

CHAPTER ONE

1. INTRODUCTION

Argel (*Solenostemma argel*) is a desert plant of traditional medical uses in the Sudan. It grows wild in the area extending from Dongola to Barber, particularly around Abu Hamad, where it is grown under irrigation (Elkamali and Khalid, 1996). Sudan is regarded as the richest source of this plant (Orange, 1982). Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos *et al.*, 1980; Kamel *et al.*, 2000; Hamed, 2001). Sulieman *et al.* (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties.

Fusariumoxysporum causes vascular wilt diseases in a wide variety of economically important crops (Beckman 1987). Vascular wilt has been a major limiting factor in the production of many agricultural and horticultural crops, including Mango.

Obviously, the fungus is one of the most hazardous diseases that widely spread. There is a limited information or lack of effective control measures of the disease. Accordingly, an effective control measures should be developed to control this devastating disease that represent real threat to fruit, forest and ornamental trees. The aim of this study was to explore the antifungal activity of alcoholic extracts of Argel and the efficacy of systemic fungicide in suppressing the growth of this fungus *in vitro* with the following objectives:

- To explore the inhibitory effect of Argel alcoholic extracts on the growth of *Fusarium* wilt of Mango.
- To evaluate *invitro* the efficacy against systemic fungicide (Amstar top) in suppressing the fungus.

CHAPTER TWO

Literature Review

Herbal medicine sometimes referred to as herbalism or botanical medicine, is the use of herbs for their therapeutic or medicinal value. A herb is a plant or a plant part valued for its medicinal, aromatic or savory 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's 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 species can be considered as important drugs in use (Mullholland, 2000).

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 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Zakia, 1988).

Ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results (Mustafa *et al.*, 1982). Crude extract solution obtained from the plant *Gordenia lutea*, showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Ahmed *et al.*, 1984). Badreledin (2006) reported that ginger oil showed antimicrobial activity against *Staphylococcus aureus*, while, ELboshra (2005) reported that *Staphylococcus aureus* was sensitive to

clove oil. The fenugreek oil was also found to inhibit *Salmonella typhimurium* (Sulieman, 2009).

Despite of the growth of global market for herbal products, following complementary and/or alternative medicines, homeopathy, health foods and natural-pharmaceuticals therapy, yet the majority of these herbs were not assessed for quality, safety and efficacy. The plant *harjal* (*Solenostemma argel*) is a member of the family Asclepiadaceae, that comprises numerous medicinal plants, like *Calotropis procera*, *Marsdenia obyssinica* 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 gastrointestinal cramps and stomach colic.

Fusarium wilt

As mentioned earlier, Fusarium wilts affect and cause severe losses on most vegetables and flowers; several field crops, such as cotton and tobacco; plantation crops, such as banana plantain, coffee, and sugarcane; and a few shade trees. Fusarial wilts are most severe under warm soil conditions and in greenhouses. Most Fusarial wilts have disease cycles and develop similar to those of the Fusarium wilt of tomato (Agrios, 2005)

Classification:

Kingdom: Fungi

Division : Ascomycota

Class : sordariomycetes

Order : Hypocreales

Family : Nectriaceae

Genus : Fusarium

Species : Fusarium oxysporum

)Snyder and Hansen, 1940.(

.2.1.2The Pathogen:

Fusarium oxysporum the mycelium is colorless at first, but with age it becomes cream-colored, pale yellow, pale pink, or somewhat purplish. The fungus produces three kinds of asexual spores. microconidia, which have one or two cells, are the most frequently and abundantly produced spores under all conditions, even inside the vessels of infected host plants. macroconidia are the typical “Fusarium” spores; they are three to five celled, have gradually pointed and curved ends, and appear in sporodochia-like groups on the surface of plants killed by the pathogen. Chlamydospores are one- or two celled, thick-walled, round spores produced within or terminally on older mycelium or in macroconidia. All three types of spores are produced in cultures of the fungus and probably in the soil, although only chlamydospores can survive in the soil for long. (Agrios, 2005.(

