Research Journal of Agriculture and Biological Sciences, 2(4): 147-152, 2006

Use of Chitin and Chitosan Against Tomato Root Rot Disease under Greenhouse Conditions

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Abstract: Root rot caused by *Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* is the most destructive disease of tomato plants. Effect of chitin and chitosan on root rot pathogens as well as their influence on soil microflora and tomato root rot disease incidence under laboratory and greenhouse conditions were studied. Chitosan at 6 g/l completely inhibited the linear growth of all tomato root rot fungi tested, while chitin has no inhibitory effect. Tomato root rot fungi were highly affected by the presence of chitin or chitosan in the soil. Their counts were decreased throughout the experiment period comparing with untreated soil. Effects of chitin on fungal counts increased gradually by prolonging the experiment period from 15 up to 45 days, while the opposite feature was observed with chitosan treatment. As for chytinolytic bacteria and actinomycetes, all concentrations of both chitin or chitosan caused high increase in total count of chitinolytic bacteria. Bacterial counts were greater in all concentrations of chitin and chitin plus chitosan than chitosan only. The bacterial counts were increased as the period of experiment increased to reach its maximum after 45 days of treatments. It was noticed that *Bacillus* spp. was the most dominant genus of isolated bacteria. Meanwhile, actinomyces was not affected with all treatments for all periods. All treatments increased the chitinase activity in tomato plants. The most effective treatment was chitin plus chitosan (6 g/kg soil), which increased the activity of enzyme more than 148% as compared with untreated plants followed by chitin or chitosan individually at 6 g/kg soil, which increased the chitinase activity more than 100%. Under greenhouse greenhouse conditions, all concentrations of chitin or chitosan were significantly reduced the tomato root rot incidence comparing with untreated plants. The highest reduction was obtained with chitosan or chitin at 6 g/kg soil as single treatments which reduced the disease incidence more than 88.0, 86.2 and 83.1% in infested soil with *R. solani*, *F. solani* and *S. rolfsii,* respectively. Combination between chitin followed by chitosan (6 g/kg soil of both) reduced the disease incidence by 94.4, 92.9 and 87.2% in infested soil with *R. solani*, *F. solani* and *S. rolfsii,* respectively. Increasing concentrations of either chitin or chitosan in combination cause more reduction in disease incidence. It could be suggested that combined treatments between chitin and chitosan might be used commercially for controlling tomato root rot diseases under field conditions.

Key words: Chitin, Chitinase, Chitosan, Microflora, Root rot, tomato

INTRODUCTION

Tomato plants is the one of most important vegetable crops in Egypt and other countries. Root rot disease caused by *Rhizoctonia solani* Kuhu.; *Fusarium solani* (Mart) Sacc. and *Sclerotium rolfsii* Sacc. is the most destructive disease of tomato[1,2]. Controlling such diseases mainly depend on fungicides treatments[3]. However, fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternative treatments for control of plant diseases are needed[4]. Chitin was reported as resistance inducer against soilborne diseases[5-7].

Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants[6-8]. Furthermore, chitosan is a safe material which has antifungal activity against many plant pathogens[9,10] Chitosan is a non-toxic compound was reported to induce resistance against soilborne fungi[11-14]. Field application of Chitosan for inducing resistance against late and early blight diseases of potato and root rot disease of lupin plants was also reported by Abd-El-Kareem *et.al*.,[15-17]. Several studies have demonstrated that over expression of chitinases and *B*-1,3-glucanase in plants is associated with enhanced resistance to various fungal pathogens[18-21].

The purpose of the present work was designed to evaluate the effect of chitin and chitosan singly or in combined treatments on the growth of tomato root rot pathogens as well as their effect on soil microflora and tomato root rot disease incidence under greenhouse conditions.

