

**Induction of Resistance in Tomato Plants
against Fusarium Crown and Root Rot Disease
by *Trichoderma harzianum* and Chitosan
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Phenolic compounds in addition to activity of polyphenol oxidase (PPO) and peroxidase (POD) were significantly increased in tomato plants in response to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) infection, whereas chitinase and -1,3 glucanase activities were significantly inhibited when compared with the healthy plant. *Trichoderma harzianum* and chitosan reflected many components of defence signals which leading to the activation of power defence system in tomato against pathogen attack. The reduction in disease incidence and severity was accompanied with remarkable increases in phenolic content and activity of polyphenol oxidase, peroxidase and glucanase in infected plant treated with *T. harzianum* and chitosan. On the other hand, chitinase activity showed insignificant increase under the application of *T. harzianum* and chitosan when compared with the infected untreated control. *T. harzianum* and chitosan treatment as root dipping in combination with chitosan foliar spray was the most effective treatment against *Fusarium* crown and root rot disease (FCRR) incidence and severity, through induction of physiological host defence mechanisms. Induction of systemic resistance against FORL is one of the main mechanisms by which *T. harzianum* and chitosan contribute to tomato plants protection.

Keywords: Chitosan, crown, root-rot, induced resistance, *Fusarium*, *T. harzianum* and tomato.

Tomato is one of the most important vegetable crops in Egypt and other countries. In the fields, tomato plant is exposed to a number of pathogens leading to dramatic losses in crop production. *Fusarium* crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) has been recorded as the most prevalent soil-borne disease of this crop in different regions of the world, resulting in high yield losses (Rattink, 1993). Although, application of fungicides is the most effective method to control tomato wilt and crown and root rot diseases, there are reports of an increasing number of fungicides resistant strains of the soil borne pathogens (Jones, 1985). In addition, some of commonly used fungicides are under review in many countries due to health risk concern (Anonymous, 1988). Therefore, great efforts to develop new effective and environmentally safe approaches for management of plant diseases are needed. Systemic acquired resistance (SAR) or induction of resistance against pathogens is a promising approach for controlling plant diseases.

Exogenous or endogenous factors could substantially affect host physiology, lead to rapid and coordinated activation of defence gene in plants normally expressing susceptibility to pathogen infection (Mandal *et al.*, 2009).

Induction of plant defence genes by prior application of inducing agents is called induced resistance (Hammerschmidt and Ku, 1995). The defence gene enzyme include peroxidase (PO), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis. Other defence enzymes include pathogenesis-related proteins such as β -1,3-glucanases and chitinases which degrade the fungal cell wall and cause lysis of fungal cell are involved also in induction of host resistance. Pradeep and Jambhale (2002) suggested that phenolic compounds and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance. Accumulation of phenolic compounds at the infection site has been correlated with the restriction of pathogen development, since such compounds are toxic to pathogens. Also, phenolic compounds may impede pathogen infection by increasing the mechanical strength of the host cell wall (Benhamou *et al.*, 2000). Houssien *et al.* (2010) concluded that salicylic acid individually or in combination with *T. harzianum* significantly increased total phenolic content in *F. oxysporum* infected tomato plants which might play an important role in resistance and defence against the pathogen. Induction of plant resistance to pathogens can be enhanced by using biological agent and/or natural substance. *Trichoderma* spp. are effective biocontrol agents for a number of soil-borne plant pathogens. Some isolates are also known for their ability to induce systemic resistance in plants against different pathogens (Harman *et al.*, 2004).

Trichoderma spp. have evolved numerous mechanisms that are involved in biological control. These mechanisms include competition for space and nutrients, antibiosis, antagonism, inhibition of pathogen enzymes and plant growth stimulation (Abd-El-Khair *et al.*, 2010). Chitosan oligomers are safe materials and have attracted attention in plant disease management due to their unique biological properties including their inhibitory effect on the growth of various pathogenic fungi and their ability to be potent elicitors of plant defence reactions (Benhamou *et al.*, 1994). When host plant treated with chitosan, pathogen growth was restricted to epidermis and outermost cortical cell layer and fungal hyphae were unable to penetrate the inner most cortical layer, due to the accumulation of high level of phenolic compounds in plant tissues (Benhamou *et al.*, 1998; Sukand and Kulkarni, 2006). Oligomers of chitosan, can protect tomato roots against *F. oxysporum* f.sp. *radicis-lycopersici* when applied to the seed or roots (Benhamou and Theriault, 1992 and Benhamou *et al.*, 1994). Induction of defence responses against the pathogen through the application of *Trichoderma* or chitosan individually is well documented. But, there is scarce information about the efficiency of these treatments in management of crown and root rot disease of tomato and the mechanism of defence or synergism when they are used together.

