**Sudan University of Science and Technology**

**College of Graduate Studies**

# Identification and Control of Fungus Wilt Disease Caused by *Fusarium oxysporum* f.sp. Ciceris in Chickpea

# تعريف ومكافحة مرض الذبول الفيوزيرمي الذي يسببه فطر *Fusarium oxysporum* f.sp .Ciceris في محصول الحمص

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in plant protection.

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**November, 2019**

**الآية**

**بسم الله الرحمن الرحيم**

**قال تعالي:**

وَإِذْ قَالَ رَبُّكَ لِلْمَلَائِكَةِ إِنِّي جَاعِلٌ فِي الْأَرْضِ خَلِيفَةً قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ قَالَ إِنِّي أَعْلَمُ مَا لَا تَعْلَمُونَ (30) وَعَلَّمَ آدَمَ الْأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلَائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلَاءِ إِنْ كُنْتُمْ صَادِقِينَ (31) قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ (32)

**صدق الله العظيم**

**الآيات (30-32 سورة البقرة)**

# Dedication

*To my father and mother*

*To my brothers and sisters*

*To all my family*

*To all my teachers*

*To all my colleagues and friends*

*With love and respect.*

## ACKNOWLEDGEMEN

First, I render my gratitude and praise to the Almighty Allah. I wish to express my sincere gratitude to my supervisor Dr: Ekhlass Hussein Mohamed for his helpful guidance, encouragement and supervision of this research.

My deepest gratitude goes to my family and my friends for their love and support. Finally, I offer my regards and blessing to all of those who supported me in any respect during the completion of this project.

Thanks are also due to the members of the department of the Plant Protection College of Agricultural Studies - Sudan University of Science and Technology for their encouragements and valuables suggestions.

I acknowledge with respect, the help rendered to me by colleagues, sincere thanks are also extend to those who helped me in various ways and encouraged me to achieve and finish my research work.

Finally yet importantly, thanks are extending to my Father, Mother and all who helping me to finish my study.

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# ABSTRACT

This study was carried out at the Plant Pathology Laboratory, Faculty of Agricultural Studies, Sudan University of Science and Technology for the purpose of isolating and identifying the fungus associated with *Fusarium oxysporum* on chickpea plant in Shambat area in Sudan University and the possibility of combating it using biological control and fungicide Till Nour using the recommended dose as standard compared to the control.The pathogen was isolated from infected chickpea parts and then the fungus was identified according to culture characteristics as *Fusarium oxysporum fs .sp*cicers. The main control method of *F.oxysporum* was chemical fungicides. However, this method has been ineffective under conditions conducive to disease development and the high cost of pesticides. The selead to development of alternative methods of controlling the pathogen .That includes the use of biological control agents, which are considered safe, easy to adopt, and environmentally friendly, one such method that can significantly contribute to controlling the disease. Trichoderma is a biological control agent that can be developed for this purpose. *Trichoderma harzianum* was tested against *F.oxysporum* and results show that *F.oxysporum* had a slower growth rate (1.282mm) compared to *T. harzianum* (3.8mm). Possible from controlledthe pathogenic fungus.It is generally found that *T. harzianum* is more effective in reducing the growth of *F.oxysporum* fungi. The results indicated that there is a significant decrease in the growth of the fungus compared to its growth with trichoderma(1.282mm). Since the effect of the pesticide is similar to the effect of the fungus *T. harzianum*, then you can use the fungus Trichoderma instead of pesticide. This strategy leads to a decrease in the rate of use of fungicides .Thusreduce their cost and increase the safety of the environment.

# ملخص الاطروحة

اجريت هذه الدراسة بمعمل امراض النبات بكلية الدراسات الزراعية -جامعة السودان للعلوم والتكنولوجيا بغرض عزل وتعريف الفطر المصاحب لمرض الزبولالفيوزيرميعلى نبات الحمص بمنطقة شمبات بالسودان وامكانية مكافحته باستخدام المكافحة البيولوجية والمبيد كستاندرد باستخدام الجرعة الموصي بها مقارنة بالشاهد. تم عزل المسبب TillNourالفطري المرضي من أجزاء الحمص المصابة ثم تم تعريف الفطر حسب الصفات المزرعية بأنه فطر (*FusariumoxysporumF.sp*.ciceris) وأوضحت الدراسة المعملية لتقييم البيئة الغذائية أن )الأمثل في درجة حرارة °28م مناسبة لنموء (PDAبيئة البطاطس ديكستروز اجار هي مبيدات الفطريات الكيميائية. *F.oxysporum*الفطر.كانت طريقة المكافحة الرئيسية لفطر ومع ذلك، فقد كان هذه الطريقة غير فعاله في ظل الظروف المواتية لتطوير المرضوالتكلفة العالية للمبيدات. وهذا يستدعي تطوير طرق بديلة للسيطرة على العامل الممرض. وهذا يشمل استخدام عوامل المكافحة البيولوجية، والتي تعتبر آمنة وسهلة التبني، وصديقة للبيئة أحد هذه الطرق التي يمكن أن تساهم بشكل كبير في السيطرة على هذا المرض. الفطر المضاد هو أحد عوامل المكافحة الحيوية التي يمكن تطويرها لهذا الغرض. Trichoderma وتشير النتايج إلى *F.oxysporum*تم اختباره ضد *Trichodermaharzianum* مقارنة بمعدل نمو 1.282mmكان معدل نموه أبطأ (*T.* )أن*F.oxysporum* *harzianum*)*mm(3.8.* بواسطةهيفات*F.oxysporu*mتم تثبيط نموهيفات *T. harzianum* *T.*التي*T.*نمت على كل جانب ممكن من الفطر المسببة للمرض. عموما وجد أن . أشارت النتائج إلى أن *F.oxysporum*أكثر فعالية في الحد من نموهيفات الفطر*harzianum* وايضا نقصًا .1)(282m m.هناك نقصًا كبيرًا في نمو الفطر مقارنةً بنموه مع الترايكوديرما بما ان مفعول المبيد مقارب لمفعول 0) .(718m mكبيرًا في نمو الفطر مقارنةً بنموه مع المبيد *اذا يمكن استخدام فطر الترايكوديرمابدلامن المبيد.* هذه الاستراتيجية *T. harzianum* فطر تؤدي الي نقصان معدلاستخدام المبيدات الفطرية وبالتالي تقليل تكلفتها وزيادة سلامة البيئة

# Chapter one

## Introduction

Chickpea (*Cicer arietinum* L.) is a very important crop that is mainly used for human and animal food (Mohammadi *et al*, 2005; Hossain *et al*, 2016), and it is the second most widely grown legume worldwide (Pang *et al*., 2017) after soybean (Varshney *et al*., 2014). This crop can be cultivated in many areas, including marginal land and low fertility areas (Esfahani *et al*., 2014), It is cultivation plays a key role in maintaining soil fertility, especially in tropical regions (Varshney *et al*., 2009),thus representing an important component of crop rotation. Current global chickpea production is approximately 13 million tons (Mt) (FAO, 2014), with an expected increase to 17 Mt in 2020(Abate *et al*., 2012). Chickpea plays important roles in the human diet (Ulukan *et al*., 2012) and agricultural systems (Varshney *et al*., 2014). The seeds are rich in fiber, vitamins, carbohydrates, mineral salts (Ulukan *et al*., 2012), unsaturated fatty acids and β-carotene (Gaur *et al*, 2012) and are a good source of protein, with a content of approximately 21% (Esfahani *et al*., 2014). Therefore, this crop plays a key role in the food security of developing countries and is an important component of subsistence agriculture (Varshney *et al*., 2014).

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. 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).

