BIOTEKNOLOGI
Volume 14, Number 1, May 2017
Pages: 1-8

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c140101

Antifungal evaluation of leaf extracts and fungicide against *Fusarium oxysporum* f.sp. *lycopersici* causal agent wilt of tomato

AMANI OSMAN ABDALAL OSMAN, IBRAHIM SAEED MOHAMED♥

Department of Plant Protection, College of Graduate Studies, Sudan University of Science and Technology, Shambat, Khartoum, Sudan. ♥email: ibrahsaeed@sustech.edu, ibrahsaeed@yahoo.com

Manuscript received: 12 September 2016. Revision accepted: 11 April 2017.

Abstract. *Osman AOA, Mohamed IS. 2017. Antifungal evaluation of leaf extracts and fungicide against* Fusarium oxysporum f.sp. lycopersici *causal agent wilt of tomato. Bioteknologi 14: 1-8. Fusarium*-wilt can attack tomato plants, and it is considered a crop threatening disease of the worldwide. Research was conducted to study the anti-fungal activity of various aqueous leaf extracts, i.e., peppermint, sweet basil plants and river red gum, beside fungicide Revus Top® on the growth of the fungi *Fusarium oxysporum* f.sp. *lycopersici*, a causal agent of wilt in tomato. In addition to an untreated-control, the experiment took three levels concentration for each aqueous plant extracts, i.e., 25, 50 and 100% and fungicide and applied to tomato plants. Their inhibitory effects were investigated in terms of retarding the fungal growth percentage. The results revealed that despite the inhibitory effect of fungicide against the fungal growth was more dominant, which range from 83.2% to 100% (no growth was recorded), all levels of concentration of aqueous leaf extracts of the three test plants significantly inhibited the growth of *Fusarium* test compared to the control treatment. Over the course of the experiment, aqueous extracts of river red gum exposed relatively high inhibition zone (44.1, 53.1 and 53.1%) followed by sweet basil (36.8, 51.5, and 54.4%) and peppermint aqueous extract as well (35.5, 39.6 and 39.6%), respectively. There is a common preference that the highest concentration of the plant extracts (peppermint, sweet basil, and river red gum 100%) and fungicide (100%) gave the significantly highest inhibition zones percent (41.9%, 48.5%, 39.3%, and 99.3%) respectively compared to the untreated control. The results showed that the increase of the dosage of aqueous leaf extracts would consistently increase their antifungal activity. Apparently, the test fungus differs in its response to the different concentrations; but overall, growth inhibition increased with the concentration. The study results may be considered promising and serve to encourage others to carry out a phytochemicals analysis of different parts of river red gum plant using different solvents so to determine the bioactive ingredient in each of these parts.

Keywords: Antifungal, plants extracts, fungicide, *Fusarium oxysporum*, tomato wilt

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), a member of the family Solanaceae, is an important crop plant in providing human nutrition. The plant is considered to have originated in South America, and it distributed to Europe in the 16th century, before being distributed around the globe (Rick 1977). Solanaceae members are important cultivated-crop plants that contain essential amino acid, vitamins, and minerals (Sainju et al. 2003). Tomatoes are rich in vitamin C content (Kanyomeka and Shivute 2005) and antioxidant substances, such as lycopene. The tomato is also believed to be an important fruit for preventing and combating cancers (Agarwal and Rao 2000). With a pleasant flavor, high nutrition value, easy to cultivate, and the ability to fruit in a range of environments, the tomato is understandably a popular vegetable plant in many countries. The estimated global annual production of tomato is 95 million Mt (FAO 2002).

Tomato cultivation takes place in Sudan, with tomato farms occupying an area greater than 36540 hectares. With good irrigation, the area has supplied tons of tomato per hectare per year and provides significant benefits for local consumption and export purposes (AOAD 2007; Dawelbeit et al. 2010).

The promising value of tomato can be hampered by the fact that the crop is threatened by several diseases. This can be a negative impact on the growth and the total production worldwide. Among these, the wilt caused by pathogenic fungi, *Fusarium*, remains a challenging task in terms of best management to overcome the disease (Rick 1976; Agrios 2000; Srinon et al. 2006). *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) W. is a fungus that specifically infects the tomato plant and has caused a significant loss of the global production yield (Rick 1979; Cal et al. 2004; Srinon et al. 2006).

In Sudan, among the diseases known limiting the production of tomato, the wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* is the most affecting one (Bhatia et al. 2004). Previous reports indicated that the disease is more severe for tomato planted in the traditional production areas characterized by the planting system on which the plant is grown on stored soil moisture after the flood waters of the Nile River subside.

Fusarium wilt of tomato has been managed primarily using resistant varieties (Jalali and Chand 1992), but a breakdown in resistance of these varieties due to the evolution of virulent races of the pathogen have undermined their importance in recent years (Haware and Nene 1982; Jiménez-Díaz et al. 1993). In fact, numerous strategies have been proposed to control this fungal

pathogen (Biondi et al. 2004; Ahmed 2011). Methods like solarization, disinfection, seed treatment with synthetic fungicides, crop rotation and mixed cropping were also in use (Sullivan 2004). However, management of seed-borne and soil-borne diseases such as wilt, caused by *Fusarium* species has remained problematic (Haware and Kannaiyan 1992; Rao and Balachadran 2002).

