Chitosan and *Trichoderma harzianum* as Fungicide Alternatives for Controlling Fusarium Crown and Root Rot of Tomato

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ABSRACT

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Tomato is one of the most important vegetable crops in Egypt and Tunisia. Fusarium crown and root rot (FCRR), caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl), is one of the most damaging soilborne disease of tomato and is becoming more common in commercial greenhouses. In the present study, effect of individual or combined application of *Trichoderma harzianum* and chitosan against Forl was assessed in vitro and in vivo. *T. harzianum* had significantly reduced the mycelial growth of the five Forl tested isolates. Chitosan applied at different concentrations (from 0.5 to 4 g/l) had also significantly decreased the mycelial growth of the pathogen and a total inhibition was obtained at the concentration 4 g/l. Under greenhouse conditions, application of *T. harzianum* and chitosan (1 g/l) as root dipping treatment combined with chitosan (0.5 g/l) as foliar spray has reduced FCRR incidence and severity by 66.6 and 47.6%, respectively. Treatments based on *T. harzianum* alone or in combination with chitosan led to an increase in the total phenols and to an enhancement of chitinase and β,1-3-glucanase activities in leaves of treated tomato plants compared with the untreated ones. The results from this study showed the possibility of using combined treatments based on *T. harzianum* and chitosan commercially as an approach for controlling FCRR on tomato.

Keywords: Chitosan, disease severity, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, tomato, *Trichoderma harzianum*.

Tomato is one of most important vegetable crops in the world and particularly in Egypt and Tunisia. Fusarium crown and root rot (FCRR) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) is becoming the most damaging soilborne disease of tomato in both countries. The disease occurs in both greenhouse and open field tomato crops and causes significant yield losses (22, 29, 34). FCRR of tomato is responsible of about 70-83% loss of tomato plants attributed to rot and basal stem decays and eventual death of heavily infected plants (34). Although application of fungicides is far the most effective method to control tomato wilt and FCRR

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disease, it can be involved in many problems due to health risk concerns and environmental pollution. Thus, there is a growing need to develop alternative approaches for the management of this pathogen. An acceptable approach that is being actively investigated involves the use of bio-agents and bio-active substances such chitosan in controlling soilborne fungi (1, 2, 7, 11, 21, 30, 40, 42, 47).

Trichoderma spp. are effective biocontrol agents against different pathogens and some isolates are also known for their ability to induce systemic resistance in plants (26). The fungus *Trichoderma*, a natural soil-inhabiting genus, has been used successfully to control FCRR of tomato (28, 29, 30, 42). The mechanisms of action of *Trichoderma* spp. include competition for space and nutrients, antibiosis, antagonism, inhibition of pathogen enzymes and plant growth enhancement (8, 27). *T. harzianum* was applied as a peat-bran preparation to the rooting medium at the transplanting time of tomato plants. Such an application resulted in significant decrease in FCRR throughout the growing season (12, 32).

Chitosan is a partly de-acetylated form of chitin, and consists of polymers of β-1,4-glucosamine subunits, with molecular weight up to 400 kDa. It is environmentally safe and non-toxic to the majority of organisms (35). Chitosan and its derivatives display antibiotic activity against microorganisms including bacteria (38, 47, 53) and fungi (40, 47, 53, 54). Chitosan can also enhance plant resistance in seeds (14, 15, 16, 36), fruits (14, 54) or leaves (54) and reduce disease caused by fungal pathogens (21, 47). Oligomers of chitosan, which are likely to be released by the action of plant encoded-chitinases from walls of invading fungi, can protect tomato roots against Forl when applied to seeds or roots (15, 36, 42). Chitosan derivatives were applied to decapitated tomato plants and evaluated for their potential to induce defense mechanisms in root tissues infected by Forl (27, 42). Field application of chitosan for inducing resistance against late and early blight diseases of potato and root rot diseases of tomato plants was also largely reported (3, 4, 5, 6).

