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Biological Control of Root Rots and Stems Canker of Tomato Plants Caused by *Rhizoctonia solani* in Saudi Arabia

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Eight antagonistic fungi; *Chaetomium* spp., *Aspergillus versicolar*, *A. terreus*, *Talaromyces* (*Penicillium*) *wortmanni*, *Epicoccum* sp., *Trichoderma viride*, *T. harzianum* and *T. hamatum* were isolated from sclerotia of *R. solani* infested tomatoes growing under greenhouse in Riyadh, Saudi Arabia. *T. hamatum*, *T. harzianum*, *T. viride* and *A. terreus* were the most antagonistic against *R. solani* by 94.44, 93.89, 92.22 and 75.22% reduction in mycelia growth, respectively. Where, *A. terreus* and *T. viride* were found to affect sclerotial viability of *R. solani*, caused 100% mortality of sclerotia. Greenhouse tests showed that the most effective treatment was the amendment of pathogen-infested soil by *T. hamatum* and *T. viride* which resulted in a disease severity of 12.50 and 12.90%, respectively compared to controls. Application of bioagents soil fungi significantly increased biomass of total fresh weight. The highest of biomass% (47.02) was observed in *T. harzianum* and the lowest biomass% (2.86) was obtained with *A. terreus*.

Key words: Stem canker, Rot root. Tomato, *Trichoderma* spp., *Aspergillus terreus*.

More than 152.96 million tons of tomatoes (*Lycopersicon esculentum*, L.) are produced worldwide on 4734356 hectares of land[1]. Tomato is a major food source and ranks fifth after wheat, barley, rice and maize [1]. About15.127 hectares are grown in Saudi Arabia, with an average yield of 35 tons per hectare [2]. *Rhizoctonia solani* Kühn AG-4 cause serious disease of some economic vegetables and crop [3]. It causes damping-off, stem canker and head rot on several vegetable crops. *R. solani* can survive as mycelium or sclerotia in the soil for a long period of time in the absence of host plants [4].

Growers rely upon the use of fungicides such as methyl bromide for control of *Rhizoctonia* diseases. However, methyl bromide has been regarded as ozone depleting material[5,6] and therefore, it is necessary to search other environmentally safe fumigant as alternatives to methyl bromide for controlling soilborne plant pathogens including *R. solani*. Biocontrol can be an effective means of control in many instances where chemical control is not available or practical[7]. Several microbial antagonists, some of which are available in commercial formulations, have shown potential for control of *R. solani* on tomato or other host crops. *T. harzianum* Rifai and *T. virens* have successfully suppressed *R. solani* in several pathosystems[8, 9].

Biological control of *Rhizoctonia* diseases has been demonstrated in some cases and represents an additional strategy that may provide effective and sustainable management. *Verticillium biguttatum*[5], *Bacillus subtilis*[4], *Pseudomonas fluorescens*[10,11], *Cladorrhinum foecundissimum*[8, 12], *Paenibacillus polymyxa*[13], have all shown some potential for disease control under the conditions studied. The main objective

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of this study was to evaluate the potential of antagonistic fungi to biologically control *R. solani*.

MATERIALS AND METHODS

Isolation and identification of isolates

Antagonistic fungi were isolated from sclerotia of *R. solani* on the root of tomato plants collected from Riyadh, Saudi Arabia. About one hundred sclerotia from each sample were divided into five sub-samples. Isolation of antagonistic fungi was carried out using the methods described by[14]. All antagonistic fungi and pathogenic fungus were identified in Mycology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt [15]. Single-spore isolates were transferred to PDA medium in tubes for preservation at 4°C. *R. solani* representing AG-4 [16] was isolated from root rot and stem canker of tomato.

Dual culture assay

Fungal antagonistic were examined for their ability to suppress the mycelial growth of *R. solani in vitro* dual culture assay on PDA [17]. The radial mycelial growth of *R. solani* against the antagonistic fungus (Ri) and that on a control plate (Rc) were measured after 2, 5 and 10 days, the mycelia growth inhibition was calculated according to the formula: (Rc-Ri)/Rc*100.

Interaction between antagonistic fungi and *R. solani*

Fungal interactions were studied in 7-day old *R. solani* cultures on 2% water agar (WA) in 9 cm Petri plates, which were inoculated with a 5 mm. disc removed from vigorously growing cultures on PDA of each antagonistic fungus. Plates were incubated at 25±2°C, After 2 weeks, 3 rectangular blocks (about 4x2cm.) from each plate were cut, mounted on glass slides, and examined for hyphal interaction between antagonistic fungi and *R. solani* by compound light microscope (400x).

