Sudan University of Science and Technology

College of Graduate Studies

Effect of Some plant extract and Chemical fungicides against *(Fusarium oxysporum f.sp. Lycopersici*) as causal agent Fusarium wilt of Tomato

تأثير بعض المستخلصات النباتية والمبيد الفطرى الكيميائي على الفطر *(Fusarium oxysporum f.sp. Lycopersici*) المسبب لمرض الذبولالفيوزارمى فىالطماطم

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

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الآية

قال تعالي:

َ السَّماءِ ماءً فَأَخْرَجْنا بِهِ نَباتَ كُلِّ شَيْءٍ فَأَخْرَجْنا
ُ حَبًّا مُتَراكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِها قِنْوانٌ دانِيَةٌ
وَجَنَّاتٍ مِنْ أَعْنابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشابِهٍ انْظُرُوا إِلى ثَمَرِهِ
إِنَّ فِي ذلِكُمْ لَآياتٍ لِقَوْمٍ يُؤْمِنُونَ (99))

صدق الله العظيم

سورة الأنعام)(99

Dedication

To my mother

To the soul of 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.

Anour

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

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Alnour

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ABSTRAC

Fusarium wilt of tomato is considered as one of the most important diseases of this crop worldwide. The present investigation was undertaken under laboratory of Plant protection Department,College of Agricultural Studies, Sudan University of Science and Technology,to study the effect of aqueous leaves extracts of Damas, Neem plants and fungicide Tilt (250 EC) on growth, of the fungi*, Fusarium oxysporum f. sp. Lycopersici* caused wilt in tomato.

Three concentration of aqueous leaves extract of damas and Neem, each of 5, 10 and 15%, and fungicide at three concentration, 5, 10, and 15% were used in addition to control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth inhibition percentage.

The results showed that all concentration of the leaves aqueous extracts of all plants tested and fungicide of significantly high inhibitory effect against the linear growth of test fungus compared to control. Moreover, concentration of each aqueous extract as well as that of fungicide reacted differently against test fungus. However, the effect of

both leaves extracts was more pronounced against test fungus than that of fungicide.

The highest concentration of the plant extracts (15%) and Tilt (15%) gave significantly higher inhibition zones percent (100%, 100%, 86.1%, and 81.1%) respectively compared to the untreated control. Among the plant extracts tested that of Damas was invariably the most effective in suppressing the fungus growth than its equivalent Neem. Generally, the results showed that the antifungal activity increase with increase in extractconcentration. Obviously, the test fungus 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 phytochemicals analysis of different parts of Damas plant using different solvents so to determine the bioactive ingredient in each of these parts.

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ملخص البحث

يعتبر مرض الذبول الفيوزيريومى فى الطماطم من اهم امراض هذا المحصول فى العالم.

اجريت هذه الدراسه فى تحت ظزوف المختبز بقسم وقاية النبات ,(معمل امراض النبات) كلية الدراسات الزراعه , جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسه تاثير المستخلص المائى لاوراق نباتات الدمس و النيم و المبيد الفطرى تلت 250EC على نموا فطر فيوواريم اوكسيسبوريم المسبب لمرض الذبول فى الطماطم.

استخدمت ثلاثه تراكيز من المستخلص المائى لأوراق الدمس و النيم ، كل (5,10,15%) وكذلك ثلاثه تراكيو من المبيد الفطرى تلت (5,10 and 15%) أضافة الى الشاهد. تم تقيم الاثر التثبيطى هذه التراكيز بتسجيل نسبه تثبيط نمؤ الفطر.

اوضحت النتائج ان كل تراكيز المستخلص المائى لاوراق النباتات المختبرة و المبيد الفطرى قد اظهرت تاثير معنوى ضد الفطر المختبر مقارنه بالشاهد. تراكيز المستخلص المائى و المبيد الفطرى قد تفاعلت كل على جده ضد الفطر المختبر مع تاثير واضح للمستخلصات المئيه من المبيد الفطرى.

التراكيز الاعلى (10,15%) فى كل من المستخلصات المائى و المبيد الفطرى اعطت اعلى نسبة تثبيط مقارنه بالشاهد (100%, 100%, 86.1%,81.1%) على التوالى . فيما بين المستخلصات المائيه المختبرة كان مستخلص الدمس دائما الاكثر فعاليه فى تثبيط نمؤ الفطر من مستحلص النيم .

عموماً اظهرت النتائج ان الفعاليه ضد الفطر تذداد بزيادة تركيزالمستخلصات . النتائج الحاليه تعتبر وعده و مشجعه للقيام بتحاليل كيميائية لمختلف اجزاء نبات الدمس باستعمال مستخلصات مختلفة لتحديد الماده الفعاله المكونه فى كل من هذه الاجزاء .

CHAPTER ONE

Introduction

Tomato (*Lycopesriconesculentum* Mill.) is member of the family solanaceae that includes also other cultivated crops such as potato, pepper, eggplant, tobacco etc… The origin of tomato is believed to be central and south America, especially Mexico, from where the crop was transferred to Europe in the 16th century , then to old world continents (Hedrick , 1919 and Rick , 1976). Tomato is an important food and cash crop for the majority of the low income farmers in the tropics (Prioret,*et al*, 1994). In fact it is considered as one of the most important and popular vegetable in many countries.

In the Sudan, the tomato is considered as one of the major vegetable crops and widely used in the processed forms as paste, ketchup, sauce and dry tomato slices. The crop presents one of the main cash vegetable crops in central and Northern Sudan. Tomato is growing successfully almost in every part of the Sudan during the winter season and in close system farming (FAO, 1999). The main producing areas of tomato (*L. esculentum* Mill.) are Central and North Sudan. Tomato is also produced in gable Mara and some parts of the main rain fed areas around villages in central clay plains and utilized as sun dried slices. Summer production of tomato which ensures high profitability because of the scarcity of the crop at that time is practiced in limited areas in Blue and White Nile and Khartoum state. The crop is recently produced under controlled greenhouses during summer season and this practice is extending rapidly every year. (FAO, 1999)

Although a number of pests and diseases (e.g. wilt diseases) are considered one of the main limiting factors for its growing, the production of tomato

was developed rapidly since 19th century. According to FAO (2005/2006) the area under tomato production has increased rapidly from 1300 hectares during 1999 to 43453 hectares in 2005/2006.

The major constraints facing the production of tomato worldwide are the losses caused by diseases, insects, nematodes and parasitic weeds. Among these, the most important are fungi, affecting roots, stems, leaves, flowers, and pods. The threat to plants from fungal infections has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, R.D. 1977). One of the main fungal pathogens that attack tomato is*Fusariumoxysporum* f.sp.Lycopersici causing Fusarium wilt disease that affects tomato production (Hanaa *et al.,* 2011).