Management of many fungal pathogens in different pathosystems through the application of *Trichoderma* or chitosan individually or in combination is well documented (5, 6, 15, 19, 42). The present study was carried out to control FCRR of tomato and to assess the induction of phenolic compounds and defense enzymes in Forl-infected tomato plants in response to the application of *T. harzianum* individually or in combination with chitosan.

The objective of the present study is to assess the effect in vitro and in vivo of individual or combined applications of *Trichoderma harzianum* and chitosan against Forl.

MATERIALS AND METHODS

Fungal and plant material.

F. oxysporum f. sp. *radicis-lycopersici* (Forl), the causal agent of tomato FCRR disease, was isolated and identified in Plant Pathology Department, National Research Center, Giza, Egypt. Five isolates were used in the present study.

One isolate of *T. harzianum* was used in this experiment and was procured from the Plant Pathology Department, National Research Center, Giza, Egypt. Stock cultures were stored on Potato Dextrose Agar (PDA) medium at 4°C.

Tomato seeds cv. Kastel rock were obtained from Vegetable Crops Research Department, Agricultural Research

Center, Giza, Egypt. This cultivar is known by its susceptibility to FCRR.

Study of the effect of *T. harzianum* and chitosan on Forl mycelial growth.

Antagonistic ability of *T. harzianum* against Forl was carried out on PDA medium using the dual culture technique (24). Five Petri dishes were used as replicates for each treatment. All plates were incubated at 25°C for 4 and 8 days. Antagonistic ability of *T. harzianum* was expressed as percent inhibition of Forl mycelial growth as compared to the untreated control.

The inhibitory effect of chitosan (Sigma Company) against Forl was tested in vitro by using five concentrations *i.e* 0.5, 1.0, 2.0, 3.0, and 4.0 g/l. Chitosan concentrations were prepared according to Benhamou *et al*. (15). Chitosan was added to conical flasks containing sterilized PDA before solidification and rotated gently then poured into sterilized Petri plates (9 cm diameter). Plates were individually challenged at the center with equal agar plugs (5 mm diameter) taken from Forl 10 days-old cultures then incubated at 25 ± 2°C. Mean colony diameter was measured when the control plates (PDA free of chitosan) reached full growth. Five plates were used for each elementary treatment.

Preparation of Forl inoculum.

A highly aggressive isolate of Forl was grown on sand maize medium. Sand and ground maize seeds were mixed in the ratio of 2:1 (w/w) and moistened to 40% moisture content. After preparation, 200 g of the medium was filled into 500 ml Erlenmeyer conical flasks and autoclaved for two hours. One ml spore suspension of Forl (10^6 conidia/ml) was added to the sand maize medium and incubated at room temperature (25-27°C) for 14 days before use.

Production of *T. harzianum* inoculum.

T. harzianum was grown in 250 ml Erlenmeyer conical flasks containing Potato Dextrose Broth at 28 ± 1°C for 8 days. Liquid culture of *T. harzianum* was homogenized in a blender (5×10^6 conidia/ml) and used for root dipping of tomato seedlings.

Management of Fusarium crown and root rot.

Two weeks after soil infestation with pathogen inoculum (5%, w/w) prepared as mentioned above, the following treatments of tomato seedlings were carried out:

(a) Root dipping (RD)

1- *T. harzianum* (RD); 2- Chitosan 0.5 g/l (RD); 3- Chitosan 1.0 g/l (RD); 4- *T. harzianum* + chitosan at 0.5 g/l (RD); 5- *T. harzianum* + chitosan 1.0 g/l (RD).

(b) Root dipping (RD) + foliar application (FA)

1- *T. harzianum* (RD) + Chitosan 0.5g/l (FA); 2- Chitosan 0.5 g/l (RD) + Chitosan 0.5 g/l (FA); 3- Chitosan 1.0 g/l (RD) + Chitosan 0.5 g/l (FA); 4- *T. harzianum* + chitosan at 0.5 g/l (RD) + Chitosan 0.5 g/l (FA); 5- *T. harzianum* + chitosan 1.0 g/l (RD) + Chitosan 0.5 g/l (FA).

