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ORIGINAL PAPER

Bio-Control Potentials of *Trichoderma* spp. Against *Sclerotium rolfsii* the Causative of Root and Crown Rot in Tomato, Common Bean and Cabbage.

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ABSTRACT

In the current study, evaluation the ability of three isolates of *Sclerotium rolfsii* isolated from tomato, bean and cabbage to infect their host plants and two non-host plants was carried out. Tomato plants were strongly affected with the three isolates of the pathogen followed by cabbage plants, whereas common bean plants were the least in this respect. Six species of *Trichoderma* were examined for their bio-control potentials against *S. rolfsii* the causative of root rot in tomato, common bean and cabbage. *In vitro* antagonistic test showed that *T. koningii* exhibited high inhibitory effect as the percentage of inhibition value was higher in case of *S. rolfsii* (tomato isolate) followed by *T. harzianum* with *S. rolfsii* (bean isolate) and *S. rolfsii* (cabbage isolate). Under greenhouse conditions; *T. koningii*, *T. viride* and *T. harzianum* showed the highest antagonistic effect against the three isolates of *S. rolfsii* in pots experiment. Microscopic examinations showed that most of *Trichoderma* spp. grew over mycelia of the tested pathogen with surrounded, coiling, lysis of hyphae and collapse of mycelium. *T. koningii* and *T. viride* exhibited the best performance regarding to the defense enzyme secretion; chitinase and β-glucanase followed by *T. harzianum*. Also, all the tested *Trichoderma* spp. produced Indole acetic acid (IAA) and Gibberellic acid (GA) as a plant growth promoting substances in variable values.

Keywords: Crown rot, enzyme; light microscopy; Root rot; *Sclerotium rolfsii*; *Trichoderma* spp.

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INTRODUCTION

Sclerotium rolfsii is an economically important pathogen on numerous crops worldwide, and commonly occurs in the tropics, subtropics and other warm temperate regions (Punja, 1985). It has wide host range, abundant growth of the pathogen and its capability of producing excessive sclerotia that may persist in soil for several years (Chet and Henis, 1972 and Punja, 1985). Hence, management of *Sclerotium rolfsii*, causing root rot of vegetable plants is difficult to be achieved chemically, in this context bio agents can be used as an alternative source for controlling soil-borne diseases since they comprise a rich source of bioactive substances (Wink, 1993). Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases (Harman *et al*. 1980; Sivan *et al*., 1984 and Coley-Smith *et al*., 1991).

Trichoderma spp. are one of the most important filamentous fungi common in soil and plant roots and can be used as an effective biocontrol agents for soil borne fungal plant pathogens and some species are also known for their abilities to control plant diseases (Gajera *et al*., 2015). *Trichoderma* species are highly opportunistic and have been isolated from a diverse range of natural and artificial substrata, which show their adaptability to various ecological conditions (Druzhinina *et al.*, 2011and 2012). *Trichoderma* species are some of the best studied fungal species which are being developed as biocontrol agents commercially (Schuster and Schmoll, 2010). *Trichoderma* spp. play an important role in three-ways interaction with plant and pathogen (Woo *et al.*, 2006; Shoresh *et al*., 2010; Viterbo and Horwitz, 2010 and Hermosa *et al*., 2012). *Trichoderma* spp. are endophytic plant symbionts that can also establish themselves in the rhizosphere and are widely used as seed treatments to control diseases and to enhance plant growth and yield (Mastouri *et al.*, 2010; Harman, 2011). *Trichoderma* spp. can control plant diseases through various

mechanisms such as antibiosis, competition, suppression, mycoparasitism, induced resistance, hypo virulence and predation (Mayee and Datar, 1988 and Tseng *et al*., 2008).

The present investigation was carried out to evaluate the bio efficacy and antagonistic effects of six *Trichoderma* spp. against root and crown rot caused by *S. rolfsii* in tomato, common bean and cabbage plants. Furthermore, determination of hydrolysis of chitin and cellulose enzymes was taken into consideration.

MATERIALS AND METHODS

Plant materials:

Three vegetable crops; tomato, common bean and cabbage cvs, Super Strain B, Paulista and Os-cross, respectively were used in the current study. Seeds of all cultivars were obtained from the Horticultural Research Institute, Agricultural Research Center, Egypt.

Isolation and identification of the associated micro-organisms:

Samples of tomato, common bean and cabbage showing typical symptoms of root and crown rot were collected from some Farms located at Beba county, Beni Sweif governorate, Egypt. The infected roots were firstly washed under running tap water, air dried, then surface disinfected by dipping in 3% sodium hypochlorite solution for 3 minutes, washed several times with sterilized distilled water and dried between two folds of sterilized filter papers. The sterilized samples were cut into small fragments and aseptically transferred onto the surface of potato dextrose agar (PDA) medium in Petri plates (9cm in diam.).Thereafter, plates were incubated at 25±2°C and they were daily visually inspected for a week. The emerged fungi were picked up and transferred onto new PDA medium. Purification of each isolated fungus was carried out using the hyphal tip technique (Hawker, 1956). Identification of the isolated fungi was conducted according to their cultural and morphological characteristics according to the descriptions of Punja and Damini (1996); Sarma *et al*. (2002) and Watanabe (2002). Identification was confirmed in Mycological Research and Disease Survey Department, ARC, Giza, Egypt. Frequency of the isolated fungi was calculated. Stock cultures were maintained on PDA slants and kept at 5°C for further studies.

Inoculum preparation of *S. rolfsii*:

Three isolates of *S. rolfsii* isolated from tomato, common bean and cabbage were used in the present study. These isolates were grown on PDA at 25±2°C for 10 days. Mycelium plug (5mm) of each tested isolate was cut using a 0.5-cm-diameter cork borer and placed in bottles containing sterilized sand corn medium (25 g washed sand, 75 g corn and enough tap water to cover the mixture in 500 ml bottles) and plugged with cotton wool and aluminum foil (Sennoi *et al*., 2010). The bottles were incubated at room temperature (25 ±2°C) and shacked regularly to support the fungus colonization. Three weeks post incubation; the inoculum was ready to use.

Soil infestation:

Inoculum of each isolate of *S. rolfsii* was mixed thoroughly with the soil of plastic pots (25cm diameter) filled with sterile substrate mix of 1:2 sand and peat moss, at the rate of 2% w/w. The infested pots were irrigated till saturation 7 days before sowing.

Pathogenicity test:

The three isolates of *S. rolfsii* were tested for their pathogenicity against their host plants from which they were isolated and two other plant species. Three healthy transplants (28 days old) of tomato Super Strain B cv., two healthy seedlings of cabbage Os-cross cv. (28 days old) and five healthy seeds of common bean Paulista cv. were planted in the infested plastic pots prepared as mentioned before, each pot served as one replicate and five replicates were used for each treatment. Healthy transplants of tomato and seedlings of cabbage in addition to seeds of common bean were planted in non-infested pots as check treatment.

