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Evaluation of Different Application Methods of Chitin and Chitosan for Controlling Tomato Root Rot Disease under Greenhouse and Field Conditions

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Abstract: Root rot disease caused by *Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* is the most sever disease attacks tomato plants. Effect of different application methods of chitin and chitosan as single and combined treatments on tomato root rot disease incidence under greenhouse and field conditions was studied. Pots containing sandy loam soil artificially infested with tomato root rot fungi, *i.e*. *R. solani, F. solani* and *S. rolfsii* were used. Three application methods of chitin and chitosan, *i.e.* soil amendment, seed bed and transplant root dip treatments were evaluated. Results indicate that the most effective methods were seed bed and soil amendments, while transplant root dip showed less effect. The highest reduction was obtained with combined treatments between chitin plus chitosan at 6 g /kg soil for both which reduced the disease incidence more than 89.1, 88.7 and 70.8% with soil amendments, seed bed treatments and transplants root dip, respectively against all tested fungi. Combined treatment between chitin and chitosan, the promising treatments in pot experiments, were applied under field conditions. Similar trend obtained in greenhouse experiments was also observed under field trials. Results indicate that, all treatments reduced the root rot incidence during two growing seasons. The highly reduction was obtained with combined treatment between chitin and chitosan at 6 g/kg soil, which reduced the disease incidence more than 91.0%. Individual treatments of chitin or chitosan at 6 g/ kg soil as well as Rhizolex-T reduced the percent of root rot incidence more than 80.7% as compared with untreated plants. As for tomato yield, combined treatment between chitin and chitosan (6g/kg soil for each) increased yield more than 66.7%, while fungicidal treatment increased tomato yield more than 32.0% at the two successive cultivation season. It could be suggested that combined treatments between chitin and chitosan considered an easily applicable method and might be used commercially for controlling tomato root rot diseases under field conditions.

Key words: Chitin, Chitosan, Root rot and Tomato.

INTRODUCTION

Tomato plants is the one of most important vegetable crops overall the world. Root rot disease caused by *Rhizoctonia solani* Kuhu., *Fusarium solani* (Mart) Sacc. and *Sclerotium rolfsii* Sacc., are the most destructive pathogens of tomato[1,2]. Controlling of plant diseases mainly depends on fungicidal treatments[3]. Therefore, alternatives to fungicidal treatments, *i.e* safety material[4] or induced resistance in plants[5] are needed. Chitin is a safe material was reported to induce resistance against soil borne diseases[6,7]. Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants[8]. Chitosan is a safe material has antifungal activity against many plant pathogens[9,10]. Moreover, Chitosan also reported to induce resistance against soilborne fungi[11-15]. Field application of chitosan for inducing resistance against late and early blight diseases of potato and root rot disease of bean and lupin plants was reported by Abd-El-Kareem *et.al*.,[15-17] and Abd-El-Kareem[14]. Abd-El-Kareem *et.al*.[7] reported that combined treatments between chitin plus chitosan as soil amendments has high effect on root rot disease incidence of tomato plants through, reducing total counts of pathogenic fungi in soil, increasing chitinolytic bacteria and increasing the chitinase activity in treated plants.

The purpose of the present work is to evaluate different application methods of chitin and chitosan against tomato root rot disease incidence under greenhouse and field conditions.

MATERIALS AND METHODS

Pathogens and plant materials: One pathgenic isolate of tomato root rot fungi, *i.e. R. solani; F. solani* and *S. rolfsii* were obtained from Plant Pathology Dept., National Research Centre, Giza, Eyppt. Meanwhile, tomato seeds cv. Kastle rock were obtained from Department of Vegetables Crops Research, Agricultural Research Centre, Giza, Egypt.

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Soil infestation with tomato toot rot 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°C±2. Plastic pots (30-cm-diameter) containing sandy loam soil were artificially infested individually with prepared fungal inoculum at the rate 3% of soil weight. Infested soil irrigated every other day and left for further study.

Greenhouse experiments:

Effect of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse conditions: Tree methods of chitin and chitosan application, *i.e.* soil amended, seed bed and transplant root dip were evaluated against tomato root rot disease incidence. Four concentrations of chitin or chitosan, *i.e.* 0, 2, 4 and 6 g per 1000 units (soil or water). Combined treatments between chitin plus chitosan was carried out using the highest rate of both.

