Sudan University of Science and Technology

College of Graduate Studies

Invitro Effect of Damas Plant Aqueous Extract and Fungicide on Growth of The *Fusarium Oxysporum* Causal Agent of wilt in Some Crops

تأثير المستخلصات المائية لنبات الدمس والمبيد الفطري على نمو الفطر فيوزاريم اوكسيسبورم المسبب لمرض الذبول في بعض المحاصيل خارج العائل

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

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الآية

قال تعالي:

الَّذِي أَنْزَلَ مِنَ السَّماءِ ماءً فَأَخْرَجْنا بِهِ نَباتَ كُلِّ شَيْءٍ
نا مِنْهُ خَضِراً نُخْرِجُ مِنْهُ حَبًّا مُتَراكِبًا وَمِنَ النَّخْلِ مِنْ
ها قِنْوانٌ دانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنابٍ وَالزَّيْتُونَ وَالرُّمَّانَ
وَغَيْرَ مُتَشابِهٍ انْظُرُوا إِلى ثَمَرِهِ إِذا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي
ذلِكُمْ لآياتٍ لِقَوْمٍ يُؤْمِنُونَ (99))

صدق الله العظيم

سورة الأنعام(99)

Dedication

To my mother

My father

My brothers and sisters

All my family

All my teachers

All my colleagues and friends

With love and respect.

Mawada

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

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Mawada

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Abstract

Fusarium oxysporium affects a wide variety host of different age Tomato, Tobacco, Legumes, Cucurbits. Sweet Potatoes, Chickpea and Banana. The present investigation was undertaken under laboratory of Plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology during November 2015 to February 2016, to study the effect of Damas plant parts (leaves, fruits, barks and roots) aqueous extracts and fungicide Score (250 EC) on growth of the fungus *Fusarium oxysporum causal* agent of wilt disease in crops. Three concentrations of aqueous leaves, fruits, barks and roots extract of Damas, each of 25, 50 and 100%, and fungicide were used in addition to control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth. The results showed that all concentration of the leaves fruits, barks and roots aqueous extracts Damas plant tested and fungicide of significantly inhibitory effect against the linear growth of *Fusarium oxysporium* compared to control. Moreover, concentration of each aqueous extract reacted differently against *Fusarium oxysporium*. However, the highest concentration of the Damas extracts (100%) gave significantly higher inhibition zones percent respectively (75.5%, 68%, 66%, and 50%) compared to the untreated control. Among the Damas parts extracts screened the fruit (75.5) was the most effective in suppressing the fungus growth than its equivalent other part. the results showed that the antifungal activity increase with increase in extract concentration. Obviously, the fungus *Fusarium oxysporium* differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration. The current results were considered promising and encouraging to carry out a photochemical analysis of different parts of Damas plant using different solvents so to determine the bioactive ingredient in each of these parts.

ملخص البحث

يوثر الفيوزيريوم اوكسسبوريوم علي اصناف متنوعه وواسعه في مختلف ألاعمار الطماطم التبغ البقوليات القرعيات البطاط الحمص والموز. أجريت هذه الدراسة تحت ظروف المختبر بقسم وقاية النبات, (معمل أمراض النبات) كلية الدراسات الزراعة , جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسة تأثير المستخلص المائي لأوراق وثمار وسيقان وجذور نبات الدمس والمبيد الفطري اسكور250EC على نموء فطر الفيوزاريم اوكسسبوريوم المسبب لمرض الذبول في المحاصيل. استخدمت ثلاثة تراكيز من المستخلص المائي لأوراق, وثمار وسيقان وجذور الدمس، كل50,25 و100%إضافةإلى الشاهد. تم تقيم الأثر التثبيطي لهذه التراكيز بتسجيل نموء الفطر. أوضحت النتائج إن كل تراكيز المستخلص المائي لأوراق وثمار وسيقان وجذور نبات الدمس والمبيد الفطري اسكور قد أظهر تأثير معنوي ضد فطر الفيوزاريم اوكسسبوريوم مقارنه بالشاهد. تراكيز المستخلص المائي و المبيد الفطري قد تفاعلت كل على حده ضد فطر الفيوزيريوم اوكسسبوريوم . التراكيز الاعلى 100 %في كل من المستخلص المائي و المبيد الفطري أعطت أعلى نسبة تثبيط مقارنه بالشاهد (75.5%, 68%, 66%,50%) على التوالي. فيما بين المستخلصات المائية المختبرة لنبات الدمس كان مستخلص ثمار الدمس(75.5)هو الأكثر فعالية في تثبيط نموء الفطر من بقيه الأجزاء. أظهرت النتائج أن الفعالية ضد الفطر تزداد بزيادة تركيزالمستخلصات. النتائج الحالية تعتبر واعده و مشجعه للقيام بتحاليل كيميائية لمختلف أجزاء نبات الدمس باستعمال مستخلصات مختلفة لتحديد المادة الفعالة المكونة في كل من هذه الأجزاء .