Thus, the present study was carried out to manage crown and root rot disease and to assess the induction of phenolic compounds and defence enzymes in

F. oxysporum f.sp. *radicis-lycopersici* infected tomato plant in response to the application of *T. harzianum* individually or in combination with chitosan.

Materials and Methods

Pathological study:

Host:

Tomato seedlings (cv. Kastel rock) were obtained from Vegetable Crops Res. Dept., Agric. Res. Centre, Giza, Egypt.

Microorganisms:

The pathogenic fungal isolate was isolated from root of tomato plants showing typical symptoms of crown and root rot disease and identified as *Fusarium oxysporum* f. sp. *Radici-lycopersici* Jarvis and Shoemaker (FORL). Identification was confirmed by Plant Pathol. Dept., National Res. Centre, Giza, Egypt. Biocontrol agent, *Trichoderma harzianum* Rifai (TH) was procured from the Plant Pathol. Dept., National Res. Centre, Giza, Egypt. Stock cultures were prepared on Potato Dextrose Agar (PDA) slants medium and stored at 4°C.

Preparation of F. oxysporum f.sp. radicis-lycopersici (FORL) inoculum:

The 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) moistened to 40% moisture content. After preparation, 200g of the medium was filled into 500ml Erlenmeyer conical flask and autoclaved for two hours. One ml spore suspension of FORL (10^6 spore/ml) was inoculated into sand maize medium and incubated at room temperature for 14 days.

Preparation of Trichoderma harzianum:

Trichoderma harzianum was grown in 250 ml Erlenmeyer conical flasks containing PD broth at $28 \pm 1^\circ\text{C}$ for 8 days. Growth culture of *T. harzianum* was homogenized in a blender (5×10^6 cfu / ml) and used for root dipping of tomato seedlings.

Management of Fusarium crown and root rot (FCRR) in greenhouse:

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

(a) *Root dipping (RD):*

Trichoderma harzianum (THRD) - Chitosan 1.0g/l (CHRD 1.0g/l) - *T. harzianum* + chitosan at 0.5g/l (THRD + CHRD 0.5g/l) - *T. harzianum* + chitosan 1.0g/l (THRD + CHRD 0.1g/l).

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

Trichoderma harzianum (THRD) + Chitosan 0.5g/l (CHFA 0.5g/l) - Chitosan 1.0g/l (CHRD 1.0g/l) + Chitosan 0.5g/l (CHFA 0.5g/l) - *T. harzianum* + chitosan at 0.5g/l (THRD+ CHRD 0.5g/l) + Chitosan 0.5g/l (CHFA 0.5g/l) - *T. harzianum* + chitosan 1.0g/l (THRD + CHRD 0.1g/l) + Chitosan 0.5g/l (CHFA 0.5g/l).

(c) Control:

Control 1 (artificially infested untreated plants) FORL- Control 2 (Healthy plants uninfested untreated plants)

Chitosan concentrations were prepared according to Benhamou *et al.* (1994). Tomato transplants cv. Kastel rock which grown in free soil treatments were dipped for 15 min in homogenized growth culture of *T. harzianum* (5×10^6 cfu / ml) or water emulsion containing 0.5 or 1.0 g/l chitosan. The treated seedlings were transplanted in plastic pots (20 cm diameter) filled with FORL infested soil. Ten pots with five seedlings were used as replicates for each treatment. Foliar application of chitosan was applied three times (100ml/pot) at interval of 10 days after transplanting.

Disease assessment:

Disease incidence and severity was evaluated 30 days from transplanting. The plants were examined for external and internal disease symptoms. As the disease expression may vary from light discoloration on roots to complete rotting of the tap root, each plant was scored on a scale from 0 to 5. According to Rowe (1980) assessment scale: 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. Disease severity (DS) was calculated using the following formula:

$$DS (\%) = \frac{(\text{Disease grade} \times \text{Number of plants in each grade})}{(\text{Total number of plants} \times \text{Highest disease grade})} \times 100$$

Biochemical studies:

All biochemical studies were carried 30 days after seedlings transplanting. Tomato leaves collected from all applied treatments as well as control treatments were used for the purpose of biochemical studies.

