Hyphal interaction between the biocontrol agents *trichoderma* spp. And *sclerotinia sclerotiorum* for suppression of common bean damping-off disease

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ABSTRACT

Hyphal interaction between the microparasite *Trichoderma harzianum* and the soil borne plant pathogen fungus *Sclerotinia sclerotiorum* were investigated in dual culture by light microscopy. Different forms of hyphal interaction between *T. harzianum* and *S. sclerotiorum* were recorded, i.e. complete surface colonization, formation of approssorium-like structure before penetration, penetration through one site of the host by more than one hyphae of the bioagent, coiling around the host hyphae through the formation of two very short branches of the hyphae tip of the bioagent and the formation of many lateral branches when the bioagent become closer to the cell.

Penetration of common bean seedlings occurred, during the first 48 hours after inoculation, through the epidermis and the outer layer of the cortex. At 72 hours after inoculation, damage extended deeper into the cortical cells. Infection took place inter-and interacellularly after 96 hours more damage occurred.

Greenhouse experiments indicated that soil treatment with *T. harzianum* together with *T. koningii* was the most efficient in controlling damping-off disease, whereas commercial promot treatment was less effective. Root system of seedling grown in infested soil treated with the bioagents was larger than in those grown in soil free of the bioagents. Length of both main and fine roots and also area of root hairs layer were higher when soil was treated with both *T. harzianum* and *T. koningii* together. The least values of root fresh and dry weight were obtained in *T. koningii* and commercial promot treatments. On the other hand soil treatment with *T. harzianum* together with *T. koningii* pronouncedly increased these values. Generally, soil treated with the bioagents significantly improved plant growth and root system parameters.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary causes severe diseases in soybean, sunflower and various vegetable and field crops in many temperate and humid regions. The high cost of fungicides and the difficulties in obtaining resistant cultivars make biological control a more interesting alternative for the suppression of this fungus (Purdy, 1979; Wipps and Budge, 1990 and Baker and Pulitz, 1996). *Trichoderma harzianum* Rifai, a filamentous soil fungus is considered an effective biocontrol agent against several economically important plant pathogenic fungi. *T. harzianum* has been shown to be effective in controlling *Sclerotinia* – forming plant pathogenic fungi (Hadar *et al.*, 1979; Dose Santos and Dhingra, 1982 and

Elad *et al.*, 1983). Elad *et al.* (1982) and Harman *et al.* (1996) found that *Trichoderma* isolates produce chitinases and gluconases when grown on live mycelium of *Sclerotium rolfsii* Sacc and *Rhizoctonia solani* Kuhn in soil. Mycoparasitism seems to play an important role in the controlling of *S. sclerotiorum* in treated fields (Abd-El-Moity and Shatla, 1981). Microparasitic interactions between *Thrichoderma* and soil borne plant pathogenic fungi have been described using light and electron microscopy (Elad *et al.*, 1982).

Trutman and Keane (1990) have demonstrated parasitism of *T. koningii* on *S. sclerotiorum* and the potential of this antagonist to control the disease caused by *S. sclerotiorum* in beans. The interaction between *T. harzianum* and sclerotia of the soil borne plant pathogen *Sclerotium rolfsii* was studied by scanning and transmission electron microscopy to assess the potential role of enzymatic hydrolysis in the antagonistic process (Benamou and Chet, 1996).

Laboratory experiments (Elad *et al.*, 1983 & 1984) as well as biocontrol field trials (Elad *et al.*, 1982) showed convincingly that some species of *Trichoderma* display the ability to attack the mycelium and sclerotia of *S. sclerotiorum*, reducing pathogen inoculum in soil. Shoots and roots of 10-day old seedlings of maize grown in sandy loam field soil were larger in the presence of *T. harzianum* than in its absence. Both main and secondary roots were increased in size and area and the root hair area was greater with *T. harzianum*. Root colonization with *Trichoderma* spp., also frequently, enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Harman *et al.*, 2004).

This investigation was, therefore, carried out in order to study: (a) the hyphal interaction between *T. harzianum* and *S. sclerotiorum* in dual culture; (b) damping-off suppression by biocontrol agents; (c) effect of biocontrol agents on root parameters of bean seedlings; (d) effect of bioagents on the growth of beans; and (e) the histopathological changes occurred in bean hypocotyls infected with *S. sclerotiorum* at different incubation periods.

MATERIALS AND METHODS

(1) Sources of the tested fungal isolates and common bean seeds tested:

Trichoderma harzianum and *T. koningii* isolates used in these studies were kindly obtained from the Research Institute of Phytopathology, Giza, Egypt.

Commercial *Trichoderma* spp. of promot (*T. koningii* 3 x 10^7/gm plus *T. harzianum* 2 x 10^7/gm) was manufacture by J. H. Biotech. Ins. Ventura,

California, USA, and used in this study at concentration of recommended dose (2 gm/kg aoil).

An *S. sclerotiorum* isolate isolated from diseased root and hypocotyl of common bean plants seedlings (*Phaseolus vulgaris* L.), collected from El-Behera governorate, was used as the target pathogen throughout this work.