In Sudan, several diseases are known to limit production of tomato, one of which Fusarium wilt caused by (*Fusariumoxysporum*f.sp. Lycopersici)is one of the most important (Bhatia *et al.,* 2004*)*. It is reported that the disease is especially serious in the traditional production areas where tomato is grown on stored soil moisture after the flood waters of the Nile River subside. In these areas.

Considering the nature of damage and survival ability of the fungus which can survive in soil up to six years in the absence of susceptible host (Haware et al., 1978 and 1986), Fusarium wilt of tomato has been managed primarily by the use of resistant varieties (Jalali and Chand, 1992). However, breakdown in resistance of these varieties due to evolution of virulent races of the pathogen have undermined their importance in recent years (Haware and Nene, 1982 and Jiménez-Díaz, et al., 1993).In Sudan tomato is becoming increasingly important for local consumption and for export. It is cultivated throughout the year under irrigation in an area that exceeds 36540 hectares with an average yield of 17.57 tons per hectare (AOAD*,* 2007). Similarly, the cultivated tomatoes

suffer greatly from *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *Lycopersici*. In fact, Fusarium wilt is one of the major yield limiting factors of tomato production in Sudan (*Bhatia et al., 2004)*. It is reported that the disease is especially serious in the traditional production areas where tomato 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)

(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*.

Presently, Anon (1994) and Ristaino *et al.,* (1997)reported that methyl bromide fumigation is used extensively for tomato production in some geographical areas in addition to reducing or eliminating soil borne diseases like Fusarium wilt . Fumigation allows more rapid transplant growth allowing for earlier harvesting and optimizes fresh markets. The use of methyl promide may be curtailed in near future and alternative chemicals are being examined.

Based on the foregoing, this study was undertaken to focus on investigation of two components for management of Fusarium wilt of Tomato caused by *Fusarium oxysporum*f.sp. Lycopersici, 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* f.sp. Lycopersici.
- To evaluate the effect of systemic fungicide on fungal growth
- To develop promising disease management components against Fusarium wilt of tomato
- Investigate some control measure. Chemical plant extract.
- Isolation and Identification of the agent.

CHAPTER TWO

LITEREATURE REVIE

2.1. Tomato plant

Tomato, (*Lycopersicon esculentum* Mill.), which belongs to family Solanaceae is one of the most popular and widely consumed vegetable grown worldwide. The tomato crop (*Lycopersicon esculentum*) originated in tropical central South America it was domesticated in Mexico and later taken to Europe (Rick, 1978). In many countries the tomato is very popular vegetable. This is because of it acceptable flavor nutritive, to fruit in a wide range of environments and the relative ease with which it can be cultivated. The production of tomato developed rapidly 19th century (Rick, 1978). The means and method of tomato production have largely changed from a hand –cultivated crop to one, which can be full mechanized.

The popularity of the crop stems from its acceptable flavors, nutritive value (high vitamin C and A), the short cycle life and high productivity (Abdelmageed *et al.*, 2003).Tomato is the major vegetable crop grown worldwide, with a production estimate of 95 million Mt *(Faostat, 2002)* and its production is concentrated in semi-arid regions (Santa-Curz *et al*., 2002).Presently, tomato is becoming increasingly important in Sudan, forlocal consumption as well as for export. It is cultivated throughout the year under irrigation in an area that exceeds 36540 hectareswithanaverageyieldof 17.57 tons per hectare (Aoad, 2007).The most important grown cultivars are the canning types such as Strain B,

Strain C, Peto86, Peto111 andCastleRock in addition to few localvarieties.

In the Sudan, tomato ranks second to onion among vegetable crops based on cultivated area. It is grown by holders who employ relatively poor management practices (Abdelmageed*et al.,* 2003) Tomato combined with peanut butter dominating the food table of most of the poor families in Sudan.

Relative to phytonutrient, the most abundant in tomatoes are the carotenoids. The antioxidant activity of Lycopene as well as several other carotenoids and their abundance in tomatoes make the crop source of antioxidant activity (Beecher, 1998).

2.1.1. Classification

- Kingdom plant
- Sub kingdom tracheobionia
- Division Magnoliopida
- Sub class Asterielae
- Order Solanacaea
- Genus Lycopesricon
- Species *Esculentum* (Mill)

2:1:2. Economic importance of tomato

The importance of tomato, both as vegetable food and cash crop cannot be over-emphasized. It is a vegetable crop of considerable economic importance in tropical and subtropical countries where high yields of tomato result in high incomes to farmers when it is cultivated on large scale (Thompson and Kelly, 1957). For its nutritional values, analysis shows that fresh (ripe) tomato contains; 13mg Ca; 27mg P; 0.5mg Fe; 3mg Na; 244mg K; 900 (I.U) of Vitamin A; 0.6mg Thiamine; 0.4mg Riboflavin; 0.7mg Niacin; and 233mg Ascorbic acid (Nonnecke, 1989).The tomato plant is versatile and the crop can be divided in to two categories ;(1) fresh market tomato (2) processing tomatoes. Tomatoes are good sourcing of vitamins (A and C) a fact that is becoming more important in modern diets.

2.1.3 Fungal diseases of tomatoes

Plant diseases constitute a major constraint to crop production often resulting in a great degree of crop losses which may range from slight to 100% (Agrios, 1969).In Sudan, cultivated tomatoes suffer from many fungal diseases such as are Fusarium wilt (*Fusariumoxysporum f. sp. lycopersici*), Verticillium wilts (*Verticillium dahliae*), powdery mildews (*Leveilula taurica*) and early and late blights, which are caused by *Alternariasolani*/*alternata* and *Phytophthorainfestans*, respectively.In fact, Fusariumwilt disease is considered one of the major agents of yield reduction of the crop (Awad, 1990 and Stone *et al.,* 2000).

2.2 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 1971; Summeral *et al*. 2003). Fusarium wilt of tomato (*Lycopersicon esculentum) c*aused by *Fusarium oxysporum f. sp. lycopersici* is a disease that causes serious economic loss (Agrios 2005). The fungus causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele (Bowers and Locke 2002).

Fusarium wilt of tomato is most severe where the crop is grown at relatively high temperature or when seasons are hot and dry (Rick, 1983). The disease is characterized by yellowing and dying of the tomato leaves progressively from the upward, and by the discoloration of the vascular tissue.

2.2.1Classification.

Kingdom: Fungi

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: Fusarium

Species:***Fusarium oxysporum f.sp. Lycopersici***

*(*Snyder & Hansen*,* 1940)

The Ascomycota fungus Schaechas emended by (Snyder and Hansen1940) 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 morphospecies(O'Donnell,and Cigelnik 1997, Waalwijk, et al, 1996) whose strains represent some of the most abundant and widespread microbes of the global soil micoflora.(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, T.R.1981).

Fusariumoxysporum 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 endophytes 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 Lemanceau1993).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.

