

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Sudan University of Science and Technology

College of Agricultural Studies

Department Of plant protection



Effect of Ginger (*Zingiber officinale*)

Ethanollic extract on the fungus in Tomato (*Fusarium oxysporium*)

تأثير المستخلص الايثانولي للجنزبيل على فطر (فيوزيريوم

اكسوسبوريم) في الطماطم

**A thesis submitted in partial of the requirements for the B.Sc (Honors)in
Plants Protection**

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الاية

بسم الله الرحمن الرحيم

قال تعالى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ بَاطَاتٍ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا
مُتَرَكَبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزُّرُّوتُونَ وَالرِّمَّانَ مُشْتَبِهًا وَغَيْرَ
مُتَشَابِهٍ انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكَ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ)

صدق الله العظيم

سورة الانعام (99)

Dedication

To my father...To my mother

To my Family...

To my teachers...

To all my friends....

With respect

Nasma

اهداء

الي من أدين لها بعمرى ومستقبلى

اليك يا رمز الصبر والحنان

اليك يا اروح معانى الحب والتضحية.....

فمنك تعلمت معنى الحياة والصبر وكبرياء النفس وطيبة القلب

اهدي اليك ثمرة جهدى.....

امى الغالية

الي الدكتورة الفاضلة اخلاص حسين محمد

الي رفقاء الدرب زملائي وزميلاتي

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ABSTRACT

This study was carried out in the laboratory of plant pathology, plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology in (2017) the objective of this study was to evaluate The efficacy of ginger (*Zingiber officinale*) ethanol extracts and (Fulldazim 50 WP) fungicides against *Fusarium oxysporum* f.sp Lycopersion in tomato in culture media Potato dextrose agar (PDA) *in vitro*. The fungi are an important causing significant reduction in yield. In the present study, the pathogenic fungi were isolated from infected plant parts .The fungi were identified based on morphological and culture characters as *Fusarium oxysporum* f.sp Lycopersion. Studies were conducted three concentrations of ginger ethanol extracts (25, 50 100%) in addition to the control treated with distilled water. The results obtained that all concentrations exhibited and has an inhibitory effects on the growth of the fungus tested compared to control (5).The highest inhibition effect was obtained at (100%) compered by control (5). Generally the inhibition zone increases with increase the concentration of the extracts. In conclusion, these studies showed that ginger contain antifungal properties that could investigate in further studies.

ملخص البحث

اجريت هذه الدراسة في معمل امراض النبات بقسم وقاية النبات كلية الدراسات الزراعية جامعة السودان للعلوم والتكنولوجيا (في اغسطس -سبتمبر 2017) لتقييم تاثير المستخلص الكحولي للجنزيبيل علي فطر فيوزوريوم اكسسوريوم في بيئة بطاطس دكستروز اجار مقارنة بمبيد فلديزيم تحت ظروف المعمل تمت تحضير المستخلص الكحولي من الجنزيبيل وقد استخدمت ثلاثة تراكيز من المستخلص الكحولي للجنزيبيل (25%-50%-100%) النتائج التي تم الحصول عليها توضح ان تاثير المستخلصات الكحوليه للجنزيبيل في كل التركيزات كانت ذات تاثير معنوى في تثبيط نمو الفطر مقارنة بالكنترول. تاثيرالتثبيط يزداد بزيادة التركيزات 100-80-60%نتيجة لذلك توضح هذه الدراسة بان الجنزيبيل يحتوى علي مواد ذات تاثيرمضاد لنمو الفطريات يمكن توضيح هذه المواد في الدراسات المستقبلية

CHAPTER ONE

INTRODUCTION

1.1 Tomato Plant:

Tomato is a tender a warm season perennial cultivated as an annual; it is an annual shrubby member of solanaceae.

In Sudan are fifteen states cultivating tomato crop, but the main products area are Gezira, Khartoum, and Nile state. Tomato cultivated in both open filed and greenhouses. It is the second popular vegetables after onion in Sudan (Abdol hafeez, et al. 2010).

In the arid to tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowing and fruiting of the plant in relation to climatic condition (Abdalla and Verkerk, 1968).

Tomato is major vegetable crop in Sudan' the major varieties of tomato growing in the Sudan are strain B' and peto 86' since they tolerate high temperature. Several bacterial diseases (caused by fungi 'and viruses) effect tomato , these include: Bacterial canker, Bacterial spots, Early blights, Anthracnose, *Verticillum* wilt, Powdery mildew And Fusarium wilt (*Fusarium oxysporum*).