Developing of fungal isolate was picked out, subcultured several times, purified using hyphal tip technique. The purified fungal isolate was maintained on PDA slants. Identification of the target pathogen was carried out according to Parmeter and Whitney (1970).

Common bean (*Phaseolus vulgaris* L.) cvs Giza 3, Giza 6 and Contendered seed samples were obtained from the Agricultural Research Center, Giza and used throughout this study.

(2) Hyphal interactions in dual culture:

Dual culture of *T. harzianum* and *S. sclerotiorum* was carried out as described by Chet *et al.* (1981) and Elad *et al.* (1983). According to this technique fungal isolate was grown on a cellophane membrane in Petri dishes with potato dextrose agar (PDA) medium. Due to its slower growth rate, *S. sclerotiorum* was inoculated two days before the antagonist. The cultures were kept in the dark at 25° C. After four to six days, pieces of cellophane membrane from the contact zone were cut and mounted on a slide, stained with cotton blue or and examined using light field phase-contract microscope.

(3) Histopathological experiments:

Twenty to twenty five days after planting, seedlings hypocotyls of Giza 6 bean cultivar were inoculated with *S. sclerotiorum*. Inoculation was carried out as described by El-Samra *et al.* (1981) and which was modified later on by El-Faham and Aboshosha (1987). According to this technique, seedlings were inoculated by wrapping hypocotyls, just below the cotyledons, with mycelial strips. Inoculated seedlings were reset in cm-tall germination rolls of filter paper, with their cotyledons above the roll taps. The rolls were placed vertically in a breaker with 2 cm of water and incubated at 20°C with a light period of 10 hrs in a growth chambers.

Serial microtome sections were prepared in inoculated hypocotyls 24, 48, 72 and 96 hours after inoculation. The technique applied in this research, described in detail by Waked (1979), is considered a modification of the procedures recommended by Brooks *et al.* (1950); Sass (1958); Berlyn and Mikschie (1976); Sections were examined using a bright field

microscope. Results of extensive microscopic observations at different stages of infection were presented in many selected photographs.

(4) Greenhouse experiments:

Sandy loam soil was autoclaved at 1.5 kg/cm^2 for 90 min and lift to aerate for 6 days before adding the inocula. Inoculum of *S. sclerotiorum* was prepared by growing on potato dextrose (PD) medium in 250 ml flasks, each contains 50 ml media. The inoculated flasks were incubated at 18-20° C for 10 days then the mycelial mats were collected and blended with tap water and used at the rate of 3 g/kg autoclaved soil.

On the other hand, *T. harzianum* and *T. koningi* were grown on a wheat bran: sawdust: tap water (3 : 1 : 4 v/v), autoclaved for 30 min at 121°C on 2 successive days (Hadar *et al.*, 1979; Elad *et al.*, 1980). The media were inoculated then incubated for 10 days. The antagonists were added to the infested soil at the rate of 5 g/kg soil. Surface sterilized of common bean seeds cultivars were planted at the rate of 10 bean seeds from for each cultivars were sawn each in plastic pot (10-12 cm) containing 250 gm of infested soil and placed in the greenhouse at 17-20° C.

Pre- and post-emergence damping-off were calculated out 20-25 days following planted. The effect of the different treatments with biocontrol agents on root system length dry and fresh weight of root and shoot systems were also studied. The tested treatments included the study of the following interaction:

1. *S. sclerotiorum*.
2. *S. sclerotiorum* and *T. harzianum*.
3. *S. sclerotiorum* and *T. koningii*.
4. *S. sclerotiorum* + *T. harzianum* + *T. koningii*.
5. *S. sclerotiorum* + Promot.

Statistical analysis

Statgraphic package was used for analysis of variance (ANOVA) to evaluate the effect of different soil treatments with biocontrol agents on the disease incidence of common bean plants caused by *S. sclerotiorum*. Fisher's least significant difference (LSD) was used to compare the means. Results with a different letter are significantly different from each other for α=0.05 (Fry, 1993). LSD = t * [(2 * Error Mean Square)/repetitions]$^{1/2}$.

RESULTS AND DISCUSSION

(1) Micoparasitic activities

Examination of the hyphal interaction of *T. harzianum* and *S. sclerotiorum* using bright field microscope showed different types of interactions between the two fungi. The diameter of *T. harzianum* hyphae was 2.1 µm while it was 6.2 µm for *S. sclerotiorum*, so the hyphae of both fungi could be easily distinguished.

By 24 hrs after contact between mycelia of *T. harzianum* and *S. sclerotiorum*, a clear zone of interaction was formed. Samples containing interaction zones on cellophane were observed under light microscopy.

T. harzianum hyphae grew along those of the host forming hayphal branches which coiled and surrounded the hyphae of *S. sclerotiorum* (Figure 1-A). These findings were in agreement with those of Jacop *et al.* (1996) and Allen (2003). Whipps (1987) also observed coiling of *Trichoderma* around the hyphae of *S. sclerotiorum* grown on PDA. Hyphae of *T. harzianum* completely colonized the surface of *Sclerotinia* hyphae (Figure 1-B). This finding was in agreement with El-Farnawany and Shama (1996) who found that the hyphae of *T. viridi* completely colonized the hypahe of *Rhizoctonia solani* during the interaction between them. Hyphae of *T. harzianum* were observed to grow in contact with the outer surface of *Sclerotinia* for some distance then extensive branches of the hyphae formed infection cushion-like structure before penetration the host cell (Figure 1-C). Similar results were observed by El-Farnawany (1996), who found that *T. harzianum* significantly affect the induction of different forms of infection cushions by *R. solani* during the process of mycoparasitism and hyphal interactions. More than one of hyphal branches of *Trichoderma* were found to penetrate directly host cell through one point on the hyphal host cell and hyphae of *Trichoderma* multiplied abundantly at the hyphal surface, forming a dense mycelium (Figure 1-D).

