African Journal of Microbiology Research Vol. 7(6), pp. 517-524, 5 February, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1902
ISSN 1996 0808 ©2013 Academic Journals

Full Length Research Paper

Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi

Al-Rahmah A. N.[1], Mostafa A. A.[1], Abdel-Megeed A.[1,2]*, Yakout S. M.[3], and Hussein S. A.[3]

[1]Botany and Microbiology Department, College of Science, King Saud University,
P. O. Box 2455, Riyadh, 11451 Kingdom of Saudi Arabia.
[2]Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.
[3]Biochemistry Department, College of Science, King Saud University,
P. O. Box 2455, Riyadh, 11451 Kingdom of Saudi Arabia.

Accepted 8 February, 2013

The fungicidal activity of five methanolic plant extracts from *Lantana camara, Salvadora persica, Thymus vulgaris*, *Zingiber officinale and Ziziphus spina-christi* were evaluated for their antifungal efficiency on tomato phytopathogenic fungi, *Fusarium oxysporum, Pythium aphanidermatum and Rhizoctonia solani*, the causative agents of tomato damping-off diseases. Three of five plant extracts were effective against thees phytopathogenic fungi. *T. vulgaris* and *Z. officinale* extracts were strongly active and showed fungistatic and fungicidal activities against the phytopathogenic fungi with minimal inhibitory concentration (MIC of 4 mg ml^{-1} and minimal fungicidal concentrations (MFC) of 8 mg ml^{-1} except *F. oxysporum* which was less sensitive and its MFC reached to 16 mg ml^{-1} of *Z. officinale* extract. On the other hand, *S. persica* extract showed a moderate antifungal activity while *L. camara* and *Z. spina-christi* were not effective against tomato phytopathogenic fungi except *P. aphanidermatum* which was completely inhibited at 10 mg ml^{-1} of L*. camara* extract. Carbendazim fungicide was more effective than all methanolic plant extracts inhibiting mycelial growth of all phytopathogenic fungi at 8 ppm and a huge concentration reached to 8 mg ml^{-1} of the effective plant extract was required to attain the same effect. Analysis of the effective plant extracts by GC/MS revealed that T*. vulgaris* extract was mainly composed by thymol (38.73%), carvacrol (19.31%), β-cimene (10.13%) and α-terpinolene (5.94%) while *Z. officinale* was mainly composed by Gingerol (46.85%), cedrene (8.39%), zingiberene (7.41%) and α-curcumene (7.32%) respectively. These effective plant extracts may contribute to development of potentially effective and environmentally safer alternative fungicide to control tomato damping- off phytopathogenic fungi.

Key words: Fungicides, plant extracts, fungitoxic properties.

INTRODUCTION

Seedlings damping-off caused by *Pythium aphanidermatum* and *Rhizoctonia solani* and Fusarium wilt caused by *Fusarium oxysporium F.* sp *Lycopersici* are regarded as the most important diseases that affect the tomato crop causing serious economic losses to the producers (Schwarz and Grosch, 2003; Song et al., 2004). The chemical control of these pathogens is responsible for the increase in the productivity and quality of the crop but it is inappropriate and nondiscriminatory use has put human and animal health at risk, as well as contaminating the environment (Pandy, 2003; Kumar et al., 2007). In an attempt to modify this condition, some alternative methods of control have been adopted. Within this context is the utilization of plant extracts which are natural sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or environmental problems at

*Corresponding author. E-mail: Ashraf812@yahoo.com. Tel: 00966562061061.

Table 1. The ethnobotanical data of the plant parts employed and extract percentage yield**.**

Plant species	Family	Common name	Plant part used	Yield extract (%)
Lantanta camara	Verbenaceae	Lantanas	L	7.68
Salvadora persica	Salvadoraceae	Arak	B	10.72
Thymus vulgaris	Lamiaceae	Thyme	L	11.05
Zingiber officinale	Zingiberaceae	Ginger	R	4.2
Ziziphus spina-christi	Rhamnaceae	Seder	L	9.43

L, leaves; B, bark; R, rhizome.

any concentration which they are used (Kim et al., 2004;Yang et al., 2010).

The biological inhibitions of different natural substances, such as plant extracts have been investigated on fungal activity. For example, there are studies evaluating the inhibitory activity of plant extract on fungi; Ushiki et al. (1996) tested suppression of soil borne plant diseases by medicinal plant extracts; Ejechi et al. (1999) studied pepper extract against tomato rot fungi; Jasso de Rodriguez et al. (2005) evaluated fungal activity of Aloe vera pulp on mycelial growth of *Rhizoctonia solani* and *Fusarium oxysporum*. They reported that this extract reduced colony growth rate of *R. solani* and *F. oxysporum* at concentration of 105 µl^{-l}. Curir et al. (2005) determined phytoalexin inhibitory effect involved in carnation against *F. oxysporum F*. sp causative agent of Fusarium wilt.