Pot experiment was carried out as the following procedures:

Soil amendment: infested soil was mixed individually with each tested concentration of chitin or chitosan, while combined treatment was carried out by addition of chitosan after 20 days of chitin treatment. Treated infested soils were filled into plastic pots (30-cm-diameter). Tomato transplant cv. Kastle rock were transplanted in treated pots.

Seed bed treatment: Tomato seeds cv. Kastle rock were sown in transplants production foam trays containing peat-moss soil mixed individually with each tested concentration of chitin or chitosan. Combined treatment was carried out by addition of chitosan after 20 days of chitin treatments.

Transplant root dip treatment: Tomato transplants cv. Kastle rock which grown in free soil treatments were dipped individually for 5 minuets in water emulsion containing different concentrations of chitin, chitosan or combined treatments between chitin plus chitosan then transplanting in infested soil

Effect of different addition intervals of chitosan after chitin as seed bed treatment on tomato root rot disease: Four intervals between addition of chitosan after chitin treatment (at 6 g/kg soil of each) were tested. Combined treatment was carried out by adding of chitosan to mixed peat-moss with chitin after 0, 10, 20 and 30 days of chitin treatment. Foam trays containing treated peat-moss soil were sown with tomato seeds cv. Kastle rock. Tomato transplant were transferred to infested pots.

All greenhouse experiments repeated twice and each treatment represented by 6 replicates, 4 transplants/pot and 6 pots asreplicates were used.

Field experiments: Seed bed treatment, the most promising treatments against tomato root rot disease in pot experiments, was applied under field conditions.

Four concentrations of chitin or chitosan as seed bed treatments, *i.e.* 0, 2, 4 and 6 g/ kg soil as single treatment in addition to combined treatment between chitin plus chitosan at 6 g/kg soil of each were applied to study their effect on root rot disease incidence and produced yield of tomato plants under field c onditions. Rhizolex-T (50% WP) at 3 g/kg soil was used as comparison treatment in this study.

Field experiment was carried out, in Experimental Farm of National Research Centre at El-Noubareia, Behera governorate. Tomato seeds cv. Kastle rock were sown in the transplanting tray containing peat-moss soil which mixed individually with each tested concentration of chitin or chitosan as described earlier. Tomato seedlings were transplanted in field after 30 days of sowing. A field experiments consisted of plots (7x10 m) each comprised of 12 rows and 50 transplants/row. Treatments were conducted in randomly complete block design with three replicates (plots) for each particular treatment as well as control.

Disease assessment: The average percentage of tomato root rot incidence was recorded until 90 days, of transplanting date. Determination of accumulated tomato yield per m^2 was calculated.

Statistical analysis: Tukey test for multiple comparisons among means was utilized[18].

RESULTS AND DISCUSSIONS

Greenhouse experiments

Effect of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse conditions: Tree application methods of chitin and chitosan, *i.e.* soil amendment, seed bed and transplant root dip were evaluated. Four concentrations of chitin or chitosan, *i.e.* 0, 2, 4 and 6 as well as the highest rate of both per 1000 units (soil or water) were tested.

Results in Table 1 indicate that the most effective applied methods were seed bed treatments and soil amendment, while transplant root dip treatment showed moderate effect. All treatments significantly reduce the disease incidence except that chitin at 2 or 4 g/L soil when applied as transplants root dip. The highest reduction was obtained with combined treatments between chitin plus chitosan at 6 g/kg soil each which reduced the disease

Table 1: Tomato toot rot incidence in response to different application methods of chitin and chitosan under greenhouse conditions

Treatment & applied dose (g/kg or g/L)	Tomato Root rot Incidence (%)								
	Application Methods								
	Soil Amendement			Seed Bed			Transplant Root Dip		
	R. solani	*F. solani*	*S. rolfsii*	*R.. solani*	*F. solani*	*S. rolfsii*	*R.. solani*	*F. solani*	*S. rolfsii*
Chitin 2	28.5b	26.5b	33.2b	20.0b	22.1b	24.0b	70.0a	65.0a	64.5a
Chitin 4	17.5c	17.2c	22.0c	14.0c	20.0c	23.0c	65.0a	65.0a	65.5a
Chitin 6	12.0d	8.5d	14.5d	11.0d	10.1d	15.0d	51.2b	50.0b	46.0b
Chitosan 2	25.0b	23.5b	30.5b	18.0b	20.0b	26.0b	55.4b	52.1b	43.5b
Chitosan 4	18.5b	15.5c	20.0c	12.5c	13.0c	21.0c	24.0c	26.0c	29.0c
Chitosan 6	11.5d	10.0d	12.5d	12.0c	10.0d	13.0d	16.5d	17.5d	22.0d
Chitin+Chitosan (6+6)	6.5e	4.0e	5.0e	6.0d	7.0d	7.0f	13.5d	15.0d	19.0d
Control	60.0 a	62.0 a	65.0 a	60.0 a	62.0 a	65.0 a	60.0a	62.0a	65.0a

