Efficacy of some Bio-agents, Chemical Inducers and Fungicides in Controlling Tomato Root Rot Disease Caused by *Rhizoctonia solani*

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Abstract

Root rot disease caused by *Rhizoctonia solani*; is one of the most common destructive tomato diseases. Many different agents *i.e.*, biological agents, chemical inducers and fungicides were investigated for their antagonistic effects on growth of *R. solani in vitro*. The obtained results showed that, maximum inhibitory effect "100%" was obtained by most tested fungicides *i.e.* Rizolex–T, Maxim-XL and Moncut followed by salicylic acid at 8mM as chemical inducers. Also, *Glomus* sp and *T. viride* as bio-agents were most effective treatments against *R. solani* where they inhibited the growth of *R. solani* by 67.22 and 64.07%, respectively followed by oxalic acid at 8 mM and K_2HPO_4 at 15 mM treatments which recorded 59.6 and 44.1%, respectively. Meanwhile, chitosan treatment was the least inhibitive treatment *in vitro*. Under greenhouse conditions, chitosan was the highest effective treatment in reducing incidence severity of tomato root rot disease as well as increased the assessed vegetative parameters. Moreover, all tested treatments clearly increased the total phenol content and activities of plant defense-related enzymes. As for tomato root anatomy, most treatments increased root diameter of treated tomato plants. Where, *T. viride* followed by *B. subtilis* treatments were the best comparing with control treatment.

Keywords: *Rhizoctonia solani* – tomato – bio-agents - chemical inducers – fungicides – total phenols - plant defense-related enzymes - root anatomy.

Introduction

Tomato (*Lycopersicon esculentum* Mill) is known as one of the most crucial vegetable crops which have tremendous popularity around the world. Egypt ranked 5th in the world tomato production which constituting 4.86% of all global production in 2014, **(FAO, 2017)**. It has been informed that tomato plants could be infected by *Rhizoctonia solani* pathogen destructively; causing damping-off, root and crown root rot diseases. Root rot disease has great economic importance where, it infects and destroys the entire root system of tomato plants, limiting their nutritional activity and affecting tomato productivity in the quantity and quality under greenhouse conditions **(Abd-El-Wahab, 2004; Saad, 2006)**. For controlling Rhizoctonia root rot disease of tomato, several management strategies were done to manage such disease. Among the most truly effective and old method for disease control is using of fungicides. However, chemical fungicides are expensive and not environmentally safe besides their hazards to the human health. Recently, *Trichoderma* spp, *Bacillus subtilis*, *Glomus* sp. and *Pseudomonas fluorescens* are considered ecofriendly biocontrol agent against a number of phytopathogens and has been marketed commercially as biopesticides, biofertilizers and soil improvements **(El-Katatny *et al*., 2006; Rini and Sulochana, 2007; El-Khallal, 2007)**. On the other hand, The phenomenon of plant resistance to pathogens can be enhanced by the application of various abiotic agents (chemical inducers) such as salicylic acid (SA), mannitol, ethephon, H_2O_2, triggering systemic resistance in plants **(El-Khallal, 2007; Abdel-Monaim 2010)**.

This study aimed to evaluate some fungicides, bio-agents and chemical inducers in controlling tomato root rot disease caused by *R. solani in vitro* and *in vivo* (under greenhouse conditions). Also, determining the activities of some plant defense-related enzymes in addition to study anatomical changes of treated tomato plants.

Materials and Methods

1. Source of the pathogenic isolate of *Rhizoctonia solani*:

The used isolate of *R. solani* in this study was isolated, identified and tested for its pathogenic ability as mentioned by **Aboelmagd, (2020)**.

2. Inoculum preparation and soil inoculation:

Inoculum of *R. solani* was prepared using autoclaved sand barley medium at rate of 1:3 **(Abd-El-moneem, 1996)**. In this respect, for preparing inoculum, sand and barley grain were mixed at rate of 1:3 (25 g clean sand + 75 g barely + 2 g sucrose + 0.1 g yeast + 100 mL water) filled in 500 mL glass bottles and autoclaved at 121°C for 20 minute on two consecutive days. Fungal discs (5 mm Ø) of the tested Rhizoctonia isolate were used for inoculation of the prepared sand barley grain medium bottles prior to incubation at room temperature (25±2°C) for 14 days. Regarding soil inoculation, under greenhouse conditions, formalin-sterilized pots (20 cm Ø) were filled with the sterilized soil (sandy clay soil 1:3 w/w), inoculated with the prepared inocula of *R. solani* at rate 3% (w/w) and then, irrigated regularly for one week before transplanting the tomato transplants. Pots containing sterilized soil only without inoculation of Rhizoctonia isolate served as control treatment. The inoculated and un-inoculated pots were irrigated regularly two times weekly and left under greenhouse conditions at 25-30ºC and 70% relative humidity (RH)

approximately. Three pots were used as replicates. Four weeks old tomato transplants cv. Super Strain B were transplanted into the previously prepared pots at rate 3 transplants/pot. Pathogenicity test and Koch's postulates of isolated *R. solani* were carried out successfully at the Plant Pathology Department, Moshtohor, Faculty of Agriculture, Benha University, Egypt.