Objectives of this present study:

Evaluate the effectiveness of ethanolic extracts of ginger rhizomes in controlling *Fusarium oxysporum* in tomato plant

CHAPTER TWO

2.1. Tomato plant

Tomato (*Solanum lycopersicum L*) is the edible, often red fruit from the plant, commonly known as a tomato plant. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While it is botanically a fruit, it is considered a vegetable, accounts for 14% of world vegetable production over 100 million metric tons/year\$ 1.6 billion market (Food and Agriculture Organization FAO, 2010). The total production year 1999 was 707715 tons and the total production of tomato for one greenhouse (350m) in Khartoum reached 5ton per season (Abdol hafeez, *et al.* 2012).

2.1.1Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Subfamliy: Solanoideae

Tribe: Solaneae

S.N: *Solanum lycopersicum (L)* (Raabe *et al* 2014)

2.1.2 Distribution:

The written literature of tomato began in 1500 when Spanish and Portuguese explorers found these plant first in Mexico and then along the west coast of South America mainly Peru, and then along on the Galapagos island, tomato is a native to Peru-Ecuador region of South America, evolving from the cherry from (*Lycopersicon esculantum* Var. *cerasiform*). (Perice, 1987).

In Sudan are fifteen states cultivating tomato crop, but the main products area are Gezira, Khartoum, and Nile state. Tomato cultivated in both open field and greenhouses. It is the second popular vegetables after onion in Sudan (Abdol hafeez, et al. 2010).

In the arid to tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowering and fruiting of the plant in relation to climatic condition (Abdalla and Verkerk, 1968)

2.1.3 Varieties:

There are around 7500 tomato varieties grown for various purposes, Heirloom tomatoes are becoming increase popular, particularly among home gardeners and organic producers, since they tend to produce

More interesting and flavourful crops at the cost of disease resistance and Productivity (Redenbaugh *et al* 1992),

The tomato is now growing worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions. Cultivated tomatoes Vary in size about 5mm in diameter, though cherry tomatoes, about the Same (1-2cm)

size as the wild tomato, up beefsteak tomatoes (10cm) Or more in diameter, the most widely grown commercial tomatoes tend To be in the (5 6cm) diameter range, Most cultivars produce red fruit, but A number of cultivars with yellow, orange, pink, purple, green, black, or White fruit are also available.Multicolored and requite striking .T0mat0es grown for canning and sauces are often Elongated, (7-9cm) long and (4-5cm) diameter, they are known as plum Tomatoes, and have a lower water content.Roma-type tomatoes are Important cultivars in the Sacramento Valley (Redenbaugh, *et al* 1992). The tomato varieties for summer season such as: Eloths and Sophie Areas in Sudan 58400fedans in1999 (Mohamed *e t al.*2003).There are other resiance type breeding in Sudan against tomato yellow leaf curl Virus includes, Sennar (1) Sennar (2), Omdurman, and Alijazeera (96)Variety Abed Allah and Somerset (98) are breeding to resist high Temperature in Sudan(Ahmed,2009).

2.1.4 Importance and Nutrition value of tomato:

Tomato is considered as an importance source of some vitamins and Mineral salt such as; vitamin B, and Riboflavin, which are considered necessary for growing, and safety of skin .The external part of fruit contains high level of vitamin C.This for red tomato, raw (per100g:energy74kg, carbohydrates 3.9g, fat 0.2, protein 0.9, vitamin5% and trace metals 3%).their also others constituents such as water94.5and lycopene 2573mg (Naika, *et al.* 2005).which are considered necessary for growing and safety of skin. The external part of fruit Contains high level of vitamin C. (Alaa Edrees, 2014).

2.1.5 Disease:

Tomato cultivars vary widely in their resistance to disease. Modern Hybrid focus on improving disease resistance over the heirloom plants. On common tomato disease is tobacco mosaic virus, so smoking or use of tobacco products are discouraged around tomato, over whether the Virus could possibly survive being burned and converted into smoke. Various forms of mildew and blight is also common tomato afflictions, Which is why tomato cultivars are often marked with combination of? Letters the refer to specific disease resistance. The most common letters are Verticillium wilt, F wilt strain I and II, Nematodes, Tobacco mosaic virus, Alternaria solani (Mourvaki, *et al* .2005).

Tomato attacks by many diseases and pest in Sudan, the important

Disease in Sudan include; Damping off-of seedling, tomato yellow leaf

Curl viruses (TYLCV), powdery mildew, and Bacterial spot, early and late

Blight and Fusarium wilt (Juha, 1996).

