JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 191-196

Suppression of the Cotton Seedlings Pathogen; *Rhizoctonia solani* by Some Plant Extracts

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(Received: 10 September 2014; accepted: 23 October 2014)

Cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts were *in vitro* evaluated against eight *Rhizoctonia solani* anastomosis groups AGs causing cotton seedlings damping-off. Experimental results were statistically analyzed and the least significant difference was used to compare means. Both of the clove and garlic extracts were significantly suppressed the growth of all tested *R. solani* AGs but the other two extracts were ineffective. The effectiveness of both clove and garlic extracts were increased as the concentration increase. The potency of such extracts were varied depending on the concentrations and *R. solani* AGs. All tested *R. solani* AGs were completely inhibited at the 1.6 % concentration of clove extract. Both of the clove and garlic could be promising as sources of natural eco-friendly phyto-fungicidal compounds for *in vivo* applications.

Key words: Medicinal plants, Phytopathogens, Fungicide, Disease control.

Numerous fungi could be attack the cotton seeds during different stages causing seed spoilage[1,2]. Some of those are capable of reducing seed viability, injuring the sowing seeds causing seed rots and seedling diseases[3,4]. Seedling diseases caused by fungi are the most widespread and devastating biotic stresses that subsequently affect cotton yield [5,6,7]. *R. solani* is one of the widest pathogen that affect cotton seedlings causing pre and/or post emergence damping-off and root rot diseases[3,8].

Although the pad effects of the chemical fungicides on the environmental pollution, the human health and the development of resistant fungal races; still the most common method to reduce yield losses caused by fungal diseases[9,10,11]. The use of not risky; alternative control strategy against phytopathogenic fungi is advised avoiding fungicidal risks[12,13].

Plant extracts from which represent a rich sources of safer and ecofriendly antimicrobial agents had effectively been used against number of pathogenic fungi[14,15]. Extracts of different plant parts including stem, root, bark, flower and leaves had been reported to possess antimicrobial properties [16,17]. Control of phytopathogenic fungi including *R. solani* using plant extracts had also been documented[18,19]. Therefore, the present study aimed to evaluate the potential of four plant extracts against eight *R. solani* AGs, causing cotton seedlings damping-off.

MATERIALS AND METHODS

Fungi

Eight *R. solani* AGs, the cause of cotton seedlings damping-off were tested in this study.

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The pathogenic isolates of *R. solani* used in this study had been confirmed in Aly *et al*., 2014 work[20], on five cotton (*Gossypium barbadense* L.) cultivars; Giza 80, Giza 86, Giza 88, Giza 90 and Giza 92 under greenhouse conditions. These *R. solani* AGs were pathogenic on the tested cotton cultivars.

Plant extracts

Cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) extracts were selected for evaluation against *R. solani* AGs in this study. One hundred grams of plant materials were homogenized in 100 ml of distilled water (1:1W/V) for 5 minutes using a blender[21]. The obtained extracts were filtered through a sheath layer, and used immediately, or stored at 4°C until use.

***In vitro* antifungal assay**

Different volumes of crude extracts were incorporated into PDA medium just before pouring in sterilized Petri dishes to obtain different concentrations[13]. Petri dishes were centrally inoculated with 5mm fungal plugs and incubated at 28±2°C for 7-10 days. Concentrations of 0.2%, 0.4%, 0.8% and 1.6% of clove extract and 5%,10%,15% and 20% of Cinnamon, garlic and ginger extracts were tested. The radial growth of the colony was measured daily and % inhibition of mycelial growth was calculated over the control.

Statistical analysis:

ANOVA of the *R. solani* AGs growth data was performed with SPSS-16 statistical package. The Least Significant Difference (LSD) was used to compare means.

RESULTS

Both of the clove and garlic extracts were significantly suppressed the growth of *R. solani* AGs, but the cinnamon (*Cinnamomum zeylanicum*) and ginger (*Zingiber officinale*) extracts were ineffective.

Analysis of variance of clove and garlic extracts showed that isolate (I), concentrations (C) and their interactions (Ix C) were all highly significant sources of variation in the inhibition of *R. solani* AGs growth (Table 1). The significant interactions (I x C) indicated that the effects of tested plant materials were varied depending on the concentration and *R. solani* AGs. Significant difference was found among AG5 & AG6 of *R. solani* at 0.4% concentration while those were insignificantly differed from each other at 0.8% concentration. Meanwhile, insignificant difference was found among AG2-2 & AG3 of *R. solani* at 0.2% concentration while those were significantly differed from each other at 0.4% & 0.8%

Table 1. Analysis of variance of the effect of different concentrations of clove and garlic extracts on linear growth of *R. solani* AGs

	Source of variation	D.F.	M.S.	F.VALUE	P > F.
Clove extract	Conc. (C)	4	10279.804	495.313	0.000
	Error	8	20.754[a]		
	Isolate (I)	7	114.827	17.836	0.000
	Error	14	6.438[b]		
	Rep.	2	3.733		
	Error				
	CXI	28	20.390	2.050	0.011
	Error	56	9.947		
Garlic extract	Conc. (C)	4	30037.208	12603.024	0.000
	Error	8	2.383[a]		
	Isolate (I)	7	38.389	9.227	0.000
	Error	14	4.161[b]		
	Rep.	2	6.008		
	Error				
	CXI	28	29.889	8.628	0.000
	Error	56	3.464		

[a] MS (C X Rep.); [b] MS (I X Rep.)

concentrations. Moreover the growths of all tested isolates of *R. solani* were completely inhibited at the 1.6% concentration of clove extract (Table 2).

