Sudan University of Science and Technology
College of Graduate Studies
Department of Plant Protection

In-Vitro Evaluation of Bioactivity of the Ethanolic Extracts of (Mesquite and Argel) Leaves and the Fungicide (Amstar Top®) against *Fusarium oxysporum*

التقييم المعملي للفعالية الحيوية للمستخلص الإيثانولي لاوراق المسكيت والحرجل والمبيد الفطري (Amstar Top®) على الفطر *F.oxysporum*

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

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الآية

قال تعالى:

(وَسِيقَ الَّذِينَ اتَّقَوْا رَبَّهُمْ إِلَى الْجَنَّةِ زُمَرًا حَتَّى إِذَا جَاءُوهَا وَفُتِحَتْ أَبْوَابُهَا وَقَالَ لَهُمْ خَزَنَتُهَا سَلَامٌ عَلَيْكُمْ طِبْتُمْ فَادْخُلُوهَا خَالِدِينَ (73) وَقَالُوا الْحَمْدُ لِلَّهِ الَّذِي صَدَقَنَا وَعْدَهُ وَأَوْرَثَنَا الْأَرْضَ نَتَبَوَّأُ مِنَ الْجَنَّةِ حَيْثُ نَشَاءُ فَنِعْمَ أَجْرُ الْعَامِلِينَ (74) وَتَرَى الْمَلَائِكَةَ حَافِّينَ مِنْ حَوْلِ الْعَرْشِ يُسَبِّحُونَ بِحَمْدِ رَبِّهِمْ وَقُضِيَ بَيْنَهُمْ بِالْحَقِّ وَقِيلَ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ(75)).

صدق الله العظيم

سورة الزمر الآيات (75.74.73)

Dedication

To my mother

To my father

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Alli

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All praises are due to Almighty Allah who gave me health and strength, and helped me tremendously to produce this work.

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ABSTRACT

This study was conducted under laboratory conditions at the Plant Pathology Laboratory in College of Agricultural Studies(CAS) during September - October 2018. The study aim is to evaluate the efficacy of Argel (*Solenostemma argel*) and Mesquite (*Prosopis juliflora*) leaves ethanolic extracts and the fungicide Amstar Top® on the *Fusarium oxysporum.* Fusarium wilt is considered as one of the most important diseases of different crops worldwide. However, the indiscriminate use of synthetic fungicides and their increasing hazards to the public health and the environment coupled with the development of resistant strains of phytopathogenic fungi has led to the use of safe alternate products. Mesquite (*Prosopis juliflora*) and, Argel (*Solenostemma argel*) leaves ethanolic Extracts and Fungicide Amstar top was tested for their antifungal potential against *F. Oxysporum.* Different concentrations i.e. 25, 50, and 100% of both plants were prepared and their in-vitro bioactivity was examined against the said fungus. Our results revealed that all the tested concentrations were found significantly (P0.05) inhibiting the growth of the fungus. The higher concentration of ethanol extract of argel (100%) caused the maximum inhibition in the diameter of the tested fungus growth by 100% followed by the concentration (50%) which inhibited the fungus growth by 86.4% and the concentration (25%) by (61.2%).The concentrations 100% of Mesquite leaves recorded inhibitory effect by (97.2%) and 50% of recorded (67.3%) respectively while the fungicide “Amstar top” inhibited the fungus growth by 100%. The inhibition zone of the three tested concentrations i.e. 25, 50, and 100% were compared with untreated control and a standard chemical (Amstar top). Our results obtained from this study conclude that Mesquite

and Argel leaves ethanolic Extracts are promising and encouraging to carry out further chemical analyses of plants to identify the effective ingredients to use it as alternatives to harmful pesticides that adversely affect human, animal and environment. On the other hand we recommend further studies for the bioactivity of the Mesquite and Argel leaves ethanolic Extracts *In-vivo* under field conditions.

ملخص البحث

أجريت هذه الدراسة بمعمل أمراض النبات التابع لكلية الدراسات الزراعية – شمبات ، جامعة السودان للعلوم والتكنولوجيا خلال(سبتمبر- اكتوبر) من العام 2018م. اهتم هذا البحث بدراسة الأثر الحيوي للمستخلص الإيثانولي لاوراق الحرجل والمسكيت على نمو الفطر موضوع الدراسة تحت ظروف المعمل. يعتبر مرض الذبول الفيوزيرمي من اهم امراض المحاصيل فى العالم. إن الاستخدام الغير مرشد لمبيدات الفطريات المصنعة ومخاطرها المتزايدة على الصحة العامة والبيئة إلى جانب تطوير سلالات مقاومة للفطريات الممرضة للنباتات قد أدى إلى استخدام منتجات بديلة آمنة. اجريت هذه الدراسه لمعرفة تاثير المستخلص الإيثانولي لاوراق الحرجل والمسكيت و المبيد الفطرى امستار توب على نمؤ فطر الفيوزيرم اوكسيسبوريم المسبب لمرض الذبول فى المحاصيل. تم تحضير تركيزات مختلفة ، 25 و 50 و 100 ٪ من مستخلص الإيثانولي لاوراق الحرجل والمسكيت و المبيد الفطرى امستار توب وتم فحص تأثيرها في المختبر ضد الفطر موضع الدراسة في 3مكررات وقورنت النتائج بالشاهد غير المعامل وكذلك بالاطباق المعاملة. ومن ثم تم تحليل البيانات إحصائيا على المستوى (P0.05). كشفت نتائج البحث أن جميع التركيزات المختبرة مؤثرة معنويا (P0.05) مثبطة لنمو الفطر. حيث كان التركيز 100% من الحرجل هو صاحب الحد الأقصى لتثبيط نمو الفطر المختبر بنسبة (100%) تبعه التركيز 50% بنسبة (86.4%) متبوعا بالتركيز 25% الذي ثبط نمو الفطر بنسبة (61.2%). بينما كان أثر المبيد الفطري (امستار توب) تثبيط نمو الفطر بنسبة 100% مقارنة بالشاهد. اما المسكيت تركيز 100% فقد سجل (97.2) اما تركيز 50% و25% فكانت القراءت (67.3 % و44.6%)على التوالي. كشفت النتائج التي تحصلنا عليها ان جميع التركيزات المختبرة في تجربتنا اعطت نتائج مبشرة مما يشجع على إجراء المزيد من التحليلات الكيميائية لأجزاء أخرى من نباتي المسكيت والحرجل لتحديد المكونات الفعالة لاستخدامها كبديل لمبيدات الافات الضارة التي تؤثر سلبًا على الإنسان والحيوان والبيئة. من ناحية أخرى، نوصي بإجراء مزيد من الدراسات للنشاط الحيوي لمستخلصات المسكيت والحرجل في الجسم الحي تحت ظروف الحقل.