At the tips of *Trichoderma* hyphae, very short branches were swollen and then coiled around the hyphae of the pathogen (Figure 1-E & F). Hyphae of *T. harzianum* grew on the surface of pathogen hyphae and their tips swelled and formed appressorium-like structure before penetration through *Sclerotinia* hyphae (Figure 1-G & H). It was also observed that growing *T. harzianum* near the host often stimulates the formation of lateral branches which tended to be in contact with outer lined cells (Figure 1-I & J). This was in agreement with the findings of Allen (2003).

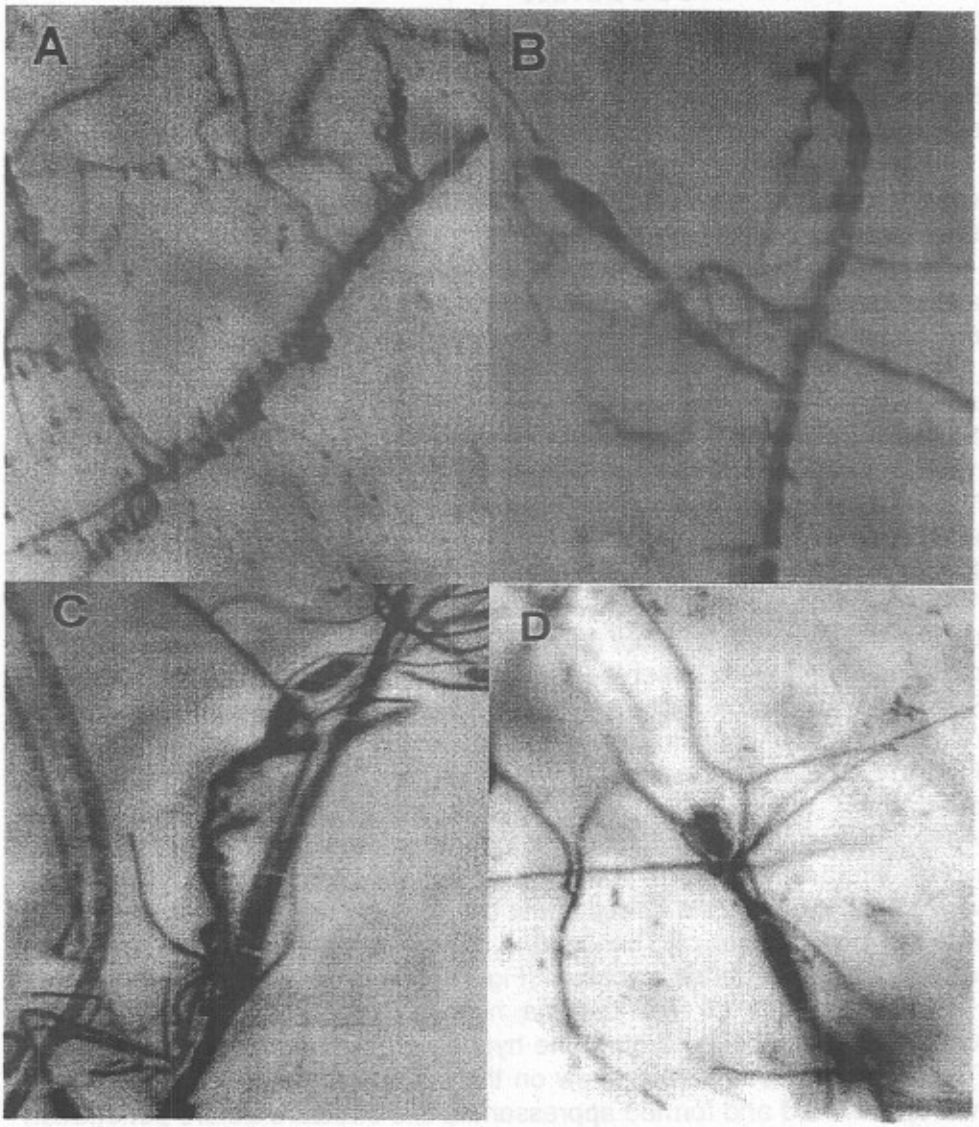

Figure (1): Different forms of hyphal interaction between *S. sclerotiorum* and *T. harzianum*. (A) Hyphae of *T. harzianum* grew on and twisted around a hypha of *S. sclerotiorum*. (B) Hypha of *Trichoderma* formed lateral branches and colonized the hyphae of the pathogen. (C) Penetration of the pathogenic host cell through the formation of infection cushion like structure. (D) Direct penetration through the formation of dense mycelium at the penetration site of the host cell.