MATERIALS AND METHODS

Source of pathogenic fungi and tomato seeds: Tomato pathogenic fungi, *i.e. R. solani; F. solani* and *S. rolfsii* as

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the causal agents of tomato root rot diseases were kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt. Meanwhile, tomato seeds cv. Kastel rock were obtained from Vegetable Crops Research Department, Agricultural Research Centre, Giza, Egypt.

Effect of chitin and chitosan on the growth of tomato root rot fungi: The inhibitory effect of chitin and chitosan (Sigma company) against tomato root rot fungi was tested *in Vitro* at four concentrations, *i.e.* 0, 2, 4 and 6 g/l. Chitin or chitosan were added to conical flasks containing sterilized PDA medium before its solidifying and rotated gently then disbanded into sterilized Petri-plates (9 cm diameter). Plates were individually inoculated at the centre with equal disks (6 mm diameter) taken from 10 days old cultures o f each *F. solani; R. solani* and *S. rolfsii*, then incubated at 25±2°C. Linear growth of tested fungi was measured when the control plates (medium free of Chitin or chitosan) reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

Effect of chitin and chitosan as soil amendment on total count of pathogenic fungi, actionmyces and chitinolytic bacteria: The influence of chitin and chitosan on some soil microflora was evaluated at four concentrations, *i.e.* 0, 2, 4 and 6 g/kg soil in addition to combined treatments between chitin and chitosan at 6 g/kg (of each) were mixed individually with previously infested soil with *R. solani*, *F. solani* or *S. rolfsii*. Plastic pots (30 cm diameter) were filled with treated infested soils, then tomato transplants cv. Kastel rock were planted. The rhizospheric microflora as a total count of pathogenic fungi, actinomycetes and chytinolytic bacteria after 15, 30 and 45 days of transplant date were estimated according to methods of Lauw and Webly[22] and Kobayashi *et.al.,*[23].

Effect of chitin and/or chitosan as soil amendments on chitinase activity of tomato plants: The same abovementioned treatments of chitin or chitosan were evaluated for their effect on chitinase activity of tomato plants as follows:

Extraction of enzyme: Chitinase activity was determined after 10 days of seedlings planted. The enzyme extracted from tomato plants and the supernatant was prepared according to method of Tuzun *et.al*.,[24]. The chitinase activity was determined by colourimetric method of Boller and Mauch[25]. Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

Effect of chitin and chitosan on tomato root rot disease under greenhouse conditions

Soil infestation with pathogenic fungi: One pathogenic isolate of each *R. solani; F. solani* and *S. rolfsii* was grown on sandy–barley medium (1:1 w:w and 40% water) for 20 days at $25^{\pm 2}$ °C. Natural sandy loam field soils were artificially infested individually with prepared fungal inoculum at the rate 3% of soil weight, then mixed thoroughly.

Soil amendments with chitin and chitosan: Chitin or chitosan at four concentrations, *i.e.* 0, 2, 4 and 6 g/kg soil were tested against tomato root rot incidence. Previously infested soil was mixed individually with each concentration tested of chitin or chitosan.

Soil amendments with combined treatments between chitin and chitosan: Infested soils with tomato root rot pathogens were mixed individually with chitin at concentrations of 0, 3 or 6 g/kg soil. Ten days later chitosan was added in alternative order to the same soil at the same concentrations.

Treated infested soils were poured into plastic pots (30 cm diameter) and irrigated every other day for ten days. Tomato transplants cv. Kastel rock were then planted at the rate of 6 transplants/pot and 6 pots/treatment were used. The percentage of tomato root rot incidence was recorded up to 50 days of transplanting date.

Statistical analysis: Tukey test for multiple comparisons among means was utilized[26].

