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Inhibitory Influence of Plant Extracts on Soil Borne Fungi Infecting Muskmelon (*Cucumis melo* L.)

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Abstract: In the present study, the antifungal activity of *Cinnamomum zeylanicum* Blume, *Trigonella foenum-graecum* L., *Eucalyptus globulus* Labill, *Eruca sativa* L. and *Allium sativum* L. extracts were investigated against soil borne pathogenic fungi. The results showed the plant extracts had inhibitory activities on the mycelial growth and spore germination of these fungi. The results exhibited *C. zeylanicum* and *E. globulus* at 1000 ppm gave the highest inhibition on the mycelial growth of the tested fungi except *Fusarium verticillioides* (Sacc.) that giving 48.5 and 54.4% inhibition, respectively. Whereas, *A. sativum* was the best extract effective against *F. verticillioides* (*F. moniliforme*) which produced 55.9% reduction in the mycelial growth. Extract of *C. zeylanicum* completely inhibited spore germination of the three *Fusarium* species tested. Also, *E. globulus* completely inhibited spore germination of *F. oxysporum* f. sp. *melonis* (Leach. and Currence) Snyd. and Hans. While, *A. sativum* gave 100% inhibition of the spore germination of *F. solani* at 1000 ppm concentration.

Key words: *Fusarium* species, cinnamon, antifungal, plant extracts

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is affected by various diseases which in turn produce heavy loss to the crop. The diseases include wilt and root rot causing by soil borne fungi. These fungi are the important that causing great reduction in the field in Egypt (El-Sheshtawi *et al.*, 2014). Fungicides are necessary to manage plant diseases and to maintain high crop yields. However, indiscriminate utilization of these fungicides has frequently resulted in adverse ecological effects, as disturbing the environmental stability of soils and making plants still more susceptible to diseases (Mancini *et al.*, 2008). Increasing public concern on environmental issues requires alternative disease management systems which are less fungicides based on naturally occurring compounds (Cuthbertson and Murchie, 2005). Chemical pesticides utilization is a very popular practice to manage several plant pathogenic fungi as compare to natural one that are prepared from plants or plant parts. Nonetheless, consumer now demands less use of synthetic fungicides as a result of the residual toxicities, pollutive nature and

non-biodegradability of agrochemicals (Avasthi *et al.*, 2005). Researchers have revealed the plant extracts as source of natural fungicides that make good efforts for new fungicides development (Arokiyaraj *et al.*, 2008; Brindha *et al.*, 2009). Meanwhile, various spices and herbs have been utilized for centuries as preservatives for foods and medicinal purposes, some of them possess antifungal potential in combination and are considered as alternatives to conservative antifungal agents (Nwaopara *et al.*, 2008). There are several studies reported that the phytochemicals of *Melia azedarach*, *Euclyptus citriodora* and *Alstonia scholaris* showed fungicidal activity against pathogenic fungi (Lloyd *et al.*, 2005). The essential oil from *Cymbopogon citratus* showed inhibition of *F. oxysporum* f. sp. *cubense* (Guimaraes *et al.*, 2011). Also, De Oliveira *et al.* (2008) showed that *Lippia sidoides* was effective in suppressing the mycelial growth of *F. oxysporum* f. sp. *cubense* with similar results imposed by carbendazin which demonstrates the possibility for utilizing these sources without losses in efficacy compared to current products. The present study was carried out to investigate the

antifungal activity of plant extracts of some plant species against soil borne pathogenic fungi isolated from diseased rhizosphere and soil of muskmelon.

MATERIALS AND METHODS

Soil samples and plant parts showing wilt and root rot characteristic were collected from the field of muskmelon. The samples were taken from Dakahlia governorate, Egypt. The plant parts were examined under microscope to confirm the presence of pathogens. An infected plant roots were cut into pieces (2-3 mm), then it is surface sterilized with 0.1% sodium hypochlorite (NaOCl) for 30 sec. The root samples were washed three times with sterilized distilled water and transferred aseptically on Potato Dextrose Agar (PDA). The plates were incubated at 25±2°C and observations were made daily for emergence of culture. After the development of the fungal colonies stock cultures were prepared using PDA in test tubes and stored at 4°C. All pathogens were isolated from infected muskmelon identified according to Barnett and Hunter (1998), Booth (1977) and Kora *et al.* (2005).

