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INDUCTION OF SYSTEMIC RESISTANCE AGAINST TOMATO ROOT ROT DISEASE UNDER GREENHOUSE CONDITIONS

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ABSTRACT: Induction of systemic resistance against tomato root rot is considered save and good strategy for controlling this serious disease which lead to delay growth and death of severely infected plants especially under protective production. Sixteen isolates of *Rhizoctonia solani* and ten of *Fusarium solani* were isolated from diseased tomato samples collected from ten Egyptian governorates. The isolated fungi were differed in their virulence on tomato plants. Kafr-El-Sheikh isolates No.16 of *R. solani* and Gharbia isolate No.8 of *F. solani* were significantly more aggressive than the others. All tested antagonistic treatments including bioagents, biocides, chemical inducers and plant oils as well as Vitavax-200 significantly reduced the disease severity under greenhouse conditions. The results also revealed that dipotassium hydrogen phosphate at 2% concentration, Bio-Arc and Bio-Zeid at recommended doses recorded the least disease severity caused by the isolated pathogens, followed by *Trichoderma koningii* (14), *T. harzianum* (15) and *Acinetobacter genospecies* (B40). Other treatments were less effective. Soaking roots of tomato seedling for two hours in most of biotic and abiotic inducers before transplanting in soil infested with the pathogenic fungi increased peroxidase, polyphenol oxidase and chitinase activities at 14 days post inoculation (dpi) to values more than 7 dpi.

Key words: Tomato root rot, biocides, *Trichoderma* spp, abiotic inducers, *Rhizoctonia solani, Fusarium solani*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetables in the world. It occupied the second rank in importance after potato in many countries **(Abd-El Kareem *et al*., 2006)**. It is considered as an important cash and industrial crop in many parts of the world **(Ayandiji and Adeniyi, 2011),** not only because of its economic importance but also its nutritional value to human diet and subsequent importance in human health (**Willcox and Amy, 2003**). It contains high amounts of lycopene which is beneficial in reducing the incidence of some chronic diseases like cancer (**Freeman and Reimers, 2010**).

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Egypt production of tomato was placed fifth with a global tomato production, which constituting 4.86% of all global production in 2014, as well as, it leads the African tomato production, which was 43.11% of total global production. Recently, Egypt production of tomato was increased and reached 8.3 million ton from 214016 hectares with average production of 77.87 ton/hectare through 2014 season **(FAOSTAT, 2017).**

Tomato plants are attacked by many viral, bacterial and fungal diseases causing serious setback to its production **(Linn and Luckmann, 1967)**. Among of these diseases, root rots caused by *R. solani* and *F. solani*. Resistance induction in plants to overcome pathogen infection is a

novel approach for controlling plant diseases. Exogenous or endogenous factors could substantially affect host physiology, leading to rapid and coordinated activation of defense-genes in plants known to express susceptibility to pathogen infection **(Mandal *et al.,* 2009)**. Moreover, induced resistance can be achieved by application of various abiotic agents (chemical inducers) such as salicylic acid, potassium salts and sorbic acid (**Akram and Anjum, 2011 ; El-Mohamedy *et al*., 2014**). In addition, field application of these inducers have increased growth, yield components and quality of fruits in many vegetable plants **(Karlidag *et al.,* 2009; Zahra *et al.,* 2010).** The objective of the present work was to evaluate the inhibitory activity of some biocides and alternatives of fungicide on the growth of *R. solani* and *F. solani* the causal pathogens of root rot disease in greenhouse.

MATERIALS AND METHODS

Isolation and Identification of Root Rot Pathogens

Samples of tomato plants exhibited root rot symptoms were collected from different locations at different governorates along Egypt for isolation of the causal pathogens of root rot (Table, 2). Samples washed under tap water and sterilized by immersing in 5% sodium hypochlorite for 2 minutes **(Waller, 1981)** and subsequently rinsed with sterile distilled water. Thin sections were placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 25±2°C, for five days. Each of the emerged fungi was picked up and purified by transferring the hyphal tip into PDA plates and identified at the Unit of Identification of Microorganisms, Plant Pathology Research Institute, ARC, Giza, Egypt according to their morphological characters using light microscope **(Gilman 1957, Booth 1971 and Singh 1982)**.

Pathogenicity Tests of the Isolated Fungi under Greenhouse Conditions

The pathogenic potential of purified 26 isolated fungi (16 *R. solani* and 10 *F. solani*) were tested on tomato Super Strain B susceptible plant cultivar in greenhouse conditions at the Unit of Identification of Microorganisms, at PPRI, ARC, Giza, Egypt. Inoculum of the *R. solani* and *F. solani* were grown on sorghum grain sand medium **(Akram and Anjum, 2011)** for 15 days at 25±2°C. Clay pots (25 cm in diameter) were soaked in 5% formalin solution for 10 min and left to dry in open air for two weeks. The inoculum of *R. solani* and *F. solani* at the rate of 3-5% (*W/W*) were mixed with the soil to ensure the distribution of the tested pathogens then watered daily for one week. Three tomato seedlings (4-5 weeks old) were transplanted into plastic pots (25 cm. in diameter) filled with infested 2 Kg of each soil mixture (clay and sand at rate of 2:1 *W/W*). In control treatment, soil was mixed with the same amount of non-inoculated sterilized sorghum grains. A set of three replicates was used for each treatment.