Recently, many efforts by recognizing antimicrobial compounds in higher plants, gave promising strategies in combating plant pathogenic diseases. Biodegradability and selective in the toxicity of such compounds are considered valuable properties for controlling some plant diseases (Schmutterer 2002).

This study was undertaken to focus on the investigation of the potentiality of higher plant extracts and synthetic fungicides for management of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*. This was done under laboratory conditions, to formulate a promising disease management approach with the following objectives: (i) To explore the antifungal potential of some higher plants aqueous crude extract against *F. oxysporum* f.sp. *lycopersici*. (ii) To evaluate the effect of systemic fungicide on fungal growth. (iii) To develop promising disease control components against *Fusarium* wilt of tomato.

MATERIALS AND METHODS

Experimental site

This study was conducted under laboratory conditions at Plant Pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology (SUST) within the period January to March 2015. The study focused on evaluating the antifungal activity of sweet basil, peppermint, and River red gum (Table 1) leaves aqueous extracts and efficacy of fungicide, Revus Top®, against *Fusarium oxysporum* f.sp. *lycopersici*.

Fungal inoculum

Random samples were collected from roots and stems of infected tomatoes plants (*Lycopersicon esculentum* Mill.) in fields at Wad RamLi area. Secured samples were put in paper bags and brought to the laboratory, where they were kept in the refrigerator for further investigations.

Isolation of the pathogen from tomato

Isolation was done from diseased roots and stem of infected tomato plants showing typical symptoms of *Fusarium* wilts. They were then cut into pieces of 0.5 to 1 cm and washed under tap water for about 5 minutes to remove soil particles. The washed pieces were dipped in 70% ethyl alcohol (5% concentration) for 2 minutes and rinsed three times in sterilized distilled water and dried on sterilized filter paper. The sterilized sections were then plated at the rate of 6 sections per plate on potato dextrose agar (PDA) medium.

Table 1. List of plant species tested for antifungal activity.

Name of plant	Family
Sweet basil (*Ocimum basilicum* L.)	Lamiaceae
Peppermint (*Mentha piperita* L.)	Lamiaceae
River red gum *(Eucalyptus camaldulensis* Dehnh.)	Myrtaceae

Identification of pathogen

The Petri dishes were incubated at 25ºC. After incubation for 7 days, growing fungus was sub-cultured on PDA medium for further purification of the fungus. Furthermore, compound microscopic examinations were carried out for Mycelia and conidia structure based on the method of (Booth key 1971) to confirm that the fungus was *Fusarium oxysporum* f.sp. *lycopersici*. Standard books and research papers were also consulted during the examination of this fungus (Aneja 2004; Rifai 1969; Barnet and Hunter 1999). The purified isolates were maintained on PDA medium for further studies.

Preparations

Preparation of Fusarium oxysporium *inoculum*

Using a cork-borer (1 cm), agar plugs were taken from the actively growing region of the mycelial growth for sub-culturing in other sterilized Petri dishes containing PDA medium. These were then left for seven days under fluorescent light at the room temperature. From these plates pure cultures of *Fusarium oxysporum* f.sp. *lycopersici* isolates were used for the experiment (Rampersad 2005).

Aqueous extract preparation

Sweet basil and River red gum leaves were collected from the Shambat area, whereas peppermint was obtained from the Omdurman Market. All samples were brought to the laboratory where they were shade dried. The samples were freed from foreign materials like stones, sand, and dust, before being kept in the laboratory for further investigation. The leaves were then milled into fine powder after previously washed with water and dried. The powdered samples were then weighted separately (25, 50 and 100 g) and placed in 75, 50 and 100 mL of sterilized distilled water respectively and placed on a shaker for 24 hrs. The extracts were then filtered overnight to obtain the concentrations 100%, 50%, and 25%.

Preparation of Revus Top® fungicides

The chemical tested was Revus Top® fungicide. Two mL was dissolved in 100 mL of sterilized distilled water and the final concentration of 25, 50 and 100 ppm was obtained by serial dilution test.

Inhibition of *Fusarium* growth

Inhibition zone technique was used in this study (Rao and Srivastava 1994). The PDA medium was amended with the required concentration from sweet basil, River red gum, peppermint, and fungicide Revus Top® before being solidified in a conical flask of 250 mL containing 100 mL of PDA medium, agitated, and poured 25 mL into a

sterilized Petri dish. Three plates were assigned for each concentration and left to solidify. The other three plates with PDA medium were left to serve as a 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* f.sp. *lycopersici* grown on PDA as described above. The inoculated Petri dishes were then incubated at room temperature, with the radial growth being measured every two days. All treatments were done in triplicates and were arranged in a Complete Randomized Design.

