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

تأثير المستخلص الكحولي لوراق الحرجل على فطر (فيوزيريوم اكسوسپوریم)

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

BY

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2017
بسم الله الرحمن الرحيم

قال تعالى:

وأضرب لهم مثال الحياة الدنيا كما أنزلت من السماوات فاختلط يدها نبات الأرض فأصبح هشيمًا نذروه ريح وكان الله على كل شيء مقدمًا

صدق الله العظيم

سورة الكهف (45)
DEDICATION

This work is dedicated to my

Father... mother

Family...

teachers...

all friends....

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 extend 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). Generally the 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 الدراسة في معمل امراض النبات، قسم وقاية النبات كلية الدراسات الزراعية
جامعة السوادان للعلوم والتكنولوجيا(في أغسطس - سبتمبر 2017) لتقييم تأثير المستخلص
الكحولي لأوراق نبات الحرجل على فطر فيوبوريم اكسبوريوم في بيئة بطارس
دكستروز اجار مقارنة بمبيد فندزيم تحت ظروف العمل. تم تحضير المستخلص الكحولي
من أوراق نبات الحرجل وقد استخدمت ثلاث تركيزات من المستخلص الكحولي لأوراق نبات
الحرجل (25%, 50%, 100%). النتائج التي تم الحصول عليها توضح ان تأثير
المستخلصات الكحولية لأوراق نبات الحرجل في كل التركيزات كانت ذات تأثير معنوي في
تثبيط نمو الفطر مقارنة بالكمترول. التأثير التثبيط يزيد بهدف تركيز 98%, 54%, 40%.

نتيجة لذلك توضح هذه الدراسة بأن أوراق نبات الحرجل تحتوي على مواد ذات تأثير
مضاد لنمو الفطريات يمكن توضيح هذه المواد في الدراسات 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).

2.1.1 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Subfamily: 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 *(Lycopersicon esculentum* 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 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 5-10 cm in diameter, though cherry tomatoes, about the same (1-2 cm) size as the wild tomato, up to beefsteak tomatoes (10 cm) or more in diameter, the most widely grown commercial tomatoes tend to be in the (5-6 cm) 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 striped fruit can also be quite striking. Tomatoes grown for canning and sauces are often elongated, (7-9 cm) long and (4-5 cm) 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 58400 fedans in 1999 (Mohamed et al. 2003). There are other resistance type breeding in Sudan against tomato yellow leaf curl viruses includes, Sennar (1) Sennar (2), Omdurman, and Aljazeera (96). Variety Abed Allah and Somerset (98) are breeding to resist high temperature 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 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 (per 100 g: energy 74 kg, carbohydrates 3.9 g, fat 0.2, protein 0.9, vitamin 5% and trace metals 3%). There are also other constituents such as water 94.5 and lycopene 2573 mg (Naika, et al. 2005), which are considered necessary for growing and safety of skin.
The external part of fruit contains light level of vitamin C (Alaa Edrees, 2014).

2.1.4 The Diseases

Tomato cultivars vary widely in their resistance to disease. Modern hybrids focus on improving disease resistance over the heirloom plants. One 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 referring 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, 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. (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


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. (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 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 oxysporum* 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
for control 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 propagules which are widely dispersed 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 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 Sudan and 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 treat gastro-intestinal cramps and stomach colic.
CHAPTER THREE

MATERIAL 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

3.1.2 **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 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 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 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 times 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.

3.2.1 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.
3.3 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*) Harigel leaves alcohol extracts on the liner growth of *Fusarium oxysporum* in culture media under laboratory conditions where temperature around 25°C.

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% reduction, the concentration 50% extracts tested reduction 60% the concentration 25% extracts tested 40% reduction compared to control 0
Table 1. Effect of Hargel leaves alcohol extracts on the liner growth of *Fusarium oxysporum* days after inoculation

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean</th>
<th>Reduction growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0</td>
<td>0%</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hargal 0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>2.3</td>
<td>54%</td>
</tr>
<tr>
<td>50%</td>
<td>3.0</td>
<td>40%</td>
</tr>
<tr>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

\[ R = \frac{A - B}{A} \times 100 \]

\( R = \) Percent reduction of *Fusarium oxysporum*

\( A = *Fusarium oxysporium* \) growth control

\( B = *Fusarium oxysporum* \) growth of Treatment
PLATES

Plate (1): Control

Plate (2): Chlamydospires
Plate (3): The growth of fungus

<table>
<thead>
<tr>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>Fungicide</th>
<th>Control</th>
</tr>
</thead>
</table>
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-derived 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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Hibberd, J. M., Bungard, R. A., Press


McNeal, J. R., Kuehl