CHAPTER ONE

INTRODUCTION

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).

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 material, 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 crop, 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 objectives:-

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

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 residue and can be recovered from any part of the plant from the deepest root to the highest flower (Booth,1971andSummeral *et al*., 2003).

2.1.1Classification

Kingdom: Fungi
Division: Ascomycota
Class: Sordariomycetes
Order: Hypocreales
Family: Nectriaceae
Genus: Fusarium
Species:*Fusarium oxysporum*
*(*Snyder & Hansen, 1940)

The Ascomycota fungus Schaech as amended by (Snyder and Hansen,1940) comprises all the species , varieties and forms recognized by (Wollenweber and Reinking, 1935) with in an infra generic grouping called section Eleganns, while the species, as defined by Snyder and Hansen, has been widely accepted for more than 50 years,(Booth, 1971 and Nelson,1983). More recent work indicates this taxon is actually a genetically heterogeneous polytypic morph species (O'Donnell and Cigelnik 1997; Waalwijk, *et. al.*, 1996) whose strains represent some of the most abundant and widespread microbes of the global soil mycoflora (Gordon and Martyn, 1997). Although this last statement has not been proven or supported by actual data. These remarkably diverse and adaptable fungi have been found in soils ranging from

the Sonoran Desert, to tropical and temperate forests, grasslands and soils of the tundra. (Stoner, 1981).

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

2.1.2 Descriptions

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 chlamydospores 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 teleomorphs 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 mycoflora, (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 a*l., 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.oxysporium* 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 probes, 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 are fumonisin and trichothecenes

2.1.4Economic Importance

Fusarium oxysporum is significal problem in many crops. It is economically damaging too many industrial crops egg, 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).

2.1.5Host 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 the following hosts *Alliums cannabis* ,*Beta vulgaris, Cucumis sativa, Phaseolus vulgaris* and *Psumist sativum.*

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 (Teetlor, 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 Symptoms

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 (Agrios,2005).Plant infected with *Fusarium oxysporum* show symptom such as chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping off The most important of these 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 plant, symptoms are more distinct between the blossoming and fruit maturation stage (Nene et al., 1991;Agrios, 1988 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 *et al* ., 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 a*l., 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 rots, 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 close, the leaves with, and the plant eventually disease. At this point the fungus invades the plants parts until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios ,2005). The resulting spores can be used as new inoculums for further spread of the fungus.

2.2 Control

2.2.1Culture control

The culture control is the only practical measure for controlling the diseases in the field. The wilt fungus is so widespread and so persistent in soils that 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 greenhouse (Agrios ,2005).

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

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

2.2.2 Botanical controls

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

Jatrophamultifida, Azadirachta indicia, Allium sativum were significantly pronounced in reducing wilt incidence in Cicerarietinum L. Mycelial growth of various Fusarium species were inhibited by the plant extracts of Adhatodavasica, Azadirachta indica ,Cinnamomum camphora, and Ocimum sanctum (Prasad and Ojha,1986);Agave Americana, Cassia nadosa(Redd 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 Hair Chand, 2004) reported that Leaf extract of *Azadirachta indica* at 100/con completely inhibited germination of pathogen spores

2.2.3 Chemical control

Presently, (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. The use of methyl promide may be curtailed in near future and alternative chemicals are being examined.

