Egyptian Journal of Phytopathology, Vol. 49, No. 2, pp 141-149 (2021)
DOI 10.21608/ejp.2021.98799.1044

ORIGINAL PAPER

Controlling of Crown and Root Rot in Tomato Caused by *Sclerotium rolfsii*

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Received: 30 September 2021 / **Accepted**: 28 December 2021 / **Published online**: 30 December 2021.

ABSTRACT

Tomato (*Solanum lycopersicum* L.) plants are susceptible to infection by *Sclerotium rolfsii*, causing damping-off of nursery seedlings as well as crown rot in adult plants**.** Effect of onion and garlic extracts, neem oil, salicylic, ascorbic, citric acids and hydrogen peroxide and some bioagents such as *Bacillus subtilis, Pseudomonas fluorescens, Saccharomyces cerevisiae* and *Trichoderma harzianum* on the linear growth of the pathogen was studied. Neem oil, salicylic acid and *P. fluorescens* came in the first rank and recorded the best values of reducing disease incidence and severity followed by *B. subtilis* furthermore, decreasing the linear growth of *Sclerotium rolfsii*. Onion extract, citric and ascorbic acids recorded the lowest values in this respect.

Keyword: Tomato, *Solanum lycopersicum*, Root rot, *Sclerotium rolfsii*, Neem oil, Salicylic acid, *Trichoderma harzianum*

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INTRODUCTION

Sclerotium rolfsii Sacc., (teleomorph, *Athelia rolfsii*) (Okabe and Matsumoto, 2003) is one of the major problems in tomato crops in the warm, moist tropical regions of the world (Fu *et al.*, 2002) causing damping-off of nursery seedlings as well as stem rot, wilting and blight in adult plants (Hyeuk and Park, 2002). Synthetic chemical fungicides have long been used to reduce the incidence of plant diseases. However, they are costly, have negative environmental effects and may induce pathogen resistance (Eid, 2013). Consequently, biological control, including the use of microorganisms or their antibiotics, offers an alternative or supplement to pesticides for the management of plant diseases without the negative impact of chemical control (Cunniff and Gilligan, 2011). *Pseudomonas* spp. were common soil bacteria easily cultured from most agricultural soils and plant rhizospheres. They have been studied in considerable detail because of their ability to promote plant growth, either by directly stimulating the plant or by suppressing pathogens (Rosas *et al.*, 2009).

Bacillus is a genus of great interest to agriculture because some of its members promote plant growth and have a wide range of antagonistic mechanisms, which means that they are able to circumvent phytopathogen defenses (De Curtis *et al.* 2010 and Sarhan and Shehata, 2014).

T. harzianum is a common soil species and is used in biological control of a variety of plant-pathogenic fungi (Marra *et al*, 2006). It was found to be an effective biological control agent for protecting a number of crop plants from damage induced by *S. rolfsii* under both greenhouse and field conditions (Zhang *et al*., 2016 and Kamel *et al*., 2020).

Antagonistic yeasts are able to colonize rapidly and grow in superficial lesions. Their faster growth rate means that they can inhibit the development of phytopathogenic fungi by successfully competing for nutrients and space. (Úbeda *et al.*, 2014). *In vivo* tests they have shown that some yeasts significantly reduced the incidence of Monilinia rot in plums by competing with the phytopathogen for nutrients and space (Zhang *et al.*, 2017).

Salicylic acid (SA) plays an important role in plant defense and is well documented for dicotyledonous plants, where it's required for basal resistance against pathogens as well as for the inducible defense mechanism and systemic acquired resistance (SAR) which confers resistance against a broad-spectrum of pathogens (Chaturvedi and Shah, 2007 and Sarhan *et al*., 2018). The effect of five antioxidants (citric acid, salicylic acid, benzoic acid, ascorbic acid and sodium citrate) on the

resistance of tomato plants (*Solanum lycopersicum*) to early blight incited by *Alternaria solani* was investigated *in vitro* and *in vivo*.

Therefore, the present study aimed to determine the efficacy of certain bioagents and inducers against crown and root rot on tomato.

MATERIALS AND METHODS

Plant materials:

1. Survey of crown and root rot in tomato:

Disease incidence and severity were determined in tomato plantations located at Beni Suef and Minia governorates. Survey was conducted in June, 2018-2019. Five samples were collected from 10 fields represented each county.

