Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 14 (4): 242-249, 2015
ISSN 1812-5387

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ppj.2015.242.249

Physical and Biochemical Integrated Management For Controlling Tomato Wilt Disease Under Field Conditions

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ARTICLE INFO
Article History:
Received: July 12, 2015
Accepted: September 19, 2015

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ABSTRACT
Physical and biochemical integrated management using soil solarization and chitin or chitosan singly or in combination for controlling tomato wilt disease under field conditions was studied. Chitin had no inhibitory effect on the growth of pathogenic fungus. On the other hand, chitosan at 6 g L^{-1} completely inhibit the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici*. Under field conditions, results indicated that maximum soil temperatures in solarized were recorded 55.2, 50.8 and 46.3°C at depths of 1-10, 11-20 and 21-30 cm of soil surface. Solarization was more effective in reducing the pathogen population. The highest reduction in total count of *F. oxysporum* f. sp. *lycopersici* was observed in mulched soil at 1-10 and 11-20 cm depths. Soil solarization and chitin at 6.0 g kg^{-1} soil, chitosan at 6.0 g kg^{-1} soil or topsin at 3.0 g kg^{-1} soil singly or in combination for controlling tomato, wilt disease under field conditions was evaluated. Results revealed that all treatments significantly reduced the disease incidence and severity during two growing seasons. The highest reduction in disease incidence and severity were obtained with combined treatments between soil solarization and chitin, chitosan or topsin which recorded 3.2-5.0% as disease incidence and 0.2-0.3% as disease severity during two growing seasons. As for tomato yield the highest increase in tomato yield was obtained with combined treatments between soil solarization and chitin, chitosan or topsin which increased the tomato yield more than 66.7, 68.9 and 66.7% during two growing seasons. All treatments significantly increased the chitinase activity of tomato plants. The most effective treatments were combined treatments between soil solarization and chitin or chitosan which increased the chitinase activity by 100.0 and 116.7%. It could be suggested that combined treatments between soil solarization and chitin or chitosan as safety materials might be used commercially for controlling tomato wilt diseases under field conditions.

Key words: Soil solarization, chitin, chitosan, tomato wilt-field conditions

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), is one of the major yield limiting factors of tomato (*Solanum lycopersicum*) which causes serious economic and yield losses. Major strategies for controlling this disease are soil fungicides (De Cal *et al*., 2000; Attitalla *et al*., 2001; Ojha and Chatterjee, 2012).

Soil solarization is an environmentally friendly method of using solar power for controlling pests such as soilborne plant pathogens including fungi, bacteria, nematodes and insect and mite pests along with weed seed and seedlings in the soil by mulching the soil and covering it with tarp, usually with a transparent polyethylene cover, to trap solar energy. It may also describe methods of decontaminating soil using sunlight or solar power. This energy causes physical, chemical and

biological changes in the soil. Soil solarization was carried out as transparent polyethylene plastic placed on moist soil during the hot summer months increases soil temperatures to levels lethal to many soil-borne plant pathogens, weeds and nematodes (Abd-El-Kareem *et al*., 2004; Culman *et al*., 2006; Farrag and Fotouh, 2010; Saied-Nehal, 2011).

Chitin and chitosan are naturally-occurring compounds that have potential in agriculture with regard to controlling plant diseases (Abd-El-Kareem and Haggag, 2014). These molecules were shown to display toxicity and inhibit fungal growth and development. They were reported to be active against viruses, bacteria and other pests. Chitosan applied as seed or soil treatments was shown to control *Fusarium* wilts in many plant species (Badawy *et al*., 2005). Chitosan induce host defense responses in both monocotyledons and dicotyledons. These responses include lignification, ion flux variations, cytoplasmic acidification, membrane depolarization and protein phosphorylation, chitinase and glucanase activation, phytoalexin biosynthesis, generation of reactive oxygen species, biosynthesis of jasmonic acid and the expression of unique early responsive and defense-related genes. In addition, chitosan was reported to induce callose formation, proteinase inhibitors and phytoalexin biosynthesis in many dicot species (Uppal *et al*., 2008; Elwagia and Algam, 2014; Mishra *et al*., 2014; Saied-Nehal, 2015).

Chitin was reported as resistance inducer against soilborne diseases (Kuchitsu *et al*., 1993; Bell *et al*., 1998). Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants (Kuchitsu *et al*., 1993; Bell *et al*., 1998; Abd-El-Kareem *et al*., 2006). Based on these and other proprieties that help strengthen host plant defenses, interest has been growing in using them in agricultural systems to reduce the negative impact of diseases on yield and quality of crops.