Calculation

The diameter of fungal radial growth was measured for every 48 hours 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 a 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

Statistical analyses

The obtained data were statistically analyzed according to the analysis of variance (ANOVA);-Duncan's Multiple Range Test was used for mean separation using SAS software for Windows Version 9.

RESULTS AND DISCUSSION

This study was conducted under laboratory conditions at Plant Protection Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology within the period January to March 2015. The study aimed to evaluate the antifungal activity of sweet basil, peppermint and River red gum leaves aqueous extracts and efficacy of fungicide, Revus Top®, against *Fusarium oxysporum* f.sp. *lycopersici*.

Effect of different concentrations two days after inoculation

The results (Table 2) showed that the leaves aqueous extracts of all plants tested, and fungicide exhibited an inhibitory effect on the fungal growth after 2 days from inoculation. The percentage inhibition ranged from 5.9% at 25% concentration of River Red Gum, to 100% inhibition achieved by 50 and 100% concentrations of fungicide. Furthermore, the percentages of fungal growth inhibition was significantly high compared to the control.

Among plant extracts, sweet basil aqueous extract at all concentrations (25, 50, and 100%) gave the highest inhibition of mycelial growth (31.9, 39.9 and 51.7%). This was followed by River red gum which gave reduction in linear growth of the fungus as (5.9, 41.3 and 43.5%) at the three concentrations (25, 50, and 100% respectively). The lowest reduction (15.5, 24.6 and 27.4%) was obtained by peppermint at the three concentrations (Table 2). Moreover, the fungicide especially at 50 and 100% concentration demonstrated 100% inhibition. However, the suppressing effect of fungicide was more pronounced (83.2, 100 and 100%) at all concentrations tested than other treatments.

Effect of different concentrations four days after inoculation

In day four after inoculation all plant extract concentrations, as well as that of the fungicide, were invariably exhibiting suppressing effects against the fungal growth. However, all concentrations of the fungicide (25, 50, and 100%) demonstrated the significantly highest inhibition zones percent (86.1, 92.5 and 99.3% respectively). This was followed by sweet basil which gave 22.7, 42.8 and 48.5 and the lowest inhibition zone percent was given by Eucalyptus at 25 and 50% concentrations (22.4 and 39.3) . Moreover the inhibitory effect from all concentrations tested was significantly different from the control (Table 3).

Effect of leaves aqueous extracts six days after inoculation

After six days from inoculation, the results (Table 4) showed that extracts of all the plants tested as well as the fungicide proved to be effective in suppressing the fungal growth. In fact, all tested concentrations of peppermint, sweet basil, River red gum and fungicide continued inducing significant inhibition zone percentages against test fungus compared to control (Table 4). Meanwhile, the River red gum aqueous extract at all concentrations tested (25, 50, and 100%) gave relatively more inhibitory effect (44.1, 53.1 and 53.1%) than sweet basil (36.8, 51.5 and 54.4%) and peppermint aqueous extract as well (35.5, 39.6 and 39.6%). Obviously, the test organism differs in its response to the different concentrations but on the whole, growth inhibition increased with increasing concentration. This inhibitory effect from all concentrations was significantly different from control.

Effect of leaves aqueous extracts eight days after inoculation

After eight days from inoculation, the results (Table 5) showed that extracts of all the plants tested as well as the fungicide maintained their suppressing effect on the fungal growth. This suppressing effect of all tested concentrations of peppermint, sweet basil, River red gum and fungicide was significantly higher than the control (Table 5). However, among all treatments, the inhibitory effect of the fungicide at all concentrations was more pronouncing than others. Moreover, the assessment of the fungicide effect on fungal growth after eight days from inoculation showed a concentration dependant differential inhibition (Table 5) where the percentage inhibition increased with increasing concentration.

Discussion

Tomato (*Lycopersicon esculentum* Mill.) is considered an important and popular plant in many countries. The global production of tomatoes doubled three times in the last 4 decades (FAO 2006). This is because of its acceptable flavor, nutritive value and ability to fruit in a wide range of environments and the relative ease with which it can be cultivated (Suarez et al. 2007). Many diseases affect tomatoes during the growing season, both in the greenhouse and field. Among these are *Fusarium* wilt disease, caused by pathogenic formae speciales of the soil-inhabiting fungus; *Fusarium oxysporum* f.sp *lycopersici.* In fact, wilt of tomato is one of the most economically important diseases world-wide (Rick 1979; Cal et al. 2004; Srinon et al. 2006).This pathogenic fungus remains to be a challenging task in terms of management (Rick 1976; Agrios 2005; Srinon et al. 2006).

In Sudan, tomato is becoming increasingly important for local consumption and for export. It is cultivated throughout the year under irrigation in an area that exceeds 36540 hectares, with an average yield of tons per hectare (AOAD 2007; Dawelbeit et al. 2010). Likewise, in Sudan, several diseases are known to limit production of tomato, of which *Fusarium* wilt caused by (*Fusarium oxysporum* f.sp. *lycopersici*) is one of the most important (Bhatia et al. 2004*).*

Table 2. Effect of different concentrations of plants leaf aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum* f.sp. *lycopersici in vitro* two days after inoculation.