2.2.2 Descriptions

Fusariumoxysporum is a common soil inhabitant. Booth (1977) stated that *F. Oxysporum. f.sp. lycopersici* has a colorless mycelium at first but with age it becomes creamy in colors pale yellow, pale pink or purplish. These colors give the characteristic culture pigment within the plant. Khune and patil (1992) 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 afoot shaped cell. They are generally producedon 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 phialides 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 inoculum 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).

2.2.3 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.oxysporium* 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, andJacobson (1989), Katan (1971).and may even protect plants or be basis of disease suppression .Larkin*, et a*l (1993), Lemanceau*, etal* (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 harmlesssaprobes, 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

2.2.4Economic Importance.

*Fusariumoxysporum*is significal problem in many crops. It is economically damaging to many industrial crops e,g, banana industryThe threat of more virulent strains or mutants that damage previously resistant crops is of major concern.(Dreistadt, S.H. and Clark, J.K. 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, E.H.2013)

Fusariumoxysporum 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).

2.2.5 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 the following hosts Allium cannabis. Beta vulgaris, Cucumis sativa, Phaseolus vulgaris and Psumi stativum.

Fusarium oxysporum is one of the major causal agents of wilt disease (Nene *et al*; 1991). The disease is prevalent in most Tomato growing countries and is a major disease. It is seed and soil borne disease .The fungal pathogen *F.oxysporium* 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).

2:2:6 Pathogenic.

*F.oxysporium*has been studied for more than 100 years. Host range of these fungi is extremely broad, and includes animals, ranging from arthropod. (Teetlor, 1983).Tohuman, (Nelson, 1994) as well as plant, including range of both gymnosperms and angiosperms. Whilecollectively, 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, H.C. 2001).

2.2.7Casual organism.

Fusarium wilt it is wide spread and most sever where tomatoes are grown at relatively high temperature or when seasons are hot and dry (Rick, 1983.). The diseases caused by fungus *Fusarium oxysporum*, and *Fusarium sp*,is one of the most prevalent tomato .the disease is characterized by yellowing and dying of the tomato leaves progressively from the upward, and by the discoloration of the vascular tissue.

2.2.8 Symptom.

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 , witheringof plant and finally death (Westerlund *et al*.,1974;Prasad and Padwich ,*et al* ., 1939).

Causes vascular wilt in tomato. The disease starts out as yellowing and drooping on one side of the plant. Leaf wilting, plant stunting, browning of the vascular system, leaf death, and lack of fruit production also occur (Snyder and Hans.2004)

2.2.9 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 crownof theplant. 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 with, and the plant eventually disease. At this point the fungus invades the plants parechymatous 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.

2.3 Control

2.3.1Culture control

The culture control is the only practical measure for controlling the diseases in the field. Thewilt 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 applicationbut it should be always practiced for greenhouse grown tomato plant (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). Most Fusarium wilts have diseases cycles and develop similar to those of the Fusarium wilt of tomato.

2.3.2 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 diseasesdevelopment, 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(Redd and Reddy,1987*); Azadirachta indicia* (Eswaramoothy *et a*l ., 1989);*Azadirachta indica, Atropha belladonna,Calotropis procera,*

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

2.3.3Chemical control.

Presently,Anon (1994) and Ristaino *et al.,*(1997)reported that methyl bromide fumigation is used extensively for tomato production in some geographical areas in addition to reducing or eliminating soil borne diseases like Fusarium wilt . Fumigation allows more rapid transplant growth allowing for earlier harvesting and optimizes fresh markets. The use of methyl promide may be curtailed in near future and alternative chemicals are being examined.

2.4 Neem Tree.

Neem is versatile tree, it is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management, environment protection and medicine. Also it has showing great promise as potential fertilizer (Abdalla, 2010).

2.4.1 Taxonomy.

Kingdom: Plantae

Division: Magnoliophyta

Order: Rutales

Suborder: Rutinease

Family: Meliaceae

Genus: Azadirachta

Species: Azadirachta indica

S.N: *Azadirachta indica* A.Juss

E.N: Neem

A.N: نيم

(Vietmeyer, 1992, and Schmutterer, 2002)

Plate .1:Neem leaves

2.4.2 Uses of Neem in pest and disease control.

Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated and is recommended for those who are sensitive to Permethrin. A known insecticide which might be irritants and also the scabies mite has yet to become resistant to Neem, so in persistent cases Neem has been shown to be very effective, there is also anecdotal evidence of its effectiveness. In treating infestations of head lice in humans, it is also very good for treating worms (soak the branches and leaves in lukewarm water and drink it). In the traditional medicine Neem trees originated on the Indian subcontinent. The Neem twig is nature's tooth brush to over 500 million people daily in India alone. Herbal medicine is the oldest form of therapy practiced to be mankind and much of the oldest medicinal use of plants seems to have been based on highly developed 'dowsing instinct' (Grigs, 1981). Siddig (1993) reported from Sudan that Neem seed water extracts at1Kg/1Liter of water repelled foliage pest of potato including *B. tabaci*, *Aphis gossypii* and *J. lybica* and yield increased to 5 ton/ ha. Mohammed (2002) reported that Neem seed showed good performance against *A. gossypii*, *B. tabaci*, and*J. lybica* on Okra. Dawood (2001) reported that Neem water extracs at 1Kg/liter water reduced the number of onion thrips at 63.5% under the field condition.

2.5Damas

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).

2.5.1 Classification

Kingdom: Plantae

Phylum: Tracheophyta

Class : Magnoliopida

Order : Myrtales

Family : Combretaceae

S. N. : *Conocarpus lancifolius* Engl.

Plate.2: *Conocarpus lancijolius* plant.

2.4.2.4Uses of damas.

Conocarpuslancifolius 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. lancijolius* 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 Damas (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 September to December2013, toevaluatethe antifungal activity of Neem and damas leaves aqueous extracts and efficacy of fungicide, Tilt 250 EC,against *Fusarium oxysporumf.sp.lycopersici*.

3.1Collections of plant samples.

Random samples were collection from infected tomato from different parts (stem, leaves, roots, soil) field were collected from (Shambat area) and put in plastic pay and leaptin refrigeration fen further brought to the laboratory for isolation, Identification of Fusarium wilt assesse of tomato and further studies.

3.2 Isolation of *Fusarium oxysporumf.sp.lycopersici*.

3.2.1 Isolation from plant material

Infected tomato plant (root and stem) showing symptom of the disease was obtained from Shambat research field. The root were cut into small part (0.5, 1.0 cm) washed thoroughly with the tap water, surface sterilized with Clorox (NaOCl) (1% concentration) for 1 minute, rinsed three times in sterilized distilled water and dried on sterilized filter paper. The sterilized

roots sections were then plated at the rate of 6 sectionsper plate on to potato dextrose agarmedium (PDA).

