BIOLOGICAL CONTROL OF DAMPING-OFF IN BROAD BEAN VARIETIES GIZA 2 AND REBAYA 40

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ABSTRACT

Seeds of broad bean varieties Giza 2 and Rebaye 40 were inoculated with potent antagonistic rhizospheric isolates belonging to Bacillus sp. Streptomyces sp. or Trichoderma sp., which proved in earlier experiments (in vitro) to be potent antagonists against F.solani, R.solani and Scl.rolfsii. Seed inoculation treatments decreased the pre-and post-emergence daming-off and increased the survival plants percentages and decreased the deleterious effect of the pathogens on the growth of survival plants as compared to their respective control (uninoculated seeds).

Seed inoculation with the three potent antagonists (Bacillus sp. + Streptomyces sp. + Trichoderma sp. isolates) was the most efficient treatment in controlling the aforementioned pathogens in soil. Multiple antagonists treatment gave lower pre- and post-emergence daming-off and higher survival plants percentages and less deleterious effect of the pathogens on the growth of survival plants than when any of the antagonists was added solely.

These results were found in both broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with the pathogens F.solani, R.solani and Scl. rolfsii.

INTRODUCTION

Fusarium solani, Rhizoctonia solani and Sclerotium rolfsii cause root-rot and damping-off to broad bean and other leguminous plants (Yu and Fang,1948; Yamamato et al., 1955; Ashour et al., 1964; Ali, 1967 and Habib,1979). These pathogens may be controlled by seed dressing with fungicides or by other means. The application of fungicides introduce the problem of environment (soil) pollution and the side effect of such chemicals on beneficial soil microflora such as nitrifiers and nitrogen-fixing bacteria. One of the other methods to control soil pathogens, is seed inoculation with potent antagonists, which will be the subject of this investigation.

The inoculation of seeds with antagonistic microorganisms against root-rot pathogens, before sowing in natural soil, has long been studied in Russia and has apparentely resulted in increased yield and control of some root-rot pathogens. This was confirmed by many investigators including Broadbent and Baker (1969) and Broadbent and Waterworth (1971).

In this investigation, broad bean seeds were heavily inoculated with rhizospheric isolates belonging to Bacillus sp., Streptomyces sp. and Trichoderma sp., which proved in previous experiments by the authors to be potent antagonists against F.solani, R.solani and Scl.rolfsii, in vitro. Inoculated seeds were sown in soil infested with the aforementioned pathogens, aiming to study the effect of seed inoculation with potent antagonists on controlling the pathogens in the rhizosphere of the two broad bean varieties Giza 2 and Rebaya 40.

MATERIAL AND METHODS

The pathogens F.solani, R.solani and Scl.rolfsii were grown on sorghum medium (Whitehead , 1975) for 15 days. An inoculum of 5%, of each of the pathogens or all of them, was added each to sterilized and unsterilized soils in 25 cm pots. Pots containing sterilized and unsterilized soil were not infested, to which was added 5% sterilized sorghum medium , served as control I. Pots were watered daily for one week, with sterilized water for the sterilized soil and unsterilized water for the unsterilized soil, then sown with seeds of broad bean varieties Giza 2 and Rebaya 40. The following treatments were carried out :

A) <u>In sterilizd soil</u> :-

1. Sterilized soil sown with surface sterilized seeds served as control I (for sterilized soil).
2. Sterilized soils inoculated with <u>F.solani</u>, <u>R.solani</u>, <u>Scl.rolfsii</u> or all of the three pathogens sown with seeds which received any of the following treatments:-
   - a. Surface sterilized seeds which served as control II (for the sterilized soil).
   - b. Surface sterilized seeds mounted with the conidia of the potent antagonistic fungus namely <u>Trichoderma</u> sp. isolate.
   - c. Surface sterilized seeds mounted with the conidia of the potent antagonist <u>Streptomyces</u> sp. isolate.
   - d. Surface sterilized seeds mounted with the potent antagonist <u>Bacillus</u> sp. isolate.
   - e. Surface sterilized seeds mounted with the three potent antagonists <u>Trichoderma</u> sp. + <u>Streptomyces</u> sp. + <u>Bacillus</u> sp., isolates).

