Effectiveness of Some Plant Extracts against *Fusarium* spp. Causing Cotton Seedlings Damping-off.

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Abstract: The antifungal activity of 4 plant extracts from cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) was *in vitro* evaluated against 10 *Fusarium* spp. causing cotton seedlings damping-off. The pathogenicity of such *Fusarium* spp. on cotton seedlings was confirmed using soil infestation technique under greenhouse conditions. Experimental results were statistically analyzed and the least significant difference was used to compare means. Some of the tested isolates were found to be virulent on the two inoculated cultivars compared with the control. Other isolates were virulent on one of the tested cultivars only. The highest virulent degrees were recorded for *F. moniliforme* on the Giza-90 cultivar and *F. oxysporum* on the Giza-86 cultivar. Most of the applied plant extracts were found to be effective in inhibiting the Fusarium growth. The efficacy of all tested plant extracts was increased as the concentration increase. The potency of such extracts were varied depending on the concentrations and *Fusarium* spp. Garlic extract, at 20% concentration exhibited more than 50% inhibition against 80% of the tested species. Meanwhile, 94% inhibitions of all tested isolates were achieved by 4% concentration of the clove extract. Both of the clove and garlic extracts were successfully effective in suppressing the Fusarium growth *in vitro*. They could be promising as a source of natural eco-friendly phyto-fungicidal compounds for *in vivo* applications.
[El-Samawaty A.M.A., Yassin M.A., Moslem M.A., Omar M.R. **Effectiveness of Some Plant Extracts against *Fusarium* spp. Causing Cotton Seedlings Damping-off**. *Life Sci J* 2013;10(4):510-515] (ISSN:1097-8135). http://www.lifesciencesite.com. 66

Keywords: Disease control, Medicinal plants, Fungicide, Phytopathogens.

1. Introduction

One of the main sources of the natural fibers and oils all over the world is the Cotton crop (*Gossypium hirsutum* L.). It is attacked by various biotic stresses that threaten its growth and production. Seedling diseases caused by fungi are the most widespread and devastating biotic stresses that subsequently affect cotton yield (Aly *et al.,* 2000; El-Samawaty, 2004; Nehl *et al.,* 2004).

Cotton seedling diseases are worldwide problem caused by a complex of microorganisms. Fungi are the widest pathogens that affect cotton seedlings causing pre and/or post emergence damping-off and root rot diseases (Omar *et al*., 2007; Aly *et al*., 2008).

Fusarium species are among the most common fungi associated with cotton seedlings damping-off and frequently isolated from diseased seedlings (Palmateer *et al*., 2004; Costa *et al*., 2005; El-Samawaty *et al*., 2008).

The application of synthetic fungicides is the most common method to reduce yield losses caused by fungal diseases (Kaewchai *et al*., 2009). Chemical fungicides, have negative effects on human health and on the environment and may result in developing resistance in plant pathogenic fungi (Calhelha *et al*., 2006; Kim and Hwang, 2007; Kaewchai *et al*., 2009). The use of plant extracts which are not risky to the human health and environment, in controlling plant diseases is a potentially powerful alternative method (Reddy *et al*., 2010; Yassin *et al*., 2013). Different plant parts including stem, root, bark, flower and leaves had been reported to possess antimicrobial properties (Fawzi *et al*., 2009; Dwivedi and Dwivedi, 2012). Plant extracts from which represent a rich source of safer and ecofriendly antimicrobial agents had effectively been used against number of pathogenic fungi (Mohana *et al*., 2008; Tagoe *et al*., 2011).

Control of phytopathogenic fungi including *Fusarium* spp. using plant extracts had also been documented (Siva *et al*., 2008; Shrestha and Tiwari, 2009). Therefore, the present study aimed to evaluate the potential of 4 plant extracts against 10 *Fusarium* spp. causing cotton seedlings damping-off. The pathogenicity of such *Fusarium* spp. on cotton seedlings ware also investigated.

2. Materials and Methods

Pathogenicity test.