Inoculum preparation of *Trichoderma* species:

Six different species of *Trichoderma* namely; *T. asperillium*, *T. harzianum*, *T. hamatium*, *T. koningii*, *T. viride* and *T. album* obtained from our stock at Vegetable Diseases Research Department, Plant Pathology Research Institute, ARC were used in the experiments. All isolates were cultured and maintained on PDA medium in an incubator at 25°C. Four days post incubation, mycelium plugs of any tested species of *Trichoderma* growing on PDA were taken using a cork borer (0.5-cm-diameter) and transferred to Czapix·s Dox broth (Allen, 1950) in flask 500 ml. The flasks were incubated in a shaken incubator at 25°C for 7-10 days.

***Trichoderma* species versus *S. rolfsii in vitro*:**

Interestingly, seven days-old pure cultures of *S. rolfsii* (three isolates) and *Trichoderma* isolates (six isolates) were used in this experiment. Discs of 5 mm were taken from each isolate and transferred to new Petri dishes (9 cm in diam.) containing PDA medium and incubated in darkness at 25±2°C for the *in vitro* test. Linear growth of the tested *Trichoderma* spp. against *S.*

rolfsii isolates was recorded by the direct confrontation approach where each Petri dish included two discs, one from the pathogen and one from the bio-agent, placed at 7 cm distance from each other. Reduction percentages of *S. rolfsii* were calculated by using the formula:

$$\% \text{ inhibition over control} = \frac{C-T}{C} \times 100$$

Where: C- mycelial growth of *S. rolfsii* in control; T- mycelial growth of *S. rolfsii* in dual plate.

***Trichoderma* species versus *S. rolfsii in vivo*:**

In the greenhouse, seeds of tomato and cabbage were sown in 84-cell seedling trays containing peat moss-vermiculite mixture (1:1). 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. Thereafter, *S. rolfsii* inoculum (5% volume) was added to each 20 cm pot containing sterilized clay: sand: peat moss (1:1:1). The pots were kept free of plants for one week to facilitate growth and colonization of the causal agent in the soil. Four weeks after sowing, seedlings of tested tomato and cabbage cultivars were transferred to transplant into the inoculated pots. Regarding common bean, all seeds were directly sown into infested 20 cm pots under greenhouse conditions. Subsequently, the desired *Trichoderma* species was added at the rate of 5ml/pot (spore suspension at a concentration 1.8 x 10^6 spore/ ml). Additionally, other plants representing each crop were allowed to grow in non-infested pots to serve as a control. The fungicide Rizolex-T, (active ingredient: Tolcofos-methyl) obtained from Sumitomo Chemical CO, LTD, Osaka, Japan. at the concentration of 2g/l was used.

Disease assessment:

Plants of each crop were evaluated up to six weeks after sowing. The plants without yellowing, root rot and crown rot were considered healthy, whereas those displayed conspicuous symptoms (yellowing, root rot and crown rot) were considered infected. The percent disease incidence (PDI) was assessed using the following formula.

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

Disease severity was recorded (% symptomatic plants) based on the progress of yellowing and root rot and rotting at the end of the experiment. Disease severity % (DS%) assessment was determined according to a 0-5 scale of Shahzad & 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 *et al*. (1995).

$$\% \text{ Disease severity} = \frac{\Sigma n \times r}{5N} \times 100$$

Where: n = number of plants in each numerical rate, N = total number of plants multiplied by the maximum numerical rate, r = 5.

Microscopic observations:

Light microscope (Leica DM1000) examination was used to study in duel culture antagonism between *Trichoderma* spp. tested and *S. rolfsii* after seven days. Photographing by microscope was done at the Vegetable Diseases Research Dept., Plant Pathology Research Institute, A.R.C, Giza. Egypt.

Biochemical activity:

Activities of chitinase (mg glucoseamine/g dry soil) were determined according to Boller and Mauch (1988). and β-glucanase was produced and determined according to the method described by Dewi *et al*. (2016).

The most active *Trichoderma* spp. isolates were tested for their quantitative capabilities to produce auxins (IAA) and gibberellins (GA) by the methods described by Glickmann and Dessoux (1995)

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

Isolation and identification of the associated micro-organisms:

Data presented in Table (1) show that four fungal species were isolated from roots and crowns of tomato, common bean and cabbage plants naturally infected with stem and crown rots. These fungi were identified according to their morphological criteria as *Sclerotium rolfsii* Sacc, *Fusarium oxysporum* (Schlecht emend. Snyder and Hansen), *F. solani* (Mart.) Sacc. and *Rhizoctonia solani* Kuhn. *Sclerotium rolfsii* was the most prevalent with tomato, bean and cabbage .The corresponding frequencies were 46.7, 57.14 and 40.0%, respectively, of the total isolates followed by *Fusarium oxysporum* with frequencies 20.0, 0.0 and 20.0%, respectively.

Meanwhile, frequencies of *F. solani* were 26.7, 28.57 and 26.7from samples of tomato,

bean and cabbage, respectively. However, *R. solani* isolated from the three vegetables was the lowest prevalent fungus (6.6, 14.26 and 13.3%), respectively.

Table (1): Fungi isolated from roots, crowns and stems of tomato, common bean and cabbage plants suffered from root and crown rot and their frequencies.

Isolated fungi	Tomato		Bean		Cabbage	
	No. fungal colony	Frequency %	No. fungal colony	Frequency %	No. fungal Colony	Frequency %
S. rolfsii	7.0 a	46.7	8.0 a	57.14	6.0 a	40.0
F. oxysporum	3.0 b	20.0	0.0 d	0.0	3.0 c	20.0
F. solani	4.0 b	26.7	4.0 b	28.57	4.0 b	26.7
R. solani	1.0 c	6.6	2.0 c	14.29	2.0 d	13.3
Total	15.0	100	14.0	100	15.0	100

- Values in the same column followed by the same letter are not significantly differences *at P < 0.5* level.

Pathogenicity test:

Infection of tomato, common bean and cabbage plants grown in soil artificially infested with each of the three isolates of *Sclerotium rolfsii* was estimated as percentage of disease incidence and severity under greenhouse conditions. Three isolates of *S. rolfsii* were isolated from each of tomato, common bean and cabbage plants and were used against their host plants (tomato, common bean and cabbage).

Data illustrated in Table (2) and Fig. (1) show that *S. rolfsii* isolated from tomato plants recorded the highest disease incidence and disease severity percentages of infection with root and crown rot on its host (tomato) than on both bean and cabbage plants, except at 50 days after sowing at which the recoded infection reached the highest disease severity in the three tested hosts. Bean plants were affected by this isolate more than cabbage plants at different periods. The infection reached its maximum in tomato, bean and cabbage plants when determined on 50 days old plants. The disease severity of root and crown rot in cabbage plants with the tested fungal isolate was lesser than that recorded from tomato and bean plants when determined after 20, 30 and 40 days after planting into infested soil (Fig. 1).

