Sudan University of Science and Technology

College of Graduate Studies

Invitro Evaluation of Neem Tree Seed Oil Against Fungal Growth of *Fusarium oxysporum. F. sp. lycopersici,* the Causal Agent of Tomato Wilt Disease.

التقييم المعملي لزيت بذرة شجرة النيم ضد نمو فطر الفيوزايريم المسبب لمرض الذبول في الطماطم.

A Thesis Submitted in Partial Fulfillment of Requirement for the M.Sc. Degree in Plant Protection.

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Feb, 2018

الآية

بسم الرحمن الرحيم

قال تعالى :

(أَوَلَمْ يَرَ الَّذِينَ كَفَرُوا أَنَّ السَّمَوَاتِ وَالْأَرْضَ كَانَتَا رَتْقًا فَفَتَقْنَاهُمَا وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ أَفَلَا يُؤْمِنُونَ)

صدق الله العظيم

سورة الأنبياء الآية(30).

Dedication

To my father and Mother

To all my family

To all my teachers

To all my colleagues and friends

With intimate love and deep thanks

Acknowledgements

I wish to deeply thank my Supervisor: **Dr. Ibrahim Saeed Mohamed**, for his the greatest support, encouragement, patience and for his continuous concern about overall period of this Study. My deepest gratitude goes to all Doctors and Associate professors of Plant protection Department and the **Co-Supervisor Prof: Hatim Juma**. My sincere thanks go to my colleagues who advise and assist me during the field experiment Ms. M.Sc: **Mawda Ibrahim** and special thanks goes to my colleagues of M.Sc. whom helped me during the experiment lab.

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Abstract

Fusarium wilt considered as one of the most important diseases of tomato in Sudan. The present investigation was undertaken under laboratory conditions to study the effect of different neem oil concentrations, and synthetic fungicide,(AmistarTop 200ml/100L Water), on growth of the fungus *Fusariumoxysporumf. sp. Lycopersici*the causal agent of wilt disease on tomato. Three concentrations of neem oil (1.5, 2.5 and 3.5 %) and fungicide as recommended dose were tested in addition to control. The assessment of theirinhibitory effect against the pathogen was expressed as fungal growth inhibition percentage after six days of treatment. The results obtained showed that neem oil at alltested concentrations (1.5, 2.5, 3.5 (%) as well as the fungicide reduced significantly ($P<0.05$) the fungal growth (36.65%, 34.59%, 40.95, and 53.59 %) respectively compared to untreated control(100%). Moreover, the fungicideat100% concentration (0.2\100ml) demonstrated the highest inhibition of fungal growth (53.59%) followed in descending order by neem oil concentrations. Among neem oil concentration, the highest one (3.5%) reduced significantly the growth of the fungus (40.59%). Generally, the results showed that the antifungal activity increase with increase in extract concentration. Obviously, the test fungus differs in response to the different concentrations.

ملخص البحث

يعتبر مرض ذبول الفيوزارمي أحد أهم أمراض محصول الطماطم في السودان. أُجريت هذه الدراسة تحت ظروف المعمل لتقييم أثر التركيزات المختلفة من زيت بذرة شجرة النيم والمبيد الفطري المصنع (امسترتوب0.2مل/100مل ماء) على نمو فطر الفيوزيريم اوكسسبوريم المسبب لمرض الذبول في الطماطم، تم اختبار ثلاثة تركيزات من النيم (1.5,2.5,3.5%) والمعدل الموصي به من المبيد الفطري بالإضافة للشاهد، تم التعبير عن اثر التثبيطي ضد المسبب المرضي كنسبة مئوية للتثبيط بعد ستة أيام من المعاملة، أوضحت النتائج المتحصل عليها ،أن زيت النيم في كل التركيزات المختبرة بالإضافة للمبيد الفطري زادت معنوياً من النسبة المئوية للتثبيط (36.65%,34.59% 40.95% , 53.59%) علي التوالي مقارنة مع الشاهد غير المعامل.أعطى المبيد الفطري أعلي نسبة مئوية للتثبيط تلاه مستخلص النيم بتركيز 3.5%. من بين مستخلصات النيم المختبرة أعطى أعلى تركيز 40.95% تثبيط. عموماً أوضحت النتائج إن النشاط المضاد لنمو الفطريات يزداد بزيادة التركيز، كما أن الفطر المختبر يختلف في إستجابته بإختلاف التركيزات.

CHAPTER ONE

INTRODUCTION

Neem (*Azadirachta indica*) tree is native to tropical South East Asia and is a member of the Mahogany family, *Meliaceae* (Okonkwo, 2004). In Sudan, Neem trees are found along the Nile bank, in towns, villages as shade and avenue tree (Siddig, 1991 and Ruskin, 1991).

The most famous product of the tree is the oil obtained from the seed kernel (Adewoye and Ogunleye, 2012). According to Ikasari and Indraswati (2008), Neem seed is a part of the Neem tree which has high concentration of oil of between 35-45%. The quality of the oil differs according to the method of processing. Neem oil is a vegetable oil pressed from the fruits and seeds of Neem plant, an evergreen tree which has found its use widely in different regions of the globe for medicinal and agricultural purposes (Kovo, 2006; Ranajit *et al*., 2002; Awad, 2003; Muñoz-Valenzuela *et al*., 2007 and Kumar *et al.,* 2006).

Peter (2000) reported that, uses of neem seed oil to include the following soap production, raw material for pesticides and cosmetics, plant protection, stock and textile protection, lubrication oil for engines, lamp oil, candle production and refining to edible oil. Kovo (2006) reported its use as an insect repellant, pesticide and fungicide. Neem Cake is the residue obtained from pure Neem seed kernels which have been crushed to extract the oil. This cake is an excellent organic fertilizer with high N-P-K values and reducing the fungus that effect the crops such as potato, tomato, specifically *fusarium oxysporum.f.sp.lycopersici* on tomato. So that Tomato *Lycopersicon esculentum* is one of the most widely cultivated food crops. It belongs to family *Solanaceae*. This family comprises about 90 genera and

2000 types of plants. Those were infected with fusarium to destruction of permeability of leaf cell by fugal toxin so that reducing the productivity. Based on the foregoing, this study was undertaken to focus on investigation of component for management of Fusarium wilt of tomato caused by *Fusarium oxysporum f.sp. Lycopersici*; Neem oil extracts and synthetic fungicides under laboratory conditions in order to formulate promising disease management approach with following study objective:

-To explore the antifungal potential of neem seed oil crude extract against *F. oxysporum f. sp. Lycopersici Invitro.*

CHAPTER TWO

LITREATURE REVIEW

2.1 Neem Tree

The neem tree was introduced in to Sudan in 1921. The tree is frequently distributed in Kassla, villages along the blue and White Nile, irrigated area of central Sudan and rain fed regions in Kordofan and Darfur (Schmutterer, 1995). Neem seed kernel contain number of chemical compounds, most important of which are Azadirachtin and Solanni in Triterpeniod fraction . The oil extracted from the seeds used to control Grey leaf spot, Powdery mildew and viruses (Stoll, 2000).