2.3: Damas

Family Combretaceae comprises about 20 genera and about 600 species found in tropical and subtropical regions of the world. The family has few genera with great economic value, a useful timber is obtained from some species belong to it and other species has medicinal importance. Damas *Conocarpus lancifolius* Engle is one of the most important species in this family (Pandey and Misra, 2008).

2.3.1 Classification

Kingdom: Plantae

Phylum: Tracheophyta

Class : Magnoliopida

Order : Myrtales

Family : Combretaceae

S. N. : *Conocarpus lancifolius Engl.*

2.3.2. Uses of damas

Conocarpus lancifolius is multipurpose; wood which is the main product is used domestically for house construction, firewood and excellent charcoal. Commercially timber was more useful formerly; it was cut and exported from Somalia to Arabia for dhow construction. Other potential uses include wood based board. Bark may be a useful source of tannins (Booth and Wickens, 1993).

The tree is evergreen and its foliage makes a good fodder, also it is a good shade and roadside tree. It is used as wind breaks around irrigated agricultural areas and for avenue planting. A drought-resistant species, *C.lancifolius* is one of the more promising trees for trials in arid areas. It is recommended for a variety of soil types including saline soils, and yields excellent charcoal and valuable wood (NAS, 1983).

Information on the importance of *C. lancifolius* in its native distribution areas relative to other species with similar wood, fuel and forage uses is lacking hence it is difficult to assess its importance. However Somali tribes owing the Dames (Tugs) dry river valleys (wades) containing *C. lancifolius* have restricted cutting because of the threat of overexploitation (Booth and Wickens, 1993)

CHAPTER THREE

MATERIALS AND METHODS

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 November 2015 to February2016,to investigate the inhibitory effect of all parts of Damas (leaves, bark, fruit, and root) aqueous extracts and efficacy of fungicide, Score250 EC, against the fungus *Fusarium oxysporum Invitro.*

3.1 Collections of plant samples

Different parts of Damas (Fruits, leaves, barks and roots) were collected from trees growing in Elshair farm project. The parts collected were cleaned from dust and strange material by hand, washed with distilled water, surface sterilized with 1% Sodium Chloride, thoroughly washed in sterilized water and dried under shade at ambient temperature, ground and powdered separately to obtain fine powder for extraction and kept till use.

3.2Preparations.

3.2.1 Preparation of plant extract

All part of Dames (leaves, barks, fruits, roots) were collected from elshair farm project and brought to dry in shade. After complete dryness plant samples were crushed separately to obtain fine powder for extraction.

3.2.2 Preparation of inoculums

The pure cultures of Fusarium oxysporum were prepared using 7 days old mycelia. The fungi was culture on PDA then Trans ferried as, aseptically to the center of Petri dishes containing PDA medium and incubated at 25c the linear growth of the fungus was assessed in cm after 72h .

3.3. Aqueous extract preparation

Aqueous extracts of each of the plant materials were prepared as recommended by (Okigbo ,2006). The obtained fine powder form different parts of Damas was weighted (100 gm.) and added to it 100 ml sterilized distilled into conical flask 250 mland then placed in a shaker for 24 hrs. The extracts were filtered under reduced pressure as crude water extract with 100% and the other concentrations were obtained diluted to subsequently 50% 25% and kept in the refrigerator to serve as stock solutions.

3.3.1: preparation of fungicide

The fungicide tested was Score of which 2ml were dissolved in 1000ml of sterilized distilled water to give 100 ppm.

The effect of each extracts was calculated as percentage of reduction in diameter of fungal growth (R) where: -

$$R = \frac{dc - dt}{dc} \times 100$$

Where R = Percentage reduction of the growth, dc= diameter of controlled growth and dt= diameter of treated growth

3.4. Effect of different parts of Damas extract on the linear growth of the Fusarium oxysporum invitro

The PDA media was amended with the required concentration from all part of Damas and fungicide score(25ml, 50 and 100ml from each) before being solidified in a conical flask of 250 ml, agitated and poured it into sterilized Petri dishes. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

The Petri dishes of each concentration were incubated at 25 C^0 for 5 days. The growth Diameter of the fungus was measured and calculated by centimeter after 3, 4 and 5 days after inoculation.