Products		Inhibition zone (%)			
Plant extract	**Concentrations**	**R1**	**R2**	**R3**	**Mean**
Peppermint	25%	28.5 (5.4)	10.3 (3.3)	7.6 (2.8)	15.5 (3.8)ef
	50%	14.2 (3.8)	17.2 (4.2)	42.3 (6.5)	24.6 (4.8)de
	100%	46 (6.8)	24.1 (5)	11.5 (3.5)	27.4 (5.1)de
Sweet basil	25%	28.5 (5.4)	17.2 (4.2)	50 (7.1)	31.9 (5.6)de
	50%	57.1 (7.6)	24.1 (5)	38.4 (6.2)	39.9 (6.3)cd
	100%	39.2 (7.6)	62 (7.9)	53.8 (7.4)	51.7 (7.6)bc
River red gum	25%	0 (0.07)	13.7 (3.8)	3.8 (2.1)	5.9 (2.2)fg
	50%	39.2 (6.3)	34.4 (5.9)	50 (7.1)	41.3 (6.4)cd
	100%	42.8 (6.6)	41.3 (6.5)	46.1 (6.8)	43.5 (6.7)cd
Fungicide	25%	92.8 (9.7)	75.8 (8.7)	80.7 (9)	83.2 (9.1)ab
	50%	100 (10)	100 (10)	100 (10)	100 (10.0)a
	100%	100 (10)	100 (10)	100 (10)	100 (10.0)a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0.0 (0.7)g
C.V. (%)					17.03
SE±					0.46
LSD					1.725

Note: Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

Table 3. Effect of leaf aqueous extracts of sweet basil, peppermint, river red gum and fungicide Revus Top® on the linear growth of *Fusarium oxysporum* f.sp. *lycopersici in vitro* four days after inoculation.

Treatment		Inhibition zone (%)			
Plant extract	**Concentration**	**R1**	**R2**	**R3**	**Mean**
Peppermint	25%	38.3 (6.2)	24.4 (5)	61.5 (7.9)	41.5 (6.4)bc
	50%	45 (6.7)	42.8 (6.6)	36.5 (6.1)	41.5 (6.5)bc
	100%	53.3 (7.3)	20.4 (4.6)	51.9 (7.2)	41.9 (6.4)bc
Sweet basil	25%	33.3 (5.3)	2 (1.6)	32.6 (5.8)	22.7 (4.2)c
	50%	53.3 (7.3)	32.6 (5.8)	42.3 (6.5)	42.8 (6.5)bc
	100%	55 (7.4)	34.6 (5.9)	55.7 (7.5)	48.5 (6.9)b
River red gum	25%	38.3 (6.2)	2 (1.6)	26.9 (5.2)	22.4 (4.3)c
	50%	40 (6.4)	20.4 (4.6)	50 (7.1)	36.8 (6)bc
	100%	56.6 (7.6)	28.5 (5.4)	32.6 (5.8)	39.3 (6.2)bc
Fungicide	25%	90 (9.5)	83.6 (9.2)	84.6 (9.2)	86.1 (a
	50%	93.3 (9.7)	91.8 (9.6)	92.3 (9.6)	92.5 a
	100%	100 (10)	97.9 (9.9)	100 (10)	99.3 a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7) d
C.V. (%)					19.71
SE±					0.42
LSD					2.120

Note: Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

Table 4. Effect of leaf aqueous extracts of sweet basil, peppermint, River red gum and fungicide Revus Top on the linear growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro six days after inoculation.

Treatments		Inhibition zone (%)			
Plant extract	**Concentrations**	**R1**	**R2**	**R3**	**Mean**
	25%	40.3 (6.4)	16.6 (4.1)	49.5 (7.1)	35.5 (5.8) d
Peppermint	50%	53.8 (7.4)	34.3 (5.9)	30.6 (5.6)	39.6 (6.3) bcd
	100%	25.9 (5.1)	35.2 (6)	58.4 (7.7)	39.9 (6.2) bcd
	25%	41.3 (6.5)	35.2 (6)	33.6 (5.8)	36.8 (6.1) cd
Sweet basil	50%	51.9 (7.2)	48 (7)	54.4 (7.4)	51.5 (7.2) bc
	100%	55.7 (7.5)	54.9 (7.4)	53.4 (7.3)	54.7 (7.4) b
	25%	49 (7)	43.1 (6.6)	40.5 (6.4)	44.3 (6.6) bcd
River red gum	50%	55.7 (7.5)	50.9 (7.2)	52.4 (7.3)	53.1 (7.3) bc
	100%	55.7 (7.5)	51.9 (7.2)	51.4 (7.2)	53.1 (7.3) bc
	25%	94.2 (9.7)	92.1 (9.6)	92 (9.6)	92.8 (9.6) a
Fungicide	50%	92.3 (9.6)	96 (9.8)	92 (9.6)	93.5 (9.6) a
	100%	99 (10)	100 (10)	100 (10)	99.7 (10) a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)e
C.V. (%)					13.04
SE±					0.40
LSD					1.090

Note: Means followed by the same letter are not significantly different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis

Table 5. Effect of different concentrations of plant leaf aqueous extracts and fungicide on the linear growth *of Fusarium oxysporum* f.sp. *lycopersici in vitro* eight days after inoculation.