2.1.1 Description

The tree is a fast growing plant that usually reaches a height of 15-20 meters. Under favorable conditions the tree grows up to approximately 35-40 meters (May, 2009). It is an ever green plant elevates extreme circumstances such as long dry periods and it might shed mostly or nearly all of its leaves with branches that spread widely. The fairly dense crown dishevels and may reach diameter of 15- 20 meters on old trees. The tree possesses all the ideal characteristics as promising botanical pest control martial; these characteristics include: being a perennial, easy to grow and not destroyed each time. It is hardy evergreen tree, which sheds its leaves only under extreme condition of heat and drought for only a short time. The tree is 5- 30 meters tall and 2.5m width, its branches spread up to 10 meters across. The tree is generally deep-rooted and with lateral roots extends radically to 15 meters. It is normally grown with 150 mm of rainfall with and optimum of 450-750 mm and has been successfully planted in regions

with up to 2000 mm rainfall. Propagation is generally by seeds, seedling, cutting and tissue culture (Ahmed and Grainge, 1984). The fruits are smooth, ellipsoidal drupe, up to 2cm long. When ripe it is yellow or greenish and comprises a sweet pulp enclosing seed. The Neem tree may live for more than two centuries (Maydell, 1986 and Ruskin, 1991).

The pest control material is easy to harvest and showed effective control against broad range of target pest. It is also easy to formulate, relatively safe to non-target organism, wildlife, and man and pose little other environmental hazards (Ahmed and Grainge, 1984). Neem Oil is used to protect plants and crops against pests. It is effective against insecticide-resistant pests, highly environmentally compatible, non-toxic to mammals and birds, and does not affect beneficial insects. Neem Oil is ideal for integrated pest management programs. We supply one of the best qualities Neem Oil available.

2.1.2 Distribution:

Currently the Neem tree is widely distribution in dry tropical and subtropical zones of Asia, America, Australia and Africa. In the Sudan Neem which was introduced in 1921 to Sudan is frequently distributed in the eastern towns, villages along the Blue and White Nile, irrigated areas of central Sudan and rain fed regions in Kordofan and Darfur (Schmutterer,1995).

2.1.3 Classification:

Kingdom: Plantae

Division : Magnoliophyta

Suborder: Rutales

Family : Meliaceae

Genus : Azadirachta

Species :Azadirachta Indica

S.N : Azadirachta indica A.Juss

E.N : Neem

(Vietmeyer, 1992 and Schmutterer, 2002).

2.1.4 Usage of Neem:

The Neem tree has become important in the global context today because it offers answers to the major concerns facing mankind. During the last years there are intensive search by many groups all over the world who showed the plant family meliaceae as one of the most promising source of compounds with pest control properties in particular. In fact, one of the widely studied plants in this family is the Neem tree *Azadirachta indica* A. Juss . It is widely used in traditional medicine, preparation of insecticides and soap manufacturing using the oil pressed from its seed kernels which containing up to 4%oil (Siddig, 1991). Neem kernels contain a number of chemical compound, most important of which are Azadirachtin and Salanin triterpeniod fraction (Margan, 1987).

2.1.5 Chemical constituent:

Neem protects itself from the multitude of pest with a multitude of pesticides ingredient ,it is main chemical ingredients of 3 or 4 related compounds, backed by up to 20 or so others that minor none the less active in a way or other. The secompounds belong to general class of natural products called triterpenes or Limonoids (Rusking, 1991). All parts of neem tree (bark, leaves, fruit leaves and seeds) have been examined and found constitute of Triterpene , which are most concentrated and accessible in the seed .The main constituents include Azadirchtin, Salanin, Meliantriol, Nimbin, Nimbidin,

Salannol acetate-3-deacetyl-solannin,4-, epoxyazaradion gedunin,nimbinen and deacetyl Nimbin (morgan,1969; Ermel *et al.,* 1984; Feuerhake, 1984; jones *et al*., 1989 and Ruskin, 1991).Neem seed oil is produced in large quantities either as end product by physical expelling of the oil from seeds or as by–product of organic solvent extraction of the seed. Neem seed can contain up to 45% oil which has been extracted in india from centuries for its medicinal properties and for soap production. The oil is known to contain a number of biologically active compounds and application of this activity in agricultural systems for crop protection is being explored , Neem seed oil is a vegetable – type oil composed primarily of glycerides (80- 95%) free fatty acids (4-20%) and small quantities of inorganic and organic salts it is hydrophopic in water and in order to emulsify it in water in application purposes, it must be formulated with appropriate surfactant s. Some usage, such as seed treatment or post harvest applications, may utilize the soil directly.

The sources quality degree of retirement and formulation of neem oils reported in the literature vary to a great extent and thus make comparison of results difficult. Some researchers reported considerable phytotoxicity caused by neem oil whereas other report on problems at efficacious rates. Consideration of research results using neem seed oil well be divided in to pathogen groups, soil borne, post-harvest and foliar. Four major soil borne pathogens, f. *oxysporum f.ciceri, Rhyizectonia solani*) were tested in liquid culture for sensitivity to various aqueous neem extracts and seed oil (Singh *et a*l., 1980). The oil was most inhibitory suggesting the presence of antifungal substances which may in fact, explain all or part of the activity noted with the use of neem cake in soil amendment. Solvent extracted seed oil was reported to be as effective as the fungicide Hymezol in protecting sugar beets against fungal damping –off pathogens in greenhouse trials but the oil exhibited some phytotocicty (Lehmann, 1991and Lehmann *et al*., 1993).