Disease Assessments

Disease severity of root rot was estimated based on a 0-5 scale depending on the degree of root browning on the whole root system whereas: 0 = no symptom, 1 = 0-25% of root browning, 2 = 26-50% of root browning, 3 = 51-75% of root browning, 4 = 76-100% of root browning, and 5= plant death (**Abdeljalil *et al.,* 2016**).

Control Studies

Isolation of microorganisms

The identified antagonistic microorganisms (fungi and bacteria) obtained from PPRI unite and were previously isolated from healthy tomato plants rhizosphere, collected from different infested fields locations mentioned before.

Greenhouse Experiments

Effect of biocides, fungicide, and bioagents, against *R. solani* and *F. solani* of tomato plants under greenhouse conditions

The antagonistic potential of the most effective bacterial and fungal isolates, tested *in vitro* was evaluated on (Super Strain B cv.) tomato plants infected with root rot nonpathogenic fungi genera *R. solani* and *F. solani*. The antagonistic fungi (10 days old) and bacteria (3 days old) were grown on (PDA) broth medium and Luria-Bertani (LB) broth medium, respectively. The antagonistic spore suspension (6×10^6 spore/ml) of fungal isolates and bacterial cell suspension (10^8 cfu/ml) were prepared

(Abou-Zeid *et al*., 2003). The effect of the biocides Bio-Arc® and Bio-Zeid®, (commercial biocides labeled on different crops in Egypt (2.5 g/liter) and the fungicide Vitavax 200 (1.5 g/L) were tested on disease incidence severity under greenhouse conditions. All tested treatments were applied as tomato root dipping for 2 hr., before transplanting seedlings in clay pots containing infested soil at the rate of 3 seedlings/pot and three replicates/treatment. The check treatment (un-infested soil) was considered. Root rot severity was estimated at 10 day intervals up to 60 days after transplanting, using a rating scale of 0 - 5 scale according to **Abdeljalil *et al.* (2016)**.

Identification of the microorganisms

The most effective antagonistic bacteria and purified fungal isolates were identified using Biolog-System technique **(Anonymous, 1993 and 2010)**, belonging to the Identification of Microorganisms Unit, PPRI, ARC, Giza, Egypt.

Effect of inducers on *R. solani* and *F. solani* infected tomato plants in greenhouse conditions

Tomato seedlings (cv. Super Strain B) were soaked for 2 hours before planting in the most effective concentrations of each tested inducers including dipotassium hydrogen phosphate, potassium sorbate 8.0%, chitosan 0.1%, salicylic acid and ascorbic acid 0.1%, as well as the clove oil and mint oil 0.5%. Three seedlings were sown in each pot contained infested soil with *R. solani* and *F. solani* and three pots were used for each treatment as replicates. In control treatment, untreated tomato seedlings were planted in infested soil. Root rot severity was estimated two months after transplanting, disease severity on collars and roots were estimated based on scale of 0-5 **(Abdeljalil *et al.,* 2016)**.

Enzymatic Studies

Determination of oxidative enzymes and hydrolytic enzymes in tomato root under greenhouse conditions

Samples from the most effective treatments in decreasing disease severity from each group such as (T14) *Trichoderma koningii and* (T15) *Trichoderma harzianum*, (B5) *Acinetobacter calcoaceticus,* (B40) *Acinetobacter genospecies,* salicylic acid, ascorbic acid, dipotassium hydrogen phosphate, chitosan, clove oil, mint oil, Bio-Arc®, Bio- Zeid® and Vitavax 200 in addition to the infected and uninfected control that were chosen for assaying the different oxidative enzymes (peroxidase, polyphenol oxidase) and hydrolytic enzymes (chitinase). The collected root tissues from each particular treatment at 7 and 14 days post inoculation with the different materials were homogenized immediately using liquid nitrogen **(Ojha and Chatterjee 2012)**. One gram of powdered sample was extracted with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged at 4°C for 20 min at 4000 rpm. The crude extract was used to estimate the peroxidase, polyphenol oxidase and chitinase activities **(Anand *et al*., 2007)**.

Peroxidase activity (PO)

Peroxidase assay (based on oxidation of pyrogallol to purpyrogallin in the presence of H_2O_2) was determined according to **Hartee (1955)**. Enzyme activity was expressed as the change in the absorbance of the reaction mixture min^{-1} g^{-1} on a fresh weight according to **Hammerschmidt *et al*. (1982)**.