Table (2): Pathogenicity test of *S. rolfsii* isolated from tomato plant on tomato, common bean and cabbage plants in pots experiments.

Source of isolate	Host	% Disease incidence				
		After 10 days	After 20 days	After 30 days	After 40 days	After 50 days
Tomato	Tomato	13.3	33.3	60.0	93.3 a	100 a
	Bean	16.0	36.0	64.0	88.0 c	100 a
	Cabbage	10.0	40.0	70.0	90.0 b	100 a
Control uninfested soil		0.00	0.00	0.0	0.0 d	0.0 d

- Values in the same column followed by the same letter are not significantly differences *at P < 0.5* level.

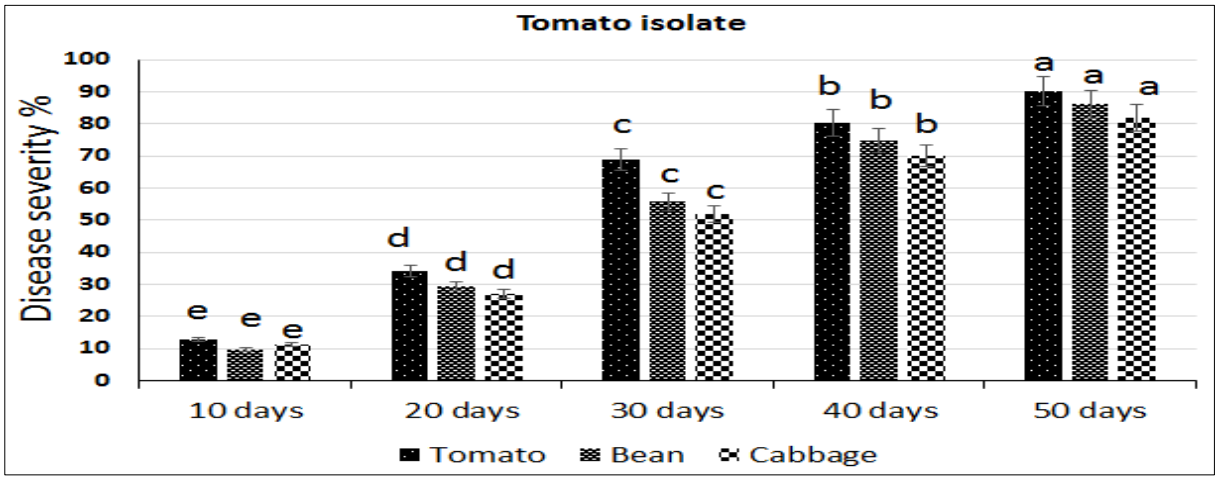


Fig. (1): Disease severity of *S. rolfsii* (tomato isolate) on tomato, common bean and cabbage plants under greenhouse conditions.

Sclerotium rolfsii (common bean isolate) was able to infect bean, tomato and cabbage plants causing different percentages of infection during different periods. Ten days after sowing in artificially infested soil with the tested fungal isolate, tomato, common bean and cabbage plants had been infected with root and crown rot (Table, 3 and Fig., 2). The disease severity percentages of root and crown rot at 10 days were 14.1, 8.3 and 9.4%, respectively (Fig. 2), for the three crops mentioned before. Whereas at 20, 30 and 40 days' plant age common bean cultivar recorded the highest percentage of disease severity with crown rot than tomato and cabbage cultivars. Interestingly, at 50 days old, plants of all tested crops were completely destroyed and recorded maximum disease incidence (100 %) and disease severity 83, 88 and 81% on tomato, common bean and cabbage respectively.

Table (3): Pathogenicity test of *S. rolfsii* isolated from common bean plant on tomato, common bean and cabbage plants in pots experiments.

Source of isolate	Host	% Disease incidence				
		After 10 days from sowing	After 20 days from sowing	After 30 days from sowing	After 40 days from sowing	After 50 days from sowing
Bean	Tomato	20.0	40.0	66.7	93.3 a	100 a
	Bean	12.0	32.0	60.0	88.0 c	100 a
	Cabbage	10.0	40.0	70.0	90.0 b	100 a
Control uninfested soil		0.0	0.0	0.0	0.0 d	0.0 b

- Values in the same column followed by the same letter are not significantly differences *at P < 0.5* level.

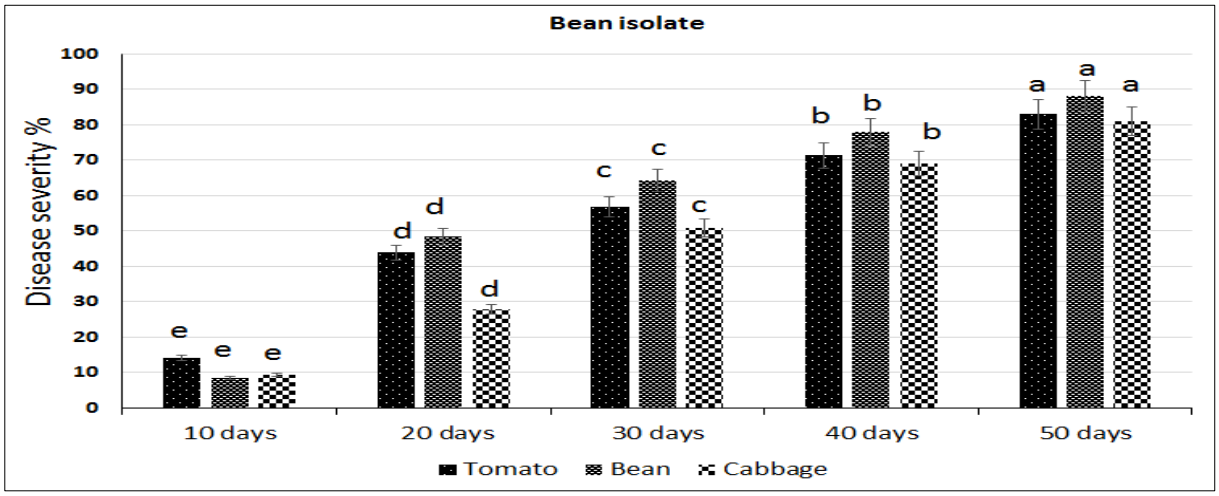


Fig. (2): Disease severity of common bean isolate of *S. rolfsii* on tomato, common bean and cabbage plants under controlled conditions.

The obtained results (Table, 4 and Fig. 3) show the same trend when the cabbage isolate of *S. rolfsii* was applied to investigate its pathogenicity on tomato, bean and cabbage cultivars. At 10 days after transplanting, tomato plants revealed disease severity (15.4%) compared to the other tested crops. Meanwhile, severity percentages of root and crown rot in case of bean and cabbage were 12.9 and 16.8%, respectively (Fig. 3). Moreover, cabbage plants showed a great increase in percentage of severity when determined from plants growing in infested soil for 20, 30 and 40 days with values 34.7, 76.8 and 82.7%, respectively. All plants showed the highest disease incidence and severity at 50 days after planting.