Preparation of plant extracts: Bark of *C. zeylanicum*, seeds of *T. foenum-graecum*, leaves of *E. globulus*, leaves of *E. sativa* and bulb of *A. sativum* were washed with distilled water. Leaves were surface sterilized with 1% sodium hypochlorite solution for 1 min followed by several washings with sterilized water. Fifty gram of plant material of each of the five test plant species was taken in 100 mL of sterilized distilled water and blended for 5 min at low speed and left for 1 h. The blended materials were then passed through muslin cloth and finally filtered through Whatman filter paper No.1 to obtain a 50% w/v stock solution.

Aqueous extract bioassays: To determine the inhibitory effects of plant extracts, selected concentrations of the plant extracts were incorporated into the PDA and poured into Petri dishes. Agar discs (0.5 cm in diameter) were placed in the center of Petri dishes containing PDA with the corresponding plant extract at different concentrations. Petri dishes were sealed with parafilm. The plates without the plant extract

were used as control. The plates were incubated for 4 days and mycelium growth was measured.

Inhibitory effect of plant extracts on spore germination:

The antifungal effect of plant extracts on conidial germination of the three *Fusarium* species used were tested using different concentrations of aqueous plant extracts by spore germination method using cavity slides (Maji *et al.*, 2005). Spore suspension of the pathogens was prepared aseptically from 7 days old pure culture. Fifty microliter of spore suspension and 50 µL of different concentrations of aqueous leaf extracts were taken on separate cavity slides. One cavity was maintained as control without adding any extract. All treatments were maintained in triplicates. And the cavity slides were incubated at ambient temperature (25±2°C) in moist chambers for 24 h. After the incubation period, observations were made under microscope to calculate the Percentage Inhibition (PI) by counting the number of spore germinated and the total number of spores in different microscopic view.

RESULTS

Inhibitory effect of plant extracts against *F. oxysporum*

f. sp. melonis (Leach. and Currence) Snyd. and Hans: The extract of *C. zeylanicum* was found highly effective in suppressing the growth of *F. oxysporum* f. sp. *melonis*. All the concentrations of the extract significantly reduced the mycelial growth of the fungus *in vitro*. There was 46.1-78.6% reduction in fungal mycelial growth due to different concentrations of the extract (Table 1). This was followed by *E. globulus* extract, *E. sativa* and *T. foenum-graecum* extract giving 69.3, 66.1 and 62.8% reduction in the mycelial growth of *F. oxysporum* f. sp. *melonis*, respectively. In case of spore germination, the maximum inhibition of spore germination was recorded in *C. zeylanicum* and *E. globulus* extract which completely inhibited spore germination at 1000 ppm concentration (Table 2). This was followed by *E. sativa* and *A. sativum* with 95.7 and 93.0% inhibition in spore germination, respectively. Significant results were also observed in *T. foenum-graecum* extract which showed average results with 54.2% inhibition in spore germination.

Table 1: Effect of plant extracts on radial growth of *F. oxysporum* f. sp. *melonis*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	R.G	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	76.0±4.5	0.0	41.0±2.2	46.1	36.3±3.9	52.2	31.0±4.5	59.2	16.3±1.7	78.6
<i>T. foenum</i>	76.0±4.5	0.0	63.3±3.9	16.7	47.0±2.2	38.2	41.0±1.4	46.1	28.3±3.3	62.8
<i>E. globulus</i>	76.0±4.5	0.0	57.8±22.9	23.9	45.3±3.9	40.4	38.3±3.8	49.6	23.3±3.3	69.3
<i>E. sativa</i>	76.0±4.5	0.0	65.5±2.4	13.8	46.0±4.9	39.5	41.0±2.9	46.1	25.8±2.6	66.1
<i>A. sativum</i>	76.0±4.5	0.0	48.0±2.2	36.8	45.8±3.3	39.7	47.3±2.6	37.8	32.5±1.7	57.2

R.G: Radial growth, Inh %: Inhibition (%)

Table 2: Effect of plant extracts on spore germination of *F. oxysporum* f. sp. *melonis*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	S.G	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	100.0±0.0	0.0	25.0±1.6	75.0	13.3±2.1	86.7	5.0±1.6	95.0	0.0±0.0	100.0
<i>T. foenum</i>	100.0±0.0	0.0	92.3±2.1	7.7	86.3±4.6	13.7	56.0±2.9	44.0	45.8±2.1	54.2
<i>E. globulus</i>	100.0±0.0	0.0	21.0±1.4	79.0	20.5±1.3	79.5	8.5±2.6	91.5	0.0±0.0	100.0
<i>E. sativa</i>	100.0±0.0	0.0	34.0±2.4	66.0	32.3±3.3	67.7	12.5±1.3	87.5	4.3±1.3	95.7
<i>A. sativum</i>	100.0±0.0	0.0	35.8±2.6	64.2	29.7±3.9	70.3	19.8±1.9	80.2	7.0±1.8	93.0