Eucalyptus leaf extracts were found to be effective against most Gram+ bacteria and can inhibit mycelial growth of *Rhizoctonia solani*, *Phytophthora*, *F. oxysporum* and many other fungi in concentrations above 20% (Ceron et al., 1999; Arora and Kaushik, 2003; Gupta and Bansal, 2003).

The extracts of *Thymus vulgaris* leaves in concentrations of 400 and 800 ppm can inhibit mycelial growth and cause degeneration of hyphae of *Pythium ultimum* and *Colletotrichum lindemuthianum* (Zambonelli et al., 1996; Ramanathan et al., 2004) while *Thymus vulgaris* essential oil exhibit broad fungitoxic spectrum against eight fungal strains including *F. oxysporum* with concentration 0.7 µl/ml (Kumar et al., 2008). The cedar leaf extract of concentrations that rangefrom 2.5 to 5% (v/v) can inhibit growth of *Opbtostoma piceae*, *Avreobasium piceae*, *Alternaria alternate* and *Gliocladium viride* (Dawson-Andoh et al., 2000). In case of ginger (*Zingiber officinale*), Arora and Kaushik (2003) screened ginger with 40 different plant extracts for their activity against soybean fungal pathogens as *F. oxysporum* and they reported that ginger inhibit mycelial growth of *F. oxysporum*. However, Singh et al*.* (1998) and Dubey et al*.* (2000) recorded fungitoxicity of ginger extract against a range of fungi among which *Rhizoctonis solani* was present. Also, compost has been used to control seedling damping-off caused by *R. solani* (Termorshuizen et al., 2007), *Pythium* (Scheuerell et al., 2005) and several species of *Fusarium* (Borrero et al., 2004).

Arak (*Salvadora persica*) has traditionally been used as tooth brushes in Arabian area as it exhibit antimicrobial effect against aerobic and anaerobic bacteria recovered from teeth with necrotic pulps (Ahmed et al*.*, 2008). Presently, the control of seedlings damping-off diseases is mainly based on the application of fungicides and the adverse effects of these fungicides on environment and human health have focused the efforts on developing environmentally safe, long lasting and effective biocontrol agents. So the objective of this work was to evaluate the fungitoxic effect of some plant extracts such as *Lantana camara*, *Salvadora persica, Thymus vulgaris, Zingiber officinale* and *Ziziphus spina-christi* against seedlings damping-off diseases of tomato caused by *Pythium aphanidermatum* and *Rhizoctonia solani* and Fusarium wilt caused by *Fusarium oxysporum in vitro*.

MATERIALS AND METHODS

Plant materials

Plant materials (leaves, rhizomes or bark) of five plant species belonging to 5 botanical families included in this study (Table 1) were collected from Riyadh region of Saudi Arabia. The collected plant was carried in polyethylene bags to the laboratory, washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite and dried in shade for 23 days. The shade–dried material of each plant species was ground into a powdered material using a blender to pass 100 mm sieve and the mince was sealed in polyethylene bags until extraction.

Preparation of plant extracts

For investigations, methanolic plant extracts were prepared by soaking 50 mg of dry powder plant material from each plant species in methanol (10 ml of methanol /mg of plant material) with stirring for 48 h and then filtered through double layers of muslin, centrifuged at 9000 rpm/min for 10 min and finally filtered again through Whatman filter paper No. (41) to remove leaf debris and obtain a clear filtrate. The filtrates were evaporated and dried under reduced pressure and temperature below 40°C. The yield of the dry residues in relation to the starting plant material was calculated.

Fungal cultures

Fungal cultures of *Pythium aphanidermatum*, *Fusarium oxysporum* and *Rhizoctonia solani* were isolated from tomato crop by hyphae

Table 2. Antifungal screening test of some methanolic plant extracts (10 mg ml^{-1}) against tomato phytopathogenic fungi.

Plant species	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
	F. o	*P. a*	*R. s*	*F. o*	*P.a*	*R. s*
Lantanta camara	47.8*±.085	0.00 ± 0.00	69.5*± 0.065	28.12	100	16.56
Salvadora persica	56.8*±0.085	18.8* ±0.085	36.3* ± 0.085	14.69	77.83	56.43
Thymus vulgaris	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100

F. o, Fusarium oxysporum; P. a, Pythium aphanidermatum; R. s, Rhizoctonia solani. Values in the same column followed by asterisk (*) are significantly different at (P = 0.05). Data are means (n = 4) ± standard error of four replicates.

point and monsporic technique (Anguiz, 1989) and preserved in slants containing potato dextrose agar (PDA) till used. The tomato phytopathogenic fungi were provided from the culture collection of Botany and Microbiology Department, King Saudi University, Riyadh, K.S.A.