2.2 Fusarium Wilt of Tomato:

Fusarium is a major genus of soil Fungi that is found in many parts of the world most species are harmless saprobes and are relatively abundant members of the soil microbial community. Some Fusarium species are economically significant due to the devastating impact they can have on crops. (Peirce, 1987)

2.2.1 Scientific classifications of Fusarium wilt of Tomato:

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Subclass: Hypocreomycetidae

Order: Hypocreales

Family: Nectriaceae

S.N: *Fusarium oxysporum f.sp. lycopersici*.

2.2.2 HOSTS of the pathogen:

The fungal pathogen *Fusarium oxysporum* affects a wide variety of hosts of any age. Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana are a few of the most susceptible plants, but it will also infect other herbaceous plants

2.2.3 Symptoms:

Fusarium oxysporum generally produces symptoms such as wilting, chlorosis, and necrosis, premature leaf drop, browning of the vascular system, stunting, and damping off. The most important of these is vascular wilt (Agrios, *et al* 2005).

Fusarium wilt starts out looking like vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting of the plant, yellowing of the lower leaves, defoliation, marginal necrosis and death of the plant. On older plants, symptoms are more distinct between the blossoming and fruit maturation stages.

The life cycle of *F. oxysporum* commences with a saprophytic phase when the fungus survives in soil as chlamydospores (Beckman & Roberts 1995). Chlamydospores remain dormant and immobile in the remains of decayed plant tissue until stimulated to Germinate by utilising nutrients that are released from extending roots of a variety of plants (Stover 1962 Beckman and Roberts 1995). Following germination, a thallus is produced from which conidia form in 6-8 hours, and chlamydospores in 2-3 days if conditions are favourable. Invasion of the roots is followed by the penetration of the epidermal cells of a host or a non-host (Beckman and Roberts 1995) and the development of a systemic vascular disease in host plants (Stover, 1970). In the advanced stages of the disease, the fungus grows out of the vascular system into adjacent parenchyma cells, producing vast quantities of conidia and chlamydospores. The pathogen survives in infected plant debris in the soil as mycelium and in all its spore forms, but most commonly as chlamydospores in the cooler temperate regions (Agrios 2005).

2.2.4 Distribution:

Overall, the distribution of *Fusarium oxysporium* is known to be COSMOPOLITAN. However, the different special forms (f.sp) of *F. oxysporum* often have varying degrees of distribution.

2.2.5 Biology:

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *f. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors- ranging from violet to dark purple- according to the strain(or special form) of *F. oxysporum*. If sporodochia are abundant, the culture may appear cream or orange in color (Smith *et al.*, 1988).

F. oxysporum produces three types of asexual spores: microconidia, macroconidia, and chlamydospores (Agrios, 1988). {Microconidia} are one or two celled, and are the type of spore most abundantly and frequently produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. {Macroconidia} are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochialike groups. {Chlamydospores} are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. These spores are either one or two celled (Agrios, 1988).

2.2.6 Epidemiology:

Fusarium oxysporum is primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 1988). It is also possible that the spores are spread by wind.

2.2.7 Management:

Because *F. oxysporum* and its many special forms affect a wide variety of hosts, the management of this pathogen is discussed in more detail in the respective summaries. In general, some effective means of controlling *F. oxysporum* include: disinfestation of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones etales., 1982; Agrios, 1988; Smith etales., 1988).

2.2.8 Control:

The control of fusarium oxysporum of tomato is important in maintaining plant vigour. Documented methods that are used in the control of the disease cultural, biological, use of resistance and use of natural products however, each method has got its own strengths and imitations.

2.2.9 Use of resistance varieties:

The most cost-effective and environmentally safe method of control is the use of resistant cultivars whenever they are available. The use of resistant varieties is the best strategy for disease control according to pritesh et al (2011), identification and utilization of tomato plant varieties resistant to the disease represents a valid alternative to the use of chemicals. However, breeding for resistance can be very difficult when no dominant gene is known. In addition, new races of pathogens overcoming host resistance can develop.