Clarified neem oil was evaluated as a postharvest treatment of apple fruit to protect against three storage pathogens mainly *Botrytis cinerea*, *penicilum* , and *Glemerlla cingulate* (Moline and Locke, 1993). The 2% emulsified neem oil treatment was moderately fungicide to *B.cinerea* and *G.cingulata* in wound –insulated fruit , but had little activity against *P. expansum*. Although neem seed oil treatment did not completely protect the wound –inoculated fruit, it did not result in about a 50%reduction in decay. Evaluation of clarified neem oil against *Rhizopus* soft rot of sweet potato in storage did not reduce the amount of rot and may have actually increased rot severity Ali, *et al* .(1992) Reported that the growth of isolates of *Penicilum Italicum* , *Alternaria* and *Spergillus niger* from rotted tomato fruit was checked by neem oil in vitro. Number of neem oil formulation have been evaluated against foliar pathogens on various crops with varying degrees of causes ,many crude neem seed oil have been reported to be phytotoxic (Chianella and Rovest, 1992) But clarified neem seed oil formulation has shown no phytotoxicity at efficacious rates (0.5 to 2.0%) several ornamental crops (Locke *et al*. ,1993). The antifungal activity of several essential oils, including neem seed oil, was demonstrated using filter paper assay discs (0.2ml oil /10mm disc) on test plates of potato dextrose agar (Kher and Chaurasia, 1977).

2.2 Tomato:

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated food crops. It belongs to family *Solanaceae* or Night shade. This family comprises about 90 genera and 2000 types of plants. Tomato pertains to genus *Lycopersicon,* which contains seven wild types. The commercial one or cultivated tomato belongs to five botanical varieties "Baily classification" Purseglove (1968). All cultivated forms of tomato belong to species *esculentum*. The origin of tomato is central and South America especially Mexico, from where tomato was transferred to Europe in the 16 century, then to the old world continents (Hedrik, 1919 and Rick, 1976). Nutritive Value:

Every 100-grams of raw tomatoes contain 93.59% water, 22 calories, 1.1 protein, 4.7 g carbohydrates, 13 mg calcium, 27 mg phosphorous, 0.5 mg ferrous, 244 mg potassium, 900value vitamin A0.06 mg thiamine, 0.04 mg riboflavin, 0.7 mg niacin, 23 mg ascorbic acid (Watt and Merril, 1963). Economic Importance: In 1985 tomatoes were grown on over 2.5 million ha over 6.3million acres and production worldwide exceeded 60 million metric tons. Production in North and Central America is accounting for 10.8 million metric tons (Jones, *et al*., 1991).

2.2.1 Tomato in Sudan:

In Sudan tomato is gaining importance; it is normally used fresh in salad or in the processed form (tomato paste). It is mainly grown during winter and autumn seasons. Out-off season tomato production during summer is limited by prevailing high temperature, strong dry winds and shortage of irrigation water. The area under tomato production has remarkably been increased. In 1999 the area located for tomato production was estimated at 13000 hectares and the amount produced about 417 thousand metric tons. In 2000, the area allocated for tomato production was 21000 hectares and the amount of production was 242 thousand metric tons. The major areas of production in the Sudan are Khartoum State, Northern Gezira and many parts of the Northern and Eastern State (Ali1, 993).Tomato is attacked by many fungal pathogens in the field resulting in losses. Fusarium wilt of tomato is one of the most important diseases, which affect all plant stages (seedling stage, flowering stage, and fruiting stage). Also, it can affect the whole plant parts, leaves and stems. Agrios (1997) reported that the disease is most destructive in warm climates and warm sandy soil of temperate region. It causes great losses, especially under favorable weather conditions. Occasionally entire fields of tomatoes are destroyed or severely damaged before a crop can be harvested.

2.2.2 Classification

Division : Magnoliopida

Sub class :Asterielae

Order :Solanacaea

S.N :*Lycopesricon esculentum* (Mill)

2.2.3 Economic importance of tomato:

The importance of tomato, as a 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.

2.3 Fungal diseases of tomato:

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, Fusarium wilt disease is considered one of the major agents of yield reduction of the crop (Awad, 1990 and Stone, *et al*., 2000).

2.3.1 Fusarium wilt:

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.

2.3.2 Classification:

Division : Ascomycota

Class : Sordariomycetes

Order : Hypocreales

Family : Nectriaceae

S.N : *Fusarium oxysporum f.sp. Lycopersici* (Snyder and Hansen, 1940).

2.3.3 Habitat:

Nelson *et al*., (1983) reported that *Fusarium oxysporum* is a Cosmopolitan soil-borne fungus with a worldwide distribution. It is one of the more commonly encountered fungus in agricultural soils, often Surviving as dormant Chlamydospores. Booth, (1971) and Walker, (1971) Noted that *F. oxysporum f.sp. Lycopersici* also readily form chlamydospores.

2.3.4 Morphology:

Windles (1992) noticed that, the fungus readily grows on artificial medium on PDA. It grows rapidly covering 9 cm diameter Petri-dish

in approximately 7 days. It produces white aerial mycelium often with pink, orange, red, blue, violet or purple pigmentation developing with age. It can also produce slimy clump of spores (sporodochia) and blue sclerotia in culture. *Fusarium oxysporum f.sp. Lycopersici* can produce three types of asexual spores, macroconidia, microconidia and chlamydospores depending on culture conditions. Macroconidia are usually produced abundantly as long, sickle-shaped, thin-walled, spores with several septa. Micro conidia are also usually abundant and are smaller single celled, oval-shaped spores. The production of microconidia on short monophialides is a distinguishing characteristic. Chlamydospores are thick-walled, round; dormant spores are formed singly or in pairs. It is typically produced in 2-4 weeks on old culture (Nelson, 1983).