2-Isolation, identification and pathogenicity:

Samples of tomato plants collected from different tomato plantations in Minia showing typical symptoms of crown and root rot, were by subjected to isolation trials following method described by Sinclair and Dhingra (2019). Identification of the developed fungi was conducted according to their cultural and morphological characteristics using the descriptions of Sarma *et al.* (2002). Identification was also confirmed by the Mycological Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Pathogenicity test was carried out at Sids Agricultural Research Station, during 2018 using sterilized pots and soil. The pots were infested with the tested isolates grown individually on sorghum grain for 10 days, at ratio of 30g/1000g soil. After 7 days, tomato transplants cv Super Gakal were transplanted in infested soil and checked up to 6 weeks for symptoms development (Roodriguez-Molina *et al*., 2003).

Disease assessment:

Plants were evaluated up to six weeks after transplanting. Disease severity was recorded based on the progress of yellowing and root rot and rotting at the end of the experiment. Plants without rotting and wilting were considered healthy, whereas those displayed conspicuous symptoms (yellowing, root rot and rotting) were considered infected.

The percent of disease incidence (PDI) was assessed using the following formula.

$$\textbf{Disease incidence \%} = \frac{\textbf{Number of infected plants}}{\textbf{Total number of plants}} \times 100$$

Disease severity % assessment was determined according to a 0-5 scale of Shahzad and Ghaffar (1992) with minor modification.

Where: 0=0, l= 0≥10, 2=10≥25. 3=25≥50 , 4=50≥75 and 5=75≥100%. it was calculated as recommended by Liu and Lu (1995).

$$\textbf{Disease severity\%} = (\Sigma\ \mathbf{n} \times \mathbf{r} / \mathbf{N5}) \times 100$$

Where:

n= number of plants in each numerical rate

N= total number of plants multiplied by the maximum numerical rate

r= 5.

3. Treatments:

Chemical substances *i.e.*, salicylic, ascorbic, citric acids, hydrogen peroxide were prepared at concentrations of 0.1, 0.2, 0.3%. Neem oil and some bioagents, *B. subtilis, P. fluorescens,* and *Saccharomyces cerevisiae* were obtained from Mycological Research and Disease Survey Department, ARC, Giza, Egypt. *Trichoderma harzianum* was supplied by Department of Microbiology. Soil, Water & Environment Res. Inst., Agricultural Research Center, Giza, Egypt.

Extraction of onion and garlic was carried out by adding 40 g of bulbs to 50 mL distilled water and then crushed to mash using pestle and mortar. These mashes were centrifuged at 2000 rpm for 20 min and the supernatants were added to 30 ml distilled water in conical flasks (100 mL). The extracts were sterilized in autoclave then stored at -20ºC till using.

4-Factors affecting linear growth of *S. rolfsii*:

4.1. Chemical treatments:

Chemical substances *i.e.*, salicylic, ascorbic, citric acids, hydrogen peroxide were added to PDA at concentrations of 0.1, 0.2 and 0.3%. Petri dishes containing tested substances were inoculated with 5 mm culture-disk taken from the edge of an actively 7-day-old culture of *Sclerotium rolfsii*. Cultures were incubated at 25ºC. Control treatments were Petri dishes containing PDA medium free from the substances tested and inoculated by the tested pathogen. After 7 days of incubation, the radial growth was measured. Five plates were used as replicates for each treatment.

4.2. Plant extracts:

Effect of some plant extracts on *S. rolfesii* linear growth was studied. Extracts of garlic and onion were added at concentrations of 5%. Neem oil was added to PDA at the same concentration. Petri dishes containing PDA plus the tested extracts individually were inoculated with 5.0 mm culture-disk taken from the edge of an actively 7-day-old culture of the tested pathogen. Petri dishes containing PDA free from

plant extracts and inoculated by the pathogen were used as control.

Cultures were incubated statically at 25° C. After 7 days of incubation, the radial growth was measured. Five plates were used as replicates for each treatment.

4.3. Bioagents:

Bacterial suspension of *B. subtilis* was cultivated in nutrient broth medium, while *P. fluorescens* was multiplied on King broth (KB) liquid medium, *T. harzianum* was maintained on PD broth medium at 26 ℃. for one week. The tested antagonists were streaked on the nutrient agar medium at periphery of Petri dish, a 5 mm disk from a 7 days-old culture of *S. rolfsii* was placed near the periphery of Petri dish directly opposite the antagonist (70 mm distance). Control plates contained nutrient agar medium streaked with sterilized distilled water instead of bioagents tested away with 70mm distance from a 5 mm disk bearing the tested pathogen. Three replicates were used for each treatment. Cultures were incubated at 25ºC for 7 days. Linear growth of the tested fungus was recorded.