Antifungal activity of plant extracts

Antifungal activity was evaluated on tomato phytopathogenic fungi using the food poisoning technique (Kumar et al., 2008). The plant extract residues were re-dissolved in 5 ml of methanol, sterilized in disposable Millipore filter (0.22 µm pores) and mixed with sterile potato dextrose agar medium (P.D.A) to obtain the final concentration of 10 mg ml^{-1} of each plant extract and then poured in sterile Petri dishes (90 mm diameter).

For control, 5 ml of Millipore-sterilized methanol was added to the PDA medium and discs of 7 mm diameter of phytopathogenic fungi were cut from the periphery of 6 days old cultures and inoculated aseptically to the center of poured Petri dishes of treatment and control sets and incubated at 25 ± 2°C for 7 days. Fungal colony diameter of treatments and control sets were measured and percentage of mycelial inhibition was calculated using the following formula:

Percentage of mycelial inhibition = [C – T / C] x 100

Where, C and T are the growth diameter (mm) in control and treatment respectively.

Fungicidal analysis of the effective plant extracts compared with reference fungicide (carbendazim)

The effective plant extracts including Thymus *vulgaris* and *Zingiber officinale* were used to determine MIC) and MFC of these effective plant extracts. Different concentrations of each plant extracts (0.0, 2.0, 4.0, 8.0 and 16.0 mg/ml) were prepared separately by dissolving their requisite amount in 5 ml of methanol, sterilized through Millipore filter and mixed with PDA medium to obtain the final concentrations. To compare efficacy of plant extracts with that of fungicide (Carbendazim) in controlling the tomato phytopathogenic fungi, different concentrations (0.0, 2.0, 4.0, 8.0 and 16.0 ppm) of Carbendazim of 98% active ingredients were prepared by mixing weighted powder of fungicide with a known volume of sterile (PDA). Fungal plugs (0.7 mm in diameter) were obtained and placed at the center of Petri dish in potato dextrose agar medium with plant extracts of various concentrations and fungicide. The cultures were incubated at 25 ± 2°C and radial growth of mycelia was measured after 6 days. Fungicidal effect of the plant extracts was measured and MIC and MFC were determined and then compared with fungicidal effect of the reference fungicide under a totally random design with four replications.

GC/GC-MS analysis of the effective plant extracts

The effective plant extracts of *T. vulgaris* and *Zingiber officinale* were analyzed through Gas Chromatography and Mass Spectroscopy (GC-MS) Varian model, 450 equipped with a flame ionization detector and quantization was carried out by the area normalization method neglecting response factors. The analysis was carried out using a VF-5MS capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The operating conditions were as follow: injection and detector temperature, of 250 and 300°C respectively; split ratio of 1 : 50; carrier gas, of Helium with flow rate of 1.0 ml/min. Oven temperature program was 50 to 300°C at the rate of 7°C/min. Mass spectrometer conditions were: ionization potential of 70 eV; mass range from m/z 40 to400 amu; electron multiplier energy, of 2000 V. The components of plant extracts were identified by comparison of their relative retention times and the mass spectra with those authentic reference compound shown in the literature (Adams, 2007) and by computer matching of their MS spectra with Wiley and Nist 8 mass spectral library.

Statistical analysis

All experiments were conducted in four replicates for each treatment and the data were reported as mean ± SE (standard error). The data were also analyzed statistically using One-way analysis of variance (ANOVA) and differences among the means were determined for significance at P ≤ 0.05 (by SPSS, 16.1 Chicago, USA).

RESULTS

Botanical and common name of the plants used and the extract percentage yield of the selected plant species are illustrated in Table 1. Five plants belonging to five different families were studied to evaluate their antifungal activities against tomato phytopathogenic fungi.

Methanolic extracts of five plant species were studied and evaluated for their antifungal activities against tomato phytopathogenic fungi (F*. oxysporum*, *P. aphanideratum* and *R. solani*). Of these, three plant extracts at 10 mg ml^{-1} were effective in suppressing the mycelial growth of tomato phytopathogenic fungi compared to non-treated control. *T. vulgaris* extract was the most effective in suppressing the mycelial growth of phytopathogenic fungi followed by *Z. officinale* and *S. persica* (Table 2). The methanolic extract of *T. vulgaris* showed complete suppression on mycelial growth of the three phytopathogenic fungi at 10 mg ml^{-1}) followed by *Z.*

Table 3. Effect of different concentrations of reference fungicide (Carbendazim) on mycelial growth of tomato phytopathogenic fungi.

