

Integrated Management of Tomato Sclerotinia Rot Disease by using the Combined Treatments between Compost, Bioagents and some Commercial Biocides

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Abstract

Efficacy of compost, bioagents and some commercial biocides individually or in combination for controlling tomato sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary under greenhouse and field conditions were studied. Results indicated that *Trichoderma album* and *Bacillus subtilis* were the most effective bioagents in inhibiting mycelial growth of *S. sclerotiorum* *in vitro*. All treatments significantly reduced disease incidence and severity of tomato white rot disease compared with untreated control. However, *T. album* and *Pseudomonas fluorescens* combined with compost reduced the disease incidence and disease severity from 100 and 77.40 in control to 11.1 & 1.5% and 11.1 & 2.5%, respectively, under greenhouse conditions. The integrated *T. album*, *P. fluorescens* and Bio-Zeid with compost recorded the highest increase of fresh weight and dry weight of shoots and roots compared with individual treatments and control. Under field conditions, adding compost to the soil pre-transplanting decreased the percentage of infection and increased yield of tomato plants compared with other treatments with compost. In this respect, the integration between *T. album* + *B. subtilis* + *Ps. fluorescens* and compost was the most effective treatment for reduced the disease incidence and disease severity. As well as, this treatment increased the fruit weight per plant. On the side, all treatments increased the phenols and flavonoids content in tomato plants. The highest increase in the total phenols and flavonoids contents were recorded with *T. album*, *P. fluorescens* and Bio-Zeid combined with compost. Also, all treatments increased peroxidase (PO), polyphenoloxidase (PPO), chitinase and β -1, 3- glucanase activities in treated tomato plants. It could be concluded, the combination between compost, bioagents and some commercial biocides might be useful as an useful tool for controlling tomato sclerotinia disease under greenhouses and field conditions.

Key words: Tomato, *Sclerotinia* rot, Bioagents, Commercial biocides, Compost, Enzymes.

1.Introduction

Egypt is one of the top tomato producers in the world. Egypt production of tomato was placed as the fifth with a global tomato production, which constituting 4.86% of all global production in 2014, as well as, it leads the Africa tomato production, which was 43.11% of total global production. Recently, tomato production of Egypt was increased and reached 8.3 million ton from 214016 hectares with average production of 38.72 ton/hectare through 2014 season [1].

Sclerotinia sclerotiorum (Lib.) de Bary is worldwide in distribution and pathogen to more than 400 plant species. This disease causes significant yield losses of various important crops including tomato [2]. The capability of sclerotia to survive for more than 4 years becomes very difficult to manage the crop from the infection of white mold fungus [3]. The major methods of controlling *Sclerotinia* disease are applying fungicides and crop rotation. However, fungicide chemicals are expensive and not all environmentally safe[2].

Development of new disease protection strategies is essential to achieve an efficient and sustainable tomato industry. *Bacillus subtilis*, *B. thuringiensis* and, *B. amyloliquefaciens* decreased *Sclerotinia* stem rot severity by 72-100% and significantly increased plant height by 52-67%, roots fresh weight by about 66-88% and aerial part weight by

47-75%, compared to *S. sclerotiorum*-inoculated and untreated control [4]. [5] found that, *T. harzianum* and *B. amyloliquefaciens* protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum*. [6] reported that, the use of *Trichoderma* spp. as bioagents induced the accumulation of some enzymes such as chitinase, peroxidase and polyphenol oxidase in treated snap bean plants. Many plant enzymes are involved in defence reactions against plant pathogens. Besides other modes of action, enzymes responsible for cell-wall degradation such as chitinases and glucanases have been associated with the ability of *Trichoderma* to control plant pathogens [7]. Oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin, while other oxidative phenols contribute in formation of defence barriers for reinforcing the cell structure [8]. Chitinase and β -1, 3 glucanase enzymes play a significant role in plant defense against fungi by hydrolyse their cell wall [9-10-11]. Addition of 10% compost to soil significantly decreased diseases as *Aphanomyces* root rot of peas; *Rhizoctonia* root rot of bean, cotton, and radish; *Sclerotinia* drop of lettuce, *Fusarium* wilt of cucumber and *Phytophthora* crown rot of pepper [12].

The objective of the present work is the controlling tomato *Sclerotinia* disease by the compatibility of compost in combination with

bioagents and some commercial biocides in the greenhouse and field conditions in addition to estimate some biochemical as response of treated plants.

2. Materials and methods

2.1 Isolation and identification of the causal organism

Diseased samples of tomato plants showing *Sclerotinia* mold symptoms were collected from EL-Behaira governorate and subjected to isolation trails. *Sclerotinia* spp. were isolated from the lesions appeared on diseased plants. The infected tissues were cut into small pieces, surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, washed for several times with sterilized distilled water, dried between sterilized filter papers and transferred directly to the PDA medium in plate 9cm. The plates were incubated for 1-2 days at $22\pm 2^{\circ}\text{C}$. The fungi grown from the lesion pieces were transferred to potato dextrose agar (PDA) slants. The fungus was purified by hyphal tip technique [13]. The purified fungal isolates were identified according to [14]. PDA slants from the fungus were kept in refrigerator at 4°C for further experiments.

2.2 Laboratory experiments

2.2.1 Effect of antagonistic fungi on the growth of *Sclerotinia sclerotiorum* in vitro

Two discs (\varnothing 5 mm) of 4 days old plain agar culture of both antagonistic fungi (3 *Trichoderma harzianum*, 2 *T. viride*, *T. hamatum*, *T. album*, *T. lignorum* and *Glomus* sp) these isolates were obtained from plant pathology Dept., Fac. of Agric., Benha Univ. Egypt and *S. sclerotiorum* were inoculated simultaneously each opposite the other 1 cm apart from the plate edge in individual plates (\varnothing 9 cm) contained 10 mL PDA medium. In control treatment, each plate was inoculated with 1 disc of mycelial growth of a given isolate of *S. sclerotiorum*. Three plates were used for each treatment. All dishes were incubated at $22 \pm 2^{\circ}\text{C}$ for 10 days. Percentage of the fungal growth reduction (X) was calculated by using the following formula suggested by [15].

$$X = G1 - G2 / G1 \times 100$$

Where: X= fungal growth reduction.

