

## BIOCONTROL OF BEAN ROOT-ROT BY *Trichoderma* SPP. AND SOLARIZATION

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### ABSTRACT

Two field experiments were conducted through 2006 and 2007 growing seasons at Giza Governorate. Selected fields were naturally infested with *Fusarium solani* and *Rhizoctonia solani*.

The ability of three bioagents to control these two root-rot pathogenic fungi, *i.e.*, *Fusarium solani* and *Rhizoctonia solani* was tested *in vitro*, under greenhouse and field conditions. All the tested bioagents, *i.e.*, *Trichoderma album*, *T. lignorum* and *T. harzianum* reduced the radial growth of the two tested pathogenic fungi and suppressed the disease incidence. The effect of soil solarization and biological application using *Trichoderma* isolates in combination form on bean root-rot disease incidence was evaluated under field conditions during two successive growing seasons 2007 and 2008. Application of soil mulching for six weeks using 50 micron thick transparent polyethylene sheets was carried out. Soil solarization reduced significantly root-rot disease of bean. Solarization and biological treatments both in combination reduced significantly root-rot incidence and increased the seed quality (100-seed weight) and quantity (seed yield/plot) during the tested seasons. The average of disease reduction was ca 65, 42 and 34% with *T. harzianum*, *T. lignorum* and *T. album*, respectively, after 60 days.

### INTRODUCTION

Beans (*Phaseolus vulgaris* L.) is an important vegetable crop in Egypt. It grows well all over Egypt except in areas suffering from salinity, alkalinity or in heavy and compact clay soils. It is also, considered to be sensitive to high and low temperatures (more than 35°C and lower than 15°C, respectively). Meanwhile, the optimum temperature for seed germination and growth is 18 to 24 °C. Moreover, it is belonging to family leguminaceae and increases the soil fertility by the nodule formation caused by *Rhizobium phaseoli*.

Soil solarization is a physical advance in the non-chemical control against many pathogens and pests. This method of control is non-expensive, non-hazardous and easy to apply in agricultural practices compared with the chemical control. Several workers reported the effect of soil solarization in reducing the incidence and severity of plant diseases caused by soil borne pathogens (Tjamos and Fravel, 1995; Ansoori and Jaliani, 1996 and El-Ghareeb *et al.*, 2002).

In recent years, the potential of using antagonistic microorganisms for the biological control of plant pathogens had been frequently discussed (Campbell, 1994; Mahmoud, 2007 and Gomah and Mahmoud, 2007).

The use of biological control with solarization provides many possibilities. The use of *T. harzianum* with solarization in fields infested with

*R. solani* has been shown to improve disease control, which delay the build up of inoculum (Tjamos and Fravel, 1995). Similarly, Yucel and Cali (1997) applied *T. harzianum* in solarized soil to control tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* and reported that disease incidence was significantly reduced by this treatment.

Fahim *et al.* (1987) found that both of *R. solani* and *Fusarium* spp. Caused damping-off to bean plants. Moreover, Faris *et al.* (1992) and Mahmoud (1992 & 1997) stated that all isolated fungi, *i.e.*, *R. solani*, *F. solani*, *Phytophthora* sp., *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Fusarium oxysporum* f.sp. *phaseoli*, *Pythium* spp. And *Verticillium* sp. from diseased roots of bean plants, were pathogenic but varied in their virulence in pre- and post-emergence damping-off.

Papvizas and Lewis (1989) and Mahmoud (1992) stated that *T. harzianum* reduced root-rot disease of bean seedlings caused by *Sclerotium rolfsii*. Benhamou and Chet (1993) showed by the electron microscope that the hyphae of *T. harzianum* surrounded with *R. solani* hyphae and caused markedly broke-down because of chitinase enzyme produced by the antagonistic fungus as well as *T. harzianum* added only to fumigated soils, suppressing plant damage by 83%. Roberti *et al.* (1993) reported that *T. harzianum* and *T. viride* were more effective than other fungi tested as seed treatments against *R. solani*.

Recently, Al-Jurifani (1996) proved that *Trichoderma lignorum* reduced the percentage of infection with *R. solani* to great extent in bean.

Therefore, the present study is designed to investigate various methods (alone or combined) for controlling bean root-rot, and it could be considered advances and future prospects in biological and physical control as parts of integrated management programs for root-rot of bean and other crops.