(c) Control

Control 1 (artificially infected and untreated plants); Control 2 (Healthy plants uninfected untreated plants).

Tomato transplants cv. Kastle rock grown in free soil treatments were dipped for 30 min in homogenized growth culture of *T. harzianum* (5×10^6 conidia/ ml) or water emulsion containing 0.5 or 1.0 g/l of chitosan. The treated seedlings were transplanted into plastic pots (20 cm diameter) filled with Forl infested soil. Ten pots with five seedlings each were used as replicates for each treatment. Foliar application of chitosan was

applied, after transplanting; three times (100 ml/pot) at 10 days interval.

Disease assessment.

FCRR incidence and severity were evaluated 45 days post-planting (DPP). Disease severity was scored by using a modified scale of Rowe (46) where 0 = no internal or external browning, 1 = no internal browning, discrete superficial lesions on tap root or stem base and root lesions at the points of emergence of lateral roots, 2 = brown tap root with slight internal browning at the tip of the tap root, 3 = moderate internal browning of the entire tap root, 4 = severe internal browning extending from tap root into lower stem above soil surface, abundant lesions on distal roots, 5 = dead plants (46).

Study of the effect of *T. harzianum* and chitosan on physiological activities of tomato plants.

Determination of total phenolic content. Total phenol content of leaves of treated and untreated as well as healthy tomato plants was determined using the Folin-Ciocalteau reagent (49). Freshly collected leaves (2 g) were homogenized in 80% aqueous ethanol with a pinch of neutral sand to facilitate crushing and the mixture was passed through a clean cloth to filter the debris. The filtered extract was centrifuged at 10,000 g for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were collected, put into evaporating dishes and evaporated to dryness at room temperature. Following evaporation, the residue was dissolved in 5 ml of distilled water. Extract was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% of sodium carbonate were added and the contents were mixed thoroughly. The color was developed and absorbance was measured at 650 ηm in a spectrophotometer (Systronics, uv-vis, 117) after 60 min. Catechol was used as a standard. The phenolic content was expressed as mg catechol/100 g of fresh weight of tomato leaves.

Determination of β-1,3glucanase and chitinase activities.

Extraction of enzymes. One gram of leaf tissue was homogenized with 0.2 M tris HCl buffer (pH 7.2) containing 14 mM of b-mercaptoethanol at the rate of 1/3 (w/v). The homogenate was centrifuged at 300 rpm for 15 min, the supernatant was used to determine the enzyme activity (51)

β-1,3glucanase activity. The method of Abeles and Forrence (9) was used to determine the β-1,3glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugar. The optical density was determined at 500 ηm using a spectrophotometer (TM, Spectronic Educatar). The β-1,3glucanase activity was expressed as mM glucose equivalent released in gram fresh weight tissue/60 min.

Chitinase activity. Colloidal chitin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugar (41). The optical density was determined at 540 ηm. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released in gram fresh weight tissues/60 min.

Statistical analyses.

All experiments were set up according to a completely randomized design. One-way ANOVA was used to analyze differences between treatments. A general linear model option of the analysis system SAS (48) was used to perform the ANOVA. Duncan's multiple

range test at $P < 0.05$ was used for mean separation (57).

RESULTS

Effect of *T. harzianum* on the mycelial growth of Forl.

Antagonistic ability of *T. harzianum* against Forl was carried out on PDA medium using the dual culture technique. Results presented in Table 1 indicate that *T. harzianum* significantly reduced the mycelial growth of all the Forl tested isolates. *T. harzianum* reduced the in vitro growth of Forl by more than 77.7%. The highest antagonistic potential of *T. harzianum* was recorded against Forl-2, Forl-3 and Forl-5 isolates where the mycelial growth was reduced by 93.3, 90.0 and 87.7%, respectively.