The Petri dishes were incubated at 25^0Cfor 7days. After incubation, isolated fungi were sub cultured on PDA medium for further purification of the fungus. Furthermore, Compound microscopic examinations were carried out for Mycelia and conidia structure based on the method of (Booth key, 1977) to confirm that the fungus is *Fusarium oxysporum* f.sp *lycopersici*.Standardbooks and research papers were also consulted during the examination ofthisfungus (Aneja, 2004). The purifiedIsolates were maintained on PDA medium for further studies.

3.2.2 Isolation from soil sample.

Soil samples, 1gm each, were collected from the roots of infected plants collected from Fusarium contaminated field. The samples were thoroughly mixed and 1gm sample was randomly selected. The obtained soil sample was added to 100 ml of sterilized distilled water to make soil suspensions. One mlof the soil suspension was uniformly spread over solidified PDA medium in Petri dish and was incubated at 25^0C. After incubation for 7 days, isolated fungi were sub cultured on solidified PDA medium for further purification of the fungus and Microscopic examinations based on the method of (Booth key, 1977) to confirm that the fungus is *Fusarium oxysporumf.sp.Lycopersici*

3.3Preparations.

3.3.1 Preparation of plant extract.

Neem and Damas leaves were collected from Shambat area and brought to the laboratory where they were shade dried. After complete dryness plant samples were crushed separately to obtain fine powder for extraction.

3.3.2 Preparation of inoculum

3.4. Aqueous extract preparation.

The obtained fine powder form each plant was weighted (5, 10 and 15 gm.) and placed in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 5 % 10% and 15% concentrations.

3.5Preparation of tilt fungicides.

The chemical tested were tilt fungicides 10ml dissolved in 100ml of sterilized distilled water to give 5 , 10, 15 ppm respectively

For this solution 5, 10, 15 were completed to 100ml by adding sterilized potato dextrose agar medium to give final concentration.

The PDA media was amended with the required concentration from neem; damasandfungicide tilt(5ml, 10 and 15ml 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 of the fungus was measured and calculated successfully after 3.4 and 5 days after inoculation.

The effect of each extracts was calculated as percentageof 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

3.5 Effect of Neem extract on the linear growth of the *Fusarium oxysporum*invitro.

The PDA media was amended with the required concentration from neem;(5ml, 10 and 15ml 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 of the fungus was measured and calculated successfully after 3.4 and 5 days after inoculation.

3.6 Effect of Damas extract on the linear growth of the *Fusarium oxysporum* invitro.

The PDA media was amended with the required concentration from damas(5ml, 10 and 15ml 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 of the fungus was measured and calculated successfully after 3.4 and 5 days after inoculation.

3.7 Effect of Tilt on the linear growth of *Fusarium oxysporum* invitro.

The PDA media was amended with the required concentration fromfungicide tilt (5ml, 10 and 15ml 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 of the fungus was measured and calculated successfully after 3.4 and 5 days after inoculation.

3.8 Experimental design.

These experiments were arranged in a Complete Randomized Design

3.10 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

RESULTS

This study which conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during the period September to December 2013 to investigate the inhibitory effect of Neem and damas leaves aqueousextracts and fungicide, Tilt 250 EC efficacy against the fungus *Fusarium oxysporum* f.sp lycopersici

4.1 Isolation and Identification from the infected sample of tomato plant

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

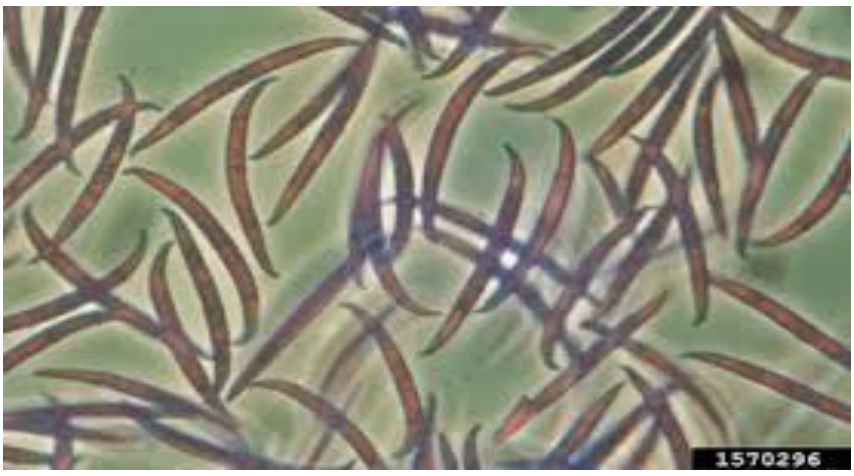
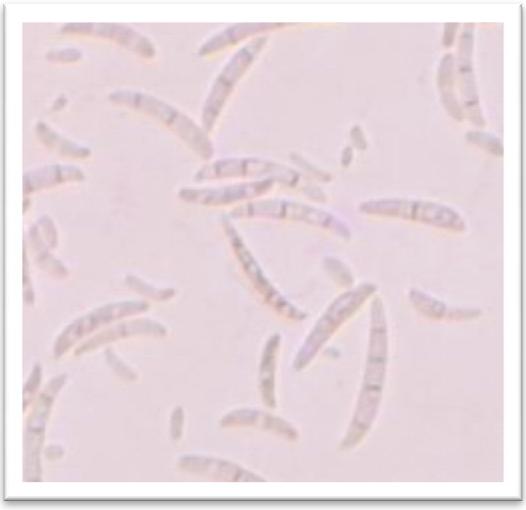


Plate (3) Fusarium oxysporum Magnification(x40):



Plat (4) spores

4.2. Effect of leaves aqueous extracts of Neem, Damas and fungicide tilt on the linear growth *of Fusarium oxysporum f.sp.lycopersici* invitro.

The results (Table 1 and Figure 1) showed that the leaves aqueous extracts of all plants tested and fungicide tilt had effects on the fungal growth after three days from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

The effect of Neem(5, 10, 15%) gives the Reduction of the growth as (37.3, 56.1, 100%) Respectively, had the effective of Damas of (5, 10, 15%) give the reduction of of the growth as (58.9, 77.8, 100%).

The Damas was more effective of the growth domeliof *Fusarium oxysporum f. sp. Lycopersici*than Neem invitro.

Moreover, the highest concentration of the plant extracts (15%) and tilt (10 and 15%) gave significantly higher inhibition zones percent (100%, 100%, 86.1 and 81.1) respectively compared to the untreated control. Among the plant extracts tested Damas was invariably the most effective in suppressing the fungus growth than its equivalent Neem (Table, 1). Generally,the results showed that the antifungal activity increase with increasing of extract concentration.

Table, 1: Effect of leaves aqueous extracts of Neem, Damas and fungicide tilt on the linear growth *of Fusarium oxysporum f. sp. Lycopersici* invitro.