G1= linear growth of the pathogen inoculated alone.

G2= linear growth of the pathogen inoculated against the antagonistic fungus.

2.2.2 Effect of antagonistic bacteria on growth of *S. sclerotiorum* in vitro

Studying the effect of antagonistic bacteria isolates (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus megaterium* and *Serratia*

marcescens) on growth of *S. sclerotiorum* were conducted as follow; individual plates (\varnothing 9 cm) contained PDA medium were streaked at one side 1cm apart from the plate edge with a loop full of the antagonistic bacteria (48 hrs- old) grown on nutrient broth medium (NB) and incubated for 24 hrs at 22°C then the same plate was inoculated at the opposite side 1cm apart from the plate edge with 5mm disc of 4-days-old plain agar culture of *S. sclerotiorum*. All plates were incubated at $22\pm 2^{\circ}\text{C}$ for 5 days [16].

2.3 Greenhouse experiments

The inoculum of *S. sclerotiorum* was grown for two weeks on sand barley medium (3:1, w:w and 40% water). Inoculum of *S. sclerotiorum* fungus was added to the potted soil at rate of 3.0% w/w, mixed thoroughly with the soil surface of each pot then watered and left for one week to insure even distribution of the inoculum.

2.3.1 Preparing inoculum of biological agents

The inoculum of *Trichoderma* (2 *Trichoderma harzianum*, 2 *T. viride*, *T. hamatum*, *T. album*, *T. lignorum*) was grown on PDA plates for 10 days at 22°C . Then transferred to sand barley medium for two weeks (3:1, w: w and 40% water). According to [17], it was grown on agar Bushnell's medium [18]. Plates containing autoclaved Bushnell's agar medium were inoculated, each with a disc 5-mm in diameter of the 7-days old mycelial growth and incubated for 7 days at 28°C . Then *Glomus* sp transferred to sand barley medium for two weeks (3:1, w: w and 40% Bushnell's broth medium). Inocula of each isolate of *Trichoderma* and *Glomus* sp. were added to the potted soil at rate of 3.0% w/w, mixed thoroughly with the soil surface of each pot (\varnothing 20 cm) then watered and left for one week to insure even distribution of the inoculum.

The biological agents were mixed manually into the soil amended with composts at rate 10 % (w/w) and incubated for 14 days in plastic pots at temperature of $18-20^{\circ}\text{C}$. Seven days before sowing, the potting were inoculated with the pathogen.

2.3.2 Effect of some antagonistic bacteria as dipping treatment on *Sclerotinia* rot

In this experiment, three antagonistic bacteria (*Pseudomonas fluorescens*, *Bacillus subtilis*, and *Serratia marcescens*) were used in this study as dipping treatment to evaluate their efficiency in controlling *Sclerotinia* rot.

Bacterial suspensions (1×10^8 cfu/mL) were prepared by dilution plate assay as described by [20]. Bacterial cells from agar cultures of each isolate were inoculated into nutrient broth (NB) and

centrifuged at 3000 rpm for 5 min., the supernatant was discarded, and the precipitate was re-suspended in 100 mL sterilized distilled water. The suspension was centrifuged again for 5 min. and the precipitate was finally suspended in sterilized distilled water. Tomato seedlings were dipped into cell suspension of any of the tested antagonistic bacteria at the rate of 5cm/L for 15 minutes according to [21]. Transplants were planted in pots amended with mixture of soil compost or soil without compost. Untreated transplants were used as control. Soil infestation was carried out as previously mentioned. Three pots were used as replicates for each treatment. Disease incidence and disease severity was determined as mentioned before.

2.3.3 Effect of some commercial biocides in controlling Sclerotinia rot

Three commercial biocides i.e. Plant guard, Blight stop, Bio-Zeid, Table 1, were used as dipping treatment at the rate of 5ml/L for Plant guard, 10ml/L for Blight stop and 5g/L for Bio-Zeid. Healthy tomato transplants of Super Strain B were dipped in each biocide solution for 5 min then raised and left to dry in air before planting. Transplants were planted in pots amended with composted soil or without compost. Untreated transplants were used as control. Soil infestation was carried out as previously mentioned before. Three pots were used as replicates for each treatment. After 45 days from planting data were recorded as mentioned before.

Table (1) List of the tested commercial biocides, their active ingredients, manufacture, and rate of use

Commercial bio- fungicides	Active ingredient	Manufacture	Rate of use/L
Blight Stop (liquid)	Mixture of <i>Trichoderma</i> spp.	Central lab. of Organic Agri. (ARC)	1L/100L water
Bio-Zeid 2.5% Powder	<i>Trichoderma album</i> (10×10 ⁶ spore/g)	Organic for biotechnology, Egypt	5 g
Plant guard L	<i>Trichoderma harzianum</i> (30×10 ⁶ spore/ml)	Bio Tec for fertilizers and biocides, Egypt	5 mL

2.3.4 Determination of enzymes activities

Leaves samples of tomato plants cv. Super Strain B hybrid that treated with different treatments under study in greenhouse were taken 30 days after transplanting. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant layer was used to determine enzyme activities [22].