Effect of chitosan on the radial growth of Forl.

The inhibitory effect of five chitosan concentrations (0.5, 1.0, 2.0, 3.0, and 4.0 g/l) against Forl was tested in vitro. Results presented in Table 2 indicate that all tested concentrations have significantly reduced the mycelial growth of Forl. Complete inhibition of the pathogen was obtained with chitosan applied at 4 g/l. The highest reduction was obtained with chitosan used at 3 g/l where the mycelial growth was reduced by up to 88.8% for all the Forl tested isolates. Used at 1.0 and 2.0 g/l, chitosan induced moderate antifungal activity.

Table 1. Effect of *Trichoderma harzianum* on the mycelial growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* isolates on PDA medium noted after 8 days of incubation at 25°C

Forl isolate	Colony diameter (mm)	Inhibition rate* (%)
Forl-1	16.0 bc	82.2
Forl-2	6.0 d	93.3
Forl-3	9.0 d	90.0
Forl-4	20.0 b	77.7
Forl-5	11.0 cd	87.7
Control	90.0 a	-

* Growth reduction as compared to the untreated control. Means followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Table 2. Effect of chitosan concentrations on the mycelial growth (mm) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* isolates on PDA medium noted after 8 days of incubation at 25°C

Forl isolate	Chitosan concentration (g/l)									
	0.5		1.0		2.0		3.0		4.0	
	D	I	D	I	D	I	D	I	D	I
Forl-1	81 c	10.0	68 c	24.4	55 b	38.8	25 b	72.2	0 b	100
Forl-2	84 b	6.6	74 b	17.7	52 bc	42.2	30 b	66.3	0 b	100
Forl-3	80 cd	11.1	66 c	26.6	47 cd	47.7	27 b	70.0	0 b	100
Forl-4	76 de	15.5	65 cd	27.7	45d	50.0	10 c	88.8	0 b	100
Forl-5	70 e	22.2	60 d	33.3	33 e	63.3	8 c	91.1	0 b	100
Control	90 a	-	90 a	-	90 a	-	90 a	-	90 a	-

D= Colony diameter (mm); I = Inhibition percentage (%) as compared to the untreated control. Means followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Management of tomato Fusarium crown and root rot disease.

T. harzianum and chitosan (concentrations 0.5 and 1.0 g/l) applied individually or as combined treatments were tested against Forl on tomato plants. Results shown in Table 3 indicate that all the tested treatments had significantly reduced FCRR incidence, noted 45 DPP, as compared to the untreated tomato plants (Control 1). The most effective treatments were *T. harzianum* + chitosan (0.5 and/or 1.0 g/l) applied as root dipping combined with chitosan (0.5 g/l) as foliar spray application where the FCRR incidence was reduced by 60.0 and 66.6%, and disease severity decreased by 47.8 and 47.6%, respectively. Root dipping treatment based on *T. harzianum* + chitosan (0.5 or 1.0 g/l) led to reduced FCRR incidence (50.0%) and severity (28.5 and 47.8%, respectively for both tested concentrations). *T. harzianum* and chitosan applied as individual treatments (without chitosan foliar sprays) caused lesser reduction in disease incidence as compared to combined treatments together with chitosan foliar sprays. Chitosan treatments were the least effective in reducing disease incidence and severity (Table 3).