The advantages of this method include saving the cost of chemical for control of the disease and enhancing cultivation of previously infested fields

2.2.10 Chemical:

Application to soil and resistant cultivars are the main approaches to control the disease (Fravel et al. 2003). However, fungicide application is often ineffective as the chemical may not reach the

Fungal propagules which are widely disseminated in the soil (Campbell 1989). In addition, new races of the pathogen have overcome host resistance and Discovery of new resistant varieties is expensive and difficult when no dominant gene is known (Fravel et al. 2003). Biological control, therefore, holds promise as a strategy for disease management

Bio control agents (BCAs) including fluorescent *Pseudomonas*, a non-pathogenic *Fusarium* strain,

Trichoderma harzianum and *T. asperellum*, have been reported to provide control of *Fusarium* wilt

(Larkin and Fravel 1998; Cotxarrera et al. 2002; Yigit and Dikilitas 2007).

2.3 Ginger plant:

Ginger (*Zingiber officinale*) is widely used around the world in

foods as a spice for centuries, it has been an important ingredient

In Chinese, Ayurvedic and Tibb-Unani herbal medicines for the

Treatment of catarrh, rheumatism, nervous diseases, gingivitis,

Toothache, asthma, stroke, constipation and diabetes (Tapsell et al. 2006).

Several reviews have appeared in the literature about this plant,

and this may reflect the popularity of the subject and its common use as

a spice and medicinal (Afzal, et al 2001 and Chrubasik, et al. 2005).

2.3.1 Scientific classifications:

Kingdom: Plantae

Class: Commelinids

Order: Zingiberales

Family: Zingiberaceae

Genus: *Zingiber*

Species: *Z. officinale officinal*

Binomial name: *Zingiber officinale*

2.3.2 Plant description:

Ginger (*Zinger officinal*), is an erect herbaceous perennial plant in Family Zingiberaceae grown for its edible rhizomes (underground stem) which is widely used as spice? The rhizome is brown, with a corky outer Layer and pale-yellow scented centre, the above ground shoot Is erect and reed-like with linear leaves that are arranged alternated on The stem, the shoot originates from a multiple bases and wraps around on other. The leaves can reach (7cm) length and (1.9cm) broad on Shorter stem and the plant produces cone shaped, pale yellow flowers The ginger plant can reach (0.6-1.2m) in height and grown as an annual Plant (9Ginger may also be referred to as true ginger, stem Ginger, garden ginger or root ginger and it is believed to have originated in the Southeast Asia (CABI Crop protection compendium, 2012).

2.3.3 Plant Distribution:

The origin of ginger is from themide-14th century, from Old English Gingerer, from Medieval Latin ginger, said to be a native of Asia Cultivated in West India Jamaica, and Africa. In2013with global Production of ginger is 2.1 million tonnes, India accounted for (33%) Followed by China (19%), Nepal, Indonesia and Nigeria (FAo, 2013)

CHAPTER THREE

MATERIAL AND METHODS

3.1. Isolation of *Fusarium oxysporum*:

3.1.2. Isolation from plant materials:

Infected tomato fruit showing symptom of the disease were obtained from Research botry in Agues, 2017. The fruits were cut into small Sections (0.5-1.0 cm), washed thoroughly with tap water, surface sterilized with Clorox (Naocl) for 5 minutes, rinsed three times in changes of sterilized distilled water and dried on sterilized filter papers. The sterilized fruits section were plated at the rate of five sections/plate onto potato dextrose agar (PDA) medium supplemented with chloramphenicol (0.05 g/L) in 9-cm petri dishes. The petri dishes were incubated at 25°C. After incubation for 7 days, isolated fungus was subcultured on PDA. When free from contamination; Isolates were maintained on PDA slants and examined visually for their growth patterns and pigmentation on the adverse side of the agar. Further microscopic examinations were carried for mycelia and conidia structure using pure of *Fusarium.oxysporum* f.sp .lycopersion was obtained by using hypha tip technique. Pure culture of the isolated fungi was transferred to PDA slants and kept in refrigerator at 4°C for further use. Sample of the obtained colonies were sub cultured by transferring small mycelia from the colony margins. Pure cultures were obtained by sub –culturing three times and slides were prepared and examined microscopically to confirm identity (x:40)

3.1.3. Identification of the pathogen:

The identification of the fungus was based on visual culture characteristics, mainly The growth patterns and pigmentation, Furthermore, microscopic examinations were carried out for mycelial and conidia structure based on method of Booth key (1977).

3.1.4. Growth Rate of the pathogen:

The pure cultures of *Fusarium oxysporum* were prepared using 7 days old mycelia. The fungus was cultured on PDA then transferred, aseptically, to the center of petri dishes containing PDA medium and incubated at 25°C. The linear growth of the fungus was assessed in cm after 48h.

3.1.5 Collection and preparing of plant materials:

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

3.1.6 Extraction Process:

The obtained fine powder from each plant was weighted (70gm.) and placed in a conical flask containing 70 ml distilled water and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 25% 50% and 100% concentrations.