Reduction percentage in mycelial growth for *S. rolfsii* was calculated using the formula:

Inhibition over control% = (C – T / C) × 100

Where:

C= mycelial growth of *S. rolfsii* in control.

T= mycelial growth of *S. rolfsii* in dual plate.

5-Manegment of crown and root rot caused by *S. rolfsii*:

The experiments were carried out at greenhouse experimental conditions at Sids Agricultural Research Station, Agricultural Research Center, Beni Suef governorate, during early summer growing season 2020 to evaluate the potential of the tested bioagents to control crown and root rot of tomato. Twenty cm-diameter clay pots each containing sterilized clay: sand: peat moss (1:1:1) were infested with inoculum of *S. rolfsii* (3% w/w). The pots were kept for one week to facilitate growth and colonization of the causal agent in the soil.

5.1. Using chemical treatments

Two groups of infested pots were transplanted by tomato seedlings (Super Gakal) either treated with the tested chemical substances for 30 min or the chemicals were drenched to the infested soil (500 ml/pot). All agriculture practices were successfully applied according to recommendations of Ministry of Agriculture and Land Reclamation where plants were watered and fertilized with NPK 19:19:19 as well as necessary microelements. Each treatment was represented by five pots.

5.2. Using plant extracts:

Tomato seedlings (Super Gakal) were soaked in onion, garlic extract and neem oil before transplanting in the infected pots. Also tested extracts (75 ml/plot) were added individually to the infested pots two days before transplanting. All agricultural practices were successfully applied according to recommendations of Ministry of Agriculture and Land Reclamation where plants were watered and fertilized with NPK 19:19:19 as well as necessary microelements. Each treatment was represented by five pots.

5.3 Using bioagents:

Infested pots were divided into two groups. The first one was treated with each of *Bacillus subtilis*, *Pseudomonas fluorescens* (3×10^8 CFU), and *Saccharomyces cerevisiae* (0.5%), respectively or *Trichoderma harzianum* (1×10^6 conidia/ml). After another one week the pots were cultivated by tomato (Super Gakal) seedlings. The second group was cultivated by tomato seedlings after root-immersing in the suspension of bioagents each alone for 30 min and then transplanted in the infested pots. Treatment with Rizolex-T was used as positive control at the recommended dose.

All agricultural practices were successfully applied according to recommendations of Ministry of Agriculture and Land Reclamation where plants were watered and fertilized with NPK 19:19:19 as well as necessary microelements. In all experiments, Rizolex-T was used as a positive control.

6- Statistical analysis:

The collected data were undergone to analysis of variance based on RCBD where statistical procedures were performed using WASP software. Least significant difference (LSD) was utilized to compare mean differences (Hoshmand, 2006).

RESULTS

1. Survey of crown and root rot in tomato:

The results indicated that Beni Suef County showed the highest disease incidence and severity in 2018 and 2019 (Table, 1). The lowest ones were recorded from Minia County in 2018, since disease severity and incidence were 22.3 and 11.3%, respectively. Whereas, in Matai 2019, disease severity and incidence were 28.0 and 19.3%, respectively. Generally, in 2019 the disease incidence and disease severity were higher than in 2018 in the two governorates.

Table (1): Occurrence of crown and root rot in tomato caused by *S. rolfsii* expressed as disease incidence (DI %) and disease severity (DS %) in different locations at Beni Suef and El-Minia governorates during 2018 and 2019.

Location		Disease occurrence			
Governorate	County	2018		2019	
		DI %	DS %	DI %	DS %
Beni Suef	El-Fashn	26.6	6.6	47.7	22.3
	Beba	37.6	14.3	40.9	32.0
	Beni Suef	48.7	22.3	59.0	41.0
	Mean	37.6	12.2	49.2	31.8
Minia	Maghagha	26.8	15.3	37.5	28.5
	Matai	26.0	11.0	28.0	19.3
	El-Minia	22.3	11.3	38.6	21.0
	Mean	25.0	12.5	34.7	22.9

2- Identification and pathogenicity:

Isolation process from naturally infected tomato plants with crown and root rot symptoms yielded 10 fungal isolates identified as *Sclerotium rolfsii* (Sr.), these isolates were designated as Sr 1 to Sr 10. According to pathogenicity test, isolates Sr 5 and Sr 10 showed the highest disease incidence and disease severity (Table, 2). They caused 90.3 and 88.3% disease incidence and 45.6 and 58.0 disease severity, respectively. The lowest disease incidence was resulted from Sr I (20.6) while Sr 2, Sr 3, Sr 6, Sr 7 and Sr 8 showed comparable disease incidence and severity.

