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

Evaluation of Garlic (*Allium Sativum* L.) Aqueous Extract and the Fungicide (Till Nour 25%EC) against *Fusarium oxysporium* f.sp. *ciceris* InVitro

تقويم فاعلية المستخلص المائي للثوم والمبيد الفطري (Till Nour 25% EC) والتأثير التثبيطي على نمو الفطر *F.oxysporium* f.sp. *ciceris* معمليا

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

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October, 2019

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الآية:

قال تعالي:

(وَهُوَ الَّذِى أَنزَلَ مِنَ السَّماءِ ماءً فَأَخرَجنا بِهِ نَباتَ كُلِّ شَىءٍ فَأَخرَجنا مِنهُ خَضِرًا نُخرِجُ مِنهُ حَبًّا مُتَراكِبًا وَمِنَ النَّخلِ مِن طَلعِها قِنوانٌ دانِيَةٌ وَجَنّـٰتٍ مِن أَعنابٍ وَالزَّيتُونَ وَالرُّمَّانَ مُشتَبِهًا وَغَيرَ مُتَشابِهٍ انظُروا إِلىٰ ثَمَرِهِ إِذا أَثمَرَ وَيَنعِهِ إِنَّ فى ذٰلِكُم لَـٰآيـٰتٍ لِقَومٍ يُؤمِنُونَ ۝99))

صدق الله العظيم

سورة الأنعام(99)

DEDICATION

إلى من أدين لها بعمري ومستقبلي... . إليك يا رمز الصبر والحنان .... إليك يا أروع معاني الحب والتضحية.... فمنك تعلمت معني الحياة والصبر وكبرياء النفس وطيبة القلب.... أهدي إليك ثمرة جهدي.... **أمي الغالية**

يامن سكنت مخيلتي وترسخت في ذاكرتي.... وبنيت مهجتي ووهبتني حياتي.... فكونت منك ذاتي .... **أبي الغالي**

شكرا لك **أخي الغالي** على بقائك بجانبي وقت حزني وشدتي دامك الله لي نعمة....

أخي الحبيب لك بعض كلمات الشكر بالرغم من أنها لا توافيك حقك فشكرا لك يا شمس حياتي

أختي... أنت نعمة من نعم ربي.... وانا يا ربي بنعمتك اكتفيت الله يحفظك ويجزيك خير الدنيا ونعيم الأخرة

لك منزلة بالروح م حل به حي... عرش الغلا لك بالمحبه لحالك ....

الي رفيق دربي وسندي بالحياة.... **خطيبي (يوسف)**

هو حكايتي التي عجزت عن الاكتمال .... ونعيمي الذي لن ينتهي وسعادتي الدائمة.... اليك **خالي (عبد الله)**

اسم أخر للحب وقطعه من الأم.... اليك **خالتي (ماجدة)**

أخوة الي ونعمة من ربي علي.... الله لا يحرمني منكم.... **(مؤمن, سمر)**

إلى رفقاء الدرب **زملائي وزميلاتي**... إلى من أضاءوا لي الظلمات بنور العلم والمعرفة **أساتذتي الأجلاء ...** وإلي كل **طالب علم**

ACKNOWLEDGEMENT

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

I would like to express my thanks to my supervisor Dr. Amin Hussein Ibrahim, Plant Protection Department CAS, SUST; who devoted most of his time to teach us on various disciplines of scientific research.

Also Special Thanks are due to my colleagues in the M.Sc. “Batch 10” who helped me during the experiments. Grateful thanks are due to all the staff of the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology.

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ABSTRACT

Chickpea(*Ciceri arietinum* L). Is an important crop in Sudan. This imperative crop is severely affected by the wilt disease of *Fusarium oxysporium* f.sp. *ciceris*. However, the indiscriminate use of synthetic fungicides and their increasing hazards to the public health and the environment coupled with the development of resistant strains of phytopathogenic fungi has led to the use of safe alternate products. In the present study, the fungus was isolated from infected Chickpea plants and cultured in PDA media. Then the cultured fungus was confirmed to be *Fusarium oxysporium* f . sp. *ciceris* when compared to a previously prepared slides at the plant pathology laboratory. The cloves of garlic (*Allium sativum* L.) aqueous extract was tested for their antifungal potential against *F. Oxysporium* f. Sp. *ciceris* at the Plant Pathology Laboratory of the College of Agricultural Studies (CAS), Sudan University of Science and Technology during 2019. Various concentrations i.e. 25, 50, and 75% of garlic cloves aqueous extract were prepared and their *invitro* bioactivity was examined against the said fungus. Our results revealed that All the tested concentrations were found significantly (P0.05) inhibiting the growth of the fungus. The higher concentration of aqueous extract (75%) caused the maximum inhibition in the diameter of the tested fungus by 100% followed by the concentration (50%) which inhibited the fungus growth by 45.4% and the concentration (25%) by 36.1 %, while the fungicide “Till Nour 25%” inhibited the fungus growth by 88.8%. The inhibition zone of the three tested concentrations i.e. 25, 50, and 75% were compared with untreated control and a standard chemical (Till Nour 25%). Our results conclude that garlic aqueous extract are promising and encouraging to carry out further chemical analyses of other parts of garlic plant to identify the effective ingredients to use as alternatives to harmful pesticides that adversely affect human, animal and environment. Finally, we recommend further

studies for the bioactivity of the garlic extracts by using other parts of the plant. More studies is also needed to examine the effect of garlic extracts on the wilt fungus “*InVivo* under field conditions.

الملخص

نبات الحمص محصول هام في السودان يتأثر بشدة بمرض الذبول الفيوزاريومي المتسبب عن الاصابة بفطر *F. oxysporium* f. sp. *Ciceris* . إن الاستخدام العشوائي لمبيدات الفطريات الاصطناعية ومخاطرها المتزايدة على الصحة العامة والبيئة إلى جانب تطوير سلالات مقاومة للفطريات الممرضة للنباتات قد أدى إلى استخدام منتجات بديلة آمنة. في هذه الدراسة ، تم عزل الفطر المسبب من نبات الحمص المصاب وتمت تنميته علي بيئة دكستروز البطاطس (PDA) . ومن ثم تم تأكيد تعريف الفطر النامي في هذه البيئة على أنه فطر الفيوزاريوم *F. oxysporium* f. sp. *Ciceris* بمقارنته بشريحة معدة مسبقا بمعمل أمراض النبات.
أجريت هذه الدراسة بمعمل أمراض النبات التابع لكلية الدراسات الزراعية – شمبات ، جامعة السودان للعلوم والتكنولوجيا خلال العام 2019م. عني هذا البحث بدراسة الأثر الحيوي للمستخلص المائي لفصوص الثوم *Allium sativum* L. على نمو الفطر موضوع الدراسة تحت ظروف المعمل. تم تحضير تركيزات مختلفة ، 25 و 50 و 75 ٪ من مستخلص مائي من فصوص الثوم ، وتم فحص تأثيرها في المختبر ضد الفطر موضع الدراسة في 5 مكررات وقورنت النتائج بالشاهد غير المعامل وكذلك بالاطباق المعاملة. ومن ثم تم تحليل البيانات إحصائيا على المستوى (P0.05). كشفت نتائج البحث أن جميع التركيزات المختبرة مؤثرة معنويا (P0.05) مثبطة لنمو الفطر. حيث كان التركيز 75% هو صاحب الحد الأقصى لتثبيط نمو الفطر المختبر بنسبة 100% تبعه التركيز 50% بنسبة 45.4% متبوعا بالتركيز 25% الذي ثبط نمو الفطر بنسبة 36.1%. بينما كان أثر المبيد الفطري ((Till Nour 25%) تثبيط نمو الفطر بنسبة 88.8% مقارنة بالشاهد. خلصت نتائجنا التي تم الحصول عليها من هذه الدراسة إلى أن جميع التركيزات المختبرة من مستخلص الثوم المائي يبشر بالخير ويشجع على إجراء المزيد من التحليلات الكيميائية لأجزاء أخرى من نبات الثوم لتحديد المكونات الفعالة لاستخدامه كبديل للمبيدات الحشرية الضارة التي تؤثر سلبًا على الإنسان والحيوان والبيئة. من ناحية أخرى، نوصي بإجراء مزيد من الدراسات للنشاط الحيوي لمستخلصات الثوم علي فطر الزبول علي نباتات الحمص تحت ظروف الحقل.

CHAPTER ONE

INTRODUCTION

Chickpea (*Ciceri arietinum L.*) is a vital source of plant derived edible protein in many countries. Chickpea also is considered as one of the most important food pulse legumes, providing a major source of low-cost protein for masses of low-income groups. Apart from being an important source of dietary protein for human consumption, this leguminous crop is also important for management of soil fertility due to its nitrogen-fixing ability (Agrios, 2005). It is cultivated on about 9.89 million hectares in the world, with an average annual production of 7.80 million tons per Year (FAO, 2002).

In Sudan, chickpea is widely grown as pulse legume crop. According to FAOSTAT (2017), the area harvested of chickpea estimated to 6851 hectare in 2017, mostly concentrated in the northern States. The average of area harvested during the period 1999-2017 is estimated as 8126.26 hectare of an average production 15430.05 Tons during the same period (Figure 1 and figure 2).

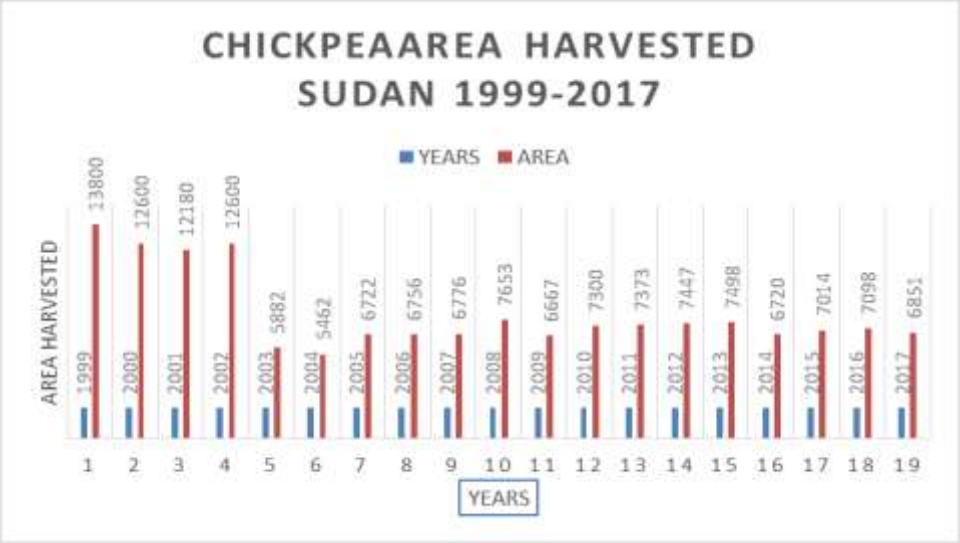

Figure 1: Chickpea production in Sudan during 1999-2017 (source FAOSTAT 2017)