Table 3. Fusarium crown and root rot of tomato in response to *Trichoderma harzianum* and chitosan based-treatments applied as root dipping and/or foliar sprays with chitosan under greenhouse conditions noted 45 days post-planting

Treatment	Foliar spray	Disease incidence and severity			
		Incidence (%)	Incidence reduction (%)	Severity	Severity reduction (%)
T. harzianum	Chitosan 0 g/l	45.0 d	40	3.2c	23.8
Chitosan 0.5 g/l		62.5 b	16.6	3.8 b	9.5
Chitosan 1.0 g/l		45.0 cd	40	3.5 b	16.6
***T. harzianum* + Chitosan 0.5 g/l**		37.5 d	50	3.0 c	28.5
***T. harzianum* + Chitosan 1.0 g/l**		37.5 d	50	2.4 d	42.8
T. harzianum	Chitosan 0.5 g/l	37.5 d	50	2.7 c	35.7
Chitosan 0.5 g/l		55.0 c	26.6	3.5 b	16.6
Chitosan 1.0 g/l		42.5 d	43.3	3.0 c	28.5
***T. harzianum* + Chitosan 0.5 g/l**		30.0 eg	60	2.4 d	42.8
***T. harzianum* + Chitosan 1.0 g/l**		25.0 g	66.6	2.2 d	47.6
Control 1 (infested soil and untreated plants)		75.0 a	-	4.2 a	-
Control 2 (non infected and healthy plants)		0	-	0	-

In each column, means followed by the same letter are not significantly different according to Duncan's multiple range test (at $P \leq 0.05$).

Effects of *T. harzianum* and chitosan on physiological activities of tomato plants.

Effects of* T. harzianum *and chitosan on phenol content. *T. harzianum* and chitosan were applied individually or as combined treatments onto tomato plants inoculated with Forl. The effects of these treatments on total phenol contents in both treated and

untreated plants were presented in Table 4. Obtained results showed that all *T. harzianum* and chitosan based-treatments had increased phenol production in tomato plants infected with Forl and treated with *T. harzianum* + chitosan as root dipping followed with foliar sprays with chitosan (0.5 g/l). Meanwhile, untreated plants showed no evident changes in their phenol content level. Dipping root system of tomato seedlings in chitosan (at 0.5 or 1.0 g/l) then their transplanting in Forl infested soil led to an increase of the total phenols in leaves of treated seedlings. This content increase reached 114.8 and 124.0 mg/100 g of fresh weight at 30 DPP as compared to 85.6 and 74.2 mg/100 g of fresh weight noted on infected and untreated plants (Table 4). The highest levels in phenol content were recorded in tomato seedlings treated with *T. harzianum* + chitosan (at 0.5 or 1.0 g/l) combined with foliar spray with chitosan (at 0.5 g/l) with 134.2 and 148.2 mg/100 g of fresh weight noted 30 DPP, respectively. The total phenol content was significantly higher in Forl infected seedlings following the application of *T. harzianum* + chitosan (at 0.5 or 1.0 g/l) combined with foliar application with chitosan (at 0.5 g/l). In our investigation, the highest reduction in disease incidence and severity was accompanied by the accumulation of maximum amounts of phenolic compounds in infected tomato leaves treated with *T. harzianum* and chitosan as root dipping in combination with chitosan as foliar spray.

Effects of* T. harzianum *and chitosan on β-1,3glucanase and chitinase activities. Results shown in Table 5 indicate that all treatments tested enhanced enzyme activity in leaves of Forl-infected tomato seedlings as compared to the control plants. *T. harzianum* + chitosan (0.5 and 1.0 g/l) applied as root dipping individually or in combination induced significant increase in chitinase and β-1,3 glucanase activities in treated plants as compared with the untreated control ones. The most effective treatments is the combination between *T. harzianum* + chitosan (0.5 or 1.0 g/l) as foliar spray where the chitinase activity increase was of about 111.1 and 122.2%, respectively. The same treatments have also increased the β-1,3glucanase activity by 146.2 and 146.2%, respectively. These combined treatments applied without additional chitosan foliar spray had also enhanced chitinase and β-1,3glucanase activities by 88.9 and 122.2% and by 76.9 and 107.7%, respectively.

Table 4. Total phenolic compounds in leaves of tomato plants inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* and treated with *Trichoderma harzianum* and chitosan as root dipping and/or foliar application of chitosan.