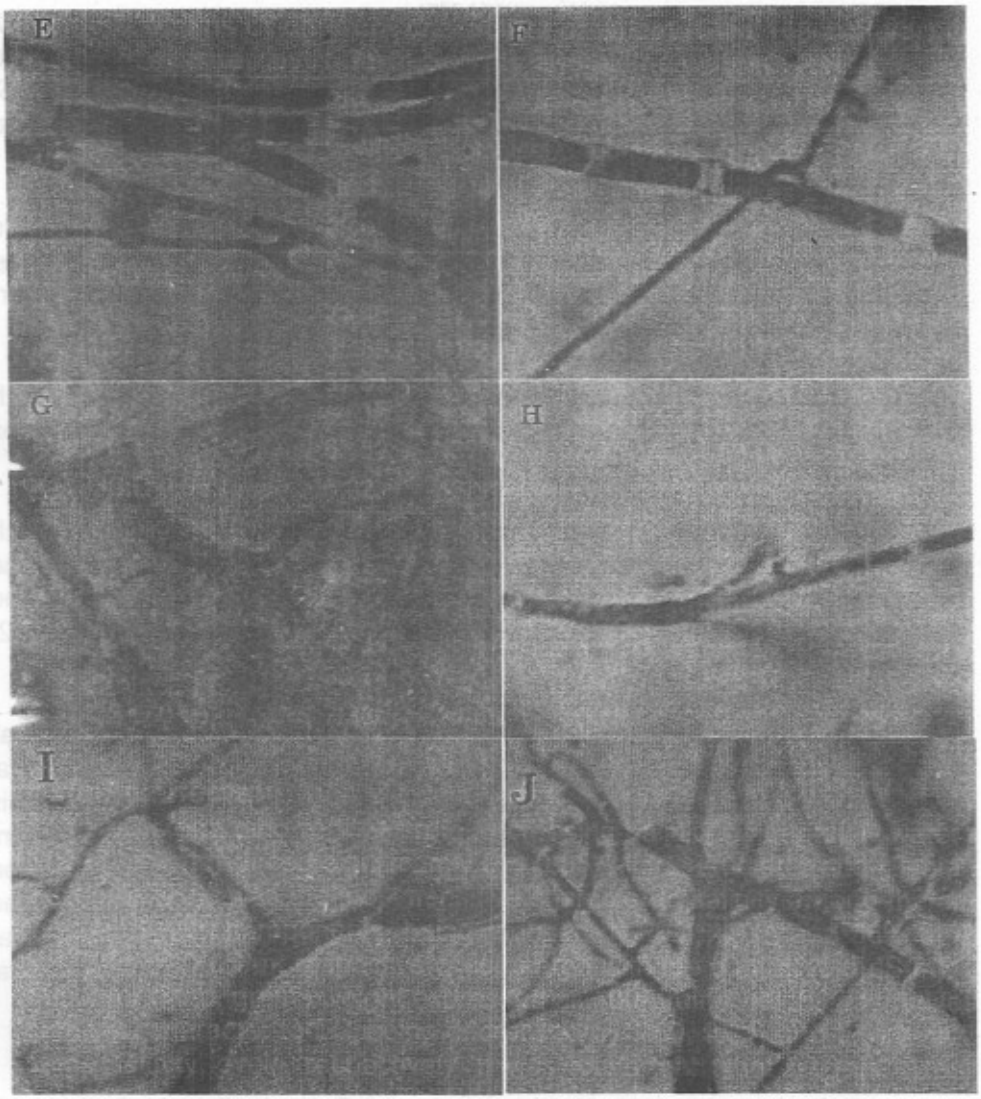

Figure (1) followed: (E) & (F) Formation of two very short branches at the end of *T. harzianum* hyphae before coiling around the hyphae of *S. sclerotiorum.* (G & (H) Formation of appressorium-like structure from the hyphal tip of *T. harzianum* against the side wall of *S. sclerotiorum* hyphae before penetration. Notice the discoloration of the host cell wall, the formation of short penetration tube from the appressorium, and the swelling of the growing tip of hyphae before the formation appressorium. (I) & (J) The stimulation of *T. harzianum* to form lateral branches when grown near *S. sclerotiorum* hyphae.

(2) Histology of bean hypocotyls inoculated with *S. sclerotiorum*

This study was carried out in order to investigate and confirm the findings of various researchers on the mode of penetration of *S. sclerotiorum* on common bean plants. Further histological deviations due to fungal invasion of different host tissues, was investigated.

a) 24 hours after inoculation

Microscopic examination of the cross sections of bean hypocotyls 24 hours after inoculation showed an active progress of fungal hyphae through the epidermal cells and the first two layers of the cortex both inter- and intracellulary (Figure 2-A).

b) 48 hours after inoculation

Cross sections showed complete destruction of epidermal cells with partial destruction of some cortical cells at the outer layer of the cortex (Figure 2-B).

c) 72 hours after inoculation

Cross sections showed that tissue discoloration extended much deeper into the cortical cells layers. Invasion of cortex by the fungal hyphae progressed both inter-, and intracellularly. Hyphae colonization and destruction of the outer layers of the cortex were clearly shown (Figure 2-C).

c) 96 hours after inoculation

After 96 hours, complete destruction of the epidermal cells and all layers of cortex occurred (Figure 2-D). However, presence of the fungal structures in vascular tissues and pith cells was rarely observed. This confirms the findings of previous studies on *R. solani* of bean (Van Etten, 1967; Dodman *et al.*, 1968 and Kenning and Hanchey, 1980) and of cotton (Selim, 1985). Rubber (1973) found that invasion by *R. solani* was limited by the epidermis or the outer layers of secondary cortex in resistant sugar beet plants, but in susceptible plants the fungus transected several vascular rings.