Treatment		Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	5	20 (4.5)	25(5.0)	66.7(8.2)	37.3(5.9)c
	10	60 (7.8)	25(5.0)	83.33(9.2)	56.1(7.3)bc
	15	100 (10.0)	100(10.0)	100(10.0)	100(10.0)a
Damas	5	60 (7.8)	50(7.1)	66.7(8.2)	58.9(7.7)bc
	10	100 (10.0)	50(7.1)	83.3(9.2)	77.8(8.8)ab
	15	100 (10.0)	100(10.0)	100(10.0)	100(10.0)a
Tilt	5	40 (6.4)	25(5.0)	33.3(5.8)	32.8(5.7)c
	10	100 (10.0)	75(8.7)	83.3(9.2)	86.1(9.3)ab
	15	60 (7.8)	100(10.0)	83.3(9.2)	81.1(9.0)ab
Control		0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)d
C.V			15.5		
SE			0.53		

Any two mean value (s) bearing different superscripts (s) are differingsignificantly (p<0-0.5).

- Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

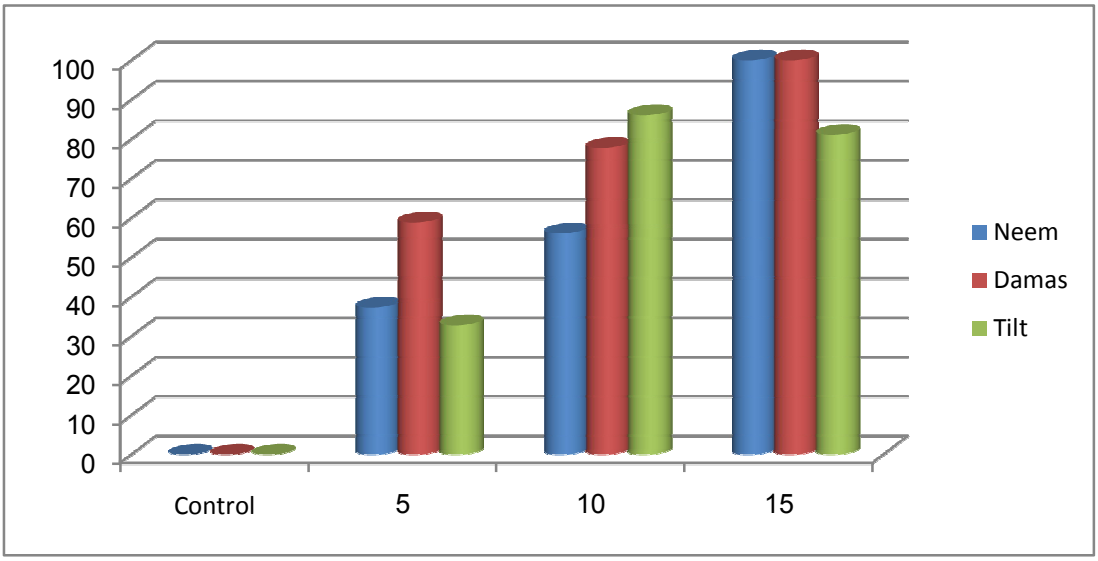


Fig.1.Effect of leaves aqueous crude extracts of Neem, Damas and fungicide tilts on the linear growth of Fusarium oxysporum *fsp. Lycopersici* invitro.

4.3. Effect of leaves aqueous extracts of Neem, Damas and fungicidetilt on the linear growth of *Fusarium oxysporum f. sp. Lycopersici* (invitro)%.

In day four after inoculation, all plant extracts 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 (15%) and tilt (15%) gave the highest inhibition zones percent (81.7%, 82.6%, and 77.9) respectively. This inhibitory effect from all concentrations tested was significantly different from control (Table, 2 and Fig. 2). Furthermore, the fungicide irrespective of concentration, (5, 10 and 15) effected significant reduction of fungal growth (58.5%, 58.9% and 77.9) respectivelycompared to control.

Furthermore, the Damas plant extract at all concentrations tested continued to be the most suppressive, followed in descending order by the fungicide and Neem.

Table, 2: Effect of leaves aqueous crude extracts of Neem, Damas and fungicide tilt on the growth of *Fusarium oxysporum fsp. Lycopersici* (invitro) %.

Treatment	Concs. (%)	Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	5	5(0.7)	35.7(6.0)	53.3(7.3)	31.3(4.7)b
	10	37.5(6.2)	64.3(8.0)	66.7(8.2)	56.2(7.5)a
	15	79.2(8.9)	71.4(8.5)	80(9.0)	63.5(8.8)a
Damas	5	83.3(9.2)	78.6(8.9)	83.3(9.2)	81.7(9.1)a
	10	75(8.7)	78.6(8.9)	83.3(9.2)	79.0(8.9)a
	15	79.2(8.9)	78.6(8.9)	90(9.0)	82.6(8.9)a
Tilt	5	58.3(7.7)	64.3(8.0)	53.3(7.3)	58.5(7.7)a
	10	50(7.1)	53.6(7.4)	73.3(6.8)	58.9(7.1)a
	15	75(8.7)	78.6(8.9)	80(9.0)	77.9(8.9)a
Control		0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)c
C.V			16.3		
SE			0.5		

Any two mean value (s) bearing different superscripts (s) are differing significantly ($p<0$-0.5).

- Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$) before analysis.

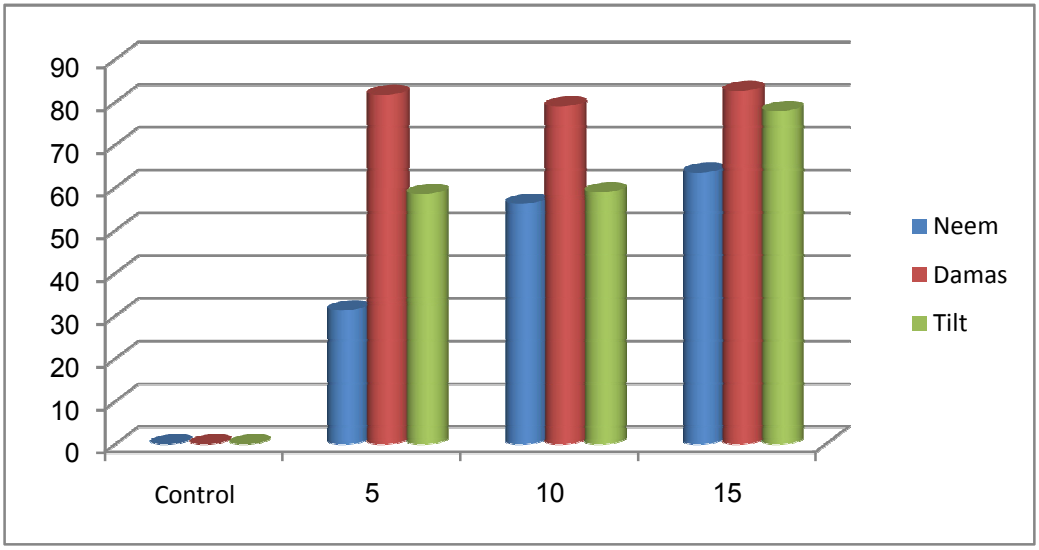


Fig.2 Effect of leaves aqueous extracts of Neem, Damas and fungicide Tilt on the linear growth of *Fusarium oxysporumf. sp. lycopersici*(invitro) %.