In Sudan, where agriculture continues to dominate economy, more than 80% of the population is engaged in crops production. Chickpea, which occupy more than 7,000 ha, is considered as one of the principal cool-season food legumes in the Sudan, besides faba bean, lentil and haricot bean, to a lesser extent, field pea and lupin (ICARDA/ARC.1995). The Crop has a significant role in the diets of the Sudanese people and contributes substantially to their income. It is gaining further importance as a source of protein. Thus, chickpea is weighed for its economical and nutritive values.

Fusarium wilt caused by *Fusarium oxysporum Schlechtend. f. sp. ciceris* is one of the most important soil borne diseases of chickpea in the Sudan and adversely affects crop stand and hence reduces crop productivity (Freigoun 1980; Hussein 1982). Yield losses can reach up to 100% in some traditional chickpea-producing areas of northern part of the Sudan that forced farmers to abandon growing chickpea. Recently, chickpea cultivation was shifted from Northern States to the Gezira State due to high market value over the heat-tolerant wheat. Although chickpea production is new to Gezira State, the crop still suffers from epidemics of wilt/root rots.

In beneficial biological agent, Trichoderma is a filamentous fungus, which has attracted the attention against various plant pathogens (Harman, *et al*., 2004). Several modes of action have been proposed to explain the biocontrol of plant pathogens by *Trichoderma*, these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities (Cook, *et al*., 1985) *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular (Faruk, *et al*., 2002,). The species of

Trichoderma have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition (Podder *et al.*, 2004) Considering these points, the present study was conducted to find out the most effective species of Trichoderma and fungicide against chickpea Fusarium wilt.

Chickpea grown in the Sudan is produced under river flood, its cultivation with pump irrigation is expanding (Belal, 1994) Khartoum State is proposed to be a promising production area. Hence the occurrence of wilt/root-rot disease complex has been observed in recent years in Khartoum area with high levels of severity (Hussein *et al*, 2002) this study was under taken with the following objectives:

1. Isolation and identification of the causal agent of Fusarium wilt of chickpea *in vitro*.
2. Evaluating the efficacy of *Trichoderma harzianum*as biocontrol agents against *Fusarium oxysporum* .f. sp *.ciceris in vitro*.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Chickpea :

Chickpea (*Cicer arietinum* L)is the world's third most important crop, among pulses, fifth among grain legumes, and 15th among grain crops of the world. It is one of the world's most important leguminous food crops. It accounts for 12% of the world pulses production, with a 1.9% annual growth rate of chickpea production during the last 20 years (Vishwadhar and Gurha, 1998), and Upadhyaya, 2003). The plant is one of the major pulse crops in West Asian and North African regions. It has great importance as food, feed and fodder. Chickpea is a major grain legume used for food from ancient days. It is one of the essential semi-arid tropical (SAT) legume crop. Chickpea is either grown during the post-rainy season on stored soil moisture (South Asia and spring-sown Mediterranean) or as a Mediterranean winter crop on in-season rainfall. In both instances the crop is exposed to terminal drought which is accompanied by rising temperatures. The South Asian crop may also experience high temperatures in the seedling phase if planted early (Berger and Turner 2007). Chickpea productivity is constrained by several a biotic stresses (Singh *et al*. 1994; Gaur et al. 2007) and temperature is one of the most important determinants of crop growth over a range of environments (Summerfield *et al*. 1990) and may limit chickpea yield (Basu *et al*. 2009). The effects of heat stress during the vegetative and reproductive growth stages using agronomic, phonological, morphological and physiological assessment has been studied in various crops such as wheat (Sharma *et al*. 2005), rice (Weerakoon *et al*. 2008) and cotton (Cottee *et al*. 2010) whilst only limited

research has been conducted in chickpea (Wang *et al*. 2006). The detrimental effects of high temperature on various growth and reproductive stages are difficult to assess when growing conditions are favorable in the short term (few days) as the plant continues vegetative growth but sets fewer pods because of indeterminate plant type (Liu *et al*. 2003). The relatively narrow genetic base of cultivated chickpea is another reason why high temperature has such a detrimental effect on growth and reproductive physiology (Abbo *et al*. 2003a). For these reasons chickpea tends to be sensitive to high temperature during the growth and reproductive stages. In general, the cool season food legumes (peas, lentil, chickpea and faba bean) are more sensitive to heat than warm season legumes (cowpea, soybean, groundnut, and pigeon pea). Among cool season legumes, chickpea is less sensitive to high temperature (Wery *et al*. 1993; McDonald and Paulsen 1997). Although chickpea is exposed to warm temperature (> 30ºC) in certain regions, limited yield loss was found at 30ºC which is higher than other cool season legumes such as field peas, faba bean and lentil (Summerfield *et al*. 1984; and Patrick *et al* 2010).

## 2.2. State of Chickpea Production:

Climates favorable for chickpea production fall into two general groupings; Mediterranean and summer dominant rainfall semi-arid subtropical climates (Berger and Turner; 2007). Chickpea production is also grouped into three regions globally: West Asia and North Africa (WANA), The Indian subcontinent region and recently emerged other regions. The details of these regions, their climate and relative intensity of the principle stresses are discussed by Berger and Turner (2007). Chickpea is extensively cultivated in the Mediterranean climate regions of northern Pakistan, Iran, Iraq, Turkey, southern and south Western Australia and the

Mediterranean basin. In these areas, chickpea is widely sown in winter at a maximum air temperature of 10ºC (Berger, 2007) and high temperature occasionally occurs during reproductive development in the spring (Iliadis; 1990). In the Indian subcontinent region and recently emerged regions (e.g. eastern, northern and southern Australia), the crop experiences cool (5 to10ºC) and frosty nights (0 to -1ºC) in the early vegetative stage and warm (20 to 27ºC) to hot (> 30ºC) air temperature during the day over the reproductive phase (Summerfield *et al*. 1984; Summerfield *et al*. 1990; Berger and Turner; 2007). During the last two decades, south Indian and eastern Australian late-sown chickpea has been exposed to heat stress in the growing season, mainly in reproductive phase. In south India, if the rainy season (kharif) is extended, then the chickpea sowing in the spring season will be delayed (Ali; 2004). This delay exposure of the crop to high temperature during the reproductive stage. In Australia, particularly in northern NSW and depending on the climatic conditions, sowing can be delayed until last week of June to reduce the incidence of Ascochyta blight (Moore and Knights; 2009). However, late sown crops may experience high temperatures during the reproductive phase. Berger and Turner; (2007) and Berger *et al*; (2011) described the global chickpea distribution based on climate analysis and current production trends. The climate analysis showed that the current chickpea growing area is under threat from increasing temperature and production may extend to cooler regions.

## 2.3. Nitrogen Fixation:

The ability of chickpea to fix atmospheric N lessens chickpea is dependence on soil-N and reinforces its role in the cropping system. Beck;,(1992)reported that, $N_2$ or N fixation in chickpea range from 0 to 176 kg/ha-1/ season, with nitrogen derived from

air ranging from 0 to 82 %, dependingon environmental variables, method of measurement, cultivars, and presence of appropriate rhizobia.

Identifying cultivars with high capacity to fix nitrogen is the ultimate goal in any programmed aimed to enhancing N2-fixation? Although, several methods, such as dry matter yield (Beck, 1992), nodule number or mass, total-Ndifferences (Edwards, *et al*1981), acetylene reduction assay, and the N-15 isotope technique (Danso, 1986) have been used to assess N2-fixation. Beck, (1992) used N-15 technique to evaluate eight chickpea cultivars for their ability to fix nitrogen, and the interaction between chickpea cultivars and rhizobial strain treatments.