3.5. Experimental design

The treatments were arranged in a Complete Randomized Block Design.

3.6. Statistical analyses

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

CHAPTER FOUR

RESULT

This study which conducted under laboratory condition of jica, College of Agricultural Studies, Sudan University of science and Technology during the period novomber2015 to February 2016 to investigate the inhibitory effect of all parts of Damas (leaves, barks, fruits, roots) aqueous extracts and fungicide, score 250 EC efficiencies against the growth of fungus *Fusarium oxysporum*.

4.1 Isolation and Identification from the infected sample:

Isolation and identification of the fungus was based on the method of (Booth, 1977) and on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these funguses (Aneja, 2004).

4.2. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum invitro* after three days from inoculation.

The results (Table 1, Table 2, and Figure 1) showed that all part of Damas (leaves, fruits, bark, roots) aqueous extracts screened and fungicide had effects on the fungal growth after three days from inoculation. Furthermore, the fungal growth inhibition was significantly high compared to the control.

Moreover, the highest concentration of the plant extracts (100%) gave significantly higher inhibition compared to the untreated control which gave(75.5%, 68%, 66%, and 50%). Among the parts of Damas extracts tested fruit was the most effective in suppressing the fungus growth than the other parts of Damas respectively which gave(75.5)in(Table 1, Table 2),the results showed that the antifungal activity increase with increasing of extract concentration.

Table 1: Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after three days from inoculation

		Growth			
Treatments	Cons.	R1	R2	R3	Mean
Leaves	25	4(2.1)	4.1(2.1)	4(2.1)	4.033(2.1)ab
	50	3.9(2.1)	4(2.1)	3.9(2.1)	3.93(2.1)ab
	100	2.5(1.7)	2.7(1.8)	3.1(1.9)	2.76(1.8)bc
Fruit	25	4(2.1)	4.1(2.1)	3.9(2.09)	4(2.09)ab
	50	2.05(1.6)	1.9(1.5)	2.2(1.6)	2.05(1.6(cd
	100	1.4(1.4)	1.2(1.3)	1.6(1.4)	1.4(1.36)d
Bark	25	3.5(2)	3.7(2)	3.3(1.7)	3.5(1.9)bc
	50	3(1.9)	2.5(1.7)	2.8(2)	2.76(1.8)bc
	100	1.6(1.4)	2(1.6)	1.7(1.4)	1.76(1.46)d
Root	25	3(1.9)	3.5(2)	3.7(2)	3.4(1.96)abc
	50	2.2(1.6)	2.6(1.8)	2.1(1.6)	2.3(1.66)cd
	100	1.7(1.4)	2.3(1.8)	2(1.6)	2(1.6)cd
Fungicide		0.3(0.9)	0(0.7)	0.4(0.7)	0.233(0.76)e
Control		5(2.3)	4.9(2.3)	5(2.3)	4.96(2.3)a
C.V				11.74	
SE				0.06	

Means in the same column with the same letter (s) are not significant at P=0.05 according to DRMT. Values between brackets were transformed to $\sqrt{x+0.5}$.