2.3.4.1 Determination of Peroxidase (PO)

Peroxidase activity was determined according to the method described by [23-24]. Peroxides activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

2.3.4.2 Determination of Polyphenoloxidase (PPO)

The polyphenoloxidase activity was determined according to the method described by [25]. Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

2.3.4.3 Determination of chitinase

Determination the activity of chitinase was carried out according to the method of [26] Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

2.3.4.4 Determination of β-1,3-Glucanase

Determination the activity of the β-1,3-glucanase was carried out according to the method of [27]. β 1-,3-glucanase was expressed as mM glucose equivalent released /g fresh weight tissue /60 minutes.

2.3.5 Determination of phenols and flavonoids activities

2.3.5.1 Determination of total phenols content

Total phenols was determined using Spectrophotometer (SPECTRONIC 20-D) at 520 nm according to the method of [28].

2.3.5.2 Determination of total flavonoids content

The flavonoid content is expressed as milligrams of rutin equivalents per gram of sample (mg RE/g) according to the method of [29].

2.3.5.3 Field Experiments

This experiment was conducted at the Experimental Farm of Faculty of Agriculture, Benha Univ., during October 2014 to January 2015. A field with fine texture soil heavily natural infested with *S. sclerotiorum* was used to evaluate disease incidence and disease severity and fruits weight as result of integrated management of white rot in tomato. Treatments showed efficiency in greenhouse was used. The experiment involved 38 treatments with three replicates in field experiments as follow: *T. album*, *P. fluorescens*, *B. subtilis*, *Glomus* sp, Bio-Zeid, *T. album* + *P. fluorescens* + *B. subtilis*, Compost, *T. album* + Compost, *P.*

fluorescens + Compost, *B. subtilis* + Compost, *Glomus* sp. + Compost, Bio-Zeid + Compost, *T. album* + *P. fluorescens* + *B. subtilis* + Compost and control.

2.4 Statistical analyses

Statistical analyses of all the previously designed experiments have been carried out according to the procedures (ANOVA) reported by [30]. Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

3. Results

Laboratory Experiments

1. Effect of antagonistic fungi on the growth of *S. sclerotiorum*.

Data in Table (2) and Fig (1) show that all antagonistic fungi reduced the growth of *S. sclerotiorum*. In this respect, *Trichoderma album* was the best antagonistic fungus in inhibiting the mycelial growth by 67.78 % followed by *Trichoderma lignorum* by 64.44 %. *Trichoderma harzianum* isolate 1, 2 and 3 and *T. viride* 1 and 2 and *T. hamatum* came the next in reducing the mycelial growth. While *Glomus* sp. resulted in 54.44% growth reduction.

Table (2) Effect of some biological agents on *S. sclerotiorum* growth.

Treatment	Mycelial growth(mm) of <i>S. sclerotiorum</i>	Efficacy %
<i>T. harzianum</i> 1	36	60.00
<i>T. harzianum</i> 2	35	61.11
<i>T. harzianum</i> 3	40	55.56
<i>T. viride</i> 1	36	60.00
<i>T. viride</i> 2	38	57.78
<i>T. hamatum</i>	37	58.89
<i>T. album</i>	29	67.78
<i>T. lignorum</i>	32	64.44
<i>Glomus</i> sp	41	54.44
Control	90	00.00
LSD 0.05 =	5.6	--

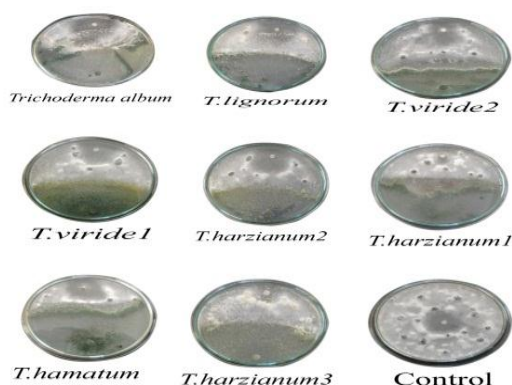


Fig (1) Effect of some species of *Trichoderma* on *S. sclerotiorum* growth

2. Effect of antagonistic bacteria on the growth of *S. sclerotiorum*

Data in Table (3) and Fig (2) show that all antagonistic bacteria reduced the growth of *S. sclerotiorum*. In this respect, *Bacillus subtilis* was

most effective as antagonistic bacteria and reduced growth of *S. sclerotiorum* 57.78% followed by *Pseudomonas fluorescens* giving 45.56% growth reduction. *Bacillus megaterium* and *Serratia marcescens* were the least effective in this respect.

Table (3) Effect of some antagonistic bacteria on the growth of *S. sclerotiorum* in vitro

Treatment	Mycelial growth(mm) of <i>S. sclerotiorum</i>	Efficacy %
<i>Pseudomonas fluorescens</i>	49	45.56
<i>Bacillus subtilis</i>	38	57.78
<i>Bacillus megaterium</i>	64	28.89
<i>Serratia marcescens</i>	64	28.89
Control	90	0.00
LSD 0.05 =	4.4	---

Greenhouse experiments

1. Effect of some antagonistic fungi as adding to soil treatment on the disease incidence, disease severity, fresh and dry weight of shoot and root in tomato.

Data in Table (4) indicate that all the tested isolates of *Trichoderma* spp. were significantly reduced white rot disease incidence and disease severity, as well as it increased fresh and dry weight of shoots and roots. bioagent treatments with compost were effective more than the individual treatment. The highest increase in shoot,

root fresh weight and shoot, root dry weight were recorded in case of treatment with *T. album* combined with compost. *Trichoderma album* combined with compost reduced the disease incidence and disease severity from 100 and 77.40 in control to 11.10 and 1.50 %, respectively, followed by *T. lignorum* with compost. All tested treatments also, increased shoot and root fresh weight, shoot and root dry weight. The least effective antagonists in this respect was *T. harzianum* (1 and 2) and *T. viride* (1 and 2) and *Glomus* sp.