- Figures with the same letter are not significantly different (P= 0.05)
-Soil amendment was carried out by treated infested soil with concentration of chitin and /or chitosan
- Seed bed treatment was carried out by treated soil free pathogen with concentration of chitin and /or chitosan
-Transplant root dip was carried out by dipping tomato roots in emulsion containing tested concentration of chitin and / or chitosan

incidence more than 89.1, 88.7 and 70.8% at soil amendments, seed bed and transplants root dip, respectively for all tested fungi. Moderate effect was observed with individual treatments of chitin or chitosan at 6 g/kg soil. They reduced the percent of diseased plants more than 77.7, 80.0 and 66.1% when applied as soil amendment, seed bed treatments and transplants root dip, respectivelyfor all tested fungi. Meanwhile, concentration of 4 g/kg soil of both treatments reduced the disease incidence more than 66.2, 64.6 and 55.4% in respective order. No significant differences between chitin treatment at 2 or 4 g/L and untreated plants when applied as transplants root dip.

Effect of different addition intervals of chitosan after chitin as seed bed treatment on tomato root rot disease: Four interval periods between chitin and chitosan treatments, *i.e.* 0, 10, 20 and 30 days were tested. Results in Table 2 indicate that all treatments reduced the root rot incidence. The most effective treatment that when the chitosan was added 30 days after chitin treatment which reduced the disease incidence by 95.7, 94.6 and 93.3% in infested soil with *R. solani, F. solani* and *S. rolfsii,* respectively. No significant difference between the other tested addition interval periods were observed.

Field experiments: Seed bed treatment the most promising and easily applicable treatments in pot experiments was applied in Experimental Farms of National Research Centre at El-Noubareia, Behera governorate during two successive cultivation seasons.

Table 2: Root rot incidence (%) in tomato plants as affected by different addition interval periods between chitin and chitosan as seed bed treatment

Intervals between Chitin and chitosan application(days)		Root rot incidence (%)		
		R. solani	*F. solani*	*S.rolfsii*
0		10.0c	8.0c	11.0c
10		9.0c	8.5c	10.0c
20		9.0c	8.5c	10.0c
30		3.0d	3.5d	5.0d
Control	Chitin (6g/kg)	16.2b	14.5b	17.5b
	Chitosan (6g/kg)	14.0b	13.0b	16.0b
	Untreated	70.5a	650.a	75.0a

Figures with the same letter are not significantly different (P= 0.05)
Chitin or chitosan at 6g / kg soil was used.

Effects on tomato root rot disease: Results in Table 3 indicate that, all treatments significantly reduce the root rot incidence during the two successive growing seasons. The high reduction was obtained with combined treatment between chitin plus chitosan (6 g/kg soil), which reduced the disease incidence more than 91.0%. Individual treatments of chitin or chitosan at 6 g/ kg soil as well as Rhizolex-T (50% wp) reduced the percent of root rot incidence more than 80.7% as compared with untreated plants. The moderate effect was obtained with chitin or chitosan at 4 g/ kg soil, which reduced the disease incidence more than 70.1%. While, concentrations at 2 g/kg soil of both treatments was less effective.

Effect on tomato yield: Results in Table 4 indicate that all treatments increased tomato yield during the two growing seasons. The high effect was observed with

Table 3: Tomato root rot incidence (%) in response to different concentrations of chitin and / or chitosan as seed bed treatment during two cultivation seasons under field conditions

	First growing season		Second growing season	
Treatment (g/kg)	Disease	Reduction (%)	Disease	Reduction (%)
Chitin 2	25.5b	30.0	31.2b	57.0
Chitin 4	18.4c	70.1	23.5c	67.6
Chitin 6	9.0d	85.4	14.0d	80.7
Chitosan 2	20.0b	67.5	28.0b	61.4
Chitosan 4	14.5cd	76.4	21.0c	71.0
Chitosan 6	11.2d	81.8	12.5d	82.8
Chitin + Chitosan(6+6)	4.0e	93.5	6.5e	91.0
Rhizolex-T 3	11.2d	81.8	14.0d	80.7
Untreated	61.5a	—	72.5a	—

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

combined treatment between chitin and chitosan, which increase tomato yield more than 66.7%. The moderate increase was obtained with individual treatments of chitin or chitosan at 6 g/kg soil, that tomato yield records more than 40.0% increase as compared with untreated plants. Treatments of chitin or chitosan at 4 g/kg soil caused increase in tomato yield estimated more than 20.0%. Meanwhile, 32.0% increase in accumulated tomato yield was recorded at Rizolex-T fungicidal treatment.