Treatments		Inhibition zone (%)			
Plant extract	**Concentrations**	**R1**	**R2**	**R3**	**Mean**
Peppermint	25%	38.2 (6.2)	19.4 (4.5)	53.3 (7.3)	37.0 (6) c
	50%	21.9 (4.7)	38.8 (6.3)	51.8 (7.2)	37.5 (6) c
	100%	58.5 (7.7)	49.2 (7.1)	40.7 (6.4)	49.5 (7) bc
Sweet basil	25%	39.8 (6.4)	40.2 (6.4)	41.4 (6.5)	40.5 (6.4) c
	50%	49.5 (7.1)	46.2 (6.8)	46.6 (6.9)	47.5 (6.9) bc
	100%	54.4 (7.4)	58.2 (7.7)	57 (7.6)	56.6 (7.5) b
River red gum	25%	38.2 (6.2)	41 (6.4)	40.7 (6.4)	40.0 (6.2) c
	50%	36.5 (6.1)	42.5 (6.6)	41.4 (6.5)	40.2 (6.4) c
	100%	43 (6.6)	41 (6.4)	45.1 (6.8)	43.1 (6.6) bc
Fungicide	25%	95.1 (9.8)	94 (9.7)	94 (9.7)	94.4 (9.7) a
	50%	93.4 (9.7)	97 (9.9)	94 (9.7)	94.9 (9.7) a
	100%	99.1 (10)	100 (10)	100 (10)	99.7 (10) a
Control		0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)d
C.V. (%)					8.25
SE±					0.38
LSD					0.9553

Note: Means followed by the same letter are not significant different according to Duncan's multiple range ($P< 0.05$). Data in parentheses transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis

Several research findings have presented strategies to control this fungal pathogen (Haware and Nene 1982; Jiménez-Díaz et al. 1993; Biondi et al. 2004; Ahmed 2011). However, management of seed-borne and soil-borne diseases such as tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* has always been problematic (Rao and Balachadran 2002). Generally, the use of synthetic fungicides considerably reduced wilt incidence in tomato, but their use is costly as well as environmentally undesirable (Song and Goodman 2001). Moreover, the use of resistant varieties is faced with the breakdown of resistance due to high pathogenic variability in the pathogen population (Kutama et al. 2011; 2013). In this context, the search for an eco-friendly way of managing *Fusarium* wilt in tomato which offers an alternative to fungicides is highly demanding.

Fortunately, progress achieved in recognizing antimicrobial compounds in plants gave more promises in combating plant pathogenic diseases. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer 2002). In fact, plants with biologically active secondary metabolites are extremely abundant, where over 80% of all known Alkaloids, Terpenoid, Phenols and other secondary metabolite were produced from them (Siddig 1993; Newman et al. 2000).

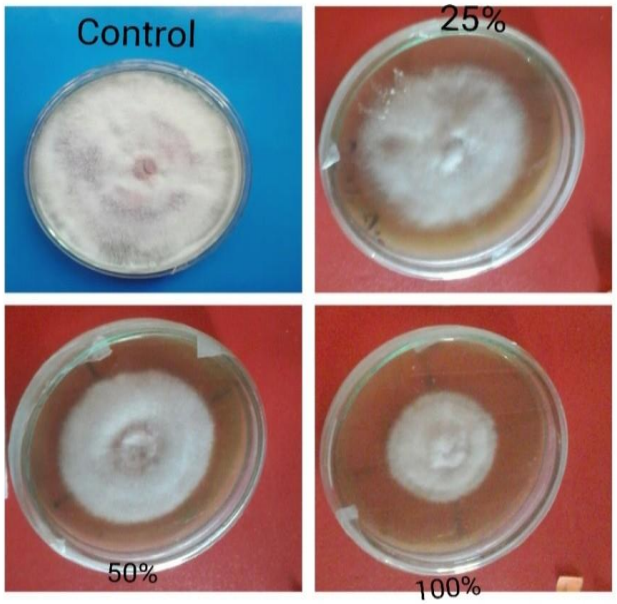

Figure 1. Effect of leaf aqueous extracts of peppermint on growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro.