B) <u>In unsterilized soil</u> :-

All the aforementioned experiments (1 and 2, a,b,c, d and, e) were carried out in non-sterile soil where seeds were inoculated without surface sterilization.

Sterile water was used for irrigation of sterile soil, and non-sterile water was used for irrigation of non-sterile soil. Three replicates were made for each treatment. Ten seeds were sown in each pot.

Germination percentages were determined after 10 days, while post-emergence damping-off was determined after one month from sowing. Pre- and post-emergence damping-off were estimated to indicate the effect of seed inoculation with the potent antagonists on reducing the damping-off in broad bean plants sown infested soil with the pathogens.

After one month from sowing, plants were removed from pots and different morphological determinations such as length, fresh and dry weight of the root and shoot systems were estimated and recorded. This was carried out to indicate the effect of seed inoculation with potent antagonists on reducing the deleterious effect of the pathogens on the growth of survival plants.

RESULTS AND DISCUSSION

Effect of seed inoculation with the efficient antagonists, Bacillus sp. Streptomyces sp. and Trichoderma sp. isolates on damping-off and the growth of broad bean plants sown in soil infested with F.solani R.solani and Scl.rolfsii :-

In previous experiments by the authors, the most efficient antagonists were selected on the basis of their ability to exhibit highest antagonism against F.solani, R.solani and Scl.rolfsii in laboratory media.

In this investigation, the selected antagonistic isolates were inoculated on broad bean seeds which will be sown in sterile and non sterile soil. Seed dressing with heavy inoculum of the efficient antagonist(s) may offer a concentration of the antibiotic(s) sufficient to inactivate the invading pathogen in the vicinity of seeds where the infection may occur. Moreover, it is easier and more practical to inoculate seeds than soil.

The inoculated seeds were sown in sterile and non sterile soil infested with the pathogens F.solani, R.solani and Scl.rolfsii. This was carried out in sterile soil infested with the pathogens to study the efficiency of the antgonist(s) in controlling the pathogen(s) in the abscence of natural soil microflora. This represents a step forward in carrying out the experiment in conditions approaching normality.

The ultimate experiment was to ascertain whether the antagonist(s) will control the pathogen(s) in natural soil, in the presence of natural soil microflora. This was accomplished by sowing the inoculated seeds in infested natural soil.

Effect of seed inoculation with the antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on reducing the damping-off in broad bean varieties Giza 2 and Rebaya 40 sown in sterile and non-sterile soil infested with F.solani:-

The effect of seed inoculation with the most efficient antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on the percentages of pre-and post-emergence damping-off and survival plants in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with Fusarium solani is presented in Table (1).

Data in Table (1) Lead to the following results :-

1. Infestation of the sterile soil with F.solani increased the pre-and post-emergence damping-off and reduced the survival plants percentages as compared to control I (uninfested soil).

This was true in both varieties. However, Rebaya 40 seemed to be more susceptible than Giza 2 to the pathogen F.solani.

On the other hand, infestation of the unsterilized soil with F.solani showed less damping-off and more survival plants as compared to infested sterile soil. This may be due to the effect of soil saprophytic microflora in moderating the virulence of the pathogen.

Table (1) : Effect of seed inoculation with the efficient antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on the percentages of pre-and post-emergence damping-off and survival plants in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with F.solani.

Treatment (Added antagonist against F.solani)	Sterilized soil						Unsterilized soil					
	Giza 2			Rebaya 40			Giza 2			Rebaya 40		
	Damping-off %		Survival	Damping-off %		Survival	Damping-off %		Survival	Damping-off %		Survival
	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %
Control I *	00.00	00.00	100.00	3.33	0.00	96.67	6.67	0.00	93.33	10.00	0.00	90.00
Control II ** (Control I+F.solani)	16.67	20.00	63.33	30.00	20.00	50.00	13.33	16.67	70.00	23.33	13.33	63.33
Trichoderma sp.	10.00	13.33	76.67	20.00	13.33	66.67	6.67	10.00	83.33	20.00	6.67	73.33
Streptomyces sp.	6.67	10.00	83.33	16.67	10.00	73.33	6.67	6.67	86.67	16.67	3.33	80.00
Bacillus sp.	6.67	10.00	83.33	16.67	6.67	76.66	6.67	3.33	90.00	16.67	0.00	83.33
Trichoderma sp. + Streptomyces sp. + Bacillus sp.	3.33	6.67	90.00	6.67	3.33	90.00	3.33	3.33	93.33	13.33	0.00	86.67

