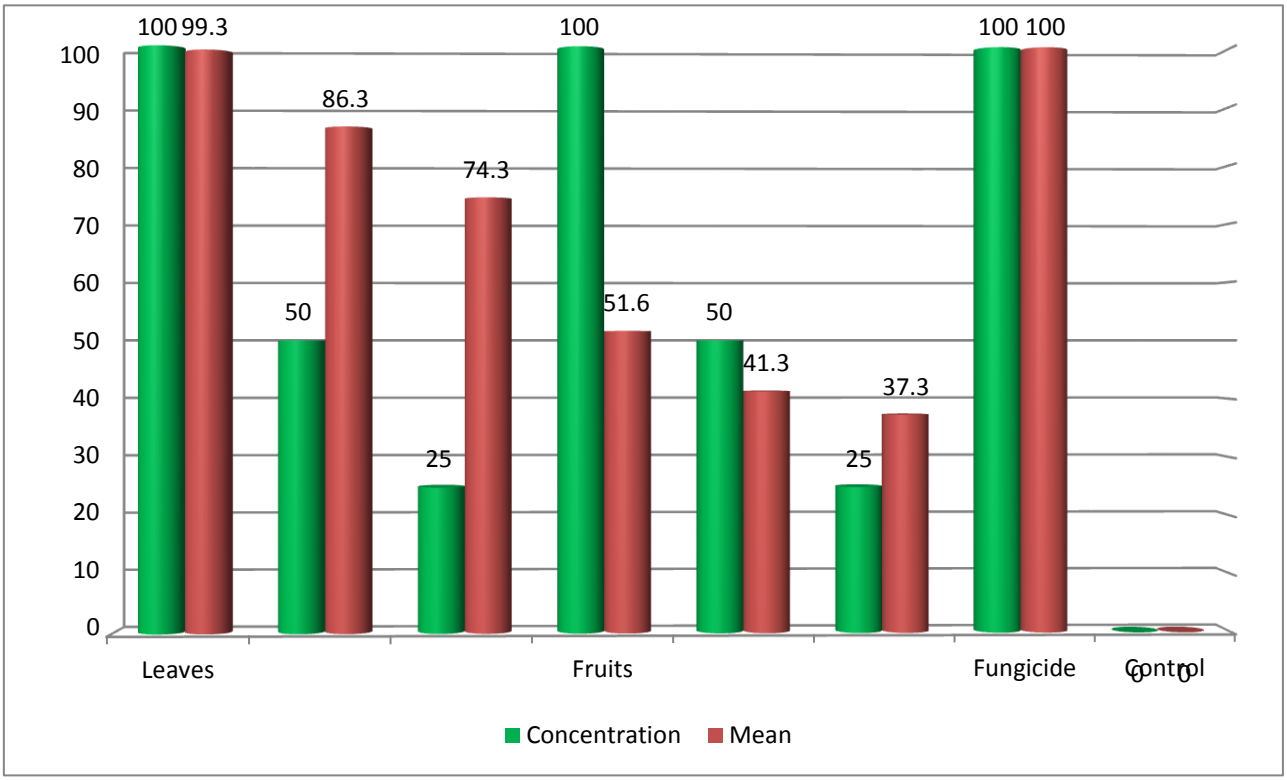

Figure: (6) Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum.f.sp.tuberosi* four days after inoculation *in vitro.*

4.5 Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum.f.sp.tuberosi* five days after inoculation *in vitro.*

In five days post inoculation, the result (Table 3 and Fig. 3 and Plates) 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 the two parts of Mesquite (25 % , 50 % and 100 %) and fungicide Amistar top® induced significantly higher inhibition against *F. oxysporum* compared to control (96.6%, 65 % and 100) respectively. However, the leaves aqueous extract at high concentration tested exhibited consistently more inhibitory effect than the fruits aqueous extract which give (96.6%) 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 (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum.f.sp.tuberosi* five days after inoculation *in vitro.*

Treatment	Concentration %	Percentage of mycelium inhibition %			
Mesquite Leaves	100	98(9.8)	98(9.8)	94(9.4)	96.6(9.6 a)
	50	82(8.2)	80(8)	78(7.8)	80(8.0 b)
	25	71(7.1)	56(5.6)	66(6.6)	64.3(6.4 c)
Mesquite Fruits	100	67(6.7)	62(6.2)	66(6.6)	65(6.5 c)
	50	46(4.6)	66(6.6)	48(4.8)	53.3(5.3 d)
	25	42(4.2)	42(4.3)	44(4.4)	42.6(4.2 e)
Fungicide		100(10)	100(10)	100(10)	100(10 a)
Control		0(0.7)	0(0.7)	0(0.7)	0(0.7 f)
LSD		0.87			
C.V (%)		7.9			
SE±		0.25			

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.