## **MATERIALS AND METHODS**

### **Laboratory experiment**

#### **Antagonistic and root-rot fungi**

Two virulent fungi strains of *Fusarium solani* and *Rhizoctonia solani* and three bioagent fungal strains of *Trichoderma album*, *T. lignorum* and *T. harzianum* were isolated by the author from root rotted and healthy plants of bean (*Phaseolus vulgaris* L.) during a previous investigation (Barakat *et al.*, 1991).

#### **Antagonistic study**

The antagonistic effect of *Trichoderma* strains against *R. solani* and *F. solani* was determined using dual culture technique (Ordentlich *et al.*, 1991). Tests were performed in Petri dishes (90 mm diam.) containing 15 ml synthetic medium consisting of: 0.2 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.9 g K<sub>2</sub>HPO<sub>4</sub>, 0.2 g KCl, 1.0 g NH<sub>4</sub>NO<sub>3</sub>, 5.0 g glucose, 0.002 g FeCl<sub>2</sub>, 0.002 g Mn Cl<sub>2</sub>, 0.002 g ZnSO<sub>4</sub>, 0.001 g thiamine hydrochloride and 20 g agar/L (Difco) at pH 6.8. Mycelial discs (5 mm diam.) were cut from a 7days-old culture of *R. solani* or *F. solani* and transferred to the synthetic medium plates 2 – 3 cm from the edge. The opposite side of medium was inoculated with a disc of 5 mm diam. of any of the bioagent fungal strains. The inoculated plates were incubated at 28 ± 2°C

for 6-7 days and then the growth reduction (GR) was calculated according to Ferreira *et al.* (1991) and the observation for overgrowth or the interactions was recorded.

#### **Biological control**

Plastic pots of 25 cm diam., containing natural unsterilized loamy soil, were artificially infested, at the rate of 5% (w/w) with the inoculum of each root-rot pathogenic fungal strains and/or bioagent strains grown on sand-sorghum grains mixture at the rate of 1 : 1 (w/w) and 40% water (Whithead, 1957) for 2 weeks at  $28 \pm 2^\circ\text{C}$ .

Pots were filled with inoculated soil with the pathogenic fungal strains individually without any of antagonistic fungi served as control 1. However, the pots of control 2 were filled with non-inoculated soil. Ten replicates were used for each particular treatment. All treatments received two irrigates weekly to stimulate the fungal growth and to ensure its distribution in the soil. Five seeds of bean Giza 3 cv. were planted in each pot. The percentage of bean root rotted plants (BRR) were recorded after 15 and 60 days from sowing and the percentage of disease reduction (DR) was also calculated as follow:

$\% \text{DR} = (N - T)/N \times 100$ ; where N = percentage of root rotted plants in infested soil with pathogenic fungi (control 1) without any antagonistic treatment. T = percentage of root rotted plants in the infested soil with pathogenic and antagonistic isolates (Barakat *et al.*, 1998).

#### **Field experiments**

Two field experiments were conducted in two successive seasons (2006 and 2007) to evaluate the effect of solarization and biological control on bean root-rot disease incidence both individually and/or in combination. The experiments were carried out in naturally heavily contaminated clay-loamy soil with root-rot pathogens at Giza Governorate, where bean has grown frequently for many years.

#### **Solarization**

On July, 2006, the experimental area was designed in factorial completely randomized block design with 5 replicates, each 3 x 3.5 m (1/400 fed.) with 6 rows/plot, all plots were irrigated to field capacity and the half of experimental plots (4 treatments x 5 replicates = 20 plots) were covered with 50 micron thick transparent polyethylene sheet for 6 weeks. Minimum and maximum degree of soil temperature was weekly measured by soil thermometer during that period at depths of 10 and 20 cm.

#### **Biological control**

On November, 2006, the biological control treatments were carried out in both solarized and non-solarized soils. The inoculum of bioagent isolates grown on sand-sorghum grain mixture for two weeks were used for soil treatment. The bioagent inoculum was incorporated at the rate of 120 g/m<sup>2</sup> to the top 20 cm of the soil surface at planting row sites. Bean seeds Giza 3 cv. were used at the rate of 3 seeds/hole and each row contained 30 holes. All plots received the traditional agricultural practices recommended by the Egyptian Ministry of Agriculture and Land Reclamation.