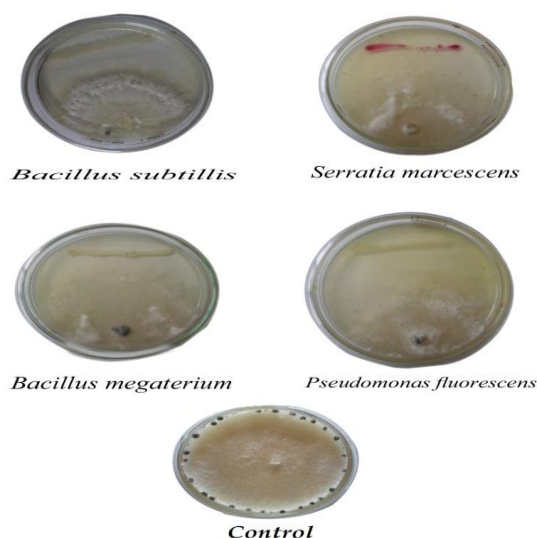


Fig (2) Effect of some antagonistic bacteria on *S. sclerotiorum* in vitro

Table (4) Effect of adding different isolates of antagonistic fungi to soil on the disease incidence, disease severity, fresh and dry weight of shoot and root in tomato.

Treatment	Without compost						With compost									
	DI		DS		FW(g)		DW(g)		DI%		DS%		FW(g)		DW(g)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
T. harzianum1	66.60	25.50	2.13	3.11	0.56	0.85	55.50	24.03	2.80	3.48	0.47	0.72				
T. harzianum2	44.40	25.36	1.21	2.18	0.33	0.58	44.40	22.03	1.30	2.32	0.19	0.43				
T. viride1	22.20	16.53	1.18	2.35	0.25	0.64	44.40	16.00	1.41	3.37	0.43	0.77				
T. viride2	33.30	25.3	1.39	1.93	0.37	0.45	22.20	12.10	2.51	3.77	0.45	0.75				
T. hamatum	55.50	27.16	2.14	2.42	0.58	0.74	22.20	2.56	1.47	2.83	0.54	0.66				
T. album	22.20	5.00	3.33	3.87	0.71	0.92	11.10	1.50	4.01	5.09	0.95	0.97				
T. lignorum	33.30	4.43	2.43	3.13	0.67	0.88	22.20	2.00	3.39	4.50	0.94	0.95				
Glomus sp	44.40	14.10	2.13	3.11	0.56	0.85	22.20	8.30	2.51	3.77	0.45	0.75				
Compost 10%	---	---	---	---	---	---	88.89	45.20	0.44	0.93	0.22	0.13				
Un-inoculated soil	0.00	0.00	2.78	3.80	0.83	0.94	0.00	0.00	2.78	3.80	0.83	0.94				
S. sclerotiorum	100	77.40	0.27	0.37	0.16	0.33	100	77.40	0.27	0.37	0.16	0.33				
LSD 0.05 =	17.60	4.15	0.44	0.27	0.21	0.10	17.60	4.15	0.44	0.27	0.12	0.10				

DI: disease incidence, **DS:** disease severity, **FW:** Fresh weight, **DW:** Dry weight

2. Effect of some antagonistic bacteria as root dipping treatment on the disease incidence, disease severity, fresh and dry weight in tomato.

Data in Table (5) show that all the tested isolates of antagonistic bacteria were significantly reduced the disease incidence and severity, as well as increased fresh and dry weight of shoots and roots. Antagonistic bacteria treatments with compost were more effective than the individual

treatment. The highest increases in shoot, root fresh and dry weight were recorded in case of *Ps. fluorescens* combined with compost. Disease incidence and disease severity were recorded in case of *Ps. fluorescens* integrated with compost by 11.10 and 2.50%, respectively, followed by *B. subtilis* combined with compost where disease incidence and disease severity recorded 22.20 and 6.76 % respectively.

Table (5) Effect of some antagonistic bacteria as dipping treatment on the disease incidence, disease severity, fresh and dry weight of shoot and root in tomato

Treatment	Without adding compost						With adding compost					
	DI%	DS%	FW(g)		DW(g)		DI%	DS%	FW(g)		DW(g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
<i>Ps. fluorescens</i>	33.30	16.46	4.01	5.09	0.95	0.97	11.10	2.50	2.80	3.48	0.47	0.72
<i>B. subtilis</i>	44.40	22.43	3.39	4.59	0.94	0.95	22.20	6.76	1.30	2.32	0.19	0.43
<i>S. marcescens</i>	44.40	25.50	0.44	0.93	0.22	0.13	22.20	12.60	1.41	3.37	0.43	0.77
Compost 10%	---	---	---	---	---	---	88.89	45.20	0.44	0.93	0.22	0.13
Un-inoculated soil	0.00	0.00	2.78	3.80	0.83	0.94	0.00	0.00	2.78	3.80	0.83	0.94
<i>S. sclerotiorum</i>	100	77.40	0.27	0.37	0.16	0.33	100	77.4	0.27	0.37	0.16	0.33
LSD 0.05 =	22.55	4.54	0.21	0.30	0.14	0.08	22.55	4.54	0.21	0.30	0.14	0.08

DI: disease incidence, DS: disease severity, FW: Fresh weight, DW: Dry weight.

4.4.5 Effect of commercial biocides as root dipping treatment on the disease incidence, disease severity, fresh and dry weight of shoot and root in tomato

Data in Table (6) illustrate that all the tested commercial biocides were significantly reduced the disease incidence and disease severity, as well as increasing the fresh and dry weight of shoots and roots. The commercial biocides with compost were effective more than the individual treatment. The highest increase in shoot, root fresh weight and

shoot, root dry weight were in case of treatment with Bio-Zeid combined with compost. Bio-Zeid with compost reduced the disease incidence and disease severity from 100 and 77.40 in control to 22.1 and 8.36 % respectively, followed by Blight stop combined with compost. However, in case of plant guard, disease incidence and disease severity recorded 33.30 and 13.60% respectively, and showed the least incidence in fresh weight and dry weight of shoot and root.