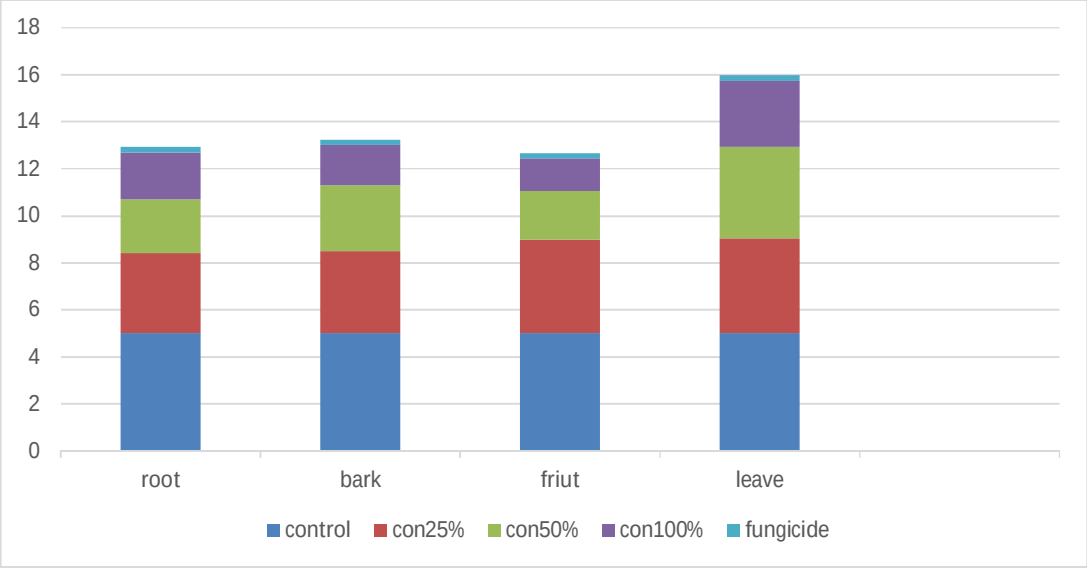

Figure 1. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after three days from inoculation

Table 2. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after five days from inoculation

Treatments	Cons.	Inhibition zone			
		R1	R2	R3	Mean
Leave	25	20.00	16.30	20.00	18.30
	50	22.00	18.30	22.00	20.70
	100	50.00	44.00	38.00	44.00
Fruit	25	20.00	16.30	22.00	19.40
	50	59.00	61.20	56.00	58.70
	100	72.00	75.50	68.00	71.80
Bark	25	30.00	24.40	34.00	29.50
	50	40.00	48.90	44.00	44.30
	100	68.00	59.18	66.00	64.40
Root	25	40.00	28.50	26.00	31.50
	50	56.00	49.00	58.00	54.30
	100	60.00	59.18	66.00	61.10
Fungicide		94.00	100.0	92.00	95.30
Control		00.00	00.00	00.00	00.00

4.3. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum invitro* after four days from inoculation

In four day after inoculation, all parts of Damas plants tested concentrations as well as that of the fungicide were invariably continued exhibiting inhibitory effects against the fungal growth. However, the highest concentration of the plant extracts (100%) gave the highest inhibition zones percent (68.57%, 42.85%,57.14 and 55.71) respectively. This inhibitory effect from all concentrations tested was significantly different from control (Table3, Table4 and Fig. 2).

Furthermore, the part fruit of Damas plant extract at all concentrations tested continued to be the most suppressive, followed in descending order by the other parts.

Table 3. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after four days from inoculation

		Growth			
Treatments	Cons.	R1	R2	R3	Mean
Leave	25	5.75(2.5)	5.9(2.6)	5.75(2.5)	5.8(2.5)ab
	50	5.25(2.4)	5.7(2.5)	5.25(2.4)	5.4(2.4)bc
	100	3.75(2.1)	4(2.1)	4.1(2.1)	3.95(2.1)ef
Fruit	25	4.5(2.2)	4.35(2.2)	4.5(2.2)	4.45(2.2)de
	50	2.9(1.8)	2.5(1.7)	2.9(1.8)	2.8(1.8)gh
	100	2.4(1.7)	2.2(1.6)	2.3(1.6)	2.3(1.63)h
Bark	25	5.8(2.5)	5.6(2.4)	5(2.3)	5.46(2.4)bc
	50	4.6(2.3)	4(2.1)	4.7(2.5)	4.43(2.3)cd
	100	3.0(1.9)	3.5(2)	3.3(1.9)	3.26(1.9)fg
Root	25	5.5(2.4)	5.7(2.5)	5.45(2.3)	5.6(2.4)bc
	50	3.5(2)	3.8(2)	3.9(2.1)	3.7(2.0)ef
	100	3.1(1.9)	3.5(2)	3.5(2)	3.4(1.9)f
Fungicide		0.9(1.1)	0(0.7)	0.8(1.1)	0.56(0.96)i
Control		7.0(2.7)	7.0(2.7)	7.0(2.7)	7.0(2.7)a
C.V				4.71	
SE				0.07	

Means in the same column with the same letter (s) are not significant at P=0.05 according to DRMT. Values between brackets were transformed to $\sqrt{x+0.5}$