Table (4): Pathogensty test of *S. rolfsii* isolated from cabbage plant on tomato, common bean and cabbage plants in pots experiments.

Source of isolate	Host	% Disease incidence after				
		10 days	20 days	30 days	40 days	50 days
Cabbage	Tomato	20.0	33.3	66.7	93.3a	100a
	Bean	12.0	36.0	64.0	88.0c	100a
	Cabbage	20.0	40.0	80.0	90.0b	100a
Control un infested soil		0.0 c	0.0	0.0	0.0d	0.0b

- Values in the same column followed by the same letter are not significantly differences *at P<0.5* level.

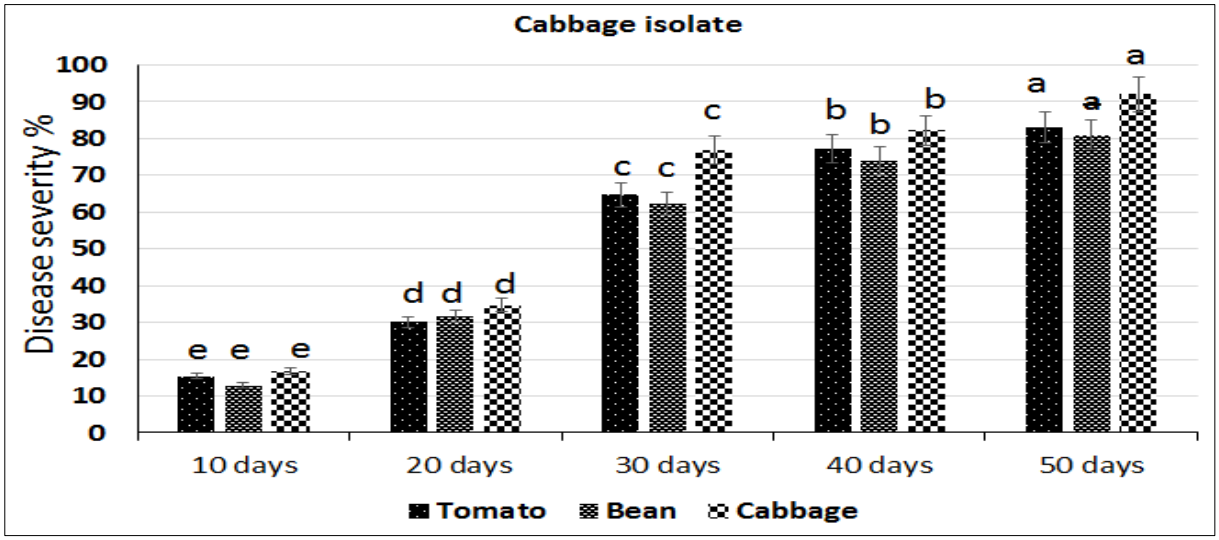


Fig. (3): Pathogenicity test of *S. rolfsii* isolated from cabbage plants on tomato, common bean and cabbage plants under greenhouse conditions.

Data obtained in Fig. (4) show the effect of variable isolates of *S. rolfsii* on tomato plants, common bean and cabbage plants. The pathogen was used to infest the potted soil. The pathogen caused the death of most plants after 40 days from planting. Also, the pathogen produced high numbers of sclerotia in the pots, which can attack different hosts under study**.**

Fig. (4): Showing the effect of *S. rolfsii* at 40 days after planting on tomato, common bean and cabbage plants.

***Trichoderma* species versus *S. rolfsii in vitro*:**

Data indicate that the effect of different *Trichoderma* spp. exhibited different levels of bioactivity against the different isolates of root and crown rot pathogen (*S. rolfsii*) (Table, 5). It is obvious that all tested strains of *Trichoderma* significantly reduced the growth of the three isolates of *S. rolfsii*. It is clear (Table, 5) that among the strains of *Trichoderma* tested, *T. viride* followed by *T. koningii* and *T. harzianum* were the most active strains against the growth of *S. rolfsii* (Tomato isolate). On the other hand, *T. harzianum* followed by *T. asperillium* and *T. viride* strains caused significant reduction in the linear growth of *S. rolfsii* (Bean isolate) (Table, 5). Regarding the effect of *Trichoderma* strains against the linear growth of *S. rolfsii* (cabbage isolates, data (Table, 5) clearly show that *T. harzianum*, followed by *T. asperillium* and *T. viride*, were the best in this respect. The obtained findings showed individual inhibition of mycelial growth patterns towards *S. rolfsii* isolated from tomato, common bean and cabbage. *S. rolfsii* growth completely filled the Petri dishes (9 cm diameter) at seven days after inoculation in check treatment. Data showed that *T. viride* and *T. koningii* reduced the mycelial growth of the pathogenic fungus (tomato isolate)compared to

the other *Trichoderma* spp. where linear growth reached 1.3 and 1.36 cm at 7 days, respectively, while mycelial growth of the pathogen reached 4.9 cm with *T. hamatium*. Furthermore, *T. harzianum* sharply decreased the mycelial growth of *S. rolfsii* isolated from common bean and cabbage, being 1.0 and 1.86 cm, respectively. The highest mycelial growth (3.56 and 4.2 cm) of common bean and cabbage isolates, respectively was recorded with *T. hamatium* treatment at seven days. Results also demonstrated that *S. rolfsii* could grow till 15 days in the presence of some bioagents like *T. hamatium* where, mycelial growth of *S. rolfsii* (tomato and cabbage isolates) completely filled out the Petri dish. Meanwhile, *S. rolfsii* bean isolate failed to grow.

Table (5): Effect of six species of *Trichoderma* on linear growth of *S. rolfsii* isolated from tomato, common bean and cabbage plants *in vitro* after 7 and 15 days.

Strains	L.G* tomato isolate		L.G bean isolate		L.G cabbage isolate	
	7 days	15 days	7 days	15 days	7 days	15 days
T. asperillium	2.46	5.46	3.20	3.20	2.66	4.20
T. harzianum	1.93	2.88	1.00	1.00	1.86	3.56
T. hamatium	4.90	9.00	3.56	3.56	4.20	9.00
T. koningii	1.36	1.60	3.43	3.43	3.43	4.60
T. viride	1.30	2.56	3.23	3.23	2.80	4.20
T. album	3.10	3.10	3.33	3.96	3.10	3.10
Control	9.00	-	9.00	-	9.00	-
LSD at 0.05	0.152		0.240		0.323	

- Values in the same column followed by the same letter are not significantly differences *at P < 0.5* level. *L.G= Linear growth of pathogen (cm).