The purpose of the present work was designed to evaluate the effect of soil solarization and chitin or chitosan singly or in combination for controlling tomato wilt disease under field conditions.

MATERIALS AND METHODS

Source of pathogenic fungus and tomato transplants: Pathogenic isolate of *Fusarium oxysporum* f. sp. *lycopersici* the causal agent of tomato wilt disease was kindly provided by Department of Plant Pathology (Project Integrated management for controlling tomato fungal diseases under Egyptian and Tunisian conditions), National Research Centre, Giza, Egypt. Tomato transplants cv. Kastel rock were obtained from the Department of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Laboratory experiments

Effect of chitin and chitosan on the linear growth and spore germination of *Fusarium oxysporum* f. sp. *lycopersici*: The inhibitory effect of chitin and chitosan (Sigma company) against *F. oxysporum* f. sp. *lycopersici* (FOL) was tested *in vitro* at four concentrations, i.e. 0, 2, 4 and 6 g L^{-1}. 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 culture of *Fusarium oxysporum* f. sp. *lycopersici* then incubated at 25±2°C. Linear growth of tested fungus was measured when the control plates (medium free of chitin or chitosan) reached full growth and the average growth diameter was calculated. As for spore germination test, spore suspension was prepared by culturing FOL on Petri-plates containing PDA medium for 20 days at 25°C. Colony Forming Units (CFU) containing hyphal fragments, microconidia, macroconidia and chlamydospores were released in sterilized water using a needle then adjusted to 10^6 CFU mL^{-1} using haemocytometers slide. One milliliter of suspension was transferred to each test tubes containing sterilized broth PD medium which treated with previous concentrations of culture filtrates. Test tubes were incubated for 24 h at 25°C. One milliliter of treated spore suspension (CFU) was examined microscopy and the average percent of spore germination was calculated. Each treatment was represented by 5 plates as replicates.

Field experiments

Efficacy of soil solarization and chitin or chitosan singly or in combination for controlling tomato wilt disease: The field experiment carried out at the Experimental Research Station of National Research Centre at El-Noubariya region, Behera Governorate, Egypt. Under field conditions, the efficacy of soil solarization and chitin or chitosan singly or in combination for controlling tomato wilt disease was studied.

The field trail conducted in 60 plots, each 12 m^2 (3 m width×4 m length) comprised of 5 rows with 4 m length and 20 transplants/row, established in naturally heavily infested soil with tomato wilt pathogens. All plots were irrigated to field capacity and 30 plots were subjected to solarization treatment which carried out by covering with 100 μm thick transparent polyethylene sheets for 4 weeks during August then removed. The other untreated 30 plots were considered as un solarized soil. Maximum and minimum degrees of soil temperature were regularly measured during that period at the depths of 1-10, 11-20 and 21-30 cm of surface soil. The average temperature at the three soil depths was calculated at the end of mulching period.

Treatments: The following treatments were applied:

- Single treatment:
  - Soil solarization for 4 weeks
  - Chitosan at 6.0 g kg^{-1} soil
  - Chitin at 6.0 g kg^{-1} soil
  - Topsin at 3 g kg^{-1} soil

- Combined treatment:
  - Soil solarization+chitosan at 6.0 g kg^{-1} soil
  - Soil solarization+chitin at 6.0 g kg^{-1} soil
  - Soil solarization+topsin at 3 g kg^{-1} soil
  - Control (Un-treated)

Application

Seed bed treatment: Tomato transplants cv. Kastle rock which grown in foam trays (209 cells) containing peat-moss soil mixed individually with chitin or chitosan at 6.0 g kg^{-1} soil as well as topsin at 3 g kg^{-1} soil. In addition to tomato transplants grown in free soil treatments in foam trays served as control.

Effect of soil mulching on population density of *Fusarium oxysporum* f. sp. *lycopersici*

Preparation of *Fusarium oxysporum* f. sp. *lycopersici* inoculum: Inoculum of *F. oxysporum* f. sp. *lycopersici* was prepared by culturing the fungus on 50.0 mL Potato Dextrose Broth (PDB) medium in 250 mL Erlenmeyer flasks for 15 days at 25°C. Inoculum of *F. oxysporum* f. sp. *lycopersici* was prepared from the growing upper solid layers which washed and blended in sterilized water. Colonies forming units (CFU) were adjusted to 10^6 CFU mL^{-1} using haemocytometers slide. Soil infestation was carried out at rate of 50 mL (10^6 CFU mL^{-1})/kg soil (Elad and Baker, 1985).

