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Biological Control of Fungal Plant Pathogens Using *Trichoderma harzanium* and *Bacillius subtilis* on Chickpea

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ABSTRACT

The pathogenic effect of *Machrophomina spp, Fusarium spp, Rhizoctonia* and *Sclerotium spp* on chickpea plants is high. The most virulent fungi are *F. oxysporum* and *M. phaseolina* followed by *S. rolfsii* and *R. solani*. Laboratory experiments revealed that using biological controls inhibits the growth of mycelium. In addition, when *T. harzianum* and *B. subtilis* were used instead it was found that they could control the pathogens, whereas the total dissolved sugars and phenols in the plant roots increased and lignin decreased. The use of biological controls increases the percentage of surviving plants and the increased nitrogen fixation increases the number of root nodes and decreases the degree of infection. Also, it is found that the treatment with harzianum fungus led to larger significant increase than the treatment where the subtilis.

INTRODUCTION

Pesticides used to treat fungi also affect humans and animals (Laws 1993), and their effect is known since 1945. In the present study, we look for alternative methods to treat plant diseases without any side effects. We discuss the use of environmentally friendly antifungal organisms that do not require highly specialized preparation methods, as is the case for chemical pesticides. Chickpea plants were infected using pathogenic fungi, such as *F. ozysporum*, *M. phaseolinea*, *R. solani* and *S. rolfsi* (see also Tabosa et al. 1989; El-Far 1998). Hwang and Chang (1989) stated that *Fusarium ozysporum* and *R. solani* are the most common pathogenic fungi found in the infected roots of plants. Laboratory studies suggest that *B. subtilis* and *T. harzianum* reduce the growth of mycelium colonies. Aydın *et al.* (2009) argued in favour of the antibacterial properties of these organisms in fungus-infected soil and concluded that the use of *T. harzianum* produced better results than *B. subtilis* and both produced results superior to the control treatment.

MATERIALS AND METHODS

1. Fungal infection and pathogenicity

Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia solani and *Sclerotium rolfsii* were purchased in testing tubes and incubated 15 days at 30 °C. Then, each species was added to well- mixed and sterilized soil. Subsequently, the soil was mixed with water, left undisturbed for seven days and then packed in polyethylene bags that were 17 cm in diameter and were immersed in 40% formaldehyde for 15 min. Each bag contained one fungal species and chickpea (*Cicer arietinum*) seeds. We examined soil samples after 30, 60 and 90 days following the procedures in Singh *et al* (2007).

2. *Trichoderma harzianum* and *Bacillus subtilis* effect on pathogenic fungi growth

The fungal pathogens were grown in potato dextrose agar (PDA) in petri dishes and incubated for 15 days at 25°C. In addition, *T. harzianum* and *B. subtilis* suspensions were added to the PDA in the petri dishes. Disks 4 mm in diameter of the fungal pathogens were cut and nursed at 28°C in three dishes, as replicates. The fungal growth in each dish was checked after 3, 5, 7 and 9 days (Pant *et al.* 2004).

3. Plant resistance

The soil was prepared, sterilized and infected by pathogens before bagged into four bags, one bag for each pathogen. Chickpea (*Cicer arietinum*) seeds were planted in each bag; one set had been soaked in the *B. subtilis* suspension and the other in the *T. harzianum* suspension for at least 20 h and left to dry. Chickpea seeds that did not undergo viral treatment were the control samples (Siddiqui 1993).

To evaluate the effect of bacterial and fungal suspension on the root rot of the chickpea plants, field conditions were replicated in the laboratory. The plants were examined after 30, 60 and 90 days of planting. All dead plants were removed and the percentage of live plants was estimated. Moreover, the degree of plant infection and number of root nodes were evaluated after 60 and 90 days, whereas the plant height and dry mass of seeds were measured after 90 days.

RESULTS

1. Fungal pathogenicity

The effect of the four fungi on *Cicer arietinum* was evaluated by enumerating the dead plants in the bags. From the data in Table 1, it is clear that the fungal pathogenicity is high despite variation in the percentages of dead plants with time and fungal species.

2. Mycelium growth in soil samples

The fungus-infected and seeded soil samples were treated with *harzianum* and *subtilis*. The data are given in Table 2 and shown and suggest the following. After three days, the treatment affected the mycelium growth of the target fungi. After five days, the growth area in all the treated fungi dishes had increased but did not exceed 1.01 cm^2 in dishes infected by *M. phaseolina* and treated with *B. subtilis*. After seven days, the growth area in dishes infected using *M. phaseolina* and treated with *B. subtilis* did not exceed 1.19 cm^2. Apparently, the treatment most affected the *M. phaseolina* fungus. After nine days, the fungi in the untreated dishes had covered the entire dish area, whereas this is not the case for the treated samples; furthermore, *B. subtilis* restricted the growth of *M. phaseolina* growth to 1.29 cm^2.