2.3.5 Disease symptoms:

Wellman (1941) described epinasty as an early symptom of tomato wilt still earlier symptom of this disease has been seen repeatedly, and it is believed to be the first above-ground indication of infection of the tomato by wilt pathogen. Foster (1946) demonstrated that the early symptom is clearing of the ultimate vein lets in the leaflets of infected tomatoes giving them a "netted" appearance. It can be seen only when leaves are viewed with transmitted light. Very early symptoms of tomato Fusarium wilt are of little importance in studying the disease as it occurs in the field but such symptoms have been of considerable value in various controlled studies in the green house and laboratory. Walker, (1971) and Jones, *et al*., (1991) showed that the symptoms often occur on mature plants after flowering and at the beginning of fruit setting. Initial symptoms can appear as slight wilting on part of the plant. Chlorotic symptoms being to appear on one side of leaf, then leaf let’s become yellow on one half of the leaf. As symptoms

progress, wilting is often associated with one side of the plant. Wilt symptoms are more commonly observed during the warmest part of the day. Cutting longitudinal section into the xylem at the base of the stem reveals a dark-brown to red discoloration. As the disease becomes more severe vascular discoloration can be observed further up the stem even extending in to the vascular bundles of the petioles. According to CPC abstracts heavily infested fields and high incidence of symptomatic plants can be observed throughout the field.Seedling also can become infected and in addition to the symptoms described on mature plants, seedling can appear stunted with periodic wilting. Infected plant shows reduced growth and premature ripening of fruits. The wilt progresses gradually upwards resulting in the death of the plant. The speed of this process greatly depends on environmental conditions influencing the growth of pathogen and transpiration of the host plant. Hors fall *et al*., (1959) reported that tomato plants affected by *F. oxysporum f.sp. lycopersici* show severe break down on vascular tissue only in the last stages of disease and then only if the plant is young. Several theories have been advanced to explain wilting of tomato plant infected with *Fusarium oxysporum f.sp*. Lycopersici. Gaumann, (1958). Suggested that wilting results from destruction of permeability of leaf cell by fungal toxin. Other investigations suggested that wilting is caused by occlusion of xylem vessels by mycelia and conidia, tyloses, gums and hydrophilic material that may form gels in vessels (Dimond, 1955). However, Gothoskar *et al.*, (1955) reported that wilting tomato plant is due to increased viscosity of tracheal fluid and eventual mechanical plugging of vessels by pectic gels. Henritta and Malcon (1963) found that, in diseased stems and petioles, the vascular bundles fail to increase in size and few vessel elements that are produced remain small and collapsed. Horsfall and Dimond (1959) reported that the blocking of the xylem

ducts, the toxins or enzymes carried along with the flow sap are responsible for wilting and there is the mechanism of their action , is still for controversy.

2.3.6 Disease cycle:

Fusarium oxysporum is abundant and active saprophytic in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al*., 1988). It is saprophytic ability to survive in the soil between crop or plant debris. The fungus can survive ether as the mycelium or as any it is 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 plant ether 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 invaded 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 plant 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 parechymatous tissue, 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.

2.3.7 Distribution:

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 inlinum spp* and *Gossypium* spp as reported. Whose strains represent some of the most abundant and widespread microbes of the global soil microflora (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). *Fusarium.oxysporium* strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin. (Rodriguezet, *et al.,* 1996) and complex carbohydrates associated with soil debris. they are also pervasive plant endophytes that can colonize plant roots Gordon, and Jacobson (1989) may even protect plants or be basis of disease suppression .Larkin, *et al* (1993), Lemanceau, *et al* (1993). Although the predominant role of these fungi in native soil may be as harmless or even beneficial plant endophytes or soil saprophytes, many strains within *Fusarium. 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, 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*.

2.3.8 Host Range:

The most important *Fusarium* wilt pathogens have wide range of host and including numerous forma special some of them contain two or several pathogenic races, causing devastating wilt diseases and many were seed borne as listed by Andersen (1974). For the following hosts *Allium cannabis, Beta vulgaris,Cucumis sativa,Phaseolus vulgaris* and *Psumi satavium*.

Katan (1971) demonstrated that *F. oxysporum* f.sp.*lycopersici* is also capable of infecting and colonizing the roots of a wide range of crops and weed hosts as parasite without inducing any visible disease symptoms. Foster and Walker (1971). Mentioned that the influence of physiochemical factors on the predisposition of the host where tomato plants were grown under variety of conditions. The roots were inoculated and the plant grown under conditions favorable to disease development was found to be predisposed to *Fusarium* wilt by:

(1) Soil and air temperatures near the optimum for host growth.

(2) low soil moisture.

(3) Short day length.

(4) Low light intensity.

(5) Nutrition low in nitrogen, low in phosphorus, high in potassium and low in pH.

2.3.9 Economic Impact:

Fusarium oxysporumf.splycopersici is significant problem in many crops .it is economically damaging to many industrial crops e,g, 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, and Pepper, and other commercial important plant are effected include basil, bean, carnation chrysanthemum, peas, and water melon (Ahmed, 2013).

Fusarium oxysporum is seed and soil borne fungal pathogen that causes Fusarium wilt disease was found to be more serious in low rain fall areas, were the weather condition are favorable for disease development (Khane,1980).

2.4 Control:

2.4.1 Cultural Control:

The cultural control is only practical measure for controlling the diseases in the field. the wilt fungus is so widespread and so persistent in soils that seed bed sterilization and crop rotation although always sound practices but are limited value .Soil sterilization is too expensive for application but it should be always practiced for greenhouse grown tomato plant (Agrios, 2005).

Moreover, use of health 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 several losses on most vegetable and flower, several crops such as cotton, Tobacco, Banana, plantain coffee, and a few shade trees. Fusarium wilts are most severe under warm soil condition and green house (Agrios, 2005).

2.4.2 Botanical Control:

The antifungal effect of certain medicinal and aromatic plant 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 very important step (Agrafotis, 2002).However, the step of validation of traditional uses of antimicrobial compounds in higher plants was studied by number of researcher. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrfotis, 2002).

The use of plant extract for controlling Fusarium wilt, cultural practices and the use of other methods are the most common strategies. The use of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to the negative impacts on the environment ,plant extract or plant essential oils have been tested against *Fusarium oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2002).

If natural plant products can reduce populations of soil borne pathogens and control diseases development, then these plant extracts have potential as environmentally safe alternative and as component in integrated pest management programs, (Chand and Singh, 2005). Reported that the plant extract ,such as Azadirachta indicia, Jatropha multifida ,Allium sativum were significantly pronounced in reducing wilt incidence in mycelia growth of various Fusarium species were inhibited by the plant extracts (prasad, and Ojha,1986). Also (Singh and Hair Chand, 2005) reported that leaf extracts of *Azadirachta*

indica at 100%can completely inhibited germination of pathogen spores.