Table (2): Pathogenicity of *S. rolfsii* isolates in tomato plants cv. Super Gakal expressed as disease incidence (DI %) and disease severity (DS %).

Isolates	DI %	DS %	Isolates	DI %	DS %
Sr 1	20.6	12.2	Sr 6	30.0	19.3
Sr 2	32.3	14.0	Sr 7	21.3	16.6
Sr 3	29.6	16.0	Sr 8	45.0	27.1
Sr 4	52.9	32.3	Sr 9	41.0	29.3
Sr 5	90.3	45.6	Sr 10	88.3	58.0

3-Factors affecting linear growth of *S. rolfsii*:

3.1. Effect of some chemical substances treatments:

Data in Table (3) show the inhibitory effect of some chemical substances on growth of *S. rolfsii*. The inhibitory effect of the tested substances was increased by increasing the concentrations. Salicylic acid treatments caused the highest effects. The mean of linear growth was 35 mm., followed by hydrogen peroxide (47.0 mm). Otherwise, citric acid and ascorbic acid recorded the lowest effect, being 55.3 and 62.3 mm, respectively. On the other hand, the positive control treatment (Rizolex-T) showed the highest reduction of linear growth, being 76.7% on the average comparing to the control.

3.2- Plant extracts:

Data in Table 4 show the effect of some plant extracts on linear growth of *S. rolfsii*. Generally, all the tested extracts have a lower inhibitory effect than the positive control treatment (Rizolex-T). Neem oil showed that linear growth was about 49.0 mm and growth inhibition reached 45.6%. Meanwhile, garlic and onion extracts resulted 66.0 and 72.0 mm linear growth and growth inhibition reached 26.7 and 20.0, respectively. Rizolex (Positive control treatment) had the highest effect in reducing the linear growth of *S. rolfsii*.

3.3 bioagents

Antagonism experiments showed a clear inhibition zone on the PDA medium in Petri plates. Results in Table (5) show that *B. subtilis* treatment recorded the highest reduction in linear growth (21 mm and 76.6% inhibition) of *S. rolfsii* followed by *P. fluorescens* (41 mm and 45.4% inhibition). Treatment with *T. harzianum* caused 52 mm linear growth and 42.2% inhibition. In general, the fungicide Rizolex-T had a comparable effect with *B. subtilis* in reducing the linear growth (20 cm). On the other hand, *Saccharomyces cerevisiae* showed the lowest effect on both the linear growth and the inhibitory effect.

Table (3): Effect of chemical substances *i.e.*, hydrogen peroxide, ascorbic, citric and salicylic acids at different concentrations on *S. rolfsii* growth *in vitro*.

Treatments (T)	Conc.%	Linear growth (mm)	Growth inhibition%
Ascorbic acid	0.1	66	26.6
	0.2	61	32.2
	0.3	60	33.3
Mean		62.3	30.7
Citric acid	0.1	58	35.5
	0.2	55	38.9
	0.3	54	40.0
Mean		55.3	38.1
Hydrogen peroxide	0.1	51	43.3
	0.2	46	48.8
	0.3	44	51.1
Mean		47.0	47.7
Salicylic acid	0.1	38	57.8
	0.2	34	62.2
	0.3	33	63.3
Mean		35.0	61.1
Rizolex-T	0.1	24	73.3
	0.2	20	77.8
	0.3	19	78.9
Mean		21	76.7
Control		90	0.0
Grand Mean		57.9	50.9
L.S.D. at 5 %		5.7	-

Table (4): Effect of garlic and onion extracts and neem oil on *S. rolfsii* growth *in vitro*.

Treatments	Linear growth (cm)	Growth inhibition%
Garlic extract	66.0	26.7
Neem oil	49.0	45.6
Onion extract	72.0	20.0
Rizolex-T	20.0	77.7
Control	90.0	0.0
L.S.D. at 5 %	14.0	-

Table (5): Effect of some bioagents, on *S. rolfsii* growth *in vitro*.