3. Effect of some tested treatments on *R. solani* growth *in vitro*:

3.1. Effect of some bio-agents:

In vitro evaluation of four Trichoderma isolates (*T. harzianum*, *T. viride*, *T. album* and *T. hamatum*), one mycorrhiza isolate (*Glomus* sp.) and two antagonistic bacteria as bio-agents (*Bacillus subtilis*, *Pseudomonas fluorescens*) was carried out using dual culture technique on PDA plates (90 mm Ø) against *R. solani*. These tested bio-agents and the *Glomus* sp. fungus **(El-Fiki *et al.*, 2001)** used in this research were kindly obtained from the fungal collections of Plant Pathology Department, Faculty of Agriculture, Benha University, Egypt. Equal discs (5 mm Ø) of grown fungal antagonists on PDA plates for 7-days were taken and placed individually for each one of the tested antagonists on PDA plates at a distance of 10 mm from the plate margin. At the opposite direction, at distance of 10 mm from the plate margin, a PDA disc inoculum (5mm Ø) obtained from 7-days old culture of *R. solani* was placed as described in the modified method of **Zlata *et al.*, (2008).** Also, the tested antagonistic bacteria were treated by the similar way using single streak technique according to **Wang *et al.*, (2003)** with some modifications, where, the single streak of *B. subtilis* or *P. fluorescens* bacterium was drawn by a loop at a distance of 10 mm from the margin of the plate, and then, the plates were incubated at 28°C for 24 hrs. At the opposite direction of Bacillus or Pseudomonas streak, an equal disc inoculum (5 mm Ø) obtained from 7-days old culture of *R. solani* isolate was placed at distance of 10 mm from the margin of the plate. Plates of control treatment were inoculated at10 mm apart from the plate edge with a single disc of *R. solani*. The inoculated plates were incubated at 25±2^{0}C and daily observed until the radius growth of *R. solani* inoculum covered whole plate in control treatment. After incubation, growth inhibition percentage (GI %) for each treatment was calculated according to **Arora and Upadhay (1978)** as follows**: GI%= ((C-T) /C) *100** where, GI% = percent of growth inhibition over control, C = radius growth of control (mm), T = radius growth of treatment (mm).

3.2. Effect of some chemical inducers:

Five chemical compounds as resistance inducers with three concentrations *i.e.* ascorbic acid, oxalic acid, salicylic acid at 2, 4 and 8 mM, dipotassium hydrogen phosphate at 5, 10 and 15 mM and chitosan at 1000, 2000 and 3000 mg/L were evaluated against *R. solani in vitro*. During this experiment, poisoned food technique was used on PDA medium according to the modified method of **Nene and Thapliyal, (1993)**. The growth inhibition percentage (GI %) of tested fungus was calculated as mentioned before**.**

3.3. Effect of some fungicides:

The fungicidal activities of 4 fungicides with 4 concentrations *i.e.* Rizolex-T 50%WP, Rovaral 41.6%SC and Moncut 25%WP each at 25, 50, 100 and 200 mg/L; Maxim-XL 3.5%FS at concentrations 0.1, 0.2, 0.4 and 0.8 mL/L were determined against the growth of *R. solani* onto PDA medium according to the method of **Dhingra and Sinclai, 1985**. The growth inhibition percentage (GI %) of tested fungus was calculated as mentioned before.

4. Control of Rhizoctonia root rot of tomato under greenhouse conditions:

The present experiment was carried out entirely on tomato plants 4-weeks old (cv. Super Strain B) in Rhizoctonia-inoculated pots (20 cm Ø) under greenhouse conditions at the Experimental Station, Moshtohor, Faculty of Agriculture, Benha University, Egypt during the growing season 2017. Rhizoctonia inoculum was multiplied on sand barley medium as previously mentioned according to **Abd-El-moneem, (1996)**. In this respect, the tested antagonists and chemical inducers which having good inhibitory effects on the Rhizoctonia root rot pathogen (*R. solani*) *in vitro* tests were tested again *in vivo* (under greenhouse conditions) to determine their control efficacy against Rhizoctonia root rot disease on tomato plants **Table (1)**. The tested fungicides were applied under greenhouse conditions only at recommended dose of each one. Before planting, tomato transplants were treated individually with each one of tested antagonists or fungicide treatments as root dipping for 5 mins., while , they were dipped in the tested chemical inducers for 2 hrs **(Abdel-Monaim *et al.*, 2012).** Also, the treatment of *Glomus* sp. was achieved as soil-inoculation treatment at rate 2%. Plants immersed only in water and planted on Rhizoctonia-inoculated pots used as control treatment. The experimental treatments were laid out in randomized complete block design with three replicates (each replicate represented by one pot and 3 plants in each pot).

Table 1. The tested concentrations of bio-agents, chemical inducers and fungicides against Rhizoctonia root rot disease on tomato plants under greenhouse conditions.

	Treatment			Used concentration
1-	Rhizoctonia-inoculated +	Biological agents	*T. harzianum*	10^7
2-			*T. viride*	10^7
3-			*T. hamatum*	10^7
4-			*T. album*	10^7
5-			*P. fluorescens*	10^8
6-			*B. subtilis*	10^8
7-			Mycorrhiza (*Glomus* sp.)	2%
8-		Chemical inducers	Ascorbic acid	8 mM
9-			Oxalic acid	8 Mm
10-			Salicylic acid	8 mM
11-			Dipotassium hydrogen phosphate	15 mM
12-			Chitosan	3000 mg/L
13-		Fungicides	Rizolex-T 50% WP	3000 mg/L (recommended dose)
14			Rovaral 41.6% SC	1500 mg/L (recommended dose)
15-			Moncut 25% WP	3000 mg/L (recommended dose)
16-			Maxim-XL 3.5% FS	1.0 mL/L (recommended dose)
17-	Rhizoctonia-inoculated only		Control	Water-dipped

4.1. Preparation the inocula of tested bio-agents:

The tested antagonistic fungi (*T. harzianum, T. viride, T. hamatum, T. album*) were grown on PDA medium and then, the spore suspension of each fungus was adjusted approximately to 10^7 spore/mL with the aid of a hemocytometer slide. The tested antagonistic bacteria (*B. subtilis* and *P. fluorescens*) were grown on nutrient broth medium **(Abd-Alla *et al.*, 2007)**. The concentration of each tested bacterial isolate was adjusted to approximately 10^8 cfu/mL using turbidity meter as mentioned by **Abdel-Kader *et al.*, (2012)**. Concerning *Glomus* sp., the fungus was grown on agar Bushnell's medium for 7 days at 28°C **(Bushnell and Haas, 1941)**. The pots (20 cm Ø) were filled with 2 Kg of sterilized soil and infested by the prepared inoculum at rate 2% of soil weight then mixed and irrigated regularly for one week to confirm distribution of the inoculum before tomato planting.