Carbendazim Concentration (ppm)	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
	F. o	***P. a***	***R. s***	***F. o***	***P. a***	***R. s***
0	86.8* ± 0.075	89.5* ± 0.05	84.8* ± 0.085	0	0	0
2	68.5* ± 0.132	52.8* ±0.165	63.3* ± 0.103	21.08	41.01	25.35
4	37.3* ± 0.125	0.00 ± 0.00	28.5* ± 0.132	57.03	100	66.39
8	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100

F. o, Fusarium oxysporum; P. a, Pythium aphanidermatum; R. s, Rhizoctonia solani. Values in the same column followed by asterisk (*) are significantly different at (P = 0.05). Data are means (n = 4) ± standard error of four replicates.

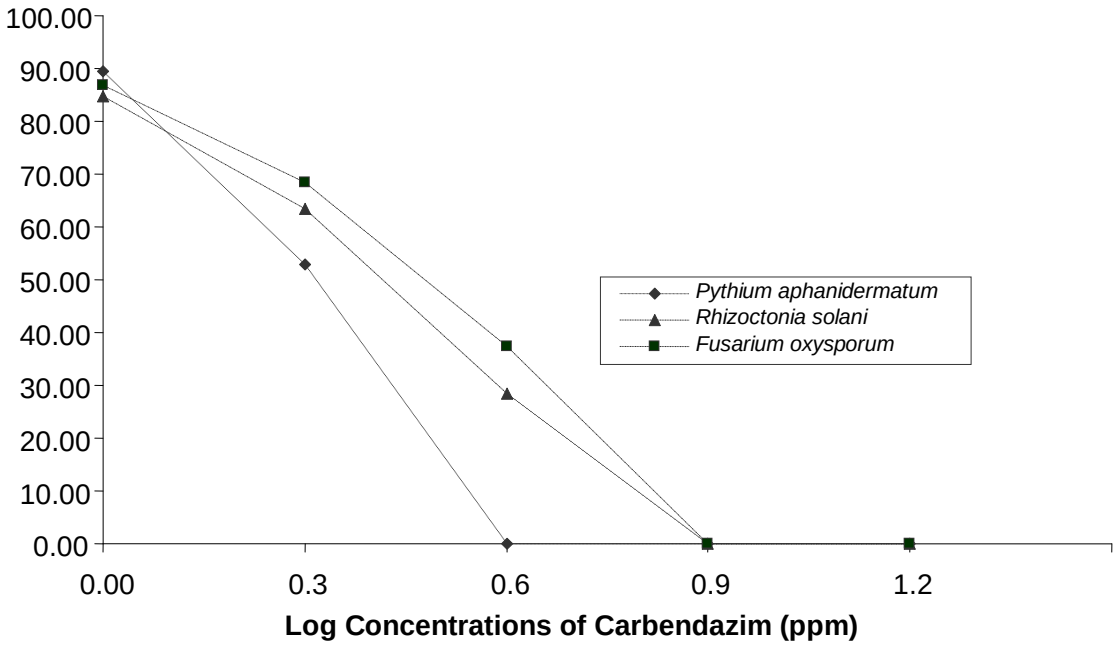

Figure 1. Effect of different concentrations of carbendazim on the colony radial growth of the tomato phytopatogenic fungi.

officinale which appear to be effective against *P. aphanidermatum* and *R. solani* suppressing completely their mycelial growth whereas *F. oxysporum* was less sensitive as its mycelial growth was inhibited to 72.18% at the same concentration.

Although, *S. persica* extract was found to be slightly effective against *F. oxysporum* inhibiting its mycelial growth to 14.69%, it was strongly effective against *P. aphanidematum* and *R. solani* suppressing their mycelial growth with 77.83 and 56.43% respectively. In contrast, *L. camara* and *Z. spina-christi* extracts were not effective against tomato phytopathogenic fungi except mycelial growth of *P. aphanidermatum* which was completely inhibited at 10 mg ml^{-1} of L. *camara* extract.

The MIC and MFC of the most effective plant extracts (*T. vulgaris* and *Z. officinale*) in comparison with carbendazim as a reference fungicide were employed by poisoned food technique to assess their fungicidal and fungistatic properties.

As illustrated in Table 3, Carbendazim shows various capabilities to suppress tomato phytopathogenic fungi on solid medium. *P. aphanidermatum* was more sensitive to carbendazim and its mycelial growth was completely inhibited at 4 ppm while *F. oxysporum* and *R. solani* were less sensitive and their mycelial growth were inhibited to 57.03 and 66.39% respectively at the same concentration.

The concentration effect of carbendazim is presented in Figure 1 where the inhibitory effect started at 2 ppm and increased in proportion to carbendazim concentration and reached to maximum in final concentration of 8 ppm.

Carbendazim was strongly effective against *P. aphanidermatum* with MIC of 2 ppm and MFC of 4 ppm while it showed fungistatic activity against *F. oxysporum*

Table 4. Effect of different concentrations of the effective plant extracts on mycelial growth of tomato phytopathogenic fungi.