## 2.4. Fungal Disease:

Chickpea productivity is limited by several diseases, of which Fusarium wilt (FW) caused by Fusarium oxysporum f. sp. ciceris, Ascochyta blight (AB) caused by *Ascochyta rabies*, and Botrytis gray mold (BGM) caused by *Botrytis cinerea* are predominant (Pande*et al*. 2007).

Among them Fusarium wilt which is a vascular disease cause damage to vascular tissue and eventually the plant die due to wilting. Ascochyta blight is a foliar disease that causes wilting and rotting of leaves. Grey mold is caused by *Botrytis cinerea* third most important disease in chickpea.

Fusarium wilt caused by *F.oxysporum* is one of the major yield limiting factors in chickpea resulting in 10 to 90% yield loss (Sharma and Muehlbauer; 2007). The disease is highly destructive and worldwide in occurrence. It has been reportedfrom almost all chickpea growing areas of the world including the Indian subcontinent, Iran, Peru, Syria, Ethiopia, Mexico, Spain, Tunisia, Turkey and US as reviewed by Sharma and Muehlbauer (2007). The persistence of the pathogen

survives in soil without a host for years have made its control more difficult. The disease to some extent can be managed by use of bio-control agentswhich provide eco-friendly control of the disease. Some bio-control agents have been reviewed by Hervas *et al*. (1997); Landa *et al*. (2001, 2004). But more economic and ecofriendly approach for Fusarium wilt management could be resistance genes (Jimenez*et al*. 1993, Sharma *et al*. 2005, Sharma and Muehlbauer, 2007) that provide natural and inherent protection against the pathogen.

## 2.5. Fusarium wilts:

Fusarium wilt is a widespread disease caused by the soil born fungal pathogen *F. oxysporium*. Fusarium wilt has been reported from diverse species from unrelated families. It is a major constraint in the production of pulse crop, chickpea and lentils in particular (Dita *et al*. 2005).The disease affects seedlings and adult plants where it causes leaf cholorses, wilting and death (Dita *et al*. 2005).

Eight physiological races of *F. oxysporum* have been identified till now (Sharma and Muehlbauer, 2007). Four of them were reported from India. Haware and Nene (1982) reported existence of these 4 races using 10 chickpea differential lines which show variable resistance and susceptibility to different races of the pathogen. Races (1, 2, 3 and 4) were found to be prevalent in India causing wide spread crop damages. The geographical distribution of races shows regional specificity for their occurrence in different regions of the world. Apart from region specificities, the eight races can also be divided into two groups based on symptomatology of infected plants i.e. yellowing syndrome and wilting syndrome.

The recesses are more important economically than the others as wilting is more devastating than the yellowing. The plants show wilting syndrome within 3-4

weeks of infestation. However, wilting and yellowing syndromes are considered race-specific (Sharma and Muehlbauer, 2007). Although the pathogen could be classified into several races, overall, genetic makeup of the pathogen is very much similar.

The pathogen penetrates through the roots until it reaches the xylem where it reduces or blocks water transport to aerial parts. The infected plants finally wilt and die. (Halila *et al*.2009). The infection process is influenced by environment especially the temperature and inoculums load. A temperature of around 25°C and inoculums load of 104–105 micro- or macro-conidia is optimum for disease development.

## 2.6 Economic Importance :

*F. oxysporum* is a seed and soil-borne fungal pathogen that causes Fusarium wilt of chickpea (Haware, 1990; Nene *et al*, 1987). Jalali and Chand (1992) reported that the major limiting factor in chickpea production is Fusarium. It was observed in wilted plants, an association between Fusarium sp. and Rhizoctonia sp.

Based on research conducted by many scientists, *F. oxysporum* f. sp. ciceris is now accepted world-wide as the causal agent of wilt (McRae, 1932; Prasad and Padwick, 1939; Booth, 1971; and Kaiser *et al*., 1994). However, the disease was reported to cause wilt in chickpea in about 33 countries of the world, causing 10–15% yield losses annually depending on environmental conditions e.g. Indian subcontinent, United States, Tunisia, Turkey, Ethiopia, Spain, and Mexico (Grewal and Paul, 1970; Singh and Dahiya, 1973; and Westerlund *et al*., 1974).

The wilt disease was found to be more serious in low rainfall areas, where weather conditions are favorable for disease development (Khan, 1980). Wilting in

chickpea can be observed after 20 to 25 days of sowing. The disease appears at seedling and reproductive stages of the crop under field conditions. The infected plants look healthy and do not show external rotting, but their roots when split vertically from the collar region to downward, show brown discoloration of the internal tissues. The main symptoms of the disease are drying and yellowing of leaves from, the base upward, improper branching, drooping of petioles and rachis, browning of vascular bundles, withering of plants and wilting of plants (Halila and Strange, 1996)

## 2.7. Symptoms:

Symptoms of the disease can develop at any stage of plant growth, and affected plants may be grouped in patches or appear spread across a field (Jiménez; Castillo *et al*, 2015).

The wilt can be observed in susceptible genotypes within 25 days after sowing in the field (designated "early wilt") (Al-taae, *et al*, 2013). However, symptoms are usually more visible in the early stages of flowering, 6 to 8 weeks after sowing and can also appear up to pudding stage ("late wilt"). Late wilted plants exhibit drooping of the petioles, rachis and leaflets, followed by yellowing and necrosis of foliage (Jiménez. Castillo *et al*, 2015).

Early wilting causes more loss than late wilting. Nevertheless, seeds from late-wilted plants are lighter, rougher and duller than those from healthy plants (Navas-Cortés, *et al*, 2000).

## 2.8. Ecology and Epidemiology :

The root diseases of chickpea are important in areas between latitudes 0°to 25° where the chickpea growing season is dry and warm. The behavior of root-infecting fungi in the soil is a complex subject. Factors important in the epidemiology of root-infecting fungi are inoculums density and path type in the soil, plant age, host resistance and its genetic potential, air and soil temperature, soil moisture, soil nutrients, and plant density.

Chickpea wilt can be observed in a highly susceptible cultivar within 25 days after sowing. Affected seedlings show drooping of the leaves and a paler color. Adult plants show typical wilt symptoms. The roots do not show external rotting, but when split vertically they display a brown discoloration of the internal tissues. Isolates of antagonist induce either a fast wilting or progressive yellowing syndrome which develops 15 to 40 days after inoculation. The fungus is seed and soil borne. Lentil, pea, and pigeon pea were identified as symptomless carrier of the fungus (Haware and Nene 1982a). It can survive in the soil in the absence of the host for at least 6 years (Haware *et al.* 1986). Several fungal pathogens, each causing seedling diseases and root rots of chickpea, have been reported. Dry root rot caused by *Rhizoctonia bataticola* is a serious disease whenever the crop is exposed to temperatures >30" C (Singh and Mehrotra, 1982). The disease development is influenced by dry soil conditions especially at flowering. The plant suddenly dries in the field. The leaves and stems of affected plants are usually straw colored. The tap root is dark and quite brittle in dry soil. The dark black sclerotia can be observed on and within host tissues. Rhizoctonia is soil borne and sclerotia, formed on the organic residue and in the host tissues become the chief source of inoculum. The pathogen has a wide host range. Collar rot caused by *Sclerotium rolfsii* is seen in wet soil and at warm temperature in the seedling stage. The pathogen continues to plague growers and cause considerable loss.

Chickpea following rice particularly suffers from collar rot infection. *F.solani*. *F. eumartii*, and *T. basicola* infected chickpea, often has severe black root rot symptoms. *Pythiumultimum* causes seed-rot and seedling blight. These four pathogens are favored by wet soil conditions and mostly attack chickpea in the seedling stage (Bowden *et al*. 1985; Trapero-Casas and Jimenez 1985).