2.4.3 Biological Control:

Currently there is no commercially available biological control products registered for the control of Fusarium dry rot. However, there are studies underway at MSU to evaluate the use of bio fungicides based on the biological control bacteria *Bacillus subtitles* (Serenade, Agra Quest) and *B. pumilus* (Sonata, Agra Quest), and the biocontrol fungus *Trichoderma harzianum* (T-22 Planter Box, Bioworks) for control of Fusarium dry rot on potato. These compounds are being evaluated both as seed treatments and for postharvest application in storage, (Haase, 2007).

2.4.4 Chemical Control:

Presently, Ristaino *et al*. (1997) reported that methyl bromide fumigation is used extensively for tomato production in some 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 bein examined such as Difenoconazole, Carbendazim 12%/ Mancozeb 63%. to be sprayed to run off on onset of disease on tomato crop as Powdery mildew and early blight in Sudan.

CHAPTER THREE

MATERIALS AND METHODS

3.1Study:

This experiment used the infected tomato plants showing symptoms of wilt of *F. oxysporium .f.spp.lycopersici*, at collage of Agricultural studies, Sudan University of science and technology in December 2017. The invitro evaluate the inhibitor effect of neem oil against the *F. oxysporium .f.spp.lycopersici* was conducted at the laboratory of the plant Pathology, at the same college.

3.2 Materials, tools and equipment used:

All materials and equipments used in this experiment were sterilized using 95% ethyl alcohol. Autoclave was used for Petri plate (glasses) sterilization. And other equipment was sterilized by UV light.

3.3 Samples collected:

Infected parts of tomato plant showing typical symptoms of wilt were collected. Thereafter, they were put in poly bags then transferred to the laboratory.

3.4 Isolation and Identification of the fungus:

The secured plant material (Stems) were cut into small bits (0. 5 to 1.0 cm) and washed well in tap water to remove the adhering dirty particles. The cut pieces were surfaces sterilized by sodium hypochlorite Clorox Naocl at 1% concentration for five minutes, rinsed three time in sterilized distilled water to remove traces of NaoCl and dried on sterilized filter paper. The sterilized stems cuttings were then plated at the rate of five cuttings per plate

on(medial) Potato Dextrose Agar (PDA) media and incubated at 28°C for 7 days of incubation. The medium was supplemented with Chloramphenicol (250mg) as antibacterial agent (Anon., 1981). The isolated fungus was sub-cultured on PDA media for further purification of the fungus. The identification of the fungi was based on visual culture characteristics of the hyphen and compound microscopic examination were also carried out for hyphen and conidia structure based on the method of (Booth, 1977). To confirm that the fungus is *F. oxysporum.* Standard books and research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified fungus was maintained on PDA for further studies.

3.5 Neem Oil:

The Neem oil was obtained from Agriculture Research Corporation, Soba Research Station.

3.5.1 Preparation of crude oil concentrations

The crude oil was prepared as recommended by Okigbo (2006). Three concentrations, 1.5%, 2.5%, and 3.5% were prepared.

3.6 Preparation of fungicide (Amistar Top ta 250 EC) concentrations

Amistar Top is a systemic fungicide; the active ingredients are (Oxistrobin 200gm\L and 125gm\L Divinolonazole). The fungicide was tested by dissolving 0.2ml in 100ml of sterilized distilled water to give hundred percentages as recommended dose. Full cooled (PDA) medium was prepared in 250ml conical flasks. Five ml of the fungicide was added full cooled to PDA medium before boring it in to plates.

3.7 Bioassay:

Inhibition zone technique was used in this study (Rao and Srivastava, 1994). To evaluate the effect of each concentration of oil and fungicide on linear fungal growth. Initially, fresh fungal growth was prepared from previously maintained culture of *Fusarium oxysporum*. Prepared PDA media was amended with the required concentration from neem oil and fungicide before being solidified in a conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 30 ml of PDA, were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

One mycelial disc (0.5ml) of the fungus was placed in the centre of PDA plates where opposite poles were marked at the back of the plate and incubated at 25°C in incubator.

The Plates of all experiment were arranged with four replication in an incubator and incubated at 25 C^0 for 3 days. The radial growth of pathogen was measured at 24 h intervals. The growth of the fungus was measured and calculated successively after 3, 4 and 5 days after inoculation. The effect of each oil concentration as well that of the fungicide on linear fungal growth 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.

3-8 Statistical Analyses

The data was statistically analyzed by (GeneStat18th edition) software computer program according to analysis of variance (ANOVA). Duncan's Multiple Range Test was used for means separation.

CHAPTER FOUR

RESULTS

4.1: Effect of neem seed oil different concentrations and Fungicide on redial growth of the fungus *in vitro* after four to six days of inoculation.

The results indicated that neem seed oil at all test concentrations as well as the fungicide reduced significantly ($P \leq 0.05$) the fungal growth compared to untreated control (Table1 and Figures). Moreover, the fungicide at concentration 100% (0.2\100ml) demonstrated the highest inhibition of fungal growth followed in descending order by neem seed oil concentrations.

Table (1): shows effect of neem oil and fungicide (Amstar top) on the linear growth (inhibition zone %) of *Fusarium oxysporum f.sp. Lycopersici Invitro.*

Concentrations	Inhibition Zone %		
	Days after inoculation		
	4	5	6
1.5	62.075(52.08)^{c}	39.89(39.09)^{c}	35.82(36.65)^{b}
2.5	77.6(61.79)^{b}	46.6(43.03)^{bc}	32.3(40.95)^{b}
3.5	80.57(65.97)^{ab}	50.55(45.56)^{b}	43(40.95)^{b}
Fungicide	68.04(67.22)^{a}	72.7(58.61)^{a}	64.42(53.59)^{a}
Control	0.07(0.00)^{d}	0.07(0.00)^{d}	0.07(0.00)^{d}
C.V%	6.2	10.7	14.5
L.S.D	4.66	6.00	7.25

Mean bearing same superscripts are not significant different at ($p \leq 0.05$).

Values in parentheses transformed using Arcsine.