The pathogenicity of 10 isolates of Fusarium represent 10 species were tested on two cotton cultivars *viz.,* Giza-86 and Giza-90. Isolates and cultivars were provided by Cotton and Fiber Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Pathogenicity test was carried out under greenhouse conditions following the soil infestation technique. The inoculums were raised in glass bottles (500 g in capacity), containing about 50g wet sorghum grains per each. The bottles were autoclaved for 30 min, aseptically inoculated with the Fusaria and incubated at 30°C until sufficient growth of the Fusarium was obtained after about 3-4 weeks. Autoclaved clay loam soil was dispensed in 10 cm diameter sterilized pots, infested with the inoculums of each isolate separately at the rate of 50 g/kg and planted with 10 non sterilized seeds per pot for each cultivar. Pots (3 for each treatment) were randomly distributed on a greenhouse bench under temperature ranged from 23 to 37±5°C. Seedlings damping-off was recorded 15-45 days after planting.

***In vitro* Antifungal assay**

The antifungal activity of 4 plant extracts from cinnamon (*C. zeylanicum*), clove (*S. aromaticum*), garlic (*A. sativum*) and ginger (*Z. officinale*) was evaluated against 10 species of Fusarium. One hundred grams of plant materials were homogenized in 100 ml of distilled water (1:1W/V) for 5 minutes using a blender (Ismaiel, 2008). The obtained extracts were filtered through a sheath layer, and used immediately, or stored at 4°C until use. Different volumes of crude extracts were incorporated into PDA medium just before pouring in sterilized Petri dishes to obtain different concentrations. Petri dishes were centrally inoculated with 2mm fungal plugs and incubated at 28±2°C for 7-10 days. The radial growth of the colony was measured daily and % inhibition of mycelial growth was calculated over the control.

Statistical analysis:

ANOVA of the data was performed with SPSS-16 statistical package. Data of growth inhibition were transformed into root square of % inhibition + 0.5 before carrying out analysis of variance to normalize and stabilize variance. The Least Significant Difference (LSD) was used to compare means.

3. Results

Pathogenicity of *Fusarium* spp.

ANOVA (Table1) showed that the Fusaria (F), cultivars (C) and their interactions (F x C) were all highly significant sources of variation in damping-off disease. The significant interactions (F x C) indicated that the virulence of the tested isolates varies depending on the tested cultivars. Relative contribution indicated that Fusarium was the most important source of variation, while (F x C) interactions was the least important topic (Figure 1). Some of the tested isolates were found to be virulent on the two tested cultivars compared with the control. Effects of these isolates were either significantly (*F. solani*) or insignificantly (*F. oxysporum*) different from cultivar to another. Other isolates such as *F. avenaceum* were virulent in one of the tested cultivars only. Meanwhile, *F. chlamydosporum* isolate was couldn't be virulent on any of the two tested cultivars (Table 2).

Table 1. ANOVA of the effects of *Fusarium* spp., cultivars and their interactions on cotton seedlings damping-off caused by *Fusarium spp.*

Source of variation	Df	Ms	F value	Sig.
Fusarium (F)	10	1949.394	19.794	0.000
Cultivar (C)	1	1751.515	17.785	0.000
F x C	10	401.515	4.077	0.001
Error	44	98.485		

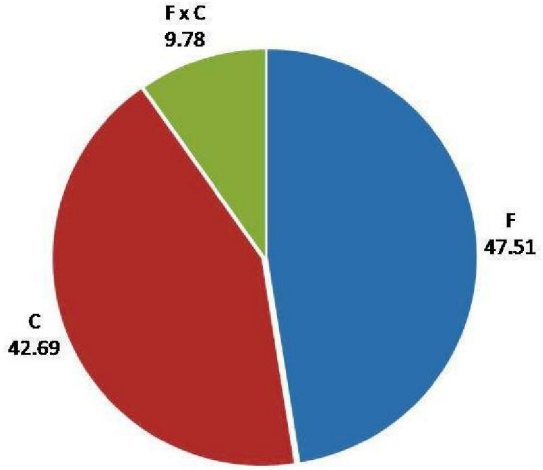

Figure 1. Relative contribution of *Fusarium* spp. (F), concentrations (C) and their interactions (FxC) to variation in Fusarium growth inhibition.