Similarly, the significance of I X C interaction, in the case of garlic extract indicated that the effects of such plant material were varied depending on the concentration and *R. solani* AGs. Significant difference was among AG1 & AG2 of *R. solani* at 10% concentration of garlic extract. They were insignificantly differed from each other at 20% concentration. Meanwhile, insignificant difference was found among AG5 & AG10 of *R. solani* at all concentration while those were significantly differed from each other at 20% concentration. Similarly, AG3 and AG5 insignificantly differed from each other at 10% concentration of garlic extract but they were significantly differed from each other at 20% concentration (Table 3).

Fig. (1) illustrated the inhibitory effect percentages of both clove and garlic extracts on *R. solani* AGs. Concentration of 1.6% of clove extract completely inhibited the growths of all tested *R. solani* AGs. More than 80% inhibition was recorded in all tested *R. solani* AGs at 0.8% concentration of clove extract. All tested *R. solani* AGs were exhibited 52.94% to 65.88% inhibition at 0.4% concentration of clove extract. More than 70% inhibition was shown at 20% concentration while 43.52%-60.0% of inhibition were recorded at15% concentration of garlic extract (Fig. 1).

DISCUSSION

Both of clove and garlic extracts used in this study had an *in vitro* antifungal activity against *R. solani* AGs, causing cotton seedlings damping-

Table 2. Effect of different concentrations of clove extract on linear growth (mm) of *R. solani* AGs

R. solani isolate	control	Concentraions (%) 0.2	0.4	0.8	1.6
AG1	85	71	32	11	5
AG1-2	85	68	35	11	5
AG2-2	85	76	40	14	5
AG3	85	78	29	9	5
AG5	85	71	30	11	5
AG6	85	67	38	12	5
AG10	85	63	32	8	5
AG4HGI	85	61	36	10	5

LSD ($p \leq 0.05$) for isolate x concentration interaction

Table 3. Effect of different concentrations of garlic extract on linear growth (mm) of *R. solani* AGs.

R. solani isolate	control	Concentraions (%) 5	10	15	20
AG1	85	74	54	45	35
AG1-2	85	71	58	44	34
AG2-2	85	72	55	44	35
AG3	85	67	53	41	29
AG5	85	64	54	43	33
AG6	85	66	51	48	31
AG10	85	64	55	44	36
AG4HGI	85	60	47	34	24

LSD ($p \leq 0.05$) for isolate x concentration interaction =3

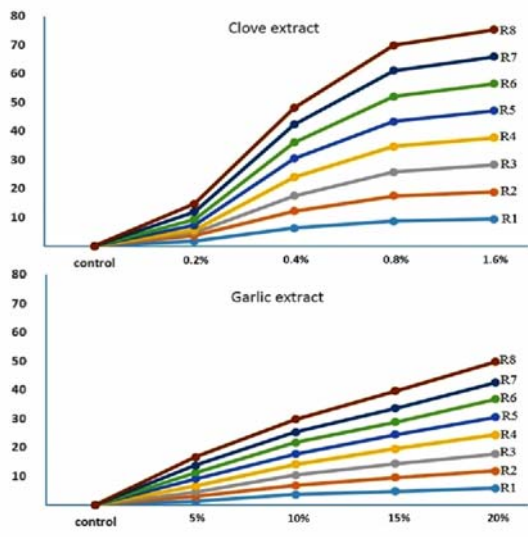

Fig. 1. The inhibitory effect percentages of different concentrations of clove and garlic extracts on the growth of *R solani* AGs; lines from R1-R8=AG1, AG1-2, AG2-2, AG3, AG5, AG6, AG10 and AG4HGI respectively.

off disease but the other two extracts were ineffective. This finding confirmed the documented antifungal activity of some plant extracts against *R. solani* [22,23.24]. Contrasting the documented results; cinnamon and ginger extracts were ineffective in controlling *R. solani* AGs in this study[25]

Role of clove (*S. aromaticum*) extract had been discussed by Al-Askar and Rashad, (2010) [24], how stated that clove extract caused complete inhibition of *R. solani* growth at concentration of 1%. The antifungal activity of clove had been attributed to the presence of the phenolic compounds; eugenol and caryophyllene that believed to possess antibacterial and antifungal properties[25,26].

Garlic (*A. sativum*) extract, was also effective against the tested isolates of *R. solani* AGs, causing cotton seedlings damping-off disease [27,28,29]. Potency of garlic extracts against several other phytopathogenic fungi had frequently been documented[26,30,31]. 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[32,33,34].

CONCLUSION

Clove and garlic extracts were successfully effective in suppressing the growth of *R. solani* AGs, causing cotton seedlings damping-off disease *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 *R. solani* AGs, and other cotton seedlings pathogenic fungi are needed.

ACKNOWLEDGMENTS

This work was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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