CHAPTER ONE

INTRODUCTION

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

Fusarium wilt is a common vascular wilt fungal pathogen, exhibiting symptoms similar to Verticillium wilt. The pathogen that causes Fusarium wilt is *Fusarium oxysporum* (Snyder and Hansen, 1940). The species is further divided into forma specialist based on host plant .These fungal generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. The most important of these is vascular wilt. Fusarium oxysporum is a common soil pathogen and saprophyte that feeds on dead and decaying organic matter. It survives in the soil debris as a mycelium and all spore types, but is most commonly recovered from the soil as chlamydospores (Snyder and Hansen, 1940). It is a major wilt pathogen of many economically important crop plants. It is a soil-borne pathogen, which can live in the soil for long periods of time, so rotational cropping is not a useful control method. It can also

spread through infected dead plant materials, so cleaning up at the end of the season is important. Members of *F. oxysporum* are present throughout the world's soils.

However, before global transportation many of the different varieties of the pathogen were isolated. Now, Global trade has spread *F. oxysporum* inoculums with the crop. A recent example of this is the spread of *Fusarium oxysporum f.sp. Cubense* which may have originated in Asia and just recently has appeared in banana producing areas in the South Pacific (Davis and Richard, 2004).In Sudan, several diseases are known to limit production of crops, One of which Fusarium wilt caused by *Fusarium oxysporum* is one of the most important diseases causing economical losses (Bhatia *et al.* 2004*)*. It is reported that the disease is especially serious in the traditional production areas. Based on the foregoing, this study was undertaken to focus on investigation of two components for management of Fusarium wilt caused by *Fusarium oxysporum*, higher plant extracts and synthetic fungicides under Laboratory conditions in order to formulate promising disease management approach with following main objective:-

1. Explore the inhibitory effect of ethanol extracts of different concentrations of Mesquite and Argel leaves against the fungus (*Fusarium* oxysporum) compared to the standard fungicide Amstar Top®.

CHAPTER TWO

LITEREATURE REVIEW

2.1 Fusarium wilt

Fusarium species causes a huge range of diseases on an extraordinary range of host plants. The fungus can be soil borne, airborne or carried in plant residues and can be recovered from any part of the plant from the deepest root to the highest flower (Booth 1971; Summeral *et al*. 2003).

2.1.1 Classification

Kingdom: Fungi

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: Fusarium

Species: Fusarium oxysporum

(Agrios, 2005 and Snyder & Hansen, 1940)

2.1.2 Fungus Description

Fusarium oxysporum is a common soil inhabitant. Booth (1977) isolated *F. oxysporum* from the tap root, lateral root, main stem, lateral branches and seed of infected plant, but not from pod bulls or leaves.

The fungus produces three types of asexual spores, micro conidia, macro conidia and Chlamydia spores. The macro conidia are straight to slightly curved, slender thin walled usually with three or four septa, of a foot shaped cell. They are generally produced on conidiophores by division. They are important in secondary infection. The micro conidia are ellipsoidal and either have no septum or single one. They are formed from phial ides in false heads by secondary infection (Agrios, 2005).

The chlamydispores are globes and have thick walls. It is formed from hyphae or alternatively by the modification of micro cells. Conidia considered as endurance organs in soil where they act as inoculums in primary infection.

The telemorph or sexual reproductive stage *of F. oxysporum* is unknown. Booth (1977) stated that the chromosome number of the fungus is (12) and the perithecial state is Gibberella but not confirmed (Agrios, 2005).

2.1.3 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). *F.oxysporum* strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin. (Rodriguez *et al* 1996) and complex carbohydrates (Christakopoulos, *et al*, 1996) Associated with soil debris. They are also

pervasive plant endophytes that can colonize plant roots (Gordon, and Jacobson, 1989; Katan 1971) and may even protect plants or be basis of disease suppression (Larkin, *et al*, 1993; Lemanceau, *et al*, 1993). Although the predominant role of these fungi in native soil may be as harmless or even beneficial plant endophytes or soil saprophytes, many strains within *F.oxysporum* complex are pathogenic to plant, especially in agricultural setting. Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these Fusarium species for example are fumonisin and trichothecenes.

2.1.4 Economic Importance of the fungus

Fusarium oxysporum is a significal problem in many crops. It is economically damaging many industrial crops eg, banana industry The threat of more virulent strains or mutants that damage previously resistant crops is of major concern. (Dreistadt, and Clark,. 2004) *Fusarium oxysporum* also causes damage to many crops of the Solanaceae such as potato, tomato, and pepper. Other commercially important plants are

affected include basil, beans, carnation, chrysanthemum, peas, and watermelon. (Ahemd, 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 (Khane, 1980).

2.1.5 Host Range

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 Andersen (1974) for example the following hosts *Alliums cannabis. Beta vulgaris, Cucumis sativa, Phaseolus vulgaris and Pisum 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).

2.1.6 Pathogenesis

F. oxysporum has been studied for more than 100 years. Host range of these fungi is extremely broad, and includes animals, ranging from arthropod. (Teetor, 1983). To human, (Nelson, 1994) as well as plant, including range

of both gymnosperms and angiosperms. While collectively, plant pathogenic *F. oxysporum* have a broad host range, individual isolates usually cause disease only on a narrow range of plant species. This observation has led to the idea of "special form" or forma specials in *F. oxysporum*. (Kistler, 2001).

2.1.7 Symptomatology

Agrios (2005) described the first symptoms appear as slight vein clearing on the outer, younger leaflets. The older leaver show epinasty caused by drooping of the petioles. Plants infected at the seedling stage usually wilt and die soon after appearance of the first symptom. Older plant in the field may wilt and die suddenly .in older plant vein clearing and leaf epinasty are followed by stunting of the plant , yellowing of the lower leaves , occasional formation of adventitious roots, wilting of the leaves and young stems , defoliation necrosis , fruit may occasionally become infected . And then it rots and drops off spotted .Roots rot after initial period of stunting. Plant infected with *Fusarium oxysporum* shows symptom such as chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping off, of which the most important is vascular wilt (Ramsamy,*et.al.*, 1996).

Fusarium wilt in the first stage is looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. On older plants, symptoms are more distinct between the

blossoming and fruit maturation stage (Nene *et al*, 1991; Agrios, 2005; and Smith, 1988). The disease can appear at any stage of plant growth, symptoms in a highly susceptible cultivar can develop any time between 25 days after sowing till as late as pudding stage (Nene, 1985). The disease occurs at seedling and flowering stage of plant growth. The symptoms which can be observed are drooping of petioles and rachis ,yellowing and drying leaves from base to upward, browning of vascular bundles improper branching , withering of plant and finally death (Westerlund *et al*.,1974; Prasad and Padwich , 1939).

2.1.8 Disease Cycle

Fusarium oxysporum is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al*.; 1988).Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium or as any of its three different spores type (Agrios, 2005). Healthy plant can be infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with it sporangial germ tube or mycelium by tips, through the wounds in the roots, or at the formation point of lateral roots (Agrios, 2005).

The mycelium grows through the root cortex intercellular. When the mycelium reaches the xylem, it invades the vessels through the xylem point, the mycelium remain in the vessel, where it usually advances upwards toward the stem and crown of the plant. As it grows the mycelium branches and produces micro conidia, which are carried upward within the vessel by the plants sap stream .When the micro conidia germinate, the mycelium can penetrate the upper wall of the xylem, enabling to be produced in the next

vessel. The fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits (Agrios, 2005).