Table 2. Effect of *Fusarium* spp., cultivars and their interactions on cotton seedlings damping-off caused by *Fusarium spp.*

	Damping-off %	
Fusarium spp.	G-86	G-90
F. anthophilium	36.67	53.33
F. avenaceum	26.67	40.00
F. chlamydosporum	26.67	23.33
F. fusarioids	20.00	30.00
F. moniliforme	56.67	80.00
F. sambucinum	36.67	50.00
F. semitectum	23.33	56.67
F. solani	46.67	76.67
F. sporotrichioides	33.33	13.33
F. oxysporum	60.00	66.67
Control	20.00	10.00

LSD (Fusarium x Concentration) interaction=16.204

Antifungal activity

Analysis of variance of all tested plant extracts showed that Fusaria (F), concentrations (C) and their interactions (F x C) were all highly significant sources of variation in the inhibition of Fusarium growth (Table 3). The significant interactions (F x C) indicated that the effects of tested plant materials were varied depending on the concentration and *Fusarium* spp. for example no significant differences were found among *F. solani* and *F. anthophilium* at 2% and 4% concentrations of clove extract. Meanwhile, significant differences were found among the same species at 0.5% and 1% concentrations (Table 4). In respect of the cinnamon extract, no significant differences were found among *F. anthophilium* and *F. oxysporum* at 5% or 20% concentrations while; significant differences were recorded at 10% and 15% (Table 5). Similar inhibitory effects were found at 20% concentration of the garlic extract against both of *F. solani* and *F. oxysporum*. Significant to highly significant differences were found among the same species at the other concentrations. However, 20% concentration of the garlic extract exhibited more than 50% inhibition against 80% of the tested species (Table 6). Regarding the ginger extract; none of the applied concentrations could inhibit the growth of *F. sambucinum*. However, ginger extract showed the minimal inhibitory effects on all tested isolates. Only 31.37% inhibition of *F. sporotrichioides* growth could be obtained at 20% concentration (Table 7).

Table 3. ANOVA of the effects of *Fusarium* spp. (F), concentrations (C) and their interactions (F x C) on the linear growth of *Fusarium spp.*

Source of variation	Df	Ms	F value	Sig.
Clove extract				
F	9	2.796	84.325	0.000
C	4	372.648	1.124E4	0.000
F x C	36	1.609	48.506	0.000
Error	100	0.033		
Cinnamon extract				
F	9	10.271	126.851	0.000
C	4	179.348	2.215E3	0.000
F x C	36	1.252	15.469	0.000
Error	100	0.081		
Garlic extract				
F	9	10.502	87.455	0.000
C	4	202.996	1.690E3	0.000
F x C	36	1.651	13.751	0.000
Error	100	0.120		
Ginger extract				
F	9	5.209	27.631	0.000
C	4	51.146	271.306	0.000
F x C	36	1.271	6.743	0.000
Error	100	0.189		

Table 4. Antifungal activity of different concentrations of clove (*S. aromaticum*) extract against *Fusarium spp.*

	Concentrations									
	Control		0.5%		1%		2%		4%	
Fusarium spp.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.
F. anthophilium	0.00	0.71	9.41	3.13	45.88	6.81	67.45	8.24	94.12	9.73
F. avenaceum	0.00	0.71	10.98	3.36	35.69	6.01	75.29	8.70	94.12	9.73
F. chlamydosporum	0.00	0.71	23.92	4.94	43.92	6.66	64.71	8.07	94.12	9.73
F. fusarioids	0.00	0.71	44.31	6.69	64.71	8.07	73.73	8.61	94.12	9.73
F. moniliforme	0.00	0.71	44.31	6.69	67.45	8.24	74.90	8.68	94.12	9.73
F. sambucinum	0.00	0.71	6.67	2.66	52.16	7.26	73.33	8.59	94.12	9.73
F. semitectum	0.00	0.71	31.76	5.68	43.92	6.66	63.14	7.98	94.12	9.73
F. solani	0.00	0.71	30.20	5.54	54.90	7.44	64.71	8.07	94.12	9.73
F. sporotrichioides	0.00	0.71	7.84	2.88	48.24	6.98	56.08	7.52	94.12	9.73
F. oxysporum	0.00	0.71	28.24	5.36	50.59	7.15	56.08	7.52	94.12	9.73

Inh.% = Inhibition% Trans. = Transformed data into root square of % Inhibition + 0.5

LSD (Fusarium x Concentration) interaction=0.294.