3.1.7 Fungicide Process:

The chemical tested were Fulladazim fungicides 10ml dissolved in 100ml of sterilized distilled water to give 5,10,15ppm respectively. For this solution 5, 10, 15 were completed to 100 ml by adding sterilized potato dextrose agar medium to give final concentration.

CHAPTER FOUR

RESULTS

The effect of ginger extracts on the linear growth of the *Fusarium oxysporum* compared to control. The three concentrations from the extract showed spectrum of fungicide activity. The results (Table1) the concentration 100% extracts tested caused 100% reduction, the dose 50% extracts tested reduction 80% the dose 25% extracts tested 60% reduction compared to control 0.

Table (1) Effect of different concentrations of Ginger ethanol extracts on

Fusarium oxysporum.

Treatments	Mean	Redaction growth
Control	5.0	0%
	0.0	
Fullidazim		
Ginger 100%	0.0	100%
50%	1.5	80%
25%	2.6	60%
C.V	26	
SE	24	

$$R = \frac{A-B}{A} * 100$$

A

R= Percent reduction of *Fusarium oxysporum*

A= *Fusarium oxysporum* growth control

B= *Fusarium oxysporum* growth of Treatment

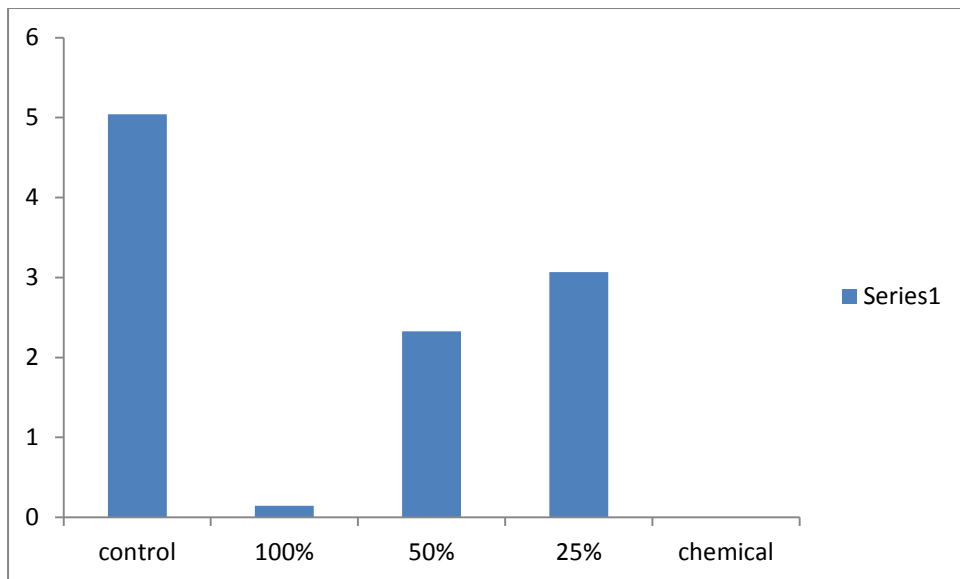
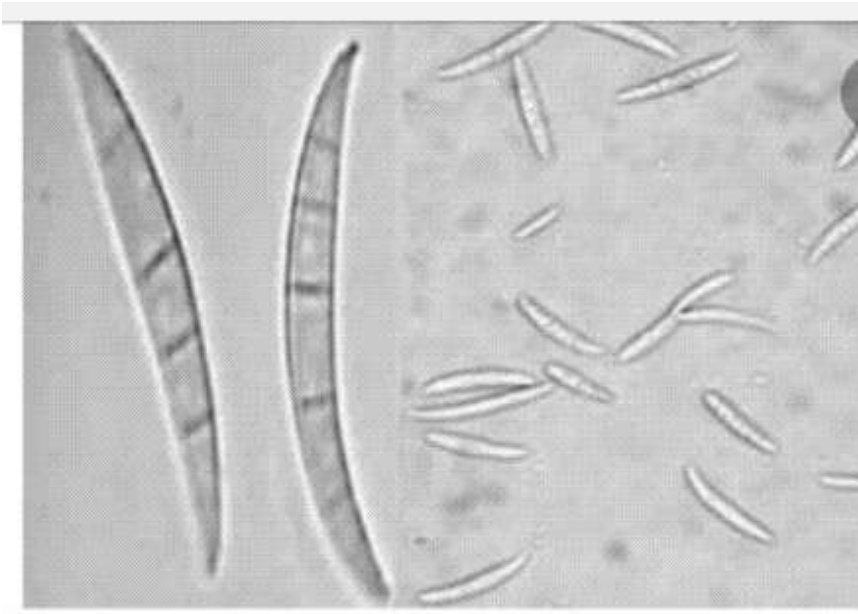
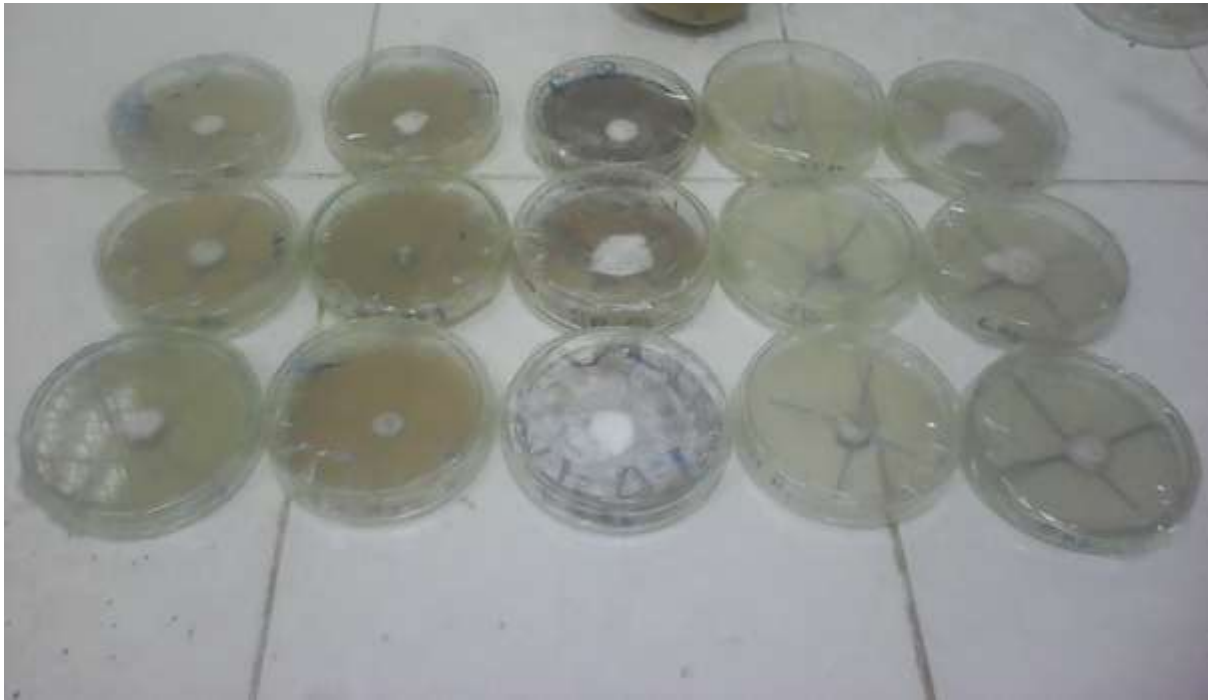