Plant extract	Concentration (mg ml^{-1})	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
		F. o	*P. a*	*R. s*	*F. o*	*P. a*	*R. s*
Thymus vulgaris	0	69.3* ±0.125	83.8* ± 0.111	76.8* ± 0.165	0	0	0
	2	48.3* ±0.189	39.3* ± 0.149	44.3* ± 0.111	30.3	53.1	42.32
	4	21.5* ±0.119	11.8* ± 0.103	15.3* ± 0.075	68.98	85.92	80.1
	8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
	16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100
Zingiber officinale	0	76.5* ±0.156	87.5* ± 0.126	81.5* ± 0.266	0	0	0
	2	65.3* ±0.180	53.5* ±0.065	46.5* ± 0.096	14.64	38.86	42.95
	4	41.3* ±0.103	32.3* ± 0.125	21.8* ± 0.138	46.01	63.09	73.25
	8	25.6* ±0.103	0.00 ± 0.00	0.00 ± 0.00	66.53	100	100
	16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100	100	100

F. o, Fusarium oxysporum; P. a, Pythium aphanidermatum; R. s, Rhizoctonia solani. Values in the same column followed by asterisk (*) are significantly different at (P = 0.05). Data are means (n = 4) ± standard error of four replicates.

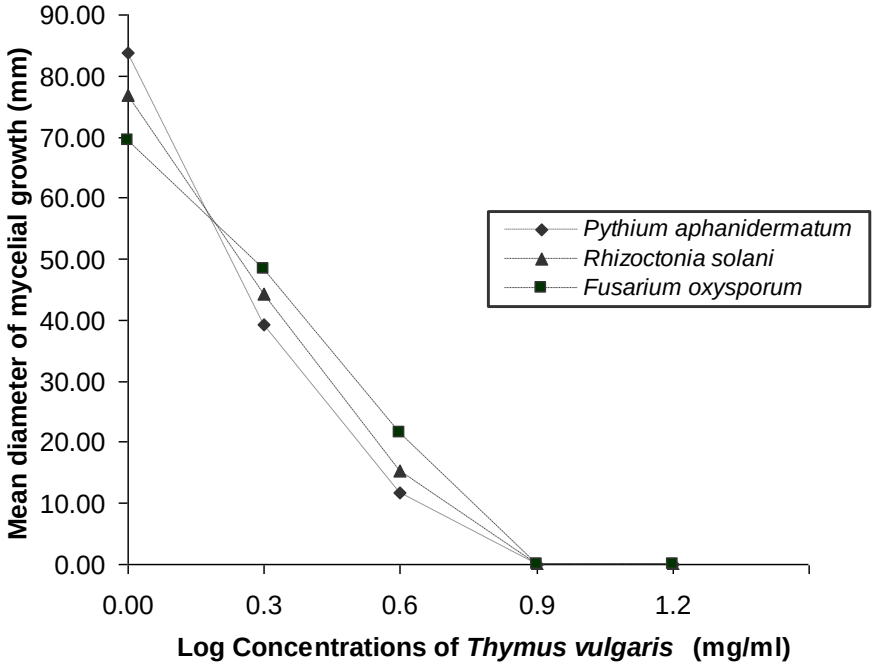

Figure 2a. Effect of different concentrations of *Thymus vulgaris* on the colony radial growth of the tomato phytopathogenic fungi.

and *R. solani* with MIC 4 ppm and MFC of 8 ppm. On the other hand, *T. vulgaris* and *Z. officinale* extracts showed antifungal activities against the tomato phytopathogenic fungi and their inhibitory effect was increased in proportion to their concentrations and reached to maximum at 8 mg ml^{-1} except *F. oxysporum* which was inhibited to 66.53% with *Z. officinale* extract at the same concentration and completely inhibited at 16 mg ml^{-1}. These inhibitions were reported to be significant for the most effective plant extracts (*T. vulgaris* and Z. *officinale*) at the level of 0.05 (ANOVA) *T. vulgaris* and *Z. officinale* extracts were strongly active and showed fungicidal and fungistatic activities against the tested phytopathogenic fungi with MIC of 4 mg ml^{-1} and MFC of 8 mg ml^{-1} except *F. oxysporum* which was less sensitive and its minimal fungicidal concentration reached to 16 mg ml^{-1} of *Z. officinale* extract (Table 4).