Soil infestation: Certain weights of field soil was sterilized with autoclave at 120°C for 1 h and artificially infested with *F. oxysporum* f. sp. *lycopersici*. Soils were mixed individually with the prepared inoculum.. Soil infestation was carried out at rate of 50 mL (10^6 CFU mL^{-1})/kg soil (Elad and Baker, 1985). Artificially infested soils were filled into cloths bags at the rate of 1 kg soil/bag and 40 bags was used. Before soil mulching cloths bags were buried into the field soil at three different levels down below the surface at depths of 1-10, 11-20 and 21-30 cm at three spots of each plot and three plots were used.

After removal the polyethylene sheets, the buried bags of each certain level in either solarized or un-solarized plots were collected and mixed into one mother composite sample for each tested fungus. Total count of pathogenic fungus in solarized and un-solarized soil compared with their count before soil mulching was estimated following the plate count technique (Porras *et al*., 2007).

Determination of total count of pathogenic fungus: Total count of pathogenic fungus was carried out according to the method described by Porras *et al*. (2007) as mentioned before. The resulting colonies were calculated as colonies per gram of dry soil and the reduction was calculated as follow:

$$\text{Reduction} = \frac{\text{No. of colonies in control-No. of colonies in treatment}}{\text{No. of colonies in control}} \times 100$$

Disease assessment: The disease incidence was recorded as percentage of wilted plants after 80 days of transplanting. Meanwhile, The disease severity was recorded by 0-4 scale as described by Biswas *et al*. (2012), where zero represents no infection and four denotes complete infection.

The scale of 0-4 of the disease severity was modified as follows:

- 0-No infection
- 1-Slight infection, which is about 25% of leaves turned yellow and wilted
- 2-Moderate infection, which is about 50% of leaves turned yellow and wilted
- 3-Extensive infection, which is about 75% of leaves turned yellow and wilted
- 4-Complete infection, which is about 100% of leaves wilted and the plants died

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

Determination of tomato yield: Accumulation of tomato yield (kg m^{-2}) for each treatment was determined.

Effect of soil solarization and chitin or chitosan singly or in combination on chitinase activity of tomato plants: The efficacy of soil solarization and chitin or chitosan singly or in combination on chitinase activity of tomato plants was studied.

Extraction of chitinase enzyme: Chitinase activity was determined after 60 days of transplanting. Extraction of enzyme from tomato leaves was done according to method of Tuzun *et al*. (1989).

Chitinase assay: Chitinase activity was determined by colourimetric method of Boller and Mauch (1988). Colloidal chitin was used as a substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as millimolar N-acetylglucose amine equivalent released/gram fresh weight tissue/60 min.

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

RESULTS

Effect of chitin and chitosan on the linear growth and spore germination of *Fusarium oxysporum* f. sp. *lycopersici*: The inhibitory effect of chitin and chitosan against *F. oxysporum* f. sp. *lycopersici* was tested *in vitro* at four concentrations, i.e. 0, 2, 4 and 6 g L^{-1}. Results in Table 1 indicate that chitin has no inhibitory effect on the growth of pathogenic fungus. On the other hand, all concentrations of chitosan had significant inhibitory effect against

Table 1: Linear growth and spore germination of *Fusarium oxysporum* f. sp. *lycopersici* as affected by different concentrations of chitin and chitosan

Treatments and concentration (g L^{-1})	*Fusarium oxysporum* f. sp. *lycopersici*			
	Linear growth (mm)	Reduction (%)	Spore germination (%)	Reduction (%)
Chitin				
2	90.0[a]	0.0	90.0[a]	0.0
4	90.0[a]	0.0	90.0[a]	0.0
6	90.0[a]	0.0	90.0[a]	0.0
Chitosan				
2	41.0[b]	54.4	37.0[b]	61.4
4	19.0[c]	78.9	17.0[c]	82.3
6	0.0[d]	100.0	0.0[d]	100.0
Control				
0	90.0[a]	-	96.0	-

Values with the same letter are not significantly different (p = 0.05)

Table 2: Average of maximum and minimum soil temperatures at different soil depths in mulched and un-mulched soil