Treatments	Linear growth (mm)	growth inhibition %
B. subtilis	21	76.6
P. fluorescens	41	45.4
T. harzianum	52	42.2
S. cerevisiae	68	24.4
Rizolex-T	20	77.8
Control	90	0.0
L.S.D. at 5 %	5.2	-

4-Manegement of crown and root rot caused by *S. rolfsii*:

4.1. Using chemical substances:

In general, the inhibitory effect was increased by increasing the tested chemical concentrations (Table, 6). Also, treatment of soaking the seedling had a higher effect on disease incidence and to somewhat lower effect on disease severity. Treatment by Rizolex-T showed the highest reduction in disease incidence and disease severity. The disease incidence was 36.1 and 46.1 and the disease severity was 13.1 and 13.5 as a result of soaking the seedlings and soil drenching, respectively.

Meanwhile, the second effective substances that reduced the disease caused by *S. rolfsii* were salicylic acid and hydrogen peroxide (Table, 6). The lowest effect was obtained as a result of ascorbic and citric acids treatments.

4.2. Using plant extracts

Data presented in Table (7) show that Neem oil recorded a high effect on reducing disease incidence and disease severity. It caused 44.4 and 66.6 % incidence and 8.13 and 11.1 disease severity as a result of soaking seedlings and soil drenching, respectively. Meanwhile the positive control treatment (Rizolex–T) still showed the highest inhibitory effect on both disease incidence and disease severity (Table, 7). It reduced the disease incidence to 11.1 and 33.33 and the disease severity to 4.53 and 5.27 in case of the soaking seedlings and soil drenching, respectively. On the other hand, onion and garlic extracts had the lowest effect on disease development.

Table (6): Effect of some chemical substances *i.e.*, ascorbic and citric acids and H_2O_2 and salicylic acids on crown and root rot in tomato plants caused by *S. rolfsii* under greenhouse conditions.

Treatments	Conc. %	Application methods (M)			
		Seedling soaking		Soil drenching	
		DI %	DS %	DI %	DS %
Ascorbic acid	0	100.0	40.1	100.0	40.3
	1	88.9	19.1	88.9	21.5
	2	75.0	14.4	88.9	10.7
	3	75.0	13.3	72.0	10.0
Mean		84.7	21.7	87.5	20.6
Citric acid	0	100. 0	39.3	100	39.3
	1	87.0	13.3	93.0	12.6
	2	86.9	10.1	90.0	12.2
	3	88.9	14.6	88.0	11.8
Mean		90.7	19.3	92.8	19.6
Hydrogen peroxide	0	100.0	39.7	100.0	39.2
	1	88.9	14.8	90.0	12.9
	2	67.8	13.3	88.2	12.2
	3	60.1	9.2	70.2	12.9
Mean		79.7	19.3	87.1	19.3
Salicylic acid	0	98.8	39.7	100.0	30.3
	1	53.3	5.9	95.2	5.2
	2	48.9	9.3	90.2	5.2
	3	48.9	12.2	90.0	9.6
Mean		62.8	16.8	93.9	12.8
Rizolex-T	0	100.0	39.3	100.0	38.5
	1	22.2	4.1	44.4	6.0
	2	11.1	4.5	23.3	4.5
	3	11.1	4.4	16.5	5.1
Mean		36.1	13.1	46.1	13.5
Grand mean (M)		70.7	18.0	81.5	17.6
L.S.D. at 5 % for: Treatments (T) Concentrations (C) Application methods (M)		T= 1.0, C= 1.0, M= 1.1, T×C = 2.1, T×M= 2.4, C×M= 2.1 T×C×M = 4.7		T= 0.5, C= 0.3, M= 0.2, T×C= 0.7, T×M= 0.5, C×M= 0.5, T×C×M= 1.1	

Table (7): Effect of application methods, onion and garlic extracts and neem oil on disease incidence (DI) and disease severity (DS) of crown and root rot in tomato plants caused by *S. rolfsii* under greenhouse conditions.