S.G: Spore germination, Inh %: Inhibition (%)

Table 3: Effect of plant extracts on radial growth of *Rhizoctonia solani*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	R.G	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	85.0±2.9	0.0	49.6±1.8	41.6	45.0±1.6	47.1	20.3±1.3	76.1	16.8±2.9	80.2
<i>T. foenum</i>	85.0±2.9	0.0	56.3±1.3	33.8	51.8±2.8	39.1	45.5±2.1	46.5	40.5±3.1	52.4
<i>E. globulus</i>	85.0±2.9	0.0	65.3±3.6	23.2	54.3±1.3	36.1	47.3±1.3	44.4	42.0±0.6	50.6
<i>E. sativa</i>	85.0±2.9	0.0	56.3±1.3	33.8	50.3±5.3	40.8	46.8±3.4	44.9	45.0±1.4	47.1
<i>A. sativum</i>	85.0±2.9	0.0	75.1±0.8	11.6	67.3±1.3	20.8	42.0±1.4	50.6	40.3±1.3	52.6

R.G: Radial growth Inh.%: Inhibition (%)

Table 4: Effect of plant extracts on radial growth of *F. verticillioides*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	R.G	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	68.0±2.2	0.0	56.3±3.1	17.2	52.8±3.3	22.4	49.3±1.3	27.5	35.0±4.1	48.5
<i>T. foenum</i>	68.0±2.2	0.0	55.3±3.1	18.7	54.0±2.2	20.6	49.0±2.9	27.9	37.0±1.8	45.6
<i>E. globulus</i>	68.0±2.2	0.0	45.2±2.1	33.5	49.0±1.4	27.9	47.0±1.4	30.9	31.0±2.9	54.4
<i>E. sativa</i>	68.0±2.2	0.0	55.0±1.6	19.1	51.0±3.3	25.0	48.0±1.6	29.4	40.5±3.4	40.4
<i>A. sativum</i>	68.0±2.2	0.0	42.8±2.1	37.1	50.0±1.8	26.5	47.5±2.4	30.1	30.0±2.6	55.9

R.G: Radial growth, Inh %: Inhibition (%)

Table 5: Effect of plant extracts on spore germination of *F. verticillioides*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	S.G	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	100.0±0.0	0.0	65.0±1.8	35.0	13.0±1.8	87.0	11.5±1.3	88.5	0.0±0.0	100.0
<i>T. foenum</i>	100.0±0.0	0.0	96.3±2.1	3.7	77.8±2.2	22.2	62.3±2.2	37.7	55.0±1.4	45.0
<i>E. globulus</i>	100.0±0.0	0.0	29.5±2.6	70.5	22.5±1.3	77.5	16.8±2.5	83.2	3.5±1.3	96.5
<i>E. sativa</i>	100.0±0.0	0.0	85.3±4.3	14.7	71.0±0.8	29.0	54.3±3.7	45.7	39.3±1.3	60.7
<i>A. sativum</i>	100.0±0.0	0.0	40.3±1.3	59.7	38.8±1.7	61.2	29.0±3.6	71.0	15.5±2.6	84.5

S.G: Spore germination, Inh %: Inhibition (%)

Inhibitory effects of plant extracts against *Rhizoctonia solani* Kühn: Data in Table 3 showed that all plant extracts had inhibitory activity against *R. solani*. The *C. zeylanicum* extract induced growth inhibition zone (80.2%) at a concentration of 1000 ppm. On the other hand, another plant extract used showed moderate reduction ranging from 47.1% (*E. sativa*) to 52.6% (*A. sativum*).

Inhibitory effects of plant extracts against *F. verticillioides* (Sacc.): *In vitro* antifungal activities of plant extracts showed that all studied plant extracts had inhibitory effect on *F. verticillioides*. The extracts obtained from the bulb of *A. sativum* showed the highest antifungal activity against the pathogen that produced 55.9% reduction in mycelial growth (Table 4). This was followed by *E. globulus* extract by 54.4% suppression in mycelial growth. Conversely, *E. sativa* extract gave the

lowest effect against *F. verticillioides* giving 40.4% reduction in mycelial growth. On the other hand, the effect of different extracts of selected plants was observed on spore germination of *F. verticillioides*. The extract of *C. zeylanicum* at 1000 ppm was found to be most effective in reducing the spore germination with 100%, followed by *E. globulus* extract with 96.5% reduction (Table 5).