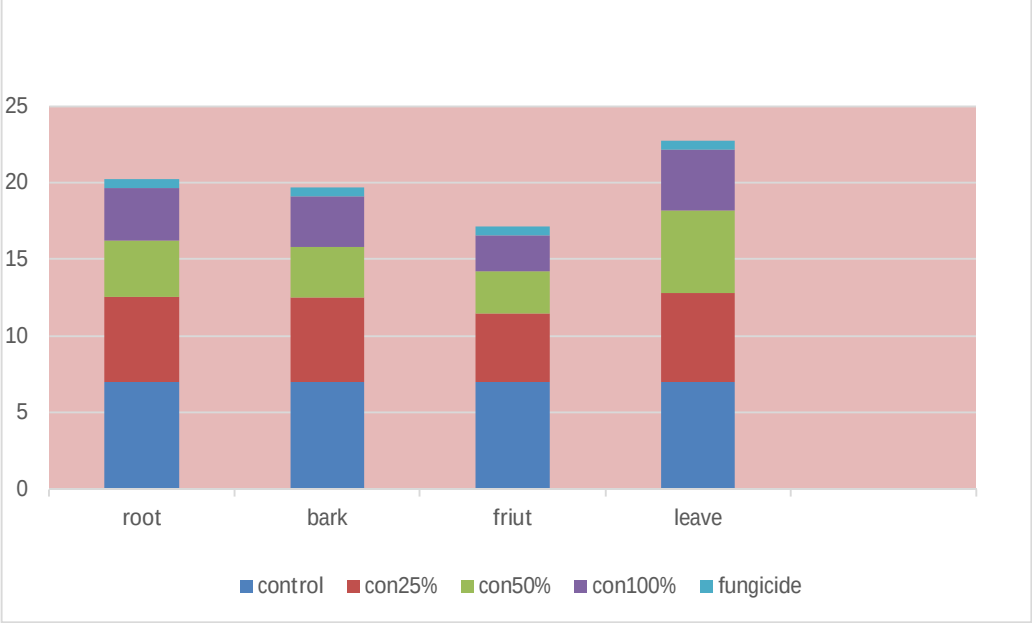

Figure 2. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after four days from inoculation

Table 4. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after four days from inoculation

		Inhibition zone			
Treatments	Cons.	R1	R2	R3	Mean
Leave	25	17.85	15.71	17.85	17.14
	50	25.00	18.5	25.00	22.83
	100	46.42	42.85	41.42	43.56
Fruit	25	35.7	37.8	35.7	36.40
	50	58.5	64.28	58.57	60.45
	100	65.71	68.57	68.24	67.50
Bark	25	17.14	20.00	28.57	21.90
	50	34.28	42.85	32.85	36.66
	100	57.14	50.00	52.28	53.14
Root	25	21.42	18.57	22.41	20.80
	50	50.00	45.71	44.28	46.66
	100	55.71	50.00	50.00	51.90
Fungicide		87.14	100.0	88.57	91.90
Control		00.00	00.00	00.00	00.00

4.4. Effect of different parts of Damas plant aqueous extracts and 4 on the linear growth *of Fusarium oxysporum invitro* after five days from inoculation

Infive day from inoculation, the results (Table5, Table6 and Figure, 3) showed that extracts of all parts of Damas plants tested proved to be effective in suppressing the fungal growth.

In fact, all tested concentrations of all parts of Damas(100, 50 and 25%) induced significantly higher inhibition against test fungus compared to control which give (63.75, 43.75,59.37 and 58.12 percent). Meanwhile, the fruit aqueous extract at high concentrations tested exhibited consistently more inhibitory effect than the other parts of Damas plant aqueous extracts which give (63.75).