## 2.9. Control Measures :

### 2.9.1, Cultural Practices :

Soil borne pathogens persist in the soil, while it is difficult to eliminate the inoculums from the field; an approach that can minimize the effects of these diseases on yield in areas where availability of land is not limiting is to avoid planting in heavily infested fields. The Fusarium wilt fungus has the ability to survive in the soil for long periods. Therefore crop rotation is not effective in reducing wilt incidence. Another method of reducing inoculums is deep plowing during the summer and removal of host debris from the field. Pre-emergence damping-off due to *Rhizoctonia bataticola* does not occur at low temperature, while the disease attack is maximal at 34°C. In India, early sowing of early-maturing cultivars with timely irrigation can avoid high temperature above 30"C during crop maturity, thereby reducing mortality. Research in the Pacific Northwest of USA shows that tillage and residue management can markedly influence the severity of root rot in pea (Kraft *et al*. 1988). Recommendations for controlling *S. rolfsii*; emphasize the importance of sanitary and cultural practices. These include rouging, increasing plant spacing, eliminating weed hosts and removing host tissues from the soil surface. Chickpea should not be sown under wet soil conditions to reduce seed rot and pre-emergence damping off.

### 2.9.2. Chickpea Seed :

It is important to make high quality chickpea seed available to farmers for sowing. A seed production program for food legumes is not taken up by most of the national programs and seed agencies. Seed should give high germination and plant vigor, and be pure and free from seed borne diseases. Emergence differs with color of the testae in chickpea. White seeded kabuli types emerge poorly in comparison with desi types which have brown or black testae (Kaiser and Hannan1983). The white testae of the kabuli types adhere loosely to the cotyledons compared to the close adherence of colored testae.

### 2.9.4. Seed Treatment :

Combined use of host resistance with fungicide results in better seedling emergence and may delay the onset of root rots. *F. oxysporium* is internally seed borne, and seed dressing with Benlate® T (30% beno my 1 + 30% thiram) at 1.5 g kg·1 seed successfully eradicates the seed borne inoculums (Haware *et al*. 1978). Seed dressing with protectant or systemic fungicides used singly or as a mixture, significantly increased seedling emergence in moderately susceptible chickpea cultivars (Jimenez and Trapero-Casas 1985).

### 2.9.5. Plant Resistance:

At ICRISAT Center, Patancheru, India, effective field screening and laboratory procedures have been developed and wilt resistance sources identified (Nene and Reddy 1987). Some of them such as ICC 12237 and 12269 have additional resistance to dry root rot and black root rot (Nene 1988). Multilocational testing for

Fusarium wilt and root rots have been carried out through active cooperation between national programs and ICRISAT, and stable resistance has been found. Over 150 wilt resistant sources are available at ICRISAT. Wilt resistance has been incorporated into high yielding desi and kabuli backgrounds. Wilt-resistant short duration kabuli cultivars ICCV 2, 3, 4, and 5 escape tenninal drought in South and Central India (Kumar *et al*. 1985). Chickpea lines, Avrodhi, BG 246, ICCC 32, and ICCC 42 were found to be resistant at several locations in India in multilocation testing. In spite of the existence of rac~ in F. oxysporum f .sp. cieeri, it has not been difficult to identify a high level of resistance that is operative at severa~ locations. Chickpea breeding programs at Culiacan and Sonora in Mexico have several advanced wilt resistant lines (Morales 1986). In California, a wilt-resistant cultivar Surutato 77 from Mexico was introduced in 1980 and presently covers most of the chickpea area. Recently two large seeded kabuli cultivars UC 15 and UC 27 have been released (Buddenhagen *et al*. 1988). Screening trials in a fusarium wilt sick plot at Cordoba. Spain in 1987 and 1989 indicated the wilt resistance in some small seeded kabuli gennplasm from ICARDA. Field screening in a wilt sick plot at Beja, Tunisia resulted in identifying the wilt-resistant chickpea cultivar Amdoun 1 in 1986.

**2.9.6. Biological Control:**

Biological control of Fusarium wilt has given encouraging results .Control may involve prior inoculation of plant with forma specials of F. oxysporum not pathogenic to crop, or use of antagonistic fungi such as *Trichoderma* spp.(Agrios,1997). Chickpea seed bacterization by *Pseudomonas* spp showed an

increased seed germination, shoot height, root length, fresh weight, dry weight and yield.

The disease suppression and/or growth enhancement along with the positive root colonization by *Pseudomonas* spp. indicates its possible use as a biocontrol agent against chickpea wilt (Kumar, 1998).

*F.oxysporum*was tested in liquid culture for sensitivity to various aqueous neem extracts and seed oil .The oil was found inhibitory suggesting the presence of antifungal substances which slowed the growth of the fungus, but did not kill it (Schmutterer, 1995).

**2.9.7. Chemical control:**

Khare*et al*. (1973) reported Benlate and Thiram to be better seed dressers in reducing chickpea wilt incidence and increasing seed yield without any harmful effects on nodulation. Verma and Vyas (1977) found benomyl, carboxin, thiabendazole and carbendazim as the superior seed dresser against chickpea wilt. Hawar*eet al*. (1978) eradicated seed-borne *F. oxysporum* f.sp. *ciceris*by treating the seeds with Benlate-T 0.15 per cent. Vishwakarma and BasuChaudhary (1982) reported that seed treatment with Benlate was found to be the most effective fungicide in reducing infection due to *F. solani* and *Rhizoctonia solani* and vitavax against *Sclerotium rolfsii*. Singh *et al.* (2003) evaluated seven fungicides, i.e. thiram, Bavistin (carbendazim), Blitox (copper oxychloride), Captaf (captan), Indofil M-45 (mancozeb+thiophanate-methyl) Ridomil (mancozeb+metalaxyl) and Kitazin (iprobenfos), against chickpea wilt *in vitro* (each at 1% concentration) and *in vivo* (as seed treatment each at 2.5 g/kg seed, except for Kitazin (1.0 ml/kg)

and as soil drenching each at 0.3%) in Pusa. Thiram and Bavistin proved the most suitable fungicides in inhibiting the fungus growth *in vitro*. These fungicides reduced the incidence of wilt when used as seed treatment and soil drenching. Both fungicides decreased disease incidence and increased grain yield under field conditions. The fungus being soil-borne remains in the soil for long periods, so it cannot be controlled by crop rotation. The seed-borne inoculums can be eradicated by seed-dressing fungicides, Benlate-T, Benomyl 30% and Thiram 30% at 1.5% (Hawar*eet al*., 1978). Thiram (0.15%) and Carbendazin (0.1%) as seed treatment against *Fusarium* wilt were found to be the most effective chemicals. Due to biological competition and environmental stresses it can survive in soil for a long period (Nikam *et al.,* 2007). Christian *et al.* (2007) reported significant effect of five fungicides and found that the highest inhibition of the fungus was acheired 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 Polyramcombi 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.

# CHAPTER THEREE

# MATERIAL AND METHOD

## 3.1. Study site:

This study was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology in February 2019. The aim of this study was to evaluate the efficacy of *Trichoderma harzianum* against the *Fusarium oxysporum f. sp. Ciceris* the causal agent of wilt disease of chickpea and to explore the methods of control under laboratory conditions where temperature around $28^0$C.

## 3.2. Materials, tools and equipment used:

All materials and equipments used in this experiment were sterilized using 95% ethyl alcohol .Autoclave was used forsterilization of Petri plate (glasses). Other equipments were sterilized by UV light.

## 3.3. Samples collected:

Infected parts of chickpea plant showing typical symptoms of wilt were collected from thereafter; they were put in poly bags then transferred to the laboratory.