RESULTS AND DISCUSSIONS

Effect of chitin and chitosan on the growth of tomato root rot fungi: The inhibitory effect of chitin and chitosan on the growth of tomato root rot fungi, i.e. *F. solani; R. solani* and *S. rolfsii* was tested. Results in Table 1 indicate that chitin has no inhibitory effect on the growth of all tested fungi. On the other hand, all concentrations of chitosan had significant inhibitory effect against tested fungi. Chitosan at 6 g/l completely inhibit the linear growth of all tomato root rot fungi. The

Table 1: Linear growth of tomato root rot fungi as affected by different concentrations of chitin or chitosan

Treatment	Concentration (g/ kg soil)	Average linear growth (mm)		
		R. solani	*F. solani*	*S. rolfsii*
Chitin	2	90.0a	90.0a	90.0a
	4	90.0a	90.0a	90.0a
	6	90.0a	90.0a	90.0a
Chitosan	2	44.0b	50.0b	62.0b
	4	23.0c	30.0c	36.0c
	6	0.0d	0.0d	0.0d
Untreated	0	90.0a	90.0a	90.0a

Figures with the same letter are not significantly different (P= 0.05)

Table 2: Effect of single or combined treatment of chitin and chitosan on reduction (%) in total counts of pathogenic fungi in soil.

		Reduction in total count of pathogenic fungi %								
		R . solani			*F. solani*			*S. rolfsii*		
		Days after treatment								
Treatment	Concentration (g/kg soil)	15	30	45	15	30	45	15	30	45
Chitin	2	10.5	20.4	30.5	8.4	17.5	32	10.2	12.4	25.2
Chitin	4	23.4	45.6	65	20.3	43.2	60	8.5	40	50
Chitin	6	24.7	50.4	63	19.5	40.2	63	12.0	33	55
Chitosan	2	50	45	40.5	40	35.5	32	28.0	28	24
Chitosan	4	70	65	60	65.2	58	54	55.5	50.2	52
Chitosan	6	75.5	75.5	65	80	78	68	64.2	60.5	63
Chitin+chitosan	6 +6	75	80	80	70.5	70.5	80	65.0	64	70

Table 3: Increase (%) in total count of chytinolytic bacteria and actinomyses in the rhizosphere of tomato plants in response to single or combined treatment of chitin and chitosan.

		Increase %					
		Actinomycetes			Chitinolytic bacteria		
		Days after treatment					
Treatment	Concentration (g/ kg soil)	15	30	45	15	30	45
Chitin	2	0	0.0	0.0	10.5	30	43
	4	0.0	2.0	2.0	15.4	52	62
	6	0.0	0.0	2.0	20	55	66
Chitosan	2	0.0	0.0	2.0	0	10	12
	4	0.0	0.0	0.0	10	20	20
	6	0.0	0.0	0.0	8	22	19
Citin + chitosan	6+6	0.0	2.0	2.0	12	53	60

moderate effect was obtained with chitosan at 4 g/l which reduced the linear growth more than 60.0% for all tested fungi as compared with untreated fungal growth medium. Meanwhile, chitosan at 2 g/l was less effective in this concern.

Effect of chitin and chitosan as soil amendment on total count of pathogenic fungi, actionmyces and chitinolytic bacteria

Effect on tomato root rot pathogenic fungi: Results in Table 2 indicate that tomato root rot fungi were highly affected by the presence of chitin or chitosan in soil, whereas their counts decreased throughout the experimental periods comparing with untreated soil. It was noticed that the influence of chitin treatment on fungal counts increased gradually by prolonging the experimental period from 15 up to 45 days, while the opposite trend was observed with chitosan treatment. Chitin treatments caused slight reduction after 15 days and increased as the experimental period increased for all fungi. While, chitosan caused higher reduction in total count of fungi after 15 days of treatments and then decreased as the period increased.

Effect on chitinolytic bacteria and actinomycetes: Results in Table 3 indicate that, all concentrations of both chitin or chitosan caused high increase in total count of chitinolytic bacteria. Bacterial counts were greater in all concentrations of chitin and (chitin+ chitosan) than chitosan only. The bacterial count was increased as the period of experiment increased to reach its maximum after 45 days of treatments. It noticed that *Bacillus* spp. was the most dominant genus of isolated bacteria. Meanwhile, actinomyces was not affected with all treatments at all experimental periods.