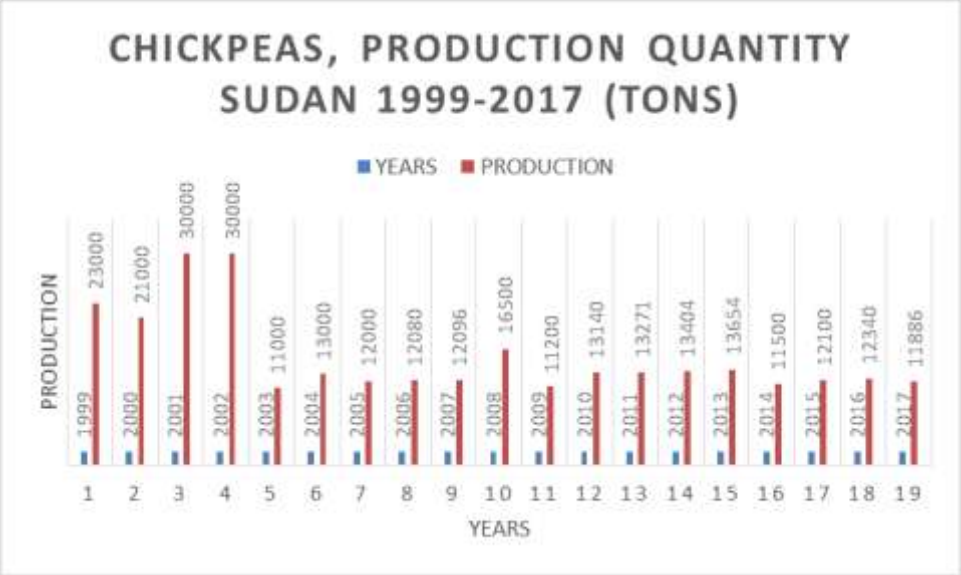

Figure 2: Chickpea harvested area in Sudan during 1999-2017 (source FAOSTAT 2017)

Low yield of chickpea attributed to its susceptibility to several fungal, bacterial, and viral diseases. Among the diseases affecting chickpea, vascular wilt caused by an important obligate biotroph *Fusarium oxysporium* f. sp. *ciceris* (Padwick). Although the disease is wide spread in the chickpea growing areas of the world, it is most prevalent in the Mediterranean Basin and the Indian subcontinent (Jalali and Chand, 1992).

In Sudan, the crop is subjected to several diseases that are known to limit production of chickpeas. *Fusarium oxysporium* f. sp. ciceris (Fusarium wilt) is the most problematic that reduces yields up to 100 % in severe cases (Ali, 1989). In fact, Fusarium wilt and root rot are the most important diseases of food legumes in the Sudan (Ali, 1989). It is reported that the disease is especially serious in the traditional production areas of Wad Hamid basin in Northern Sudan, where chickpea is grown on residual soil moisture after the flood waters of the River Nile subside. In these areas, farmers do not adhere to crop rotation and the crop at the

post-flowering stage is often subjected to moisture stress in years of low flood (Faki *et al*., 1992; Ali, 1996).

F. oxysporium f.sp. *ciceris* is a highly virulent pathogen which causes diseases in many hosts including potato, tomato, cucurbits ,legumes (chickpea),and banana are a few of the most susceptible plants, but it will also affect other herbaceous plants (Pan Germany,2010).

Fusarium wilt epidemics cause significant annual losses of chickpea yields which, account for 10% to 15% of the total yield and sometimes escalate to 100% under conditions favorable for the disease to develop (Navas-Cortés *et al.*, 2000). *F. oxysporium* f. sp. *ciceris* infects chickpea at seedling as well as at flowering and pod forming stages (Grewal, 1969), with more incidence at flowering and pod forming stages if the crop is subjected to sudden temperature rise and water stress (Chaudhry *et al*., 2007).

Following infection of host roots, the fungus enters the xylem tissues and spreads rapidly up through the vascular system, becoming systemic in the host tissues, and may directly infect the seed (Cho and Muehlbauer, 2004). Translocation of water and nutrients is severely prevented by blockage of vessels, resulting in stomata closure, wilting and death of leaves, often followed by death of the whole plant (Cho and Muehlbauer, 2004). Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rough and dull than those from healthy plants (Haware and Nene, 1980). *F. oxysporium* f. sp. *ciceris* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for up to 6 years (Singh *et al*., 2007).

Resistance breeding programs primarily manage the disease, but high pathogenic variability and mutability limit the sustainability and effectiveness of any naturally selected resistance against the pathogen (Nimalkar *et al*., 2006). Management of Fusarium wilt with fungicides is uneconomical and difficult to achieve because of the soil and seed-borne nature of the pathogen (Ahmad *et a*l., 2010). Moreover, the application of fungicides causes groundwater pollution, loss of nontarget beneficial flora and evolving fungicidal resistance variants of the pathogen. (Ahmad *et a*l., 2010)

Similarly, amending soil with plant extracts significantly reduces Fusarium wilt in the field (Chand and Singh, 2005). Therefore management of *Fusarium oxysporium* of chickpea should be based on strategies that combine the use of additive or synergistic combinations of biotic, cultural, and chemical control measures (Landa *et al*., 2004).

However, excessive usage of fungicides damages the environment and human health. Plant derived pesticides and plant metabolites have shown as the best substitutes of synthetic pesticides. They have less harmful impacts on environment and less hazardous to users in contrast to chemical fungicides (Chand and Singh 2005).

There are reports that crude extracts of many plant species namely Neem, datura ,jatropha, argel, calotropis and garlic (Chand and Singh 2005).

Garlic (*Allium sativum*), family Amaryllidaceae, known to possess antimicrobial and medicinal derivatives (Lewis, *et al.,* 2003; Younis, *et al.,* 2010).

Keeping in view the bioactive potential of *A. sativum,* this study was designed to explore its potential for the control of *F.oxysporium* the cause of Fusarium wilt disease of chickpea.

Objectives of the study

The aim of this research work is to

- isolate the causal fungus from chickpea stem.
- determine antifungal activity of aqueous garlic extract on fungus at different concentrations and to determine the minimum inhibitory concentration of aqueous extract of garlic. To explore the antifungal potential of plant aqueous crude extract against *F. oxysporium* f. sp.*ciceri in vitro*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Chickpea

Chickpea is considered as one of the most important food pulse legumes, providing a major source of low-cost protein for masses of low- income groups.

2.1.1 Classification

Kingdom: *Plantae*

Division*:* Magnoliophyta

Class: *Magnoliopsida*

Order*: Fabales*

Family: *Fabaceae*

Subfamily: *Faboideae*

Genus: *Ciceri*

Species: *Ciceri arietinum*

Ciceri arietinum L. belongs to the Dicotyledonous crops, which are the most important family with an annual world market value. Furthermore, chickpea was second in importance to cereals among world food production (Winter *et al*., 2003).

2.1.2 The importance of chickpea

Chickpea belongs to the family Fabaceae (Leguminosae) (Rajput *et al.*, 1996). It is an important crop among the major leguminous crops of Sudan and the world ranking first in South Asia and Mediterranean basin. About 75% of the world

production is contributed by India (Saxena, 1990; FAO, 2007). Whereas, according to Robertson *et al.*, (1995) chickpea ranked second in the world after dry peas food legume. It is a cool season annual pulse crop which is grown in subtropical, tropical and temperate regions of the world. (Emenky *et al.*, 2008). It has high protein digestibility and richer in phosphorus and calcium than other pulses. It possesses higher fat content, fiber and protein (Emenky *et al.*, 2008).

In Sudan, chickpea is one of the principal cool-season food legumes as the crop has a significant role in the diets of the Sudanese people and contributes to the economy of the country. Moreover, the crop is gaining further importance as a source of protein for the majority of the population who cannot afford animal products because of escalating prices.

2.2 Chickpea Problems

Nene and Reddy (1987) reported more than 50 pathogens so far from different chickpea growing countries. Nene (1982) also found chickpea blight (*Ascochyta rabiei* (Pass) Lab.) and wilt (*F. oxysporium* f. sp. *ciceris*) as the most important diseases of chickpea, including dry root rot (*Rhizoctonia bataticola*, *Macrophomina phaseolina*), black root rot (*Fusarium solani*), grey mould (*Botrytis cinerea*), Phytophthora root rot (*Phytophthora megasperma*), *Pythium* seedling and seed rot (*Phythium ultimum*) and stunt (Pea roll virus). Among all diseases, *Fusarium* wilt is supposed to be one of the most important disease depending on the conducive conditions, availability of inoculum and cultivation of susceptible host.

In Sudan chickpea is also suffers from various biotic factors including wilt and root rot diseases with different infection incidence (Mohamed *et al.*, 2018*)*.

2.2.1 Chickpea wilt (*Fusarium oxysporium* f. sp. *ciceris*)

Fusarium oxysporum is a seed and soil-borne fungal pathogen that causes fusarium wilt of chickpea (Haware, 1990; Nene and Reddy, 1987).

2.2.1.1Classification

Kingdom*: Fungi*

Phylum: *Ascomycota*

Class: *Sordariomycetes*

Order: *Hypocreales*

Family*: Nectriaceae*

Genus: *Fusarium*

Species: *Fusarium oxysporium* f.sp. *ciceri*

Chickpea wilt has been reported almost all over the world. The first report of this fungus was described in India (Butler, 1918). The pathogen is either soil-borne or seed –borne and it belongs to *Fusarium* spp. (McKerral, 1923; Kraft *et al.*, 1994). *Fusarium* wilt has a worldwide occurrence (Jalali and Chand, 1992). Khune and Patil (1992) studied *F. oxysporium* f. sp. *ciceris* isolation from the tap root, lateral roots, main stem, lateral branches and seed of infected chickpea, but not from pod hulls or leaves.

2.2.1.2 Fungal description

Booth (1977) reported that *F.oxysporium* f.sp. *ciceris* (Padwick) is with colourless mycelium at first, but it becomes creamy in colour with age, in addition it may be pale yellow, pale pink or purplish. These colours give the characteristic culture pigmentation.

The fungus produces three types of asexual spores, micro conidia, macro conidia and chlamydospores. The macro conidia are straight to slightly curved, slender, thin walled usually with three or four septa, a foot shaped basal cell and a tapered and curved apical cell. They are generally produced from phialides on conidiophores by basipetal division. They are important in secondary infection.

The micro conidia are ellipsoidal and either have no septum or a single one. They are formed from phialides in false heads by basipetal division and are important in secondary infection. The chlamydospores are globosely and have thick walls. They are formed from hyphae or alternatively by the modification of hyphal cells. Micro conidia considered as endurance organs in soils where they act as inocula in primary infection. Teleomorph or sexual reproductive stage, of the fungus is unkown.