Inhibitory effect of plant extracts against *Sclerotinia sclerotiorum* (Lib.) de Bary: The activity of the plant extracts against the mycelial growth of *S. sclerotiorum* is presented in Table 6. It was noticed that out of five plant extracts tested, leaf extract of *E. globulus* (62.0%) showed maximum inhibitory effect against the mycelial growth of *S. sclerotiorum* followed by *C. zeylanicum* extract (55.7%). On the other hand, *T. foenum-graecum* produced the lowest effect against *S. sclerotiorum* with 30.0% reduction in mycelial growth.

Table 6: Effect of plant extracts on radial growth of *Sclerotinia sclerotiorum*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	R.G	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	79.0±2.4	0.0	54.8±1.0	30.6	49.0±2.2	38.0	39.8±1.7	49.6	35.0±3.4	55.7
<i>T. foenum</i>	79.0±2.4	0.0	73.8±3.3	6.6	70.0±1.4	11.4	62.0±2.4	21.5	55.3±2.5	30.0
<i>E. globulus</i>	79.0±2.4	0.0	57.0±2.9	27.8	51.0±2.8	35.4	43.8±3.8	44.6	30.0±1.8	62.0
<i>E. sativa</i>	79.0±2.4	0.0	77.8±1.7	1.5	70.3±1.0	11.0	62.3±6.2	21.1	51.0±3.7	35.4
<i>A. sativum</i>	79.0±2.4	0.0	73.3±2.5	7.2	62.3±1.7	21.1	59.0±2.2	25.3	49.3±1.5	37.6

R.G: Radial growth, Inh %: Inhibition (%)

Table 7: Effect of plant extracts on radial growth of *F. solani*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	R.G	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)	R.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	62.0±1.6	0.0	43.0±1.6	30.6	34.3±1.7	44.7	22.5±5.4	63.7	18.0±2.2	71.0
<i>T. foenum</i>	62.0±1.6	0.0	51.0±1.4	17.7	45.0±2.2	27.4	34.0±2.2	45.2	29.8±1.7	51.9
<i>E. globulus</i>	62.0±1.6	0.0	58.8±1.7	5.2	40.0±0.8	35.5	33.8±1.7	45.5	21.0±1.8	66.1
<i>E. sativa</i>	62.0±1.6	0.0	54.5±1.3	12.1	50.3±2.6	18.9	41.5±2.9	33.1	35.8±2.6	42.3
<i>A. sativum</i>	62.0±1.6	0.0	47.8±1.7	22.9	31.0±3.3	50.0	30.8±3.6	50.3	27.0±2.2	56.5

R.G: Radial growth, Inh %: Inhibition (%)

Table 8: Effect of plant extracts on spore germination of *F. solani*

Plant extract	0 ppm		10 ppm		100 ppm		500 ppm		1000 ppm	
	S.G	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)	S.G (Means±SD)	Inh (%)
<i>C. zeylanicum</i>	100.0±0.0	0.0	71.0±3.7	29.0	62.3±3.4	37.7	22.3±1.9	77.7	0.0±0.0	100.0
<i>T. foenum</i>	100.0±0.0	0.0	97.5±1.3	2.5	82.0±2.4	18.0	81.0±4.3	19.0	70.3±5.0	29.7
<i>E. globulus</i>	100.0±0.0	0.0	81.3±1.7	18.7	56.8±2.9	43.2	14.3±4.1	85.7	1.8±0.5	98.2
<i>E. sativa</i>	100.0±0.0	0.0	85.8±3.8	14.2	10.5±1.3	89.5	8.5±3.1	91.5	8.3±1.7	91.7
<i>A. sativum</i>	100.0±0.0	0.0	60.5±2.6	39.5	40.5±3.3	59.5	6.8±2.2	93.2	0.0±0.0	100.0

S.G: Spore germination, Inh %: Inhibition (%)

Inhibitory effect of plant extracts against

***Fusarium solani* (Mart.) Sacc:** It is evident from Table 7 that *C. zeylanicum* extract showed antifungal activity against *F. solani* with 71.0% reduction in mycelial growth followed by *E. globulus* extract that produced 66.1% reduction in mycelial growth of the pathogen. Oppositely, the extract of *E. sativa* exhibited moderate activity against *F. solani* with 42.3% reduction. On the other hand, the extracts of *C. zeylanicum* and *A. sativum* at 1000 ppm completely suppressed spore germination. Also, the extract of *E. globulus* and *E. sativa* highly effective on spore germination with 98.2 and 91.7% inhibition in spore germination of *F. solani*, respectively (Table 8). Conversely, the *T. foenum-graecum* extract produced the least reduction in spore germination with 29.7%.