Effect of chitin and/or chitosan as soil amendments on chitinase activity of tomato plants: Results in Table 4 indicate that all treatments increased the chitinase activity. The most effective treatment was chitin plus

Table 4: Chitinase activity of tomato plants as affected by grown in soil treated with different concentrations of chitin and chitosan under greenhouse conditions

		Soil infestation					
		R. solani		*F.solani*		*S. rolfsii*	
Treatment	Concentration (g/kg soil)	Chitinase activity	Increase (%)	Chitinase activity	Increase (%)	Chitinase activity	Increase (%)
Chitin	2	4	60	5	100	4.5	80
	4	4.2	68	5.2	108	5.0	100
	6	5.4	116	5.5	120	5.0	100
Chitosan	2	4	60	4.5	80	4.2	68
	4	4.5	80	5	100	4.3	72
	6	5	100	5.5	120	5.5	120
Chitin+chitisan	6+6	6.2	148	6.5	160	6.3	152
untreated	0	2.5	-	2.5	-	2.5	-

Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes

shitosan (6 g/kg soil), which increased the activity of enzyme between 148 and 160% as compared with untreated plants, followed by chitin or chitosan individually at 6 g/kg soil, which increased the chitinase activity more than 100%, while other treatments have moderate effect.

Effect of chitin and chitosan on tomato root rot disease under greenhouse conditions: Results in Table 5 indicate that all concentrations of chitin or chitosan significantly reduced the tomato root rot incidence comparing with untreated soil. Data also, show that the percentage of root rot incidence decreased significantly by increasing concentrations of either chitin or chitosan. The highest reduction was obtained with chitosan or chitin was at 6 g/kg soil treatment which reduced the disease incidence more than 88.0, 86.2 and 83.1% in infested soil with *R. solani*, *F. solani* and *S. rolfsii,* respectively followed by concentration at 4 g/kg of both which reduced the disease incidence more than 74.6% as compared with untreated plants. Meanwhile chitin or chitosan at 2 g/kg showed moderate effect.

Effects of combined treatments between chitin and chitosan as soil amendments for controlling tomato root rot under greenhouse conditions: Results in Table 6 indicate that significant reduction was observed for tomato root rot incidence when soil was treated with both or each of chitin and chitosan. Combination treatment as chitin followed by chitosan (6 g/kg soil for each) reduced the disease incidence by 94.4, 92.9 and 87.2% in infested soil with *R. solani*, *F. solani* and *S. rolfsii,* respectively. Increasing concentrations of either chitin or chitosan in combination cause more reduction in disease incidence. Combined treatments between chitin at 3 or 6 and chitosan at 6 or 3 g/kg soil, respectively reduced the root rot disease more than 75.8% for all tested fungi. Meanwhile single treatment showed moderate effect.

Table 5: Root rot incidence in tomato plants as affected by different concentrations of chitin or chitosan as soil amendments under greenhouse conditions.

		Tomato root rot incidence %		
Treatment	Concentration (g/ kg soil)	*R. solani*	*F. solani*	*S. rolfsii*
Chitin	2	24.5b	23.4b	35.5b
	4	12.0c	15.0 c	19.2c
	6	8.0c	8.8d	13.5d
Chitosan	2	22.0b	26.0b	35.5b
	4	11.5c	16. 5c	17.0c
	6	8.4c	9.0d	11.5d
Untreated	0.0	70.0 a	65.0a	80.1a

Figures with the same letter are not significantly different (P= 0.05)

Table 6: Effect of combined treatment between chitin and chitosan as soil amendments on tomato root rot diseases under greenhouse conditions

Treatment				
		Root rot diseases %		
Chitin (g/kg soil)	Chitosan (g/kg soil)	*R. solani*	*F. solani*	*S. rolfsii*
Chitin 3	0	25.0b	23.0b	32.0b
	3	16.2c	13.0c	22.0c
	6	10.0d	9.0cd	14.0d
Chitin 6	0	12.5cd	14.0c	21c
	3	10.5d	9.0cd	17.0d
	6	4.5e	5.0d	9.0e
0.0	0	80.0a	70.5a	70.4a
	3	24.4b	20.0b	30.0b
	6	10.2d	12.0c	18.0cd