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 colose, the leaves wither, and the plant eventually died. At this point the fungus invades the plants parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios2005). The resulting spores can be used as new inoculums for further spread of the fungus.

2.2 Control

2.2.1 Cultural control

The cultural control is the only practical measure for controlling the disease in the field. The wilt fungus is so widespread and so persistent in soils that seedbed sterilization and crop rotation although always sound practices but are of limited value. Soil sterilization is too expensive for application but it should be always practiced for greenhouses (Agrios2005).

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

As mentioned above, Fusarium wilts affect and cause severe losses on most vegetable and flowers, several field crops such as cotton, Tobacco, banana, plantain, coffee, sugarcane and a few shade trees. Fusarium wilts are most severe under warm soil conditions and green houses (Agrios, 2005).

2.2.2 Botanical control

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

The use of plant extracts for controlling Fusarium wilt, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Plant extracts or plant essential oils have been tested against *F. oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2000). If natural plant products can reduce populations of soil borne pathogens and control diseases development, than these plant extracts have potential as environmentally safe alternatives and as component in integrated pest management programs. Chand and Singh (2005) Reported that the plant extracts, VIZ *Calotropis procera*, *Eucalyyptus globulens*, *Jatropha multifida*, *Azadirachta indicia*, *Allium sativum* were significantly pronounced in reducing wilt incidence in *Cicer arietinum* L. Mycelial growth of various Fusarium species were inhibited by the plant extracts of *Adhatoda vasica*, *Azadirachta indica* ,*Cinnamomum camphora*, and *Ocimum sanctum* (Prasad and Ojha,1986); *Agave americana*,

Cassia nadosa (Reddy and Reddy,1987*); Azadirachta indicia* (Eswaramoothy *et al* ., 1989); *Azadirachta indica*, *Atrophabelladonna,Calotropisprocera, Eucalyptus amgdalline, Ailanthus exclsa and Lantana camera* (Bansal and Rajesh, 2000; Nwachukku and Umechuruba (2001).

Also (Singh and Chand, 2005) reported that Leaf extract of *Azadirachta indica* at 100/con completely inhibited germination of pathogen spores.

2.2.2.1 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. (Felker *et al*, 2003). Prosopis spp grow in arrays of environments and are not restricted by soil type, pH, salinity or fertility (Babiker, 2006).

2.2.2.1.1 Characteristics 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).

2.2.2.1.2 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, *et al*, 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).

2.2.2.1.3 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 food stuffs 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).

2.2.2.2 Argel

Argel (*Solenostemma argel*) is a desert plant of traditional medical uses in the Sudan. It grows wild in the area extending from Dongola to Barber, particularly around Abu Hamad, where it is grown under irrigation (Elkamali and Khalid, 1996). Sudan is regarded as the richest source of this plant (Orange, 1982). Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos *et al.*, 1980; Hamed, 2001). Sulieman *et al*. (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties.

The farmers in Kassala State put argel shoots in porous jute sacks in the irrigation canals to be leached by water. The water was effective in controlling aphids and white flies in summer tomatoes and Egyptian bull worm in okra respectively (Unpublished observation). In a pilot field experiment on Brassica nigra, some peripheral plots were severely infested by aphids. The infestation caused stunting of shoots and delayed flowering compared to non-infected plots. However, upon treatment with argel as a soil additive, or a spray of shoot water extract or a combination of soil

additive and spray, the vegetative growth was restored in all plots after pest disappearance and the plants flowered within 10-15 days after treatments. The inflorescence was abnormally thick and profusely branched in plants that received the combined treatment suggesting a growth-regulator-like effect and indicating the efficiency of argel as a pesticide (Abdelwahab, 2002).

2.2.3 Chemical control

Anon (1994) reported that methyl bromide fumigation is used extensively 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.

2.2.3.1 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.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Location

This study was conducted in the Laboratory of Plant Pathology Department of Plant Protection, College of Agricultural Studies, Shambat, Sudan University of Science and Technology during the period September, to October (2018) to evaluate the antifungal effect of different concentrations of Mesquite (Leaves) and argel .ethanolic extracts and efficacy of fungicide Amstar top® against the fungus *Fusarium oxysporum*.

3.2 Equipments, Tools and Materials used in the Study

Injections cabinet	Incubator Laminar flow
Marker pens	Compound microscope
Needle	Autoclave
Slide	Sensitive balance
Petri-dishes	Conical flasks
Aluminum foils	
Gloves	
Registration form	
Potato Dextrose Agar (PDA).	
Mesquite leaves	
Argel leaves	
Soap	
Ethanol 80%	
Filter paper	

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

3.3 Source of materials:

Mesquite (leaves) was collected from trees growing in the premises of the College of Agriculture Studies, Shambat and argel bought from market. The fungicide (Amstar top) was obtained from the laboratory of Plant Protection Department College of Agricultural Studies.

3.4 Preparations

3.4.1 Preparation of extracts:

Extraction was carried out according to method described by Sukhdev et. al. (2008) where 50 g of each sample was extracted by soaking in 750 ml of ethanol 80% for about seventy two hours with continuous filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract was then exposed to air till complete dryness. Three concentrations were then obtained viz, 100%, 50% and 25% for both plants.

3.4.2 Preparation of the fungus inoculums:

The pure culture of *fusarium oxysporum* was prepared using 7 days old mycelia. The fungi was cultured on Potato Dextrose Agar media (PDA) then transferred as , aseptically to the center of petri dishes containing PDA medium and incubated at 25Co 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

The inhibition zone technique was used in this study according to (Rao and Srivastava, 1994). The PDA media was amended with the required concentration from Mesquite and Argel 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 which cut by a sterile cork-borer (5 mm) from an edge of an actively growing culture of the fungus *Fusarium oxysporum*. The inoculated Petri dishes were then incubated in an incubator and the radial growth was measured every day.

3.6 Calculation

The diameter of growth was measured every 24 hours, by taking the average of two crossed dimensions for each disc in the Petri dish. The radial growth was then calculated as a percentage from the Petri dish diameter (9.0 cm). The effect of each extract concentration on linear fungal growth was calculated as percentage of inhibition in diameter of fungal growth related to the Control diameter according the following equation:

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

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 with three replicates, the obtained data was statistically analyzed by "Statistix8" software computer program according to analysis of variance (ANOVA). The Least Significant Difference (LSD) Test was used for mean separation.

CHAPTER FOURE
RESULT

4.1 Laboratory Experiment

This study was conducted under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology, during September, to October 2018, to evaluate the inhibitory effect of different concentrations of mesquite leaves , Argel leaves and efficacy of fungicide Amstar stop® on the fungus growth.

4.2 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro*

4.2.1 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* four days after inoculation

The results in (Table 1, Figure 1) shows that after three days of inoculation all the applied concentrations of Argel leaves and Mesquite leaves extracts induced significant suppression on the radial diameter of the test fungus ($P \geq 0.05$) compared to untreated control (0.0%). However, the 100% concentrations of Argel leaves, Mesqutite leaves and the standard fungicide Amstar Top were found to be the most effective (100%, 97.2% and 100%) respectively. as it inhibited the growth up to 100% compared to control. The concentration 50% of Argel leaves were also significantly retarded the growth of the target fungus by 86.4%, followed by the 50% concentration of Mesquite leaves by 67.3%. The results showed that the antifungal activity increases with the increase of the extract concentration.