4.4.Effect of leaves aqueous extracts of Neem, Damas and fungicide tilt on the linear growth of *Fusarium oxysporum f.sp. Lycopersici*(invitro) %.

After five days from inoculation the results (Table, 3 and Figure, 3) showed that extracts of all the plants tested as well as the fungicide proved to be effective in suppressing the fungal growth.

In fact, all tested concentrations of Neem, Damas and fungicide induced a significantly higher inhibition zones percentage against test fungus compared to control (Table, 3). Meanwhile, the Damas aqueous extract at all concentrations tested (5, 10 and 15%) give (61.9, 59.3 and 58. percent) respectively exhibited more inhibitory effect than the Neem aqueous extract (5, 10, and 15%) gives (6.5, 32.4 and 46.4%)respectively.

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

Table.3. Effect of leaves aqueous extracts of Neem,Damas and fungicide tilt on the linear growth of *Fusariumoxysporumf. sp. Lycopersici* (invitro) %.

Treatment	Concs. (%)	Inhibition zone (%)			
		R1	R2	R3	Mean
Neem	5	3.1(1.9)	5.3(2.4)	11.1(3.4)	6.5(2.6)d
	10	18.8(4.4)	39.5(6.3)	38.9(6.3)	32.4(6.0)c
	15	50(7.1)	42.1(6.5)	47.2(6.9)	46.4(6.8)bc
Damas	5	53.1(7.3)	60.5(7.8)	61.1(7.8)	58.3(7.6)ab
	10	62.5(7.9)	57.9(7.6)	57.9(7.6)	59.3(7.7)ab
	15	59.4(7.7)	63.2(8.0)	63.2(8.0)	61.9(7.9)a
Tilt	5	46.9(6.9)	39.4(6.3)	39.4(6.3)	41.9(6.5)c
	10	40.6(6.4)	44.7(6.7)	44.7(6.7)	43.3(6.6)c
	15	68.8(8.3)	71.1(8.5)	71.1(8.5)	70.3(8.4)a
Control		0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)e
C.V			9.2		
SE			0.45		

Means followed by the same letter are not significant different at (P< 0.05)