The calculated mycelial growth inhibation percentage showed that *T. viride*, *T koningii* and *T. harzianum* increased inhibition percentage of *S. rolfsii* (tomato isolate) at seven days, being 85.5, 84.9 and 78.5, respectively (Figs. 5 and 6). *T. album* inhibited mycelial growth of *S. rolfsii* (tomato isolate), being 65.5% at 7 days. *T. harzianum, T. asperillium, T. viride, T. koningii* and *T. hamatium* inhibited the mycelial growth of *S. rolfsii* (bean isolate) with 88.9, 64.4, 64.1, 61.9 and 60.4%, respectively. On the other hand, the inhibition percentages with cabbage isolate achieved 79.3, 70.4, 68.9, 65.5, 61.9 and 53.3%, respectively, with *T. harzianum, T. asperillium, T. viride, T, album, T. koningii* and *T. hamatium*.

In general, the obtained results showed that all *Trichoderma* spp. inhibited mycelial growth *of S. rolfsii* isolated from tomato, bean and cabbage *in vitro* at 7 days with different inhibition percentages.

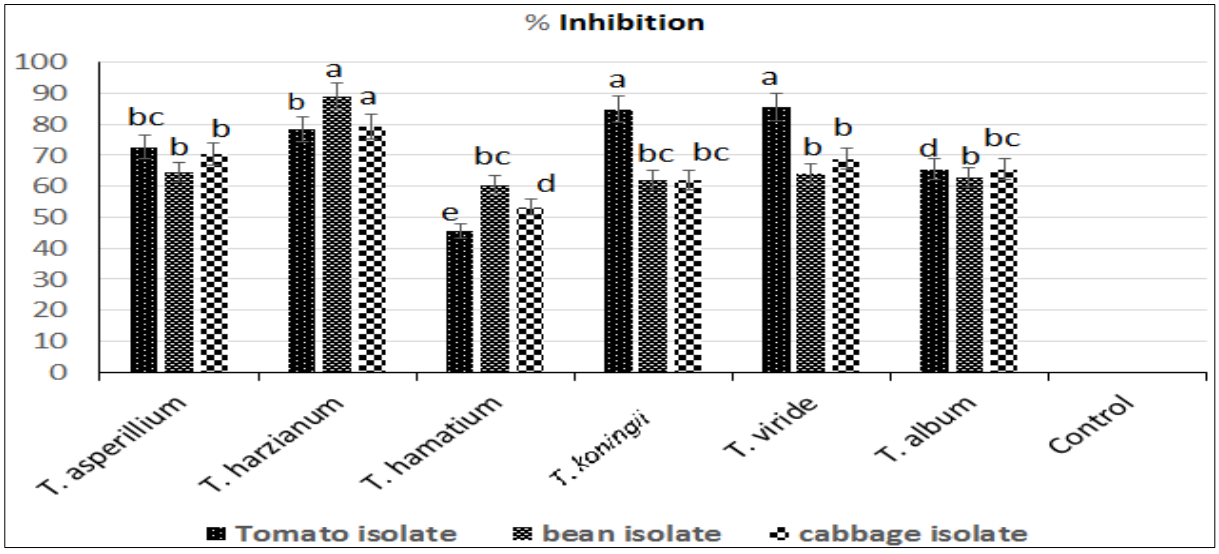


Fig. (5): Inhibition% of mycelial growth of *S. rolfsii* in Petri plates due to *Trichoderma* strains after 7 days incubation.