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

Table 5. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after five days from inoculation

Treatments	Cons.	Growth			
R1			R2	R3	Mean
Leave	25	6.15(2.6)	6.1(2.6)	6.2(2.6)	6.15(2.6)b
	50	5.56(2.5)	5.9(2.5)	5.8(2.5)	5.8(2.5)bc
	100	4.35(2.2)	4.5(2.2)	4.7(2.3)	4.51(2.2)d
Fruit	25	4.9(2.3)	4.9(2.3)	5.3(2.4)	5.0(2.3)cd
	50	3.7(2.05)	3.5(2)	3.35(2)	3.5(2.02)cd
	100	3(1.87)	2.9(1.8)	3.3(1.9)	3.06(1.85)f
Bark	25	6.65(2.6)	6.1(2.6)	6.4(2.6)	6.21(2.6)b
	50	6(2.5)	6(2.5)	5.7(2.5)	5.9(2.5)bc
	100	3.35(1.9)	4(2.1)	3.9(2.09)	3.71(2.03)ef
Root	25	5.7(2.5)	6.25(2.6)	6.1(2.6)	6.016(2.56)b
	50	3.9(2.1)	4.5(2.2)	5(2.3)	4.46(2.2)de
	100	3.35(2)	4(2.1)	3.7(2)	3.68(2.03)ef
Fungicide		1.4(1.4)	0(0.7	0.8(1.1)	0.73(1.06)g
Control		8(2.9)	8(2.9)	8(2.9)	8(2.9)a
C.V					4.80
SE					0.07

- Means in the same column with the same letter (s) are not significant at P=0.05 according to DRMT.
- Values between brackets were transformed to $\sqrt{x+0.5}$.

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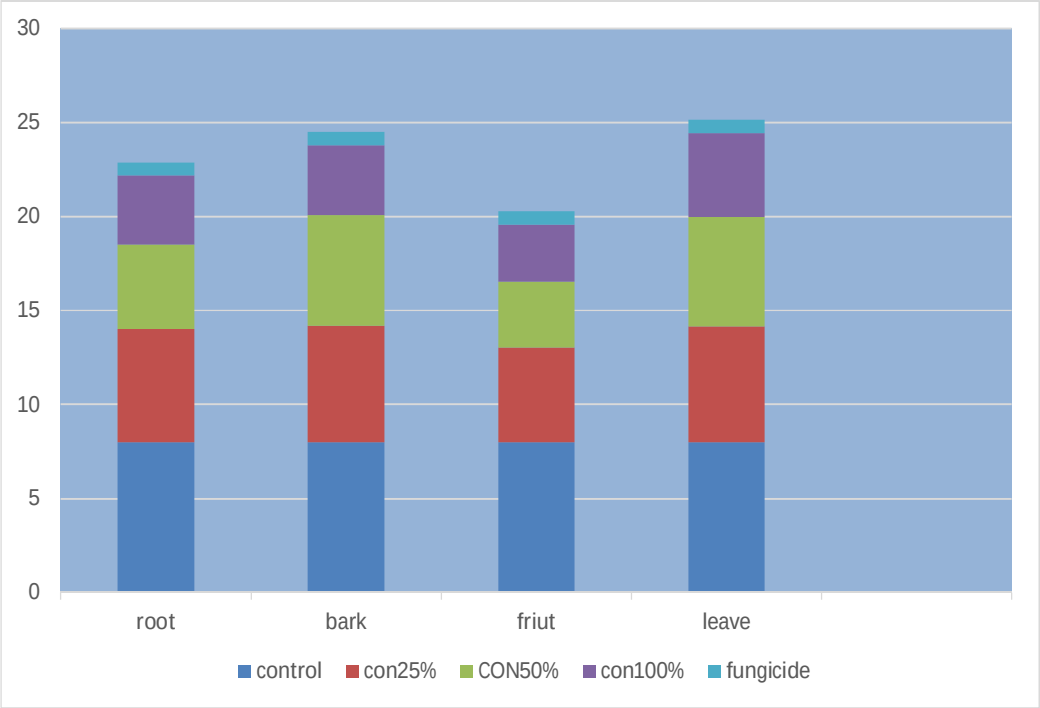

Figure 3. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after five days from inoculation

Table 6. Effect of different parts of Damas plant aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum in vitro* after four days from inoculation

	Inhibition zone				
Treatments	Cons.	R1	R2	R3	Mean
Leave	25	23.12	23.75	22.50	23.12
	50	29.37	26.25	27.50	27.70
	100	45.62	43.75	41.25	43.54
Fruit	25	38.75	38.75	33.75	37.08
	50	53.75	56.25	58.12	56.04
	100	62.50	63.75	58.12	61.45
Bark	25	33.25	23.75	20.00	25.66
	50	25.00	25.00	28.75	26.25
	100	59.37	50.00	51.25	53.54
Root	25	28.75	21.87	23.75	24.79
	50	51.25	43.75	37.50	44.16
	100	58.12	50.00	53.75	53.95
Fungicide		82.50	100.0	90.00	90.83
Control		0.000	0.000	0.000	00.00

CHABTER FIVE

DISCUSSION

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 the following hosts *Alliums cannabis*, *Beta vulgaris, Cucumis sativa, Phaseolus vulgaris* and *Psumist sativum*.