* Control I = uninfested soil.

** Control II = Soil infested with F.solani.

2. Inoculation of the seeds with any of the antagonists then sowing in infested sterile soil decreased the percentages of pre-and post-emergence damping-off and increased the survival plants percentage as compared to control II (soil infested with the pathogen F.solani without inoculating any antagonist). Such effects were more obvious when the isolate Bacillus sp. was the inoculated antagonist. However, inoculation with the three antagonists Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates showed the lowest pre-and post-emergence damping-off and the highest survival plants percentages as compared to their respective percentages when any of the antagonists was inoculated alone. This was true for both broad bean varieties Giza 2 and Rebaya 40.

   In case of unsterilized soil, inoculation of seeds with any of the antagonists showed the same trend as in sterile soil. The pre-and post-emergence damping-off decreased and survival plants percentages increased as compared to control II. The highest percentages of survival plants were recorded when the three antagonists were inoculated on Giza 2 and Rebaya 40 seeds as compared to the respective values when any of the antagonists was inoculated solely.

Effect of seed inoculation with the antagonists, Trichoderma sp. Streptomyces sp. and Bacillus sp. isolates on reducing the damping off in broad bean varieties Giza 2 and Rebaya 40 sown in sterile and non-sterile soil infested with Rhizoctonia solani.

The effect of seed inoculation, with the most efficient antagonists namely Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates, on the pre-and post-emergence damping off and survival plants percentages in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with Rhizoctonia solani is presented in Table (2).

Table (2) : Effect of seed inoculation with the efficient antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on the percentages of pre-and post-emergence damping-off and survival plants in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with R.solani.

Treatment (Added antagonist against R.solani)	Sterilized soil						Unsterilized soil					
	Giza 2			Rebaya 40			Giza 2			Rebaya 40		
	Damping-off %		Survival	Damping-off %		Survival	Damping-off %		Survival	Damping-off %		Survival
	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %	Pre-emergence	Post-emergence	plants %
Control I *	00.00	00.00	100.00	3.33	00.00	96.67	6.67	00.00	93.33	10.00	00.00	90.00
Control II ** (Control I+R.solani)	36.67	16.67	46.66	43.33	16.67	40.00	20.00	10.00	70.00	26.67	13.33	60.00
Trichoderma sp.	33.33	6.67	60.00	36.67	3.33	60.00	16.67	3.33	80.00	23.33	10.00	66.67
Streptomyces sp.	30.00	3.33	66.67	33.33	6.67	60.00	13.33	6.67	80.00	23.33	6.67	70.00
Bacillus sp.	30.00	6.67	63.33	23.33	10.00	66.67	13.33	10.00	76.67	20.00	13.33	66.67
Trichoderma sp. + Streptomyces sp. + Bacillus sp.	16.67	3.33	80.00	20.00	6.67	73.33	10.00	3.33	86.67	16.67	3.33	80.00

* Control I = uninfested soil

** Control II = Soil infested with R.solani

Data in Table (3) Lead to the following results :-

1. Infestation of the sterilized and unsterilized soil with Scl.rolfsii (Table 3) showed moderate damping-off incidence which was lower than R.solani (Table 2) and higher than F.solani (Table 1).
2. Inoculation of seeds with the antagonists, Trichoderma sp., Streptomyces spand Bacillus sp. isolates then sowing in sterilized and unsterilized soil reduced the damping -off and increased the survival plants percentages for both broad bean varieties as compared to their respective control (soil infested with Scl.rolfsii without inoculating any antagonist). Streptomyces sp. and Bacillus sp. isolates seemed to be more effective antagonists than Trichoderma sp. isolate in controlling the damping-off caused by Scl.rolfsii. However, inoculation with the three antagonists was the most effective treatment in controlling Scl.rolfsii than when any of the antagonists was inoculated alone.