Determination of total phenol content:

Total phenol content of tomato leaves of infected treated and non treated as well as healthy tomato plants was determined using the Folin - Ciocalteu reagent (Singleton and Rossi 1965). 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 rpm for 15 min and the supernatant was saved.

The residue was re-extracted twice and supernatants were collected and evaporated to dryness at room temperature. Following evaporation the residue was dissolved in 5 ml of distilled water. Known volume of the extract (100 μ l) was diluted to 3 ml with distilled water and 0.5 ml of Folin - Ciocalteu reagent was added.

After 3 min, 2 ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance was measured at 650 nm in a spectrophotometer cuvette (Systronics, uv-vis, 117) after 60 min. Catechol as a standard was used. The phenolic content was expressed as mg catechol/100g fresh weight of tomato leaves.

Determination of polyphenol oxidase:

Tomato leaves (2 g) were collected and washed thoroughly with running tap water then by distilled water. The surface was wiped off with filter paper. To extract the enzyme, leaves were ground separately with a pinch of neutral sand in 6.0 ml of sodium phosphate buffer (0.1 M at pH 7.0). The extracts were obtained by filtering off the debris with a clean cloth and centrifuged at 3,000 rpm for 15 min in a refrigerated centrifuge. The supernatants were recovered and kept in a glass, in an ice bath until assayed. Enzyme assay was done according to the method of Sadasivam and Manickam (1996). Three milliliters of 0.1 M sodium phosphate buffer at pH 7.0 and 2.0 ml of the enzyme extract were mixed in a spectrophotometer cuvette (Systronics, uv-vis, 117). The mixture was immediately adjusted to zero absorbance. Catechol of 0.01 M (1.0 ml) in 0.1 M phosphate buffer was added above mixture and the reactants were quickly mixed. Enzyme activity was recorded as the change in absorbance at 495 nm per /minute / g tissue after the addition of catechol.

Determination of peroxidase:

Tomato leaves (4.0 g) were homogenized in 20 ml of chilled distilled water at 0°C. A pinch of neutral sand was added to facilitate crushing. The extracts were obtained by filtering off the debris with a clean cloth and centrifuged at 3,000 rpm for 15 min at 4°C. The supernatants were collected and used as enzyme source and kept in ice-bath until assayed. A peroxidase enzyme assay was done according to the method of Mahadevan and Sridhar (1982). Freshly prepared 5 ml of pyrogallol reagent (prepared by mixing 10 ml of 0.5 M pyrogallol and 12.5 ml of 0.66 M phosphate buffer at pH 6.0) and 1.5 ml of enzyme extract were mixed in spectrophotometer cuvette (Systronics, uv-vis, 117) and the mixture was immediately adjusted to zero absorbance, and 0.5 ml of 1% H₂O₂ solution was added to it. Enzyme activity was recorded as the change in absorbance per / minute / g tissue at 430 nm after the addition of the substrate.

Determination of -1,3glucanase:

One gram of leaf tissue was homogenized with 0.2ml of Tris HCl buffer (pH 7.2) containing 14 mM of -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 enzyme activities (Tuzan *et al.*, 1989). The method of Abeles and Forrence (1970) was used to determine -1, 3 glucanase activity. Laminarin was used as substrate and dinitro salicylic acid as reagent to measure reducing sugar. The optical density was determined at 500 nm using SPECTRONIC EDUCATAR^{-TM} spectrophotometer.

-1,3 glucanase activity was expressed as mM glucose equivalent released /gram fresh weight tissues/60 min .

Determination of Chitinase activity:

The method of chitinase extraction was similar to that of -1, 3 glucanase extraction. Colloidal chitin was used as substrate and dinitro salicylic acid as reagent to measure reducing sugar (Monreal and Reese, 1969). The optical density was measured at 540 nm. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released /gram fresh weight tissues/60 min.

Statistical analysis:

Tukey test for multiple comparisons among means was utilized after Neler *et al.*, (1985).

Results*Management of Fusarium crown and root rot (FCRR) disease incidence:*

Trichoderma harzianum separately or in combination with chitosan at 0.5 or 1.0 g/l applied as root dipping or in combination with chitosan at 0.5 g/l applied foliar application were significantly effective for controlling Fusarium crown and root rot (FCRR) disease of tomato plants caused by *F. oxysporum* f.sp. *radicis-lycopersici* (Table 1). Incidence and severity of FCRR disease were significantly reduced in all treatments when compared with the infected untreated seedling (infected untreated control). *T. harzianum* in combination with chitosan as root dipping at 1.0g/l and foliar application of seedlings at 5.0g/l was the best treatment for controlling the disease. Disease incidence and severity recorded the lowest values at this treatment (12.5% and 1.6, respectively), when compared with 75% and 4.2 in the infected untreated control.