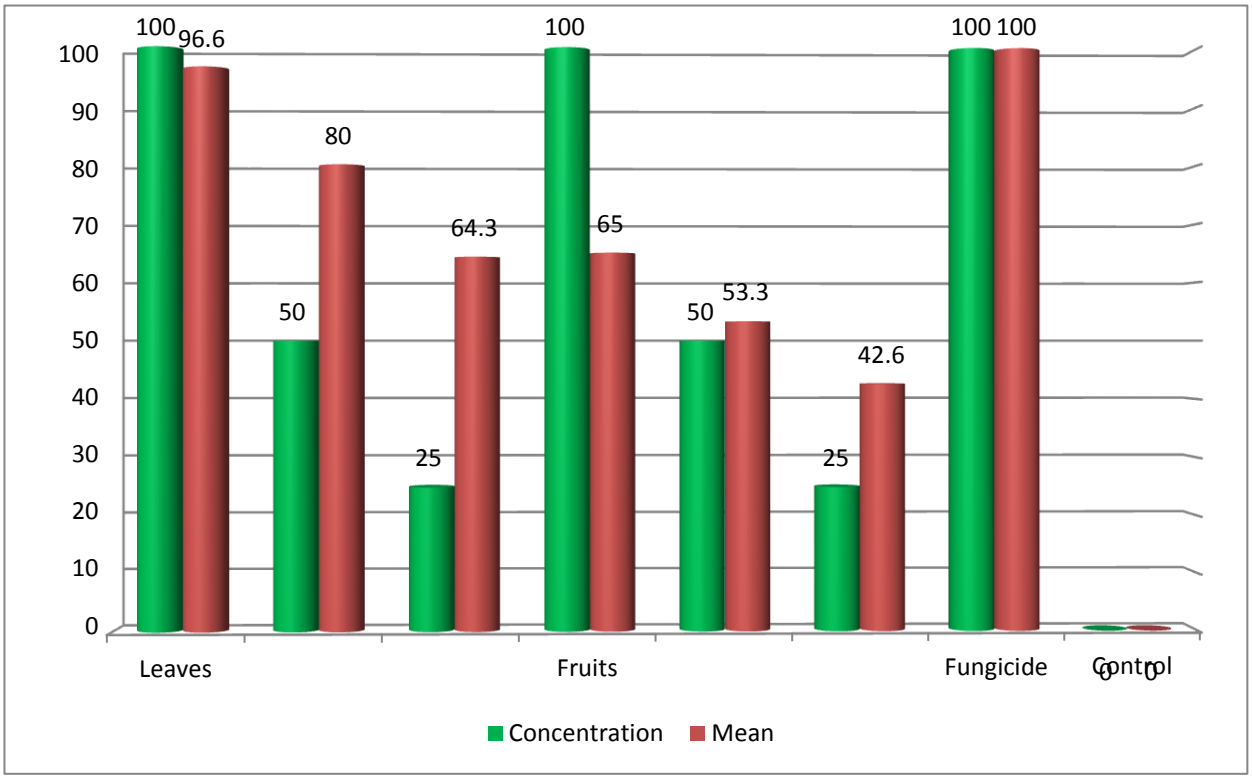

Figure: (7) Effect of Mesquite different Parts (Leaves and Fruits) aqueous Extracts and Fungicide Amistar Top® on radial growth of *Fusarium oxysporum* five days after inoculation *in vitro*.

Plate: (1) Effect of aqueous extracts of mesquite leaves on the growth of *Fusarium oxysporum.f.sp.tuberosi* in vitro five days post inoculation.