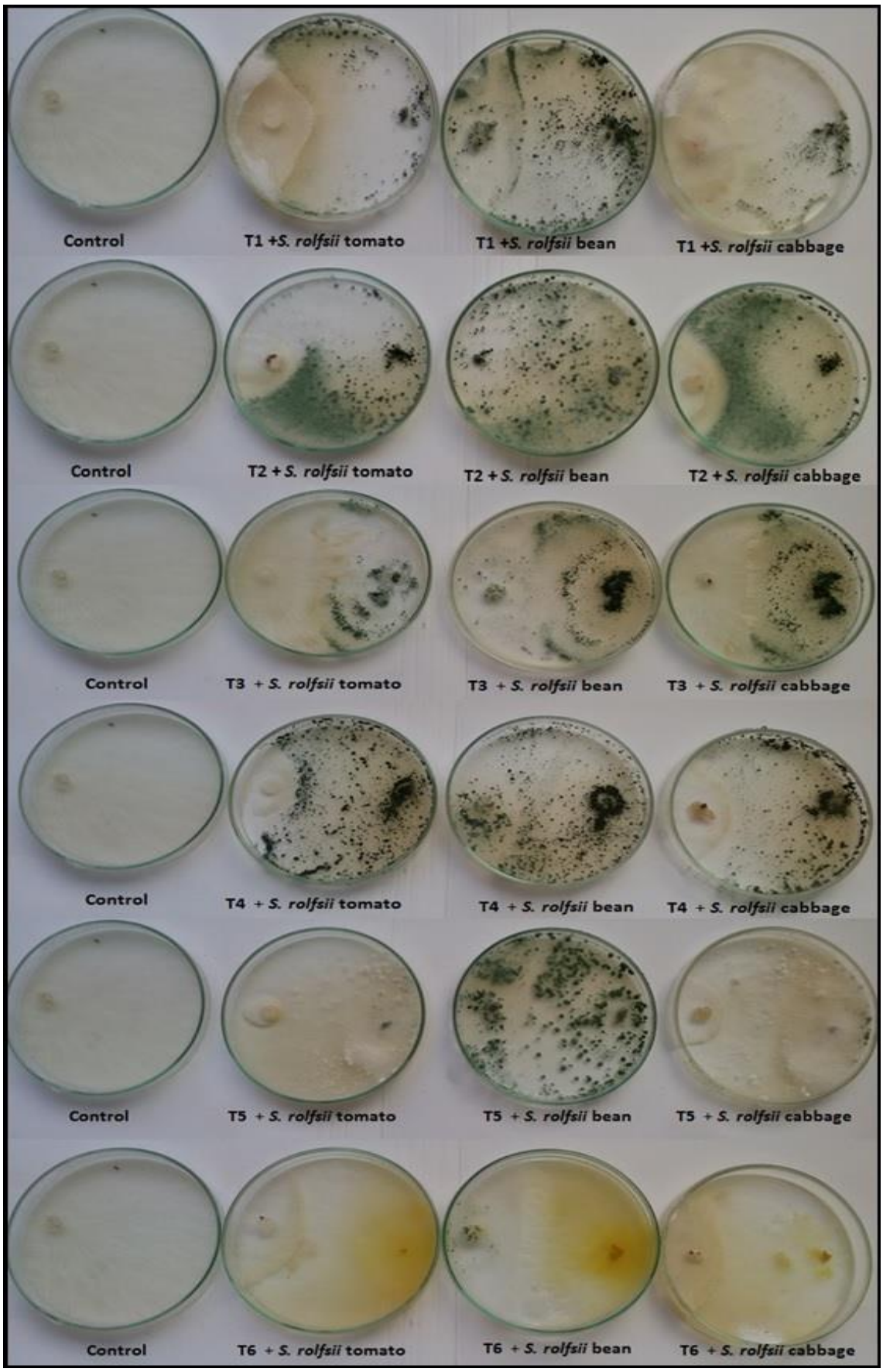
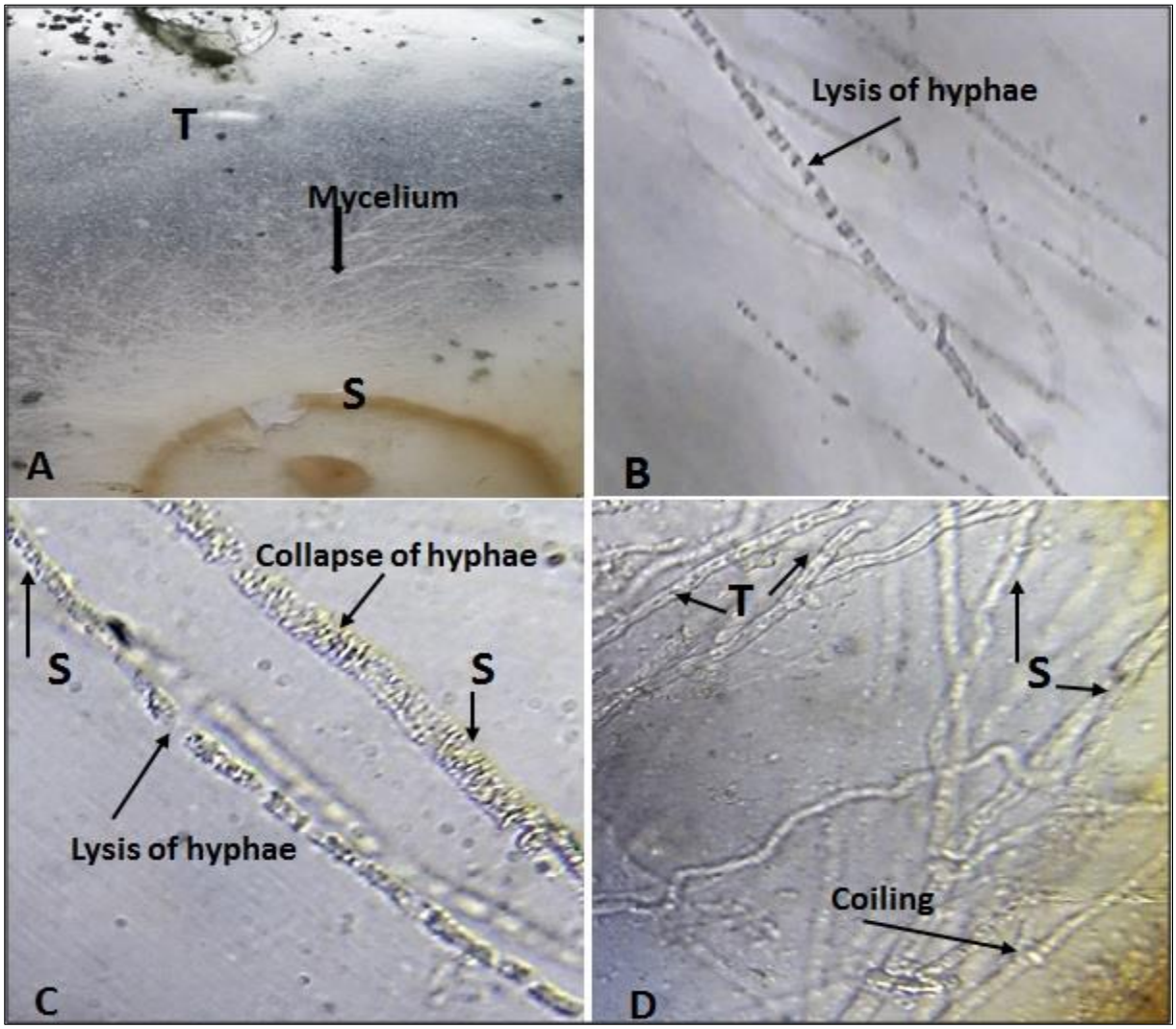


Fig. (6): Inhibition% of mycelial growth of *S. rolfsii* isolates in Petri plates due to Trichoderma strains, *i.e. T. asperillium* (T1), *T. harzianum* (T2), *T. hamatium* (T3), *T. koningii* (T4), *T. viride* (T5) *and T. album* (T6)

Microscopic characterization of *Trichoderma* spp. inhibiting *S. rolfsii*:

The intersection effect between antagonists and the tested pathogen was examined under microscope after 7 days of incubation The Trichoderma isolates antagonized the pathogen as observed in microscopic observations. The strong antagonism was observed due to *T. viride* (T5) when grew over mycelia of the tested pathogen with surrounded coiling, lysis of hyphae and collapse of mycelium structure formation followed by disintegration and disruption of mycelia of the pathogen (Fig. 7).



S = *Sclerotium rolfsii* hyphae, T = *Trichoderma* spp. hyphae

Fig. (7): Microscopic characterization of *Trichoderma* isolates for *in vitro* growth inhibition of the pathogen *S. rolfsii* after 7 days incubation 10×(A), 200× (B and D) and 400x (C). (A) = growth of pathogen with *Trichoderma* spp. (B and C) = Lysis and collapse of the fungal hypha by *Trichoderma* spp. and (D) = Coiling hypha of *Trichoderma* spp. on *S. rolfsii*.

***Trichoderma* species versus *S. rolfsii in vivo*:**

Data presented in Table (6) illustrate the ability of all bio agents in controlling the causal of root and crown rot (*S. rolfsii*) on tomato, common bean and cabbage plants. *T. koningii* and *T. harzianum* showed the lowest percentage of disease incidence, being 26.7 and 27.3 without significant deferences. Also, data of disease incidence clearly showed that *S. rolfsii* cabbage isolate was more virulent than *S. rolfsii* tomato isolate and bean isolate.

Regarding to the interaction between *Trichoderma* spp., the three isolates of *S. rolfsii* and the tested plants; *T. harzianum* gave the best results in controlling the pathogen in case of *S. rolfsii* tomato isolate, being 26.7, 20.0 and 20.0% disease incidence on tomato, bean and cabbage, respectively. Also, *T. harzianum* achieved the

lowest disease incidence with cabbage isolate 20.0, 20.0 and 30.0%, respectively, in tomato, bean and cabbage plants. While, the fungicide (Rhizolex T) recorded the lowest disease incidence with the three isolates in case of tomato, bean and cabbage plants.

Table (6): Effect of six species of *Trichoderma* on *S. rolfsii* disease incidence percentage on tomato, common bean and cabbage plants under greenhouse conditions.