The concentration effect of the most effective plant extracts (*T. vulgaris* and Z. *officinale*) on mycelial growth is shown by plotting their logarithm concentration against mycelial growth of the phytopathogenic fungi (Figure 2a, b). Growth inhibitions of phytopathogenic fungi were

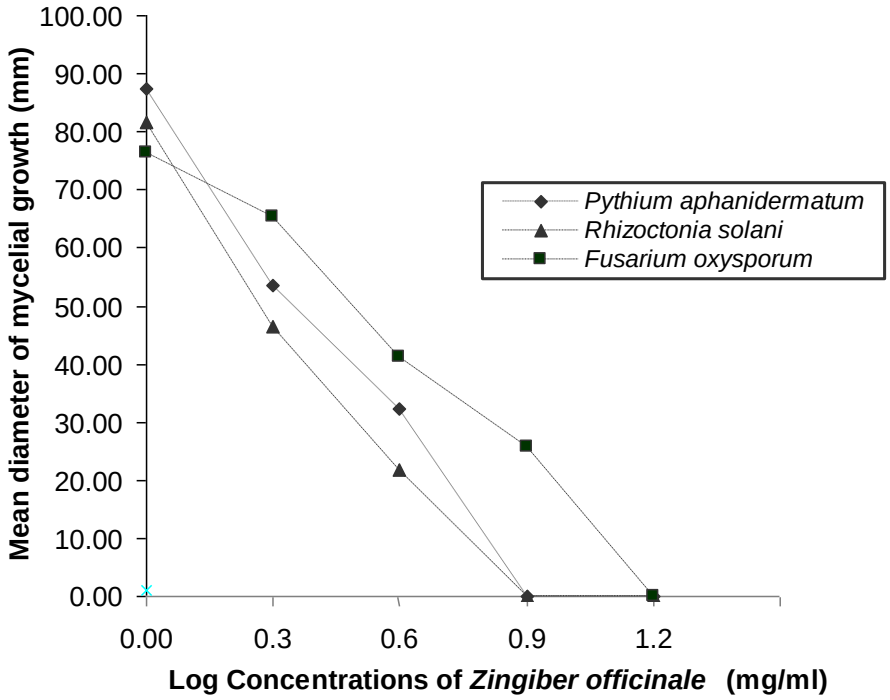

Figure 2b. Effect of different concentrations of *Zingiber officinale* on the colony radial growth of the tomato phytopatogenic fungi.

observed and increased with concentration reaching to maximum at 8 mg ml^{-1} except for *F. oxysporum* which was completely inhibited at 16 mg ml^{-1} of *Z. officinale* extract.

The main constituents of the effective plant extracts (*T. vulgaris* and *Z. officinale*) identified by GC- MS spectrometric analysis are summarizied in Table 5 according to their retention indices (RI) and percentage composition. *T. vulgaris* extract was composed by thymol (38.73%), carvacrol (19.31%), β-cimene (10.13%) and α-terpinolene (5.94%) while *Z. officinale* was mainly composed by Gingerol (46.85%), cedrene (8.39%), zingiberene (7.41%) and α-curcumene (7.32%) respectively.

DISCUSSION

The control of seedlings damping-off diseases of tomato is mainly based on the application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties. However, fungicide application has resulted in the accumulation of residual toxicity in soil and vegetables, increase environmental pollution and alter the biological balance in the soil by decimating non-target and beneficial microorganisms. Adverse effects of chemical fungicides on the environment and human health are burning issues and there is a need to search for a new fungicides eco-friendly in nature. Five methanolic plant extracts were screened *in vitro* at 10 mg ml^{-1} to evaluate them to control tomato phytopathogenic fungi (*Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium oxysporium*). Assays showed that, three plant extract provided a significant inhibition of mycelial growth of the phytopathogenic fungi and their sensitivity to a given plant extract varied greatly. *T. vulgaris* extract showed complete suppression on colony growth *of F. oxysporum, P. aphanidermatum* and *R. solani* followed by extract of *Z. officinale* which appear to be effective against *P. aphanidermatum* and *R. solani* and less effective against *F. oxysporum*. These results are in accordance with that of Ramanathan et al. (2004) and Jung et al. (2003). On the other hand, *S. persica* extract was found to be effective against *P. aphanidrematum* and *R. solani* inhibiting their mycelial growth to 77.83 and 56.43% and slightly inhibited mycelial growth of *F. oxysporum* to 14.69 respectively. Although, *L. camara* was found to be effective in controlling *P. aphanidermatum* at 10 mg ml^{-1}, it was ineffective in controlling the other tested fungal species and higher concentrations more than 10 mg ml^{-1} were required to be effective. A variation on fungitoxicity of the concerned plant extracts against phytopathogenic fungi may be due to considerable variations in their constituents and variation in fungal species itself (Manoranjitham et al., 2001; Narayana Bhat and Shukla, 2001). The study of MIC and MFC of the fungitoxicants compared with reference fungicide are necessary to evaluate their efficacy in suppressing mycelia growth of

Table 5. Phytochemical composition of the most effective plant extracts and their relative contents (%).