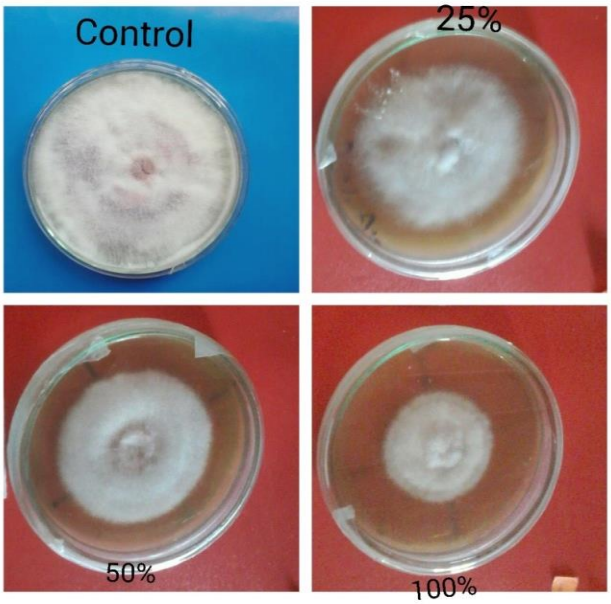

Figure 3. Effect of leaf aqueous extracts river red gum on the growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro.

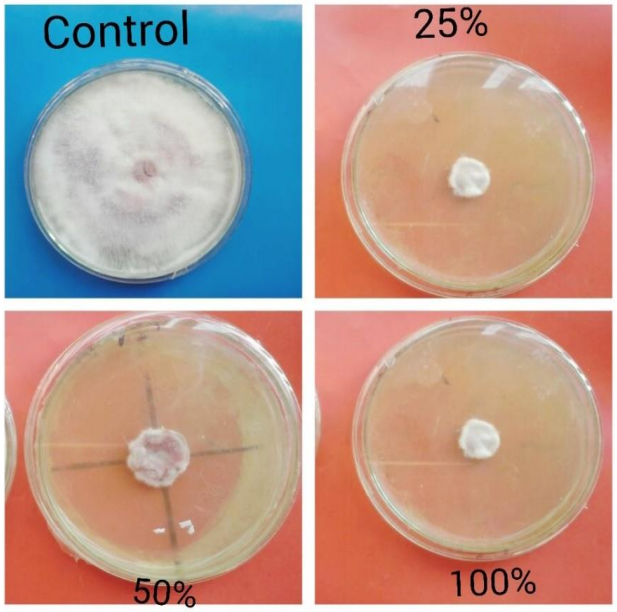

Figure 2. Effect of leaf aqueous extracts of sweet basil on the growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro.

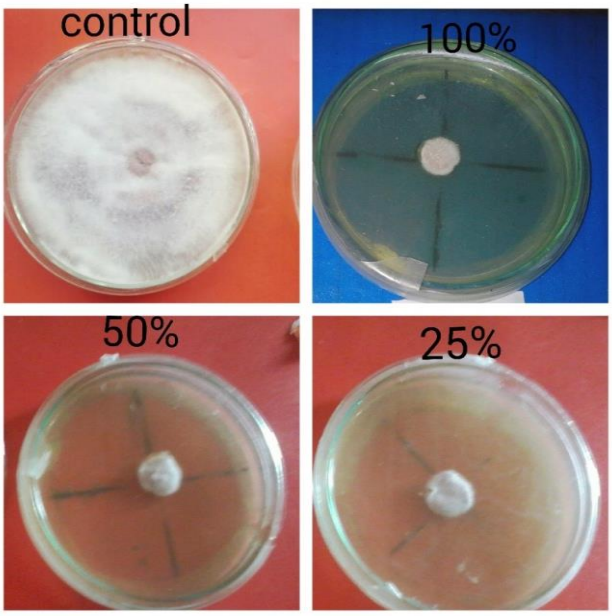

Figure 4. Effect of leaf aqueous extracts fungicide (Revus Top®) on the growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro.

The results (Tables 2-5) revealed that the sweet basil, peppermint, and River red gum leaves aqueous extracts and fungicide, Revus Top®, solution consistently and throughout the course of the experiments exhibited an inhibitory effect on the 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; Ergene et. al. 2006; Kiran and Raveesha 2006; Okigbo and Ogbonnaya 2006; Shariff et. al. 2006). In fact, this finding agrees with Muntasir (2014) who tested the bioactivity of sweet basil extract against fungi and demonstrated its suppressing effect on the fungal growth in vitro. Bansal and Rajesh (2000) also reported the antifungal effect of River red gum.

As demonstrated by various research, there is considerable interest in the use of peppermint for controlling various fungal diseases in plants (Kalemba

2003; Soković et al. 2009). Similar results were found in Moghtader (2013), who tested the effect of essential oil of its *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*. These well-known Bicarbonates are widely used in the food industry and were found to suppress several fungal diseases of cucumber plants.

The data presented in this study showed that the use of sweet basil in vitro expressed an inhibitory effect against the mycelial growth of *Fusarium oxysporium*, and the percentage zone of inhibition was significantly higher than the control. The obtained results were in line with that of Katooli et al. (2012) who tested the antifungal activity of River red gum (*Eucalyptus camaldulensis*) essential oil evaluated on suppressed the mycelial growth of postharvest pathogenic fungi, *Penicillium digitatum, Aspergillus flavus, Colletotrichum gloeosporioides* and soilborne pathogenic fungi, *Pythium ultimum, Rhizoctonia solani*, and *Bipolaris sorokiniana* pathogenic fungi.