Isolate Source(B)	Host (C)	Treatment (A)								Mean (BC)	Mean (B)
		*T1	T2	T3	T4	T5	T6	F	Control		
Tomato isolate	Tomato	46.7	26.7	40.0	26.7	33.0	26.7	20.0	100.0	39.98	37.16
	Bean	32.0	20.0	36.0	24.0	28.0	36.0	16.0	100.0	36.50	
	Cabbage	30.0	20.0	30.0	20.0	30.0	30.0	20.0	100.0	35.00	
	Mean(AB)	36.23	22.23	35.33	23.57	30.33	30.9	18.6	100.0		
Bean Isolate	Tomato	40.0	33.3	20.0	26.7	40.0	46.7	13.3	100.0	40.00	38.17
	Bean	36.0	36.0	32.0	28.0	20.0	32.0	12.0	100.0	37.00	
	Cabbage	40.0	40.0	30.0	30.0	20.0	30.0	10.0	100.0	37.50	
	Mean(AB)	38.67	36.43	27.33	28.23	26.67	36.2	11.7	100.0		
Cabbage Isolate	Tomato	33.3	20.0	33.3	26.7	33.3	40.0	13.3	100.0	37.49	39.00
	Bean	32.0	20.0	32.0	28.0	32.0	36.0	16.0	100.0	37.00	
	Cabbage	40.0	30.0	40.0	30.0	40.0	40.0	20.0	100.0	42.50	
	Mean(AB)	35.10	23.33	35.10	28.23	35.10	38.6	16.4	35.10		
All over Mean	Tomato	40.0	26.7	31.1	26.7	35.4	37.8	15.5	100.0	39.15	--
	Bean	33.3	25.3	33.3	26.7	26.7	34.7	14.7	100.0	36.83	
	Cabbage	36.7	30.0	33.3	26.7	30.0	33.3	16.7	100.0	38.33	
	Mean(A)	36.7	27.3	32.6	26.7	30.7	35.3	15.6	100.0		
LSD at 0.05		A=0.456, B= 0.288, C= 0.288, AxB= 0.794, AxC=0.794, BxC= 0.484 and AxBxC = 1.379									

* T1= *T. asperillium*, T2= *T. harzianum*, T3= *T. hamatium*, T4= *T. koningii*, T5= *T. viride*, T6= *T. album* and F= fungicide Rizolex-T.

Data in Table (7) show that the highest antagonistic effect was obtained due to the application of *T. koningii*, *T. viride* and *T. harzianum* with significant differences where disease severity recorded 19.06, 19.69 and 21.5%, on the average, respectively. On the other hand, the highest disease severity was due to using either *T. asperillium* or *T. hamatium* that recorded 28.91 and 28.64% on the average, respectively, compared to inoculated and untreated control which exhibited 91.44% disease severity. The three isolates of *S. rolfsii* exhibited a clear effect on the three tested hosts when treated by *Trichoderma* spp. where disease infection recorded 33.6, 33.07 and 32.54% on the average, respectively without significant differences among them.

Regarding to the interaction between *Trichoderma* spp. and the three isolates of *S. rolfsii*, plants were grown in soil treated with *T. harzianum* under greenhouse conditions, it was clear that cabbage isolate revealed the lowest disease severity, being 15.97% followed by *T. koningii* and *T. harzianum* under infection with tomato isolate, being 16.17 and 17.07%, respectively (Table, 7). While, the highest disease severity (42.43%) was recorded with *T. hamatium* under infection with tomato isolate. Furthermore, each of the three isolates of *S. rolfsii* (tomato, common bean and cabbage isolates) recorded the highest infection value with its host crop. *Trichoderma* spp. and tested crops interacted positively where tomato plants treated with *T. koningii* and common bean or cabbage plants treated with *T. viride* recorded the lowest percentages of disease severity, being 18.4, 18.7 and 18.7, respectively. Application of *T. hamatium* with tomato plants grown in soil infested with *S. rolfsii* (common bean isolate) recoded the lowest disease severity percentage (10.3%) followed by (13.5%) due to *T. album* application with cabbage plants grown in soil infested with tomato isolate.

Biochemical activity:

Interestingly, both of *T. koningii* and *T. viride* exhibited the best performance regarding the defense enzymes secretion, chitinase and glucanase (1.76 and 1.54 U) (0.265 and 0.246 U), respectively, followed by *T. harzianum* (1.45 and 0.241 U), respectively. While, *T. asperillium* and *T. album* produced moderate amounts of both enzymes (0.96 and 0.87 U) and (0.193 and 0.201 U), respectively. On the other hand, *T. hamatium* secreted slight amounts, *i.e.*0.82 and 0.186 U), respectively (Table 8).

Regarding the assay of plant growth promoting substances; all the tested *Trichoderma* spp. produced indole acetic acid (IAA) and

gibberellic acid (GA) in variable values. *Trichoderma viride* exhibited the best performance production of IAA 5.71 µg/ml followed by *T. harzianum* and *T. koningii* recorded 4.12 and 4.01 µg/ml, respecticly. Regarding to GA production by *T. koningii*, *T. esperillium* and *T. viride* achieved 22.15, 16.4 and 15.93 µg/ml, respectively.

Table (7): Effect of six species of *Trichoderma* on *S. rolfsii* disease severity percentage on tomato, common bean and cabbage plants under greenhouse conditions.

Isolate Source(B)	Host (C)	Treatment (A)								Mean (BC)	Mean (B)
		*T1	T2	T3	T4	T5	T6	F	Control		
Tomato isolate	Tomato	32.8	17.7	72.2	15.3	22.8	14.9	11.2	96.0	38.8	33.60
	Bean	26.3	18.2	32.1	17.3	19.3	27.3	13.4	92.0	33.2	
	Cabbage	21.4	15.3	23.0	15.9	19.3	13.5	10.3	93.0	28.8	
	Mean(AB)	26.83	17.07	42.43	16.17	20.47	18.6	11.6	93.7		
Bean Isolate	Tomato	29.3	30.4	10.3	17.8	23.7	27.3	10.7	90.3	32.7	33.07
	Bean	30.7	31.3	24.7	18.3	13.8	21.9	12.7	94.1	33.5	
	Cabbage	31.7	32.7	23.2	19.9	13.9	20.3	13.8	88.9	32.9	
	Mean(AB)	30.57	31.47	19.40	18.67	17.13	23.2	12.4	91.1		
Cabbage Isolate	Tomato	29.7	13.8	23.2	22.3	18.3	22.9	12.3	87.7	31.1	32.54
	Bean	29.9	14.3	24.2	21.8	23.0	24.4	16.4	88.0	32.2	
	Cabbage	28.4	19.8	24.9	22.9	23.1	27.8	15.5	93.0	34.3	
	Mean(AB)	29.33	15.97	24.10	22.33	21.47	25.0	14.7	89.7		
All over Mean	Tomato	30.6	20.6	35.2	18.4	21.6	21.7	11.4	91.3	34.2	
	Bean	28.9	21.2	27.0	19.1	18.7	24.5	14.2	91.4	33.0	
	Cabbage	27.1	22.6	23.7	19.5	18.7	20.5	13.2	91.6	32.0	
	Mean(A)	28.91	21.50	28.64	19.06	19.69	22.3	12.9	91.4		
$LSD_{at0.05}$	A=0.968, B= 0.639, C= 0.639, AxB= 1.676, AxC=1.676, BxC= 1.093 and AxBxC = 2.905										

* T1= *T. asperillium*, T2= *T. harzianum*, T3= *T. hamatium*, T4= *T. koningii*, T5= *T. viride*, T6= *T. album* and F= fungicide Rizolex-T.