Table 1. Percentage of disease incidence and severity in infected tomato plants treated with *Trichoderma harzianum* and chitosan

Treatment *	Crown and root rot disease incidence and severity			
	DI (%)	Reduction (%)	Severity (%)	Reduction (%)
FORL+ TH	32.5c	56.6	3.0c	28.5
FORL+ CHR D 0.1 g/l	38.5cd	48.6	3.4cd	19.0
FORL+TH+CHR D 0.5g/l	22.5d	70.0	2.8c	33.3
FORL+TH+CHR D 1.0/l	22.5d	70.0	2.2d	47.6
FORL+ TH+CHFA 5.0 g/l	25.0e	66.6	2.6c	38.1
FORL+CHR D 0.1g/l+CHFA 5.0g/l	33.0c	56.6	3.0c	28.1
FORL+TH+CHR D 0.5g/l+CHFA 5.0g/l	17.5 f	76.6	2.1d	50.0
FORL+TH+CHR D 1.0 g/l+CHFA 5.0 g/l	12.5 g	83.3	1.6e	61.9
FORL (control ₁) **	75.0b	0.0	4.2b	0.0
Healthy plant (control ₂) ***	0.0a	0.0	0.0a	0.0

* FORL: *F. oxysporum* f.sp. *radicis-lycopersici*; TH: *T. harzianum*; CHR D: Chitosan as root dipping; CHFA: Chitosan as foliar application; DI: Disease incidence.

** FORL (control₁): Infected untreated.

*** Healthy plant (control₂): Uninfected untreated.

- Means followed by the same letter are not significantly different at P 0.05.

Biochemical studies:

Results clearly show that the pathogen infection lead to the accumulation total of phenolic compounds. Phenolic compounds in infected and healthy tissues reached to 85.6 and 74.2 mg/100 g fresh weight of tomato leaves, respectively (Table 2). Also the activity of both polyphenol oxidase (1.88 unit ml⁻¹) and peroxidase (1.6 unit ml⁻¹) increased significantly due to infection when compared with the healthy plant (0.7 unit ml⁻¹ and 0.64 unit ml⁻¹, respectively). The application of *T. harzianum* separately or in combination with chitosan enhanced the production of phenolic compounds as well as the activity of both enzymes compared to untreated infected or untreated healthy seedlings. The highest levels of phenol content (148.2 mg/100 g fresh weight of tomato leaves) and activity of both polyphenol oxidase (3.8 unit ml⁻¹) and peroxidase (3.4 unit ml⁻¹) were recorded in tomato leaves treated with *T. harzianum* as root dipping + chitosan at 1.0g/l as root dipping followed with chitosan foliar spray at 0.5g/l.

Table 2. Accumulation of phenolics and activity of polyphenol oxidase and peroxidase enzymes extracted from tomato leaves infected with *F. oxysporum* f.sp. *radicis-lycopersici* and treated with *T. harzianum* and chitosan

Treatment*	Phenolics		Polyphenol oxidase		Peroxidase	
	mg/100g (F wt)	Change (%)	Activity unit ml ⁻¹	Change (%)	Activity unit ml ⁻¹	Change (%)
FORL+ TH	96.1d	12.2	0.88a	53.1	1.4b	12.5
FORL+ CHRd 0.1 g/l	92.4d	7.9	0.80a	53.4	1.2	25.0
FORL+TH+CHRd 0.5 g/l	114.8e	34.1	2.10b	11.7	2.4c	50.0
FORL+TH+CHRd 1.0 g/l	124.4 f	45.3	3.16d	68.0	2.7c	68.7
FORL+ TH+CHFA 5.0 g/l	102.0f	19.1	0.92a	51.0	1.8b	12.5
FORL+ CHRd 0.1 g/l +CHFA 5.0 g/l	98.4d	14.9	0.88	53.1	1.4d	12.5
FORL+TH+CHRd 0.5 g/l+CHFA 5.0 g/l	134.2g	56.7	2.48c	31.9	2.8c	75.0
FORL+TH+CHRd 1.0 g/l+CHFA 5.0 g/l	148.2g	85.6	3.68d	95.7	3.4d	112.5
FORL (control ₁) **	85.6a	---	1.88b	---	1.6b	---
Healthy plant (control ₂) ***	74.2b	---	0.70a	---	0.64a	---

*, ** & *** As described in footnote of Table (1).