There are four genera of fungi that cause vascular wilts: Ceratocystis, Ophiostoma, Fusarium, and Verticillium. Each of them causes disease on several important crop, forest, and ornamental plants. Ceratocystis causes the vascular wilt of oak trees (C. fagacearum), of cacao, and of eucalyptus. Ophiostoma causes the vascular wilt of elm trees, known as Dutch elm

disease (*O. novo-ulmi*). *Fusarium* causes vascular wilts of vegetables and flowers, herbaceous perennial ornamentals, plantation crops, and the mimosa tree (silk tree). Most of the wilt causing *Fusarium* fungi belong to the species *Fusarium oxysporum*. Different host plants are attacked by special forms or races of the fungus. The fungus that attacks tomato is designated *F. oxysporum* f. sp. *lycopersici*; cucurbits, *F. oxysporum* f. sp. *conglutinans*; banana, *F. oxysporum* f.sp. *cubense*; cotton, *F. oxysporum* f. sp. *Vasinfectum*; carnation, *F. oxysporum* f. sp. *dianthii*; and so on. (Agrios, 2005). *Fusarium* wilt is a common vascular wilt fungal pathogen exhibiting symptoms similar to verticillium wilt. The pathogen that causes *Fusarium* wilt is *Fusariumoxysporum* (Snyder and Hansen, 1940).

The fungus can survive in the soil as mycelium or as spores in the absence of its hosts. If a host is present, mycelium from germinating spores penetrates the host roots, enters the vascular system (xylem) in which it moves and multiplies, and causes the host to develop wilting symptoms. For the fungus to be successful in infecting the plant, it must mobilize different sets of genes for early plant–host signaling, attachment to root surface, enzymatic breakdown of physical barriers, defense against antifungal compounds of the host, and inactivation and death of host cells by fungal toxins.(George N. Agrios,2005).

CHAPTER THREE MATERIALS AND METHODS

3.1. Study location:

This study was conducted in the Laboratory of Plant Pathology Department of Plant Protection, College of Agricultural Studies, Shambat, Sudan University of Science and Technology during the October to November to evaluate the antifungal effect of alcoholic extract of Argel ethanol extracts and efficacy of fungicide Amstar top® against the growth of the fungus *Fusarium oxysporum p.f mangphrae in vitro*

3.2. Equipments, Tools and Materials used in the Study:

- Incubator Laminarflowcabinet
- Autoclave Compound microscope
- Needle Injection
- Slide Marker pen
- Petri-dishes Conical flask
- Sensitive balance Aluminum fowl
- Gloves Face mask
- Regestration form Camera
- Potato Dextrose Agar (PDA).
- Mesquite root Mesquite leave
- Mesquite park Soap
- Ethanol 95% Medical cotton
- Filter paper
- Fungicide Amstar Stop®
- All Tools, which used in the experiments, were sterilized.

3.3. Collection of samples

Samples of *harjal* (*Solenostemma argel* L.) leaves were obtained from Khartoum Bahre local market during 2018. The samples were taken from retailer's stores. The leaves were freed from foreign materials like stones, sand and dust, before kept in the lab for further investigation. The leaves and stems of Argel were then washed with water, dried, and milled using laboratory mill into fine powder.

Aqueous extract of the powder (50g/500ml) was used to prepare different concentrations (0.00, 50 and 100% extract).

3.4. Preparations

3.4.1. Preparation of extract:

Extraction was carried out according to method described by [Sukhdev et. al. \(2008\)](#): 50 g of leaves and stems sample was extracted by soaking in 750 ml 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness.

3.4 .2. Preparation of inoculums:

The pure culture of *fusarium oxysporum* were prepared using 7 days old mycelia. The fungi was cultured on PDA then transferred aseptically to the center of Petri dishes containing PDA medium and incubated at 25⁰c the linear growth of the fungus was assessed in cm after 72 hrs

3.4.3. Preparation of fungicide:

The chemical tested was Amstar Top fungicide. Two ml was dissolved in 100 ml of sterilized distilled water.