2.2.1.3 Symptoms of *Fusarium* wilt on chickpea

Fusarium wilt of chickpea is seed-borne. Seeds harvested from wilted plants when mixed with healthy seeds can carry the wilt fungus to new areas and can establish the disease in the soil to economic threshold levels within three seasons (Pande *et al.*, 2007). The disease also observed at seedling and flowering stages of plant growth. The symptoms which can be observed are drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally complete loss of the plant (Westerlund *et al.*, 1974). The pathogen was also isolated by Mohamed (2013) in Sudan and described similarly.

Pathogen gets entry into xylem vessel and invades whole vascular system, inducing symptoms of yellowing and wilting. In the absence of host plant the pathogen can survive up to six years in soil and plant debris (Haware, 1993).

Chauhan (1962) reported that, the initial symptoms of the disease due to pathogen infection to be vein clearing of leaves and decrease in the chloroplast and starch formation in mesophyll cells. Whereas, Erwin (1957) characterized chickpea wilt by yellowing of leaves and necrosis of the xylem. Leaves of the wilted plants turned grayish green, then became dull yellow and wilted. The xylem and pith become darkened and discolored. Moreover, internal discoloration of pith and xylem can be seen if stem and root of the wilted plants split vertically (Saxena and Singh, 1987). The disease resulted in reduced plant population, reduced spear size and sub-optimal yield (Ravikumar *et al.*, 2007)

The fungus attack the root system made its way through epidermis, cortex and finally penetrates into the xylem vessels of the tap root from where it spread further. As a result, the lateral roots wither off. In many cases xylem vessels had been found to contain fungus mycelium, interfering with normal translocation of the sap. Seeds harvested from wilted plants were lighter in weight, rough (wrinkled surface) and dull in colour as compared with those obtained from healthy plants (Murumkar and Chavan,1985).

2.2.1.4 Disease cycle of *Fusarium oxysporium* f. sp. *ciceris*

Fusarium oxysporium is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith *et al*., 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types – macro conidia, micro conidia and chlamydospores (Agrios, 2005).

Healthy plants can become infected by *F. oxysporium* f. sp. *ciceris* if the soil is contaminated with the fungus. The fungus can invade a plant with its either

sporangial germ tube or mycelium by invading the plants' roots. The roots can be infected directly through the root tips, through wounds in the roots, or at the formation point of lateral roots (Agrios, 2005). Once it has been inside the plant, the mycelium grows through the root cortex intercellular. When the mycelium reaches the xylem, it invades the vessels through the xylems' pits. At this stage, the mycelium remains in the vessels, where it usually advances upwards toward the stem and crown of the plant. As it grows, the mycelium branches and produces micro-conidia, which are carried upward within the vessel by the plants sap stream. When the micro-conidia germinate, the mycelium can penetrate the upper wall of the xylem vessel, enabling more micro-conidia 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 plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. At this point the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios, 2005). The resulting spores can then be used as new inoculums for further spread of the fungus

2.2.1.5 Survival and mode of dissemination of *F. oxysporium* f.sp *ciceris*

The pathogen is seed and soil borne, facultative saprophyte, in the absence of susceptible host; it can survive up to six years in the soil (Haware *et al.,* 1992). When the inoculum is developed in the soil, it is difficult to check the disease or eliminate the pathogen except by following crop rotation for more than six years (Gupta, 1991; Haware and Nene, 1982).

From mycelium, spores or sclerotia, the fungus spreads in the soil to a small extent, the other mode of dissemination of the diseases are farm equipment, trans planters, tubers, seeds, cuttings of infected plant and in some cases wind blown spores and conidia. The life cycle of the pathogen has a parasitic and saprophytic phases (Backman and Turner,1989). The chlamydospores of the pathogen remained viable throughout the high temperature in the summer months during the non-cropping period in naturally infected roots of chickpea at soil depth of 5, 10 and 15cm. The fungus can not survive in the roots at soil surface (Sharma and Gupta, 1987). The exudates released from root allows mycelium growth and germination of chlamydospores in the presence of host plant (Schroth and Hildebrand, 1964) whereas, the fungus can survive by formation of chlamydospores in the absence of host plant (Schippers and Van Eck, 1981).

2.3 Management

Management of Fusarium wilt in chickpea would be best achieved if those disease control measures are used within an integrated management strategy where by their use is combined either simultaneously or in a sequence (Haware *et al*., 1990)

2.3.1 Cultural management

Cultural management is only practical measure for controlling the diseases in the field. The wilt fungus is widespread and so persistent in soils, so seed bed sterilization and crop rotation should be practiced although they are of limited value (Agrios, 2005).

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

Prevent spreading of the pathogen to disease free areas by using clean tools and equipment (Agrios,1997; jones *et al*.,1997).

2.3.2 Biological control

Recently, biological control of Fusarium wilt seems to be successful. Such control methods include prior inoculation of plants with nonpathogenic strains of *F. oxysporium* or the use of antagonistic fungi, such as Trichoderma and Gliocladium, *Pseudomonas fluorescens*. Although promising, none of these methods have been used for control of Fusarium wilt in practice so far (Agrios, 2005).

2.3.3 Botanical control

The medicinal and antimicrobial activities of extracts from plants are gaining attention of researchers worldwide. The modern medicine has its own advantages and side effects, so the plant based products are getting more popularity, as they are safe to use, and comparatively easily available and cheap. Many extracts possess antifungal activity (Siripornvisal, *et al.* 2009). Plant extracts and essential oils are effective in plant pathogens (Tripathi, *et al*., 2008). Apart from the use of plant based products in medicine, the usage of these extracts in plant protection also now becoming popular throughout the world (Tzortzakis, *et al.*, 2007; Dellavalle, *et al.* 2011). 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 have been tested against *Fusarium oxysporium* species for inhibitor effect (Bowers, and Locke, 2000).

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; Singh and Hair Chand, 2004). Garlic is one among the important earliest known medicinal plants (Lewis, *et al.* 2003; Younis, *et al.* 2010).

Garlic *(Allium sativum*) is a species in the onion genus, *Allium*, its close relatives include the onion, shallot, leek chive , and Chinese onion.

2.3.3.1 Garlic tree

Being an important food spice plant, it has significant role in disease prevention and control, many of the diseases can be cured with garlic (Yousuf, *et al.*, 2010). It has been used since long time against human pathogens, but studies are less regarding the usage of garlic against plant pathogens. Some earlier works (Russel, *et al.* 1977; Obagwu, *et al.*, 1997; Garcia, 1999; Obagwu and Korsten, 2003; Kanan and Al-Najar, 2008) deals with the action of garlic against pathogens.

2.3.3.2 Classification

Kingdom: plantae

Clade: Angiosperms

Clade :Monocots

Order: Asparagales

Family: Amaryllidaceae

Subfamily: Allioideae

Genus: Allium

Species: A.sativum

2.3.3.3 Origin and major types

Allium sativum grows as wild or cultivated species. The "wild garlic", "crow garlic and "field garlic ", there are at least 120 cultivars originating from central Asia, making it the main center of garlic biodiversity (Kamenetsky *et al.,* 2005)

2.3.3.4 Uses

Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking (Katzer, 2009).

Fresh or crushed garlic yields the sulfur-containing compounds alliin, ajoene, diallyl polysulfides, vinyldithiins, S-allylcysteine, and enzymes, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds (Block Eric 2010.). Singh *et al* (1990) reported that a compound derived from garlic (*A. sativum* L) ajoene, inhibited spore germination of some fungi, namely, *Alternaria solani, A. tenuissima, A.triticina, Colletotricum sp.,Curvularia sp., Fusarium oxysporium, F. semetictum, and F.udum,* which cause serious diseases in some important crop plants in India. On the other hand they suggested that It is quite likely that the compound may be useful in controlling these diseases under field conditions. Another auther, Abdalla (2017) also reported

a noticeable inhibition of *Aspergillus niger* growth using minced garlic cloves of a Sudanese varierty.

2.3.4 Chemical control

Christian *et al.* (2007) reported significant effect of five fungicides and found that the highest inhibition of the fungus was achieved with Cabindazim, Benomyl, and Captan. Whereas, Joseph (2003) reported Benomyl (0.3 g per L) combined with 1% (w/v) garlic extract effective against *Colletotrichum capsici.* Ayyub (2001) evaluated eleven fungicides and found Benlate, Folicar and Derosal, as the most effective against mycelial growth of Fusarium wilt. Moderate response was observed in case of Topas-l00 and Tilt, whereas, Daconil, Antracol, Apron and Polyram combi in these studies were found least effective. Elfatih *et al.* (2002) use Tecto-TM and Quinolate Pro seed-dressing fungicides for control of chickpea wilt and found neither decreased or increased seedling emergence in the wilt infected plot of farmer's fields.

However, another inhibiting fungicide Till Nour (Propiconazole 250EC) is also used as antifungal compound against the powdery mildew fungus in tomato. Till Nour is a demethylation inhibiting (DMI) fungicide recommended for the control of various important plant diseases. The safety period on tomatoes is 3 days as written on the chemical container.

CHAPTER THREE

MATERIALS AND METHODS

This study was conducted under laboratory conditions at plant pathology laboratory , College of Agricultural studies " (CAS) Shambat", Sudan University of science and Technology in 2019. It was intended to investigate the antifungal activity of crude aqueous extracts of Garlic and efficacy of fungicide (Till Nour 25 %EC),against *Fusarium oxysporium* f.sp. *ciceris*.

3.1 Materials, Tools and Equipment Used

All materials and equipment used in this experiment were sterilized using 95% ethyl alcohol. The autoclave was used for glassware and media sterilization. UV light was used to sterilize other equipment.

3.2 Samples Collection

Random samples of infected chickpea showing typical symptoms of Fusarium wilt were collected from CAS fields. Samples were kept in paper sacks brought to laboratory for isolation and identification of *Fusarium oxysporium* f. sp. *Ciceris* the causal agent of chickpea wilt as well as for further studies.

3.3 Isolation of *F. oxysporium* f. sp. *Ciceris* from Chickpea Stem

Infected chickpea stems showing symptoms of Fusarium wilt disease were used. These stems were cut into small pieces (0.5, to 1.0 cm). Then they were washed thoroughly with tap water, and surface sterilized with Clorox (Naocl) (1%) for 5 minutes. Then these pieces were rinsed in sterilized distilled water (3 times) and dried on sterilized filter paper. The sterilized pieces were then plated at the rate of

5 pieces per plate on to potato dextrose agar medium (PDA). The inoculated Petri-dishes were incubated at room temperature (25^{o} C) for 7days.

The growing fungus were sub cultured on PDA medium for further purification. Furthermore, compound microscopic examinations were carried out for Mycelia and conidia structure based on the method of Booth (1977). *F. oxysporium* identification was confirmed with the support of already prepared slides of *F. oxysporium* at the plant pathology laboratory. Texts and research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified isolates were maintained on PDA medium for further studies.