4.2. Preparation of the tested chemical inducers:

Resistance inducer compounds *i.e.* ascorbic acid, oxalic acid, salicylic were prepared at concentrations of 8 mM for each one, meanwhile, dipotassium hydrogen phosphate and chitosan were used at 15 mM and 3000 mg/L, respectively.

4.3. Preparation of the tested fungicides:

The tested fungicides *i.e.* Rizolex-T, Rovaral, Moncut and Maxim-XL, were prepared for application at concentrations of 3000 mg/L, 1500 mg/L, 3000 mg/L and 1.0 mL/L, respectively.

5. Disease assessments, vegetative and yield parameters:

The disease incidence and disease severity percentages of Rhizoconia root rot of tomato were recorded at 60-days post transplanting under greenhouse conditions, respectively. Disease incidence percentage (DI%) was calculated and expressed in percentage scale by using the following formula: **DI%**= (D/T) X 100, where, (D) = Number of diseased plants; (T) = Total observed plants. Disease severity percentage (DS %) was calculated using the disease scale of **Abdeljalil *et al.*, (2016)** as follows: 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, 5 = plant death. Under greenhouse condition, fresh and dry weight/plant (g), plant height (cm) and root length (cm) were recorded.

6. Determination of some bio-constituents:

6.1. Total phenol content:

For total phenol content determination, leaves samples were extracted separately by using the method suggested by **Simons and Ross (1971)**. The total phenol content in extracts was determined by Folin–Ciocalteu reagent method as modified by **Bary and Thorpe (1954)**, and were calculated for each treatment as milligrams of catechol per one gram dry weight (mg cat/g DW) according to standard curve of catechol. Efficacy percentage (Efficacy %) was calculated for comparing all tested treatments with control treatment as following: **Efficacy%=** treatment-control /control*100.

6.2. Plant defense-related enzyme activities:

Leaves samples were taken from each particular potted treatment under greenhouse condition for determining the plant defense-related enzyme activities. The crude leaf enzyme extract was prepared as recommended by **Tuzun *et al.*, (1989)**. The activity of peroxidase enzyme (PO) was measured as described by **Allam and Hollis (1972)**, and was calculated for each treatment as the change in absorbance at 425 nm per 15 min per gram fresh weight (Δ_{425}/15 min/g FW) Polyphenoloxidase activity (PPO) was determined according to **Matta and Dimond (1963)**, and was calculated as (Δ_{420}/30 min/g

FW). Chitinase activity was determined by the method of **Ried and Ogryd-Ziak (1981)**, and was expressed as mM N-acetylglucoseamine equivalent per gram fresh weight per 60 min. Efficacy percentage was calculated as mentioned before.

7. **Anatomical changes in roots of treated tomato plants:**

It was intended to carry out a comparative microscopical examination on plant material (roots) which showed the most positive response of plant growth to tested treatments with control. Sixty days post-planting, the tested materials included root samples taken basically 20 cm apart from the root tips. Specimens were killed, fixed and embedded in paraffin wax (58-60°C) according to the method described by **Johansen, (1940)**. Section cross 15µ thick were carried out by the rotary microtome, double-stained with fast green-safranin **(Nassar and El-Sahhar, 1998).** Sections were redden to detect histological manifestations of the noticeable responses resulted from the tested treatments compared to the control treatment and photomicrographed. The following anatomical characters were determined for each treatment; root diameter (µm), epidermal layer thickness (µm), cortex thickness (µm), number of cortex layers, mean thickness of cortex layers (µm), phloem thickness of the vascular bundle (µm), cambium thickness (µm), xylem thickness in the vascular bundle (µm), diameter of the widest xylem vessel in the vascular bundle (µm), pith layer thickness (µm). The increasing/reduction percentage (± %) over the control treatment was calculated for each estimated character; **±%** = control-treatment/control*100.

8. Statistical analyses:

Statistical analyses of all the previously designed experiments have been carried out according to the procedures (ANOVA) reported by **Snedecor and Cochran (1989)**. Treatment means were compared by the least significant difference test "LSD" at 5% level of probability.

Results and Discussion

1. Effect of some bio-agents on growth of *R. solani in vitro*:

Data in **Table (2)** show that all used antagonists reduced the growth of *R. solani* compared with control. Among Trichoderma species, *T. viride* was the best antagonistic fungus for reducing the mycelial growth and caused the highest reduction (64.07%) followed by *T. harzianum*, *T. album* and *T. hamatum* with 63.70, 62.59 and 60.37, respectively. Meanwhile, *Glomus* sp. caused 67.22% growth reduction. Among the two bacterial antagonists**,** *B. subtilis* was more effective in reducing the growth of respectively **(Amara, *et al.*, 1996; Roberti and Selmi, 1999)**.