	Soil temperature (°C)			
	Mulched soil		Un-mulched soil	
Soil depth (cm)	Maximum	Minimum	Maximum	Minimum
1-10	55.2	40.0	40.5	28.2
11-20	50.8	36.7	37.4	26.0
21-30	46.3	34.6	33.1	22.5

Table 3: Percentage reduction in pathogenic fungus at three depths as affected with mulched and un-mulched soil under field conditions

Treatments	Soil depths (cm)	Reduction in pathogenic fungus (%)
Mulched soil	1-10	78.4
	11-20	71.4
	21-30	60.1
Un-mulched soil	1-10	23.4
	11-20	17.9
	21-30	7.8

F. oxysporum f. sp. *lycopersici*. chitosan at 6 g L^{-1} completely inhibit the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici*. The highest reduction was obtained with chitosan at 4 and 6 g L^{-1} which reduced the linear growth and spore germination by 78.9, 82.3 and 100% in both linear growth and spore germination, respectively. Meanwhile, chitosan at 2 g L^{-1} was less effective.

Effect of soil solarization on soil temperatures: Average of maximum and minimum soil temperatures in solarized and non-solarized soil was recorded during solarization period. Results in Table 2 indicate that maximum soil temperatures in solarized were recorded 55.2, 50.8 and 46.3°C at depths of 1-10, 11-20 and 21-30 cm of soil surface.

Meanwhile in un-solarized soil recorded 40.5, 37.4 and 33.1°C as maximum soil temperatures at three depths, respectively.

Effect on total count of pathogenic fungus: The population density of *F. oxysporum* f. sp. *lycopersici* at three soil depths either in mulched or un-mulched soil was determined. Results in Table 3 indicate that fungal population decreased in both mulched and un-mulched soils at the end of experimental period. However, solarization was more effective in reducing the pathogen population. The highest reduction in total count of *F. oxysporum* f. sp. *lycopersici* was observed in mulched soil at 1-10 and 11-20 cm depths. As for the lower soil depth, 21-30 cm, the pathogen population was reduced by 60.1%. The same trend was also noticed in fallow un-mulched soil at the three similar depths, i.e., 23.4, 17.9 and 7.8%, respectively.

Efficacy of soil solarization and chitosan, chitin or topsin singly or in combination for controlling tomato wilts disease: Soil solarization and chitosan at 6.0 g kg^{-1} soil, chitin at 6.0 g kg^{-1} soil or topsin at 3.0 g kg^{-1} soil singly or in combination for controlling tomato wilt disease under field conditions was evaluated. Results in Table 4 reveal that all treatments significantly reduced the disease incidence and severity during two growing seasons. The highest reduction in disease incidence and severity were obtained with combined treatments between soil solarization and chitin, chitosan or topsin which recorded 3.2-5.0% as disease incidence and 0.2-0.3 as disease severity during the two growing seasons. Meanwhile, single treatments show moderate effect.

Efficacy of integrated treatments between soil solarization and chitosan, chitin or topsin on tomato yield under field conditions: Results in Table 5 soil solarization and chitosan at 6.0 g kg^{-1} soil, chitin at 6.0 g kg^{-1} soil or topsin at 3.0 g kg^{-1} soil singly or in combination on tomato yield under field conditions was evaluated. Results in Table 5 reveal that all treatments significantly increased the tomato yield during the two growing seasons. The highest increase in tomato yield was obtained with combined treatments between soil solarization and chitin, chitosan or topsin which increased the tomato yield more than 66.7, 68.9 and 66.7% during the two growing seasons. Meanwhile, single treatments show moderate increase.

Effect of soil solarization and chitosan, chitin or topsin singly or in combination on chitinase activity of tomato plants: The efficacy of soil solarization and chitosan, chitin or topsin singly or in combination on chitinase activity of tomato plants was studied.

Results in Table 6 indicate that all treatments significantly increased the chitinase activity of tomato plants. The most effective treatments were combined treatments between soil solarization and chitosan or chitin which increased the

Table 4: Efficacy of integrated treatments between soil solarization and chitosan, chitin or topsin on tomato wilt disease under field conditions