Table (8): Biochemical activity of six *Trichoderma* spp. to produce lytic enzymes and plant growth promoters.

Bioagents	Biochemical activity			
	Chitinase U	β-glucanase U	IAA µg/ml	GA µg/ml
T. asperillium	0.96 c	0.193 d	3.66 c	16.40 b
T. harzianum	1.45 b	0.241 b	4.12 b	13.08 c
T. hamatium	0.82 c	0.186 d	3.40 c	6.96 de
T. koningii	1.76 a	0.265 a	4.01 b	22.15 a
T. viride	1.54 b	0.246 b	5.71 a	15.93 b
T. album	0.87 c	0.201 c	2.86 d	8.54 d

- Values in the same column followed by the same letter are not significantly differences *at P < 0.5* level.

DISCUSSION

Sclerotium rolfsii occurs in soil as a saprotroph and attacks living plants. *Sclerotium rolfsii* is a soil-borne fungus, facultative parasite and Omni pathogenic organism which occurs worldwide and infects more than 500 plant species (Aycock. 1966 and Punja, 1985) including tomato, cucumber, brinjal, soybean, maize, groundnut, bean, watermelon, etc. This pathogen propagates by sclerotia under favorable conditions. After germination, sclerotia may cause chlorosis and wilting of entire plants (Yaqub and Shahzad, 2005). This study was conducted on three isolates of *S. rolfsii*, each isolate not only had high effect on the host from which it was isolated but also it affected the other tested plants in the experiment. At first ten days after planting, tomato plants were strongly affected with the three isolates followed by cabbage plants while, bean plants exhibited less sensitivity to this procedure.

Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases (Coley-Smith *et al*., 1991). Control of soil borne plant pathogens including *Sclerotium rolfsii* can be achieved by different fungicides, soil fumigants (Methyl bromide) and bioagents. Because of the concern regarding the toxicity of

fungicides, there is a general trend to reduce the amounts applied to soil.

In general, data showed that all the six tested *Trichoderma* spp. inhibited mycelial growth of *S. rolfsii* isolated from tomato, bean and cabbage *in vitro* after 7 days of incubation with different inhibition percentages. Pathogen isolates from tomato and cabbage plants were able to continue their growth and decrease inhibition percentages till 15 days while, bean isolate did not show this character. These results are in harmony with those recorded by Elad (2000) and Ahmed (2013). The pronounced antifungal activity of *Trichoderma* spp., may be attributed to some lytic enzymes, which act as fungal cell-wall-degrading agents such as N-acetyl- ß-D-glucosedeaminidase, chitinase, ß-1,3 gluconase, chitobiosidase and protease (Abo-Elyousr *et al*., 2014 and Ahmed *et al*., 2016).

Results showed the contact between *Trichoderma* isolates and *S. rolfsii* in dual culture after 15days. Not all *Trichoderma* isolates were able to grow on *S. rolfsii* colony except isolates *T. asperillium* (T1) and *T. hamatium* (T3). All *Trichoderma* isolates grew toward *S. rolfsii* colony and failed to grow over the pathogen, in the case of tomato and cabbage isolates. The results revealed that Trichoderma isolates (T1, T2, T3, T4, T5 and T6) when used against S. *rolfsii* common bean isolate formed branches that coiled around the hyphae of the pathogen causing lysis of the infected hyphae . The penetration and growth of all *Trichoderma* isolates inside the hyphae of *S. rolfsii* were photographed. The ability of *Trichoderma* spp. to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of Trichoderma, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan & Chet, 1986). Increased growth response of several plants including vegetables, following the application of *Trichoderma* spp. to pathogen-free soil has been documented (Chang *et al.*, 1986; Baker, 1989 and Kleifeld and Chet, 1992).Yedidia *et al*. (2001) suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of the fungal plant association. In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. Increased growth response has been demonstrated by several other investigators (Anusuya and Jayarajan, 1998 and Altomare *et al*., 1999) who demonstrated the ability of *T. viride* and *T. harzianum* to solubilize in soluble tri calcium phosphate *in vitro*.

Microscopic examinations of the inhibition zone displayed different interactions between the pathogen and antagonists, due to the mycoparasitism. Interactions such as parallel growth of the antagonist alongside the pathogen hyphae, coiling of the antagonists around the pathogen, formation of appressorium-like structures and hyphal collapsing (disintegration) were observed in all paired cultures. Similar observations regarding the hyphal coiling and lysis of different *Sclerotium* species as a result of direct mycoparasitism of *Trichoderma* spp. were previously reported by Barnett and Binder (1973), Elad *et al*. (1983), and Singh and Islam (2010). Zivkovic *et al*. (2010) have also reported direct mycoparasitism (coiling, penetration and parallel growth) of *T. harzianum* against *Colletotrichum acutatum* and *C. gloeosporioides*. Comparable results regarding the direct mycoparasitic activity (hyphal coiling, parallel growth and lysis) of *Trichoderma* spp. were observed against *Sclerotium rolfsii* (Jegathambigai *et al*., 2010) and *Sclerotinia sclerotiorum* (De Figueiredo *et al*., 2010).

Trichoderma koningii and *T. viride* exhibited the best performance as producers for the defense enzyme chitinase and glucanase, followed by *T. harzianum*. While, *T. asperillium* and *T. album* produced moderate amount of both enzymes. On the other hand, *T. hamatium* secreted slight amount. These findings concur with reports that chitinase plays a role in the degradation of hyphal tips of *S. rolfsii* by *Serratia marcescens* and the ability of *Escherichia coli* transformed with the chi A gene from *Serratia marcescens* to reduce disease caused by *S. rolfsii* in beans (Ordentlich, *et al* 1988 and Shapira, *et al* 1989). Also, (Zhihe, *et al.*, 1998) reported that the parasitic activity of *T. viride* is mediated by its excretion of a variety of enzymes including cellulases, chitinases and antibiotics such as gliovirin

CONCLUSION

The results of this study indicated that biological control could be considered the best alternative and may be helpful, especially against soil borne pathogens. *Trichoderma* spp. could control different isolates of *S. rolfsii* on different host plants and reduce disease incidence. The beneficial effects of *T. viride* and *T. harzianum* are due to direct mycoparasitism on the pathogenic fungi.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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