Viability of Rhizoctonia solani-sclerotia inoculated with antagonistic fungi

Effect of antagonistic fungi on the germination of *R. solani* sclerotia was determined [18, 19]. For *R. solani* sclerotia production, plates were incubated (25 ± 2 °C; 8 h light) for 4 weeks to allow for sclerotia production. All sclerotia were manually harvested from plates using sterilized forceps. Sclerotia were dried overnight in a laminar flow hood [6]. Fifty sclerotia were used for each antagonistic fungus and control. Sclerotia viability index was calculated [19].

Effect of culture filtrates of antagonistic fungi on radial growth of *R. solani*

The effect of culture filtrates of selected antagonistic fungi on the radial growth of pathogen was determined by the addition of cell free culture filtrates on agar medium. For this 50 ml of potato dextrose broth was inoculated with 1 ml of spore suspension (10^5cfu/ml) of the selected isolates. Antagonistic fungi cells were harvested from stationary phase culture after 10 days. The culture filtrates were centrifuged at 10.000 rpm/min. The culture filtrates were sterilized by passing through a 0.22 ¼m filter. The filters were added to pre cooled potato dextrose agar medium before pouring into Petri plates. Each plate was inoculated with mycelia disc of the pathogen cut with a sterile cork borer (5mm. diameter). The inoculated plates were incubated at 25±2°C, 5 replicates were used for each treatment. The colony diameter in each concentration was recorded after 2, 3 and 5 days. The pathogen inoculated on PDA medium without any culture filtrate served as control.

Effect of antagonistic fungi on disease severity caused by *R. solani*

Fungal isolates were isolated from sclerotia of *R. solani* infested tomato growing under glasshouse, were from the collection of Riyadh, Saudi Arabia. Three, 5-mm-diameter agar disks of all isolates from the margin of actively growing cultures on PDA were transferred to a 1-liter flask containing 100 g of twice autoclaved wheat bran and 100 ml of deionized water. The cultures were grown for 10 days on the lab bench at approximately 25±2ºC then formulated separately into Pesta granules [20]. Sterilized plastic pots (25×30 cm.) were filled with 4 Kg of pasteurized soil mix (clay: sand: compost, 2:1:1). For application of antagonistic fungi, the pot mix was adjusted to approximately 65% moisture content then amended with 45 g of Pesta granules (8.52×10^5cfu/g) of antagonistic fungi. After 1 day from antagonistic application, *R. solani*, was inoculated in pots (1g inoculum/1kg soil (1×10^6 cfu/gm)).Two treatments were used as controls, transplanted tomato was left untreated by antagonistic fungi as an inoculated control and one treatment was absolute control (10 replicates/treatment). Severity of root rot and

stem canker was recorded after 4 weeks from inoculation[21]. Also, severity index was calculated[22].

Evaluation of plant growth-promoting effect of antagonists under greenhouse conditions

The bioagents were evaluated for their effect of plant growth promoting in the second greenhouse experiment [23]. Plant Height plants, root length, and fresh weights of whole plants were measured. Biomass increase was calculated, the following formula:

Biomass increase = [(fresh weights of plants treated with antagonist-fresh weights of control plants)/fresh weights of tomato plants] ×100%.

Statistical analysis

Data collected from all experiments were statistically analyzed using the Statistic Analysis System Package (SAS institute, Cary, NC, USA). Differences between treatments were studied using Fisher's Least Significant Difference (LSD) test and Duncan's Multiple Range Least [24]. All analysis were performed at P 5 % level.

RESULTS

Dual culture assay

Among the 8 fungal isolates isolated from Riyadh greenhouse *T. hamatum*, *T. harzianum* and *T. viride*, respectively, were the most effective

Table 1. Effect of some antagonistic fungi against *R. solani* (dual culture assay) and hyphal interaction.

		Days						
		2		5		10		Hyphal
	Treatments	R.G.	Inh.%	R.G.	Inh. %	R.G.	Inh.%	interaction
1.	*Chaetomium* spp.	4.28^{b}	9.90	4.33^{b}	50.96	4.88^{b}	45.78	+
2.	*A. versicolar*	3.80^{c}	20.00	4.25^{c}	51.87	4.13^{c}	54.11	+
3.	*A. terreus*	4.33^{b}	8.84	2.90^{d}	67.16	2.23^{d}	75.22	+
4.	*T. wortmanni*	3.83^{c}	19.37	4.38^{c}	50.40	4.80^{b}	46.67	+
5.	*Epicoccum spp.*	4.10^{bc}	13.86	4.35^{c}	50.74	4.08^{c}	54.67	-
6.	*T. viride*	2.65^{d}	44.21	2.23^{e}	74.75	0.70^{e}	92.22	+
7.	*T. harzianum*	2.88^{d}	39.37	2.10^{e}	76.22	0.55^{e}	93.89	+
8.	*T. hamatum*	1.80^{e}	62.11	1.78^{e}	79.84	0.50^{e}	94.44	+
	Control	4.75^{a}	0.00	8.83^{a}	0.00	9.00^{a}	0.00	
	LSD at 5%	0.35		0.62		0.31		