Table 1: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* four days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0%(1.1)	0.0%(1.2)	0.0%(1.5)	0.0%(1.2)[a]
Argel 25%	45.4%(0.6)	58.3%(0.5)	80.0%(0.3)	61.2%(0.4)[b]
Argel 50%	90.9%(0.1)	75.0%(0.3)	93.3%(0.1)	86.4%(0.1)[c]
Argel 100%	100%(0.0)	100%(0.0)	100%(0.0)	100.0%(0.0)[d]
Mesquite 25%	27.2%(0.8)	33.3%(0.8)	73.3%(0.4)	44.6%(0.6)[b]
Mesquite 50%	63.6%(0.4)	58.3%(0.5)	80%(0.3)	67.3%(0.4)[b]
Mesquite 100%	100%(0.0)	91.6%(0.1)	100%(0.0)	97.2%(0.03)[d]
Fungicide Amstar Top	100%(.00)	100%(0.0)	100%(0.0)	100%(0.0)[d]
C.V%	17.2			
SE±	0.05			
LSD	0.08			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x+0.05}$ before analysis

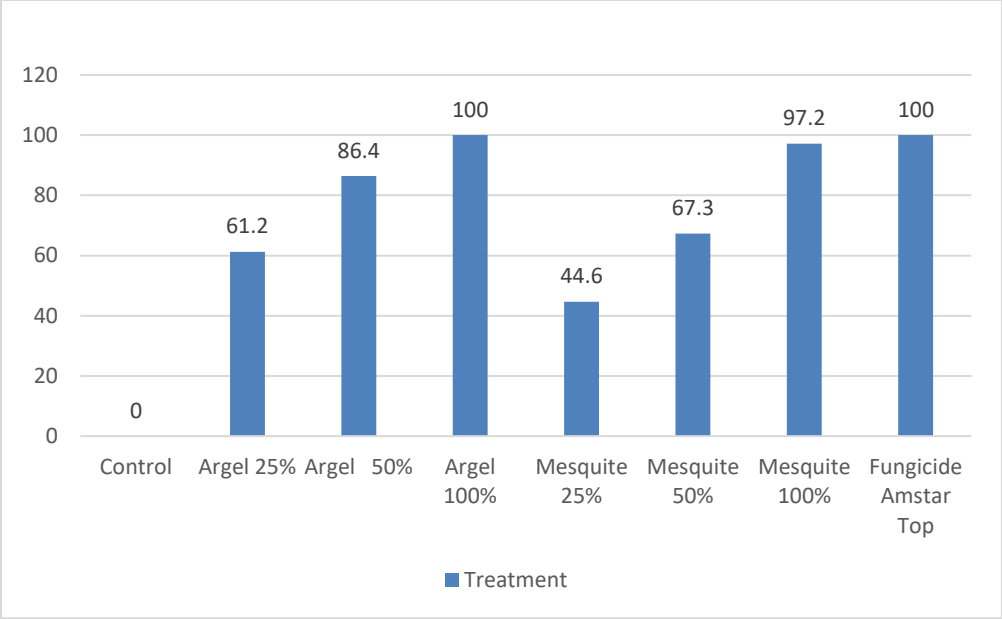

Figure 1 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* four days after inoculation

4.2.2 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* five days after inoculation

Table 2, Figure 2 shows that all the ethanol extracts of Argel leaves and Mesquite leaves tested against *F. oxysporum* as well as the fungicide continues their inhibitory effect in the fifth day after inoculation significantly ($P \geq 0.05$). The percentage of fungal growth inhibition was significantly high compared to the untreated control (0.0%). The highest inhibitory effect (100%) is recorded from the 50%, 100% concentrations of Argel leaves as well as from the standard fungicide, while the other concentrations 100% of Mesquite leaves recorded inhibitory effect by (80.7%) and 25% of Argel leaves recorded (66.2%) respectively. Still the inhibitory effect was increasing according to the increase in the concentration.

4.2.3 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* six days after inoculation

The results (Table 3, Figure 3) shows that sixth days after inoculation all the applied concentrations of Argel leaves and Mesquite leaves extracts induced significant suppression on the radial diameter of the test fungus ($P \geq 0.05$) compared to untreated control(0.0%). However, the 100%, 50% and 25% concentrations of Argel leaves and the standard fungicide Amstar Top were found to be the most effective (88.0%, 74.5%, 74.5% and 100%) respectively, compared to untreated control. The concentration 100% of Mesquite leaves was also significantly retarded the growth of the target fungus (64.3%), followed by the 25% and 50% concentrations by (49.1% and 57.5% respectively). However, the increasing of the inhibitory effect went in the same trend as it use in the previous days.

Table 2: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* five days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0%(1.3)	0.0%(1.4)	0.0%(1.5)	0.0%(1.4)[a]
Argel 25%	61.5%(0.5)	57.1%(0.6)	80%(0.3)	66.2%(0.4)[c]
Argel 50%	92.3%(0.1)	100%(0)	100%(0)	97.4%(0.03)[e]
Argel 100%	92.3%(0.1)	100%(.00)	100%(0.0)	97.4%(0.03)[e]
Mesquite 25%	23.0%(1)	28.5%(1)	40%(0.9)	30.5%(0.9)[b]
Mesquite 50%	61.5%(0.5)	64.2%(0.5)	60%(0.6)	61.9%(0.5)[c]
Mesquite 100%	76.9%(0.3)	78.5%(0.3)	86.6%(0.2)	80.7%(0.2)[d]
Fungicide Amstar Top	100%(0.0)	100%(0.0)	100%(0.0)	100%(0.0)[e]
Argel 25%	61.5%(0.5)	57.1%(0.6)	80%(0.3)	66.2%(0.4)[c]
C.V%	10.7			
SE±	0.03			
LSD	0.05			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x+0.05}$ before analysis