Generally, uses of synthetic fungicides considerably reduces the impact of this disease. In this study, the fungicide Revus Top® consistently inhibited the radial mycelial growth of *Nattrassia mangiferae* and its suppressing effect was pronounced at all concentrations tested throughout the time of the investigation. These results confirm that which were reported by Themis et al. (2005), who indicated the effectiveness of fungicides against other fungi that infect limb dieback of figs in California.

The leavf aqueous extracts of all plants tested exhibited an inhibitory effect on fungal growth. Thus, the two components plus fungicide (Revus Top®) could be applied as part of an integrated approach to control *Fusarium* wilt in tomato.

The sweet basil plant leaf aqueous extract exhibited a more inhibitory effect than that of the river red gum and peppermint. This finding is the first one of its kind in Sudan in the in vitro of *Fusarium* wilt control in tomato, which suggests more investigation is needed.

The screened concentrations of river red gum, sweet basil and peppermint leaves aqueous 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 in tomato.

REFERENCES

Agarwal S, Rao A. 2000. Tomato lycopene and its role in human health and chronic diseases. CMAJ 163 (6): 739-744.

Agrafiotis DK, Bone R, Salemme FR. 2002. Soil method of gee rating chemical compounds having desired properties. US patent 6: 434-490 August 13.

Agrios GN. 2000. Significance of plant disease. In: Agrios GN. (ed.), Plant Pathology. Academic Press, London.

Agrios GN. 2005. Plant Pathology. Elsevier, California, U.S.A.

Ahmed M. 2011. Management of *Fusarium* wilt of tomato by soil amendment with *Trichoderma koningii* and a white sterile fungus. Indian J Res 5: 35-38.

Aneja KR. 2004. Experiments in Microbiology. Plant pathology and Biotechnology. 4th ed. New International (p) Limited publishers, India.

AOAD. 2007. Arab Agricultural Statistics Yearbook. Arab Organization for Agricultural Development (AOAD), Khartoum.

Bansal KR, Rajesh KG. 2000. Evaluation of plant extracts against *Fusarium oxysporum*, wilt pathogen of fenugreek. Ind Phytopathol 53 (1): 107-108.

Barnett HL, Hunter BB. 1999. Imperfect fungi. J Adv Biosci Biotech 5 (10): Septemper 29.2014.

Bhatia P, Ashwath N, Senaratna T, Midmore D. 2004. Tissue culture studies of tomato (*Lycopesricon esculentum*). Plant Cell Tiss Organ Culture 78 (1): 1-21.

Biondi N, Piccardi R, Margheri MC, Rodolfi L, Smith GD, Tredici MR. 2004. Evaluation of *Nostoc strain* ATCC 53789 as a potential source of natural pesticides. Appl Environ Microbiol 70: 3313-3320.

Boothkey. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew.

Cal A, Larena I, Sabuquillo P, Melgarejo P. 2004. Biological control of tomato wilts. Recent Res Dev Crop Sci 1: 97-115.

Correll JC. 1991. The relationship between formae speciales, races and vegetative groups in *Fusarium oxysporum*. Phytopathology 81: 1061-1063.

Dawelbeit, SE Salih, FM Dahab, OA, Ahmed, EH. 2010. Irrigated Agriculture in Sudan 2: Main Crops Consuming Fertilizers and the Role of Education in Optimizing Fertilizer Use. International Potash Institute Research Findings: e-ifc No. 23.

Einhellig FA. 1995. Mechanism of action of allelochemicals in allelopathy. In: Inderjit, Dakshini KMM, Einhellig FA (ed.). Allelopathy: Organisms, Processes and Applications. American Chemical Society, Washington, D.C.

Ergene A, Guler P, Tan S, Mirici S, Hamzaoglu E, Duran A. 2006. Antibacterial and antifungal activity of *Heracleums phondylium* sub sp. artvinense African J Biotechnol 5: 1087-1089.

FAO. 2002. FAO Database, Food and Agriculture Organization, Rome

FAO. 2006. FAO production yearbook, Basic Database unit, Statistic Division, FAO, Rome.

Foster S. 1990. Peppermint, Mentha piperita. In: Botanical Series; American Botanical Council: Austin, TX

Gordon, T.R. Okamoto, D., Jacobson, D.J. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f.sp. melonis and other species of *Fusarium*. Phytopathology 79: 1095-1100.

Handique AK, Singh HB. 1990. Antifungal action of Lemongrass oil on some soil borne plant pathogens. Indian Performer 34 (3): 232-234.

Haware MP, Kannaiyan J. 1992. Seed Sci Technol 20: 597-601.

Haware MP, Nene. 1982. Races of *Fusarium oxysporum* f.sp. Ciceri Plant Dis 66: 809-810.

Jalali YL, Chand H. 1992. Chickpea wilt. In: plant diseases of International Importance. Vol. 1. Diseases of Cereals and Pulses. U.S.