Compound	Plant extract			
	Thymus vulgaris		*Zingiber officinale*	
	RI	%	RI	%
α-Thujane	8.135	2.31	-	-
α-Terpinolene	8.631	5.94	-	-
β-Olimene	9.742	4.73	-	-
Bornyl acetate	11.020	6.12	-	-
Piperitinene oxide	11.631	2.13	-	-
α-Gurjunene	12.473	1.72	-	-
ρ-Cimene	13.027	10.13	-	-
Borneol	15.321	3.27	-	-
Carvacol	21.173	0.90	-	-
Thymol	22.430	38.73	-	-
Carvacrol	23.251	19.31	-	-
Bisabolene	26.651	3.71	-	-
α-Curcumene	-	-	21.230	7.32
Zingiberene	-	-	21.475	7.41
α-Farnesene	-	-	21.587	1.07
Cedrene	-	-	22.004	8.39
±Nerolidol	-	-	22.580	2.71
β-Guaiene	-	-	23.943	1.86
β-Eudesmol	-	-	24.341	4.37
β-Cedren-9- α-ol	-	-	24.888	6.71
α-Bisabolene epoxide	-	-	26.626	7.64
Longipinocarvol	-	-	27.454	5.34
Gingerol	-	-	33.250	46.85

RI, Retention time.

the phytopathogenic fungi. *T. vulgaris* extract was strongly active against the tomato phytopathogenic fungi and its MIC with MFC were comparatively lower than that of *Z. officinale.* However, carbendazim 98% fungicide was the most effective fungitoxicant suppressing growth of phytopathogenic fungi than extracts of *T. vulgaris* and *Z. officinale* as mycelial growth of the three phytopathgenic fungi were completely inhibited at 8 ppm while a huge concentration of 8 mg ml^{-1} was required for plant extracts to attain the same effect. GC/MS analysis of the effective plant extracts showed that phenolic compounds as thymol, carvacrol, β-cimene and α-terpinolene present in *T. vulgaris* extract as well as other phenolic compounds such as Gingerol, cedrene, zingiberene and α-curcumene present in *Z. officinale* extract play the vital role in growth inhibition of phytopathogenic fungi. Gingerol, cedrene and zingiberene were determined as the most effective antimicrobial component in *Z. officinale* extract (Mostafa et al., 2011) while thymol and carvacrol were determined as the effective antimicrobial compounds in *T. vulgaris* extract (Al-Rahmah et al., 2011; Boyraz and Ozcan, 2006). Some researchers have suggested that antimicrobial components of the plant extracts cross the cell membrane interacting with the enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces change in the cell and ultimately their death (Pane et al., 2011 and Omidbeygi et al., 2007). Other researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur causing cell death (Burt, 2004).

The present study indicates that application of plant extracts as biocontrol agents was found to be effective in controlling tomato damping-off diseases and *T. vulgaris* and *Z. officinale* extracts may be an attractive alternative for the use of natural product to control tomato phytopathogenic fungi avoiding chemical fungicide application.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University, for funding the work through the research group project No RGP-VPP-184.

REFERENCES

Adams RP (2007). Identification of Essential Oils Components by Gas Chromatography and Mass Spectroscopy. Allured Publishing Corp., Carol Stream, IL. USA.

Ahmed SS, El-Gengaihi SE, Ibrahim ME, Schnug E (2008). Preliminary phytochemical and propagation trial with *Salvadora persica* L. Agric. For. Res. 58:135–138.

Al-Rahmah AN, Mostafa AA, Abel-Megeed A (2011). Antifungal and antiaflatoxigenic activities of some plant extracts. Afr. J. Microbiol. Res. 5 11:1342–1348.

Anguiz R (1989). Anatomosis groups pathogenicity and other characteristics of *Rhizoctonia solani* isolated from potatoes in Peru. Plant Dis. 73:199–201

Arora C and Kaushik RD (2003). Fungicidal activity of plants extracts from Uttaranchal hills against soybean fungal pathogens. Allelopathy J. 112:217–228.

Borrero C, Trillas MI, Ordovas J, Tello JC, Aviles M (2004). Predictive factors for the suppression *Fusarium* wilt of tomato in plant growth media. Phytopathology 94:1094–1101.

Boyraz N, Ozcan M (2006). Inhibition of phytopathogenic fungi by essential oil, hydrosol, grond material and extract of summer savory Satureja hortensis L. growing wild in Turkey. Int. J. Food Microbiol.107:238–242.

Burt S (2004). Essential oils; their antimicrobial properties and potential applications in foods – a review. Int. J. Food Microbiol. 94:223–253.

Ceron S, Pina RE, Avila de Moreno C, Gil AL, Numpaque PV, Villate CR, Lerena HG (1999). In vitro application of extracts of eucalyptus, garlic, calendula, black berry herb, chamomile and nettle for the control of fungus *Sclerotium capivorum*. Fitopathologia. Colombiana, 23(1-2):68–71.

Curir P, Dolci M, Galeotti F (2005). Aphytoalexin-like flavonol involved in the carnation *Dianthus caryophyllus- Fusarium oxysprum* f. sp. dianthi phathosystem. J. Phytopathol. 153:65–67.