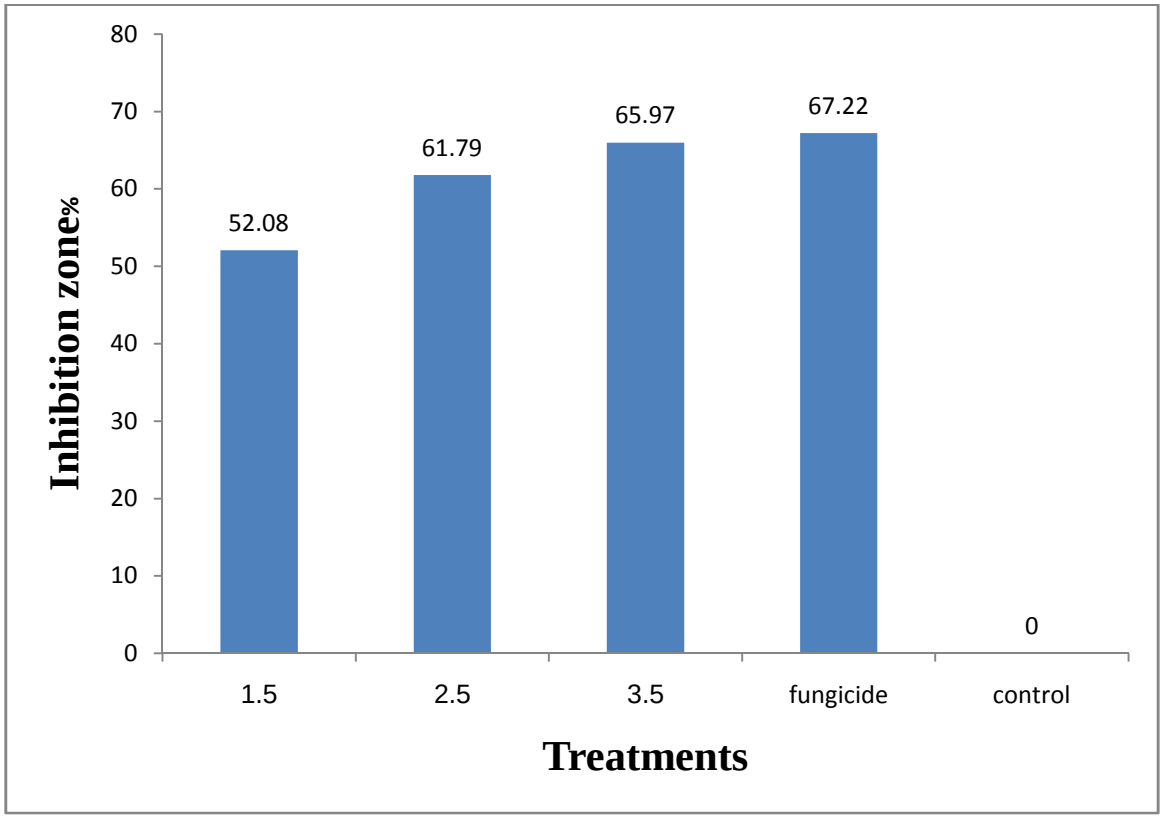

Figure 1. Effect of neem seed oil and fungicide on inhibition zone percentage of *Fusarium oxysporum f.sp*. after four days of inoculation *invitro.*

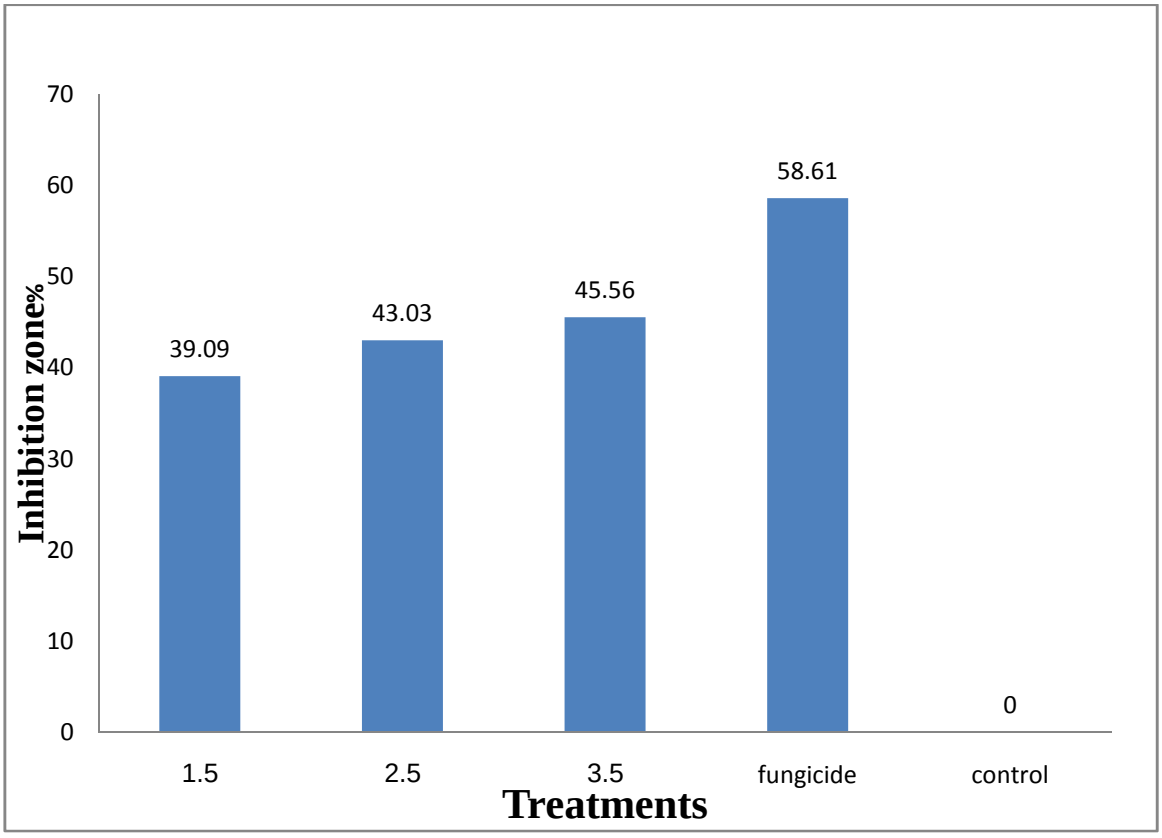

Figure 2. Effect of neem seed oil and fungicide on inhibition zone percentage of *Fusarium oxysporum f.sp.* after five days of inoculation *invitro*.