Table 2. Effect of some bio-agents on growth *R. solani in vitro*:

Treatment	Mycelial growth (mm)	GI%*
T. album	33.67	62.59
T harzianum	32.67	63.70
T. hamatum	35.67	60.37
T. viride	32.33	64.07
***Glomus* sp.**	29.50	67.22
Psedomonas fluorescens	48.33	46.30
Bacillus subtilis	39.33	56.30
Control	**90.00**	**0.00**
LSD at 0.05	**2.74**	

*** GI % =** Growth inhibition percentage = ((control-treatment) /control) *100.

R. solani than *p. fluorescens*. *Glomus* sp. as arbuscular mycorrhizae is considered a promising tool for plant protection against plant pathogens. The inhibitory effect of *Glomus*. sp. against *R. solani* could be discussed in light that, *Glomus* sp. has the ability to produce antifungal or oxidative enzymes **(Pozo and Azcon-Aguilar, 2007)** such as chitinase **(El-Khallal, 2007)**. The inhibitory effect of Trichoderma to *R. solani* could discussed also in light that Trichoderma species produce extracellular enzymes, antifungal metabolites and antibiotics **(El-Katatny *et al.*, 2006; Montealegre *et al.*, 2010)**. Also, *B. subtilis* and *P. fluorescens* are playing important role in controlling the soil-borne pathogens by producing the antibiotics and sidrophores,

2. Effect of some chemical inducers on growth of *R. solani in vitro*:

Results in **Table (3)** illustrate that the linear growth of *R. solani* was significantly decreased by the most tested chemical inducers compared with control. Reduction in linear growth was increased by increasing concentration of most tested chemical. Salicylic acid (SA) at concentration 8 mM completely inhibited mycelial growth of *R. solani* followed by salicylic acid at concentration 4 mM, oxalic acid at concentration 8 mM and K_2HPO_4 at 15 mM (88.9%, 59.6%, 44.1%), respectively. On the other hand, K_2HPO_4 treatment at concentration 5 mM was the lowest effective treatment (1.1%). These results agree with those reported by **Kataria *et al.*, (1997)** and **Abdel-Ghany, (2008**) who found that, salicylic acid and ascorbic acid, reduced growth of *R. solani in vitro*.

Table 3. Effect of some chemical inducers on growth of *R. solani in vitro*:

Treatment	Conc.	Mycelial Growth (mm)	GI%
Salicylic acid	2mM	70.0	22.2
	4mM	10.0	88.9
	8mM	0.0	100.0
Ascorbic acid	2mM	86.7	3.7
	4mM	70.0	22.2
	8mM	53.5	40.6
Oxalic acid	2mM	80.8	10.2
	4mM	74.0	17.8
	8mM	36.3	59.6
Dipotassium hydrogen phosphate (K_2HPO_4)	5mM	89.0	1.1
	10mM	80.0	11.1
	15mM	50.3	44.1
Chitosan	1000 mg/L	90.0	0.0
	2000 mg/L	90.0	0.0
	3000 mg/L	90.0	0.0
Control		**90.0**	**0.0**
LSD at 5%	**Chemical**	**2.47**	
	Conc.	**2.35**	
	Interaction	**5.75**	

Also, **Shalaby *et al.*, (2001)** recorded that, potassium salicylate, oxalic acid and salicylic acid inhibited the growth of *M. phaseolina in vitro*. **El-Ganaieny *et al.*, (2002)** recorded that some antioxidants (potassium salicylate, oxalic acid, salicylic acid or ascorbic acid) at different concentrations (2 - 10 mM) has a significant effect on growth reducing of *Fusarium oxysporum*, *F. solani* and *F. moniliforme in vitro*.

3. Effect of some fungicides on growth of *R. solani in vitro*:

Results in **Table (4)** reveal that, the tested fungicides significantly inhibited the growth of *R. solani* compared with control. The inhibitory effect of the tested fungicides was increased with increasing the fungicide concentrations.

Table 4. Effect of some fungicides on growth of *R. solani in vitro*:

Treatment	Conc.	Mycelial Growth (mm)	GI%
Rizolex –T	25 mg/L	0.00	100.00
	50 mg/L	0.00	100.00
	100 mg/L	0.00	100.00
	200 mg/L	0.00	100.00
Rovral	25 mg/L	13.00	98.56
	50 mg/L	11.76	98.70
	100 mg/L	9.00	99.00
	200 mg/L	0.00	100.00
Maxim –XL	0.1 ml/L	0.00	100.00
	0.2 ml/L	0.00	100.00
	0.4 ml/L	0.00	100.00
	0.8 ml/L	0.00	100.00
Moncut	25 mg/L	0.00	100.00
	50 mg/L	0.00	100.00
	100 mg/L	0.00	100.00
	200 mg/L	0.00	100.00
Control		**90.00**	**0.00**
LSD at 5%	**Fungicide**	**0.12**	
	Conc.	**0.29**	
	Interaction	**0.22**	

All concentrations of the fungicides *i.e.* Rizolex -T, Maxim -XL and Moncut inhibited completely the growth of the fungus, while the high concentration of Rovral exhibited the same trend. These results are agree with those found by **Alharbi, (2015)** who reported that the fungicide Rizolex-T incited great inhibitions on linear growth of *F. solani* and *R. solani in vitro*. The fungicides; Tachigaren 30%, Monceren 25%, Aracur 72.2%, Topsin M 70%, Hymexate 30% and Moncut 25% showed the greatest effectiveness, inhibiting mycelia growth of *F. solani* and *R. solani* isolates *in vitro* **(Amini and Sidovich, 2010** and **Kimar *et al.*, 2011)**

4. Determination of tomato root rot incidence, severity and some growth parameters in treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* under greenhouse conditions:

Data in **Table (5)** declare that all tested treatments were effective in reducing the disease incidence (DI%) and disease severity (DS%), as well as increased fresh and dry weight of shoots and roots, plant height and root length compared to the positive control treatment (*R. solani*-inoculated soil only). Using of bio-agents gave the best results where, *T. viride*, *Glomus* sp. and *B. subtillis* recorded 33.33% and 6.67% of DI% and DS%, respectively. Also, chemical inducer treatments recorded a significant reduction in DI% and DS% where, salicylic acid and ascorbic acid treatments were the best in reducing DI% (22.22% and 44.44%, respectively) and DS% (4.45% and 13.33%, respectively). Also, the tested fungicides recorded a significant reduction in DI% and DS% where, Rizolex-T and Rovral treatments were the best in reducing DI% (33.33% and 44.44%, respectively) and DS% (6.67% and 13.33%, respectively).