Jimenez-Diaz RM, Alcala-Jimenez AR, Hervas A, Trapero Casas JL. 1993. Pathogenic Variability and host resistance in the *Fusarium oxysporum* f.sp. *ciceris*/*Cicer arietinum* pathosystem. In: Proc 3rd Eur Semin *Fusarium* Mycotoxins, Taxonomy, Pathogenicity and Host Resistance. Radzikov, Poland.

Kalemba D, Kunicka A. 2003. Antibacterial and antifungal properties of essential oils. Curr Med Chem 10 (10): 813-829.

Kanyomeka L, Shivute B. 2005. Influence of pruning on tomato production under controlled environments. Agricultura Tropica et Subtropica 32 (2): 79-81.

Katooli N, Maghsodlo R, Honari H, Razavi SE. 2012. Fungistatic activity of essential oil of thyme and eucalyptus against of postharvest and soilborne plant pathogenic fungi. Global J Med Plant Res 1 (1): 1-4.

Kiran B, Raveesha AK. 2006. Antifungal activity of seed extract of *Psoralea corylifolia* L. Plant Dis Res 20: 213-215.

Kutama AS, Auyo MI, Umar S, Umar ML. 2013. Reduction in growth and yield parameters of sorghum genotypes screened for loose smuts in Nigerian Sudan Savanna. World J Agric Res 1 (5): 185-192.

Kutama AS, Emechebe AM, Aliyu BS. 2011. Field evaluation of some inoculation techniques on the incidence and severity of sorghum head smut (Sporisorium reilianum) in Nigerian Sudan savanna. Biol Environ Sci J Trop 8 (3): 292-296.

Moghtader M. 2013. In vitro antifungal effects of the essential oil of *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*. African J Plant Sci 7 (11): 521-527.

Muntasir A. 2014. Third conference of pest management in Sudan 2-3 February CPRC-ARC Wed Madani.

Newman DJ Cragg GM, Snader KM. 2000. The influence of natural products upon drug discovery. Natural Prod Rep 17 (3): 215-234.

Okigbo RN, Ogbonnaya UO. 2006. Antifungal effects of two tropical plant leaf extracts (*Ocium gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. African J Biotechnol 5 (9): 727-731.

Rampersad SN. 2005. a study of dicotyledonous weed species as hosts of Potato Yellow Mosaic Trinidad Virus (PYMTV). Plant Pathol J 4 (2): 157-160.

Rao AV, Balachandran B. 2002. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr Neurosci 5 (5): 291-309.

Rao GP, Srivastava AK. 1994. Toxicity of essential oils higher plants against fungal pathogens of sugarcane. In: Rao GP, Gillasple AG, Upandhaya PP, Bergamin A, Agnihotri VP, Chen CT (eds.). Current Trend in Sugarcane pathology. International Books and Periodicals Supply Service, Pitampura, Delhi.

Rick CM, Fobes JF, Holle M. 1977. Genetic variation in *Lycopersicon pimpinellifolium*: Evidence of evolutionary change in mating systems. Plant Syst Evol 127: 139-170.

Rick CM. 1976. Tomato. In: Simmonds NY (ed.). Evaluation of Crop Plants. Longman, London.

Rick CM. 1979. Biosystematic studies in *Lycopersicon* and closely related species of *Solanum*. In: Hawkes JG, Lester RN, Skelding AD (eds.) The Biology and Taxonomy of the Solanaceae. Academic Press, New York.

Rifai MA. 1969. Revision of the genus *Tricoderma*. Mycol Pap 116: 1-56.

Sainju MU, Dris R, Singh B. 2003. Mineral nutrition of tomato. Food Agric Environ 1 (2): 176-183.

Schmutterer H (ed.). 2002. The neem tree: source of unique natural product for integrated pest management medicine, industry, and other purpose. 2nd ed. VCH verlags gesllschaft, Wenham, Germany.

Shariff N, Sudarshana MS, Umesha S, Hariprasad P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. African J Biotechnol 5: 946-950.

Siddig SA. 1993. Evaluation of neem seed and leaf water extracts and powder from the control of insect pest insect in the Sudan. Agric Res Crop Tech Bull No. 6. Khartoum, Sudan.

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, van Griensven LJLD. 2009. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. Molecules 14: 238-249.

Song F, Goodman RM. 2001. Molecular biology of disease resistance in rice. Physiol Mol Plant Pathol 59: 1-11.

Srinon W, Chuncheen K, Jirattiwarutkul K, Soytong K, Kanokmedhakul S. 2006. Efficacies of antagonistic fungi against *Fusarium* wilt disease of cucumber and tomato and the assay of its enzyme activity. J Agric Technol 2 (2): 191-201.

Suárez-Estrella F, Vargas-Garcia C, Lopez MJ, Capel C, Moreno J. 2007. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f.sp *melonis*. Crop Prot 26: 46-53.

Sullivan P. 2004. Sustainable management of soil-borne plant disease-soil systems guide. National Sustainable Agriculture Information Service. Fayetteville, Arkansas.

Themis J, Michailides DP, Morgan D, Reyeslt H. 2005. Etiology Management of limb Dieback of Figs in California. Project report. http://ucanr.edu/datastoreFiles/391-292.pdf