## 3.4. Isolation and Identification of the fungus:

The secured plant material (roots and stems) were cut in to small bits (0.5 to 1.0 cm) and washed well in tap water to remove the adhering dirty particles. Then they were cut to pieces were surfaces sterilized by sodium hypochlorite Naocl at 1% concentration for five minutes.

Rinsed three times in sterilized distilled water to remove traces of Naocl and dried on sterilized filter paper. The sterilized stems cutting were then plated at the rate of five cutting per plate on Potato Dextrose Agar (PDA) media and incubated at 25ºC for 7 days of incubation. The media was supplemented with

Plate .1 Show the symptom of *Fusarium oxysporum .f.sp.cicers*. in chickpae

Chloramphenicol (250mg) as antibacterial agent (Anon., 1981). The isolated fungus was the 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 microscope examination were also carried out for hyphen and conidia structure based on the method of Booth,(1977) .To confirm that the fungus is *Fusarium oxysporum f. sp. Ciceris*. Standard books and research paper were also consulted during the examination of this fungus (Aneja, 2004).

The purified fungus was maintained PDA for further studies.

The fungal antagonist *Trichoderma harzianum* was isolate from soils. The efficacy of antagonists against the pathogen was initially evaluated on potato dextrose agar (PDA). Discs (0.8 mm diameter) of seven-day-old culture of bio-agents were inoculated opposite to disc of the tested fungus (seven days old culture) in the same plate. Both organisms were placed in such a manner that they would get equal opportunity for growth. The experiments were conducted with four replications for each treatment, while control plates were inoculated only by tested fungus. Plates were then incubated at 27±1°C. Observation were recorded after seven days of inoculation including area covered by the *T. harzianum* and the pathogen then percent of inhibition was calculated using the biologicl controlagainst the *F. oxysporum* on PDA according to the poison food technique24. Completely randomized design was adapted.

## 3.5. Preparation of Fungicide (Till Nour 25% EC) :

Till Nour is systemic fungicide the active ingredients are (Propiconazole 250 gm\1).The fungicide was tested by dissolving 0.6 in 250 ml of sterilized distilled water to give hundred percentages as recommended dose. Full cooled (PDA) medium was prepared in 250 ml conical flasks 0.6 ml of the fungicide was added in five plates.

## 3.6. Extraction procedures:

Potato Dextrose Agar (PDA) 10 g were placed in 250 ml flasks. Distill water. The antibacterial Chloromycetin capsules were used to suppress bacterial contamination. Aliquots of the extract certified medium (20 ml each) was poured into sterilized glass Petri dish. After solidification of the medium, discs (0.8 mm

diameter) cut from 7 days old *F. oxysporum* culturewere transferred, aseptically, each placed in center of a Petri containing PDA medium for7days. The Petri dishes were incubated at $25^{o}$ C. Each treatment was replicated three times. Treatment effects were assessed as in 3, 5 and 7 days later. Percent growth inhibition was calculated using the formula developed by Jagtap and Sontakke (2007):

$$I = \frac{C - T}{C} \times 100 \qquad (1)$$

Where: I = Percent inhibition, C = Growth of test f. oxysporum in control medium in cm and. T = Growth of test T.harzianum in the respective treatment in cm.

### 3.6.1. Effects of *Trichoderma harzianum* against the growth of *F. oxysporum* in vitro *:*

The experiment was laid out in Petri dishes containing sterilized PDA. Half of the solidified medium was inoculated with the fungus from 7 days old culture as in 0.8 mm discs. The second half was inoculated with a *Trichoderma harzianum*. Thus, both organisms would get equal opportunities for growth. The plate which containing the test fungus only was included as control. The individual treatments were replicated three times. The Petri dishes were incubated at 25oC and fungal growth was estimated daily and percent growth inhibition was calculated as the formula in **3.6.**

### 3.6.2. Effect of Fungicides :

The fungicidewas tested *in vitro* to evaluate the efficacy of the growth of the fungus employing the Poisoned food technique. Till Nourfungicidewas used25%

EC. One dilution of each product was used; 0.6mm of fungicide was added to 250 ml of sterilized distilled water and was consider100% concentration. From this concentration 2 and 4- fold dilutions were prepared by adding the required amount of sterilized PDA medium in 250ml conical flask to give a final respective concentration of 100% of the original. The content of each flask was poured into three sterilized Petri-dishes and left to solidify. Subsequent to medium solidification a disc of the fungus was placed at the center of each Petri dish. Petri dishes containing PDA, similarly inoculated with the fungus served as control and treatment effects were assessed and calculated as the formula in **3.6.**

## 3.7. Statistical Analysis Procedure:

The data collected was statistically analyzed according to Gen stat One-way Completely Randomized.

# CHAPTER FOUR

# RESULTS

## 4.1. Isolation and Identification of the Fungus:

The results, showed that the fungus identified based on morphological and cultural characters as *Fusarium oxysporum f.sp.* ciceris.

## 4.2. Effect of *Trichoderma harzianum*on the growth of *F. oxysporum*:

The results indicate that *F. oxysporum* had slower growth (0.576m) than *T.harzianum* (2.162m). Growth of *F. oxysporum* was inhibited by encroachment *T.harzianum which* grew on all possible side of the pathogenic fungus in plate to suppress further growth of pathogen. The results in (Table 1) showed that the growth of the fungal *F. oxysporum* decreased by the growth of *Trichoderma harzianum* after three days of inculcation 1.33 – 2.672m respectively.

Five day after inoculation *Trichoderma harzianum* suppressing effects against the fungal growth was 1.238 – 2.94m.

Seven days after inoculation the growth of the fungus *F. Oxysporum* decreased by the growth of *Trichoderma harzianum* (1.282 – 3.344m). Generally, *T. harzianum* was found to be most effective in checking the growth of F. oxysporium over control. The result indicated that there was a significant decrease ($p \leq 0.05$) in the mycelial growth of the fungus compared to the redaction of the growth of *F. oxysporum* with *Trichoderma harzianum*.