Polyphenol oxidase activity (PPO)

Activity of PPO was determined following **Mayer *et al.* (1965)** method. The activity was expressed as changes in absorbance at 495 nm min^{-1} g^{-1} fresh weight of tissue.

Chitinase activity

Chitinase activity was assayed according to **Miller (1959)**. The enzyme activity was expressed as µmoles GLc NAc g/ml at 575 nm^{-1} g^{-1} fresh weight of tissue.

Preparation of Dinitrosalicylic (DNS) Reagent

Quantities of 1 g of DNS, 200 mg of crystalline phenol and 50 mg of sodium sulphite were dissolved simultaneously in 1% solution of NaOH by stirring. The reagent was stored in a stopper bottle at 4°C. The reagent deteriorates during storage due to atmospheric oxidation of the sulphite present.

Statistical Analysis

Statistical analysis was done using analysis of variance, (ANOVA) according to **Sneddecor and Cochran (1980)**.

Table 1. Active ingredients of fungicide, biofungicides and inducer chemical structure inducers and the most active Trichoderma isolates

Tested product	Active ingredient	Rate
Bio- Arc®	*Bacillus megaterium* 6% (*W/W*)	2.5 g/l
Bio- Zeid®	*Trichoderma album* 2.5 % (*W/W*)	2.5 g/l
Vitavax 200	Carboxin + Thiram WP 75%	1.5 g/l
Salicylic acid	$C_7 H_6 O_3$	0.1% *W/V*
Ascorbic acid	$C_6H_8O_6$	0.1% *W/V*
Dipotassium hydrogen phosphate	$K_2 H PO_4$	8.0% *W/V*
Potassium sorbate	$C_6H_7KO_2$	8.0% *W/V*
Chitosan	Natural polysaccharide	0.1% *W/V*
Mint oil	Menthol, 48%	0.5% *W/V*
Clove oil	Eugenol, 90–95%	0.5% *W/V*
***Trichoderma koningii* (T14)**	Fungal sp.	10^7spore/ml
***Trichoderma harzianum* (T15)**	Fungal sp.	10^7spore/ml
***Acinetobacter calcoaceticus* (B5)**	Bacterial sp.	10^8 cfu cell/ml
***Acinetobacter genospecies* (B40)**	Bacterial sp.	10^8 cfu cell/ml

RESULTS

Isolation and Identification of Root Rot Disease Pathogens

The isolated fungi, recovered from root-rot symptoms of tomato plants collected from various locations were purified and identified as *Rhizoctonia solani* Kuhn and *Fusarium solani* Mart.

Pathogenicity Test of the Isolated Fungi under Greenhouse Conditions

Table 2 reveal that the 16 isolates of *R. solani* are being aggressive causing the highest severity of root rot. Isolate No. 16 obtained from Kafer El-Sheikh (Sakha) was significantly the most aggressive that gave the highest D.S (5.00). However, isolate No. 7 obtained from El-Minya produced the least D.S recording 2.83.

Results in Table 3 show that the ten isolates of *F. solani* caused low disease severity compared to *R. solani* isolates. The isolates No. 7 and 8 recovered from El-Qualubia (Miet-Kinana) and El-Gharbia (Tanta) sample, respectively were more significantly aggressive than the others isolated from the concerned governorate, that gave the highest D.S (4.50 and 4.50), respectively. However, the least virulent isolate was No. 5 obtained from Giza (Badrasheen), that exhibit the least D.S (3.00).

Biocontrol Studies

Identification of the antagonistic microorganisms

The most effective bacterial and fungal isolates proved to be of highly antagonistic ability *in vivo* were identified as *Acinetobacter calcoaceticus* (B5), *Acinetobacter genospecies* (B40), *Trichoderma koningii* (T14) and *Trichoderma harzianum* (T15) using Biolog-System technique.

Effect of biocides and fungicide in control of root rot

In greenhouse conditions trail, the most five active antagonistic isolates of Trichoderma fungi, bacteria and two biocides (Bio-Arc®, Bio-Zeid®) compared to Vitavax 200 fungicide were examined for their control effects against tomato

Table 2. Pathogenicity of *Rhizoctonia solani*, causing root rot of tomato seedling under greenhouse conditions using scale (0-5)

Isolate	Governorate	Location	Root rot D.S*
1	**Giza**	Badrasheen	4.83
2	**Behaira**	Nubaria	3.50
3	**Qualubia**	Miet-Kinana	4.50
4	**Qualubia**	Kaha	4.50
5	**Garbia**	Gmaiza	4.00
6	**Monofia**	Sadaat	3.83
7	**Minya**	Dayrout	2.83
8	**Minya**	Samalout	3.83
9	**Sharkia**	Menia-Elamh	3.67
10	**Monofia**	Shebin -El kom	3.50
11	**Ismailia**	Ismailia	4.33
12	**Qualubia**	Toukh	3.50
13	**Garbia**	Tanta	3.00
14	**Faiuom**	Snoors	3.33
15	**Faiuom**	Faiuom	3.83
16	**Kafr-El Sheikh**	Sakha	5.00
Control			1.00
LSD at 5%			1.080