Discussion

Results of this study revealed that *Fusarium* spp. which frequently isolated from diseased cotton plants (Aly *et al*., 2004; Costa *et al*., 2005) were pathogenic on the cotton seedlings of the two tested cultivars (Palmateer *et al*., 2004; Aly *et al*., 2008). The incidence of seedlings damping-off in the commercial cotton cultivars was varied according to fungal isolates and cultivated germplasm (Aly *et al.*, 2008; El-Samawaty *et al*., 2012).

This study proves *in vitro* antifungal activity of the tested plant extracts against *Fusarium* spp. causing cotton seedlings damping-off disease. This finding confirmed the documented antifungal activity of many plant extracts against *Fusarium* spp. (Yasmin *et al*., 2008; Rathod *et al*., 2010; Boulenouar *et al*., 2012).

Table 5. Antifungal activity of different concentrations of cinnamon (*C. zeylanicum*) extract against *Fusarium spp.*

	Concentrations									
	Control		5%		10%		15%		20%	
Fusarium spp.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.
F. anthophilium	0.00	0.71	8.24	2.94	25.49	5.10	38.43	6.24	57.65	7.62
F. avenaceum	0.00	0.71	0.00	0.71	14.90	3.91	21.57	4.69	43.14	6.60
F. chlamydosporum	0.00	0.71	7.84	2.85	18.82	4.39	40.78	6.42	46.27	6.83
F. fusarioids	0.00	0.71	10.20	3.26	25.49	5.10	48.63	7.01	55.69	7.49
F. moniliforme	0.00	0.71	15.29	3.97	17.25	4.20	42.75	6.57	48.63	7.00
F. sambucinum	0.00	0.71	0.00	0.71	5.88	2.53	8.63	2.98	17.65	4.26
F. semitectum	0.00	0.71	6.67	2.67	13.33	3.70	22.35	4.78	44.31	6.69
F. solani	0.00	0.71	10.59	3.32	29.41	5.47	36.47	6.08	59.61	7.75
F. sporotrichioides	0.00	0.71	20.00	4.52	34.51	5.91	42.75	6.57	53.33	7.34
F. oxysporum	0.00	0.71	8.63	2.98	16.08	4.06	30.98	5.60	61.57	7.88

Inh.% = Inhibition% Trans. = Transformed data into root square of % Inhibition + 0.5
LSD (Fusarium x Concentration) interaction=0. 46.

Table 6. Antifungal activity of different concentrations of garlic (*A. sativum*) extract against *Fusarium* spp.

	Concentrations									
	Control		5%		10%		15%		20%	
Fusarium spp.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.
F. anthophilium	0.00	0.71	20.39	4.56	38.82	6.27	49.41	7.06	55.69	7.49
F. avenaceum	0.00	0.71	12.55	3.57	24.31	4.97	40.00	6.36	56.86	7.57
F. chlamydosporum	0.00	0.71	12.94	3.61	23.92	4.92	39.22	6.30	59.61	7.75
F. fusarioids	0.00	0.71	16.86	4.16	29.02	5.43	54.12	7.39	67.45	8.24
F. moniliforme	0.00	0.71	15.29	3.96	34.90	5.95	47.84	6.95	58.04	7.64
F. sambucinum	0.00	0.71	0.00	0.71	4.71	2.25	28.63	5.39	41.18	6.45
F. semitectum	0.00	0.71	7.45	2.81	12.94	3.64	21.18	4.65	44.31	6.69
F. solani	0.00	0.71	3.53	1.84	8.63	2.98	25.88	5.13	50.59	7.15
F. sporotrichioides	0.00	0.71	20.00	4.52	38.04	6.20	46.27	6.84	61.57	7.88
F. oxysporum	0.00	0.71	35.69	6.00	45.10	6.75	48.24	6.98	50.59	7.15

Inh.% = Inhibition% Trans. = Transformed data into root square of % Inhibition + 0.5
LSD (Fusarium x Concentration) interaction=0.561.