3.4 Source of Plant Material

Garlic bulbs cloves were obtained from local market and then brought to the laboratory where they were kept without shading or drying until they used for extraction.

3.5 Preparation of Crude Aqueous Extract of Garlic Bulbs (Cloves)

Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo ,(2006). The collected Garlic bulbs (cloves) were cleaned by cutting the heads of the bulbs or any other signs on them. Then the cloves samples were crushed separately to obtain concentrated suspension of garlic extracts. 200 gm of garlic bulbs were placed in a conical flask(500 ml) each, and then completed to 300 ml sterilized distilled water. The mixture was then placed in a shaker for 16hrs. Three concentrations of aqueous extracts was then obtained (25% 50% and 75%) after the extracts were filtered.

A standard systemic fungicide "Till Nour (25%Ec) a.i. propiconazole 250 gm/L" (Plate 1) was used as a positive control with a dose of 0.6 ml in 125 ml of PDA medium.

Plate (1): Fungicide "Till Nour 25% EC"

3.6 Test Procedure

Inhibition zone technique was used in this study according to (Rao and Srivastava, 1994), to evaluate the effect of each concentration of garlic extract and fungicide on linear fungal growth. Initially, fresh fungal growth was prepared from previously maintained culture of *Fusarium oxysporium* f.sp.*ciceris.*

Prepared PDA media was amended with the required concentration of garlic extract and fungicide before being solidified in a 250 ml conical flask, agitated and poured into sterilized glass petri dishes. Five plates, containing 125 ml of PDA, were assigned for each concentration and left to solidify. The other five plates with PDA medium were served as untreated.

One mycelia disc (o.8 ml) of the fungus was placed in the center of the petri dish where opposite poles were marked at the back of the plate and incubated at 25^{o} C for 2 days. The radial growth of the pathogen was measured at 24hrs intervals. The growth of the fungus was measured and calculated successively after 3, 4,5 ,6,7 and 8 days after inoculation . The effect of each garlic concentration as well as that of the fungicide on linear fungal growth was calculated as percentage reduction of the fungal growth (R) where:

R=dc-dt\ dc *100

Where;R=percentage reduction of the growth,

dc=diameter of “control” growth and;

dt=diameter of “treated” growth.

3.7 Experimental Design

The experiment was arranged in a Complete Randomized Design (CRD). The data was statistically analyzed using a computer software program (Statistic8) according to analysis of variance (ANOVA). The Least Significant Difference (LSD) was calculated and used for comparisons between means (Appendix 3).

CHAPTER FOUR

RESULTS

4.1. Location

This study was conducted under laboratory conditions at the Plant Pathology laboratory (CAS, SUST) during 2019, to investigate the inhibitory effect of the aqueous extract of Garlic Cloves and the fungicide Till Nour 25% against the fungus *F. oxysporium* f. sp. *ciceris*.

4.2. Isolation and Identification of the Fungus

In the current study, the fungus was isolated from the infected stems of chickpea. The fungus identification was performed according to the shape of spores and conidia. *F.oxysporium* f.sp. *ciceris* (Padwick) is with colorless mycelium at first, but it becomes creamy in color with age, in addition it may be pale yellow, pale pink or purplish. These colors give the characteristic culture pigmentation. The fungus produces three types of asexual spores. Micro conidia that are ellipsoidal and either have no septum or a single one, they are the typical "Fusarim" spores, normally they are three to five celled, have gradually pointed and curved ends. The macro conidia that are straight to slightly curved, slender, thin walled usually with three or four septa, a foot shaped basal cell and a tapered and curved apical cell. The chlamydospores are globosely and have thick walls.

The isolated fungus as seen under the microscope shows similar description; accordingly, the isolated fungus was confirmed to be *F. oxysporium* f. sp. c*iceris* (Plate 2).

Plate (2): The isolated fungus conidia as seen under the microscope

4.3. The Antifungal Potential Of Garlic (*A. sativum)* Cloves Extracts

4.3.1 The antifungal potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* three days after inoculation *in vitro*

Different concentrations of aqueous e xtracts of Garlic Cloves were prepared (25%, 50%, and 75%) and tested for their effect on the radial growth of the fungus *F. oxysporium* f. sp. *ciceris* in PDA media under laboratory conditions. The fungus radial growth was measured three days after inoculation (Table 1).

The results (Table 1, Figure 3) shows in three days of inoculation all the applied concentrations of garlic cloves extracts induced significantly suppressed the radial diameter of the test fungus (P0.05). However, the 75% concentration and the standard fungicide Till Nour 25% were found to be the most effective, as it inhibited the growth up to 100% as compared to control. The concentration 50% was also significantly retarded the growth of the target fungus between 87.1, followed by the 25% concentration by 63.3 (Table1, Figure 3).

The results showed that the antifungal activity increases with the increase of the extract concentration.

Table (1) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* three days after inoculation *in vitro.*

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
Garlic Cloves	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)[c]
	50	100 (0.7)	100 (0.7)	95 (0.73)	83.3 (0.77)	57.1 (0.89)	87.1 (0.76)[c]
	25	50 (0.89)	66.7 (0.84)	66.7 (0.84)	50 (0.89)	85.7 (0.77)	63.8 (0.85)[b]
Fungicide	Till Nour25%	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)[c]
Control	Untreated	0 (1.1)	0 (1.1)	0 (1.1)	0 (1.1)	0 (1.1)	0 (1.1)[a]
LSD	0.0549						
CV (%)	5.09						
SE±	0.0263						

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

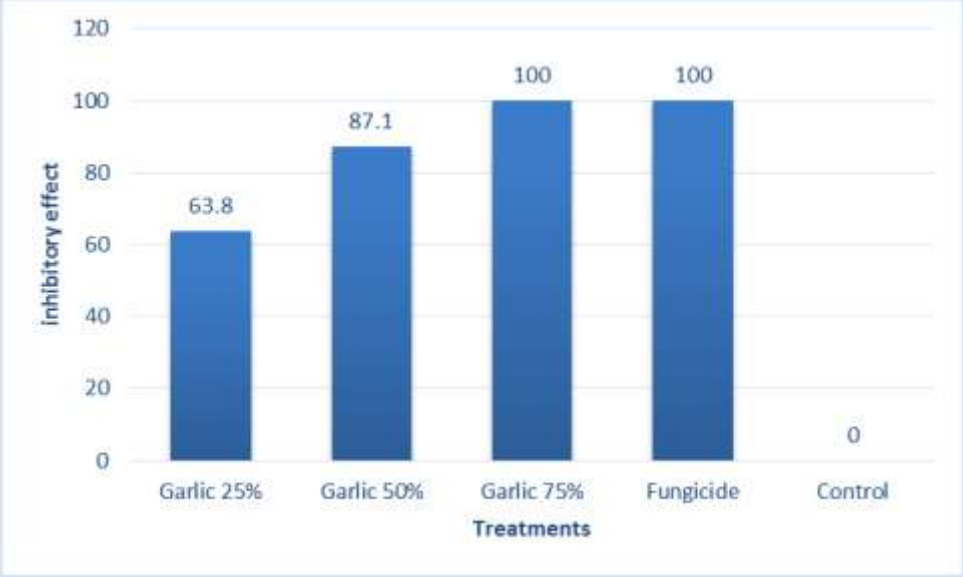

Figure (3) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* three days after inoculation *in vitro.*

4.3.2 The antifungal potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* four days after inoculation *in vitro*.

The results in (Table2, Figure 4) show that all the concentration aqueous extracts of Garlic cloves tested against *F. oxysporium* f.sp. *ciceris* as well as the fungicide continues their inhibitory effect in the fourth day after inoculation significantly (P0.05). The percentage of fungal growth inhibition was significantly high compared to control. The highest inhibitory effect (100%) is still recorded from the "75%" concentration as well as from the standard fungicide, while the other two concentrations "50%" and "25%" recorded inhibitory effect by "60%" and "43.4%" respectively. As before the inhibitory is still increasing according to the increase in the concentration.

4.3.3 The antifungal potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* Five days after inoculation *in vitro.*

Five days post inoculation the results (Table 3, Figure 5) show that all the concentrations and the fungicide tested were significantly (P0.05) different from one another. Still the Concentration "75%" recording the highest inhibitory effect by "100%". On the other hand, the "50%" recorded an inhibitory effect by "61.9%" while the concentration "25%" was slightly increased to record an inhibitory effect of "49.4% .The fungicide "Till Nour25%" recorded a sudden decrease in its inhibitory effect and the fungus started to grow in all the replications "93.8%" instead of "100%", as shown in (Table 3 and Figure 5). However, this inhibitory effect from all concentrations and the fungicide, was significantly (P0.05) different from control.

Table (2) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* four days after inoculation *in vitro*.

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
Garlic Cloves	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)[d]
	50	66.7 (0.95)	66.7 (0.95)	66.7 (0.95)	58.3 (1)	41.6 (1.1)	60 (0.99)[c]
	25	41.7 (1.1)	41.7 (1.1)	41.7 (1.1)	41.7 (1.1)	50 (1.05)	43.4 (1.09)[b]
Fungicide	Till Nour25%	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)[d]
Control	Untreated	0 (1.3)	0 (1.3)	0 (1.3)	0 (1.3)	0 (1.3)	0 (1.3)[a]
LSD	0.0407						
CV (%)	3.21						
SE±	0.0195						

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

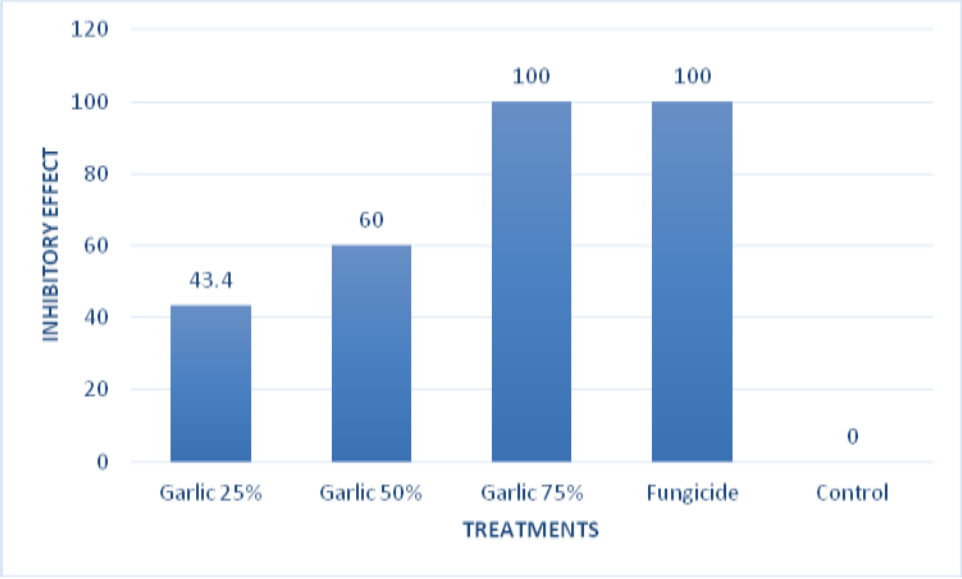

Figure (4) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* four days after inoculation *ni vitro*.