3.5. Inhibition of Fusarium growth:

Inhibition zone technique was used in this study (Rao and Srivastava, 1994). The PDA media was amended with the required concentration from Mesquite , Peppermint and fungicide Amstar top® before being solidified in a conical flask of 250 ml containing 100ml of PDA medium, agitated and poured 25 ml into each sterilized Petri dish. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

Each solidified medium was then inoculated centrally by a fungal growth disc cut by a sterile cork-borer (5 mm) from an edge of an actively growing culture of the fungus *Fusarium oxysporum* grown on PDA as described above. The inoculated Petri dishes were then incubated at room temperature and the radial growth was measured every two days. All treatments were done in triplicates and were arranged in a Complete Randomized Design.

3.6 Calculation:

Every 48 hours, the diameter of growth was measured by taking the average of two crossed dimensions for each disc in the Petri dish. The radial growth was then calculated as a percentage from the diameter (9.0 cm) of the glass Petri dish. The effect of each extract concentration on linear fungal growth was calculated as percentage of inhibition in diameter of fungal growth:

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times 100$$

Where:-

dc = Average increase in mycelial growth in control.

dt = Average increase in mycelial growth in treatment.

3.7. Experimental Design and Statistical Analysis:

The experiment was arranged in a Complete Randomized Design, the obtained data was statistically analyzed by MSTATC software computer program according to analysis of variance (ANOVA). Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

This study which conducted under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology, during January to February 2018 was to confirm that *Fusarium oxysporum* and to explore the antifungal potentials of different parts of mesquite plant and efficacy of fungicide Amstar stop® against the fungus. The results cover effect of Argel extracts on growth of *Fusarium oxysporum* and confirmation of the causal agent.

Effect of Argel Extracts and Fungicide Amstar top on radial growth of *Fusarium oxysporum* in vitro three days after inoculation:

The results (Table 1 and 2) showed that the Argel alcohol extracts at all concentration tested and fungicide (Amstar top) had negative effects on the fungal growth after three days and continued so until day seven from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

Moreover the highest concentration of Argel extract (100%) gave significantly higher inhibition (1.2) compared to the untreated control which gave (1.92). The results showed that the antifungal activity increase with increasing of extracts concentration.

Table, 1 Effect of Argel concentrations and fungicide on growth of the Fungus

	Mean	Lower	Upper
Treatment			
control	1.923	1.837	2.009
fungicid	1.000	0.914	1.086
Hargel 100%	1.208	1.122	1.294
Hargel 25%	1.328	1.242	1.414
Hargel 50%	1.251	1.165	1.337

Table, 2. Duncan's multiple range test

Treatments	Mean	
fungicide	1.000	a
Hargel 100%	1.208	b
Hargel 50%	1.251	b
Hargel 25%	1.328	b
control	1.923	c

CHAPTER FIVE

DISCUSSION

Generally, use of synthetic fungicides considerably reduce wilt incidence but their use is costly as well as environmentally undesirable (Song and Goodman, 2001). Moreover, the use of resistant varieties is faced with breakdown of resistance due to high pathogenic variability in the pathogen population (Kutama et al., 2011; 2013). In this context, the searches for an eco-friendly way of managing Fusarium wilt which offers an alternative to fungicides is highly demanding.

The disease may cause greater losses of some crops. Crop losses attributed to Fusarium has been estimated to an average of 25% (Powelson *et al.*, 1993). Fusarium species are also important to the consumer because some, Fusarium spp produce mycotoxins, one of such toxins is trichothecene which is an inhibitor of eukaryotic protein synthesis and can pose serious health problem to man and animals (Beremaid et al., 1991.)