(3) Greenhouse experiments

Soil inoculation with *T. harzianum* and *T. koningii* and infested with *S. sclerotiorum* was carried out using sterilized sandy loam soil. Common bean cultivars Giza 3, Giza 6 and Contendered were used in these experiments. Percentages of pre- and post-emergence damping-off (pre-EDO & post-EDO) were estimated 20-25 days following planting for all treatments used. Moreover, the total infection percentages were also estimated.

From results shown in Table 1 and Figure 3, it was evident that *S. sclerotiorum* was highly pathogenic to the all the tested bean cultivars; i.e.

Giza 3, Giza 6 and Contendered, where total infection % values were 86.7, 90 and 93.40%, respectively.

Generally, all tested biological control treatments significantly decreased TI% values, however, soil treatment with *T. harzianum* together with *T. koningii* proved to be the most efficient treats in reducing total infection % (9.96, 28.90 and 26.58% in Giza 3, Giza 6 and Contendered, respectively) compared with the other tested biological control treatments.

Reasonable reduction in disease incidence was obtained with *T. harzianum* treatment but in rates still lower than previous treatment. The least values of disease incidence were obtained when infected soil was treated with the *T. koningii* or promot treatments. In general, incidence of pro-EDO in all the tested treatments was more frequent than post-EDO. These results were approached by Jacob Inbar *et al.* (1996), who showed that in the greenhouse experiments, *Trichoderma* in a peat-bran preparation was incorporated into the rooting mixture resulted in a significant reduction in disease incidence was achieved in sunflower. Kleifeld and Chet (1992) have demonstrated the presence of *Trichoderma* inside the root cortex of treated plants (pepper, bean, radish, tomato and cucumber). Harman *et al.* (2004) found that seed treatment with *T. harzianum* which results in colonization of plant roots but little or no colonization of shoot or leaves had substantial effect on growth and disease expression in maize. Moreover, recent studies have indicated that *Trichoderma* spp. Induce localized or systemic resistance in plants (De Meyer *et al.*, 1998; Yeldidia *et al.*, 1999 and Harman *et al.*, 2004). Steinmetz and Schonbeck (1994) also reported that horticultural use of conifer brake amended with *T. harzianum* in controlling pre-emergence damping-off of pea caused by *Pythium altimum.*

Effect of *T. harzianum* and *T. koningii* on some root parameters of bean seedlings:

The effect of soil treatment with each of *T. harzianum* and *T. koningii* or their mixture and the commercial bioproduct promot application on root size and seedlings growth of bean cultivar (Giza 6) under greenhouse conditions was studied. Results presented in Table (2) clearly indicated that root system from soil treated with *Trichoderma* and infested with *S. sclerotiorum* had larger root than similar seedlings in the absence of bioagents. Root systems from soil treated with *T. harzianum* and infested with *Sclerotinia* were nearly twice as longer as those from soil infested with *Sclerotinia* alone.