To evaluate long-term effects of soil solarization on bean root-rot disease incidence, this experiment was repeated at the same location on

November, 2007, without polyethylene sheet coverage. All experimental plots were periodically examined and the numbers of root rotted plants were recorded after 15 and 60 days from sowing. In addition, seed yield/plot and 100 seed weight were estimated, and data were statistically analyzed according to Steel and Torrie (1960).

## RESULTS AND DISCUSSION

The antagonistic effect of *T. album*, *T. lignorum* and *T. harzianum* on linear growth of bean root rot pathogenic fungi, *i.e.*, *F. solani* and *R. solani*, was determined *in vitro*. The antagonistic test is considered a simple approach for understanding a small sector of biological role in the disease control. *T. harzianum* restricted the radial growth on dual culture plates and subsequently overgrew the mycelium of the bean root-rot pathogenic strains on these plates. Moreover, it showed high antagonistic effect against *R. solani* and *F. solani* (Table 1), resulting in significant reduction of their growth (GR), recording 26.69 and 22.70%, respectively. Within 6 – 7 days of incubation, the *F. solani* growth and all the available surface of the Petri dish involved with *R. solani* was covered by sporulating hyphae of *T. harzianum*, respectively. However, *T. lignorum* covered half or less of the available surface for *R. solani* and *F. solani*, respectively. In contrast, *T. album* did not overgrow the growth of *F. solani*. Many studies had shown the potential of *Trichoderma* spp. as bioagents of soilborne plant pathogens (Papavizas, 1985 and Chet, 1987).

Production of volatile and non-volatile antibiotics (Dennis & Webster, 1971 a & b and Claydon *et al.*, 1987) and competition for nutrients (Ahmed and Baker, 1987) have been implicated. Moreover, mycoparasitism is also considered of major importance in the antagonistic activity of *Trichoderma* spp. (Dennis and Webster, 1971 c). It could be concluded that one and/or more from the previous factors play an important role in the antagonistic action of *Trichoderma* spp. against root-rot fungi.

**Table (1): Hyphal interaction (HI) and percentage growth reduction (GR%) of *R. solani* and *F. solani* as affected by *Trichoderma* spp.**

Bioagent	Antagonistic effect			
	<i>F. solani</i>		<i>R. solani</i>	
	HI	GR%	HI	GR%
<i>T. album</i>	-	14.30	+	15.65
<i>T. lignorum</i>	+	15.25	++	17.42
<i>T. harzianum</i>	+++	22.70	++++	26.69
L.S.D. at 5%	-	7.14	-	9.12

- HI = Hyphal interaction.  
 GR% = Percentage of radial growth reduction.  
 - = No growth.  
 + = overgrowth less of half of the available surface.  
 ++ = overgrowth half of the available surface for the pathogen.  
 +++ = overgrowth all of available surface for the pathogen.  
 ++++ = overgrowth all of available surface for Petri dish.

The antagonistic efficiency of *Trichoderma* spp. isolates to reduce bean root-rot disease incidence was evaluated under greenhouse conditions (Table 2). Greenhouse experiment revealed that tested *F. solani* and *R. solani* strains are pathogenic to bean plants during 15 and 60 days.

They could attack germinated seeds, seedlings and adult bean plants, *R. solani* strains was significantly aggressive than *F. solani*. The percentage of bean root rotted plants (BRR) was 19.37 and 17.34 after 15 and 60 days from seed sowing in soil infested with *R. solani*, respectively. However, it was 18.12 and 12.99% in soil infested with *F. solani* after the same two periods, respectively.

All the tested of *Trichoderma* spp. reduced significantly the percentage of bean root rotted plants (BRR) after 15 and 60 days from sowing. *T. harzianum* was more efficient against the average decrease percentage of bean root rotted plants (BRR) after 15 and 60 days being 13.01 and 8.02, respectively, as compared with those obtained by un-inoculated soil with antagonistic strains (control 1), being 25.91 and 23.69, respectively. It also recorded the highest percentage of disease reduction (DR) being 49.51 and 65.50. No root rotted plants were observed in (control-2) during the experimental period. These results confirm those reported by many investigators (Osman *et al.*, 1986; Sivan and Chet, 1986 and Ordeutlich *et al.*, 1991). The linkage between mycoparasitism, lytic activity and biocontrol potential of *T. harzianum* has been well established for *R. solani* (Ridout *et al.*, 1988), *Sclerotium rolfsii* (Elad *et al.*, 1982) and *Fusarium* spp. (Sivan and Chet, 1986).