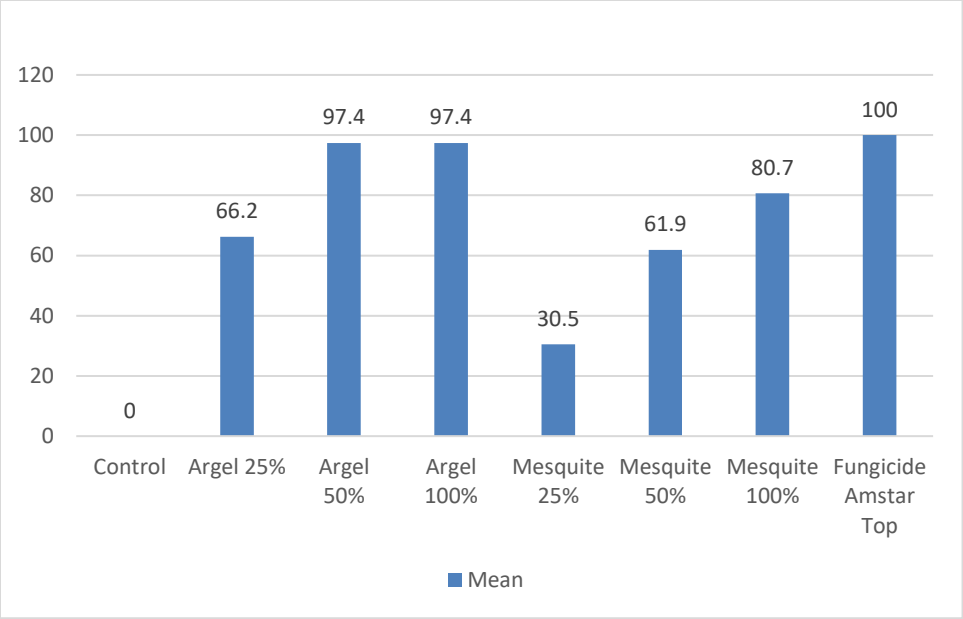

Figure 2 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* five days after inoculation

Table 3: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* six days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0 %(2)	0.0%(1.9)	0.0%(2)	0.0%(1.9)[a]
Argel 25%	75.0%(0.5)	68.4%(0.6)	80.0%(0.4)	74.5%(0.5)[c]
Argel 50%	75.0%(0.5)	73.6%(0.5)	75.0%(0.5)	74.5%(0.5)[c]
Argel 100%	85.0%(0.3)	78.9%(0.4)	100%(0)	88.0%(0.2)[d]
Mesquite 25%	45.0%(1.1)	47.3%(1)	55.0%(0.9)	49.1%(1.0)[b]
Mesquite 50%	65.0%(0.7)	52.6%(0.9)	55.0%(0.9)	57.5%(0.8)[b]
Mesquite 100%	65.0%(0.7)	57.8%(0.8)	70.0%(0.6)	64.3%(0.7)[bc]
Fungicide Amstar Top	100%(0)	100%(0)	100%(0)	100%(0.0)[e]
C.V%	11.7			
SE±	0.05			
LSD	0.07			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x+0.05}$ before analysis

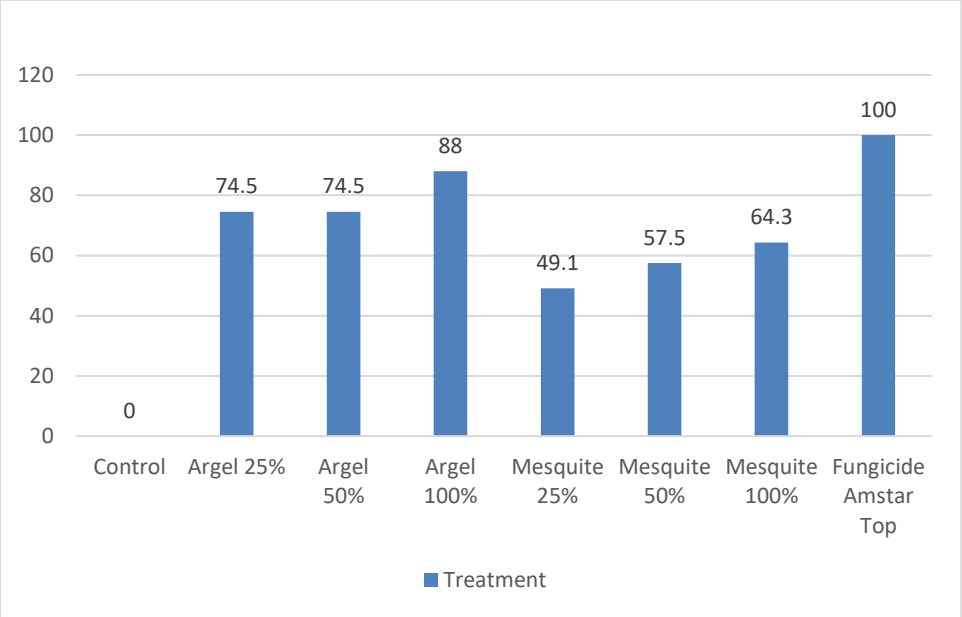

Figure 3 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of Fusarium oxysporum in vitro s ix days after inoculation

4.2.4 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* seven days after inoculation

The results in (Table 4, Figure 4) shows that after seven days of inoculation all the applied concentrations of Argel leaves and Mesquite leaves extracts significantly suppressed the radial diameter of the test fungus ($P \geq 0.05$) compared to untreated control (0.0%). However, the 100%, 50% and 25% concentrations of Argel leaves and the standard fungicide Amstar Top were found to be the most effective (87.0%, 73.8%, 65.7% and 100%) respectively, compared to untreated control. The concentration 100% of Mesquite leaves was also significantly retarded the growth of the target fungus (63.8%), followed by the 25% and 50% concentrations of Mesquite leaves by (26.2% and 44.2%).The inhibitory effect following its same trend.

4.2.5 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* eight days after inoculation

Eight days post inoculation the results in (Table5, Figure5) shows that all the concentrations and the fungicide tested were significantly ($P \geq 0.05$) different from one another compared to untreated control (0.0%). The Concentration 100% of Argel leaves recording the highest inhibitory effect by (82.1%). while the 50% recorded an inhibitory effect by (79.7%), while the concentration 25%. slightly increased to recorded an inhibitory effect (64.1%). The Mesquite leaves concentrations 100%, 50% and 25% recorded an inhibitory effect by (62.8%, 43.6% and 32.4% respectively). The fungicide Amstar top recorded (100%). Whereas, the Mesquite leaves extracts decrease in its inhibitory effect through times.

Table 4: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* seven days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0% (2.1)	0.0% (2)	0.0% (2)	0.0% (2.33)[a]
Argel 25%	57.1% (0.9)	65.0% (0.7)	75.0% (0.5)	65.7% (0.7)[c]
Argel 50%	71.4% (0.6)	75% (0.5)	75% (0.5)	73.8% (0.53)[c]
Argel 100%	81% (0.4)	80% (0.4)	100% (0)	87.0% (2.7)[d]
Mesquite 25%	28.6% (1.5)	25% (1.5)	25% (1.5)	26.2% (1.5)[b]
Mesquite 50%	47.6% (1.1)	50% (1)	35% (1.3)	44.2% (1.13)[b]
Mesquite 100%	71.4% (0.6)	65% (0.7)	55% (0.9)	63.8% (0.73)[c]
Fungicide Amstar Top	100% (0)	100% (0)	100% (0)	100% (0)[e]
C.V%	12.3			
SE±	0.06			
LSD	0.08			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x+0.05}$ before analysis

