

Sudan University of Science and Technology

College Of Studies And Scientific Research



Effect of Hargel (Solenostemma argel) leaves ethanolic extract against (Fusarium oxysporium)

تاثير المستخلص الكحولي لاوراق الحرجل على فطر (فيوزيريوم المستخلص الكحولي لاوراق الحرجل على فطر

A thesis Submitted in Partial Fulfilment of the Requirements of the Degree for B.Sc. (Agric.) in Plant Protection

BY

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بسو الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ وَٱضْرِبْ لَهُمُ مَّثَلُ ٱلْحَيَوْةِ ٱلدُّنَيَا كَمَآءٍ أَنزَلْنَهُ مِنَ ٱلسَّمَآءِ فَٱخْلَطَ بِهِ-نَبَاتُ ٱلْأَرْضِ فَأَصْبَحَ هَشِيمًا نَذَرُوهُ ٱلرِّيَحُ وَكَانَ ٱللَّهُ عَلَى كُلِّ شَىْءٍ تُمَقْنَدِرًا ﴾ حدق الله العظيم

سورة الكهف (45)

DEDICATION

This work is dedicated Tomy

Father... mother

Famíly...

teachers...

all fríends....

With respect

Eans

ACKNOWLEDGEMENTS

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

I would like to express my thanks to my supervisor Dr. Ekhlas Hussein Mohamed. 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 colleague's sincere thanks are also extended to those who helped me in various ways and encouraged me to achieve and finish my research work. Last but not least, thanks are extended to my friend's family, specially my parents and brothers for financing my studies and helping me in many ways to finish my study.

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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 Hagel (Solenostemma argel) leaves 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 Hargel 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. The highest inhibition effect was obtained at 100% (98) compared by control (5). Generallythe inhibition zone increases with increase the concentration of the extracts. In conclusion, this study showed that Hargel leaves contain antifungal properties that could investigated in further studies.

ملخص الاطروحة

اجريت هذه الدراسه في معمل امراض النبات، قسم وقايه النبات كلية الدراسات الزراعيه جامعه السودان للعلوم والتكنولوجيا (قي اغسطس - سبتمبر 2017) لتقيم تاثير المستخلص الكحولي لاوراق نبات الحرجل على فطر فيوزيوريم اكسسبوريوم في بيئه بطاطس دكستروز اجار مقارنة بمبيد فلدزيم تحت ظروف المعمل تم تحضير المستخلص الكحولي من اوراق نبات الحرجل وقد استخدمت ثلاث تراكيز من المستخلص الكحولي لاوراق نبات الحرجل (25%،50%، 100%). النتائج التي تم الحصول عليها توضح ان تاثير معنوي في المستخلصات الكحوليه لاوراق نبات الحرجل في كل التركيز ات كانت ذات تاثير معنوي في تثبيط نمو الفطر مقارنة بالكنترول. التاثير التثبيط يزيد بزياده تركيز 8%,40% (40%).

نتيجه للذلك توضح هذه الدر اسه بان اور اق نبات الحرجل تحتوي علي مواد ذات تاثير مضاد لنمو الفطريات يمكن توضيح هذه المواد في الدر اسات المستقبلية.

CHAPTER ONE INTRODUCTION

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

In Sudan they 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 diseases that affect tomato, these include, Bacterial canker, Bacterial spots, Early blights, Anthracnose, Verticillum wilt and Powdery mildew Hence the occurrence of Fusarium wilt disease the causal agent is (*Fusarium oxysporum*) has been observed in recent years in Khartoum area with high levels of severity. The thesis is therefore intended to:

1/ Evaluate the efficacy of ethanolic extracts of Harjal leaves 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)*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-equador regain of south America, evolving from the cherry from (Iycopersicon 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 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)

2.1.2 Varieties

There are around 7500 tomato varieties grown for various purposes, Heirloom tomatoes are becoming increasingly popular, particularly among home gardeners and organic producers, since they tend to producemoreinteresting 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.Cultiated tomatoes vary insize about5mmin diameter, thorugh cherry tomatoes, aboutthe 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) diameterrange.

Most cultivars produce red fruit, buta number of cultivars with yellow, orange, pink, purple, green, black, orWhite fruit are also available.Multicolored and striped fruit can also beQuite striking .TOmatoes grown for canning and sauces are oftenelongated, (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 *et al*.2003).There are other resiance type breeding in Sudan against tomato yellow leaf curlViruses includes, Sennar (1) Sennar (2), Omdurman, andAlijazeera (96).Variety Abed Allah and Somerset (98) are breeding to resist highTemperature in Sudan(Ahmed,2009).