Fig. (1)Effect of different concentrations of Ginger ethanol extracts on *Fusarium oxysporum*.



Plate(1): chlamydospores

Plate(2) : The growth of fungus



25%

100%

control

Fungicid

50%

CHAPTER FIVE

DISCUSSION

The antifungal effects of crude medicinal plant extracts Ginger was determined by *in vitro* study using Ethanol as solvents. Three concentration of Ginger plants extracts were used (25.50, 100%) as antifungal activity against *Fusarium oxysporum*. Plants are one of the most important sources of medicine. Plant derived compounds (Phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. The results of the experiment revealed that the ginger extract was more effective.

This finding corroborates the notion plant. Plants are one of the most important sources of medicine. Plants-detived compounds have been attracting much interest as natural alternatives to synthetic compounds.

The present investigations revealed that *in vitro* of *Fusarium oxysporum* was significantly checked by ethanolic extracts of ginger at all concentration. Results showed that, ginger had the highest antifungal activity against *Fusarium oxysporum* as it inhibited 100% the radial fungus growth (100%).They investigated the strong inhibition potential of ginger is attributed to fact that it contains over 400 different compounds a mixture of both volatile and non – volatile chemical constituents such Zingerone, shogaols and gingerols. Abd Malek *et al* .,(2005) Our result is in agreement with the study of Ogbebor *et al* .,(2007) .

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