Treatment	Foliar spray	Total phenol [mg/100 g fresh weight of tomato leaves			
		0 DPP	10 DPP	20 DPP	30 DPP
T. harzianum	Chitosan 0 g/l	72.8 a	88.1 d	92.2 e	96.1 e
***T. harzianum* + Chitosan 0.5 g/l**		69.4 a	94.8 c	107. 2 d	114. 8 d
***T. harzianum* + Chitosan 1.0 g/l**		71.4 a	102.4 b	116.2 c	124.4 c
T. harzianum	Chitosan 0.5 g/l	72.2 a	92.4 c	95.6 e	102.0 e
***T. harzianum* + Chitosan 0.5 g/l**		71.0 a	118.2 a	128.2 b	134.2 b
***T. harzianum* + Chitosan 1.0 g/l**		72.8 a	123.6 a	140.0 a	148.2 a
Control (untreated plant + infested soil)		71.2 a	74.8 e	77.2 f	85.6 f
Control (healthy plant)		68.0 a	72.2 e	73.0 f	74.2 g

For each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$). DPP: Days post-planting.

Table 5. Chitinase and β-1,3glucanase activities in tomato leaves inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* affected by *Trichoderma harzianum* and chitosan based-treatments applied alone or in combination

Treatment	Foliar spray	Chitinase		β-1,3 glucanase	
		Activity 20 DPP	Increase * (%)	Activity 20 DPP	Increase * (%)
T. harzianum	Chitosan 0.0 g/l	1.6 b	77.8	2.1 d	61.5
***T. harzianum* + Chitosan 0.5 g/l**		1.7 b	88.9	2.3 d	76.9
***T. harzianum* + Chitosan 1.0 g/l**		2.0 a	122.2	2.7 bc	107.7
T. harzianum	Chitosan 0.5 g/l	1.7 b	88.9	2.2 d	69.2
***T. harzianum* + Chitosan 0.5 g/l**		1.9 a	111.1	3.2 a	146.2
***T. harzianum* + Chitosan 1.0 g/l**		2.0 a	122.2	3.2 a	146.2
Control (infested soil)		0.9 c	-	1.3 e	-

* Increase as compared to the control. For each column, means followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$). DPP: Days post-planting.

DISCUSSION

Tomato is an economically important crop and may be infected by different pathogens. FCRR is a serious infecting tomato and is becoming more common in commercial productions (22, 29, 34). Farmers tend to use huge amounts of chemicals leading to human, animal and environmental problems. Therefore, effective and environmentally safe management approaches are increasingly needed.

In this study, the biocontrol agent *T. harzianum* and chitosan were used individually or as combined treatments for controlling FCRR on tomato as well as to assess their potential to induce phenolic compounds and defense enzymes production in Forl-infected tomato plants. The results clearly demonstrated that *T. harzianum* has significantly reduced the in vitro growth of all Forl isolates. This inhibition is attributed to the multiple mechanisms of action of *Trichoderma* that acts as mycoparasite. In fact, this biocontrol agent defects its host by sugar-lectin linkage and excrete extracellular lytic enzymes such as β-1,3glucanases, chitinase, protease and/or lipase (55, 56). *Trichoderma* has a substantial ability to suppress a wide range of pathogenic fungi by various mechanisms including the production of cell wall degrading enzymes (27, 33). On the other hand, complete inhibition of the pathogen was also obtained with chitosan applied at 4 g/l. The inhibitory effect of chitosan against root rot and wilt pathogens was reported by many authors (1, 3, 15, 16, 25, 21, 42, 47). Furthermore, chitosan was reported to induce resistance in tomato plants against root rot diseases when applied as seed treatment, root dipping, foliar application or soil amendment (6, 16, 20, 42). In this respect, two models have been proposed to explain the antifungal activity of chitosan; firstly, the activity of chitosan is related to its stability while interfering with the plasma membrane function (39) and secondly, the interaction of chitosan with fungal DNA and RNA (31).