Table (3) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* five days after inoculation *in vitro.*

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
Garlic Cloves	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)^{e}
	50	68.4 (1.05)	70 (1.04)	63.2 (1.1)	57.9 (1.14)	50 (1.22)	61.9 (1.11)^{c}
	25	47.4 (1.22)	50 (1.22)	42.1 (1.26)	47.4 (1.22)	60 (1.14)	49.4 (1.2)^{b}
Fungicide	Till Nour25%	94.7 (0.77)	90 (0.84)	94.7 (0.77)	94.7 (0.77)	95 (0.77)	93.8 (0.78)^{d}
Control	Untreated	0 (1.55)	0 (1.58)	0 (1.55)	0 (1.55)	0 (1.58)	0 (1.56)^{a}
LSD	0.0536						
CV (%)	3.77						
SE±	0.0257						

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

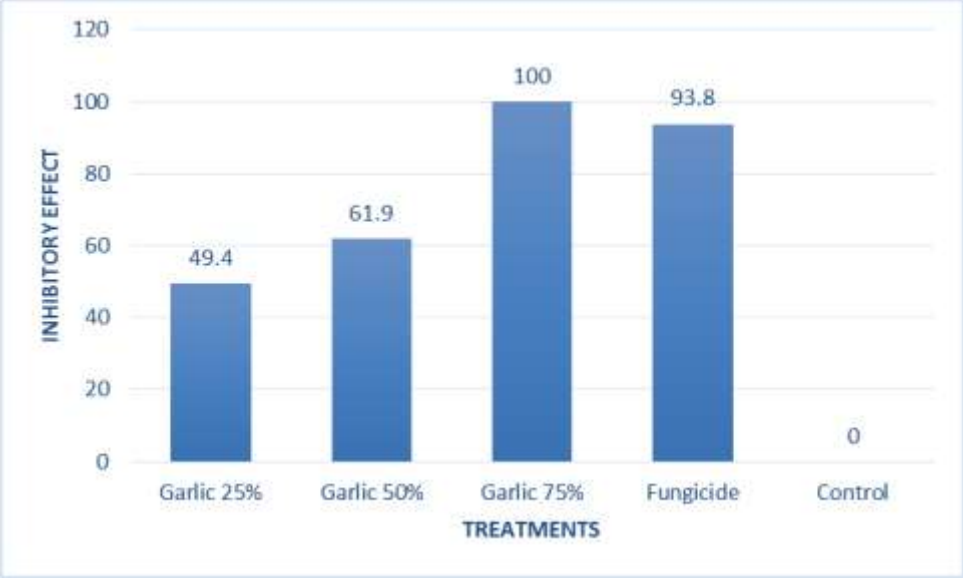

Figure (5) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* five days after inoculation *in vitro*.

4.3.4 The antifungal potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* six days after inoculation *in vitro*.

The same trend was continued after six days of inoculation (Table 4 and Figure 6). All the concentrations tested was found to be significantly (P0.05) different when compared to control and between treatments. The concentration "75%" continued to be the highest inhibitory effect (100%). On the other hand, both concentrations "50%" and "25%" continued to decrease their effect as they recorded "57.6% and 49.2 % inhibition in the radial growth of the fungus, respectively. However, the fungicide resumes its decrease in the inhibitory effect, recording "90.1%" suppression on the radial growth instead of "93.8%) in the readings of the previous day.

4.3.5 The antifungal potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* seven days after inoculation *in vitro*.

The results in (Table 6 and Figure7) shows significant (P0.05) inhibition of the radial growth of the tested fungus compared to control, seven days after inoculation. In addition, significant difference was recorded between treatments one another. The concentration "75%" continues suppressing the fungal growth completely. While the other two concentrations "50%" and "25%" showing less reduction on the radial growth of the fungus (44.8% and 35.9% respectively), but still they considered statistically significantly differ from other treatments when compared to untreated control. The fungicide Till Nour 25%EC, continued adecrease in its inhibitory effect "88.02 %" compared to the previous readings on the six day after inoculation "90.1%".

Table (4) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* six days after inoculation *in vitro*.

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)^{e}
Garlic Cloves	50	69.2 (1.14)	66.7 (1.18)	53.8 (1.3)	50 (1.34)	48.1 (1.38)	57.6 (1.27)^{c}
	25	46.2 (1.38)	48.1 (1.38)	46.2 (1.38)	46.2 (1.38)	59.3 (1.26)	49.2 (1.36)^{b}
Fungicide	Till Nour25%	88.5 (0.89)	88.8 (0.89)	92.3 (0.84)	92.3 (0.84)	88.8 (0.89)	90.1(0.87)^{d}
Control	Untreated	0 (1.8)	0 (1.8)	0 (1.8)	0 (1.8)	0 (1.8)	0 (1.8)^{a}
LSD	0.0713						
CV (%)	4.52						
SE±	0.0342						

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

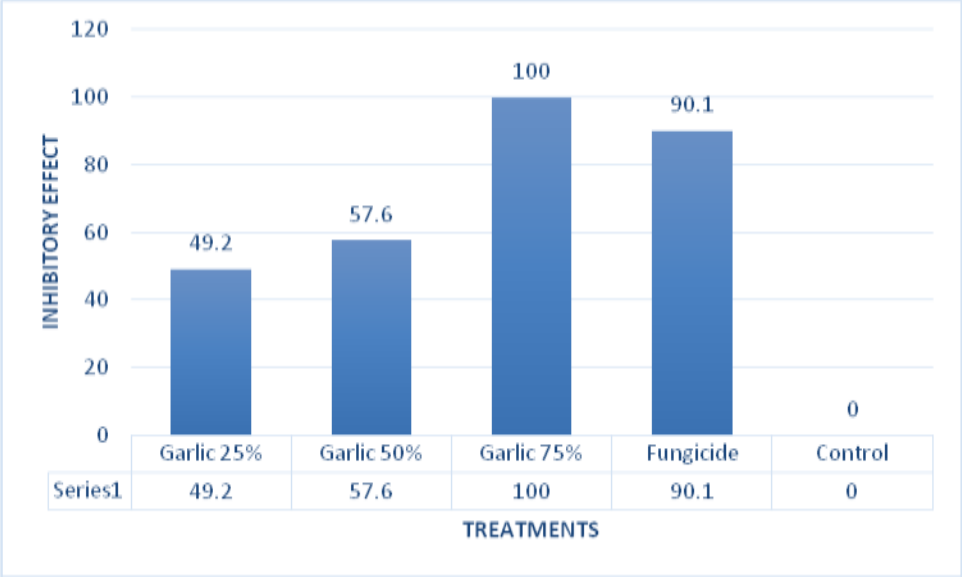

Figure (6) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* six days after inoculation *in vitro*.

Table (5) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* seven days after inoculation *in vitro.*

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
Garlic Cloves	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)^{e}
	50	61.8 (1.34)	47.1 (1.52)	42.4 (1.55)	36.4(1.61)	36.4 (1.61)	44.8 (1.53)^{c}
	25	38.2 (1.61)	35.3 (1.64)	33.3 (1.64)	36.4 (1.61)	36.4 (1.61)	35.9 (1.62)^{b}
Fungicide	Till Nour25%	85.3 (1)	88.2 (0.95)	90.9 (0.89)	90.9 (0.89)	84.8 (1)	88.02 (0.95)^{d}
Control	Untreated	0 (1.97)	0 (1.97)	0 (1.95)	0 (1.95)	0 (1.95)	0 (1.96)^{a}
LSD	0.0740						
CV (%)	4.15						
SE±	0.0355						

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

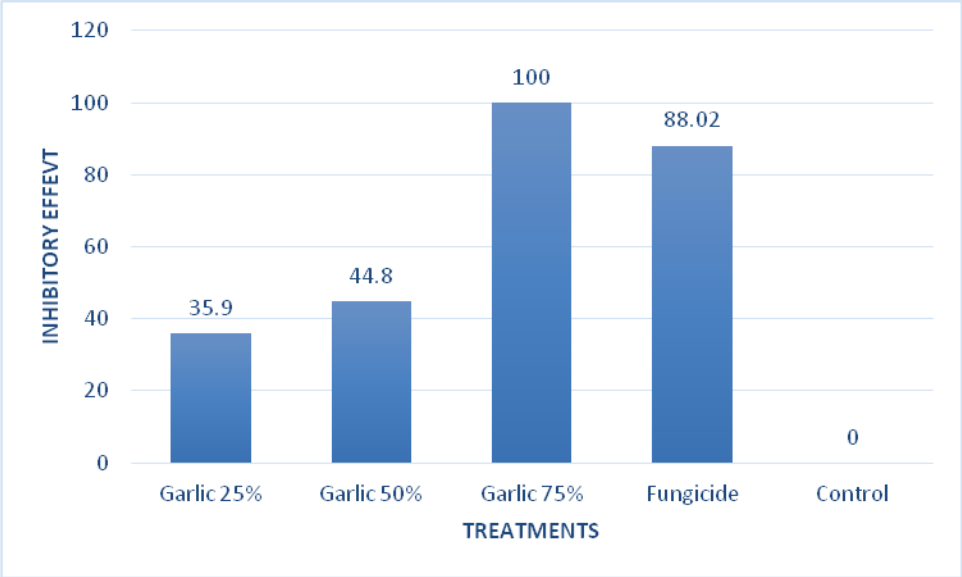

Figure (7) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* seven days after inoculation *in vitro.*

4.3.6 The antifungal Potential of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide Till Nour 25%EC on radial growth of *F. oxysporium* f.sp. *ciceris* eight days after Inoculation *in vitro.*

Eight days after inoculation the results revealed that the antifungal activity of aqueous extracts of Garlic (*A.* sativum) cloves proved a significant reduction on the radial growth of the tested fungus *F. oxysporium* f.sp. *ciceris*. However, the highest inhibitory zone (100% and 88.8%) was obtained by the "75% concentration of garlic cloves" and "the fungicide Till Nour 25%EC"respectively. On the other hand an inhibition also (45.4% and 36.1%) of the tested fungus was also recorded by the "50%" and "25% concentrations respectively. However, all the applied concentrations (25%, 50%, and 75%,) of the aqueous extracts were significantly suppressed the radial diameter of the tested fungus (P0.05) compared to the control (Table 6 and Figure 8).

The fungicide Till Nour 25%EC was surprisingly showed a complete inhibition on the first four days after inoculation, but instead; from day five on, its effect on the fungus growth was decreased (Table1s 3,4,5 and 6).