❖ Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

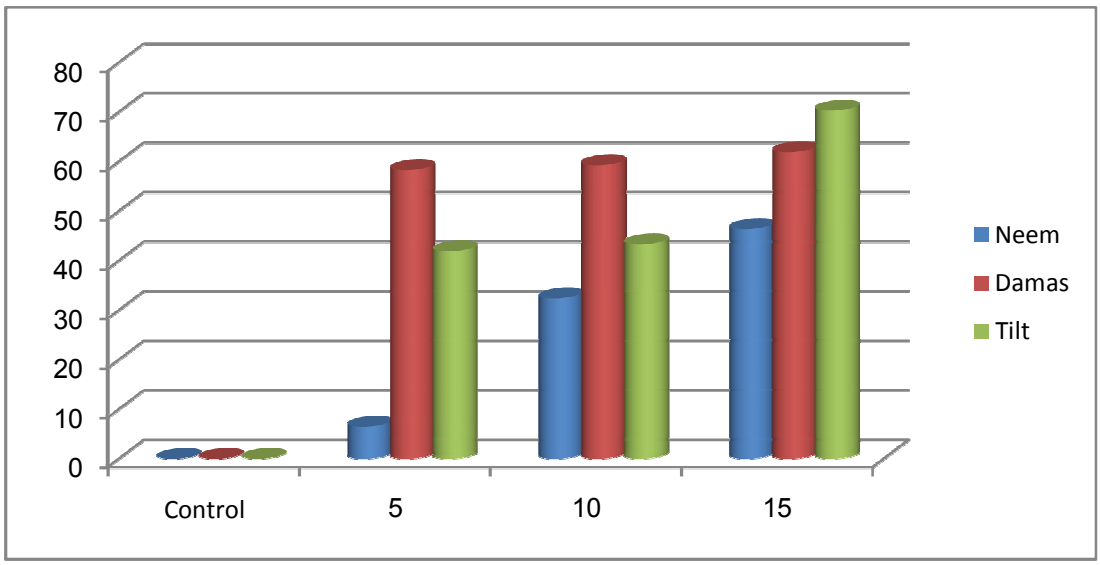
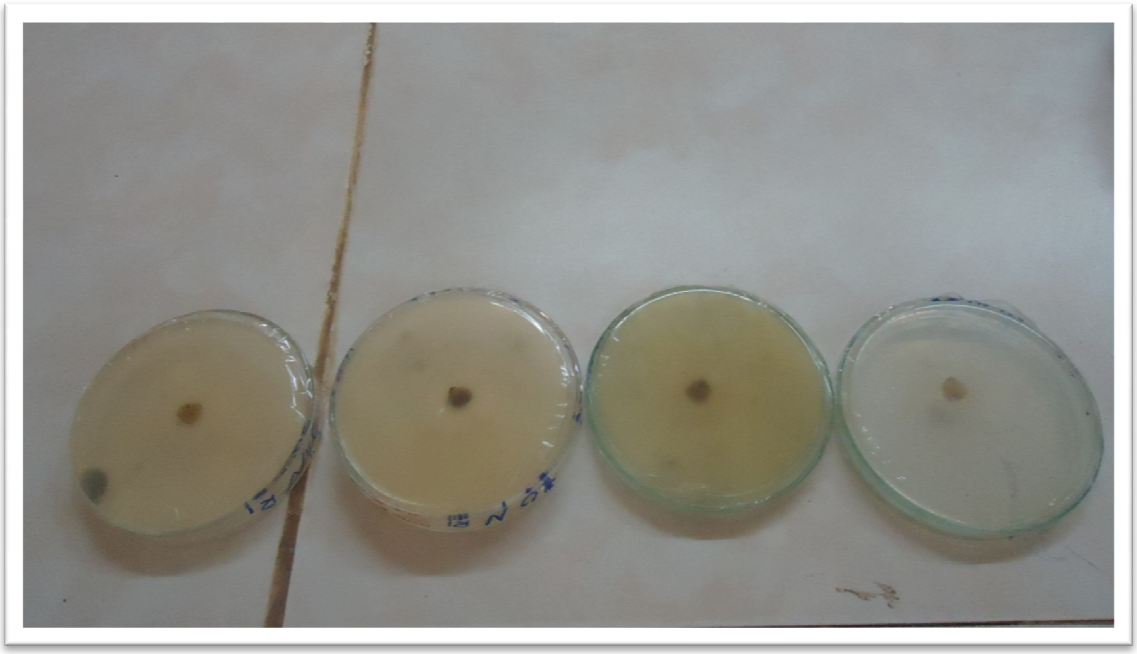
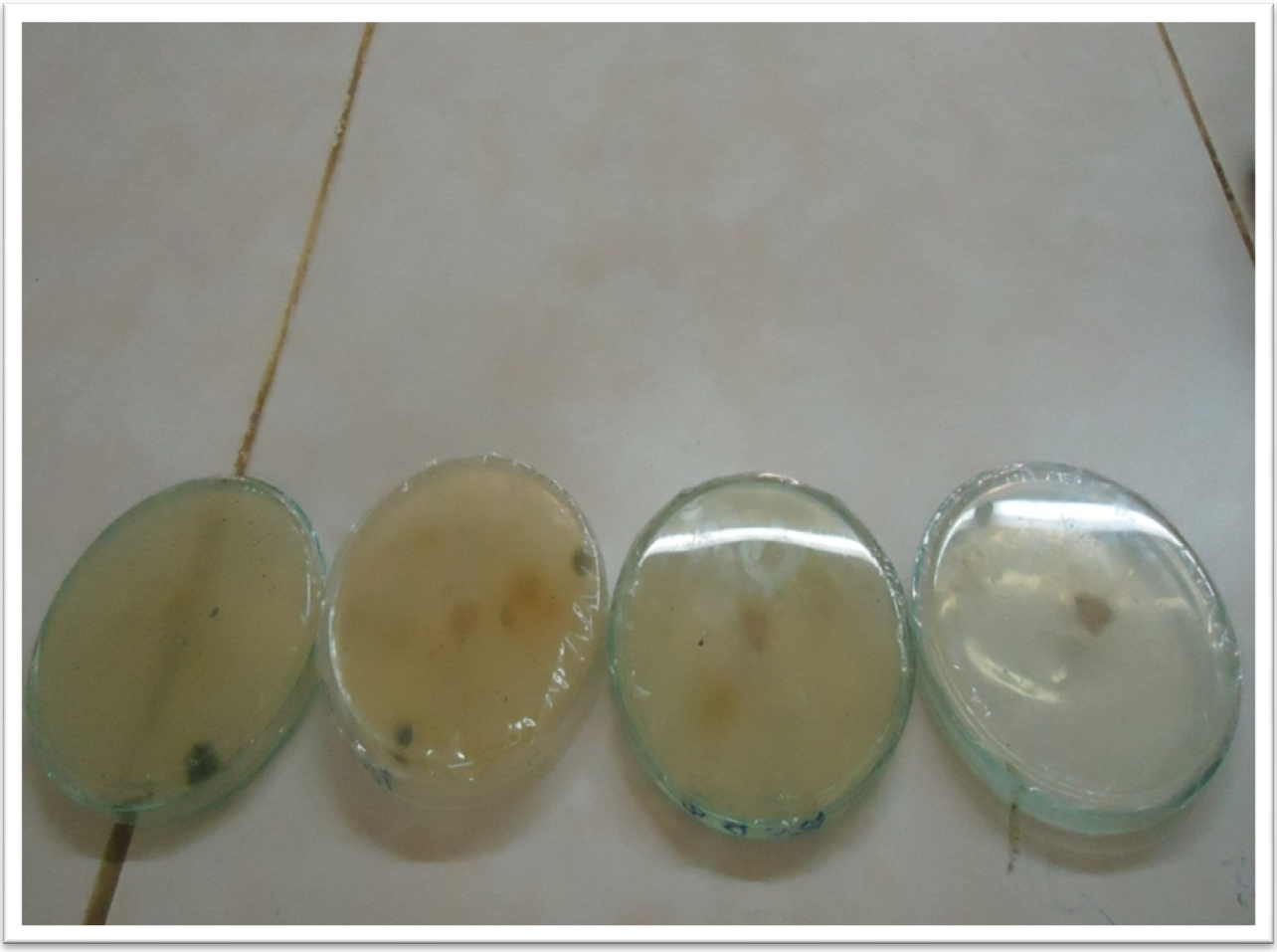


Fig.3Effect of leaves aqueous extracts of Neem, Damas and fungicide Tilt on the linear growth of *Fusarium oxysporum f.sp. Lycopersici*.(invitro) %