Table 5. Effect of some treatments on Rhizoctonia root rot incidence, severity and some growth parameters in treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* under greenhouse conditions:

Treatment	DI%	DS%	Fresh weight (g)		Dry weight (g)		Plant height (cm)	Root length (cm)
			Shoot	Root	Shoot	Root		
T. album	55.56	11.11	26.84	4.19	5.14	1.03	30.00	8.00
T. viride	33.33	6.67	29.31	4.60	5.61	1.12	31.33	9.27
T. hamatum	66.67	13.33	24.54	3.76	4.70	0.91	28.50	6.83
T. harzianum	44.44	8.89	28.65	4.52	5.49	1.10	30.67	9.23
***Glomus* sp.**	33.33	6.67	31.55	4.64	6.04	1.13	33.33	9.37
P. fluorescens	55.65	15.55	26.17	4.20	5.01	1.02	28.00	8.00
B. subtilis	33.33	13.33	27.5	4.22	5.27	1.03	29.33	8.5
Mean	**46.04**	**10.79**	**27.79**	**4.30**	**5.32**	**1.05**	**30.17**	**8.46**
Salicylic acid	22.22	4.45	33.06	4.36	6.92	1.06	31.00	9.67
Ascorbic acid	44.44	13.33	26.56	3.90	5.56	0.95	27.00	7.23
Oxalic acid	55.56	24.44	24.04	3.56	5.04	0.86	29.00	6.67
K_2HPO_4	66.67	35.55	20.09	2.64	4.21	0.64	26.17	5.57
Chitosan	66.67	31.11	22.67	3.40	4.52	0.83	25.00	6.60
Mean	**51.11**	**21.78**	**25.28**	**3.57**	**5.25**	**0.87**	**27.63**	**7.15**
Rizolex-T	33.33	6.67	30.33	4.02	6.32	0.98	32.33	9.63
Rovral	44.44	13.33	28.29	3.81	5.93	0.93	29.00	7.13
Moncut	66.67	20.00	22.31	3.23	4.67	0.79	25.67	5.50
Maxim-XL	55.56	15.55	26.77	3.64	5.89	0.88	28.00	8.73
Mean	**50.00**	**13.89**	**26.93**	**3.68**	**5.70**	**0. 90**	**28.75**	**7.75**
Control	**100.0**	**91.66**	**3.52**	**1.22**	**0.74**	**0.30**	**18.33**	**1.97**
LSD at 5%	**37.55**	**9.96**	**2.71**	**0.51**	**0.55**	**0.13**	**3.21**	**0.92**

Concerning the effect of tested treatments on vegetative parameters, the same trend was noticed where; all tested treatments increased the vegetative parameters. The tested bio-agents followed by fungicides and chemical inducers gave best results, respectively comparing with the control treatment. Similar results were obtained by **Toua *et al*., (2013)** who recorded that *P. fluorescens* significantly reduced the disease incidence and severity on tomato plants. **Alharbi, (2015)** found that Rizolex-T fungicide showed significant decrease in disease incidence followed by treatments with *T. harzianum*, *T. viride*, *P. chlororaphis* and *P. eruginosa* under greenhouse conditions. All treatments enhanced the dry weight of shoot and root systems. **Mazen, (2004)** mentioned that salicylic acid gave a significant protection against *R. solani* infection with low percentage of pre- and post-emergence damping off and high percentage of survival plants. Also, **He and Wolyn (2005)** noticed that SA-treated plants exhibited enhanced systemic resistance, with a significant reduction in disease severity of the

asparagus roots inoculated with *F. oxysporum* f. sp. *asparagi*, compared with untreated plants. **El-Mohamedy *et al.*, (2014)** studied the effect of potassium salts, salicylic acid and sorbic acid on *R. solani*, *F. solani* and *Sclerotinia rolfsii* that attacking tomato plants under greenhouse and field conditions. They found that, all tested inducers had significantly protected tomato plants against tested pathogens compared to the untreated control. **Waheed *et al.*, (2014)** stated that tomato seed treatment with salicylic acid led to a significant increment in percentage of seeds germination, plant height, fresh and dry weight of plant as compared to control treatment under greenhouse conditions.

Determination of total phenol content and activities of some plant defense-related enzymes in treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* under greenhouse conditions:

Data in **Table (6)** indicate that, all tested treatments *i.e.* biological agents, chemical inducers and tested fungicides affected positively total phenol content compared with control treatment. The highest increases in the total phenols were induced by *T. viride* (276.41%) followed by *T. harzianum* (183.43%) respectively. While *T. hamatum* was the least effective one (120.96%). Also, *Glomus* sp. as mycorrhizae treatment caused highest total phenol content (223.27%).Considering the chemical inducers; the same Table shows that all tested chemical inducers increased the total phenol content. The highest increase in the total phenols was induced by salicylic acid (499.68%) followed by ascorbic acid (480.96%) and oxalic acid (279.52%) meanwhile, K_2HPO_4 and chitosan were the least effective (226.37, 99.12%), respectively. Also, results indicate that the tested fungicides affected positively on the total phenol content comparing with control treatment. The highest increase in the total phenols was induced by Rizolex-T (352.91%) followed by Maxim XL (335.70%) and Rovral (281.04%) meanwhile, Moncut was the least effective one (218.57%). These results could be discussed in light the findings of **Chérif *et al.*, (2007)** and **Mohamed *et al.*, (2007)** who reported that, resistance could be exhibited on treated plants as a result of accumulation of various phenolic compounds and the activation of chitinases, ß-1,3-glucanases, peroxidases and polyphenoloxidases and key enzymes in the phenylpropanoid and isoflavonoid pathways may play an essential role in the control of diseases and resistance to pathogenic attack in plants. Also data in **Table (6),** reveal that, all tested treatments positively increased the activities of PO, PPO and chitinase enzymes in leaves of tomato plants compared with control (*R. solani*).