The effect of soil solarization and biological application on bean root-rot during two bean successive seasons 2006 and 2007 are presented in Table (3). The combined treatments were conducted to provide, for an even wider spectrum of disease suppression and perhaps most importantly, the use of solarization with integrated pest management (IPM.) program.

The efficacy of solarization in disease control was extended to the second bean treatment growing season 2007. Moreover, an increase in the yield was also observed as a result of biological control and/or solarization treatments. Data in Table (3) reveal that the percentage of root-rot in plants were significantly reduced from 19.81 and 13.40 after 15 and 60 days after sowing in non-solarized un-inoculated soil (control-2), respectively, to 13.81 and 8.28 after the same two periods, respectively, in solarized un-inoculated soil (control-1) as a reflection of soil solarization during 2006 growing season. These effects were extended to the 100-seed weight and seed yield/plot, which was significantly increased in solarized un-inoculated soil, being 60.44 g and 2560 g/plot, respectively. However, they were 58.24 g and 2141 g/plot in non-solarized un-inoculated one (control 2).

The average soil temperature during mulching period ranged between 38 – 41°C at upper 10 cm of soil. Meanwhile, the average ranged between 38 – 41°C at 20 cm of soil depth.

The biological control application provided significant reductions in root-rot disease incidences. All treated plots by antagonistic bioagents strains and non-solarized reduced significantly the percentage of dead plants after 15 and 60 days from sowing.



*Trichoderma harzianum* reduced the percentage of dead plants from 19.81 and 13.40 in control (2) to 13.87 and 8.47, respectively, after the same mentioned periods. The 100-seed weight and seed yield/plot also, were significantly increased and recorded 63.72 g and 2751 g/plot, respectively.

The soil solarization physical treatment as well as biological control by means of some antagonistic agents in combined treatments proved to be an effective means for controlling bean root-rot disease. These treatments reduced significantly the root-rot disease incidence when compared with the individual treatment of soil solarization alone and/or the use of antagonistic bioagents during both tested successive growing seasons. Data presented in Table (3) show that the combined treatments proved to be an effective means, which have short effect for controlling bean root-rot.

The second season 2007 results confirmed the effect of combined treatments on reducing root-rot disease incidence, increased yield quantity and improved quality. Several workers reported the success of soil solarization in reducing plant diseases caused by soil borne pathogens and increasing the produced yield as well (Greenberger *et al.*, 1987; Abdel-Kader and Barakat, 1992 and El-Ghareeb *et al.*, 2002).

Moreover, biological control treatment in solarized soil have a good effect, as control measure, for soil borne plant pathogens beside the beneficial side effects on plant growth and obtained yield (Elad *et al.*, 1980 and Greenberger *et al.*, 1987). Katan (1980) proposed the mechanism of biological control to be operated during and after soil solarization through: a) reduction of pathogen population or activity by antagonistic microorganisms whose effect is enhanced by the control heating process. B) a shift in the biological equilibrium of the soil in favour of microorganisms that prevent reinfestation by the pathogen and build up of its population during the season. These suggestions may explain the extended effect of soil solarization treatment for both tested successive cultivation seasons, in the present study.

It could be concluded that these results represent the effect of two important factors (physical and biological treatments), which may be introduced for integrated disease management programmes. Further studies are also necessary to improve *T. harzianum* strains and its establishment in the soil under adverse field conditions.