D.S*: Disease severity scale ranging from 0-5 according to **Abdeljalil *et al.* (2016)**

Table 3. Pathogenicity of *Fusarium solani* causing root rot of tomato seedling under greenhouse conditions

Isolate	Governorate	Location	Root rot D.S
1	**Monofia**	**Shebin -El kom**	3.17
2	**Sharkia**	**Menia-Elamh**	3.67
3	**Faiuom**	**Snoors**	3.83
4	**Beni Swief**	**Ehnycia**	3.67
5	**Giza**	**Bdrasheen**	3.00
6	**Qualubia**	**Kaha**	3.50
7	**Qualubia**	**Miet-Kinana**	4.50
8	**Garbia**	**Tanta**	4.50
9	**Garbia**	**Gmaiza**	4.33
10	**Ismailia**	**El Tall-Al Kaber**	4.17
Control			1.00
LSD at 5%			0.441

D.S*: Disease severity scale ranging from 0-5 according to **Abdeljalil *et al.* (2016)**.

root rot. Results in Table 4 indicat that treated tomato seedlings with bacterial and antagonists decreased root rot severity compared to control. Fungicides (Vitavax 200), Bio- Arc® and Bio-Zeid® were the most effective for controlling root-rot that recorded 1.66, 2.2 and 2.33 and 1.0, 2.0 and 2.33 against *R. solani* and *F. solani*, respectively. Meanwhile, all tested bioagent were effective in decreasing disease severity of root-rot. *Trichoderma koningii* (T14) showed low root rot severity followed by T15, on the other hand, B40 and B5 showed (2.66, 3.0, 3.33 and 3.33) and (2.33, 2.33, 2.66 and 3.0) against both pathogens *R. solani* and *F. solani*, respectively.

Effect of different inducer resistance against *R. solani* and *F. solani* of tomato plants

In greenhouse conditions trial, dipotassium hydrogen phosphate, potassium sorbate, salicylic acid, ascorbic acid, clove oil, mint oil, and chitosan were tested for improving resistance against *R. solani* and *F. solani*. Results in Table 5 show that dipotassium hydrogen phosphate (8.0% concentration) was the most effective salt in controlling tomato root rot caused by both *R. solani* and *F. solani* that recorded (1.6 and 1.3) respectively. On the other hand, salicylic acid at 0.05% concentration was the most effective organic acid which recorded (3.33 and 3.0) respectively. Regarding plant oils, Mint oil (0.5%) was more effective than clove oil in controlling tomato root rot. Meanwhile, the best effective Polysaccharide derivative (chitosan) at 0.1% recorded (4.0 and 3.33), respectively, compared to control. It is clear from the obtained results that potassium sorbate was the least effective on the disease incidence caused by both fungi genera.

Enzymes Study

Effect of soaking tomato roots with biotic and abiotic resistance inducers on enzyme activities in soil infested with *F. solani* and *R. solani* under greenhouse conditions

In greenhouse conditions trial, tomato roots were soaked in suspensions of biotic and abiotic inducers and transplanted in pots infested with *F. solani* and *R. solani*. The activities of oxidative enzymes; peroxidase, polyphenol oxidase, and hydrolytic enzyme (Chitinase) were determined 7 and 14 days after soaking with inducers.

Peroxidase activities

Results in Table 6 reveal that, the activity of peroxides increased greatly at 7 days in inoculated roots with *R. solani* and treated with *Acinetobacter calcoaceticus* and *Trichoderma koningii* followed by Bio-Zeid®. The *A. calcoaceticus* was the best effective in increasing the activity of peroxides at both 7 and 14 days in inoculated roots with *F. solani* compared with other treatments. Moreover dipotassium hydrogen phosphate, salicylic acid and ascorbic acid treatments greatly increased the activity of peroxidase at 7 days in roots inoculated with *F. solani*. Meanwhile, the activity of peroxidase was greatly increased in both inoculated roots with *F. solani* and *R. solani* , at 14 days, after soaking tomato roots with the most of tested inducers. The highest activity of peroxidase was recorded at 14 days in roots inoculated with *R. solani* after soaking of tomato roots with salicylic acid followed by with mint oil then by dipotassium hydrogen phosphate compared with other treatments. While, increased activity of peroxidase, at 14 days, with *F. solani* infected plants, was recorded in roots soaked with salicylic acid and Bio-Arc® compared with other treatments. On the other hand, the least peroxidase activity was recorded at 7 and 14 days, in roots infected with *R. solani* and soaked with ascorbic acid and *A. calcoaceticus*. *T. harzianum* and *A. genospecies* treatment also reduced the activity of peroxidase at the same periods, respectively in roots inoculated with *F. solani*. Generally, most of biotic and abiotic treatments in tomato roots inoculated with *R. solani* increased peroxidase active to values more than those inoculated with *F. solani*.