R.G. = radial growth (Cm.) Inh.%= inhibition%

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table 2. Viability of *R. solani*-sclerotia inoculated with antagonistic fungi.

	Treatments	Percentage of sclerotia according to the number of emerging hyphae					Viability index sclerotia
		0	1-5	6-10	11-25	>25	
9.	*Chaetomium spp.*	38	36	12	14	0	25.50
10.	*A. versicolar*	50	30	12	8	0	19.50
11.	*A. terreus*	100	0	0	0	0	0.00
12.	*T. wortmanni*	12	18	10	58	2	55.00
13.	*Epicoccum spp.*	50	20	14	14	0	22.50
14.	*T. viride*	100	0	0	0	0	0.00
15.	*T. harzianum*	80	14	6	0	0	6.50
16.	*T. hamatum*	90	4	4	0	0	3.00
	Control	4	4	6	20	66	85.00

Table 3. Effect of culture filtrates of antagonistic fungi on radial growth of *R. solani*

	Treatment	Days					
		2		3		5	
		R.G.	Inh. %	R.G.	Inh. %	R.G.	Inh. %
17.	*Chaetomium spp.*	2.76[a]	0.58	3.92[abc]	3.92	7.70[bc]	6.10
18.	*A. versicolar*	2.70[a]	2.74	3.82[abcd]	6.37	7.60[bc]	7.32
19.	*A. terreus*	2.64a[b]	4.90	3.76[abcd]	7.84	6.50[d]	20.73
20.	*T. wortmanni*	2.76[a]	0.58	4.00[ab]	1.96	7.90[ab]	3.66
21.	*Epicoccum spp.*	2.40c[d]	13.54	3.46[bcd]	15.20	6.60[d]	19.51
22.	*T. viride*	2.32[c]	16.43	3.26[d]	20.10	6.40[d]	21.95
23.	*T. harzianum*	2.32[c]	16.43	3.36c[d]	17.65	6.50[d]	20.73
24.	*T. hamatum*	2.40[cd]	13.54	3.46[abcd]	15.20	6.80[d]	17.07
	Control	2.78[a]	0.00	4.08[a]	0.00	8.20[a]	0.00
	LSD at 5%	0.26		0.56		0.45	

R.G. = radial growth (Cm.) Inh.%= inhibition%.

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

Table 4. Effect of antagonistic fungi on disease severity and growth of tomato infected with *R. solani*.

	Treatment	Disease severity%	Severity index	Plant height (cm.)	Root length (cm.)	Total fresh weight (kg)	Biomass %
25.	*Chaetomium spp.*	14.00[d]	14.00[d]	81.44[d]	17.70[b]	0.991[cde]	14.40[cde]
26.	*A. versicolar*	37.50[b]	36.00[a]	78.34[e]	15.10[c]	0.972[cde]	12.76[cde]
27.	*A. terreus*	18.00[cd]	28.76[c]	78.31e	14.58[c]	0.886[ed]	2.86[de]
28.	*T. wortmanni*	38.40[b]	30.44[c]	80.22[d]	17.50[b]	0.976[ecd]	12.60[e]
29.	*Epicoccum spp.*	24.00[c]	33.56[b]	83.28[c]	18.38[b]	1.010[cd]	16.92[cd]
30.	*T. viride*	12.90[d]	12.00[e]	87.30[b]	21.50[a]	1.160[ab]	34.26[ab]
31.	*T. harzianum*	14.80[d]	8.44[f]	89.58[a]	22.10[a]	1.270[a]	47.02[a]
32.	*T. hamatum*	12.50[d]	7.20[f]	86.00[b]	21.01[a]	1.070[bc]	30.32[bc]
	Non-infested Control	0.00[e]	0.00[g]	78.30[e]	14.20[c]	0.866[e]	0.00[f]
	Control	89.40[a]	0.00[g]	-	-	-	-
	LSD at 5%	6.86	1.80	1.42	1.68	0.13	14.88

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

antagonists against *R. solani* by 94.44, 93.89 and 92.22%, respectively (Table 1). These were followed by *A. terreus* giving 75.22% reduction in mycelia growth of *R. solani*. On the other hand, *Cheatomium* spp. and *T. wortmanni* were less effective against *R. solani*, with inhibition zones of 45.78 and 46.67%, respectively.