DISCUSSION

The present study demonstrated that the plant extract such as *C. zeylanicum*, *T. foenum-graecum*, *E. globulus*, *E. sativa* and *A. sativum* had considerable effect on the growth rate and spore germination of soil borne pathogenic fungi. The extract of *C. zeylanicum* indicated considerable antifungal activity against mycelial and spore germination of soil borne fungal pathogens. Our results agree with those obtained by Monteiro *et al.*

(2013) who demonstrated that *C. zeylanicum* was effective at 500 ppm against the mycelial growth and germination of conidia of *Botrytis cinerea* and *Alternaria alternata*. Hadi and Kashefi (2013) stated that *C. zeylanicum* extract was the most effective against *F. oxysporum* followed by *Mentha piperita*, *Allium hirtifolium* and *A. sativum*. Al-Taisan *et al.* (2014) found that the cinnamon oil at 10 ppm completely inhibited the mycelial growth of *S. sclerotiorum* and the minimum inhibition concentration was 2 ppm. This high activity of *C. zeylanicum* could be attributed to the presence of Cinnamic aldehyde (57.73%) and 2-propenal, 3-phenyl (16.20) (Hadi and Kashefi, 2013). Boniface *et al.* (2012) studied the antimicrobial activity of cinnamon oil. The results proved the essential oil had fungicidal activity against *Penicillium digitatum* and *F. oxysporum*.

Eucalyptus globulus extract was very potent against all the selected pathogenic fungi. Tabanca *et al.* (2001) found that the *E. globulus* oil exhibited a very strong activity against the fungus *Candida albicans* in all concentrations.

Results of the present study indicates that the tested extracts showed fungicidal activity against the tested pathogens and can be exploited as natural fungitoxicant to manage the growth of pathogenic fungi and thus reduce the dependence on the fungicides. This high antifungal activity of *E. globulus* extract may be attributed

to the presence of some compounds. The major component was 1, 8-cineole (85.8%), β -pinene (7.2%) and β -myrcene (1.5%). Other compounds identified in the extract and oil of *E. globulus* obtained were β -pinene, limonene, α -phellandrene, λ -terpinene, linalool, pinocarveol, terpinen-4-ol and α -terpineol. The *E. globulus* oil consisted mostly of oxygenated monoterpenes and monoterpene hydrocarbons (Damjanovic-Vratnica *et al.*, 2011).

In the present study, it was noticed that the extract of *A. sativum* revealed considerable antifungal activity against the tested pathogens. Okigbo *et al.* (2009) evaluated the fungicidal effects of *A. sativum* against some phytopathogenic fungi; *Penicillium oxalicum*, *F. solani*, *M. phaseolina* *Botryodiplodia theobromae*, *F. oxysporum* and *Aspergillus niger*. *Syzygium aromaticum* and *A. sativum* showed 100% inhibition of the mycelial growth of *A. niger* at 20% concentration (Avasthi *et al.*, 2005). The obtained results revealed *A. sativum* had effective inhibition the mycelial growth of all tested pathogenic fungi and spore germination of the three *Fusarium* species. *Allium sativum* bulbs extract had antifungal activity against the mycelial growth of *Fusarium pallidoroseum* (Jacob and Sivaprakasam, 1994; Appleton and Tansey, 1975). Bowers and Locke (2000) showed that the antifungal activity of *A. sativum* extract against the mycelial growth and spore germination of *Fusarium solani* f. sp. *melongenae*. This fungicidal activity of *A. sativum* possibly related to organosulphur compound including allicin (Hovana *et al.*, 2011). These compounds showed better antifungal activity than both antibiotics streptomycin and ampicillin (Ilic *et al.*, 2012). Also, Durairaj *et al.* (2009) stated that allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis. Furthermore, garlic extract is known to inhibit cell wall synthesis because it inhibits transpeptidation enzymes involved in the cross-linking (Durairaj *et al.*, 2009).

CONCLUSIONS

Because the extracts of *C. zeylanicum* (cinnamon), *T. foenum-graecum* (fenugreek), *E. globulus* (eucalyptus), *E. sativa* (rocket) and *A. sativum* (garlic) are found effective against the growth and spore germination of the test organisms. Therefore, this study suggests that the extracts of these spices would be helpful in treating diseases in plants caused by soil borne pathogens. In conclusion, the findings of this study confirmed that plant extracts can be used as natural fungicides against the growth of pathogenic fungi and thus reduce the dependence on the synthetic fungicides.

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