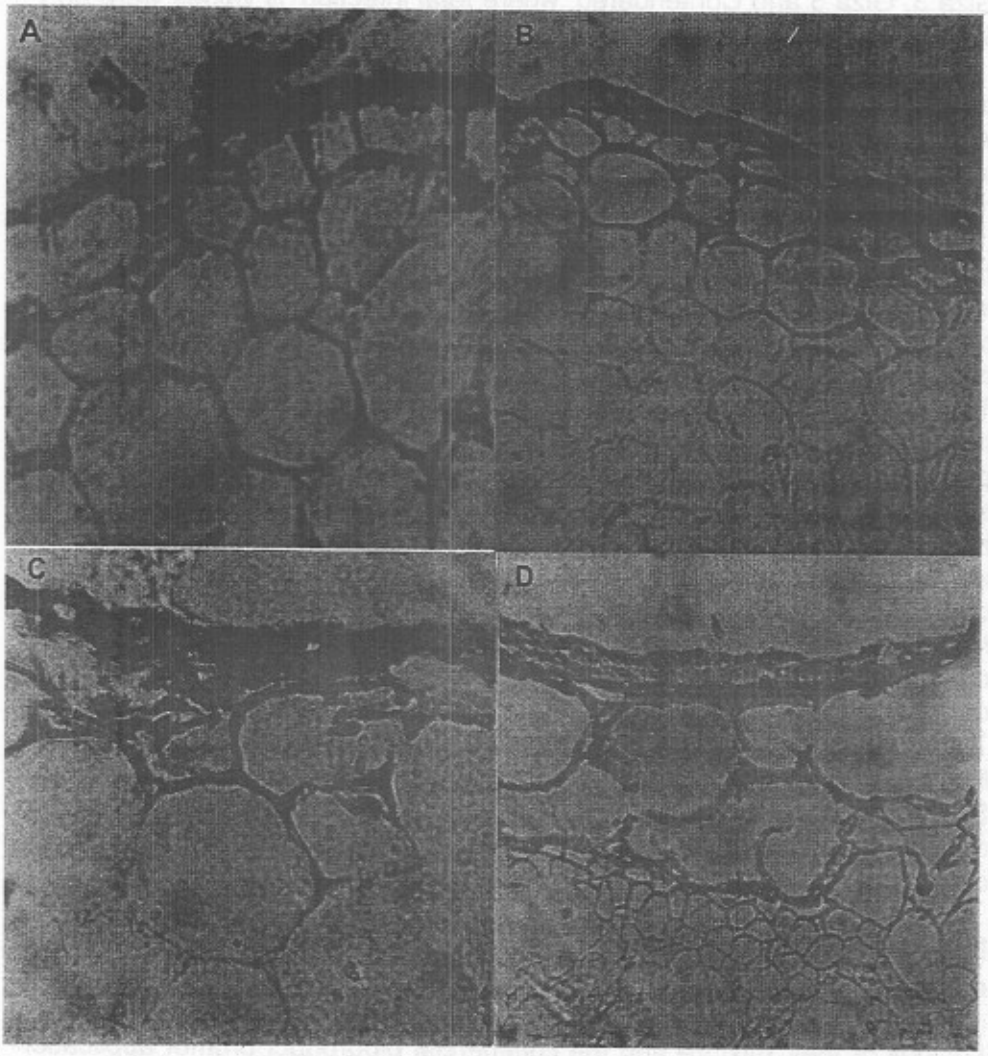

Figure (2): Histology of common bean seedlings hypocotyls inoculated with *S. sclerotiorum* after 24, 48, 72 and 96 hours after inoculation. (A) a cross section through bean hypocotyl 24 hours after inoculation showing hyphal invasion of epidermal cells and intercellular development through outer layers of cortical cells. (B & C) cross sections through bean hypocotyl 48 & 72 hours following inoculation showing complete destruction of the epidermal cells, partial destruction of some cortical cells (C). (D) a cross section through bean hypocotyl 96 hours following inoculation showing hyphae colonization and completely destruction of all cortical cells and endodermis layer.