Table 7. Antifungal activity of different concentrations of ginger (*Z. officinale*) extract against *Fusarium* spp.

	Concentrations									
	Control		5%		10%		15%		20%	
Fusarium spp.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.	Inh.%	Trans.
F. anthophilium	0.00	0.71	0.00	0.71	3.53	1.84	9.41	3.12	9.80	3.19
F. avenaceum	0.00	0.71	0.00	0.71	2.35	1.56	8.24	2.93	13.33	3.70
F. chlamydosporum	0.00	0.71	0.00	0.71	0.00	0.71	4.71	2.25	10.98	3.36
F. fusarioids	0.00	0.71	0.00	0.71	6.27	2.59	9.80	3.20	26.27	5.17
F. moniliforme	0.00	0.71	0.00	0.71	0.00	0.71	3.53	1.84	10.20	3.22
F. sambucinum	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71	0.00	0.71
F. semitectum	0.00	0.71	0.00	0.71	6.27	2.52	8.24	2.93	11.76	3.45
F. solani	0.00	0.71	0.00	0.71	4.31	1.99	11.37	3.43	18.04	4.30
F. sporotrichioides	0.00	0.71	0.00	0.71	6.27	2.59	20.39	4.56	31.37	5.63
F. oxysporum	0.00	0.71	0.00	0.71	2.75	1.64	10.98	3.37	13.73	3.76

Inh.% = Inhibition% Trans. = Transformed data into root square of % Inhibition + 0.5
LSD (Fusarium x Concentration) interaction=0.704.

The activity of cinnamon (*C. zeylanicum*) extract against *Fusarium* spp. (Gende *et al*, 2008; Fawzi *et al*., 2009; Yasmin *et al*., 2008), could be attributed to the presence of cinnamaldehyde, that inhibits amino acid decarboxylase activity. It is also highly electro-negative compound interferes in biological processes involving electron transfer and react with nitrogen-containing components, such as proteins and nucleic acids, and therefore inhibit fungal growth (Gupta *et al*., 2008). Cinnamic acid and eugenol in cinnamon also serve as antimicrobial compounds (Ranasinghe *et al*., 2002).

Clove (*S. aromaticum*) extract also found to be very active against the tested *Fusarium* spp.

(Bowers and Locke, 2000). This activity could be attributed to the presence of eugenol and caryophyllene. They are phenolic compounds known to possess antibacterial and antifungal properties (Ayoola *et al.,* 2008).

Regarding the garlic (*A. sativum*) extract, it was also effective against *Fusarium* spp. (Rathod and Pawar, 2012; Yassin *et al*., 2013). Garlic antifungal activity could be attributed to allicin that decomposes into several effective compounds, such as diallylsulphide, diallyldisulphide, diallyltrisulphide, allyl methyl trisulphide, dithiins and ajoene, that serve as antimicrobial agents (Harris *et al*., 2001; Jabar and Al- Mossawi, 2007).

Phenolic compounds such as gingerol, cedrene, zingiberene in ginger (*Z. officinale*) extract were determined as the most effective antimicrobial components; which play the vital role in growth inhibition of phytopathogenic fungi (Mostafa *et al*., 2011; Al-Rahmah *et al*. 2013). Although, ginger has been reported to use successfully in controlling *Fusarium* spp. (Minz *et al*., 2012), effective inhibition couldn't be obtained by any of the concentrations used in this study.

Conclusion

Clove and garlic extracts were successfully effective in suppressing the Fusarium growth *in vitro*. These extracts could be promising as a source of natural eco-friendly phyto-fungicidal compounds for *in vivo* applications. Confirmations of the *in vivo* efficacy of these extracts against *Fusarium* spp. and other cotton seedlings pathogenic fungi are needed.

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-VPP-298.

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10/4/2013