Polyphenol oxidase activity

Results in Table 7 show that, the activity of Polyphenol oxidase enzyme was increased in roots infected with *R. solani* at 7 days after treatment with Bio- Zeid® and *A. calcoaceticus* followed by dipotassium phosphate. *A. calcoaceticus* and dipotassium phosphate were more effective inducers as it recorded the highest increase in polyphenol oxidase activity at 7 days interval in roots infected with *F. solani*

Table 4. Effect of biocides, fungicide, bacterial and Trichoderma isolates on tomato root rot disease severity caused by *R. solani* and *F. solani* under greenhouse conditions

Treatment	Root rot D.S	
	R. solani	*F. solani*
***Acinetobacter calcoaceticus* (B5)**	3.33	3.00
Bacteria sp. (B7)	4.66	3.33
Bacteria sp. (B38)	5.60	4.00
Bacteria sp. (B49)	3.66	3.33
***Acinetobacter genospecies* (B40)**	3.33	2.66
***Trichoderma* sp. (T3)**	3.33	2.66
***Trichoderma* sp. (T8)**	3.33	3.00
***Trichoderma* sp. (T10)**	3.66	2.66
***Trichoderma koningii* (T14)**	2.66	2.33
***Trichoderma harzianum* (T15)**	3.00	2.33
Bio- Arc®	2.20	2.00
Bio- Zeid®	2.33	2.33
Vitavax-200	1.66	1.00
Control (infected)	5.50	5.00
Control (uninfected)	0.00	0.00
LSD at 5%	0.48	0.35

D.S*: Disease severity scale ranging from 0-5 according to **Abdeljalil *et al.* (2016)**.

Table 5. Effect of inducer resistance (chemicals, oils and Polysaccharide) on root rot disease incidence caused by *R. solani* and *F. solani* under greenhouse conditions

Inducer		Root rot D.S	
		R. solani	*F. solani*
(Salt)	**Dipotassium hydrogen phosphate**	1.60	1.30
	Potassium sorbate	5.50	3.66
(Acid)	**Salicylic acid**	3.33	3.00
	Ascorbic acid	4.00	4.00
(Plant oils)	**Clove oil**	4.00	3.00
	Mint oil	3.33	3.00
polysaccharide	**Chitosan**	4.00	3.33
Control (infected)		5.50	5.00
Control (uninfected)		0.00	0.00
LSD at 5%		0.57	0.51

D.S*: Disease severity scale ranging from 0-5 according to **Abdeljalil *et al.* (2016)**.

Table 6. Effect of biotic and abiotic inducers tested on peroxidase activity of tomato roots inoculated with *F. solani* and *R. solani*

Treatment	Enzyme activity (as optical density)			
	R. solani		*F. solani*	
	7 days	14 days	7 days	14 days
Acinetobacter calcoaceticus	2.258	2.048	2.515	2.400
Acinetobacter genospecies	0.864	2.424	0.647	0.272
Trichoderma koningii	1.082	3.035	0.620	0.767
Trichoderma harzianum	2.65	2.544	0.476	0.548
Dipotassium hydrogen phosphate	0.895	4.843	0.748	0.808
Salicylic acid	0.530	5.939	0.723	1.100
Ascorbic acid	0.293	3.712	0.713	0.878
Clove oil	0.679	4.075	0.531	0.651
Mint oil	0.740	5.035	0.647	0.812
Chitosan	0.794	3.584	0.671	0.904
Bio- Arc®	0.756	4.800	0.698	1.086
Bio- Zeid®	1.572	3.824	0.512	0.952
Vitavax 200	0.716	4.768	0.699	1.005
Control (infected)	0.440	2.200	0.322	0.544
Control (uninfected)	0.261	2.080	0.212	0.424
LSD at 5%	0.323	0.727	0.221	0.136

Table 7. Effect of biotic and abiotic inducers on polyphenol oxidase activity in inoculated roots with *F. solani* and *R. solani*