Fungal interaction between antagonistic fungi and *R. solani*

All fungi, after the coiling around of *R. solani*, hyphae penetrated host cell walls and grew within the hyphae (Table 1). While, *Epicoccum* sp. did not penetrated the hyphae of *R. solani*.

Viability of *R. solani*-sclerotia inoculated with antagonistic fungi

The effect of antagonistic fungi on sclerotia viability is presented in Table 2. Treatment of sclerotia by *A. terreus* and *T. viride* caused loss of viability of the sclerotia and no mycelia growth from these sclerotia (100% mortality). When the sclerotia were treated with *T. hamatum* and T. harzianum, the sclerotia remained viable and mycelial germination of the sclerotia were observed when they were transferred to the PDA medium free of antagonistic fungi giving 3.00 and 6.50 viability index of sclerotia, respectively while

control was 85.00. On the other hand, treatment of sclerotia with *T. wortmanni* and *Chaetomium* spp. gave the least result (55.00 and 25.50%) when compared with control.

Effect of culture filtrates of antagonistic fungi on radial growth of *R. solani*

Culture filtrates from *T. viride*, *T. harzianum* and *A. terreus* produced the highest inhibition for *R. solani* with 21.95 and 20.73% in mycelia growth. This was followed by *Epicoccum* spp. with 19.51% reduction in radial growth (Table 3). On the other hand, *T. wortmanni* produced the least reduction in mycelia growth by 3.66% when compared with control.

Effect of antagonistic fungi on disease severity caused by *R. solani*

All antagonistic fungi affected disease severity, *T. hamatum* and *T. viride* were the best antagonistic fungi for reducing disease severity of root rot and stem canker of tomato caused by *R. solani* with 12.50 and 12.90%, respectively when compared with controls (Table 4). This was followed by *Chaetomium* spp. that giving 14.00% disease severity. Conversely, *T. wortmanni* and *A. versicolar* were the lowest affecting disease severity with 38.40 and 37.50% disease severity, respectively.

Effect of antagonistic fungi on plant growth-promoting of tomato plants.

Data in Table 4 showed that all antagonistic fungi significantly ($P < 0.05$) increased biomass, indicating their growth-promoting effect on tomato. These bioagnts led to significantly higher fresh weights (ranging from 0.886 to 1.160 kg) than control (0.866 kg). *T. harzianum*, *T. viride* and *T. hamatum* resulted in significant increased in plant height, root length, and fresh weight compared to control. *T. harzianum*, *T. viride* and *T. hamatum* increased biomass by 47.02, 34.26 and 30.32%, respectively.

DISCUSSION

In Saudi Arabia, several vegetable crops suffer major losses due to *R. solani*. *R. solani* survives in soil by means of melanized mycelium and sclerotia [25, 26]. Sclerotia remain viable for several years and are considered as an important source of primary infection. In our study, it was noticed that *T. hamatum*, *T. harzianum* and *T. viride*, were the most effective antagonists fungi against mycelia growth and sclerotia viability of *R. solani* (Table1-3). These findings are consistent with [9, 13, 27, 28]. Who proved that the *Trichoderma* spp. as bioagents against *R. solani* in different crops. This highly antifungal activity of *Trichoderma* spp. probably related to some mode of actions. Antibiotic production, mycoparasitism and the production of cell wall-degrading enzymes are considered as the actions involved in biocontrol of pathogen [29, 30, 31]. During direct contact, lectins in the host's cell wall can induce coiling of the Trichoderma around the host hyphae and mycoparasite can produce appressorium-like structures to destroy the pathogen [30]. According to Zeilinger and Omann [30], enzymes production and infection structure formation are induced responses activated by the diffusible factor. Many interactions of Trichoderma with plant pathogens, such as Trichoderma had grown parallel to pathogen, grown along the pathogen and grown around the pathogen [32]. Normal degradation of pathogen mycelia would take place after penetrating with appressorium-like structures and they had observed the growth of *Trichoderma* hyphae within the pathogen [32].