Table(6) Effect of some commercial biocides as root dipping treatment on the disease incidence, disease severity, fresh and dry weight of shoot and root in tomato.

Treatment	Without Compost						With Compost					
	DI%	DS%	FW (g)		DW (g)		DI%	DS%	FW(g)		DW(g)	
			Root	Shoot	Root	Shoot			Root	Shoot	Root	Shoot
Plant guard	33.30	26.63	1.17	1.23	0.48	0.53	33.30	13.60	2.10	2.33	0.53	0.65
Bio-Zeid	66.60	16.93	2.78	2.86	0.74	0.87	22.10	8.36	3.29	3.49	0.84	0.92
Blight stop	55.50	21.93	2.20	2.74	0.67	0.72	22.20	9.16	2.24	2.43	0.80	0.85
Compost 10%	---	---	---	---	---	---	88.89	45.20	0.44	0.93	0.22	0.13
Un-inoculated soil	0.00	0.00	2.78	3.80	0.83	0.94	0.00	0.00	2.78	3.80	0.83	0.94
<i>S. sclerotiorum</i>	100	77.40	0.27	0.37	0.16	0.33	100	77.40	0.27	0.37	0.16	0.33
LSD 0.05 =	37.03	3.28	0.24	0.18	0.11	0.05	37.03	3.28	0.24	0.18	0.11	0.05

DI: disease incidence, DS: disease severity, FW: Fresh weight, DW: Dry weight

2- Effect of the tested treatments on enzyme activity

2.1 Effect of treating tomato plants with some antagonistic fungi as soil adding on the activity of peroxidase (PO), polyphenoleoxidase (PPO),

chitinase and β -1,3 glucanase enzymes under greenhouse conditions

Data in Table (7) show that all treatments were positively increased the activities of PO, PPO and chitinase enzymes in leaves of tomato plants comparing with control treatment. In this respect, the highest effective treatment on PO, PPO chitinase and β -1,3 glucanase enzymes was that expressed in the combinatio between *T. album* with compost where the recorded efficacy was 242.30, 142.36 151.78 and 395.00% respectively. However, the highest activity of PO, PPO and chitinase and β -1,3 glucanase in case of soil inoculated with *Glomus* sp. combined with compost was 296.92%, 103.25% 40.89% and 65.80%. In general, most of the other treatments of integration between biological agents and compost were moderately effective in this respect but all of them were more effective than control treatment.

2.2 Effect of treating tomato plants with some antagonistic bacteria as root dipping on the activity of peroxidase (PO), polyphenoleoxidase (PPO), chitinase and β -1,3 glucanase enzymes under greenhouse conditions

Data in Table (8) illustrate that all treatments were affected positively the activities of PO, PPO and chitinase enzymes in leaves of tomato plants comparing with control treatment. In this respect, the highest effective treatment on PO and PPO enzymes was recorded when combined the *Ps. fluorescens* and compost 297.62 and 208.70%, respectively. On other hand, the combined of *B. subtilis* and compost was the highest effective on Chitinase and β -1,3 glucanase where the recoded efficacy was 57.50 % and 200.83%, respectively. In general, most of the other treatments of combination between bacteria and compost were moderately effective in this respect.

Table (7) Effect of treating tomato plants with some antagonistic fungi as soil adding on the activity of peroxidase (PO), polyphenoleoxidase (PPO), chitinase and β -1,3 glucanase enzymes under greenhouse conditions.

Treatment	PO	PPO	Chitinase	β -1,3 glucanase	Efficacy %			
					PO	PPO	Chitinase	β -1,3 glucanase
T. harzianum 1	32.87	6.12	19.98	2.39	63.15	4.97	78.39	99.16
T. harzianum 2	22.81	11.25	19.83	2.49	13.25	92.96	77.05	107.50
T. viride 1	21.69	4.86	19.56	2.61	7.65	16.63	74.64	117.5
T. viride 2	32.64	8.73	15.56	1.71	62.01	49.74	38.92	42.5
T. hamatum	33.34	11.52	17.78	2.80	65.48	97.59	58.57	133.30
T. album	39.31	12.42	22.95	5.27	95.18	113.03	104.90	339.16
T. lignorum	32.64	12.52	20.86	3.83	62.06	114.75	86.25	219.16
Glomus sp	45.78	9.67	18.42	1.54	127.30	65.86	64.46	28.33
Compost 10%	25.87	5.40	9.72	2.38	28.41	7.37	13.60	98.33
T. harzianum 1+ Compost	47.38	7.65	15.70	2.55	135.21	31.21	40.17	112.50
T. harzianum 2+ Compost	35.49	7.97	21.24	2.66	76.21	36.70	89.64	121.66
T. viride 1+ Compost	36.32	6.12	12.66	2.89	80.27	4.97	13.03	140.83
T. viride 2+ Compost	47.85	9.52	13.70	1.43	137.58	63.29	22.32	19.16
T. hamatum + Compost	42.82	11.52	15.06	3.24	137.58	97.59	34.46	170.00
T. album + Compost	68.94	14.13	28.20	5.94	242.30	142.36	151.78	395.00
T. lignorum + Compost	56.16	11.79	25.95	5.58	65.54	102.22	131.69	365.00
Glomus sp + Compost	79.94	11.85	15.78	1.99	296.92	103.25	40.89	65.80
Un-inoculated soil	16.25	5.31	7.92	2.32	19.3	8.91	29.60	93.33
S. sclerotiorum	20.15	5.83	11.25	1.20	0.00	0.00	0.00	0.00

Table (8) Effect of treating tomato plants with some antagonistic bacteria as root dipping on the activity of peroxidase (PO), polyphenoleoxidase (PPO), chitinase and β -1,3 glucanase enzymes under greenhouse conditions.