Treatment	Enzyme activity (as optical density)			
	R. solani		*F. solani*	
	7 days	14 days	7 days	14 days
Acinetobacter calcoaceticus	2.656	1.664	0.8505	0.723
Acinetobacter genospecies	0.454	1.776	0.558	0.312
Trichoderma koningii	0.550	1.600	0.572	0.354
Trichoderma harzianum	2.040	1.560	0.604	0.420
Dipotassium hydrogen phosphate	2.176	5.186	0.654	0.424
Salicylic acid	1.978	4.251	0.630	0.558
Ascorbic acid	0.504	2.416	0.548	0.422
Clove oil	2.110	3.899	0.434	0.261
Mint oil	0.560	3.129	0.563	0.434
Chitosan	0.621	1.840	0.514	0.422
Bio- Arc®	1.136	3.352	0.592	0.543
Bio- Zeid®	3.051	3.086	0.540	0.432
Vitavax 200	1.022	3.328	0.476	0.442
Control infected	0.725	0.956	0.577	0.530
Control uninfected	0.400	0.884	0.346	0.236
LSD at 5%	0.454	0.609	0.021	0.021

followed by salicylic acid compared with uninfected control. The activities of polyphenol oxidase were increased in roots inoculated with *R. solani* at 14 days interval in roots soaked with most of biotic and abiotic treatments. The highest activity of polyphenol oxidase was recorded at 14 days interval in roots inoculated with *R. solani* and soaked in dipotassium phosphate and salicylic acid followed by clove oil. However, the maximum treatment activity of polyphenol oxidase with *F. solani* was recorded. *A. genospecies* and salicylic acid was also increased at 14 days followed by Bio-Arc® compared with uninfected control and other treatments.

On the other hand, the least activity of polyphenol oxidase was recorded at 7 and 14 days of inoculated roots with *R. solani* soaked with *A. genospecies* and *T. harzianum*, respectively, compared with uninoculated control and other treatments. The least activity of polyphenol oxidase was recorded at 7 and 14 days in infected roots with *F. solani* soaked with clove oil compared with other treatments. All biotic and abiotic inducers increased activities of polyphenol oxidase enzyme at 7 days in infected roots with *F. solani* more than at 14 days. Generally, most of biotic and abiotic treatments of tomato roots inoculated with *R. solani* increased polyphenol oxidase activities to values more than inoculated with *F. solani.*

Chitinase activity

Results in Table 8 reveal that soaking tomato roots with different biotic and abiotic agents affect the activity of a chitinase in soaked roots. In this respect, all biotic and abiotic agents increased the activity of chitinase at 14 days of roots inoculated with either *F. solani* or *R. solani* greater than those after 7 days post inoculation with inducers. The maximum activity of chitinase was recorded after 7 and 14 days in roots inoculated with *R. solani* and soaked in dipotassium hydrogen phosphate followed by *T. harzianum* if compared with the other agents and control one. At 7 days, the highest activity of chitinase was recorded with dipotassium hydrogen phosphate in roots inoculated with *F. solani* followed by salicylic acid. While, clove oil was more effective inducer as it recorded the highest increase in chitinase activity at 14 days roots inoculated with *F. solani* followed by salicylic acid. On the other hand, the least activity of chitinase was recorded at 7 days in roots inoculated with *R. solani* and soaked with *A. genospecies* and ascorbic acid if compared with control and other agents. However, the least activity of chitinase at 14 days was observed in *R. solani* inoculated roots and soaked with either *A. genospecies* or *A. calcoaceticus*. Meanwhile, *T. harzianum* and *A. genospecies* treatments reduced the activity of chitinase at 7 and on 14 days in *F. solani* inoculated roots compared with control and other agents.

DISCUSSION

The results of the current study idicated that 16 fungal isolates of *R. solani* and 10 of *F. solani* the causal pathogens of root-rot disease were isolated from diseased tomato plants of twelve governorates. These findings conformed with pervious one obtained by **Haggag and El-Gamal (2012),** who reported that *R. solani* and *F. solani* isolates were the most common soilborne pathogenic fungi isolated from tomato plants at different governorates in Egypt.

Obtained isolates of *R. solani* and F. *solani* were aggressive causing the highest disease severity of root rot. The results showed that Kafer El-Sheikh (Sakha) isolate No. 16 and Gharbia (Tanta) isolate No. 8, respectively were most aggressive isolates that gave the highest disease severity. The least virulent isolates were of Gharbia (Tanta) isolate No. 13 and Giza (Badrasheen) isolate No. 5, respectively. These results are in harmony with those obtained by **Mohammed *et al.* (2016)** and **Rashid *et al.* (2016)** who reported that *R. solani*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicis-lycopersici, F. solani* and *F. semitectum* have a pathogenic effect on tomato plants under greenhouse conditions.