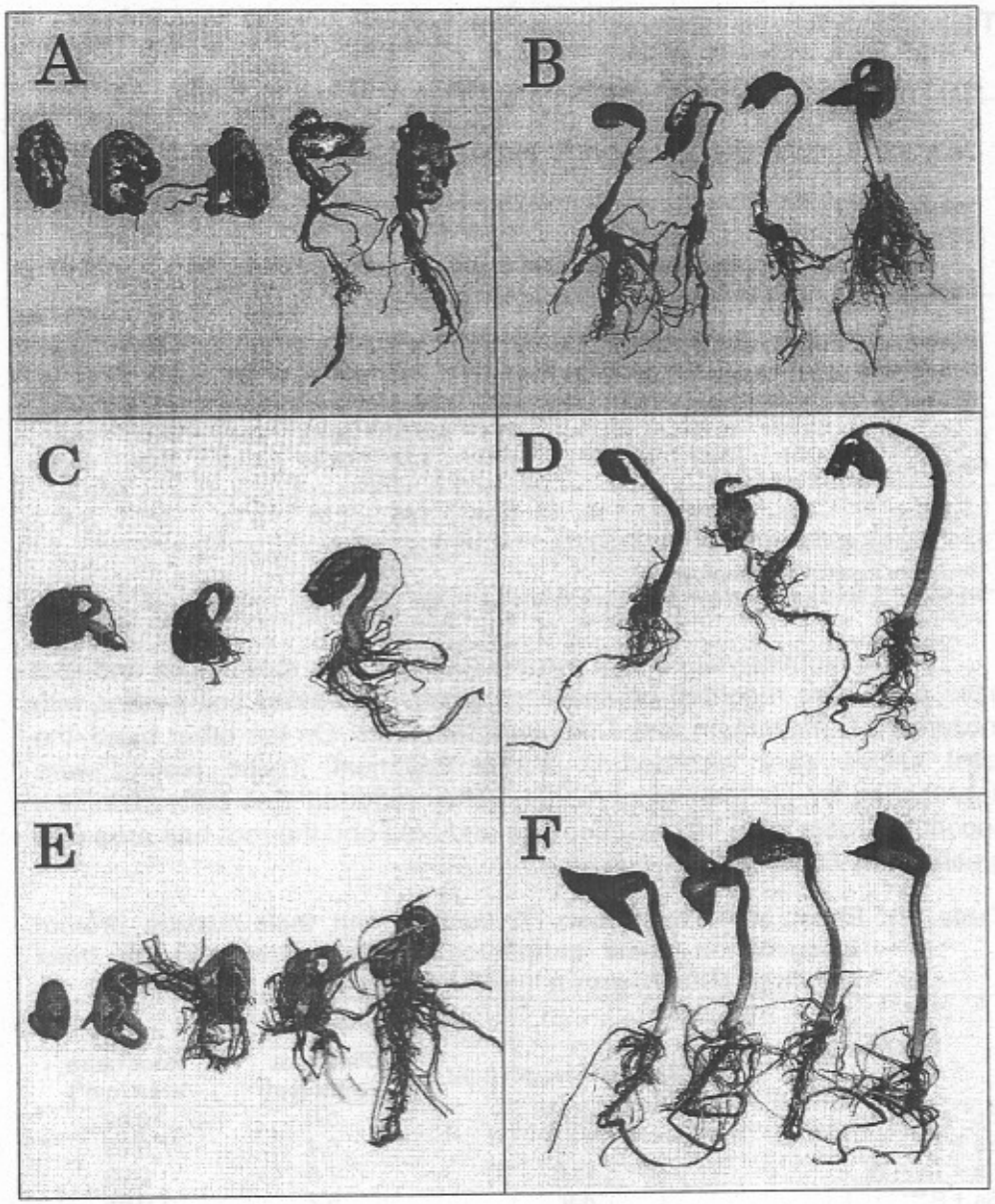

Figure (3): Effect of soil treatment with *T. harzianum* on the incidence of *S. sclerotiorum* damping-off. (A & B) pre- and post-EDO incited by *S. sclerotiorum* grown in soil. (C & D) pre- and post-EDO incited by *S. sclerotiorum* and inoculated with the bioagent *T. harzianum*. (E & F) pre- and post-EDO in uninfected untreated soil (control).

Table (1): Effect of different treatments with biocontrol agents and the commercial product promot on suppression of *Sclerotinia* damping-off on some common bean cultivars:

Soil treatment	Bean cultivars								
	Giza 3			Giza 6			Contendered		
	Emergence damping-off								
	Pre-	Post-	Total	Pre-	Post-	Total	Pre-	Post-	Total
Ss* + Th	9.96*	3.32	13.28**	29.96	16.60	46.56	9.96	29.96	39.92
Ss + Tk	36.64	29.96	66.60	39.98	40.00	79.98	39.89	16.60	56.49
Ss + Th + Tk	6.64	3.32	09.96	13.30	16.60	29.90	16.60	9.98	26.58
Ss + P***	39.98	33.30	73.28	46.70	39.98	86.68	42.12	16.60	58.72
Ss	46.70	40.00	86.70	50.00	40.00	90.00	46.70	46.70	93.40
Uninoculated soil	6.64	3.32	06.96	3.32	3.32	06.64	0.00	0.00	00.00
L.S.D. $_{0.05}$	4.98	5.65	7.38	6.90	3.55	6.30	5.12	3.7	5.90

* Ss = *S. sclerotiorum* Th = *T. harzianum* Tk = *T. koningii* P= promot
** Values are mean of three replicates.
*** Promot : *T. koningii* 3 x 10^7/gm plus *T. harzianum* 2 x 10^7/gm (2 g/kg soil).

The highest values of the main root and fine root length and root hairs area were recorded on seedlings grown in infested soil treated with mixture of *T. harzianum* and *T. koningii* bioagents. On the other hand, the least values were recorded in promot treatment. These results were approached by Harman *et al.* (2004). They reported that both main and secondary roots were increased in size and area and the root hair area was greater with *Trichoderma.*

Table (2): Effect of *T. harzianum*, *T. koningii* and their mixture promot bioagent on some morphological root parameters of bean seedlings (Giza 6) grown in soil infested with *S. sclerotiorum*:

Soil treatment	Root parameters		
	Main root length (cm)	Fine root length (cm)	Root hairs area (cm^2)
Ss* + Th	10.8**	8.0	0.92
Ss + Tk	7.6	6.4	0.69
Ss + Th + Tk	11.6	7.8	0.98
Ss + P***	8.9	5.2	0.68
Ss	5.2	3.4	0.64
Uninoculated soil	9.0	7.8	0.85
L.S.D. $_{0.05}$	2.95	2.15	0.19

* Ss = *S. sclerotiorum* Th = *T. harzianum* Tk = *T. koningii* P= promot
** Values are mean of three replicates.
*** Promot : *T. koningii* 3 x 10^7/gm plus *T. harzianum* 2 x 10^7/gm (2 g/kg soil).

Effect of *Trichoderma* spp. and *Sclerotinia* spp. on the growth of bean seedlings:

Effect of treatment with biocontrol agents on dry and fresh weight or root and shoot systems of bean plants (20 to 25 days after planting) in soil infested with *S. sclerotiorum* was studied. Table (3) clearly indicated that treatment of soil with *Trichoderma* tended to increase the growth of root and shoot system among all tested bean cultivars. The highest values of dry and fresh weight of bean seedlings were obtained in the treatment in which soil was inoculated with *T. harzianum* and *T. koningii* together, whereas the least values were recorded promot treatment. Infestation of soil with *S. sclerotiorum*, generally, reduced shoot and root system weight of bean plants in all tested cultivars.