Numerous research findings have presented a number of strategies to control this fungal pathogen ((Haware and Nene, 1982; Jiménez-Díaz, et al., 1993; Biondi et al., 2004 and Ahmed, 2011.)

Generally, management of seed-borne and soil-borne diseases such as Fusarium spp. always had been problematic (Haware, 1992) and (Rao and Balachadran, 2002). Based on the fact that botanical insecticides possess great advantages over synthetic pesticides (Karunyal, 2000; Abdel Moneim, et al., 2009 and Mawda, 2015) in being more environmentally friend and accepted by the majority of the farmers, governmental organizations and decision makers.

The results (Tables 1 and 2) revealed that the Argel alcoholic extracts and fungicide, Amstar top®, solution consistently and throughout the course of the experiments exhibited an inhibitory effect on mycelial radial growth of the fungus with significantly higher inhibition reduction growth percent compared to control. Similar studies which explored the effect of extracts of many higher plants and their essential oils have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Agrafotis, 2002; Okigbo and Ogbonnaya, 2006; Shariff et. al., 2006; Ergene et. al., 2006; Kiran and Raveesha, 2006 .(

In fact, this finding is in agreement with Shimaa , Huda (2016) who tested the bioactivity of Mesquite extract against fungi and demonstrated its suppressing effect on the fungal growth in vitro. also Similar results were obtained by Fadl Elmola et al., (2010). who reported that the extracts of Mesquite different plant parts were highly effective in suppressing bacterial growth. Also Zainal et al., (1988). reported that *P . juliflora* contain antimicrobial compounds.

CONCLUSIONS

In conclusion, the findings presented in this study indicate promising potentials of Argel, as sources of new antifungal in future that help in management of plant fungal diseases ..The Argel alcohol extracts of all concentration tested exhibited an inhibitory effect on fungal growth. Thus these components plus fungicide (Amstar top) could be applied as part of an integrated approach to control *Fusarium* wilt.

-The screened concentrations of Argel alcohol extracts differ in their reactions to test fungus. Likewise the test organism responded differently to

the different concentrations of extracts. This variability in response which expressed by test organism may be used to adjust an optimum dose for controlling Fusarium wilt.

RECOMMENDATIONS:

1. Further studies and research on Argel trees according to the current results aimed at knowledge of the chemical resistance of the Argel plant using different solvents

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Table	Treatment
rep.	3
d.f.	10
l.s.d.	0.1215

Stratum standard errors and coefficients of variation

Variate: Fungi_growth_2

d.f.	s.e.	cv%
10	0.0668	5.0

```

32 DELETE [REDEFINE=yes] _mean, _rep, _var, _resid, _rdf, _scode
33 AKEEP [FACTORIAL=9] Treatment; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid; STATUS=_scode
34 IF _scode.IN.!(1,2)
35 AKEEP [FACTORIAL=9] #_resid; DF=_rdf
36 CONFIDENCE [METHOD=individual; PROB=0.05] MEANS=_mean;
REPLICATION=_rep; VARIANCE=_var;\
37 DF=_rdf

```

Individual 95.0% confidence intervals

Equal number of observations per mean. (Input as scalar.)

MEAN, LOWER, UPPER are tables.

	Mean	Lower	Upper
Treatment			
conrol	1.923	1.837	2.009
fungicid	1.000	0.914	1.086
Hargel 100%	1.208	1.122	1.294
Hargel 25%	1.328	1.242	1.414
Hargel 50%	1.251	1.165	1.337

```

38 AMCOMPARISON [METHOD=duncan; DIRECTION=ascending; PROB=0.05]
Treatment

```

Duncan's multiple range test

Treatment

	Mean	
fungicid	1.000	a
Hargel 100%	1.208	b
Hargel 50%	1.251	b
Hargel 25%	1.328	b
conrol	1.923	c

```
39 ELSE
40 PRINT !t('Multiple comparisons available only if all components of the term',\
41 'are estimated with equal efficiency and in the same stratum.);\
42 JUST=left
43 ENDIF
```