Treatments (T)	Application methods (M)			
	Seedling soaking		Soil drenching	
	DI %	DS %	DI %	DS %
Garlic extract	77.80	13.67	88.90	16.23
Neem oil	44.40	8.13	66.60	11.10
Onion extract	100.00	14.07	100.00	16.63
Rizolex-T	11.10	4.53	33.33	5.27
Control (untreated)	88.90	39.73	100.00	39.20
Mean (M)	64.44	16.03	77.77	17.69
L.S.D. at 5 %	T= 3.40, M= 2.15, TM= 4.81		T= 0.84, M= 0.53, TM= 1.19	

4.3. Using bioagents

Data in Table (8) focus on the potentialities of bioagents to reduce the disease incidence and inhibit the progress of disease compared with the untreated control when they were used individually under greenhouse conditions.

respectively in the control treatment. *P. fluorescens* came in the second rank and recorded the best value on reducing disease Among all treatments, the fungicide Rizolex-T was the most efficient in this regard which recorded the lowest disease incidence. It significantly delayed the progress of disease to 4.53 and 5.27% comparing to 39.7 and 39.2 in case of seedling soaking and soil drenching, incidence and severity followed by *B. subtilis*. The lowest protection was detected in case of treatment with *S. cerevisiae*.

Table (8): Effect of some bio-agents on disease severity and disease incidence of crown/ root rot in tomato plants caused by *S. rolfsii* under greenhouse conditions.

Treatments (T)	Application methods (M)			
	Seedling soaking		Soil drenching	
	DI %	DS %	DI %	DS %
B. subtilis	13.00	55.57	15.13	66.67
P. fluorescens	10.37	44.43	12.59	55.57
T. harzianum	13.67	66.67	15.13	77.80
S. cerevisiae 0.5 %	15.87	88.90	15.50	100.00
Rizolex-T	4.53	11.10	5.27	33.33
Control (untreated)	39.73	88.90	39.20	100.00
Mean (M)	15.93	62.51	16.54	75.01
L.S.D. at 5 %	T= 3.02, M= 1.51, TM= 4.27		T= 0.70, M= 0.35, TM= 0.99	

DISCUSSION

Plant diseases are a significant cost component in crop production. Traditionally, the approaches to dealing with disease in agricultural ecosystems include breeding resistant varieties of the crop's species, hygiene to prevent the spread of contaminated soil or seed and fungicides to kill potentially infecting fungi. However, increasing concerns about the effects of fungicides in the environment and residues in food have resulted in deregistration of a number of fungicides. The need to replace these has increased interest in biological control of plant diseases in recent years. (O'Brien, 2017). *Sclerotium rolfsii* is a saprophytic soil-borne fungus, occurs worldwide and infects more than 500 plant species including tomato, cucumber, soybean, maize, groundnut, bean, watermelon, etc. (Yaqub and Shahzad, 2005; El-El-Ashmony *et al*., 2017 and Kamel *et al*., 2020). Many experiments have been undertaken to reduce using fungicides because of its environmental pollution and may contribute to the production of fungicide-resistant strains.

In the present study, Salicylic acid caused pronounced reduction in the linear growth of *S. rolfsii* followed by hydrogen peroxide. The effect of these substances was reported previously (Galal and Abdou, 1996 and Abdou and Gala, 1997). Also, neem and other essential oils of plants had been studied before to clarify their effect on pathogen growth (Chung, 2006, Dandare *et al*., 2014; Campos *et al.,* 2016 and Osman *et al.,* 2017). *Trichoderma harzianum, P. fluorescens, Bacillus subtilis,* yeasts and other microorganisms are previously used as bioagents for several pathogens (Harman, 2000; Paul and Sharma, 2006; Ahmed *et al*., 2016; El-El-Ashmony *et al*., 2017; Sarhan, 2018 and Kamel *et al*., 2020).

Some of tested treatments applied as seedling soaking or soil drench reduced losses caused by crown and root rot on tomato plants compared to the control. Seedling soaking treatment of tomato plants in bioagents suspensions was the most effective in reducing the disease incidence and disease severity than soil drench method. These results are in harmony with those previously recorded by Ahmed *et al*. (2016); El-Ashmony *et al.* (2017); Sarhan *et al*., (2018) and Farag and Aref (2019). The seed or seedling treatment by biocontrol agents is more applicable methods to control root pathogens because other methods need much higher amount of inocula, the bioagents are able to establish itself in rizosphere and colonize the developed growing roots (Tsahourduo and Thanassoulpoulls, 2002; Kamel *et al*., 2020 and Ketta *et al*., 2021).

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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