Table 6. Effect of some treatments on total phenol content and activities of some plant defense-related enzymes in treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* under greenhouse conditions:

Treatment	Total phenols		Oxidative enzymes				Chitinase	
			Polyphenol oxidase		Peroxidase			
	mg cat/g DW	Efficacy%	Δ420/30 min/g FW	Efficacy%	Δ425/15 min/g FW	Efficacy%	mM aga/g FW/60 min*	Efficacy%
T. album	27.73	120.96	9.27	243.33	19.8	307.41	14.78	211.16
T. viride	47.24	276.41	14.22	426.67	25.65	427.78	39.34	728.21
T. hamatum	32.63	160.00	8.73	223.33	14.97	208.02	11.88	150.11
T. harzianum	35.57	183.43	10.89	303.33	22.02	353.09	16.96	257.05
***Glomus* sp.**	40.57	223.27	12.69	370.00	30.45	526.54	18.94	298.74
P. fluorescens	28.22	124.86	12.48	362.22	14.16	191.36	16.83	254.32
B. subtilis	30.58	143.67	13.41	396.67	20.13	314.20	17.49	268.21
Mean	**34.65**	**176.08**	**11.67**	**332.22**	**21.03**	**332.63**	**19.46**	**309.69**
Salicylic acid	75.26	499.68	17.91	563.33	37.2	665.43	34.19	619.79
Ascorbic acid	72.91	480.96	16.83	523.33	28.05	477.16	21.78	358.53
Oxalic acid	47.63	279.52	4.59	70.00	25.95	433.95	21.05	343.16
K_2HPO_4	40.96	226.37	3.87	43.33	12.18	150.62	19.67	314.11
Chitosan	24.99	99.12	4.32	60.00	20.04	312.35	20.79	337.68
Mean	**52.35**	**317.13**	**9.50**	**252.00**	**24.68**	**407.90**	**23.50**	**394.65**
Rizolex-T	56. 84	352.91	12.78	373.33	29.1	498.77	30.10	533.68
Rovral	47.82	281.04	5.40	100.00	21.84	349.38	14.26	200.21
Moncut	39.98	218.57	3.96	46.67	16.8	245.68	9.17	93.05
Maxim-XL	54.68	335.7	5.04	86.67	18.66	283.95	9.70	104.21
Mean	**47.49**	**297.06**	**6.80**	**151.67**	**21.60**	**344.45**	**15.81**	**232.79**
Control	**12.55**	**0.00**	**2.7**	**0.00**	**4.86**	**0.00**	**4.75**	**0.00**

*** mM aga/g FW/60 min** = mM N-acetylglucoseamine equivalent per gram fresh weight per 60 min; **Efficacy%=** ((treatment-control) /control) *100.

In this respect, the highest activity of PO enzyme activity recoded by salicylic acid treatment followed by *Glomus* sp. and Rizolex-T fungicide where, they recorded efficacy %; 665.43, 526.45 and 498.77%, respectively. Also, salicylic acid treatment followed by *T. viride* and Rizolex-T treatments which recorded the highest efficacy% of PPO enzyme activity (563.33, 426.67 and 373.33%, respectively). Meanwhile, *T. viride* followed by salicylic acid and Rizolex-T treatments gave best results of chitinase efficacy% (728.21, 619.79 and 533.68%, respectively). Similar results were obtained by **Kamalakannan *et al.*, (2004)** who demonstrated that in addition to direct antagonism, the biocontrol agents also increase the activity of various defense-related enzymes and chemicals in response to pathogen infection. Moreover, peroxidase and polyphenoloxidase enhanced formation of lignin, while other oxidative phenols contribute in formation of defense barriers for reinforcing the cell structure **(Avdiushko *et al.*, 1993)**. Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms (**Pena and Kuc, 1992)**. Also, chitinase and β-1, 3-glucanase enzymes plays an important role in plant defense against fungi by hydrolyze their cell wall **(Tian *et al.*, 2006; Imran *et al.*, 2007** and **Barilli *et al.*, 2010)**.

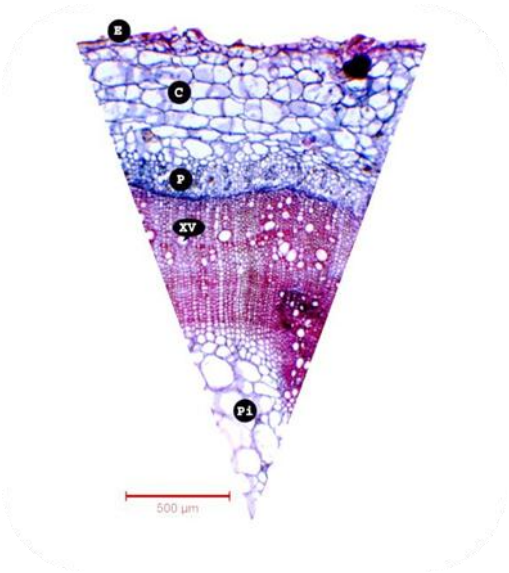
5. Anatomical changes in roots of treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* infection under greenhouse conditions:

Microscopical measurements of specific histological characters at the specimens of the main root of tomato plants root-treated with *T. viride*, *B. subtilis*, salicylic acid, Rizolex-T fungicide or soil-drenched with *Glomus* sp. and those of control treatment were recorded in **Table (7)** and illustrated in **Fig. (2)**. Data in **Table (7)** and **Fig. (2)** reveal that most of treatments increased the root diameter of treated tomato plants. In this respect, *T.viride* followed by *B. subtilis* treatment recorded the highest value over the control treatment by 32.9 and 19.6%, respectively. This increase was recorded in all examined tissues expect the cortex layers thickness of *Glomus* sp. treatment, which showed a distinguished decrease by 12.3% less than the control treatment. Concerning to phloem thickness, the drenched soil treatment with *Glomus* sp. followed by root-treated tomato plants with Rizolex-T fungicide treatment recorded the highest value of the phloem thickness over the control treatment (88.5 and 76.7%, respectively). As for cambium thickness, the treatments of *T. viride* followed by salicylic acid, *Glomus* sp. and *B. subtilis* recorded the highest value over the control treatment (55.6, 30.4, 29.0 and 28.6%, respectively). Considering to xylem thickness and diameter of the widest xylem vessel located in the vascular bundle, *T. viride* and salicylic acid treatments were the best in this respect. The root cells have to comply with two tasks. On the one hand, the root is dedicated to the acquisition of water and mineral nutrients. For this, the epidermis of roots has to be maximally permeable. On the other hand, the root surface has to be protected from pathogens. To comply with these two seemingly contradictory requirements, the root has specialized cell layers for nutrient absorption and protection. Several biotic and abiotic factors can influence internal root system architecture, including plant growth promoting microorganisms "PGPM" **(Lee and Lee, 2015; Chowdappa, *et al.*, 2013)**. Also, organic molecules like salicylic acid, able to activate plant metabolism improving plant performance **(Bulgari *et al.*, 2015).**The predominant genera of PGPM are Bacillus, Trichoderma and Glomus mycorrhiza. PGPM modify internal root system architecture and the structure of root tissues mainly through their ability to interfere with the plant phytohormonal balance pathways responsible for cell origination and differentiation involved in regulating plant root development; auxin, cytokinin, ethylene, and to a lesser extend gibberellin, and abscisic acid **(Dodd, *et al.*, 2010)**. Many investigators have been reported the protective role of PGPM for plants against phytopathogens by activating induced systemic resistance (ISR) plant defense responses **(Desoignies, *et al.*, 2012; Weller, *et al.*, 2012; García-Gutiérrez, *et al.*, 2013)**. One of the consequences of ISR is thus the reinforcement of the cell wall through enhanced lignin synthesis and callose apposition **(Kovats, *et al.*, 1991; Strömberg and Brishammar)**, which restricts the progression of phytopathogens through plant tissues. Consequently, the uptake of minerals and water, and thus the growth of the whole plant, can be increased. As well, inoculation of an arbuscular mycorrhizal (AM) fungus and *B. subtilis* can promote plant growth, enhance AM colonization, and increase plant biomass and nutrient uptake from the soil **(Awasthi, *et al.*, 2011)**. Some investigators confirmed the present findings using salicylic acid on other plant crops; for instance, **Maddah, *et al.*, (2007)** stated that foliar application with salicylic acid at the concentration of 0.1 mM increased paranchyma and sclerenchyma tissues in stem and increased xylem tissue in roots of chickpea plants. **Gomaa, *et al.*, (2015)** worked on plants of *Lupinus termis* and found that salicylic acid at 75 ppm increased diameter of the main stem and all included tissues (epidermis, cortex, fiber strands, phloem and xylem). It has been discovered that salicylic acid is essential in the signal transduction for inducing systemic acquired resistance against some pathogenic infections **(Gaffney, *et al.*, 1993; Vernooij, *et al.*, 1994)**. All illustrated data being in harmony with the present findings.

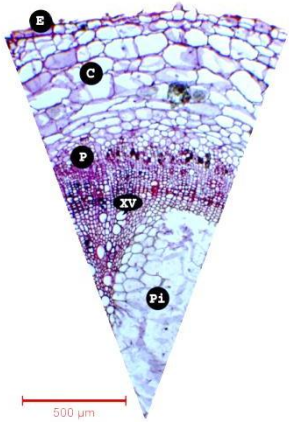
Table 7. Anatomical changes in roots of treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* infection under greenhouse conditions:

Treatment	Extra vascular stellar ground tissue measurements*									
	RD	**±%**	**ET**	**±%**	**CT**	**±%**	**CLN**	**±%**	**CLT**	**±%**
T. viride	4642.0	32.9	51.3	16.9	493.2	9.0	6.0	0.0	82.2	9.0
B. subtilis	4178.1	19.6	60.9	38.7	533.7	18.0	7.0	16.7	76.2	1.1
***Glomus* sp.**	4014.6	14.9	47.6	8.4	462.7	2.3	7.0	16.7	66.1	-12.3
Salicylic acid	4047.1	15.8	52.8	20.3	488.6	8.0	6.0	0.0	81.4	8.0
Rizolex-T	4084.3	16.9	57.4	30.8	460.3	1.7	6.0	0.0	76.7	1.7
Control	3494.1	0.0	43.9	0.0	452.4	0.0	6.0	0.0	75.4	0.0
Treatment	**Vascular stele measurements**									
	PhT	**±%**	**CamT**	**±%**	**XT**	**±%**	**DWX**	**±%**	**PiT**	**±%**
T. viride	96.7	32.5	113.1	55.6	997.3	51.4	83.0	115.6	1811.6	44.7
B. subtilis	120.0	64.4	93.5	28.6	759.2	15.3	47.6	23.6	1870.0	49.4
***Glomus.* sp.**	137.6	88.5	93.8	29.0	1084.9	64.7	47.3	22.9	1319.2	5.4
Salicylic acid	123.2	68.8	94.8	30.4	1096.3	66.5	74.4	93.2	1619.2	29.4
Rizolex-T	129.0	76.7	82.4	13.3	830.3	26.1	58.2	51.2	1727.0	38.0
Control	73.0	0.0	72.7	0.0	658.6	0.0	38.5	0.0	1251.7	0.0