Table (6) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* eight days after inoculation *in vitro*.

Treatment/concentration%		Percentage of mycelium inhibition %					Mean
Garlic Cloves	75	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)	100 (0.7)^{e}
	50	63.4 (1.41)	48.8 (1.61)	41.5 (1.7)	36.6 (1.76)	36.6 (1.76)	45.4 (1.65)^{c}
	25	43.9 (1.67)	31.7 (1.82)	31.7 (1.82)	39.02 (1.73)	34.1 (1.79)	36.1 (1.76)^{b}
Fungicide	Till Nour25%	85.4 (1.05)	90.2 (0.95)	90.2 (0.95)	92.7 (0.89)	85.4 (1.05)	88.8 (0.98)^{d}
Control	Untreated	0 (2.14)	0 (2.14)	0 (2.14)	0 (2.14)	0 (2.14)	0 (2.14)^{a}
LSD	0.1032						
CV (%)	5.40						
SE±	0.0495						

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

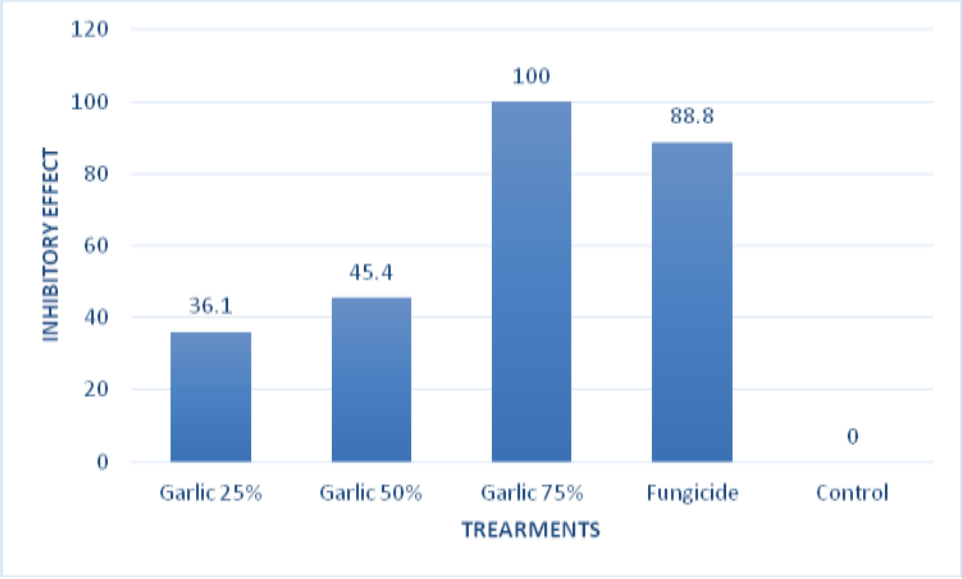

Figure (8) Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* eight days after inoculation *in vitro*.

Plate (3) : Effect of Garlic (*A. sativum)* cloves aqueous extracts and Fungicide (Till Nour 25%) on radial growth of *F. oxysporium* f.sp. *ciceris* eight days after inoculation *in vitro*.

CHAPTER FIVE

DISCUSSION

The use of fungicides, generally considerably reduce wilt incidence but their high cost beside their effects on the environment was undesirable (Song and Goodman, 2001), in addition, the resistance varieties is also could be faced with breakdown of resistance due to the high pathogenic variability in the pathogen population (Kutama *et al*, 2011; 2013). In this context, the searches for an eco-friendly way of managing Fusarium wilt which offers an alternative to fungicides is highly demanding. Wilt diseases may cause greater losses of some plantations. Fusarium wilts causes crop losses, estimated to an average of 25%.

Based on the fact that botanical insecticides possess great advantages over synthetic pesticides , this study is conducted to evaluate the bioactivity of aqueous extract from Garlic (*A. sativum*) cloves and fungicide Till Nour 25% EC on the growth of *F. oxysporium* f.sp. *ciceris* under laboratory conditions.

Our results recorded that the isolated fungus from the stems of wilting diseased chickpea plants is confirmed to be *F. oxysporium* f. sp. *ciceris.* The fungus identification was confirmed with the support of already prepared slides of *F. oxysporium* at the plant pathology laboratory. Texts and research papers were also consulted during the examination of this fungus. (Aneja, 2004).

Moreover, our results also, revealed that the aqueous extract of Garlic (*A. sativum*) cloves at all concentration (25%, 50% and 75%) and fungicide, Till Nour 25% EC throughout the course of the experiments exhibited an inhibitory effect on mycelial radial growth of the tested fungus with significantly higher inhibition reduction in growth percent compared to control.

The most effective suppression was recorded by the “75%” concentration by a 100% inhibition. This result could be a sign that this concentration worked with a fungicidal effect. Our findings, however, is in accord with the previous studies of Singh *et al* (1979) where they used Garlic leaf extract against *F. oxysporium* f. sp. *ciceris*. On the other hand Misra and Dixit (1976) have proved the fungicidal effect of Garlic leaf extracts against different fungi.

Our study also revealed that the fungicide Till Nour 25% EC showed a sudden growth of the treated fungus five days after inoculation. This might be due to the short safety period of the fungicide that obviously has a fungistatic effect on the growth of the fungus.

CONCLUSIONS

In conclusion, the findings presented in this study indicate promising potentials of Garlic (*A. sativum* L.) as it proved to be a source of antifungal effect that helps in management of plant fungal diseases. The Garlic cloves aqueous extracts exhibited an inhibitory effect on fungal growth. Thus Garlic plus the fungicide can be applied as part of an integrated approach to manage Fusarium Wilt.

- The aqueous extract of Garlic cloves at all concentrations and the fungicide Till Nour 25% EC, exhibited inhibitory effect on the radial mycelial growth of the tested fungus (*F. oxysporium* f. sp. *ciceris*). The percentage zone of inhibition was significantly high compared to control.

RECOMMENDATIONS

1. Further studies can be conducted to test other Garlic parts extracts against *Fusarium spp* and other fungal diseases effect of.
2. Further studies is also can be conducted to test the Garlic cloves under the field conditions (*In Vivo*).
3. Additional chemical studies were required to identify and isolate the active ingredient which is responsible for *Fusarium* growth inhibition.

REFERENCES

Abdalla, E.M. (2017). Potential Antifungal Activity of fresh garlic cloves (*Allium sativum* L) from Sudan. *Journal of Biotechnology Research*. 3(11). 106-109.

Agrios, G.N. (2005). Plant Pathology**,** *5th Edition, Elsevier Acad. Press***,** pp: 163-164.

Ahmad, M. A., Iqbal S. M. , Ayub N., Ahmad Y., Akram A (2010). Identification of resistant sources in chickpea against Fusarium wilt. *Pak. J. Bot.* 42: 417-426.

Ali, M.E.K. (1989). A Review of Wilt and Root rot diseases of food legumes. *Hudeiba Research Station***.**

Aneja, K.R. (2004). Experiments in Microbiology.Plant pathology and Biotechnology Fourth edition, New international (p) Limited publishers, India – 121-128.

Ayoub, M. A. (2001). Evaluation of chickpea germplasm, Fungitoxicants, organic and inorganic materials for the management of wilt (*Fusarium oxysporium* f. sp. *Ciceris).* Ph D thesis, Univ. Agri. Faisal Abad**.**

Backman, P.A. and Turmer, J.T. (1989). Plant response and disease control following seed inoculation with Bacillus subtilis. *In proceedings beltwide Cotton Products Research Conference (Book 2), J.M. Brown (ed.)* National Cotton Council of American, Memphis, 16-17.

Block, E. (2010). Garlic and other Alliums:The lore and the science.*Royal society of chemistry .ISBN 978-0-85404-190-9.*

Booth, C. (1977). Fusarium Laboratory guide to the identification of the major species. *Commonwealth Mycological Institute, Kew, Surrey***.** PP 325.

Bowers and J. C. Locke (2000). Effects of botanical extracts on the population density of Fusarium oxysporium in soil and control of Fusarium wilt in the greenhouse**.** *Plant Disease***,** 84: 300-305.

Butler, E.J. (1918). Fungi and diseases of plants**.** *Bishen Singh Mahendra Pal Singh, New Connaugh Place., Dehradun Periodical Experts. 42-D, Vivek Vihar, Delhi,* 32, 547 pp. (reprinted, 1973).

Chand H., Singh, S. (2005). Control of chickpea wilt (*Fusarium oxysporium* f sp *ciceri*) using bioagents and plant extracts**.** *Indian J. Agric. Sci***.** 75: 115-116.

Chaudhry MA, Ilyas MB, Muhammad F, Ghazanfar MU (2007). Sources of resistance in chickpea germplasm against Fusarium wilt**.** *Myco path* 5: 17-21**.**

Chauhan, S.K. (1962). Physiologic variation in F. orthoceras App. Wr. Var. ciceris Padwick causing wilt of gram. *Proc. Natn. Acad. Sci. Sec. B.*22:78-84.

Cho S, Muehlbauer FJ (2004). Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (Cicer arietinum L.). *Physiol. Mol. Plant Pathol***. 64: 57–66.**

Christian L.; Morel, N. and Luiz R. (2007). In vitro susceptibility to fungicides by invertebrate-pathogenic and saprobic fungi. *Myco. pathologia.* 164: 39-47**.**

Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Rizza MD. (2011.) Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria spp*. *Chilean Journal of Agricultural Research.* 71:231-239.

Elfatih, M., Ali, K., Inanaga, S. and Sugimoto, Y. (2002). Sources of resistance to Fusarium wilt of chickpea in Sudan**.** *Phytopathol. Mediterr***,** 41, 163–169.

Emenky, F.A.O. and Khalaf, A.S.(2008). Effect of sowing depths and seed size of some winter cultivars of chickpea (Cicer arietinum L.) on field emergence and vegetative growth**.** *Res. J. Seed Sci*.**,** 1(1):11-18.

Erwin, D.C. (1957). Fusarium and Verticillium wilt diseases of *Ciceris arietinum* L. *Phytopathology***.**47: 10.

FAOSTAT 2002.

FAOSTAT 2007.

FAOSTAT 2018.

Faki, H. H.; Sheikh Mohamed, A. I. and Ali, M. E. K. (1992). Adaptation of Chickpea in Sudan. *In: Proceedings of the international workshop on adaptation of Chickpea in the WANA region, 9-12 Nov.1992***.** ICARDA, Syria.

Garcia RP. (|1999). Laboratory evaluation of plant extracts for the control of Aspergillus growth and alfatoxin production. Proceeding of the Japanese Association of Mycotoxicology. *Horticultural Abstracts* 60:6543p.