Treatment	PO	PPO	Chitinase	β -1,3 glucanase	Efficacy %			
					PO	PPO	Chitinase	β -1,3 glucanase
Ps. fluorescens	44.22	11.52	16.98	1.79	119.56	97.59	51.60	49.16
B. subtilis	27.34	10.26	17.64	1.48	35.74	75.98	50.89	23.30
S. marcescens	23.45	8.01	12.60	1.25	16.43	37.39	12.5	4.16
Compost 10%	25.87	5.40	9.72	2.38	28.41	7.37	13.6	98.33
Ps. fluorescens + Compost	80.11	18	13.68	3.56	297.62	208.7	22.14	196.6
B. subtilis + Compost	75.81	13.23	16.90	3.61	276.41	126.92	57.50	200.83
S. marcescens + Compost	60.81	10.26	11.50	1.98	201.90	75.98	2.67	65
Un-inoculated soil	16.25	5.31	7.92	2.32	19.30	8.91	29.60	93.33
S. sclerotiorum	20.15	5.83	11.25	1.20	0.00	0.00	0.00	0.00

2.3 Effect of treating tomato plants with some commercial biocides as root dipping on activity of peroxidase (PO), polyphenoleoxidase (PPO), chitinase and β -1,3 glucanase enzymes under greenhouse conditions

Data in Table (9) indicate that all treatments were affected positively on the activities of peroxidase (PO), polyphenoloxidase (PPO), chitinase and β -1,3 glucanase in leaves of tomato plants comparing with control treatment. In this respect, the highest effective

treatment on PO, PPO, chitinase and β -1,3 glucanase was that expressed on the combined between Bio-Zeid with compost where the recorded efficacy was 171.8, 145.12 103.48 and 410.00%, respectively followed by Blight stop + Compost 76.56, 112.30, 75.53 and 292.50%, respectively. Plant guard + Compost showed the least effective in this respect. In general, most of the other treatments of integration between biological agents and compost were moderately effective in this respect.

Table (9) Effect of treating tomato plants with some commercial biocides as root dipping on the activity of peroxidase (PO), polyphenoleoxidase (PPO), chitinase and β -1,3 glucanase enzymes under greenhouse conditions.

Treatment	PO	PPO	Chitinase	β -1,3 glucanase	Efficacy %			
					PO	PPO	Chitinase	β -1,3 glucanase
Plant guard	21.97	8.23	13.08	2.25	9.08	41.16	16.78	87.50
Bio-Zeid	10.24	13.49	16.94	4.41	49.15	131.38	51.25	267.50
Blight stop	26.11	10.08	14.52	2.40	29.64	72.89	29.64	100.25
Compost 10%	25.87	5.40	9.72	2.38	28.41	7.37	13.6	98.33
Plant guard + Compost	32.95	11.07	17.20	3.54	63.60	89.23	53.57	195.00
Bio-Zeid + Compost	54.75	14.34	22.79	6.12	171.8	145.12	103.48	410.00
Blight stop + Compost	35.56	12.42	19.66	4.71	76.56	112.30	75.53	292.50
Un-inoculated soil	16.25	5.31	7.92	2.32	19.30	8.91	29.6	93.33
<i>S. sclerotiorum</i>	20.15	5.83	11.25	1.20	0.00	0.00	0.00	0.00

3. Effect of the tested treatments on the flavonoids and phenols activity

3.1 Effect of adding some biological agents to the soil on activity of flavonoids and total phenols of tomato plants under greenhouse conditions

Data in Table (10) show that all treatments of biological agents in soil inoculated with the pathogenic fungus *S. sclerotiorum* were affected positively on the activities of flavonoids and total

phenols in leaves of tomato plants in compared with control treatment. In this respect, the highest effective treatment on flavonoids and total phenols was that expressed on the combined between *T. album* with compost where the recorded efficacy was 303.68 and 767.89%, respectively. In general, most of the other treatments of combined between biological agents and compost were moderately effective.

Table(10) Effect of adding some bio-agents as soil treatment to tomato plants on the activity of flavonoids and total phenols under greenhouse conditions

Treatment	Flavonoids	Total phenols	Efficacy %	
			Flavonoids	Total phenols
<i>T. harzianum</i> 1	2.07	7.25	8.94	105.9
<i>T. harzianum</i> 2	2.91	4.39	53.15	24.71
<i>T. viride</i> 1	1.62	9.24	-14.73	162.5
<i>T. viride</i> 2	2.74	5.26	44.21	49.4
<i>T. hamatum</i>	2.07	17.64	8.94	401.13
<i>T. album</i>	6.66	23.52	250.52	568.18
<i>T. lignorum</i>	6.10	18.42	221.05	423.40
<i>Glomus</i> sp.	1.45	4.78	-23.68	35.79
Compost 10%	2.18	5.56	14.73	57.95
<i>T. harzianum</i> 1+ Compost	3.24	10.36	70.52	194.31
<i>T. harzianum</i> 2+ Compost	4.87	6.44	156.31	82.95
<i>T. viride</i> 1+ Compost	2.52	12.04	32.63	242.04
<i>T. viride</i> 2+ Compost	3.75	7.84	97.36	122.72
<i>T. hamatum</i> + Compost	2.18	11.76	14.73	234.09
<i>T. album</i> + Compost	7.67	30.55	303.68	767.89
<i>T. lignorum</i> + Compost	6.94	32.43	52.26	261.73
<i>Glomus</i> sp + Compost	3.69	6.86	94.21	94.88
Un-inoculated soil	2.52	4.41	32.63	25.28
<i>S. sclerotiorum</i>	1.90	3.52	0.00	0.00

3.2 Effect of treating tomato plants with some bacteria as root dipping on the activity of flavonoids and total phenols under greenhouse conditions

Data in Table (11) illustrate that all tested treatments of compost and bacteria in soil inoculated or un-inoculated with the pathogenic fungus *S. sclerotiorum* and compost affected positively on the activities of flavonoids and total

phenols in leaves of tomato plants comparing with control treatment. In this respect, the highest effective treatment on flavonoids and total phenols was *B. subtilis* combined with compost where it recorded 64.73 and 336.90%, respectively, followed by *Ps. fluorescens* with compost. In general, most of the other treatments of combined between biological agents and compost were moderately effective in this respect.