Effect of seed inoculation with the antagonists ; Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on reducing the damping-off in broad bean varieties Giza 2 and Rebaya 40 sown in soil infested with F.solani, R.solani and Scl.rolfsii :-

The effect of seed inoculation with the potent antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates on the percentages of pre-and post-emergence damping-off and survival plants in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with the three pathogens F.solani, R.solani and Scl.rolfsii is presented in Table (4).

Table (3) : Effect of seed inoculation with the efficient antagonists, Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates, on the percentages of pre-and post-emergence damping-off and survival plants in broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with Sclerotium rolfsii.

Treatment (Added antagonist against Scl.rolfsii)	Sterilized soil						Unsterilized soil					
	Giza 2			Rebaya 40			Giza 2			Rebaya 40		
	Damping-off %		Survival plants %	Damping-off %		Survival plants %	Damping-off%		Survival plants %	Damping-off%		Survival plants %
	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
Control I *	00.00	00.00	100.00	3.33	00.00	96.67	6.67	00.00	93.33	10.00	00.00	90.00
Control II ** (Control I+Scl. rolfsii)	26.67	23.33	50.00	30.00	26.67	43.33	13.33	16.67	70.00	16.67	16.67	66.66
Trichoderma sp.	20.00	16.67	63.33	23.33	16.67	60.00	13.33	13.33	73.34	16.67	13.33	70.00
Streptomyces sp.	16.67	13.33	70.00	20.00	16.67	63.33	10.00	10.00	80.00	13.33	10.00	76.67
Bacillus sp.	16.67	13.33	70.00	16.67	20.00	63.33	13.33	10.00	76.67	16.67	10.00	73.33
Trichoderma sp. + Streptomyces sp. + Bacillus sp.	6.67	13.33	80.00	10.00	13.33	76.67	6.67	10.00	83.33	13.33	6.67	80.00

* Control I = Uninfested soil.
** Control II = Soil infested with Scl.rolfsii.

Data in Table (4) show the following results :-

1. Infestion of the soil with the three pathogens F.solani, R.solani and Scl.rolfsii increased geatly the pre-and post-emergence damping - off, but survival plants percentages greatly decreased in both broad bean varieties, as compared to control, (uninfested soil). The increase in damping -off and the decrease in survival plants percentages, due to infestation were lower in non-sterile soil than in sterile soil. This may be due to the effect of soil microflora present in the non-sterile soil which may attenuated the invasion of the pathogens.

2. The inoculation of the seeds with any of the antagonists decreased the pre-and post-emergence damping-off and increased survival plants percentages as compared to control III (No antagonist was added to seeds sown in soil infested with the three pathogens). This trend was observed in both broad bean varieties sown in sterile and non-sterile soil. Among the investigated antagonists, Bacillus sp. isolate seemed to show the highest antagonism against the pathogens. However the inoculation with the three antagonists Trichoderma sp., Streptomyces sp. and Bacillus sp. isolates gave the lowest percentages of damping-off and the highest percentages of survival plants. Seed inoculation with multiple antagonists showed that the antagonists intensified the antagonistic activity of each other against the investigated pathogens.

Table (4) : Effect of seed inoculation with the efficient antagonists; Trichoderma sp., Streptomyces sp. and Bacillus sp. on the percentages of pre-and post-emergence damping-off and survival plants in two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with the three pathogens (F. solani, R. solani and Scl. rolfsii).