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).

In Sudan, several diseases are known to limit production of crop, 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 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).

Numerous research findings have presented a number of strategies to control this fungal pathogen (Haware and Nene, 1982; Jiménez-Díaz*, et al*., 1993). However, management of seed-borne and soil-borne diseases such as wilt caused by *Fusarium oxysporum* has always been problematic (Rao and Balachadran, 2002; Haware and Kannaiyan, 1992). 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 in crops which offers an alternative to fungicides is highly demanding.

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 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 (Kelang, 2001).

In this study, the Damas plant different parts aqueous extracts were investigated for their bioactivity against Fusarium wilt. The data (Tables 1-3 and Figures 1-3) revealed that all Damas plant parts (leaves, fruit, bark, and root) aqueous extracts screened consistently exhibited an inhibitory effect on fungal growth with significantly high inhibition zones percent. This findinds is in agreement with Satish*et.al*., (1999); Okigbo and Ogbonnaya(2006); Shariff *et a*l., (2006); Ergene*et.al*.,(2006); Kiran and Raveesha(2006) and Mohana and Raveesha(2006) who explored the effect of extracts of many higher plants and reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials. More recent results were also demonstrated by Saad *et al.,* (2014) where they demonstrated the antibacterial and antifungal activities of the methanol extract of Damas aerial parts using disk diffusion method. Similar results were also obtained by Ahmed (2014) who studied the Alkaloid extract of *Conocarpus lancifolius* Engl. against some Clinical Pathogens.

Conclusions

- The leaves, fruit, bark and root aqueous extracts of Damas plant tested exhibited an inhibitory effect on fungal growth. *Fusarium oxysporum* this component plus fungicide (score) could be applied as part of an integrated approach to control Fusarium wilt.
- Among the Damas plant parts aqueous extract in high concentration exhibited inhibitory effect than the others.
- The screened concentrations of all part of Damas aqueous extracts differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of extracts. This variability in response which expressed by test organism may be used to adjust an optimum dose for controlling Fusarium wilt.

Recommendations:

Based on the foregoing results the following studies were recommended;

- To further investigate the antimicrobial properties in a group of medicinal plants against targets organism to determine their potentials as botanical pesticides,
- To carry out a photochemical analysis of different parts of Damas plant using different solvents so as to determine the bioactive ingredient in each of these parts.

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APPENDIXS

Appendix 1

Table 1: Analysis of variance table (One way ANOVA table):

Variable (3day)

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	13	6.050	0.465	10.858	0.0000
Within	28	1.200	0.043		
Total	41	7.250			

Coefficient of Variation = 11.74%

Appendix 2

Table 2: Analysis of variance table (One way ANOVA table):

Variable (4day)

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	13	7.656	0.589	60.332	0.0000
Within	28	0.273	0.010		
Total	41	7.930			

Coefficient of Variation = 4.71%

Appendix 3

Table 3. Analysis of variance table (One way ANOVA table).

Variable (5day)

A N A L Y S I S O F V A R I A N C E T A B L E

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	13	7.821	0.602	51.725	0.0000
Within	28	0.326	0.012		
Total	41	8.147			

Coefficient of Variation = 4.80%

r3. The materials and equipment used in this study are listed below.

3.1 Equipments.

Needle	laminar
Petri dishes (9cm)	Autoclave
Conical flasks	Incubator
Desiring cylinder	Carbora
Sensitive balance	Centrifuge
Gloves	Camera
Marker pen	Medical cotton

3.2 Materials.

Potato dextrose agar

fungicide 250 EC

Damas Leaves, fruit, bark, root

Distilled water