In the greenhouse trial, more promising results were obtained by using *T. harzianum* and chitosan (0.5 and 1.0 g/l) as combined treatments against Forl. The results indicated that treating inoculated tomato plants with chitosan and *T. harzianum* had significantly reduced FCRR incidence as compared to the control. The most effective treatments in reducing both disease incidence and

severity were *T. harzianum* + chitosan (0.5 and/or 1.0 g/l) applied as root dipping combined with foliar spray with chitosan (0.5 g/l). These results are in agreement with other findings (7, 11, 20, 42, 23). It is interesting to note that present findings clearly demonstrated that using chitosan in combination with T. *harzianum* to control FCRR enhanced more the ability of *T. harzianum* to inhibit Forl and consequently disease especially when chitosan was applied as foliar additional spray. The efficiency of *T. harzianum* and/or chitosan in controlling the disease was also reported in other references (28, 29, 30, 42). *T. harzianum* and chitosan activated host defense genes leading to physical and biochemical changes in plant cells involved directly or indirectly in disease suppression. These changes included accumulation of phenol compounds and increasing activity of host defense enzymes (13, 14, 15, 16, 31, 50, 52). The present results also indicated that all *T. harzianum* and chitosan based-treatments increased phenol production in tomato plants infected with Forl and treated with *T. harzianum* + chitosan applied as root dipping combined with foliar sprays with chitosan (at 0.5 g/l). Meanwhile, untreated plants showed no noticeable changes in their phenol content. In this respect, Benhamou *et al*. (15) reported that chitosan induced systemic resistance to Forl in tomato seedlings by triggering a hypersensitive-like response at sites of fungal entrance and stimulating rapid accumulation of newly formed macromolecules such as 1,3glucans, phenols, and lignin like compounds. Ojha and Chatterjee (45) reported that application of *T. harzianum* and salicylic acid stimulated the formation of total soluble phenols in host tissues. It was concluded that the increase in phenolic content was positively proportional to the degree of plant resistance against pathogens (10). Phenolics seem to inhibit disease development through different mechanisms involving accumulation of phenolics at the infection site to isolate the pathogen, inhibition of extracellular fungal enzymes, inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complexation, protein insolubilisation), and antioxidant activity in plant tissues (18). Beckman (13) concluded that the efficiency of phenolic compounds in reducing diseases may be attributed to their effect on host defense pathways and in signaling for host defenses more than the direct toxic effect on the pathogen. In addition, *T. harzianum* + chitosan (at 0.5 and 1.0 g/l) applied as root dipping individually or in combination are found to increase chitinase and β-1,3glucanase activities in treated plants as compared to the untreated control ones. These results are in accordance with many other findings highlighting that *T. harzianum* and chitosan based-treatments enhance these enzymatic activities in many plants since chitinase and β-1,3glucanase are involved in the hydrolysis of the fungal cell walls (27, 37).

The current study revealed that inducing plant defense mechanisms by applying *T. harzianum* and chitosan particularly in combination could provide protection of tomato plants against FCRR disease.

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RESUME

El-Mohamedy R.S.R., Abdel-Kareem F., Jabnoun-Khiareddine H. et Daami-Remadi, M. 2014. Le chitosane et *Trichoderma harzianum* comme alternatives aux fongicides pour lutter contre la fusariose des racines et du collet de la tomate. Tunisian Journal of Plant Protection 9: 31-43.