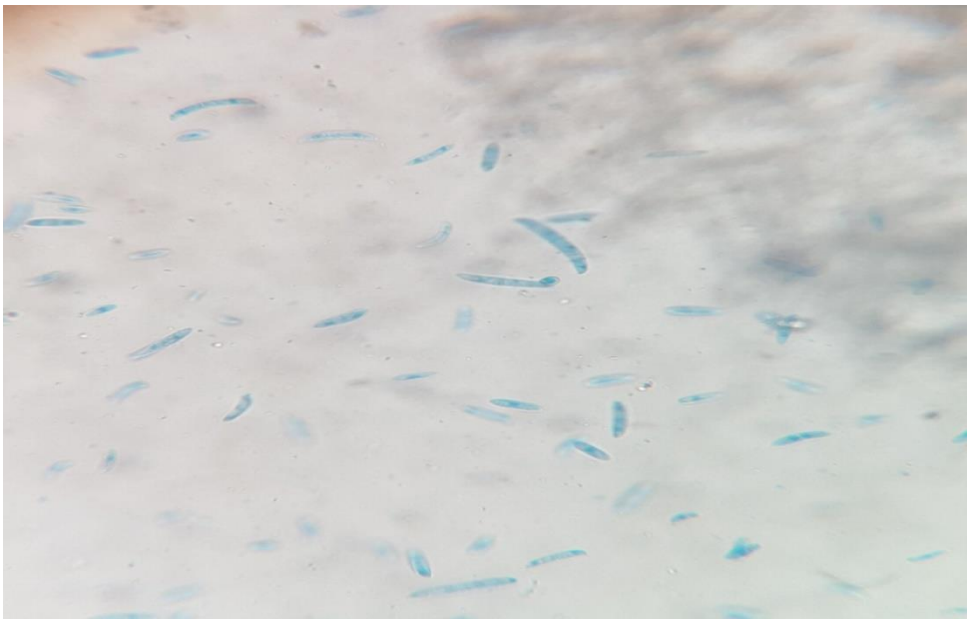

Plate***2: Fusarium oxysporum f.*sp. ciceris under Microscope**

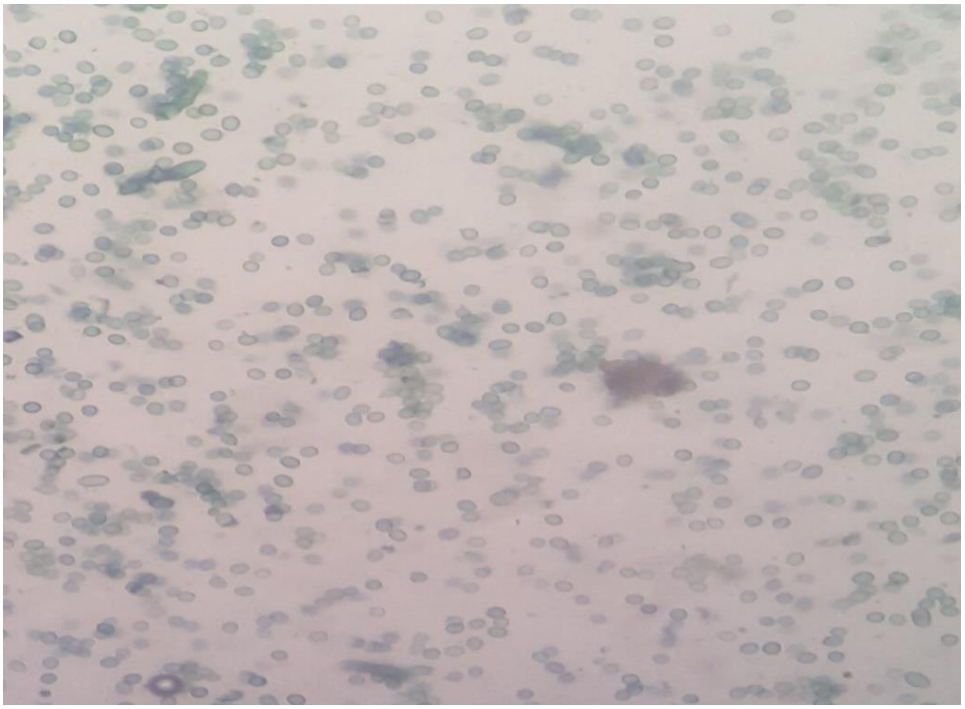

Plate3: ***Trichoderma harzianum* underMicroscope**

## 4.3. Effects of fungicides (Till Nour) on liner growth of *Fusarium oxysporum:*

The results indicated that there was a significant decrease($p \leq 0.05$)in the mycelia growth of the fungus treated with the fungicide cumbered with the control (untreated).

The results in (Table 1) showed that the growth of the fungus *F.oxysporum* decreased by the fungicide after three days of inculcation. The reductions of the hyphae growth was (0.186 – 1.33m) respectively.

Five day after inoculation the fungicide suppressed the fungal growth by (0.494 – 1.238m)

Seven days after inoculation the growth of the fungal *F.oxysporum* decreased by the fungicide was(0.718 – 1.282mm).

**Table 1 Effects of *Trichoderma harzianum*and the chemical (Till Nour) against *Fusarium oxysporum*in chickpea one, three, five and seven days after inoculation.**

| Treatment | Day 1 | Day 3 | Day 5 | Day7 |
|---|---|---|---|---|
| ***Fusarium oxysporum*** | **0.676** | **1.702** | **2.852** | **3.402** |
| ***Trichodermaharzianum*** | **2.082** | **3.508** | **3.74** | **3.8** |
| ***Fusarium+Trichoderma*** | **0.576** | **1.33** | **1.238** | **1.282** |
| ***Trichoderma+ Fusarium*** | **2.162** | **2.672** | **2.94** | **3.344** |
| **Till Nour** | **0** | **0.186** | **0.494** | **0.718** |
| **l.sd** | **0.1288** | **0.4375** | **0.2989** | **0.3198** |
| **CV** | **8.88** | **17.64** | **10.06** | **9.66** |
| **Se** | **0.0617** | **0.2098** | **0.1433** | **0.1533** |

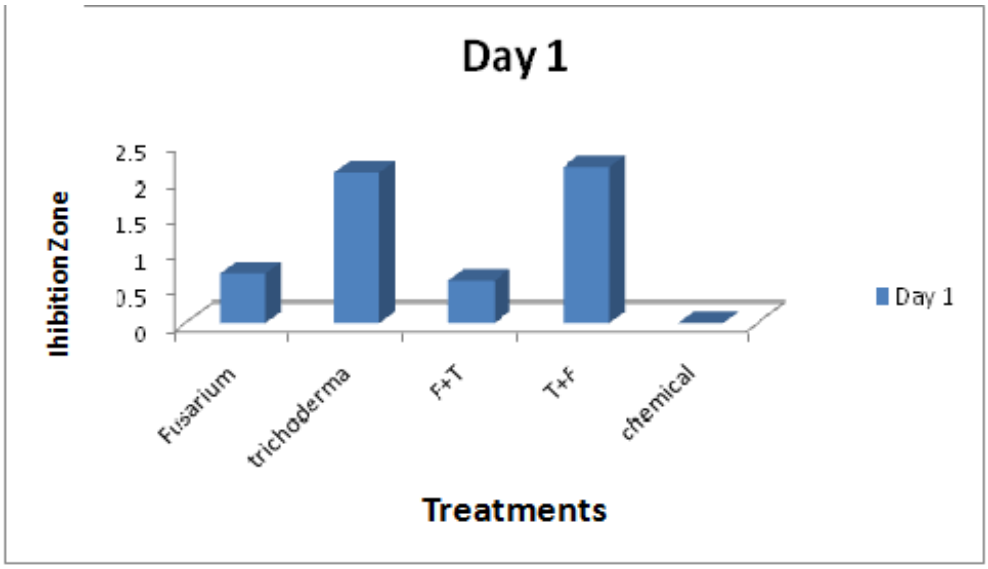


**Figure 1: Effects of Trichoderma harzianum and the chemical (Till Nour) against *Fusarium oxysporum* in chickpea one day after inoculation.**

- ***F :Fusarium oxysporum.***
- ***T :Trichoderma harzianum.***
- ***Chemical: Till Nour .***