2.1.3 Impotence and Nutrition value of tomato

Tomato is considered as an importance source of some vitamins and Mineral salt suchas; vitamin B, andRiboflavin, 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, carbohydrates3.9g, 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 fruitContains light level of vitamin C (Alaa Edrees, 2014).

2.1.4 The Diseases

Tomato cultivars vary widely in their resistance to disease. ModernHybrids focus on improving disease resistance over the heirloom plants.One common tomato disease is tobacco mosaic virus, so smoking or useof tobacco products are discouraged around tomato, over whether the virus could possibly survive being burned and converted into smoke.Various formed 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 areVerticilliumwilt, F wilt strain Iand II, Nematodes, Tobacco mosaic virus, Alternaria solani (Mourvaki, *et al* .2005).Tomato attacks by many diseases and pest in Sudan, the important Disease in Sudaninclude; Damping off-of seedling, tomato yellow leaf Curlviruses (TYLCV), powderymildew, Bacterialspot, early and lateBlight and Fusariumwilt (Juha, 1996).

2.2 Fusarium wilt of Tomato

Fusarium is a major genus of soil Fungithat 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. (Abdol Hafeez, *et al* 2012)

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. (Raabe, et al 2014)

2.2.2 HOSTS of the pathogen

The fungal pathogen *Fusariumoxysporum* 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. (Agrios, *et al*2005).

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 necrosisand 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). Chlamydosporesremain dormant and immobile in the remains of decayed plant tissue until stimulated toGerminate by utilising nutrients that are released from extending roots of a variety ofplants (Stover 1962 Beckman and Roberts 1995). Following germination, a thallus isproduced from which conidia form in 6-8 hours, and chlamydospores in 2-3 days ifconditions are favourable. Invasion of the roots is followed by the penetration of theepidermal cells of a host or a non-host (Beckman and Roberts 1995) and the developmentof 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 ininfected plant debris in the soil as mycelium and in all its spore forms, but mostcommonly 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 macro conidia. 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

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: disinfestations of the soil and planting material with fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones elates., 1982; Agrios, 1988; Smith etales., 1988).

2.2.8 Controls

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.

2.2.8.1 Use of resistance

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

forcontrol of the disease and enhancing cultivation of previously infested fields.

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 prop gules which are widely disin 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 nonpathogenic 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 Harjal Plant

Kingdom: Plantae Order: Gentianales Family: Apocynaceae Sub Family: Asclepiadoideae Genus: Solenostemma Species: *Solenostemma argel* Harjal medicine sometimes referred to as herbalism or botanical medicine is the use of herbs for their therapeutic or medicinal value. An herb is a plant or a plant part valued for its medicinal, aromatic or savoury qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Shelef, 1983)

It has been estimated that approximately 80% of the world inhabitants rely mainly on traditional medicines for their primary health care; where plant based systems still play a vital role in health care. In developed countries, plant drugs are also extremely important, currently at least 119 chemicals derived from plant spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of food. Early cultures also recognized the value of using spices and herbs in preserving food and for their medicinal value (Shelef,1983).Scientific experiments since the late 19 century have documented the antimicrobial properties of some spices, herbs, and their components(Zakia, 1988).

The plant Harjal (Solenostemma argel) is a member of the family Asclepiadaceae that comprises numerous medicinal plants, like calotropis procera, Marsdenia obyssinicna and Huernia mecrocarpa,known for their cardiac activity. Harjal grows naturally in the northern parts of the Sudanand extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria) and the Saudi Arabia (Ahmed, 2004). Harjal leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treatgastro-intestinal cramps and stomach colic.

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CHAPTER THREE

MATRIAL AND METHOD

This study which conducted under laboratory conditions was carried out at Plant pathology laboratory of plant protection department college of Agricultural studies Sudan University of science and technology to evaluate the inhibitory effect of Hargel leaves alcohol extracts against *Fusarium oxysporum*.

3.1 Isolation of Fusarium oxysporum

Fusarium oxysporum is the causal agent of tomato wilt

3.1.2Isolation 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 5minutes, rinsed three time 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 25C. After incubation for 7days, 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 40 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 time and slides were prepared and examined microscopically to confirm identity (x:40)

3.1.3 Identification of the pathogen

The indention 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 7days old mycelia. The fungus was cultured on PDA then transferred, aseptically, to the canter of petri dishes containing PDA medium and incubated at 25c. The linear growth of the fungus was assessed in cm after 48h.

3.2 Collection and preparing of plant materials

Hargel 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.

3.2.1Extraction 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.