Dawson-Andoh BE, Lovell R, Kamdem DP (2000). Inhibitory and compatibility effect of essential oils on supstain and biological control fungi. J. Essent. Oil Res. 12:509–515.

Dubey NK, Tripathi P, Singh HB (2000). Prospects of some essential oils as antifungal agents. J. Med. Arom. Plant Sci. 22:350–354.

Ejechi BO, Nwafor OE, Okoko FJ (1999). Growth inhibition of tomato-rot fungi by phenolic acid and essential oil extracts of pepper fruit *Dennetia tripetala*. Food Res. Int. 32:395–399.

Gupta RK, Bansal RK (2003). Comparative efficacy of plant leaf extracts and fungicides against *Fusarium oxyporum* Schlecht inducing fenugreek wilt under pot house condition. Ann. Biol. 191:35–37.

Jasso de Rodriguez D, Hernandez-Castillo D, Rodriguez-Carcia R, Angulo-Sanchez JL (2005). Antifungal activity *in vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Ind. Crops. Prod. 21:81–87.

Jung JW, An KN, Jin YL, Park RD, Lim KY, Kim TH (2003). Biological control of damping-off caused by *Rhizoctonia solani* using chitinase producing *Paenibacillus illinoisensis* KJA-424. Soil Biol. Biochem. 35:1261–1264.

Kim YM, Lee CH, Kim HG, Lee HS (2004). Anthraquinones isolated from *Cassia tora Leguminosae* seed show an antifungal property against phytopathogenic fungi. J. Agric. Food Chem. 52:6096–6100.

Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK (2008). Assessment of *Thymus vulgaris L*. essential oil as a safe botanical preservative against post harvest fungi infestation of food commodities. Innovat. Food Sci. Emerg. Technol. 9:575–580.

Kumar R, Dubey NK, Tiwari OP, Tripathi YB, Sinha KK (2007). Evaluation of some essential oils as botanical fungitoxicants for the protection of stored food commodities from infestation. J. Sci. Food Agric. 87:1737–1742.

Manoranjitham SK, Prakasam V, Rajappan K (2001). Biocontrol of damping-off of tomato caused by *Pythium aphanidermatum*. Ind. Phytopathol. 54:59–61.

Mostafa AA, Al-Rahmah AN, Adel-Megeed A (2011). Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. J. Med. Plant. Res. 517:4231–4238.

Narayana Bhat M, Shukla BK (2001). Evaluation of some leaf extracts against *Pythium aphanidermatum in vitro* and pot culture. Ind. Phytopathol. 54:395–397.

Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H (2007). Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control 18:1518–1523.

Pandy R (2003). Pesticides and sterility. Everyman's Sci. 38:84–86.

Pane C, Spaccini R, Piccolo A, Scala F, Bonanomi G (2011). Compost amendments enhance peat suppressiveness to *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor*. Biol. Control 56:115–124.

Ramanathan A, Marimuthu T, Raguchander T (2004). Effect of plant extracts on growth in *Pythium aphanideramtum*. J. Mycol. Plant Pathol. 34:315–317.

Scheuerell SJ, Sullivan DM, Mahaffee WF (2005). Suppression of seedling damping-off caused by *Pythium ultimum*, *P. irregulare* and *Rhizoctonia solani* in container media amended with a diverse range of Pacific Northwest compost sources. Phytopathol. 95:306–315.

Schwarz D, Grosch R (2003). Influence of nutrient solution concentration and a root pathogen *Pythium aphanidermatum* on tomato root growth and morphology. Sci. Hortic. 97:109–120.

Singh SP, Rao GP, Upadhyaya PP (1998). Fungitoxicity of essential oils of some aromatic plant against sugarcane pathogens. SugarCane 2:14–17.

Song W, Zhou L, Yang C, Cao X; Zhang L, Liu X (2004). Tomato fusarium wilt and its chemical control strategies in hydroponic system. Crop Prot. 23:243–247.

Termorshuizen AJ, Van Rijin E, Van der Gaag DJ, Alabouvette C, Chen Y, Lagerlof J, Malandrakis AA, Steinberg C, Zmora-Nahum S (2007). Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. Soil Biol. Biochem. 38:2461–2477.

Ushiki J, Hayakawa Y, Tadano T (1996). Medicinal plants for suppressing soil borne plant diseases. 1. Screening for medicinal plants with antimicrobial activity in roots. Soil Sci. Plant Nutr. 42:423-426.

Yang X, Ma X, Yang L, Yu D, Qian Y, Ni H (2010). Efficacy of *Rheum officinale* liquid formulation on cucumber powdery mildew. Biol. Cont. 522:167–173.

Zambonelli A, Zechini-D'aulerio A, Bianchi A, Albasin A (1996). Effect of essential oils on phytopathogenic fungi *in vitro*. J. Phytopathol. 144:491–494.