Infection with *F. oxysporum* f.sp. *radicis-lycopersici* significantly could inhibit chitinase and -1,3glucanase activities when compared with healthy plant (Table 3). When the infected plant treated with *T. harzianum* individually or in combination with chitosan there was insignificant increase in chitinase activity when compared with the infected plant, whereas this increase was significantly less than that in healthy plant. On the other hand, activity of -1,3glucanase increased significantly in the infected plant treated with *T. harzianum* and chitosan particularly when chitosan applied as root dipping at 1g/l and as foliar spray. At this treatment -1,3glucanase recorded 3 unit ml⁻¹ compared with 1.3 and 1.9 unit ml⁻¹ in infected and health plant, respectively.

Table 3. Activity of Chitinase and -1,3glucanase extracted from tomato leaves infected with *F. oxysporum* f.sp. *Radicis-lycopersici* and treated with *T. harzianum* and chitosan

Treatment *	Chitinase		-1,3glucanase	
	unit ml ⁻¹	Change (%)	unit ml ⁻¹	Change (%)
FORL+ TH	1.6a	77.8	2.1b	61.5
FORL+ CHRDR 0.1 g/l	1.4a	55.5	1.8cd	38.4
FORL+TH+CHRDR 0.5 g/l	1.7a	88.9	2.3b	76.9
FORL+TH+CHRDR 1.0 g/l	2.0a	122.2	2.7cd	107.7
FORL+ TH+CHFA 0.5 g/l	1.7a	88.9	2.2b	69.2
FORL+ CHRDR 0.1 g/l +CHFA 0.5 g/l	1.6a	60.0	2.0d	53.8
FORL+TH+CHRDR 0.5 g/l+CHFA 0.5g/l	1.9a	111.1	2.5d	92.3
FORL+TH+CHRDR 1.0 g/l+CFA 0.5 g/l	2.0a	155.5	3.0d	130.7
FORL (control ₁) **	0.9a	----	1.3a	--
Healthy plant (control ₂) ***	2.2b	---	1.9b	--

*, ** & *** As described in footnote of Table (1).

Discussion

One of the major problems concerning the production of food crops is the difficulty of control plant diseases to maintain the high quality and yield which the producer and consumer expect. There is a worldwide trend to explore new alternatives that control plant diseases, giving priority to methods that are efficient, reliable, and safe for environment. Induce host resistance by using bio agents and inducer natural materials seems to be one of alternatives to substitute, or at least to decrease the use of fungicides in plant disease management (Durrant and Dong, 2004 and Cavalcanti *et al.*, 2007).

In the present investigation, the obtained data indicate that all *T. harzianum* and chitosan treatments caused significant reduction in tomato crown and root rot disease incidence and severity caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL). The efficiency of *T. harzianum* and chitosan in controlling *Fusarium* crown and root rot disease of tomato was supported by other workers (Hibar *et al.*, 2007 and Nawar, 2005). *Trichoderma harzianum* and chitosan activated host defense genes led to physical and biochemical changes in plant cells, which were not favorable for disease development. These changes included accumulation of phenolic compounds and increasing activity of host defense enzymes. Our results are in accordance with those reported by (Benhamou and Theriault, 1992; Benhamou *et al.*, 1994; Benhamou *et al.*, 1998 and Sukand and Kulkarni, 2006). In our investigation the highest reduction in disease incidence and severity was accompanied the accumulation of maximum amount of phenolic content in infected tomato leaves treated with *T. harzianum* and chitosan as root dipping in combination with chitosan as foliar application. Benhamou *et al.* (1994) reported that chitosan induced systemic resistance to *Fusarium* infection in tomato seedlings by triggering a hypersensitive-like response at sites of fungal entrance and stimulate rapid accumulation of newly formed macromolecules such as 1,3 glucans,