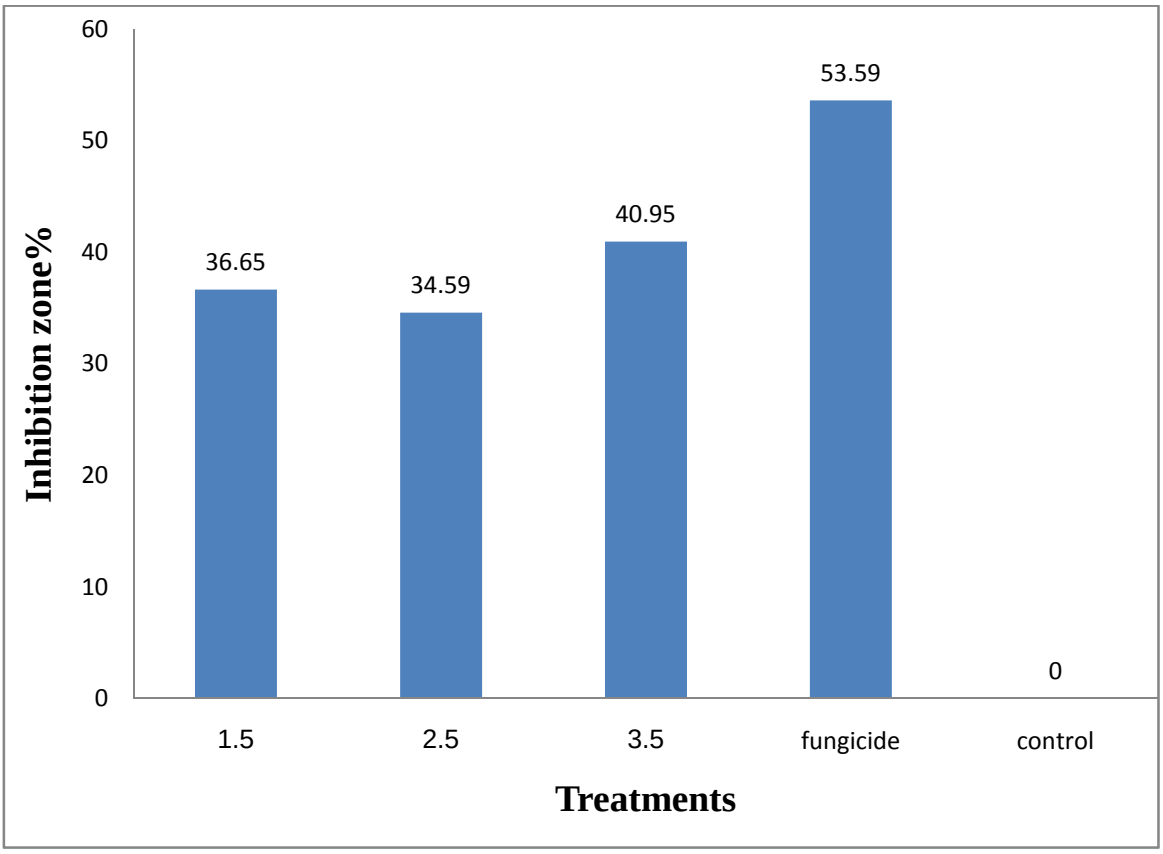

Figure 3. Effect of neem seed oil and fungicide on inhibition zone percentage of *Fusarium oxysporum f.sp.* after six days of inoculation *invitro*.

CHAPTER FIVE

DISCUSSION

The non-rational uses of synthetic pesticide have caused serious problems to human and animal health in addition to their negative impact on environment. These problems include contamination of the biosphere, toxicity to man, animal and beneficial insects and other non target organisms. This have drawn the attention of the researchers and public to adopt new pest management strategies based on safe alternate products of low environmental persistence, highly specific, cheep, available and biodegradable . This was further highlighted by Agrafotis (2002), who reported that the development of new different antimicrobial agents more safe is very important step. The aim of this study was to explore the antifungal activity of neem seeds oil and the efficacy of systemic fungicide in suppressing the growth of the *F. oxysporum* f.sp. *Lycopersici* the causal agent of wilt in tomato (*L. esculentum* Mill.) which is one of the most popular commercial vegetable rich in vitamins A, B, and C grown throughout the world . In the Sudan, the tomato is considered as one of the major vegetable crops and cash earning. The results obtained revealed that all concentrations (3.5, 2.5 and1.5%ml) of neem oil as well as the standard dose of fungicide (0.2ml/100ml) inhibited the growth of the fungus *F. oxysporum f.sp. Lycopersici* with significantly high inhibition zones percent (43%, 52%, 57%, and 80%) respectively compared to untreated control. This result is in line with that obtained by Abdelghany, (2014) and (William.(2008) who reported that the antifungal activity of *A. indica* extract. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials Okigbo and Ogbonnaya (2006); Shariff *et al.*, (2006) reported that neem tree parts contain a number of chemical compounds, most important of which are Azadirachtin. The Azadirachtin and

other biochemically active component of neem collected from different sites vary in their effect (Eemel *et al*., 1986).

However, in field of plant protection, Neem used to control: Grey leaf spots, powdery mildew and viruses (Stoll, 2000). Similar results were obtained by 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.* The results obtained from the use of fungicide on the fungus showed that the fungicide expressed suppressive ability on the growth of the Fusarium with significantly high inhibition zones percent 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 fungicide against *Fusarium oxysporum* where he found that fungicide induced 100% inhibition against the fungus when applied at 100ppm after 7days of exposure. Similar finding were also obtained by Mohammed (2005) who found that fungicides when applied at 10ppm against *Drechslera hawaiiensis* induced 100% inhibition after 4 days. This study also demonstrated that test fungus responded differently to the different concentrations (3.5, 2.5,and1.5ml/%) of neem oil. This variability in response which expressed by the fungus to different concentrations of Neem oil 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 the ones which obtained by (Reem, 2012; Alhadi 2013 and Faiza, 2013).

Conclusion:

Neem oil at all test concentrations exhibited an inhibitory effect *invitro* on fungal growth and there are still less than the Amistar top fungicide (standard).