Figures with the same letter are not significantly different (P= 0.05)

Tomato plants is the one of most important vegetable crops overall the world which seriously attacked by soilborne fungi, *i.e. Rhizoctonia solani, Fusarium solani*

and *Sclerotium rolfsii* inducing root rot diseases. Some natural and safe alternatives have been reported for replacing usages fungicides for controlling such diseases. In this regards, Chitin and chitosan the safe materials which were reported to induce resistance against soilborne diseases[6-8,14]. In the present study, under *in vitro* conditions chitin at concentrations of 2, 4 and 6 g/l showed no inhibitory effect against tested fungi, while chitosan at 6 g/l completely inhibit the linear growth of all tested fungi. The inhibitory effect of chitosan against pathogenic fungi was reported by Hirano *et.al*.,[9] and Abd-El-Kareem[14]. In this respect, two models have been proposed to explain the anti-fungal activity of chitosan *i.e.* the activity of chitosan is related to its ability to interfere with the plasma membrane function[27] and the interaction of chitosan with fungal DNA and RNA[28]. Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants[6,8]. Also, chitosan was reported to induce resistance against soilborne fungi[11-14,17]. Similar results were also recorded in the present study, the high reduction was obtained with chitosan or chitin at 6 g/kg soil which reduced the disease incidence more than 88.0, 86.2 and 83.1% for tomato plants grown in soil infested with *R. solani*, *F. solani* and *S. rolfsii,* respectively. On the other hand, combined treatments between chitin and chitosan at 6 g/kg soil of each, caused dramatic reduction in the percent of diseased plants, whereas it reduced the disease incidence more than 87.2% as compared with untreated plants. It is obvious that chitosan in the present study has inhibitory effect against pathogenic root rot fungi, it completely inhibit the linear growth of all tested fungi at 6 g/l, moreover, it reduced their total count down more than 40% when applied as soil amendment. These results lead to suggestion that chitosan could considered as a antifungal substances[10,17,27]. Furthermore, Chitosan treatments resulted in increasing chitinase activity more than 100% as compared with untreated plants. This might explain its ability to be potent elicitors of plant defense reactions. In this respect, many investigators reported that chitosan treatment caused induce resistance and increase enzymes activities in many plants[14,15].

In this respect, B-1,3-glucanases and chitinases are able to hydrolyze B-1,3-glucan and chitin, respectively, the major components of fungal cell walls[29,30]. The apparent reduction of disease incidence with chitin treatments may have been due to direct effect against tomato root rot fungi through chitin decomposition which releases volatile such as ammonia that suppress some soilborne fungi[8,31] and increase specific chitinolytic microflora in soil with chitin amendments[32]. In present study results indicate that the total count of root rot fungi was decreased and its reduction increased by prolonging experiment time up to 45 days of treatments and this decline was correlated with increasing of chitinolytic bacteria. Increasing of specific chitinolytic microflora in soil with chitin amendments was also reported by Bell *et.al*.,[7], Godoy *et.al*.,[32] and Mian *et al.*,[33]. On the other hand, the recorded reduction in root rot incidence in present study might be due to chitin treatments elicitor defense response in plants[6]. Also, the recorded results indicate that chitin treatments caused high increase in chitinase activity. In this respect Kuchitsu *et.al*.,[6] reported that chitin fragments appear to elicit host responses through rabid and transient membrane depolarization. Moreover, chitin was reported to be used as soil fertilizer[5,34].

On the light of the present study, it could be suggested that the use of natural safe materials, *i.e.* chitin and chitosan as fungicides alternatives is considered one of low cost and effective applicable methods for controlling such soilborne plant pathogens causing tomato root rot diseases.

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