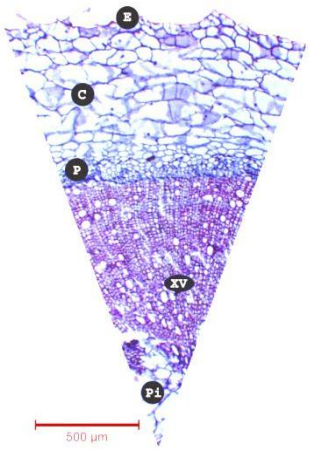
***Extra vascular stellar ground tissue** = tissues outside the vascular stele (vascular cylinder)**, ±%** = the increasing /reduction percentage over the control treatment (±% = (control-treatment)/control*100); **RD=** Root diameter (µm)**; ET=** Epidermal layer thickness (µm)**; CT=** Cortex thickness (µm)**; CLN=** Cortex layer number (µm)**; CLT=** Cortex layers thickness (µm)**; PhT=** Phloem thickness (µm)**; CamT=** Cambium thickness (µm)**; XT=** Xylem thickness (µm)**; DWX=** Diameter of the widest xylem vessel in the V.B.(µm)**; PiT=** Pith layer thickness (µm).



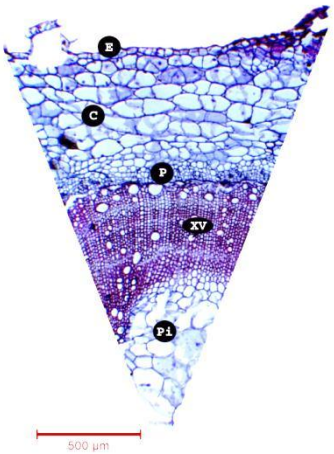
T. viride



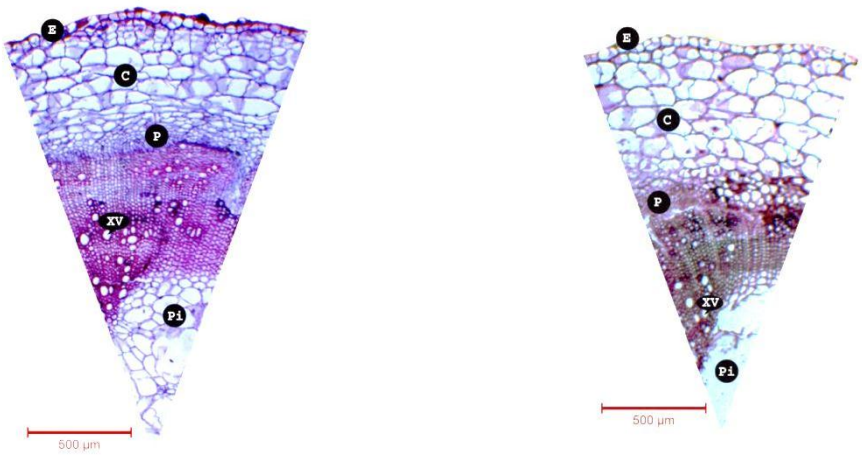
B. subtilis



***Glomus* sp.**



Salycilic acid



Rizolex-T **Control**

Figure (2): Anatomical changes in roots of treated tomato plants with bio-agents, chemical inducers and fungicides to control *R. solani* infection under greenhouse conditions.**E**= epidermis; **C**= cortex; **P**= phloem; **XV**= xylem vessels; **Pi**= pith.

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كفاءة بعض العوامل الحيوية والمحثات الكيماوية والمبيدات الفطرية فى مكافحة مرض عفن جذور الطماطم الذى يسببه فطر ريزوكتونيا سولانى

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مرض عفن جذور الطماطم الناتج عن الإصابة بفطر *R. solani* واحداً من أمراض الطماطم الأكثر شيوعا والمدمرة للنبات والمحصول. تم استخدام العديد من عوامل المكافحة للمرض ودراسة تأثيرها المثبط للفطر فى المعمل والصوبة، ومن أهم هذه العوامل: العوامل الحيوية والمحثات الكيميائية والمبيدات الفطرية. وقد أظهرت النتائج التي تم الحصول عليها أن أقصى تأثير مثبط "100%" تم الحصول عليه بواسطة معظم المبيدات الفطرية المختبرة مثل ريزوليكس– تى وماكسيم ومونكت يليه معاملة حمض السالسيليك عند تركيز 8 مللى مول. كانت معاملة ميكورهيزا جنس جلومس وتريكوديرما فيريدى أكثر المعاملات تأثيرا ضد ريزوكتونيا سولانى حيث سجلتا نسبة تثبيط لنمو الفطر وصلت 67.22 و 64.07 ٪ ، على التوالي تليها معاملة حمض الأكساليك عند تركيز 8 مللى مول و فوسفات البوتاسيوم احادية الهيدروجين عند تركيز 15 مللى مول حيث سجلا نسبة تثبيط 59.6 و 44.1 ٪ ، على التوالي، بينما معاملة الشيتوزان سجلت أدنى تأثير في هذا الصدد تحت ظروف المعمل. وفي الوقت نفسه، تحت ظروف الصوبة، معاملة الشيتوزان سجلت أعلى تأثير فى خفض نسبة وشدة الإصابة التي تم تقييمها بالإضافة إلى زيادة القياسات الخضرية. علاوة على ذلك، فقد أظهرت جميع المعاملات المختبرة زيادة واضحة فى محتوي الفينولات الكلية، نشاط الإنزيمات المرتبطة بالمقاومة. كذلك ، أدت معظم المعاملات الى زيادة فى قطر جذر نباتات الطماطم تشريحياً. حيث كانت معاملة تريكوديرما فيريدى يليها معاملة باسليس ساتلس هى الأفضل مقارنة بمعاملة الكنترول.