phenols and ligninlike compounds. Ojha and Chatterjee (2012) 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 (Abo-Elyousr *et al.*, 2009). Phenolics seem inhibits 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 (Chérif *et al.*, 2007). Beckman (2000) 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. Direct correlation between levels of phenolic compounds, polyphenol oxidase (PPO), peroxidase (POD) and glucanase and the resistance to pathogens due to the application of the tested inducers, is frequently observed in this investigation. These results agree with the general speculation, that when plant cells are recruited into stress, there is a switch from the normal primary metabolism to a multitude of the secondary defense pathway and activation of novel defense enzymes and genes take place (Tan *et al.*, 2004). Results obtained by Abd-El-Khair *et al.* (2010) showed that the levels of chitinase, peroxidase and polyphenol oxidase activities highly increased in *Trichoderma* treated bean plant compared with untreated plants. Liu *et al.* (2007) concluded that chitosan treatment induced a significant increase in the activities of polyphenol oxidase (PPO) and peroxidase (POD), and enhanced the content of phenolic compounds in tomato fruits, thus providing protection against both gray and blue mould diseases. The combined application of salicylic acid and *T. harzianum* in *Fusarium* infected tomato plants, enhanced the activities of both PPO and POD and increased the accumulation of phenolic compounds (Ojha and Chatterjee, 2012).

Increased peroxidase activity upon infection might be required for an additional deposition of lignin around the lesions induced by pathogens. Hassan *et al.* (2007) recorded lowest percentages of chocolate spot disease severity when peroxidase activity increased. Furthermore, the phenol oxidizing enzymes oxidize phenols to quinones, which are known to be more reactive and have more antimicrobial activity than the phenols, already exist in plants. Therefore, these enzymes may be directly involved in stopping pathogen development (Melo *et al.*, 2006). Significant increase of endogenous salicylic acid, intercellular chitinase and glucanase activities are examples of induced resistance response in groundnut-treated chitosan (Sukand and Kulkarni 2006). These results agree with our results because chitinase and glucanase activities increased in infected tomato tissues treated with *T. harzianum* individually or in combination with chitosan when compared with the infected untreated control. However, chitinase activity appeared to be inhibited significantly when compared with the healthy seedlings, this may explain the incomplete protection of tomato plant against FCRR disease by using *T. harzianum* and chitosan The remarkable increases of phenolic compounds and activity PPO, POD and glucanase in tomato leaves after exposing to *T. harzianum* and chitosan as seedling root dipping in this study, indicate that the host response is truly systemic and that the physiological

state of the plant has been altered. This finding is in agreement with that reported by Reddy *et al.* (1999) and Panina *et al.* (2007). Naturally all plants are enriched with defense genes. These genes are quiescent and require the appropriate stimulation signals to activate them. It has been reported that biocontrol agents and natural materials activate latent plant defense mechanisms in response to pathogen infection (Kamalakkannan *et al.*, 2004). The current study shows that inducing plant defense mechanisms by applying *T. harzianum* and chitosan particularly in combination could provide promising integrated alternative in suppression of *Fusarium* crown and root rot disease of tomato plants.

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المقاومة المستحثة في نباتات الطماطم ضد
مرض عفن التاج والجذور الفيوزاريومي
باستخدام الفطر ترايكودرما هارزيانم والكيثوزان

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أدت إصابة نبات الطماطم بمرض عفن التاج و الجذور الفيوزارمى الذى يسببه
Fusarium oxysporum f.sp. *radicis-lycopersici* الى حدوث زياده
فى تراكم المواد الفينولية وكان مصاحبا لها زياده فى نشاط كل من أنزيمى عديد
الفينولات المؤكسد و البيروكسيديز مع حدوث انخفاض ملحوظ فى نشاط كل من
أنزيمى الكيتينيز و الجلوكينيز.
T. harzianum و الكيتوزان لمقاومة المرض كان هناك أنخفاضا ملحوظا فى
نسبة حدوث المرض وشدة الإصابة مقرونا بأستحثاث تراكم المواد الفينولية
وكذلك أستحثاث نشاط كل من أنزيمات عديد الفينولات المؤكسد و البيروكسيديز
والجلوكينيز مع تثبيط نشاط انزيم الكيتينيز وذلك عند المقارنه بالنبات المصاب
والغير معامل. وتعتبر المعاملة المزدوجه التى يتم فيها غمر جذور البادرات فى
T. harzianum و الكيتوزان مع رش الأوراق بالكيتوزان من أفضل المعاملات
التي أدت الى أنخفاض بنسبة حدوث المرض وشدة الإصابة وحماية النبات
الإصابة بالمرض بصورة كبيره وذلك من خلال أستحثاث المقاومه الجهازية للعائل
والتي تشمل المواد الفينولية وانزيمات المقاومه فى العائل مثل أنزيمات عديد
الفينولات المؤكسد و البيروكسيديز و الجلوكينيز.