Root rot diseases caused by *Rhizoctonia solani, Fusarium solani and Sclerotium rolfsii* are the most destructive pathogens of tomato[1,2]. Chitin and chitosan are the safe materials which reported to induce resistance against soilborne diseases[6,7,14]. Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants[6, 14].

Also, chitosan was reported to induce resistance against soilborne fungi[7,11-14]. In the present study, three application methods of chitin and chitosan, i.e. soil amendment, transplant root dip and seed bed treatments against tomato root rot disease incidence were evaluated. The obtained results indicate that the most effective methods were seed bed treatments followed by soil amendments, while transplant root dip showed less effects. The high reduction was obtained with the combined treatments between chitin plus chitosan at 6 g/kg soil for both which reduced the disease incidence more than 89.1, 89.2 and 70.8%, respectively. On the other hand, the most effective interval period was chitosan application after 30 days of chitin treatment which reduced the disease incidence by 95.7, 94.6 and 93.3% in infested soil with *R. solani, F. solani* and *S. rolfsii,* respectively. Also, under field conditions all treatments reduced the root rot incidence during two successive growing seasons. It was observed that the great results obtained with combined treatment between chitin and chitosan at 6 g/kg soil, which reduced the disease incidence more than 91.0% and increased tomato yield more than 66.7%. Individual treatments of chitin or chitosan at 6 g/kg soil as well as Rhizolex-T) 50% wp(reduced the percent of root rot incidence more than 80.7% and increased tomato yield more than 40.0% as compared with untreated plants.

In this concern, similar results were recorded by many investigators with various crops. Chitosan treatment induced resistance against late and early blight diseases of potato and root rot disease of bean and lupin plants under field conditions[14-17]. Furthermore, chitosan has different properties, *i.e.* its inhibitory effect against pathogenic fung[19] and its ability to be potent elicitors of plant defense reactions[14,20].

In this respect Abd-El-Kareem *et. a*l.,[7] reported that chitosan at 6 g/L completey inhibit the linear growth of all tomato root rot fungi and reduced the total count of pathogenic fungi.

Two models have been proposed to explain the antifungal activity of chitosan, the first, its activity is related to the ability to interfere with the plasma membrane function[19]. While the second, the interaction of chitosan with fungal DNA and RNA[20]. Chitosan induced resistance, through increase the chitinase activity. Abd-El-Kareem *et.al.*[7] reported that chitosan at 6 g/kg soil caused high increase in chitinase activity of tomato plants. Treatment various plants with chitosan induced resistance and increased enzymes activities[7,15,17,21].

On the other hand reduction of tomato root rot disease with chitin treatments may have been due to direct effect against tomato root rot fungi that chitin decomposition releases volatiles such as ammonia which suppress some soilborne fungi[22,23] and increasing specific chitinolytic microflora in soil when chitin is used as amendments[24,25}. In this regard, Abd-El-Kareem *et a*l.,[7]

reported that the total count of root rot fungi was decreased in soil amendment with chitin and this decline was correlated with increasing of chitinolytic bacteria. Increasing of specific chitinolytic microflora in soil with chitin amendments was also reported by Godoy *et al.*,[2], Mian *et al.,*[26] and Bell *et al.*,[8].

The observed reduction in tomato root rot incidence in present study might be attributed to indirect effect of chitin treatments and its elicitor defense response in plants[6]. 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[27,28].

The present results indicate that chitin treatments increased tomato yield under field conditions .Induced resistance against root rot disease using chitin treatments was reported by Kuchitsu *et al .,*[6] and Bell *et al .,*[8]. The observed increase in tomato yield in the present study might be due to the reduction in disease incidence and promotion of plant growth as influenced by fertilizer effect of chitin and chitosn[28].

It could be suggested that combined treatments between chitin and chitosan might be used commercially as easily, safely and applicable method for controlling tomato root rot diseases under field conditions.

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