Treatment (Added antagonist against the three pathogens)	Sterilized soil						Unsterilized soil					
	Giza 2			Rebaya 40			Giza 2			Rebaya 40		
	Damping-off %		Survival plants %	Damping-off %		Survival plants %	Damping-off%		Survival plants %	Damping-off%		Survival plants %
	Pre-emergence	Post-emergence		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
Control I *	00.00	00.00	100.00	3.33	00.00	96.67	6.67	00.00	93.33	10.00	00.00	90.00
Control III *** (Control I+3 pathogens)	46.67	30.00	23.33	53.33	33.33	13.33	23.33	20.00	56.67	33.33	23.33	43.33
Trichoderma sp.	36.67	30.00	33.33	43.33	26.67	30.00	20.00	20.00	60.00	30.00	20.00	50.00
Streptomyces sp.	26.67	13.33	60.00	36.67	13.33	50.00	20.00	13.33	66.67	26.67	16.67	56.66
Bacillus sp.	26.67	10.00	63.33	30.00	13.33	56.67	16.67	13.33	70.00	23.33	16.67	60.00
Trichoderma sp. + Streptomyces sp. + Bacillus sp.	13.33	6.67	80.00	16.67	6.67	76.66	10.00	6.67	83.33	16.67	10.00	73.33

* Control I = Uninfested soil. *** Control III = Soil infested with the 3 pathogens.

Effect of Seed Inoculation with the Antagonists; *Trichoderma* sp.; *Streptomyces* sp. , and *Bacillus* sp. isolates on the Growth of Survival Plants of Broad bean Giza 2 and Rebaya 40 Sown in Soil Infested with *F. solani*, *R. solani* and *Scl. rolfsii* :

The effect of seed inoculation with the potent antagonists, *Trichoderma* sp., *Streptomyces* sp., and *Bacillus* sp. isolates, on the growth of survival plants of two broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with *F. solani*, *R. solani*, and *Scl. rolfsii* or all of the three pathogens is recorded in Tables (5,6,7 and 8).

Data in Tables 5,6,7 and 8 lead to the following results:

– Inoculation of the seeds with the potent antagonists, *Trichoderma* sp., *Streptomyces* sp. and *Bacillus* sp., isolates; almost increased the length, fresh and dry weight of root and shoot systems of survival plants for both broad bean varieties sown in sterile and non-sterile soil infested with any or all of the pathogens as compared to their respective values of control.

– The inoculation of seeds with the three antagonists almost increased the plant growth than when any of the antagonists was inoculated solely.

– The results show that multiple antagonists are more effective than single antagonist in controlling the disease and affecting the growth of survival plants.

Table (5): Effect of seed inoculation with the potent antagonists, <u>Trichoderma</u> sp. , <u>Streptomyces</u> sp., and <u>Bacillus</u> sp. isolates on the growth of survival plants after 30 days from sowing broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with <u>F</u>. <u>solani</u>.

Treatment (Added antagonist against <u>F.solani</u>)	Broad bean variety	Sterilized soil							Non-sterilized soil						
		Root system			Shoot system			R/S *	Root system			Shoot system			R/S *
		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)	
Control I	Giza 2	14.10	2.58	1.03	38.50	4.35	1.35	0.76	10.30	2.58	1.13	33.40	4.60	1.61	0.70
	Rebaya 40	12.10	2.10	0.86	35.60	3.62	1.19	0.72	10.20	2.26	0.90	34.10	4.51	1.49	0.66
Control II (control I+ <u>F.solani</u>)	Giza 2	12.40	2.00	0.78	35.60	3.90	1.17	0.60	9.30	2.22	0.87	30.80	4.51	1.34	0.65
	Rebaya 40	9.40	1.79	0.70	32.20	3.45	1.04	0.57	8.20	2.20	0.78	31.60	4.51	1.30	0.60
<u>Trichoderma</u> sp.	Giza 2	11.60	2.38	0.94	31.20	3.92	1.28	0.73	9.40	2.32	0.93	30.90	4.50	1.36	0.68
	Rebaya 40	11.80	2.08	0.83	32.90	3.79	1.14	0.72	10.10	2.24	0.86	31.50	4.48	1.35	0.63
<u>Streptomyces</u> sp.	Giza 2	10.82	2.24	0.90	36.90	4.06	1.[illegible]2	0.74	10.20	2.31	0.94	31.50	4.50	1.38	0.68
	Rebaya 40	11.06	2.05	0.81	34.10	3.52	1.07	0.75	9.20	2.30	0.88	33.40	4.47	1.34	0.65
<u>Bacillus</u> sp.	Giza 2	14.30	2.40	0.96	34.40	4.20	1.26	0.76	9.70	2.25	0.90	31.60	4.48	1.3[illegible]	0.67
	Rebaya 40	14.70	1.97	0.79	36.00	3.81	1.20	0.66	10.20	2.16	0.86	32.60	4.44	1.32	0.65
<u>Trichoderma</u> sp. +<u>Streptomyces</u> sp.	Giza 2	12.78	2.25	0.96	34.40	4.27	1.29	0.75	10.80	2.64	1.06	33.50	4.56	1.17	0.72
+ <u>Bacillus</u> sp.	Rebaya 40	13.66	2.81	0.83	36.20	3.86	1.15	0.72	10.55	2.26	0.91	33.90	4.48	1.38	0.67