Recommendations:

Based on the results findings the flowing will be recommended:

1. Further investigate (studies) of the antimicrobial properties in a group of botanicals against target organisms to determine their potentials properties so far.
2. Studies of a phytochemicals analysis to be carry out of different neem seeds form difference sites to determine the variants bioactive ingredient in each location.

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APPENDICES

Appendix:1

Analysis of variance table (one way ANOVA TABLE) Effect of neem seed oil different concentrations and Fungicide (Amster Top) on redial growth of the fungus in vitro after four days fter inoculation.

Variate: growth /ml

Source of variation	d.f	s.s	m.s	V.r	Fpr
Concentrations	4	1 2771.903	3192.076	341.77	<.001
Residual	13 (2)	121.451	9.342		
Total	17 (2)	12189.236			

Coefficient of variation = 6.2%

Appendix: 2

Analysis of variance table (one way ANOVA TABLE) Effect of Neem oil different concentrations and Fungicide on redial growth of the fungus in vitro after five days from inoculation.

variant: growth /ml

Source of variation	d.f	s.s	m.s	V.r	Fpr
Concentrations	4	7798.95	1949.74	122.92	<.001
Residual	15	237.92	15.86		
Total	19	8036.87			

Coefficient of variation = 10.7%

Appendix: 3

Analysis of variance table (one way ANOVA TABLE) Effect of Neem oil different concentrations and Fungicide on redial growth of the fungus in vitro after six days from inoculation.

Variant: growth /ml

Source of variation	d.f	s.s	m.s	V.r	Fpr
Concentrations	4	6366.91	1591.73	68.76	<.001
Residual	15	5.4075	0.3605		
Total	19	6714.13			

Coefficient of variation = 14.5%

Appendix 4.

Plate 1. Preparation of medium, neem oil and fungicide

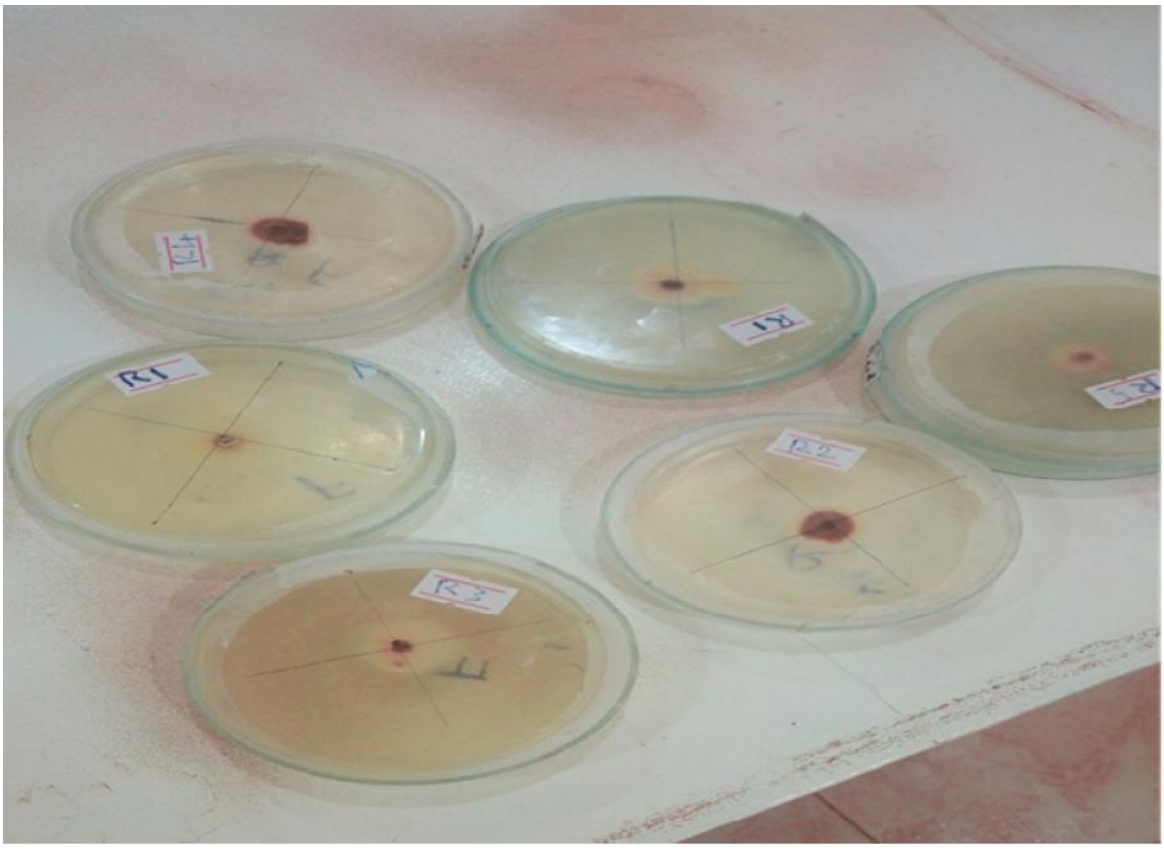

Plate 2. The effect of different concentration of Neem seeds oil and fungicide (Aster top).

Plate 3. The treatment under incubation.

Appendix 5.

Table(1): Effect of neem seed oil and fungicide (Amster top) on growth of *fusarium oxysporum f.sp.lycopersici* after four days after inoculation *invitro*.

Concentrations (%)	Inhibition Zone %			
	R1	R2	R3	R4
1.5	7.3	9	9	6.5
2.5	4.25	5.25	5	4.25
3.5	2.75	4.5	5	4
Fungicide	3.25	0	0	3
Control	21	22	20	21

Table 2:Effect of neem seed oil different concentrations and fungicide on radial growth of *fuusariu oxysporium .f.sp. lycopersici*. invitro after five days after inoculation.

Concentration (%)	**Inhibition Zone (%)**			
	R1	R2	R3	R4
1.5	16	16	18	12
2.5	16	13	13	13
3.5	16	13	12	10
Fungicide	5	7	7	9
Control	26	26	26	25

Table 3:Effect of neem seed oil different concenterations and fungicide Amstr top on radial growth of *fusarium oxysporium .f.sp.lycopersici*.in vitro after six days after inoculation.

Treatment	Inhibition zone %			
Concentration (%)	R1	R2	R3	R4
1.5	19	19	20	14
2.5	20	18	18	19
3.5	16	17	17	13
Fungicide	5.5	12	12	10
Control	27	28	27	29