Table (3): Effect of different biocontrol treatments on dry and fresh weight of common bean plants (20-25 days after planting) inoculated with *S. sclerotiorum*:

Soil treatment	Root system						Shoot system					
	Dry weight (g)			Fresh weight (g)			Dry weight (g)			Fresh weight (g)		
	Bean cultivars*											
	G 3	G6	C	G 3	G6	C	G 3	G6	C	G 3	G6	C
Ss** + Th	0.288***	0.216	0.200	0.660	0.522	0.484	0.312	0.310	0.250	2.22	2.00	1.860
Ss + Tk	0.128	0.110	0.099	0.362	0.350	0.302	0.280	0.252	0.233	1.96	1.49	1.400
Ss + Th + Tk	0.328	0.305	0.288	0.650	0.680	0.590	0.328	0.316	0.282	2.37	2.16	2.060
Ss + P****	0.099	0.082	0.059	0.310	0.296	0.262	0.233	0.228	0.200	1.80	1.75	1.680
Ss	0.088	0.069	0.460	0.266	0.218	0.200	0.029	0.022	0.016	1.66	1.38	0.122
Uninoculated soil	0.568	0.412	0.383	1.000	0.850	0.730	0.368	0.353	0.298	3.18	2.54	2.060
L.S.D. $_{0.05}$	0.035	0.033	0.720	0.091	0.081	0.069	0.057	0.074	0.49	0.270	0.297	0.380

* Cultivars: G 3 = Giza 3, G 6 = Giza 6 and C = Contendered.
** Ss = *S. sclerotiorum* Th = *T. harzianum* Tk = *T. koningii* P= promot
*** Values are average of three replicates.
**** Promot : *T. koningii* 3 x 10^7/gm plus *T. harzianum* 2 x 10^7/gm (2 g/kg soil).

Data of Table (3) also indicated that common bean cv. Giza 3 showed the highest values of dry and fresh weight. The differences in dry and fresh weight values of shoot and root systems between *T. koningii* and promot treatments were insignificant. These results were in agreement with Lo *et al.* (2000) and Yeldidia (2001), who reported that *Trichoderma* spp. have a variety of effects upon plants, including direct control of fungal pathogen, enhancement of plant growth and nutrition, and induced systemic resistance. Harman *et al.* (2004) also reported that increase

growth probably was due to direct stimulation of plant growth in addition to effects of bioagents.

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الملخص العربـــــى

التداخل الهيفى بين الطفيل الفطرى تريكودرما و الفطر سكلورنينيا إسكيلروشيورم لمقاومة مرض الذبول الطرى فى الفاصوليا

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أظهر الفحص الميكروسكوبى حدوث التداخل الهيفى بين الفطر تريكودرما هيرزيانم والطفيل الممرض المحمول بالتربة سكلوروتينيا إسكليروشيورم ، فى بيئة مزدوجة ، فى أشكال مختلفة منها: الإستعمار السطحى الكامل ، الإلتفاف حول هيفا الطفيل الممرض ، تكوين ما يشبه عضو الإلتصاق قبل عملية الإختراق ، الإختراق بأكثر من هيفا فردية معاً خلال نقطة واحدة على إحدى خلايا هيفا العائل (الطفيل الممرض) ، الإلتفاف حول خلية الطفيل من خلال تكوين فرعين قصيرين جداً فى نهاية الهيفا وتكوين عديد من الفروع الجانبية عند إقتراب الفطر الحيوى من الطفيل الممرض.

ولقد أوضحت الدراسة التشريحية لبادرات نباتات الفاصوليا حدوث الإختراق خلال الـ ٤٨ ساعة الأولى من العدوى و ذلك خلال البشرة السطحية والطبقة الخارجية من القشرة ، كما شوهد إمتداد الضرر بين وداخل خلايا طبقة القشرة و ذلك بعد ٧٢ ساعة من العدوى. ويمتد الضرر عمقاً مع طول الوقت ليشمل كل خلايا طبقات القشرة بعد ٩٦ ساعة من العدوى.

ولقد أوضحت دراسات الصوبة الزجاجية أن معاملة التربة بفطرى التريكودرما هيرزيانم والتريكودرما كونيننجى معاً كانت أكثر فعالية فى السيطرة على المرض وذلك بالمقارنة بالبروموت التجارى الأقل فعالية فى مقاومة المرض. ولقد تميز المجموع الجذرى لبادرات الفاصوليا بكبر حجمه فى التربة المعاملة بالتريكودرما والمعداه بالفطر سكلوروتينيا و ذلك عن مثيلتها من البادرات المنزرعة فى تربة خالية من الفطر الحيوى. وكما كانت قيم طول الجذر الرئيسى وكذا الجذور الدقيقة ومنطقة الشعيرات الجذرية عالية و ذلك فى التربة المعاملة بالتريكودرما بكلتا نوعيها معاً. وكانت أقل قيمة للوزن الطازج والجاف للمجموع الجذرى فى التربة المعاملة بفطر تريكودرما كونيننجى و كذلك فى التربة المعاملة بالبروموت التجارى ، بينما كانت أعلى قيمة فى التربة المعاملة بفطرى تريكودرما هيرزيانم وتريكودرما كونيننجى معاً. وبصفة عامة أدى معاملة التربة بالفطر الحيوى *T. harzianum* إلى تحسين نمو النبات وكذلك الإبعاد القياسية للمجموع الجذرى.