Under greenhouse study Trichoderma formulation (Pesta) treatment controlled *R. solani*, severity of the infection was reduced and improved the plant growth. Growth improvement of plants could be due to the synergistic activity of *Trichoderma* spp. on host plant. Trich*o*derma spp. might have colonized around the root and increased the root biomass and helped to increase the availability of nutrients. Biocontrol agents might have interacted with the plant for exchange metabolites and that could cause significant changes in plant metabolism [31]. When *Trichoderma* spp. colonizes plant roots, they invade only in the surface layers of the root, further penetration can be controlled by the plant defense reactions. Therefore, Trichoderma spp. is usually a virulent irrespective of their intrinsic ability to attack plants [33]. Even though some *Trichoderma* spp. grows only on roots, the plant defense reactions can become systemic and protect the entire plant from a range of pathogens and diseases. Besides, the root colonization increases the growth of the entire plant and thus results in an increase in plant biomass. Symbiotic association with rhizosphere

of the plant helps to surmount abiotic stresses and improve nutrient uptake [33].

Also, it was found that *Trichodema* spp. as plant growth promoting, giving the tallest plants, roots, and giving the highest total fresh weight and biomass%. The secondary metabolites such as auxin like compounds (Harzianic acid) or auxin inducing substances by Trichoderma plant interaction might be a reason for the improved growth [34]. Thus, nitrogen uptake, growth and yield response of crop plants were positively influenced by Trichoderma treatment [34] and *Trichoderma* spp. could solubilized of phosphates and micronutrients which affected plant-growth promoting [3].

A. terreus was established to affect sclerotial viability when sclerotia of the pathogen were treated with conidia of the hyperparasite. The fungus caused 100% mortality of sclerotia. *A. terreus* was able to colonize sclerotia of *R. solani*. Plasmolysis was evident in some of the host cells infected by *A. terreus*. *A. terreus* parasitized sclerotia, which hyphae of the antagonist grew abundantly on the sclerotial surface, forming dense forest of conidiophores. At a more advanced stage of parasitism, *A. terreus* completely destroyed the sclerotial cells and penetrated and collapsed the modularly tissue [35]. For a mycoparasite to be considered a successful biocontrol agent, it should be effective against resistant survival structures of plant pathogens [14]. These results herein obtained showed that *A. terreus* was able to exploit sclerotial tissue of *R. solani*. *A. terreus* occurs in tropical and subtropical zones and has a worldwide distribution on different soil. One very common habitat is the rhizosphere of plants [36]. *A. terreus* was found to be the most strongly cellulolytic species of the genus [37]. This fungus produces a large number of specific metabolites, including the nephrotoxin citrinin, the neurotoxins citroviridin, patulin, terrain, terreic acid and geodin [36] and several other compounds.

In our study, we noticed *Chaetomium* spp. that gave moderate inhibition in growth of *R. solani*. This result agrees with [38] who found that *Chaetomium* spp. could suppressed the mycelia growth of some pathogenic fungi. The ability of *Chaetomium* spp. to produce the antibiotic chaetomin (an epithiodiketopiperazine) in liquid culture was correlated with efficacy to suppress *Pythium* damping-off of sugar beet in heat-pasteurized soil [38]. This further supports the suggestion that chaetomin production in soil plays an important role in antagonism of *C. globosum* against *P. ultimum*. Four strains of *C. globosum* produced inhibition zones in Cochliobolus sativus cultures [39], and that the production of antifungal compounds by the isolates was positively correlated with the antagonism against *C. sativus* on wheat plants. Also, chaetoviridins A and B play an important role in the antagonism of *C. globosum* against several plant pathogens [11]. The formulation (Pesta) of *Chaetomium* spp. decreased disease severity with 14.00% and increased the biomass by 14.40%. This result is similarly reported by [40] who stated that registered bio-fungicide formulated from *C. cupreum* could decrease disease incidence of tomato wilt and also increase its yield. The result from bio-agent formulation of *Chaetomium* spp. in this study is also supported by [34]. As a result of bio-agent formulation *Chaetomium* sp. supported the previous work of [41] which showed that *Chaetomium* bio-products formulated from *C. globosum* and *C. cupreum* as powder formulation could control bud rot and basal stem rot of bottle palms caused by Thielaviopsis paradoxa in the field and reduce disease incidence by 75%. As seen in the report of [41] that showed that the biological products consist of *Chaetomium* sp. in biopellet and biopowder formulations which when applied to the soil could suppress the growth of *F. oxysporum* f. sp. *lycopersici* and reduce infection rate in tomato.

CONCLUSION

Treatment with single isolates *Trichoderma* species and *A. terreus* reduced disease progress to different extents while the treatment with *A. vesciolar* and *T. wortmanni* produced the lowest levels of disease progress.

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