	Tomato wilt disease			
	First season		Second season	
Treatments	Disease incidence	Disease severity	Disease incidence	Disease severity
Single treatment				
Soil solarization	12.0	0.50	14.50	0.54
Chitosan (6 g kg^{-1} soil)	10.0	0.50	13.30	0.45
Chitin (6 g kg^{-1} soil)	11.4	0.40	14.00	0.50
Topsin (3 g kg^{-1} soil)	11.0	0.50	14.00	0.50
Integrated treatment				
Soil solarization+chitosan (6 g kg^{-1} soil)	3.2	0.20	4.00	0.20
Soil solarization+chitin (6 g kg^{-1} soil)	4.0	0.20	4.30	0.30
Soil solarization+topsin (3 g kg^{-1} soil)	4.0	0.30	5.00	0.20
Control	46.0	0.80	48.00	0.70

Values with the same letter are not significantly different (p = 0.05)

Table 5: Efficacy of integrated treatments between soil solarization and chitosan, chitin or topsin on tomato yield under field conditions

	Tomato yield (kg $plot^{-1}$)			
	First season		Second season	
Treatments	Yield	Increase (%)	Yield	Increase (%)
Single treatment				
Soil solarization	6.0^{b}	50.0	6.3^{b}	40.0
Chitosan (6 g kg^{-1} soil)	5.2^{c}	30.0	5.4^{c}	20.0
Chitin (6 g kg^{-1} soil)	5.0^{c}	25.0	5.4^{c}	20.0
Topsin (3 g kg^{-1} soil)	4.5^{d}	12.5	5.0^{d}	11.1
Integrated treatment				
Soil solarization+chitosan (6 g kg^{-1} soil)	7.3^{a}	82.5	7.6^{a}	68.9
Soil solarization+chitin (6 g kg^{-1} soil)	7.2^{a}	80.0	7.5^{a}	66.7
Soil solarization+topsin (3 g kg^{-1} soil)	7.1^{a}	77.5	7.5^{a}	66.7
Control	4.0^{e}	-	4.5^{e}	-

Values with the same letter are not significantly different (p = 0.05)

Table 6: Efficacy of integrated treatments between soil solarization and chitosan, chitin or topsin on chitinase activity of tomato under field conditions

	Chitinase activity	
Treatments	Activity	Increase (%)
Single treatment		
Soil solarization	4.0^{d}	33.3
Chitosan (6 g kg^{-1} soil)	5.8^{b}	93.3
Chitin (6 g kg^{-1} soil)	5.8^{b}	93.3
Topsin (3 g kg^{-1} soil)	4.0^{d}	33.3
Integrated treatment		
Soil solarization+chitosan (6 g kg^{-1} soil)	6.5^{a}	116.7
Soil solarization+chitin (6 g kg^{-1} soil)	6.0^{a}	100.0
Soil solarization+topsin (3 g kg^{-1} soil)	4.5^{c}	50.0
Control	3.0^{e}	

Values with the same letter are not significantly different (p = 0.05)

chitinase activity by 116.7 and 100.0%. Meanwhile, single treatments of chitin or chitosan resulted in increasing chitinase activity by 93.3%. Other treatments show moderate increase. While, solarization alone or topsin alone increase chitinase activity by 33.3% only.

DISCUSSION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), is one of the major yield limiting factors of tomato (*Solanum lycopersicum*) which causes serious economic and yield losses (De Cal *et al*., 2000; Attitalla *et al*., 2001; Ojha and Chatterjee, 2012).

Chitosan applied as seed or soil treatments was shown to control *Fusarium* wilts in many plant species (Badawy *et al*., 2005; Saied-Nehal, 2015). Chitosan induce host defense responses in both monocotyledons and dicotyledons (Uppal *et al*., 2008; Elwagia and Algam, 2014; Mishra *et al*., 2014). In the present study results revealed that chitin had no inhibitory effect on the growth of pathogenic fungus. On the other hand, chitosan at 6 g L^{-1} completely inhibit the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici*. In this respect, Kulikov *et al*. (2006) reported that the antimicrobial activity increased with the increase in chitosan molecular weight and seems to be faster on fungi and algae than on bacteria. Fungicidal activity of chitosan has been documented against various species of fungi and oomycetes (Vasyukova *et al*., 2000; El-Mohamedy *et al*., 2013). The minimal growth-inhibiting concentrations varied between 10 and 5,000 ppm (Rabea *et al*., 2005). Some of the derivatives also repressed spore formation at rather high concentrations (Badawy *et al*., 2005). Recently, Palma-Guerrero *et al*. (2009) demonstrated that chitosan is able to permeabilize the plasma membrane of *Neurospora crassa* and kills the cells. In general, chitosan is able to reduce the *in vitro* growth of a number of fungi and oomycetes (Palma-Guerrero *et al*., 2008). For instance, chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and necrotrophic pathogens, such as