3.3 Fungicide Process

The chemical tested were Fulldazim 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 Harjal leaves extracts on the liner growth of the *Fusarium* oxysporum compared to control. The aim of this study is to investigate the antifungal activities of (*Solenostemma argel*)) Hargel leaves alcohol extracts on the liner growth of *Fusarium oxysporum* in culture media under laboratory conditions where temperature around $25C^{\circ}$

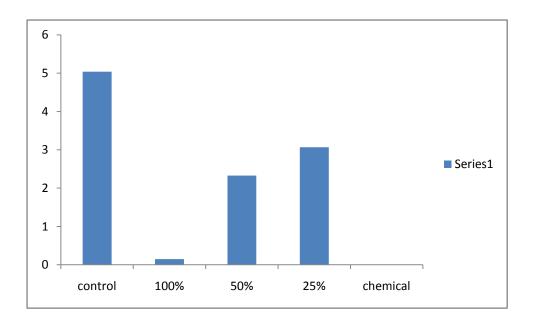
The three concentrations form the extract showed spectrum of fungicide activity. The results in (Table 1) showed that the concentration 100% extracts tested caused 100% redaction, the concentration 50% extracts tested redaction 60% the concentration 25% extracts tested 40% redaction compared to control 0

Table1. Effect of Hargel leaves alcohol extracts on the linergrowth of *Fusarium oxysporum* days after inculcation

| Concentration | Mean | Redaction growth |
|---------------|------|------------------|
| Control | 5.0 | 0% |
| | 0.0 | |
| Chemical | | |
| Hargal | 014 | 98% |
| 100% | 2.3 | 54% |
| 50% 25% | 3.0 | 40% |
| .CV | 26 | |
| LSD | 24 | |

R = A - B / A * 100

- R= Percent reduction of *Fusarium oxysporum*
- A= Fusarium oxysporium growth control
- B= Fusarium oxysporum growth of Treatment



PLATES

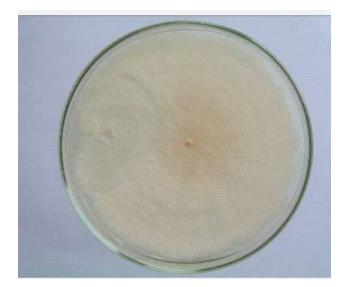


Plate (1): Control

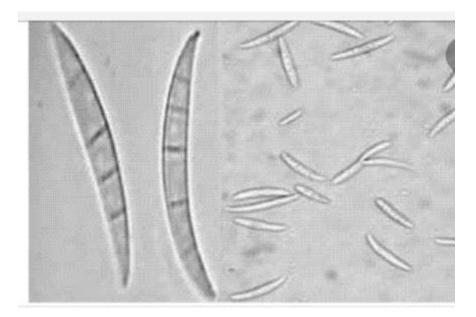
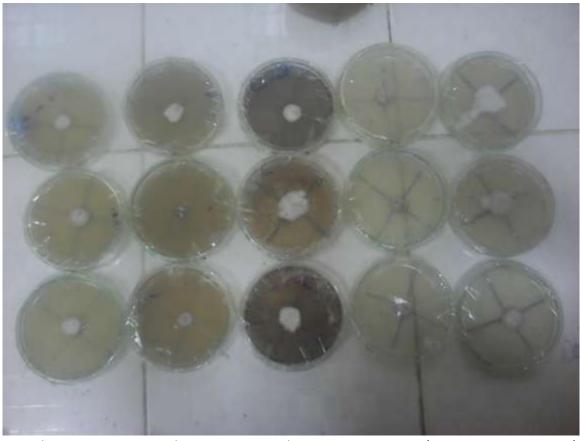


Plate (2): Chlamydospores

Plate (3): The growth of fungus



100% 50% 25% Fungicide Control

CHAPTER FIVE

DISCUSSION

The antifungal effects of crude medicinal plant extracts Argel leaves was determined by *in vitro* study using water as solvents. Three concentrations of Harjal plants extracts were used (25.50 and 100%) as antifungal activity against *Fusarium oxysporum*. The results of the experiment revealed that the Harjal extract was more effective. This finding corroborates the notion plant. Plants are one of the most important sources of medicine. Plants-detived compounds (phytochemicals) 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 Harjal at all concentration. Results showed that, Hargel had the highest antifungal activity against *Fusarium oxysporum*as it inhibited 98% the radial fungus growth (100%). In fact this finding is in agreement with (). Who tested the effects of Hargel leave alcohol extracts on the linear growth of *Fusarium oxysporum* in tomato.

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