Table(11) Effect of treating tomato plants with some bio-agents bacteria as root dipping on the activity of flavonoids and total phenols under greenhouse conditions.

Treatment	Flavonoids	Total phenols	Efficacy %	
			Flavonoids	Total phenols
Ps. fluorescens	2.39	11.27	25.78	90.95
B. subtilis	2.57	9.24	35.26	262.50
S. marcescens	2.07	7.15	8.94	103.12
Compost 10%	2.18	5.56	14.73	57.95
P. fluorescens + Compost	3.024	15.38	59.15	183.80
B. subtilis + Compost	3.13	9.99	64.73	336.9
S. marcescens + Compost	2.85	8.23	50.00	133.18
Un-inoculated soil	2.52	4.41	32.63	25.28
S. sclerotiorum	1.90	3.52	0.00	0.00

3.3 Effect of treating tomato plants with some commercial biocides as root dipping on the activity of flavonoids and total phenols under greenhouse conditions

Data in Table (12) show that adding of all treatments of vegetarian compost and biocides in soil inoculated or un-inoculated with the pathogenic fungus *S. sclerotiorum* and compost were affected positively on flavonoids and total phenols content

in leaves of tomato plants comparing with control treatment. In this respect, the highest effective treatment on flavonoids and total phenols was that expressed in the combined Bio-Zeid with compost where the recorded efficacy was 44.21 and 657.10%, respectively. Plant guard+Compost and Blight stop + Compost were increased significantly only the total phenols where the recorded efficacy was 336.93 and 573.57%, respectively.

Table (12) Effect of treating tomato plants with some commercial biocides as root dipping on the activity of flavonoids and total phenols under greenhouse conditions

Treatmen	Flavonoids	Total phenols	Efficacy %	
			Flavonoids	Total phenols
Plant guard	1.17	5.26	38.10	49.43
Bio-Zeid	2.02	10.87	6.31	208.80
Blight stop	1.68	7.44	11.57	111.36
Compost 10%	2.18	5.56	14.73	57.95
Plant guard + Compost	1.84	15.38	3.15	336.93
Bio-Zeid + Compost	2.74	26.65	44.21	657.10
Blight stop + Compost	1.96	23.71	3.15	573.57
Un-inoculated soil	2.52	4.41	32.63	25.28
S. sclerotiorum	1.90	3.52	0.00	0.00

5.7 Field Experiment

This experiment was carried out under field conditions in season 2016. The best treatments which reduced the Sclerotinia rot disease incidence and disease severity under greenhouse were chosen and applied in tomato against *S. sclerotiorum*.

5.7.1 Effect of interaction between treatments with compost on tomato white rot diseases:

Data in Table (13) illustrate that the tested treatments were significantly reduced disease incidence, disease severity and increased fruits weight per plant. Adding compost to the soil pre-transplanting was decreased the percentage of infection and it was increased yield of plants compared with control treatment. Combined

between *T. album* + *B. subtilis* + *Ps. fluorescens* with compost was the most effective treatment and reduced disease incidence and disease severity

compared with control. As well as, increased fruit weight per plant to 3.85 kg compared with 2.10 kg in control.

Table (13) Effect of integration between biological agents and compost on tomato white rot disease incidence, disease severity and fruits yield as well as weight kg/plant under field conditions during 2016 season

Treatment	Without adding compost			With adding compost		
	% Disease incidence	% Disease severity	Fruits weight /plant with kg	% Disease incidence	% Disease severity	Fruits weight /plant with kg
T. album	20.83	11.10	2.95	20.83	9.70	3.05
Ps. fluorescens	20.83	9.70	2.58	16.75	7.50	3.13
B. subtilis	25.00	10.60	2.61	16.75	9.10	3.32
Glomus sp.	33.33	16.60	2.07	16.66	9.90	3.10
Bio-Zeid	20.83	10.70	2.98	20.83	9.70	3.49
T. album + B. subtilis + Ps. fluorescens	8.33	6.80	3.44	4.16	2.30	3.85
Compost 10%	---	---	---	62.53	30.70	2.96
Control	70.83	35.40	2.10	70.83	35.40	2.10
LSD 0.05 =	13.12	2.83	0.25	11.52	2.71	0.32

4. Discussion

Trichoderma album was the best antagonistic fungus in inhibiting the mycelial growth of *S. sclerotiorum* followed by *Trichoderma lignorum*. However, *Glomus* sp. was the least effective in this respect. On the other side, *Bacillus subtilis* was the best antagonistic bacteria in reducing growth of *S. sclerotiorum* followed by *Pseudomonas fluorescens*. These results agree with the results of [31-32-33].

Indeed, a successful biocontrol agent is generally equipped with several attributes which often promotes plant growth as efficiently as it inhibits fungal growth by efficient root colonization, phytohormone production and nutrient competition [34]. [5] showed that *T. harzianum* and *B. amyloliquefaciens* inhibited the growth and production of mycelia and sclerotia of *S. sclerotiorum*. *T. harzianum* and *B. amyloliquefaciens* appeared to exhibit mycoparasitism and antibiosis respectively, *in vitro*. [35] reported that, *Trichoderma harzianum*-8, *T. atroviride* PTCC5220 and *T. longibrachiatum* PTCC5140 showed high growth inhibition of two phytopathogenic isolates of *S. sclerotiorum* (S1 and S2) *in vitro*.