In this trial, five fungal *Trichoderma* and bacterial antagonistic isolates, two biocides (Bio- Arc®, Bio- Zeid®) and one fungicide (Vitavax200) as control were tested for their antagonistic potential effects against root rot disease. Vitavax200, Bio- Arc®, Bio- Zeid® were the most effective in controlling *R. solani* and *F. solani* root-rot disease recording least disease severity followed by T4, T5, B5 and B1,

Table 8. Effect of different biotic and abiotic inducers on chitinase activity in inoculated roots with *F. solani* and *R. solani* at different periods under greenhouse conditions

Treatment	Enzyme activity			
	R. solani		*F. solani*	
	7 days	14 days	7 days	14 days
Acinetobacter calcoaceticus	0.0049	0.0066	0.0076	0.0097
Acinetobacter genospecies	0.0043	0.0051	0.0041	0.0049
Trichoderma koningii	0.0049	0.0266	0.0082	0.0092
Trichoderma harzianum	0.0114	0.0629	0.0040	0.0051
Dipotassium hydrogen phosphate	0.0194	0.0752	0.0076	0.0097
Salicylic acid	0.0070	0.0252	0.0075	0.0102
Ascorbic acid	0.0043	0.0114	0.0070	0.0084
Clove oil	0.0087	0.0287	0.0061	0.0123
Mint oil	0.0067	0.0234	0.0049	0.0054
Chitosan	0.0043	0.0106	0.0051	0.0053
Bio- Arc®	0.0052	0.0245	0.0057	0.0071
Bio- Zeid®	0.0086	0.0156	0.0051	0.0064
Vitavax 200	0.0063	0.0075	0.0048	0.0052
Control (infected)	0.0052	0.0109	0.0047	0.0043
Control (uninfected)	0.0045	0.0096	0.0046	0.0039
LSD at 5%	0.00355	0. 00322	0.00205	0.00221

respectively. These results are in agreement with **Al-Sohaibani *et al*. (2011)** and **Ismail (2017)** who reported that two biocides (Bio- Arc®, Bio-Zeid®) were significantly effective in controlling root-rot disease incidence of tomato compared with control. Bio-Zeid® was the most effective in this regard compared with other biocides.

In this trial, different inducers *i.e*., dipotassium hydrogen phosphate, potassium sorbate, salicylic acid, ascorbic acid, clove and mint oils, and chitosan were tried against root rot disease caused by *R. solani* and *F. solani in vivo*. All tested treatments significantly reduced root rot severity caused by the pathogenic isolates. Dipotassium hydrogen phosphate was the most effective treatment in controlling root rot disease, followed by salicylic acid as organic acid and mint as plant oil. Chitosan was the best effective polysaccharide derivative in control compared to check. Meanwhile, potassium sorbate was the least effective treatment against the two pathogenic fungi. These results are in agreement with the previous findings of **Ragab *et al.* (2012)** who reported that chemically induced plant resistance against root rot pathogens infecting many crops, as salicylic acid signaling the systemic expression of a broad spectrum and long-lasting resistance that is being efficient against fungi and bacteria. Chemical inducers might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes and other metabolites (**Abdel-Monaim *et al.*, 2011**). Some chemical inducers have a direct antimicrobial effect and is involved in cross-linking in cell walls, induction of gene expression, hypersensitive cell death, phytoalexin

production and induced systemic resistance **(Abdel-Monaim, 2010).** Chitosan is known to induce reactions locally and systemically that involve the activation and accumulation of defenses-related antimicrobial compounds and proteins **(Bhaskara *et al.,* 1999).**

Although chitosan could decrease plant disease through direct toxicity or chelation of nutrients and minerals from pathogens. Also, chitosan affect biopolymer properties, this compound can also form physical barriers around the penetration sites of pathogens, preventing them from spreading to healthy tissues **(Chung *et al.,* 2003)**. The obtained results indicated that soaking tomato roots in different biotic and abiotic inducers stimulate some defense mechanisms including the activity of oxidative enzymes as peroxidase and polyphenol oxidase and hydrolytic enzymes as chitinase, related to plant defense mechanism against pathogens infection caused by *F. solani* and *R. solani* compared with infected and uninfected checks. These results are in agreement with those reported by **Seo *et al.* (2012) and Surekha *et al.* (2014)** denoting that, the use of *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. as bioagents induced of defense enzymes such as chitinase as mention before. Also, biocides Bio- Arc®, Bio- Zeid® and Plant guard as currently reported along with plant extracts *Eucalyptus globules*, *Allium cepa* and *Mentha viridis* gave the highest valuse in enzyme activity **(Ayoubi and Soleimani, 2015 ; Ismail, 2017)**. Treated tomato transplants and basil seeds with different inducers (oxalic acid, salicylic acid, ascorbic acid, potassium chloride and dipotassium phosphate) resulted in an increase in peroxidase and chitinase activity **(Al-Sohaibani *et al.*, 2011)**. Induced defense reactions in plants are highly correlated with enzymatic responses, including accumulation of peroxidase, polyphenol oxidase chitinases, β-1,3-glucanases and phenolic compounds, induction of lignification and synthesis of phytoalexins **(Sekiguchi *et al.,* 1994)**. Oxidative enzymes, as peroxidase and polyphenol oxidase enhance formation of lignin, oxidation of phenols to more toxic quinones, while other oxidative phenols contribute in formation of defense barriers for cell structure (**Avdiushko *et al.*, 1993**).