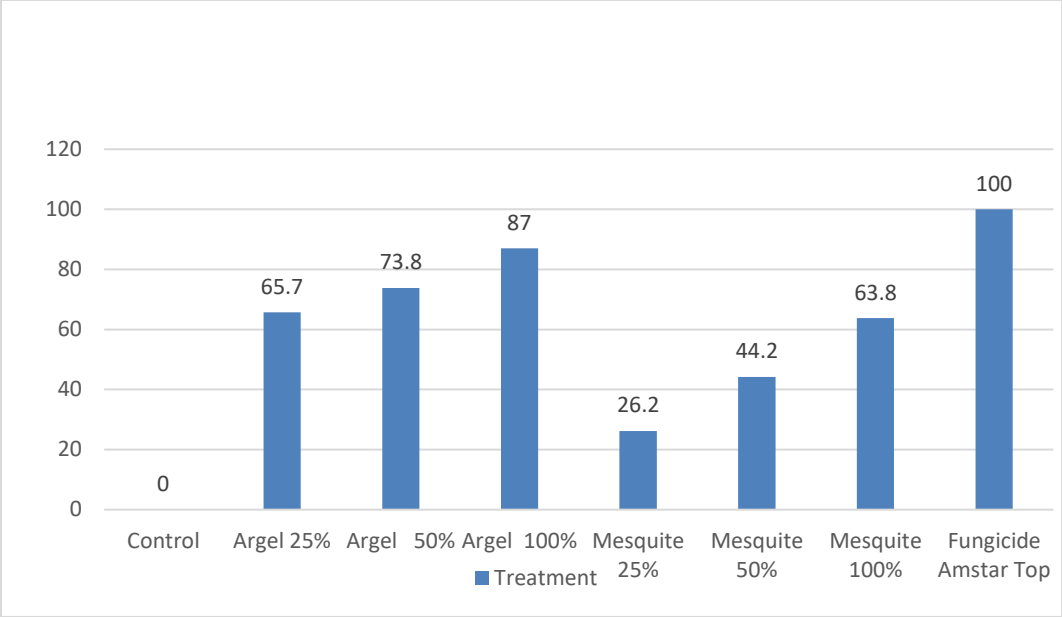

Figure 4 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* seven days after inoculation

Table 5: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* eight days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0%(3.1)	0.0%(3)	0.0%(2.8)	0.0%(2.9)[a]
Argel 25%	58.0%(1.3)	66.6%(1)	67.8%(0.9)	64.1%(1.06)[d]
Argel 50%	77.4%(0.7)	83.3%(0.5)	78.5%(0.6)	79.7%(0.6)[e]
Argel 100%	80.6%(0.6)	80.0%(0.6)	85.7%(0.4)	82.1%(0.5)[e]
Mesquite 25%	32.2%(2.1)	36.6%(1.9)	28.5%(2)	32.4%(2.0)[b]
Mesquite 50%	48.3%(1.6)	43.3%(1.7)	39.2%(1.7)	43.6%(1.6)[c]
Mesquite 100%	67.7%(1)	60.0%(1.2)	60.7%(1.1)	62.8%(1.1)[d]
Fungicide Amstar Top	100%(0)	100%(0)	100%(0)	100%(0.0)[f]
C.V%	5.3			
SE±	0.03			
LSD	0.04			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x}+0.05$ before analysis

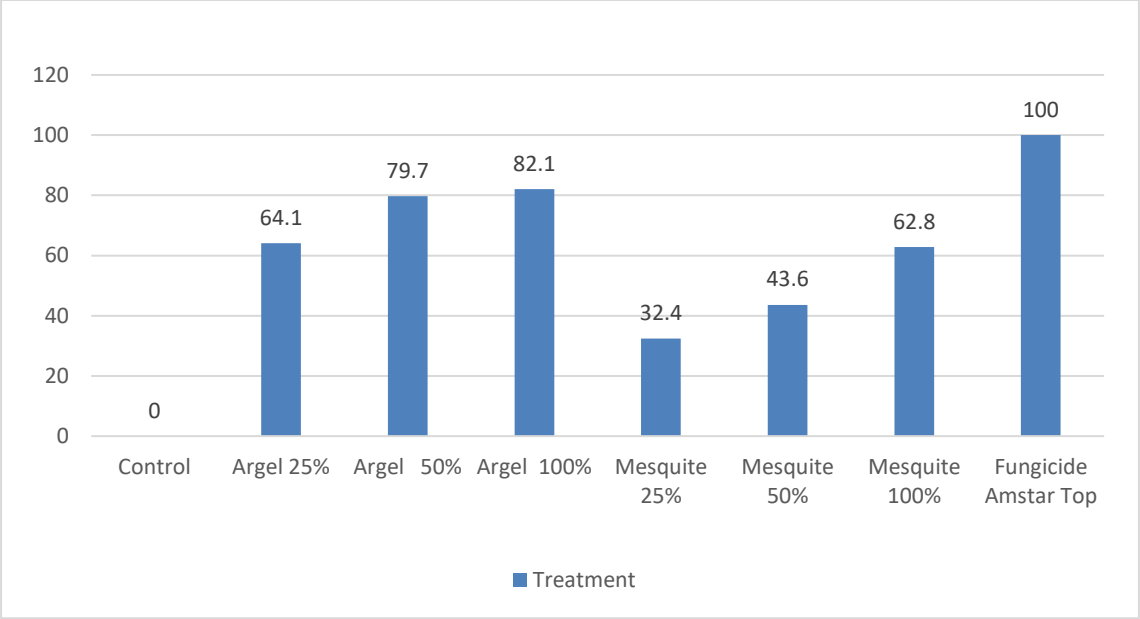

Figure 5 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of Fusarium oxysporum in vitro eight days after inoculation

4.2.6 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* nine days after inoculation

After nine days post inoculation the results in (Table 6, Figure 6) shows that all the concentrations and the fungicide tested were significantly ($P \geq 0.05$) different from one another compared to untreated control (0.0%). The Concentration 100% of Argel leaves recording the highest inhibitory effect by (80.0%) and the 50% concentration recorded an inhibitory effect by (78.0%) while the concentration 25%. slightly increased to record an inhibitory effect by (63.2%). The Mesquite leaves concentrations 100%, 50% and 25% recorded an inhibitory effect by (62.7%, 47.6% and 33.9% respectively). The fungicide Amstar top recorded (100%).

Table 6: Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* nine days after inoculation

Treatments	R 1	R 2	R3	Mean
Control	0.0%(3.5)	0.0%(3.4)	0.0%(3.1)	0.0%(3.3)[a]
Argel 25%	57.1%(1.5)	64.7%(1.2)	67.7%(1)	63.2%(1.2)[d]
Argel 50%	74.2%(0.9)	82.3%(0.6)	77.4%(0.7)	78.0%(0.7)[e]
Argel 100%	77.1%(0.8)	82.3%(0.6)	80.6%(0.6)	80.0%(0.6)[e]
Mesquite 25%	28.5%(2.5)	44.1%(1.9)	29.0%(2.2)	33.9%(2.2)[b]
Mesquite 50%	54.2%(1.6)	50.0%(1.7)	38.7%(1.9)	47.6%(1.7)[c]
Mesquite 100%	68.5%(1.1)	64.7%(1.2)	54.8%(1.4)	62.7%(1.2)[d]
Fungicide Amstar Top	100%(0)	100%(0)	100%(0)	100%(0.0)[f]
C.V%	6.75			
SE±	0.03			
LSD	0.06			