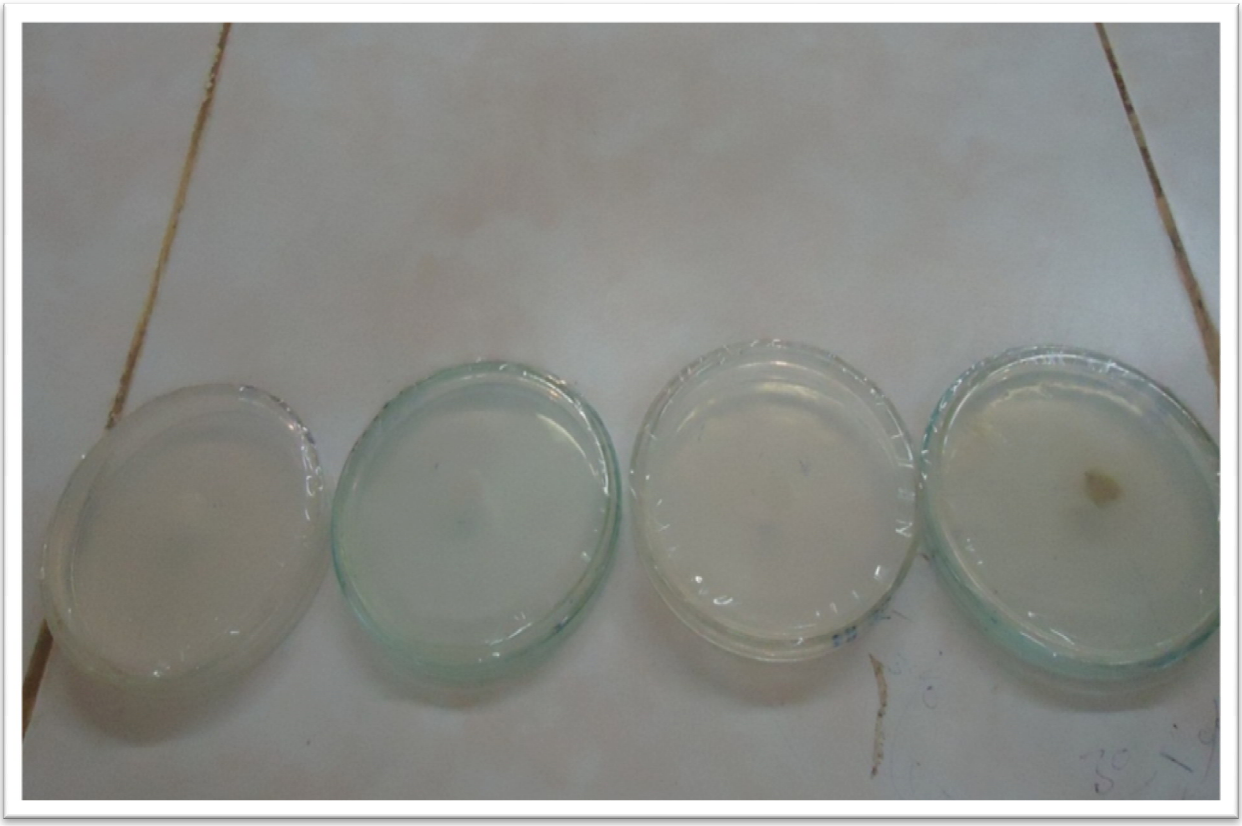
5% 10% 15%Control

Plat (5) Effect of leaves aqueous extracts of Neem on the growth of *Fusarium oxysporum* invitro



5% 10% 15%Control

Plat (6) Effect of leaves aqueous extracts of Damas on the growth of *Fusarium oxysporum* invitro



5% 10% 15% Control

Plat (7) Effect of Tilt fungicides Controlon the growth of *Fusarium oxysporum* invitro.

Plat (8) Streaking in vascular tissue of tomato (*Ly*

CHABTER FIVE

DISCUSSION

The production of tomato (*Lycopersiconesculentum*Mill.) is of worldwide importance as the crop being one of the most popular commercial vegetable rich in vitamins A, B, and C grown throughout the world (Suarez *et al.*, 2007). Many diseases affect tomatoes during the growing season, both in greenhouse and field (Peratta, 2001). Among these are Fusarium wilt disease, caused by pathogenic formae specials of the soil-inhabiting fungus; *Fusariumoxysporumf. sp.lycopersici*. In fact, thefungusis one of the most important known pathogen of tomato plant (Suarez *et al.,* 2007). The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal/reduced or even total loss/ absent crop yield Agrios, 2005.

In this study, the data (Tables 1-3 and Figures 1-3) revealed that the Damas leaves aqueous extracts consistently exhibited an inhibitory effect on fungal growth with significantly higher inhibition zones percent. This is agree with (Satish *et.al*.,1999; Okigbo and Ogbonnaya, 2006; Shariff *eta*l.,2006; Ergene *et.al*. 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006) which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials More recent results were demonstrated by Saad*et al.,* (2014) where they demonstrated the antibacterial and antifungal activities of the methanol extract of Damas (*Conocarpuslancifolius* Engl.) aerial parts using disk diffusion method. Similar results were also obtained by Ahmed (2014) who

studied the Alkaloid extract of *Conocarpuslancifolius* Engl. against Some Clinical Pathogens.

Neem tree (*Azadirachtaindica*) as well is one of the most known plants for its multiuse in controlling insect pests and diseases. In this study the results revealed that the Neem leaves aqueous extracts suppressed the fungal growth*Fusarium oxysporum f.sp. Lycopersici* with significantly high inhibition zones percent (%) compared to control. In this regards there are numerous examples and studies confirmed that higher plants contain bioactive ingredient (s) with very good potential for plant diseases control. Neem tree (*Azadirachta indica*), seed kernel contains a number of chemical compounds, most important of which are Azadirachtin and salanin in triterpenoid fraction (Stoll, 2000). Agree with Hanaa *et al.,* (2011) who found that treatment of tomato plants with Neem aqueous extracts reduced the percentage of Fusarium wilt disease incidence to the level of 25.5% and 27.8% after 6 weeks of infection respectively. Moreover, the inhibitory effect and control efficacy of plant extract under greenhouse condition had been also tested by Bowers and Lock (2000) against *F. oxysporum* species.

The efficacy of Tilt against *Fusarium oxysporum f.sp. Lycopersici*as the causal acquit of Tomato wilt disease in vitro at 10% induce the reduction of the growth after 6 days 100 applied at 15 pp. Compared to control (Tables 1-3 and Figures 1-3). This finding is in line with the observations reported by Abdelgader (2005) on efficacy of Tilt against *Fusarium oxysporum* where he found that tilt induced 100% inhibition against *Fusarium oxysporum* when applied at 100ppm after 7days of exposure. Similar finding were also revealed with Mohammed (2005) who found that tilt when applied at 10ppm against *Drechslera hawaiiensis* induced 100% inhibition after 4 days.

The current study also demonstrated that the Damas leaves extract exhibited more inhibitory effect than that of the Neem. This could be attributed to the high concentration of the bioactive inhibiting compound in the Damas plant leaves than in the Neem. Moreover, the data on concentrations from each plant leaves aqueous extract exhibited different inhibitory abilities on fungal growth. The 15 % leaves aqueous extract concentration from the two plants was the most suppressive followed in a descended order by 10% and 5%. Likewise the test organism responded differently to the different concentrations of extracts. This variability in response which expressed agrees test organism to different Damas and Neem extracts was also reported by Aiyelaagbe (2001). In his investigation, he explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree. However, these results confirmed that obtained agree with(Mohammed, R 2012; Mohammed, A 2013 andAhmed,F.A.A 2013).

Conclusions

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

- The Damas plant leaves aqueous extract exhibited more inhibitory effect than that of the Neem. This finding is the first one of its kind in Sudan in the invitro of Fusarium wilt control in Tomato which suggests more investigation to be carried out in this area.

- The screened concentrations of Damas and Neem leaves 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 in Tomato.

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 phytochemicals analysis of different parts of Damas plant using different solvents so as to determine the bioactive ingredient in each of theseparts.

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APPENDIXS

Appendix 1

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

Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Between	9	214.654	23.850	17.906	0.0000
Within	20	26.640	1.332		
Total	29	241.294			

Appendix 2

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

Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	9	191.754	21.306	15.394	0.0000
Within	20	27.680	1.384		

Total 29 219.434

Coefficient of Variation = 16.29%

Appendix 3

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

Degrees of Sum of Mean

Freedom Squares Square F-value Prob.

Between 9 168.588 18.732 59.404 0.0000

Within 20 6.307 0.315

Total 29 174.895

Coefficient of Variation = 9.23%

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

Filter paper Microscope

Gloves Camera

Marker pen Medical cotton

3.2 Materials.

Potato dextrose agar

Tilt fungicide 250 EC

Infected plant

Neem leaves

Damas Leaves

Distilled water

Sodium hydrochloride (1%)