* R = Root dry weight   S = Shoot dry weight.

Table (6): Effect of seed inoculation with the potent antagonist, <u>Trichoderma</u> sp., <u>Streptomyces</u> sp. and <u>Bacillus</u> sp. isolates on the growth of survival plants after 30 days from sowing broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with <u>R. solani</u>.

Treatment (Added antagonist against R.solani)	Broad bean Variety	Sterilized soil							Unsterilized soil						
		Root system			Shoot system			R/S *	Root system			Shoot system			R/S *
		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)	
Control I	Giza 2	14.10	2.58	1.03	38.50	4.35	1.35	0.76	10.30	2.28	1.11	31.40	4.60	1.61	0.70
	Rebaya 40	12.10	2.10	0.86	35.80	3.62	1.19	0.72	10.20	2.26	0.90	34.10	4.51	1.19	0.66
Control II (control I + R.solani)	Giza 2	7.50	1.74	0.65	27.50	3.20	1.20	0.54	8.80	2.08	0.77	25.80	3.84	1.22	0.62
	Rebaya 40	6.50	1.53	0.62	24.70	2.53	1.14	0.54	8.70	2.11	0.72	25.80	3.84	1.25	0.60
Trichoderma sp.	Giza 2	6.50	2.13	0.85	27.70	3.35	1.24	0.69	9.60	2.22	0.88	29.60	4.18	1.27	0.68
	Rebaya 40	7.90	1.79	0.72	30.00	3.28	1.16	0.62	9.00	2.15	0.81	30.10	3.95	1.22	0.66
Streptomyces sp.	Giza 2	8.70	1.85	0.79	28.30	3.49	1.20	0.66	9.10	2.20	0.88	26.90	4.96	1.38	0.64
	Rebaya 40	7.80	1.71	0.68	27.80	2.96	1.18	0.58	9.20	2.18	0.87	29.40	4.02	1.25	0.65
Bacillus sp.	Giza 2	9.00	1.81	0.72	28.80	3.50	1.25	0.58	9.50	2.20	0.88	27.5	3.89	1.28	0.69
	Rebaya 40	10.20	1.72	0.69	33.40	3.24	1.17	0.59	8.20	2.16	0.86	29.30	3.83	1.23	0.70
Trichoderma sp. +Streptomyces sp. + Bacillus sp.	Giza 2	12.50	2.12	0.85	35.38	3.65	1.29	0.66	10.10	2.31	0.95	32.50	4.19	1.19	0.60
	Rebaya 40	10.90	1.83	0.75	31.68	3.48	1.22	0.63	9.80	2.24	0.89	30.80	4.26	1.32	0.67

* R = Root dry weight. S = Shoot dry weight.

Table (7): Effect of seed inoculation with the potent antagonists, <u>Trichoderma</u> sp., <u>Streptomyces</u> sp. and <u>Bacillus</u> sp. isolates on the growth of survival plants after 30 days from sowing, broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with <u>Scl. rolfsii</u> .