Dissimilar letters on the "Mean" column show significant differences at P0.05. Data in the parentheses transformed using square root transformation $\sqrt{x+0.05}$ before analysis

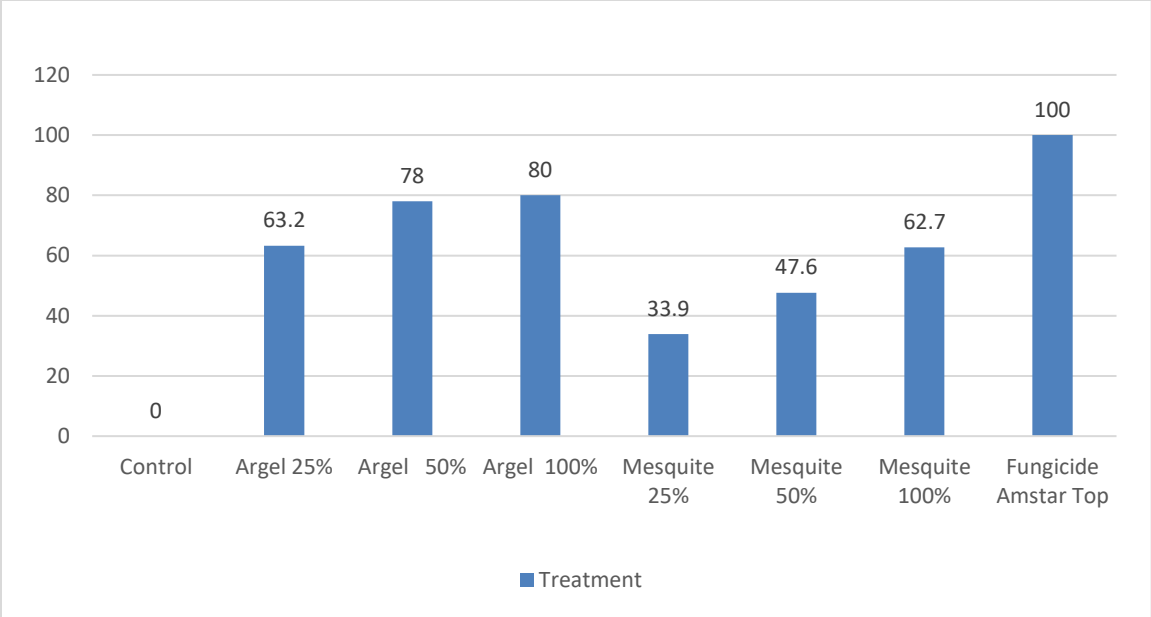

Figure 6 Effect of Mesquite leaves, Argel leaves Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum in vitro* nine days after inoculation

CHAPTER FIVE

DISCUSSION

Fusarium wilt is one of the major yield limiting factors of corps production in many countries (Nene and Reddy, 1987; and Haware, 1990 ;).

In Sudan, Fusarium wilt caused by *Fusarium oxysporum* is one of the most important diseases in crops (Bhatia *et al.*, 2004*)*. It is reported that the disease is especially serious in the traditional production areas where crops is grown on stored soil moisture after the flood waters of the Nile River subside. In these areas, farmers do not adhere to crop rotation and the crop at the post-flowering stage is often subject to moisture stress in years of low flood (Ali, 1996 and Faki *et al*., 1996).

Historically, numerous phytochemicals have been isolated from different plants which are now being prescribed by medical practitioners all around the world (Newman, *et al*., 2000). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and fungicides that are effective at very low concentrations. On the other hand botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Klosterman, R.E. (2001).

In this study, the Argel leaves and Mesquite leaves ethanolic extracts were investigated using different concentration for their bioactivity against

Fusarium oxysporum. The data (Tables 1-6 and Figures 1-6) revealed that all Argel and Mesquite plants (leaves) ethanolic extracts exhibited an inhibitory effect on fungal growth with significantly high inhibition zones percent.

The present study investigated the effect of Argel leaves ethanolic extract on growth of fungus *F. oxysporum*. From the result it was found that Argel leaves extract were highly effective against fungus, although the effect of Argel leaves was increased in its inhibitory effect through times. The inhibitory was increasing according to the increase in the concentration. Antifungal activity of other plants are well documented (Alicia, 1981). In Sumatra (Indonesia), 114 plant species extracts were assayed for their antibacterial activity (Ahmed, 2002). About 82% of the extracts were active against bacteria; *Staphaureus*, while 35% of them were active against *E.coli* and about 20 of the extracts inhibited growth of the tested fungi (*Saccharomyces cerevisiae* and *Fusarium oxysporum*).

The mesquite leaves ethanolic extract and fungicide, Amstar top®, solution consistently and throughout the course of the experiments exhibited an inhibitory effect on radial growth of the fungus with significantly higher inhibition reduction growth percent compared to untreated 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). The Mesquite leaves extracts recorded a decrease in its inhibitory effect through times. The inhibitory was increasing according to the increase in the concentration. This finding is in agreement with Mohammed and Omer (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.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

- In conclusion, the findings presented in this study indicate promising potentials of Mesquite (*Prosopis juliflora*) and, Argel (*Solenostemma argel*) leaves ethanolic Extracts as it proved to be a source of antifungal effect against the fungus. Furthermore, this antifungal effect will definitely help in management of plant fungal diseases. The exhibited inhibitory effect of Mesquite and Argel leaves ethanolic extracts on fungal growth; in addition to the standard fungicide (Amstar Top) can be applied as part of an integrated approach to manage Fusarium Wilt.
- The Mesquite and Argel leaves ethanolic Extracts at all concentrations and the fungicide Amstar top ®, exhibited inhibitory effect on the radial mycelial growth of the tested fungus (*F. oxysporum)*. The percentage zone of inhibition was significantly high compared to control.

6.2 Recommendations

1- Further studies can be conducted to test other parts of Mesquite and Argel extracts against the said fungus and other fungi.

2- Further studies is also can be conducted under field conditions (*In-vivo*) to control wilt diseases on different crops, using the Mesquite and Argel leaves ethanolic extracts.

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Appendices

Appendix 1a: Original Data

REP	TREAT	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
1	Control	1.1	1.3	2	2.1	3	3.5
2	Control	1.2	1.4	1.9	2	2.6	3.4
3	Control	1.5	1.5	2	2	2.5	3.1
1	H 25%	0.6	0.5	0.5	0.9	1	1.5
2	H 25%	0.5	0.6	0.6	0.7	0.7	1.2
3	H 25%	0.3	0.3	0.4	0.5	0.6	1
1	H 50%	0.1	0.1	0.5	0.6	0.7	0.9
2	H 50%	0.3	0	0.5	0.5	0.5	0.6
3	H 50%	0.1	0	0.5	0.5	0.5	0.7
1	H100%	0	0.1	0.3	0.4	0.6	0.8
2	H100%	0	0	0.4	0.4	0.6	0.6
3	H100%	0	0	0	0	0.2	0.6
1	M25%	0.8	1	1.1	1.5	1.9	2.5
2	M25%	0.8	1	1	1.5	1.9	1.9
3	M25%	0.4	0.9	0.9	1.5	1.8	2.2
1	M50%	0.4	0.5	0.7	1.1	1.4	1.6
2	M50%	0.5	0.5	0.9	1	1.5	1.7
3	M50%	0.3	0.6	0.9	1.3	1.5	1.9
1	M100%	0	0.3	0.7	0.6	0.9	1.1
2	M100%	0.1	0.3	0.8	0.7	1.2	1.2
3	M100%	0	0.2	0.6	0.9	1.1	1.4
1	Fung	0	0	0	0	0	0
2	Fung	0	0	0	0	0	0
3	Fung	0	0	0	0	0	0