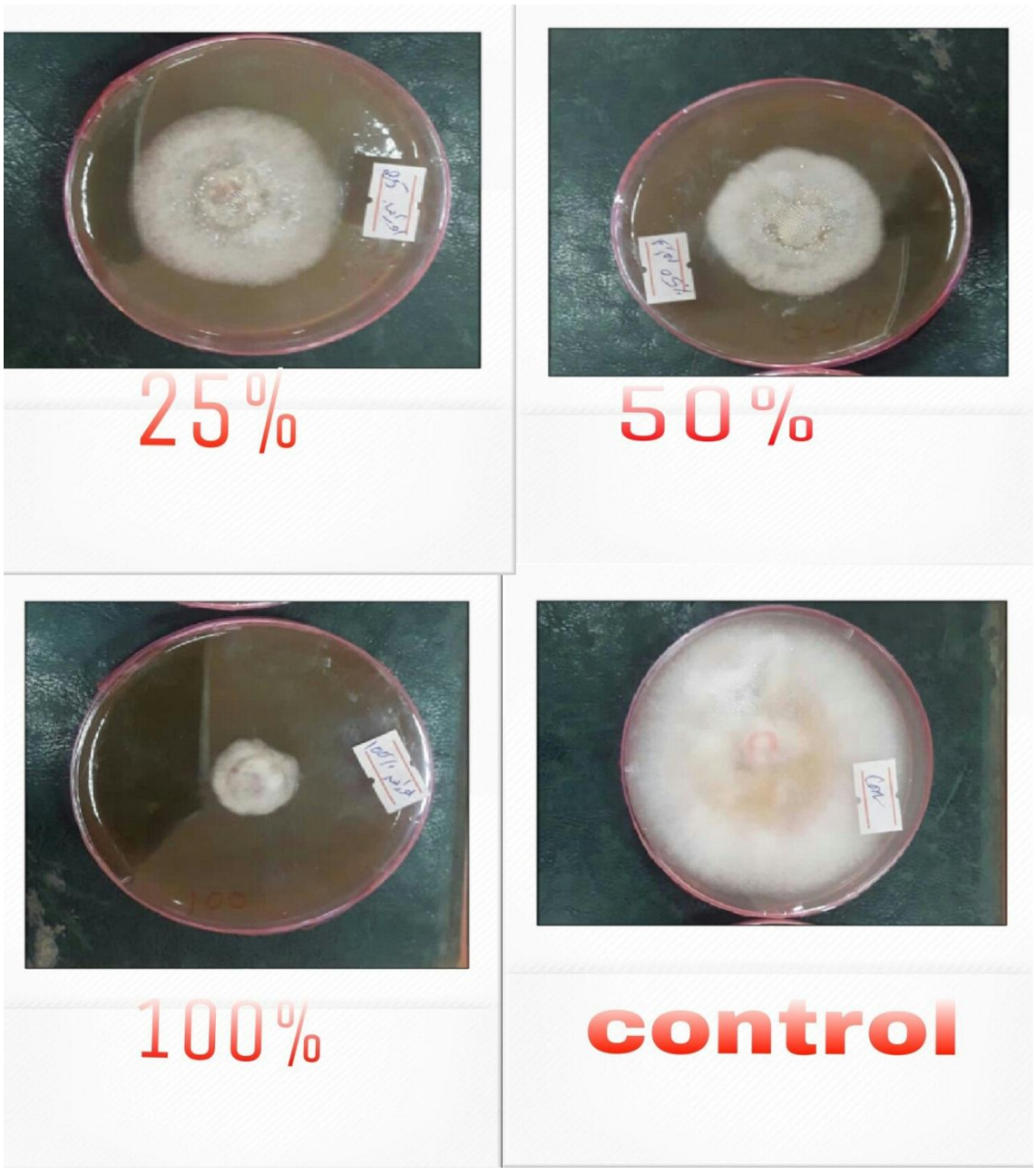

Plate: (2) Effect of aqueous extracts of mesquite fruits on the growth of *Fusarium oxysporum .f.sp.tuberosi* in vitro five days post inoculation.