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**مقاومة عفن جذور الفاصوليا باستخدام التراكودرما والطاقة الشمسية**  
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تم إختبار ثلاث عزلات لأنواع من الفطر تراكودرما هي تراكودرما ألبم وتراكودرما ليجنورم وتراكودرما هارزيانم على تثبيط نمو الفطرين فيوزاريوم سولاني وريزوكتونيا سولاني المسببان لمرض عفن جذور الفاصوليا وقد أوضحت النتائج المعملية أن جميع عزلات الفطر تراكودرما لها القدرة على تثبيط نمو الفطرين الممرضين. أيضاً أوضحت النتائج المتحصل عليها من تجارب الصوبة والحقل إنخفاض نسبة النباتات المصابة بإعفان الجذور نتيجة استخدام عزلات الفطر تراكودرما. وكان متوسط نسبة خفض الإصابة بالمرض 65 ، 42 و34% مع تراكودرما هارزيانم وتراكودرما ليجنورم وتراكودرما ألبم على التوالي ، وذلك بعد 60 يوماً من الزراعة. أجريت تجربة لدراسة تأثير تعقيم التربة بالطاقة الشمسية على الإصابة بمرض عفن جذور الفاصوليا خلال شهري يوليو وأغسطس من عام 2006 ولمدة 6 أسابيع باستخدام شرائح البولي إثيلين بسك 50 ميكرون وذلك في حقل موبوء بمرض عفن جذور الفاصوليا حيث أوضحت النتائج أن تعقيم التربة باستخدام الطاقة الشمسية يؤدي إلى إنخفاض نسبة الإصابة بالمرض وزيادة المحصول.

إتضح أيضاً أن المعاملة المزدوجة خلال موسمی 2006 و2007 التي شملت تعقيم التربة بالطاقة الشمسية واستخدام المقاومة الحيوية أدت إلى نتائج جيدة في إختزال المرض وزيادة المحصول معنوياً.



Table (2): Effect of *Trichoderma* spp. on bean root-rot disease incidence under greenhouse conditions

Bioagents (B)	<i>Fusarium solani</i>				<i>Rhizoctonia solani</i>				Average			
	BRR%		DR%		BRR%		DR%		BRR%		DR%	
	15 days	60 days	15 days	60 days	15 days	60 days	15 days	60 days	15 days	60 days	15 days	60 days
<i>T. album</i>	18.12	13.43	24.97	29.98	19.37	17.20	30.00	39.00	18.75	15.32	27.49	34.49
<i>T. lignorum</i>	16.91	12.08	29.98	37.02	17.71	15.22	36.00	46.01	17.31	13.65	32.99	41.52
<i>T. harzianum</i>	13.28	7.29	45.01	61.99	12.73	8.74	54.00	69.00	13.01	8.02	49.51	65.50
Control 1	24.15	19.18	--	--	27.67	28.19	--	--	25.91	23.69	--	--
Average	18.12	12.99			19.37	17.34						
Control 2	Natural soil mixed with sterilized sand-sorghum mixture at the same rate (BRR = 00.00)											
	BRR = Bean root-rotted plants (%).				DR = Disease reduction (%).							
L.S.D. at 5% for:			15 days				60 days					
Bioagents (B)			3.97				5.46					
Bean pathogenic isolates (F)			1.02				3.26					
B x F			2.13				2.17					

Table (3): Effect of soil solarization and biological control application on bean root-rot disease incidence under field conditions during 2006 and 2007 growing seasons

Soil treatment (S)	Bioagent (B)	2006				2007			
		% root rotted plants		100 seed weight (g)	Seed yield (g/plot)	% root rotted plants		100 seed weight (g)	Seed yield (g/plot)
		15 days	60 days			15 days	60 days		
Solarized soil	<i>T. album</i>	8.32	6.63	64.25	2815	18.49	17.12	65.18	3275
	<i>T. lignorum</i>	7.91	5.77	65.11	2945	17.25	16.38	65.51	3280
	<i>T. harzianum</i>	6.80	6.21	66.83	3016	16.02	15.28	66.18	3316
	* Control 1	13.81	8.28	60.44	2560	20.54	18.41	62.43	3146
Non solarized soil	<i>T. album</i>	16.05	11.52	59.61	2590	20.54	19.14	59.82	3180
	<i>T. lignorum</i>	14.85	10.72	60.92	2630	19.24	18.60	60.21	3260
	<i>T. harzianum</i>	13.87	8.47	63.72	2751	16.91	16.10	60.21	3290
	** Control 2	19.81	13.40	58.24	2141	24.16	21.75	56.87	3017
* control 1	= solarized un-inoculated soil (%).								
** control 2	= non-solarized un-inoculated soil (%).								
L.S.D. at 5% for:									
Solarization (S)		5.20	3.40	3.34	136	1.12	1.29	3.55	143
Bioagents (B)		1.03	2.62	3.19	183	1.16	1.49	2.14	198
S x B		0.95	0.34	4.06	219	1.43	1.26	1.59	278