Appendix 1b: Transformed data using the equation {NX (targeted) = SQRT(x+0.5)}

TREAT	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
Control	1.072381	1.161895	1.431782	1.466288	1.746425	1.884144
Control	1.118034	1.204159	1.396424	1.431782	1.627882	1.857418
Control	1.24499	1.24499	1.431782	1.431782	1.596872	1.774824
H25%	0.806226	0.74162	0.74162	0.974679	1.024695	1.24499
H25%	0.74162	0.806226	0.806226	0.866025	0.866025	1.118034
H25%	0.591608	0.591608	0.67082	0.74162	0.806226	1.024695
H50%	0.387298	0.387298	0.74162	0.806226	0.866025	0.974679
H50%	0.591608	0.223607	0.74162	0.74162	0.74162	0.806226
H50%	0.387298	0.223607	0.74162	0.74162	0.74162	0.866025
H100%	0.223607	0.387298	0.591608	0.67082	0.806226	0.921954
H100%	0.223607	0.223607	0.67082	0.67082	0.806226	0.806226
H100%	0.223607	0.223607	0.223607	0.223607	0.5	0.806226
M25%	0.921954	1.024695	1.072381	1.24499	1.396424	1.596872
M25%	0.921954	1.024695	1.024695	1.24499	1.396424	1.396424
M25%	0.67082	0.974679	0.974679	1.24499	1.360147	1.5
M50%	0.67082	0.74162	0.866025	1.072381	1.204159	1.284523
M50%	0.74162	0.74162	0.974679	1.024695	1.24499	1.322876
M50%	0.591608	0.806226	0.974679	1.161895	1.24499	1.396424
M100%	0.223607	0.591608	0.866025	0.806226	0.974679	1.072381
M100%	0.387298	0.591608	0.921954	0.866025	1.118034	1.118034
M100%	0.223607	0.5	0.806226	0.974679	1.072381	1.204159
Fung	0.223607	0.223607	0.223607	0.223607	0.223607	0.223607
Fung	0.387298	0.223607	0.223607	0.223607	0.223607	0.223607
Fung	0.223607	0.223607	0.223607	0.223607	0.223607	0.223607

Index 1c: Statistical Analysis – Complete Randomized Design

Completely Randomized AOV for DAY1

Source	DF	SS	MS	F	P
TREAT	7	2.20825	0.31546	32.1	0.0000
Error	16	0.15711	0.00982		
Total	23	2.36536			

Grand Mean 0.5750 CV 17.23

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.10188
Effective cell size 3.0

Completely Randomized AOV for DAY2

Source	DF	SS	MS	F	P
TREAT	7	2.74235	0.39176	85.3	0.0000
Error	16	0.07347	0.00459		
Total	23	2.81582			

Grand Mean 0.6286 CV 10.78

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.12906
Effective cell size 3.0

Completely Randomized AOV for DAY3

Source	DF	SS	MS	F	P
TREAT	7	2.66917	0.38131	42.6	0.0000
Error	16	0.14325	0.00895		
Total	23	2.81242			

Grand Mean 0.8059 CV 11.74

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.12412
Effective cell size 3.0

Completely Randomized AOV for DAY4

Source	DF	SS	MS	F	P
TREAT	7	3.19883	0.45698	38.8	0.0000
Error	16	0.18840	0.01177		
Total	23	3.38723			

Grand Mean 0.8783 CV 12.36

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.14840
Effective cell size 3.0

Completely Randomized AOV for DAY5

Source	DF	SS	MS	F	P
TREAT	7	4.14926	0.59275	76.8	0.0000
Error	16	0.12350	0.00772		
Total	23	4.27276			

Grand Mean 0.9922 CV 8.85

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.19501
Effective cell size 3.0

Completely Randomized AOV for DAY6

Source	DF	SS	MS	F	P
TREAT	7	4.92195	0.70314	125	0.0000
Error	16	0.08998	0.00562		
Total	23	5.01194			

Grand Mean 1.1103 CV 6.75

At least one group variance is near zero,
variance-equality tests cannot be computed.

Component of variance for between groups 0.23250
Effective cell size 3.0

Index 1d: LSD

LSD All-Pair wise Comparisons Test of DAY1 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.1451	A
M25%	0.8382	B
H25%	0.7132	B
M50%	0.6680	B
H50%	0.4554	C
Fung	0.2782	D
M100%	0.2782	D
H100%	0.2236	D

Alpha 0.05 Standard Error for Comparison 0.0809
Critical T Value 2.120 Critical Value for Comparison 0.1715
There are 4 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY2 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.2037	A
M25%	1.0080	B
M50%	0.7632	C
H25%	0.7132	C
M100%	0.5611	D
H100%	0.2782	E
H50%	0.2782	E
Fung	0.2236	E

Alpha 0.05 Standard Error for Comparison 0.0553
Critical T Value 2.120 Critical Value for Comparison 0.1173
There are 5 groups (A, B, etc.) in which the means

are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY3 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.4200	A
M25%	1.0239	B
M50%	0.9385	B
M100%	0.8647	BC
H50%	0.7416	C
H25%	0.7396	C
H100%	0.4953	D
Fung	0.2236	E

Alpha	0.05	Standard Error for Comparison	0.0773
Critical T Value	2.120	Critical Value for Comparison	0.1638

There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY4 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.4433	A
M25%	1.2450	B
M50%	1.0863	B
M100%	0.8823	C
H25%	0.8608	C
H50%	0.7632	C
H100%	0.5217	D
Fung	0.2236	E

Alpha	0.05	Standard Error for Comparison	0.0886
Critical T Value	2.120	Critical Value for Comparison	0.1878

There are 5 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY5 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.6571	A
M25%	1.3843	B
M50%	1.2314	C
M100%	1.0550	D
H25%	0.8990	E
H50%	0.7831	EF
H100%	0.7042	F
Fung	0.2236	G

Alpha 0.05 Standard Error for Comparison 0.0717
Critical T Value 2.120 Critical Value for Comparison 0.1521
There are 7 groups (A, B, etc.) in which the means
are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY6 by TREAT

TREAT	Mean	Homogeneous Groups
Control	1.8388	A
M25%	1.4978	B
M50%	1.3346	C
M100%	1.1315	D
H25%	1.1292	D
H50%	0.8823	E
H100%	0.8448	E
Fung	0.2236	F

Alpha 0.05 Standard Error for Comparison 0.0612
Critical T Value 2.120 Critical Value for Comparison 0.1298
There are 6 groups (A, B, etc.) in which the means
are not significantly different from one another.