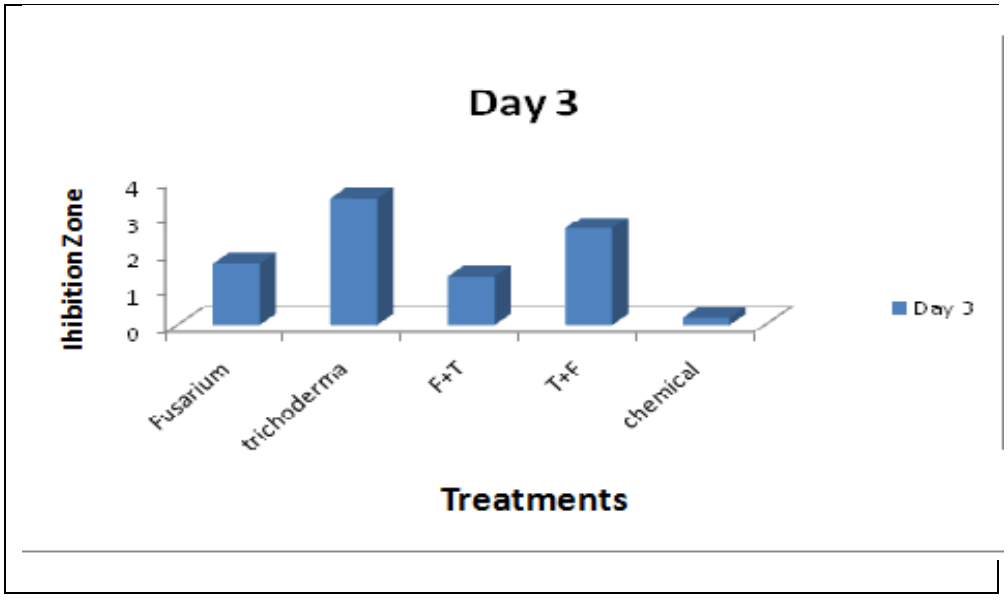


**Figure 2: Effects of *Trichoderma harzianum* and the chemical (Till Nour) against *Fusarium oxysporum* in chickpea three days after inoculation.**

- ***F : Fusarium oxysporum.***
- ***T :Trichoderma harzianum.***
- ***Chemical: Till Nour.***

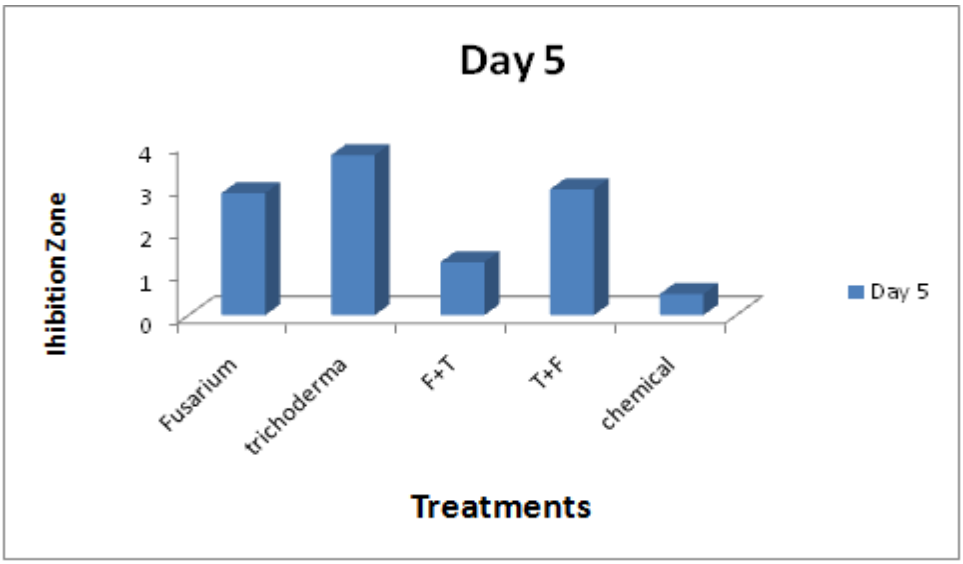


**Figure 3: Effects of *Trichoderma harzianum* and the chemical (Till Nour) against *Fusarium oxysporum* in chickpea five days after inoculation.**

- ***F : Fusarium oxysporum.***
- ***T :Trichodermaharzianum.***
- ***Chemical: Till Nour.***

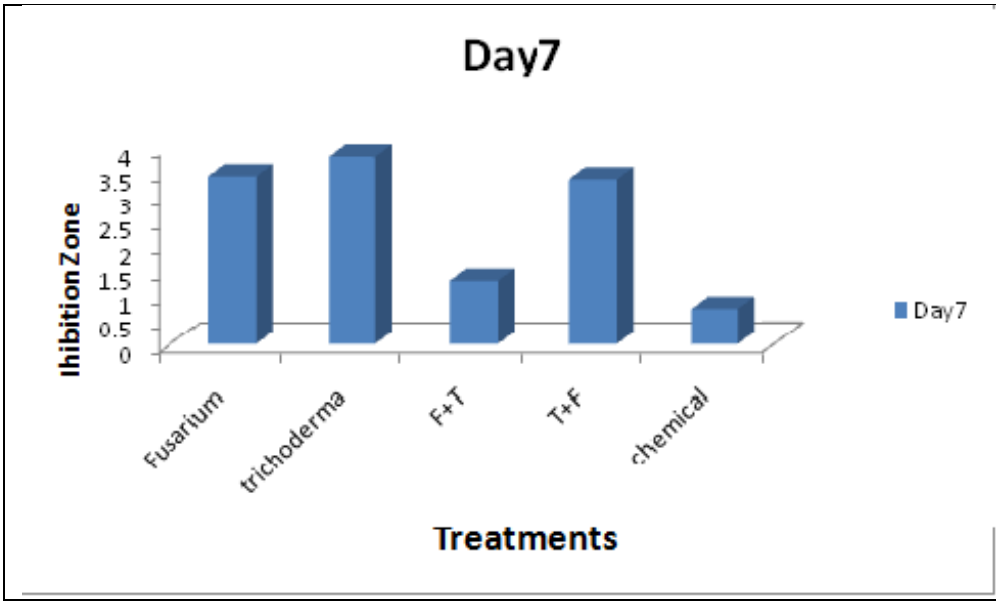


Figure 4:Effects of *Trichoderma harzianum* and the chemical (Till Nour) against *Fusarium oxysporum* in chickpea seven days after inoculation.

- ***F : Fusarium oxysporum.***
- ***T :Trichoderm harzianum.***
- ***Chemical: Till Nour.***

# CHAPTER FIVE

# DISCUSSION

Wilt disease is an economically important disease on chickpea in Sudan. This work is a participation aiming at solving the problem of wilt disease in Sudan. The estimation of yield losses by individual pests, weed or diseases ranged from 5 to 10% in temperate regions and from 50 to 100% in tropical regions (van Emden, 1988). This means that the wilt of chickpea can cause considerable losses in tropical areas like the Sudan. Isolation of the fungus was carried out from wilted chickpea plant collected from the collage of Agriculture studies, Sudan University of science and technology. The identification depended on laboratory investigation. Biological control may be defined as the reduction in inoculums density or disease producing activity of pathogen in its active or dormant state, by one or more organisms naturally or through manipulation of environment and host of the antagonists (Baker and Cook, 1974). Biocontrol agents are widely regarded by the general public as "natural" and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms. Moreover, knowledge concerning the behavior of such antagonists is essential for their effective use.

The *Trichoderma harzianum* was tested against *F. oxysporum* f. sp. ciceris in laboratory experiments .The results indicated that the *Trichoderma harzianum* significantly ($P \leq 0.05$) inhibited the growth of *F. oxysporum*.

The results indicated that *F. oxysporum* had slower growth than *Trichoderma harzianum* .Growth of *F. oxysporum* was inhibited by encrourgement of *Trichoderma harzianum.* Trichoderma grew on the possible sides of the

pathogenic fungus (Fusarium) in the plates and suppressed further growth of the pathogen .The pathogens grew fully in the control after 7 days. Rapid growth of *Trichoderma harzianum* is an important advantage in competition with plant pathogenic fungi for space and nutrients.