Grewal JS (1969). Important fungal disease of Cicer arietinum in India**.** *Pulse Improvement Project Seminar Report held at Karaj Agricultural College, University of Tehran & USDA, January 7-9, 1969***.** pp. 35-40**.**

Gupta, O. M. (1991). Symptomless carrier of chickpea vascular wilt pathogen F. oxysporium f. sp ciceris**.** *Legume Research Deptt.. Univ. Jabalpur 482004. Madhya Pradesh, India. Pl. Path. J. N. Agri* 14:193-194.

Haware, M.P. and Nene, Y.L. (1980). Influence of wilt at different growth stages on yield loss of chickpea**.** *Trop. Grain Legume Bull***.** 19:38–40**.**

Haware, M. P. and Nene, Y. L. (1982). Races of *Fusarium oxysporium f. sp. ciceri. Plant Dis***.** 66:809-810**.**

Haware, M. P., jimenez-Diaz, R. M., Amin, K. S., Phillips ,j. c., Halila, M. H. (1990). Integrated management of wilt and root rots of chickpea.In: Chickpea in the Nineties **(***Ed.), ICRISAT(International Crops Research Institute for the Semi Arid Tropics). ICRISAT center ,Patancheru, India***,** PP. 129-133.

Haware, M. P.; Nene, Y. L.; Pundir, R.P.S. and Narayana, J. (1992). Screening of world chickpea germplasm for resistance to Fusarium wilt**.** *Field Crops Res***.** 30: 147– 154**.**

Haware, M. P. (1993). Fusarium diseases of crops of India**.** *Indian Phytopath.* 46:101-109**.**

Jalali BL, Chand H (1992). Chickpea wilt. In: Plant Diseases of International Importance**.** *(Singh US, Mukhopadhyay AN, Kumar J, Chaube HS, ed.), Vol. I. Prentice Hall, Englewood Cliffs, NJ, USA***.** pp. 429– 444**.**

Joseph, O. (2003). Control of brown blotch of bambara groundnut with garlic extract and Benomyl**.** *Phytoparasitica***.** 31:207-209**.**

Kamenetsky,R.;london shafir ,l.;Khassanov,F.;et al. (2005). Diversity in fertility potential and organo -sulphur compounds among garlics from central Asia **.** *Biodivers conserv***.**14(2):281-295**.**

Kraft, J.M., Haware, M.P., Jimenez-Diaz, R.M., Bayaan, B. and Harrab, (1994). Screening techniques and sources of resistance to root rots and wilts in cool season food legumes. p. 268–289. In F.J. Muehlbauer and W.J. Kaiser (ed.) Expanding the production and use of cool season food legumes, Kluwer Academic Publ., Dordrecht, the Netherlands.

Kanan GJ, Al-Najar RA (2008). In vitro antifungal activities of various plant crude extracts and fractions against citrus postharvest disease agent Penicillium digitatum. *Jordan Journal of Biological Sciences*. 1:89-99.

Katzer , G. (2009). Garlic **(***Allium sativum* l.**).** *Retrieved December 2,2012***.**

Khune, N. N. and Patil, M. J. (1992). Transmission studies of Fusarium wilt by isolated *F. oxysporium* f. sp. *ciceris* from various plant parts of chickpea. *New Agri. Deptt. Pl. Path. Postgraduate Inst. P.K.V., Alola, India.* 3:227-229**.**

Kutuma, A. S., Emechebe, A. M. and Aliyu, B. S. (2011). Field Evaluation of Some inoculation Techniques on the Incidence and Severity of Sorghum Head Smut (*Sporisorium reilianum*) in Nigerian Sudan Savanna. *Bio. Environ. Sci. J. Tropics.* 8(3): 292-296).

Kutuma, A. S., Auyo, M. I. Umar, S. and Umar, M. L. (2013). Reduction in Growth and Yield Parameters of Sorghum Genotypes Screened for Loose Smuts in Nigerian Sudan Savanna. *World J. Agric. Res.* 1(5): 185-192.

Landa, B. B., Navas-Cortes J. A., Jimenez-Diaz R. M. (2004). Integrated management of Fusarium wilt of chickpea with sowing date, host resistance and biological control**.** *Phytopathology* 94: 946-960**.**

Lewis, W., Elvin-Lewis M. (2003). Medical Botany: Plants Affecting Human Health**.** *2nd edition. New York: Wiley***.** 255-257.

Misra, S. B., and S. N. Dixit. (1976). Fungicidal Spectrum of the Leaf Extract of *Alium sativum* L. *Indian Phytopathology.* 29, 448-449.

McKerral, A. (1923). A note on Fusarium wilt of gram in Burma and measures taken to combat it. *Indian Agric. J.* 28: 608-613**.**

Mohamed, E. H. (2013). Management of Chickpea wilt disease caused by *F. oxysporium* f. sp. c*iceris.* Thesis.

Mohamed, O. E., S. Ahmed, M. Singh and N. E. Ahmed. (2018). Association of crop production practices on the Incidence of wilt and Root Rot Diseases of Chickpea in the Sudan **.** *Arab Journal of Plant Protection***,** 36(1):21-26.

Murumkor, C.V. and Chavan, I.D. (1985). Physiological changes in chickpea level infected by Fusarium wilt**.** *Biovegyanam***.** 11(1):118-120**.**

Navas-Cortes, J.A.; Alcala-Jimenez, A.R.; Hau, B. and Jimenez-Diaz, R.M. (2000). Influence of inoculum density of races 0 and 5 of *F. oxysporium* f. sp. *ciceris* on development of Fusarium wilt in chickpea cultivars. *Eur. J. Pl. Pathology* 106:135-146**.**

Nene, Y. L. and Reddy, M. V. (1987). Chickpea diseases and their control. *In: The Chickpea, (M. C. Saxena, K. B. Singh, ed.), CABI Publishing, CAB Int., Wallingford, UK***,** 233–270.

Nene, Y.L. and Thaplyal, P.N. (1982). Fungicides in plant disease control. **Oxford** *and IBH Publi. Comp., New Delhi***,** pp.507.

Nimalkar SB, Harsulkar AM, Giri AP, Sainani MN, Franceshi V, et al. (2006). Differentially expressesd gene transcripts in roots of resistant and susceptible chickpea plant (*Cicer arietinum* L.) upon *Fusarium oxysporium* infection. *Physiol. Mol. Plant Pathol***.** 68: 176–88**.**

Obagwu J, Emechebe AM, Adeoti AA (1997). Effects of extracts of garlic (*Allium sativum*) bulb and neem (*Azadirachta indica*) seed on the mycelial growth and sporulation of Collectotrichum capsici. *Journal of Agricultural Technology*. 5:51-55.

Obagwu J, Korsten L (2003). Control of citrus green and blue moulds with garlic extracts. *European Journal of Plant Pathology*. 109:221-225.

Okigbo RN, Ogbonnanya OU. (2006). Antifungal effects of two tropical plants extracts *Ocimum gratissimum* and *Afromaomum melegueta* on post-harvest yam *Discorea spp* rot**.** *African Journal of Biotechnology***.** 5:727-731**.**

PAN Germany (2010). Biocides-risks and alternatives. *Hamburg. Hyperlink*:http://www.pangermany.org/download/biocides/biocides_risks_and_alternatives.pdf.

Pande, S., Rao, J.N. and Sharma, M. (2007). Establishment of the Chickpea Wilt Pathogen *F. oxysporium* f. sp. *ciceris* in the Soil through Seed Transmission. *Plant Pathol. J.* 23(1): 3-6.

Prasad, A.K. and Ojha, N.L. (1986). Antifungal evaluation of leaf extracts for the control at some cucurbitaceous fruit rot diseases. *Indian Phytopathol.* 39 :153**.**

Rao, G. P., AgnihortriC. T., Chen and Srivastava A. K. (1994). Toxicity of oils higher plants against fungal pathogens of sugar cane, current trend in sugar cane pathogens. *Pitampura, Delhi*.

Rajput, M.T., Syed, S.H. and Khursheed, M.K. (1996). In: *Plant Taxonomy*, pp. 214-227**.**

Ravikumar, R.L., Patil, B.S., Soregaon, C.D. and Hegde, S.G. (2007). Genetic evidence for gametophytic selection of wilt resistant alleles in chickpea**.** *Theor. Appl. Genet***.,** 114, 619–625**.**

Robertson, L.D., Singh, K.B. and Ocampo, B. (1995). A catalog of annual wild Cicer species. ICARDA, Aleppo, Syria**.**

Russel P. E., Mussa A. E. A. (1977). The use of garlic (Allium sativum) extracts to control foot rot of Phaseolus vulgaris caused by Fusarium solani f. sp. phaseoli. *Journal of Agriculture***.** 12:27-35.

Saxena, M.C. and Singh, K.B. (1987). The chickpea *published by C.A.B. Int. ICARDA.* pp: 250-252**.**

Saxena, M. C. (1990). Problems and potential of chickpea production in the nineties. *p. 13– 27. In H.A. van Rheenen and M.C. Saxena (ed.)* Chickpea in the nineties: Proceedings of the Second International Workshop on Chickpea Improvement. 4– 8 Dec. 1989. *ICRI- SAT, Patancheru, India***.**

Schippers, B. and Van Eck, W.H. (1981). Formation and survival of chlamydospores in Fusarium: Diseases, Biology and Taxonomy *(Nelson, P.E., Tousson, T.A. and Cook, R.J. eds.) plenum, NY. and London***.** pp: 21-34.

Schroth, M. N. and Hildebrand, D. C. (1964). Influence of plant exudates on root infecting fungi. *Annu. Rev. Phytopathol***.** 2: 101-132**.**

Sharma, B. L. and Gupta, R. N. (1987). Survival of F. oxysporium f. sp. ciceris causing wilt of Bengal gram uring summer month**.** *Indian Phytopath***.,** 36:261-262**.**

Singh G, Chen W, Rubiales D, Moore K, Sharma YR, Gan Y (2007). Diseases and their management. In Chickpea Breeding and Management *(Eds Yadav, Redden, Chen and Sharma). CAB International* pp. 497-519**.**

Singh, K.B. and Ocampo, B. (1997). Exploitation of wild Cicer species for yield improvement in chickpea**.** *Theor. Appl. Genet***.** 95: 418–423**.**

Singh, U.P, V.N. Pandey, K.G.Wagner, and K.P. Singh. (1990). Antifungal activity of ajoene, a constituent of garlic (*Allium sativum* L). *Canadian Journal of Botany.* 68(6): 1354-1356.

Singh, U. P., Pathak, K. K., Khare and Singh, R. B. (1979). Effect of Leaf Extract of Garlic on *Fusarium oxysporium* f. sp. *ciceri , Sclerotinia* and on Gram Seeds. *Mycologia* 71 (3): 556-564.