Under greenhouse all the tested isolates of *Trichoderma* spp. was significantly reduced sclerotinia rot disease incidence and disease severity, as well as it was increased fresh weight and dry weight of shoots and roots. Integrated treatments of bioagents with compost were effective more than the individual treatment. The highest increase in shoot, root fresh weight and shoot, root dry weight of tomato plants were in case of treatment with *T. album* combined with compost.

Also, all the isolates of antagonistic bacteria significantly reduced sclerotinia rot disease incidence and severity, as well as increased fresh and dry weight of shoots and roots of tomato plants.

The highest reduction in disease incidence and disease severity and the highest increase in shoot, root fresh and dry weight were obtained from *Ps. fluorescens* combined with compost. Bio-Zeid integrated with compost was reduced the disease incidence and disease severity from 100 and 77.40% in control to 22.1 and 8.36 %, respectively, followed by Blight stop. Soil organic amendments serve as a source of nutrients that result in an increase in the soil microbial population, which may influence disease suppression [36]. This suggests that toxic substances from organic matter and mycoparasitism by the biocontrol agents may have contributed to the enhancement of control of *S. sclerotiorum*. These results came in agreement with the results obtained by [37-38] they found that, *T. harzianum* and *B. amyloliquefaciens* protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum*. The efficacy of *T. harzianum* and *B. amyloliquefaciens* compared with two commercial products, Plant Shield and Soil Gard in the control of *S. sclerotiorum* was similar or slightly lower depending on the crop plant. Several mechanisms, by which *Trichoderma* spp. influences plant development have been suggested, such as production of growth hormones, solubilization of insoluble minor nutrients in soil [39] and increased uptake and translocation of less available minerals [40- 41]. Uptake of certain minerals, such as P and N, is of key importance considering their role in plant growth [42-43]. Promotion of growth and yield by *Trichoderma* spp. may be a result of increased root area, allowing the roots to explore larger volumes of soil and by this to access nutrients, increased solubility of insoluble compounds as well as increased availability of micronutrients [39-44]. However, initially *Trichoderma* must be able to establish an interaction with the root system. The ability of

Trichoderma species to colonize the root system of a plant depends also on the plant species. Availability of water in the soil may play a key role in facilitating establishment and effectiveness of *Trichoderma* in the soil [45]. Among the positive effects of *Trichoderma* on different plants that have been noted over the past five to ten years in studies conducted by other authors [46]. The results reported by [47] clearly illustrated that some *Trichoderma* secondary metabolites are directly involved in the *Trichoderma*-plant interactions, and particularly that the compound 6PP may be considered to act as an auxin-like compound and/or may act as an auxin inducer. [4] found that *Bacillus subtilis* B10 (KT921327) and B14 (KU161090), *B. thuringiensis* B2 (KU158884), *B. amyloliquefaciens* B13 (KT951658) and B15 (KT923051), and *Enterobacter cloacae* B16 (KT921429) suppressed myceliogenic germination of sclerotia and improved germination of bacterized tomato seeds as compared to the untreated controls. The screening of their disease-suppressive and plant growth-promoting abilities revealed 72-100% decrease in Sclerotinia stem rot severity and significant increments in plant height by 52-67%, roots fresh weight by about 66-88% and aerial part weight by 47-75%, compared to *S. sclerotiorum* inoculated and untreated control.

Trichoderma album with compost and *B. subtilis* combined with compost were the highest effective bio-treatments and expressed the highest increase in flavonoids and total phenols in tomato plants. Among commercial biocides Bio-Zeid was the highest effective one and when combined with compost were increased the flavonoids and total phenols content in tomato leaves compared with control. These obtained results could be interpreting in light the findings of [49] who found that, treating cucumber seeds with *T. viride* or *B. subtilis* or citric acid increased the total phenols content in cucumber plants sown in soil inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* compared with untreated plants. [50] found that, applying the *Pseudomonas fluorescens* 9 and 10 by bio-primed seed of faba bean treatment enhanced the accumulation of total phenols and flavonoids. In addition, [51] mentioned that the anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals or chelating process.

Under field conditions, adding compost to the soil pre-transplanting decreased the percentage of infection and increased yield of tomato plants compared with un-amended treatments with compost. Integration between *T. album* + *B. subtilis* + *Ps. fluorescens* and compost was the most effective treatment and reduced disease incidence and disease severity of *S. sclerotiorum* As well as, increased fruit weight per plant. It is known that synergisms between the actions of biological and

chemical fungicides may exist in which the effects of one treatment favor the other [52]. Nevertheless, it may be possible to develop further arrangements between antagonists and chemical fungicides or improved strategies that will enhance white mold control and increase the death rate of sclerotia in soil, with benefits to producing crops in areas infested by *S. sclerotiorum*. The effects of organic amendments, suggests that both chemical and biological components of compost-amended soils can contribute to disease suppression [53-54]. The addition of decent quality of compost is essential for increasing soil organic matter and providing nutrients for the crop. Further, increasing organic matter results in a more extensive and varied microbial community, resulting in suppression of soil-borne pathogens and improved plant health. [55] recorded that, under field conditions combining the fungicide Folicur with compost has enhanced the control of white rot of onion and bulb yield compared with using each treatment alone. [56] revealed that the combination of Chitosan and CuSO₄ increased phytoalexin production. Addition of 10% compost to soil significantly decreased diseases such as Aphanomyces root rot of peas; Rhizoctonia root rot of bean, cotton, and radish; Sclerotinia drop of lettuce, Fusarium wilt of cucumber and Phytophthora crown rot of pepper [12]. The association of methods of control may increase the efficiency of white mold management with subsequent yield increases, demonstrating its suitability and efficacy to manage the disease.

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