Our data are supported by Cook and Baker (1989) and Deacon and Berry (1992). Generally all Trichoderma spp significantly inhibited the growth of *F .oxysporum*. Maximum reduction in colony growth was induced by *T. harzianum* which was significantly ($P \leq 0.05$) superior over the other species. The antagonistic effect can be attributed to diffusible substances (antibiosis) secreted by the antagonists or due to their direct effect on the target pathogens. Concerning the antagonistic activity of Trichoderma isolates, our data are supported by Thangavelu *et al*., (2004) who found that *T. harzianum* was effective in inhibiting the growth of Fusarium *invitro*. Hervas*et.al* (1998) showed that *Trichoderma harzianum* effectively suppress wilt disease of chickpea caused by *Fusarium oxysporum*. The results obtained in respect of Trichoderma similar to those reported by Upadhyay*et al*., (2003) who had reported antagonists Trichoderma spp. as inhibitory to *F. oxysporum*. The present results are in agreement with the earlier results obtained by Gurha (2002), Pan and Bhagat (2007) and Vishwanath*et al*., (2008). *T. harzianum* isolate showed higher antagonism as compared to the other Trichoderma tested. This shows variability of efficacy of isolates and hence, the selection of better strains will be more useful for production and promotion of biological control. These isolates completely overgrew the pathogens and suppressed it within 7 days of inoculation.

The results on the efficacy of Till Nour tested *in vitro* demonstrated their significant inhibitory effect on the fungus mycelial growth. Analysis of variance

showed significant ($P \leq 0.05$) interaction between the chemical and reduction of mycelial growth of *F. oxysporum*.

Preliminary evaluation of the comparative effect of fungicide on the mycelial growth of *F. oxysporum* revealed that the effectiveness of fungicides in inhibiting the mycelial growth of the pathogen varied. This result is in agreement with those of Kovacikova (1970) and Jagtap and Sontakke (2007).

## Conclusions:

- *Trichoderma harzianum* is less costly and more environmentally friendly treatment than the fungicides.
- *T.harzianum and fungicide Till Nour* inhibited growth of the *F.oxysporum* f.sp ciceris, the causal agent of Chickpea significantly.

## ***Recommondation:***

- This experiment should be conducted in the greenhouse and field to ensure results.
- The fungus extract (*Trichoderma* harzianum) must be made in powder form so that farmers can easily use it.

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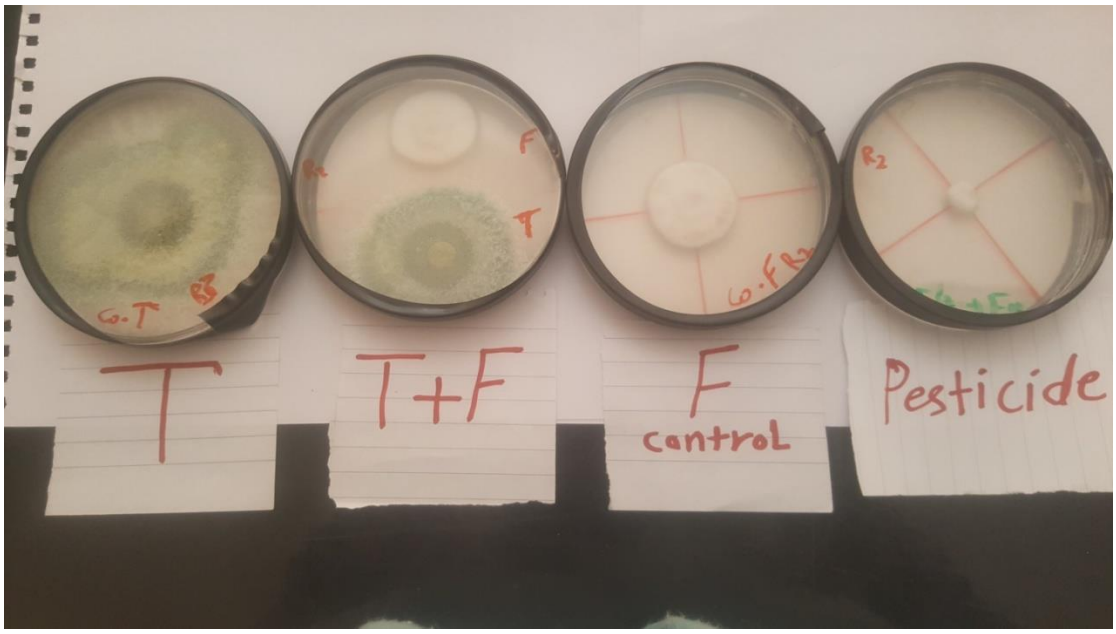


**Plate 4 Effects of *Trichodermaharzianum* and the chemical (Till Nour) against *Fusarium oxysporum*in chickpeathree days after inoculation.**

- *F:Fusariumoxysporum*..
- *T: Trichoderma harzianum.*
- Pesticide: Till Nour.

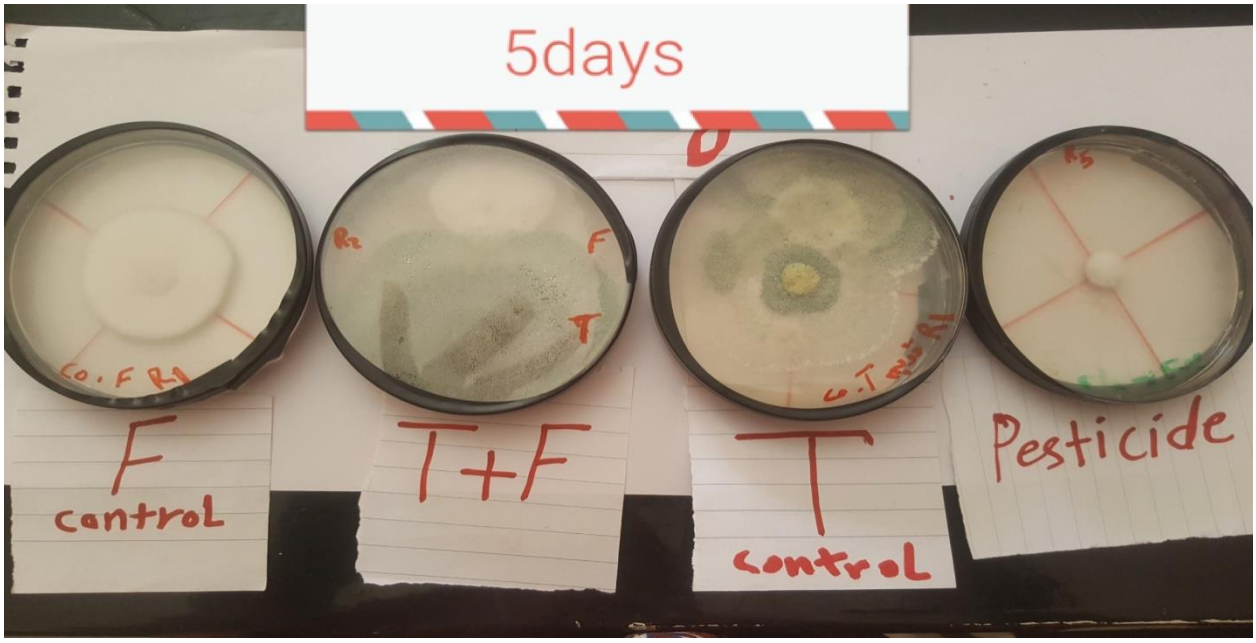


Plate 5: Effects of *Trichoderma harzianum* and the chemical (Till Nour) against *Fusarium oxysporum* in chickpea five days after inoculation

- *F:Fusariumoxysporum*..
- *T: Trichoderma harzianum.*
- Pesticide: Till Nour.


Plate .6 Effects of *Trichoderma harzianum* and the chemical (Till Nour) against *Fusarium* o*xysporum* in chickpea seven days after inoculation.

- *F:Fusariumoxysporum..*
- *T: Trichoderma harzianum.*
- Pesticide: Till Nour.