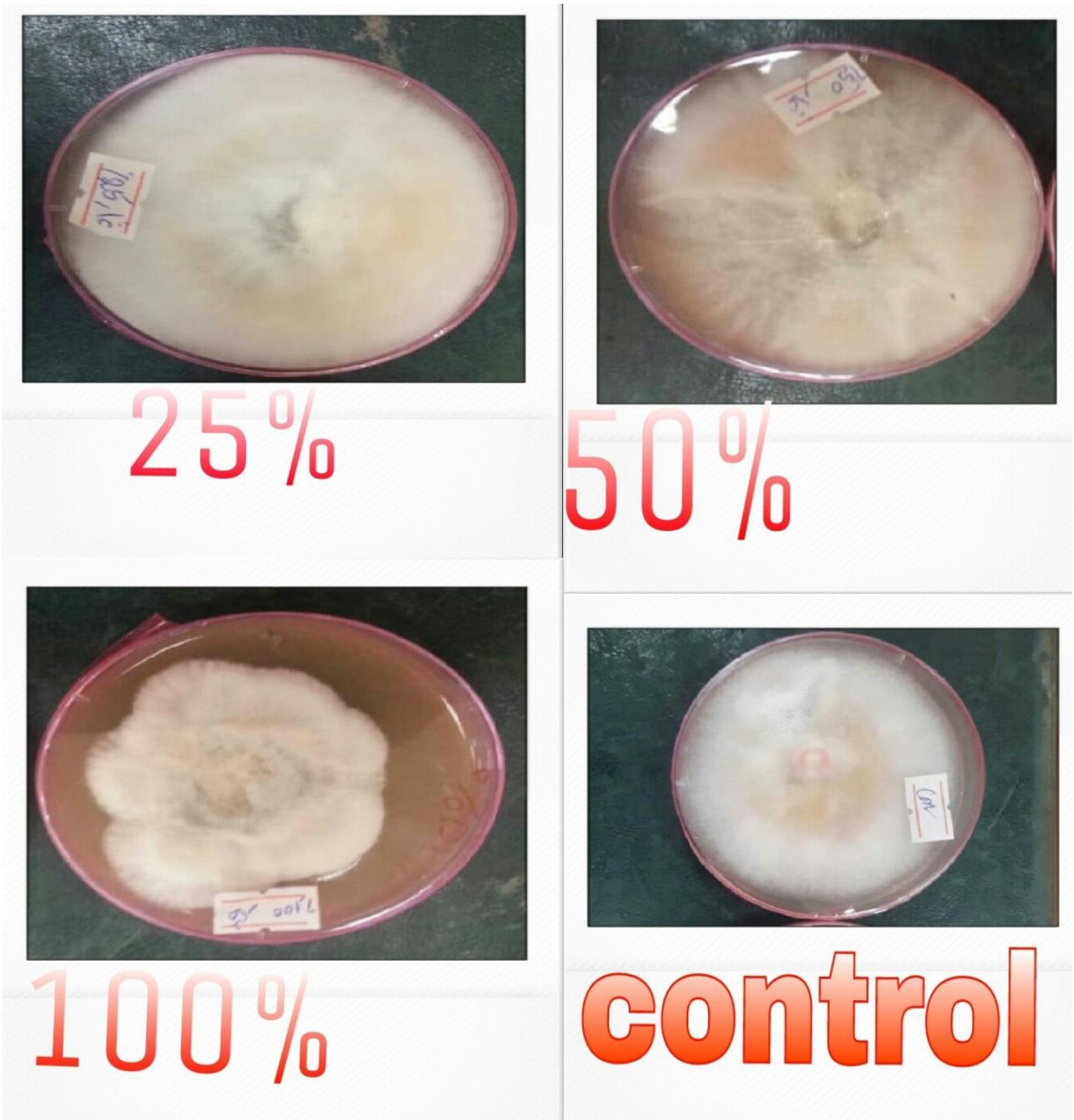

Plate: (3) Effect of fungicide (Amistar top®) on the growth of *Fusarium oxysporum .f.sp.tuberosi* in vitro five days post inoculation.

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).

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 study is conducted to evaluate the bioactivity of aqueous extracts from some parts of mesquite (leaves and fruits) and fungicide (Amistar top®) on growth of *Fusarium oxysporum f. sp. tuberosi* under laboratory conditions**.**

The results revealed that the Mesquite parts (leaves, and Fruits) aqueous extracts at all concentration (25% ,50% and 100%) and fungicide, Amistar Top®, solution consistently and throughout the course of the experiments exhibited an inhibitory effect on mycelial radial growth of *Fusarium oxysporum f. sp. tuberosi* with significantly higher inhibition reduction in growth percent compared to control.