Peroxidase activity was shown to increase due to treatment tomato roots with biotic and abiotic inducers compared with untreated control. The highest increase of peroxides activity was recorded in inoculated plants with *R. solani* and soaking tomato roots with biocides (B5, T40 and Bio-Zeid®) and chemicals (salicylic acid, mint and dipotassium hydrogen phosphate) at both 7 and 14 days after inoculation. Meanwhile, B5 was the best effective one in increasing the activity of peroxides at both intervals in roots inoculated with *F. solani* followed by chemicals as dipotassium hydrogen phosphate, salicylic and ascorbic acids in both periods, respectively. Results obtained in the current study are in agreement with those reported by **Adam *et al.* (1999)** who mentioned that peroxidase plays an important role in biosynthesis of ethylene, regulation of auxin and plant cell wall components *viz*., lignin, suberin and wall thickening as part of defense response to pathogens particularly fungi. Peroxidases are involved in the lignification, polymerization of hydroxy proline-rich glycoproteins, and regulation of cell wall elongation **(Yoshida *et al.*, 2003)**. The highest polyphenol oxidase activity was obtained when tomato was inoculated with *R. solani* and soaked tomato roots in Bio-Zeid®, B1 and chemicals as dipotassium phosphate, salicylic acid and clove oil at 7 and 14 days after inoculation with inducer treatments, respectively. Meanwhile, soil infested with *F. solani* and tomato roots soaked in B1, dipotassium phosphate, salicylic acid, B5 and Bio-Arc® exhibit the highest polyphenol oxidase activity at 7 and 14 days, respectively. These results are in agreement with **Newman *et al.* (2011)** who mentioned that polyphenol oxidase involved in the lignification and catalyzing the oxygen dependent oxidation of phenols to quinines, protection of tissue from damage and infection by pathogenic fungi.

Chitinase activity was considerably increased by time after inoculation. The highest value of enzyme activity was recorded up to the 14 days in infected tomato plants previously inoculated with *R. solani* and *F. solani*, when treated by either dipotassium hydrogen phosphate or T15 and clove oil and salicylic acid, respectively. Also, **Velazhahan *et al.* (2003)** reported that chitinases hydrolyze chitin which is major

component of fungal cell walls, leading to direct inhibition of growth of several fungi. Chitosan affects various physiological responses like plant immunity, defense mechanisms involving various enzymes such as, polyphenol oxidase, tyrosine ammonia lyase and antioxidant enzymes **(Katiyar *et al*., 2015)**.

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استحثاث المقاومة الجهازية ضد مرض عفن جذور الطماطم تحت ظروف الصوبة

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يعتبر استحثاث المقاومة ضد أعفان جذور الطماطم إحدى الإستراتيجيات الجيدة والآمنة لمقاومة مثل هذا المرض الخطير الذى يؤدى إلى تأخر نمو النباتات أو موتها في حالة الإصابة الشديدة خاصة تحت ظروف الزراعة المحمية, تم عزل ١٦ عزلة من الريزوكتونيا سولانى و١٠ عزلات من فيوزاريوم سولانى من عينات نباتات طماطم مصابة تم جمعها من عشر محافظات مصرية، اختلفت هذه العزلات فى شراستها المرضية, كانت أشد العزلات شراسة هي العزلة رقم ١٦ للريزوكتونيا سولانى المعزولة من محافظة كفر الشيخ، وكذلك العزلة رقم ٨ للفيوزاريوم سولانى المعزولة من محافظة الغربية، انخفضت شدة المرض معنويا عند استخدام عوامل المقاومة الحيوية، المبيدات الحيوية، المستحثات الكيماوية، الزيوت النباتية، المبيد الفطرى فيتافاكس٢٠٠ تحت ظروف الصوبة، أوضحت النتائج أيضا أن ٢% فوسفات الهيدروجين ثنائي البوتاسيوم والبيوأرك، والبيوزيد بالتركيزات الموصى بها خفضت الشدة المرضية للمسببات وتلى ذلك ترايكوديرما ماكوننجى (T14)، ترايكوديرما هارزيانم (T15) وأسينتوباكتر جينوسيشى (B40) وكانت باقى المعاملات أقل منهم فاعلية. زاد نشاط إنزيمات البيروكسيديز، البولى فينول أوكسيديز والشيتينيز عند غمر جذور شتلات الطماطم في محاليل العوامل الحيوية وغير الحيوية سابقة الذكر قبل زراعتها في التربة الملوثة بالمسببات المرضية وكان هذا التأثير ناتج بعد ١٤ يوم من المعاملة مقارنة بالتقدير بعد ٧ أيام من المعاملة.

الكلمات الإسترشادية: القدرة المرضية، مرض عفن الجذور، المبيدات الحيوية، المستحثات الحيوية وغير الحيوية.

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