La tomate est l'une des plus importantes cultures légumières en Egypte et en Tunisie. La fusariose des racines et du collet de la tomate, causée par *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) est l'une des maladies telluriques les plus destructives de la tomate et elle est devenue très commune dans les cultures sous serre. Dans la présente étude, l'effet de *Trichoderma harzianum* appliqué d'une façon individuelle ou combiné au chitosane contre Forl a été évalué *in vitro* et *in vivo*. *T. harzianum* a significativement réduit la croissance mycélienne des cinq isolats de Forl testés. Le chitosane appliqué à différentes concentrations (de 0,5 à 4,0 g/l) a aussi significativement réduit la croissance mycélienne du pathogène et une inhibition totale a été obtenue à la concentration 4 g/l. Sous les conditions de serre, l'application de *T. harzianum* et du chitosane (1,0 g/l) par trempage racinaire combiné avec le chitosane (0,5 g/l) par pulvérisation foliaire a réduit l'incidence et la sévérité de FCRR par 66.6% et 47.6%, respectivement. Le traitement à base de *T. harzianum* appliqué seul ou en combinaison avec le chitosane a entraîné une augmentation des teneurs en phénols totaux et une stimulation des activités chitinase et β,1-3-glucanase dans les feuilles des plants de tomate traités comparés aux non traités. Les résultats de cette étude ont montré la possibilité d'utiliser des traitements combinés à base de *T. harzianum* et de chitosane à l'échelle commerciale comme approche pour lutter contre la fusariose des racines et du collet de la tomate.

Mots clés: Chitosane, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, tomate, *Trichoderma harzianum*, sévérité de la maladie.

ملخص

المحمدى، رياض صدقي رياض وفريد عبد الكريم وهيفاء جبنون-خيار الدين وماجدة الدعمى-الرمادي. 2014. استخدام الفطر *Trichoderma harzianum* والكيتوزان كبدائل للمبيدات الفطرية في مكافحة مرض تعفن الجذور والتاج الفيوزاري على الطماطم. Tunisian Journal of Plant Protection 9: 31-43.

تعتبر الطماطم من أهم المحاصيل الخضرية في مصر وتونس. ويمثل مرض تعفن التاج والجذور الفيوزاري المتسبب عن الفطر *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl) أحد أخطر الأمراض المتسربة من التربة التى تصيب الطماطم، خاصة في الزراعات تحت البيوت الحامية. فى هذا البحث، تم اختبار تأثير الفطر *Trichoderma harzianum* والكيتوزان على نمو Forl وذلك تحت ظروف المخبر/المعمل والبيت المحمي. لقد قلص الفطر *T. harzianum* ومادة الكيتوزان من النمو الغزلي لدى خمس عزلات من الفطر Forl المجربة. لقد حد استعمال الكيتوزان باعتماد تركيزات مختلفة (من 0.5 إلى 4 غ/ل) بصفة معنوية من النمو الغزلي للفطر وقد تم الحصول على تثبيط كامل باستعمال التركيز 4 غ/ل. أما تحت ظروف البيت الحامي، فقد مكنت معاملة شتلات الطماطم بغمس الجذور في فطر *T. harzianum* والكيتوزان (1.0 غ/ل) ثم رشها بالكيتوزان (0.5 غ/ل)، من التخفيض في نسبة حدوث مرض تعفن الجذور والتاج وكذلك في شدة الإصابة بالمرض بنسبة تصل إلى 66.6 و 47.6% على التوالي. كما مكنت المعاملات المرتكزة على استعمال *T. harzianum* بصورة منفردة أو مدمجة مع الكيتوزان من الزيادة في مستوى الفينولات الكلية وكذلك من التحثيث في إفراز أنزيمات الدفاع الطبيعية مثل chitinase وβ,1-3-glucanase في أوراق نباتات الطماطم المعاملة مقارنة بالشاهد. تشير نتائج هذه الدراسة إلى إمكانية دمج استعمال الفطر *T. harzianum* مع الكيتوزان كطريقة بديلة لمكافحة مرض تعفن التاج والجذور الفيوزاري على الطماطم.

كلمات مفتاحية: كيتوزان، طماطم، شدة المرض، *Fusarium oxysporum* f. sp. *radicis-lycopersici*، *Trichoderma harzianum*.

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