Smith, I. M.; J. Dunez; D. H. Phillips; R. A. Lelliott and S. A. Archer, eds. (1988). European handbook of plant diseases**.** *Blackwell Scientific Publications: Oxford***.** 583pp**.**

Siripornvisal S, Rungprom W, Sawatdikarn S. (2009). Antifungal activity of essential oils derived from medicinal plants against grey mould (*Botrytis cinerea*). *Asian Journal of Food and Agro-Industry***.** (Special Issue): 229-223. 2**.**

Song F. and Goodman, R. M. (2001). Molecular Biology of Disease Resistance in Rice. *Physiology and Molecular Plant Pathology*, 59: 1-11.

Tripathi P., Dubey N. K., Shukla A. K. (2008). Use of some essential oils as postharvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. *World Journal of Microbiology and Biotechnology***.** 24:39-46**.**

Tzortzakis, N. G. and Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cymbopogon citrates* L.) essential oil against key postharvest pathogens**.** *Innovative Food Science and Emerging Technologies.* 8:253-258.

Westerlund, F.V.; Campbell, R.N. and Kimble, K.A. (1974). Fungal root rot and wilt of chickpea in California. *Phytopathology*, 64: 432–436**.**

Winter, M. (2003). Embeddedness, the new food economy and defensive localism**.** *Journal of Rural Studies* 19, 23–32.

Younis F, Mirelman D, Rabinkov A, Rosenthal T. Sallylmercapto-captopril (2010) A novel compound in the treatment of Cohen-Rosenthal diabetic hypertensive rats. *Journal of Clinical Hypertension*.12:451-455.

Yousuf S, Ahmad A, Khan A, Manzoor N, Khan LA. (2010). Effect of diallyldisulphide on an antioxidant enzyme system in Candida species**.** *Canadian Journal of Microbiology***.** 56:816-821.

Appendices

Appendix 1a: Origin Data

REP	TREAT	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
1	G25%	0.3	0.7	1	1.4	2.1	2.3
2	G25%	0.2	0.7	1	1.4	2.2	2.8
3	G25%	0.2	0.7	1.1	1.4	2.2	2.8
4	G25%	0.3	0.7	1	1.4	2.1	2.5
5	G25%	0.1	0.6	0.8	1.1	2.1	2.7
1	G50%	0	0.4	0.6	0.8	1.3	1.5
2	G50%	0	0.4	0.6	0.9	1.8	2.1
3	G50%	0.03	0.4	0.7	1.2	1.9	2.4
4	G50%	0.1	0.5	0.8	1.3	2.1	2.6
5	G50%	0.3	0.7	1	1.4	2.1	2.6
1	G75%	0	0	0	0	0	0
2	G75%	0	0	0	0	0	0
3	G75%	0	0	0	0	0	0
4	G75%	0	0	0	0	0	0
5	G75%	0	0	0	0	0	0
1	TL	0	0	0.1	0.3	0.5	0.6
2	TL	0	0	0.2	0.3	0.4	0.4
3	TL	0	0	0.1	0.2	0.3	0.4
4	TL	0	0	0.1	0.2	0.3	0.3
5	TL	0	0	0.1	0.3	0.5	0.6
1	CO	0.6	1.2	1.9	2.6	3.4	4.1
2	CO	0.6	1.2	2	2.7	3.4	4.1
3	CO	0.6	1.2	1.9	2.6	3.3	4.1
4	CO	0.6	1.2	1.9	2.6	3.3	4.1
5	CO	0.7	1.2	2	2.7	3.3	4.1

Appendix1b: Transformed data using the equation {NX (targeted) = SQRT(x+0.5)}

REP	TREAT	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6
1	G25%	0.89	1.1	1.22	1.38	1.61	1.67
2	G25%	0.84	1.1	1.22	1.38	1.64	1.82
3	G25%	0.84	1.1	1.26	1.38	1.64	1.82
4	G25%	0.89	1.1	1.22	1.38	1.61	1.73
5	G25%	0.77	1.05	1.14	1.26	1.61	1.79
1	G50%	0.71	0.95	1.05	1.14	1.34	1.41
2	G50%	0.71	0.95	1.05	1.18	1.52	1.61
3	G50%	0.73	0.95	1.1	1.3	1.55	1.7
4	G50%	0.77	1	1.14	1.34	1.61	1.76
5	G50%	0.89	1.1	1.22	1.38	1.61	1.76
1	G75%	0.71	0.71	0.71	0.71	0.71	0.71
2	G75%	0.71	0.71	0.71	0.71	0.71	0.71
3	G75%	0.71	0.71	0.71	0.71	0.71	0.71
4	G75%	0.71	0.71	0.71	0.71	0.71	0.71
5	G75%	0.71	0.71	0.71	0.71	0.71	0.71
1	TL	0.71	0.71	0.77	0.89	1	1.05
2	TL	0.71	0.71	0.84	0.89	0.95	0.95
3	TL	0.71	0.71	0.77	0.84	0.89	0.95
4	TL	0.71	0.71	0.77	0.84	0.89	0.89
5	TL	0.71	0.71	0.77	0.89	1	1.05
1	CO	1.05	1.3	1.55	1.76	1.97	2.14
2	CO	1.05	1.3	1.58	1.79	1.97	2.14
3	CO	1.05	1.3	1.55	1.76	1.95	2.14
4	CO	1.05	1.3	1.55	1.76	1.95	2.14
5	CO	1.1	1.3	1.58	1.79	1.95	2.14

Appendix 1c: Statistical Analysis – Complete Randomized Design

Completely Randomized AOV for DAY1

Source	DF	SS	MS	F	P
TREAT	4	0.42906	0.10726	62.0	0.0000
Error	20	0.03460	0.00173		
Total	24	0.46366			

Grand Mean 0.8176

CV 5.09

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.02111

Effective cell size 5.0

TREAT	Mean
CO	1.0600
G25%	0.8460
G50%	0.7620
G75%	0.7100
TL	0.7100

Observations per Mean 5

Standard Error of a Mean 0.0186

Std Error (Diff of 2 Means) 0.0263

Completely Randomized AOV for DAY2

Source	DF	SS	MS	F	P
TREAT	4	1.29200	0.32300	340	0.0000
Error	20	0.01900	0.00095		
Total	24	1.31100			

Grand Mean 0.9600

CV 3.21

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.06441

Effective cell size 5.0

TREAT	Mean
CO	1.3000
G25%	1.0900
G50%	0.9900
G75%	0.7100
TL	0.7100

Observations per Mean 5

Standard Error of a Mean 0.0138

Std Error (Diff of 2 Means) 0.0195

Completely Randomized AOV for DAY3

Source	DF	SS	MS	F	P
TREAT	4	2.37604	0.59401	360	0.0000
Error	20	0.03296	0.00165		
Total	24	2.40900			

Grand Mean 1.0760

CV 3.77

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.11847

Effective cell size 5.0

TREAT	Mean
CO	1.5620
G25%	1.2120
G50%	1.1120
G75%	0.7100
TL	0.7840

Observations per Mean 5

Standard Error of a Mean 0.0182

Std Error (Diff of 2 Means) 0.0257

Completely Randomized AOV for DAY4

Source	DF	SS	MS	F	P
TREAT	4	3.52514	0.88129	301	0.0000
Error	20	0.05848	0.00292		
Total	24	3.58362			

Grand Mean 1.1952

CV 4.52

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.17567

Effective cell size 5.0

TREAT	Mean
CO	1.7720
G25%	1.3560
G50%	1.2680
G75%	0.7100
TL	0.8700

Observations per Mean 5

Standard Error of a Mean 0.0242

Std Error (Diff of 2 Means) 0.0342

Completely Randomized AOV for DAY5

Source	DF	SS	MS	F	P
TREAT	4	5.23706	1.30926	416	0.0000
Error	20	0.06300	0.00315		
Total	24	5.30006			

Grand Mean 1.3524

CV 4.15

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.26122

Effective cell size 5.0

TREAT	Mean
CO	1.9580
G25%	1.6220
G50%	1.5260
G75%	0.7100
TL	0.9460

Observations per Mean 5

Standard Error of a Mean 0.0251

Std Error (Diff of 2 Means) 0.0355

Completely Randomized AOV for DAY6

Source	DF	SS	MS	F	P
TREAT	4	6.92766	1.73191	283	0.0000
Error	20	0.12248	0.00612		
Total	24	7.05014			

Grand Mean 1.4484

CV 5.40

At least one group variance is near zero,

Variance-equality tests cannot be computed.

Component of variance for between groups 0.34516

Effective cell size 5.0

TREAT	Mean
CO	2.1400
G25%	1.7660
G50%	1.6480
G75%	0.7100
TL	0.9780

Observations per Mean 5

Standard Error of a Mean 0.0350

Std Error (Diff of 2 Means) 0.0495

Appendix 2a: LSD

LSD All-Pair wise Comparisons Test of DAY1 by TREAT

TREAT	Mean	Homogeneous Groups
CO	1.0600	A
G25%	0.8460	B
G50%	0.7620	C
G75%	0.7100	C
TL	0.7100	C

Alpha 0.05 Standard Error for Comparison 0.0263

Critical T Value 2.086 Critical Value for Comparison 0.0549

There are 3 groups (A, B, etc.) in which the means are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY2 by TREAT

TREAT	Mean	Homogeneous Groups
CO	1.3000	A
G25%	1.0900	B
G50%	0.9900	C
G75%	0.7100	D
TL	0.7100	D

Alpha 0.05 Standard Error for Comparison 0.0195

Critical T Value 2.086 Critical Value for Comparison 0.0407

There are 4 groups (A, B, etc.) in which the means are not significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY3 by TREAT

TREAT	Mean	Homogeneous Groups
CO	1.5620	A
G25%	1.2120	B
G50%	1.1120	C
TL	0.7840	D
G75%	0.7100	E

Alpha 0.05 Standard Error for Comparison 0.0257

Critical T Value 2.086 Critical Value for Comparison 0.0536

All 5 means are significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY4 by TREAT

TREAT	Mean	Homogeneous Groups
CO	1.7720	A
G25%	1.3560	B
G50%	1.2680	C
TL	0.8700	D
G75%	0.7100	E

Alpha 0.05 Standard Error for Comparison 0.0342

Critical T Value 2.086 Critical Value for Comparison 0.0713

All 5 means are significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY5 by TREAT

TREAT	Mean	Homogeneous Groups
CO	1.9580	A
G25%	1.6220	B
G50%	1.5260	C
TL	0.9460	D
G75%	0.7100	E

Alpha 0.05 Standard Error for Comparison 0.0355

Critical T Value 2.086 Critical Value for Comparison 0.0740

All 5 means are significantly different from one another.

LSD All-Pair wise Comparisons Test of DAY6 by TREAT

TREAT	Mean	Homogeneous Groups
CO	2.1400	A
G25%	1.7660	B
G50%	1.6480	C
TL	0.9780	D
G75%	0.7100	E

Alpha 0.05 Standard Error for Comparison 0.0495

Critical T Value 2.086 Critical Value for Comparison 0.1032

All 5 means are significantly different from one another.