Treatment (Added antagonist against Scl.rolfsii)	Broad bean variety	Sterilized soil							Non-sterilized soil						
		Root system			Shoot system			R/S*	Root system			Shoot system			R/S*
		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)		Length (cm)	Fresh weight (gm)	Dry weight (gm)	Length (cm)	Fresh weight (gm)	Dry weight (gm)	
Control I	Giza 2	14.10	2.58	1.03	38.50	4.35	1.35	0.76	10.30	2.28	1.13	33.40	4.60	1.61	0.70
	Rebaya 40	12.10	2.10	0.86	38.50	3.62	1.19	0.72	10.20	2.26	0.90	34.10	4.51	1.49	0.66
Control II (control I + Scl.rolfsii)	Giza 2	8.40	1.80	0.72	28.10	3.60	1.24	0.58	8.50	1.98	0.80	26.80	4.35	1.35	0.59
	Rebaya 40	7.10	1.62	0.60	28.70	3.26	1.08	0.55	7.40	1.92	0.75	27.10	4.20	1.28	0.58
<u>Trichoderma</u> sp.	Giza 2	10.80	1.86	0.78	34.10	4.04	1.26	0.62	10.20	2.04	0.81	31.40	4.40	1.41	0.60
	Rebaya 40	8.50	1.69	0.68	33.40	3.52	1.08	0.62	8.50	2.02	0.81	32.50	4.36	1.32	0.61
<u>Streptomyces</u> sp.	Giza 2	11.30	2.39	0.94	35.20	3.84	1.31	0.72	10.10	2.16	0.89	31.30	4.51	1.45	0.61
	Rebaya 40	10.00	1.95	0.78	32.80	3.47	1.12	0.70	9.50	2.01	0.82	32.40	4.36	1.39	0.62
<u>Bacillus</u> sp.	Giza 2	12.50	2.47	0.97	36.30	4.15	1.32	0.74	9.90	2.11	0.93	31.80	4.49	1.44	0.65
	Rebaya 40	10.70	1.98	0.81	35.70	3.54	1.13	0.72	9.20	2.09	0.87	32.50	4.35	1.38	0.63
<u>Trichoderma</u> sp. +<u>Streptomyces</u> sp. + <u>Bacillus</u> sp.	Giza 2	13.10	1.47	0.99	37.70	4.28	1.34	0.74	10.20	2.26	0.98	33.20	4.52	1.49	0.66
	Rebaya 40	12.80	2.03	0.84	38.50	3.59	1.16	0.72	9.90	2.21	0.90	33.90	4.41	1.42	0.64

* R = Root dry weight. S = Shoot dry weight.

Table (8): Effect of seed inoculation with the potent antagonists, *Trichoderma* sp., *Streptomyces* sp. and *Bacillus* sp. isolates on the growth of survival plants after 30 days from sowing broad bean varieties Giza 2 and Rebaya 40 sown in sterilized and unsterilized soil infested with *F. solani* , *R. solani* and *Scl. rolfsii*.