Fusarium oxysporum, *Botrytis cinerea*, *Monilina laxa*, *Alternaria alternata* and *Pythium aphanidermatum* (El Hassni *et al*., 2004). The mechanism by which chitosan affects the growth of several pathogenic fungi has not been fully elucidated, but several hypotheses have been postulated, first: its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (El Hassni *et al*., 2004). Second the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis (Vasyukova *et al*., 2000), third the chelating of metals, spore elements and essential nutrients (Rabea *et al*., 2005). Forth the interaction of chitosan with fungal DNA and RNA (Palma-Guerrero *et al*., 2008) and five the malformation of fungal mycelial. Chitosan is not only effective in inhibition the growth of the pathogen fungi, but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells (Barka *et al*., 2004). Moreover, El Hassni *et al*. (2004) reported that, chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea*. Furthermore, Bautista-Banos *et al*. (2006) revealed that by microscopic observation of fungi treated with chitosan, it can affect the morphology of the hyphae.

On the other hands, chitosan induce host defense responses against several plant diseases (Uppal *et al*., 2008; Elwagia and Algam, 2014; Mishra *et al*., 2014; Saied-Nehal, 2015). In the present study, under field conditions results revealed that the highest reduction in disease incidence and severity were obtained with combined treatments between soil solarization and chitin, chitosan or topsin which recorded 3.2-5.0% as disease incidence and 0.2-0.3% as disease severity during two growing seasons. As for tomato yield the highest increase in tomato yield was obtained with combined treatments between soil solarization and chitin, chitosan or topsin which increased the tomato yield more than 66.7, 68.9 and 66.7% during two growing seasons.

All treatments significantly increased the chitinase activity of tomato plants. The most effective treatments were combined treatments between soil solarization and chitin or chitosan which increased the chitinase activity by 100.0 and 116.7%. In this regard, chitosan has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests (Badawy *et al*., 2005; Faoro *et al*., 2008; Elwagia and Algam, 2014). It has also been used to increase yield and tuber quality of potatoes (Kowalski *et al*., 2006). Chitosan had different properties i.e. had inhibitory effect against pathogenic fungus and had ability to be potent elicitors of plant defense resistance.

Chitin was reported as resistance inducer against soil borne diseases (Kuchitsu *et al*., 1993, Bell *et al*., 1998; Abd-El-Kareem and Haggag, 2014). Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants (Kuchitsu *et al*., 1993; Bell *et al*., 1998). In the present study, results indicated that under field conditions results revealed that the highest reduction in disease incidence and severity were obtained with combined treatments between soil solarization and chitin, chitosan or topsin and increased tomato yield. Also, combined treatments between soil solarization and chitin or chitosan increased the chitinase activity by 100.0 and 116.7%.

Soil solarization was carried out as transparent polyethylene plastic placed on moist soil during the hot summer months increases soil temperatures to levels lethal to many soil-borne plant pathogens, weeds and nematodes (Abd-El-Kareem *et al*., 2004; Culman *et al*., 2006; Farrag and Fotouh, 2010; Saied-Nehal, 2011). In the present study, under field conditions results revealed that the highest reduction in disease incidence and severity were obtained with combined treatments between soil solarization and chitin, chitosan or topsin and increased tomato yield. All treatments significantly increased the chitinase activity of tomato plants. The most effective treatments were combined treatments between soil solarization and chitin or chitosan.

In the current study, the population density of FOL was determined in artificially infested soil at three soil depths either in mulched or un-mulched soil.

Reduction in disease incidence and increase in obtained yield due to soil solarization were reported by many investigators (Osman *et al*., 1986). Pullman *et al*. (1981) presented a detailed study on thermal death of four soil-borne plant pathogens as affected by time and temperature of the treatment. They reported that *R. solani* was found to be killed at 50°C in only 10 min as exposure time. In the present study, the recorded maximum soil temperature measured at 1-10, 11-20 and 21-30 cm of soil depths in mulched soil reached 55.2, 50.8 and 46.3°C in average during 6 weeks of mulching period. Several investigators reported the effect of soil solarization in reducing the incidence and severity of plant diseases caused by soil-borne pathogens (Abd-El-Kareem *et al*., 2004; Culman *et al*., 2006; Saied-Nehal, 2011).

It could be suggested that combined treatments between soil solarization and chitin or chitosan as safety materials might be used commercially for controlling tomato wilt diseases under field conditions.

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