The leaves extract is more effective suppression than fruits extract on fungus growth at all concentration. 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). In fact, this finding is in agreement with (Abdelrahaman, 2016 and Harown, 2016) they are 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 (FadlElmola *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 indicated promising potentials of Mesquite, (*Prosopis juliflora*) as they proved to be a source of antifungal effect that helps in management of plant fungal diseases. The leaves aqueous extracts of the two 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 aqueous extracts of the two parts of Mesquite plant and fungicide Amistar Top®, at all concentrations, exhibited inhibitory effects against the radial mycelia growth of the tested fungus (*F. oxysporum f. sp. tuberosi)*. The percentages zone of inhibition was significantly high compared to the Control. Among the two parts of Mesquite, leaves and fruits at all concentrations tested (25 %, 50 % and 100%) 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 aqueous extracts differ in their reactions to test F *oxysporum f. sp. tuberosi*, 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 was lower effect.

RECOMMENDATIONS

1 - The Extracts of Leaves and Fruits of Mesquite trees can be tested against other fungal plant diseases such as *Fusarium oxysporum f. sp. tuberosi* .

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

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APPENDICES

Appendix 1: ANOVA

A) Variable 3. (Inhibition in third day after inoculums)

The SAS System

The ANOVA Procedure

Class Level Information

Class Levels Values

Treatment 8

Concentration F100 F25 F50 Fung L100 L25 L50

Number of observations 24

Dependent Variable: pctgerm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	222.5060192	31.7865742	77.66	<.0001
Error	16	6.5491387	0.4093212		
Corrected Total	23	229.0551578			

R-Square	Coeff Var	Root MSE	pctgerm Mean
0.971408	9.920769	0.639782	6.448917

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	7	222.5060192	31.7865742	77.66	<.0001

Tests (LSD) for pctgerm

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.409321
Critical Value of t	2.11991
Least Significant Difference	1.1074

Means with the same letter are not significantly different.

Grouping	Mean	N	treatment
A	10.0240	3	Fung
A			
A	10.0240	3	L100
A			
A	9.0320	3	L50
B	6.7430	3	L25
B			
B	6.3263	3	F100
C	4.6420	3	F50
C			
C	4.1000	3	F25
D	0.7000	3	Con

B) Variable 4. (inhibition in fourth day after inoculums)

The SAS System

The ANOVA Procedure

Class Level Information

Class Levels Values

Treatment 8

Concentration F100 F25 F50 Fung L100 L25 L50

Number of observations 24

Dependent Variable: pctgerm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	233.0154706	33.2879244	38.78	<.0001
Error	16	13.7343080	0.8583943		
Corrected Total	23	246.7497786			

R-Square	Coeff Var	Root MSE	pctgerm Mean
0.944339	14.80291	0.926496	6.258875

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	7	233.0154706	33.2879244	38.78	<.0001

Tests (LSD) for pctgerm

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.858394
Critical Value of t	2.11991
Least Significant Difference	1.6037

Means with the same letter are not significantly different.

Grouping	Mean	N	tremen
A	10.0240	3	Fung
A			
A	9.9520	3	L100
A			
B A	8.6953	3	L50
B			
B	7.5103	3	L25
C	5.2053	3	F100
C			
C	4.1730	3	F50
C			
C	3.8110	3	F25
D	0.7000	3	Cont

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

The SAS System

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Treatment	8	
Concentration	F100 F25 F50 Fung L100 L25 L50	

Number of observations 24

Dependent Variable: pctgerm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	193.9926067	27.7132295	108.49	<.0001
Error	16	4.0872307	0.2554519		
Corrected Total	23	198.0798373			

R-Square	Coeff Var	Root MSE	pctgerm Mean
0.979366	7.907317	0.505423	6.391833

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Treatment	7	193.9926067	27.7132295	108.49	<.0001

Tests (LSD) for pctgerm

NOTE: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.255452
Critical Value of t	2.11991
Least Significant Difference	0.8748

Means with the same letter are not significantly different.

Grouping	Mean	N	treatment
A	10.0240	3	Fung
A			
A	9.7113	3	L100
B	8.0423	3	L50
C	6.5247	3	F100
C			
C	6.4343	3	L25
D	5.3740	3	F50
E	4.3240	3	F25
F	0.7000	3	Cont

Appendix 2: Equipment's, 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 foil
Gloves	Face mask
Registration form	Camera
Measuring cylinder	Centrifuge
Shaker	Infested Potato Plant Fruits
Water bath	Medical cotton
Potato Dextrose Agar (PDA)	Soap
Mesquite leaves	Clorox
Mesquite Fruits	Ethanol 95%
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®

Fungicide Amistar Top®

Mesquite Plant different Parts

(Leaves and Fruits)