3. Plant survival and treatment

From Table (3), it is seen that after 30, 60 and 90 days the percentage of surviving chickpea plants is higher in the treated soil samples than in the control soil samples. Furthermore, *B. subtilis* is quite successful in increasing the percentage of surviving plants in fungus-infected soil after 30 days and less so after 60 and 90 days. *T. harzianum* is also highly successful but the percentage of plants surviving the fungal infection after 30, 60 and 90 days is variable.

4. Long-term behaviour of treated chickpea plants in fungus-infected soil

It is clear from Table 4 that *B. subtilis* and *T. harzianum* minimize the degree of infection relative to the control samples after 60 days, with *B. subtilis* offering improved protection to the chickpea plants in the fungus-infected soil.

After 60 and 90 days (Table 4 and 5), the roots of chickpea plants show increased rot compared to the control samples. The level of protection offered by *B. subtilis* and *T. harzianum* is similar.

The plant height and mass of dry roots in soils infected by fungi and treated by *B. subtilis* and *T. harzianum* is higher than that in the control samples. This applies to the 60- and 90-day observations. Similarly to the case of root rot, the level of protection offered by *B. subtilis* and *T. harzianum*, as exemplified by the similar plant heights and dry root masses, is equivalent. Note, however, that the dry root masses of plants treated with *B. subtilis* and infected by *M. phaseolina* are nearly 50% higher than the rest after 60 and 90 days.

To evaluate seed quality, we looked at the mass of 100 seeds in fungus-infected soils after 90 days (Table 5). The mass of 100 seeds treated with *B. subtilis* and *T. harzianum* is substantially higher than the control samples; the highest difference is been in soils infected by *F. oxysporum*.

Finally, we examined the total dissolved sugars, phenols and lignin in the roots of the chickpea plants (Table 6). The total dissolved sugars in the roots of chickpea plants were higher than in the control samples. The treatment with *B. subtilis* and *T. harzianum* has produced differences in the total dissolved sugars; the highest value was recorded in the plants treated with *T. harzianum* and infected by *M. phaseolina*. Similarly to sugars, the phenols are high in the treated samples. The higher values are observed in the samples treated with *T. harzianum* regardless of fungal species infection. In contrast, lignin is higher in the control samples than in the treated samples.

DISCUSSION

The fungal species used in this study cause the roots of the chickpea plants to rot, an observation congruous with previous studies (e.g. El-far 1998; Tabosa 1989). Furthermore, organisms, such as *T. harzianum* and *B. subtilis* reduce the growth of the mycelium in the fungal species used and increase the plant survival rate in fungus-infected soil samples. Moreover, *harzianum* is more effective than the *subtilis* in treating plants in fungus-infected soils. The treated plants exhibited increased root nodes owing to increased nitrogen fixation (see also Mahmood and Siddiqui 1995). Pant *et al.* (2004) found that there were fewer pathogens in the rhizosphere after treating the plants with *T. harzianum*. Furthermore, these authors also associated the increased nitrogen fixation with the increased number of root nodes in each plant and found that the total dissolved sugars in the roots of the chickpea plants were higher for *T. harzianum* than for *B. subtilis*. Finally, the phenolic compounds and lignin were higher in the plants treated with both *T. harzianum* and *B. subtilis*.

Table 1: Fungal pathogenicity

Fungus	Dead chickpea plants (%)		
	After 30 days	After 60 days	After 90 days
F. oxysporum	14.5	59.3	67.6
M. phaseolina	24.5	51.2	35.1
R. solani	13.5	43.8	52
S. rolfsii	26	34.3	55

Table (2): Treatment and fungal growth

Treatments	Third day					Fifth day					Seventh day					Nineth day				
	F. oxysporum	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages
B. subtilis	2..22	0.87	4.59	7.01	3.69	7.46	1.01	12.56	25.74	11.69	10.89	1.19	12.56	34.9	14.89	13.66	1.29	12.59	34.9	15.6
T. harzianum	2.22	3.14	4.34	7.1	4.2	8.01	7.07	10.28	24.44	12.45	11.27	12.56	14.55	29.59	16.99	14.95	19.63	14.55	33.55	20.67
Control	0.87	1.77	4.9	7.88	3.86	10.49	7.07	19.63	34.8	17.99	17.89	38.47	63.59	46.15	41.52	63.59	63.59	63.59	63.59	63.59
Averages	1.77	1.92	4.61	7.35		8.65	5.05	14.15	28.33		13.35	17.41	30.23	36.88		30.23	30.23	30.23	44.02	

5% value of L.S.D.:

	Third day	Fifth day	Seventh day	Nineth day
For fungi:	0.51	3.51	4.02	3.52 .52
For treatments:	0.44	3.04	3.48	3.05
For treatments X fungi treatments:	0.88	6.07	6.96	6.09

Table (3): Plant survival rate and treatment of fungus-infected soils

Treatments	**The planting percentage after 30 days**				**The percentage of the remaining plants after 60 days of the agriculture**				**The percentage of the whole remaining plants after 90 days of the agriculture**			
	F. oxysporum	*M. phaseaolina*	*R. solani*	*S. rolfsii*	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*
B. subtilis	100	87.5	100	100	87.5	71.	75	75	57.1	60	50	50
T. harzianum	87.5	75	75	62.5	75.1	66.	66.6	60	100	75	75	66.6
Control	87.5	75	87.	75	42	50	57.1	66.6	33.3	66.6	50	50

Table (4): Degree of infection, root rot, plant height and dry root mass in fungus-infected and treated soils after 60 days

Treatments	The rate of injury degree					The number of nodes for each plant					The plant height (cm)					The weight of the dry roots				
	F. oxysporum	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages
B. subtilis	51.6	51.1	41.5	50.1	48.7	8	8	11	8	8.7	18.2	17.3	18	14.8	17	0.30	0.61	0.32	0.36	0.39
T. harzianum	66.4	58.5	58.6	66.7	62.5	5	9	10	8	8	18	14.5	15	13.3	15.2	0.37	0.30	0.26	0.22	0.28
Control	70.3	65.1	65.1	75	68.9	3	3	4	4	4.6	13.5	12	10	10.5	11.5	0.08	0.09	0.10	0.04	0.07
Averages	62.7	55	55			5.3	6.6	8.3	6.6		16.5	14.6	14.3	12.8		0.25	0.33	0.22	0.2	

5% value of L.S.D.:	The rate of injury degree	The number of nodes for each plant	The plant height (cm)	The weight of the dry roots
For fungi:	5	1.6	2	0.02
For treatments:	4.3	1.4	1.7	0.03
For treatments X fungi:	8.7	2.8	3.4	0.04

Table (5): The effect of using two www woo the vital resistance ways on some measurements of chickpeas product after 90 days of the agriculture in soil prepared with four tested fungi

Treatments	The rate of injury degree					The number of rot roots for each plant					The plant height (cm)					The weight of the dry roots					The weight of 100 seeds (gram)				
	F. oxysporum	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages
B. subtilis	54.1	59.9	47.9	51	53	8.5	8.2	9	8	8.4	20.1	20.2	20.5	18.2	19.7	0.37	0.53	0.34	0.39	0.4	105	50	49	90	73.5
T. harzianum	51.6	47.9	43.2	40	45	10	9.5	10	9.2	9.6	23.9	24.4	23.9	23.1	23.8	0.47	0.58	0.53	0.48	0.51	106	76	67	59	77
Control	73	70.2	70.1	77	72	3.5	3.7	3.5	5	4.2	14	12.2	10.7	12.3	12.3	0.09	0.12	0.16	0.12	0.12	23	25	15	13	19
Averages	59.5	59.3	53.7	57 56		7.3	7.1	7.3	7.4		19.3	18.9	18.3	17.8		0.31	0.41	0.34	0.33		78	50.3	43.6	54	

5% value of L.S.D.:

For fungi:	5.2	1	1.6	0.05	14
For treatments:	4.5	0.8	1.4	0.04	12.2
For fungi X treatments:	8.9	1.7	2.9	0.09	24.3

Table (6): The effect of using two ways of the antimicrobial on the content of chickpeas roots of some vital compounds in a soil infected by tested fungi

Treatments	Totally dissolved sugars (milligram- glucose)					Whole phenolic (milligram- glucose)					Lignin (milligram- glucose)				
	F. oxysporum	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages	*F. oxysporum*	*M. phaseaolina*	*R. solani*	*S. rolfsii*	Averages
B. subtilis	0.59	0.45	0.57	0.51	0.53	0.48	0.57	0.67	0.71	0.61	0.44	0.47	0.47	0.53	0.49
T. harzianum	0.73	0.75	0.55	0.69	0.68	0.85	0.83	0.79	0.64	0.78	0.39	0.35	0.35	0.48	0.43
Control	0.04	0.03	0.02	0.05	0	0.06	0.08	0.08	0.07	0.07	0.67	0.56	0.56	0.66	0.59
Averages	0.45	0.41	0.38			0.46	0.49	0.51			0.5	0.46	0.46	0.56	

5% value of L.S.D.:			
For fungi:	0.08	0.07	.00.09
For treatments:	0.07	0.06	0.08
For treatments X fungi	0.	0.11	0.14

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