Treatment	Broad bean variety	Sterilized soil							Non-sterilized soil						
		Root system			Shoot system			B/S[x]	Root system			Shoot system			R/S[x]
		Length (cm)	Fresh weight (gm)	Dry	Length (cm)	Fresh weight (gm)	Dry		Length (cm)	Fresh weight (gm)	Dry	Length (cm)	Fresh weight (gm)	Dry	
Control I	Giza 2	14.10	2.58	1.03	38.50	4.35	1.35	0.76	10.30	2.28	1.13	33.40	4.60	1.61	0.70
	Rebaya 40	12.10	2.10	0.86	35.80	3.62	1.19	0.72	10.20	2.26	0.90	34.10	4.51	1.49	0.66
Control III (control I + 3 pathogens)	Giza 2	6.80	1.30	0.52	25.20	3.32	0.99	0.53	7.20	1.74	0.71	26.20	3.72	1.19	0.59
	Rebaya 40	5.90	1.12	0.43	23.00	2.54	0.86	0.50	6.80	1.69	0.64	26.10	3.59	1.18	0.54
Trichoderma sp. +3 pathogens	Giza 2	9.20	1.87	0.77	25.80	3.63	1.18	0.65	9.70	2.01	0.81	30.40	3.94	1.21	0.67
	Rebaya 40	8.50	1.90	0.74	26.10	3.43	1.09	0.67	8.75	1.89	0.77	26.20	3.71	1.19	0.65
Streptomyces sp. +3 pathogens	Giza 2	11.80	2.05	0.63	32.30	3.93	1.23	0.67	10.10	2.11	0.83	31.30	4.03	1.24	0.67
	Rebaya 40	10.30	2.04	0.80	28.70	3.39	1.12	0.71	9.30	2.09	0.83	32.70	3.98	1.20	0.65
Bacillus sp. +3 pathogens	Giza 2	12.10	2.07	0.83	34.10	3.82	1.21	0.69	9.60	2.07	0.81	30.50	3.96	1.22	0.66
	Rebaya 40	11.20	1.67	0.70	31.60	3.06	1.05	0.71	8.90	2.03	0.80	31.30	3.89	1.18	0.68
3 Antagonisés +3 pathogens	Giza 2	13.20	2.26	0.90	36.60	3.58	1.07	0.71	10.20	2.15	0.89	32.10	4.33	1.39	0.68
	Rebaya 40	10.90	2.08	0.83	[illegible]	3.14	1.16	0.72	9.30	2.11	0.86	33.60	4.28	1.26	0.68

x R = Root dry weight S= Shoot dry weight.

Multiple antagonists intensify the antagonistic activity of each other because it may possess different antagonistic mechanisms against the pathogens. In addition, the use of multiple antagonists result in stable protection of plants against the pathogens. An antagonist may be sensitive to the effect of natural soil microflora, while the others are insensitive and achieve the control of the disease.

DISCUSSION

Pathogenisity:

The three investigated pathogens namely *F. solani* , *R. solani* and *Scl. rolfsii* proved to be pathogenic to both broad bean varieties. However, Rebaya 40 seemed to be more susceptible than Giza 2. Among investigated pathogens , *R. solani* was the most virulent. This result is in agreement with El-Arosi *et al*. (1970); El-Shanawani (1973) and Habib (1979).

Inoculation of the soil with the three pathogens (*F. solani* + *R. solani* + *Scl. roflsii*) gave higher pre- and post-emergence damping-off and lower survival plants percentages than when any of the pathogens was inoculated solely. This was found in both broad bean varieties , cultivated in sterile or non-sterile soil. It seems that the pathogens intensified the virulence of each other.

The investigated pathogens were more virulent in the sterilized soil than unsterilized soil. This is in agreement with many earlier investigators including Alexander (1961) and Fahim et al. (1967). Soil saprophytes compete with or antagonize the soil pathogens and slow their rate of spread.

Biological control of the pathogens by seed inoculation with the antagonists:

1- Seed inoculation with the antagonist Trichoderma sp.isolate:

Seed inoculation with the antagonist Trichoderma sp.isolate almost reduced the pre- and post-emergence damping-off and increased survival plants percentages, as compared to their respective control, for both broad bean varieties sown in sterilized or unsterilized soil infested with F. solani, R. solani, Scl. rolfsii or all of the three pathogens.

Trichoderma inoculation to Giza 2 seeds, sown in natural soil infested with the 3 pathogens (F.solani - R.solani + Scl.rolfsii), reduced the pre-emergence damping-off from 23.33% (control) to 20% and increased survival plants percentages from 56.67% (control) to 60%. In case of Rebaya 40, the pre-emergence damping-off decreased from 33.33% (control) to 30% and post-emergence from 23.33% (control) to 20% and survived plants increased from 43.33% (control) to 50%.

In sterile soil, the same trend was obtained, but the pathogens showed greater virulence in sterile than non-sterile soil; and the antagonist Trichoderma sp. isolate almost showed greater antagonism in the sterile soil than the non-sterile soil. This result is logic since soil microflora moderate the virulence of the pathogens and the antagonism of the antagonist.

Trichoderma sp. isolates, from soil, were found to antagonize soil-borne pathogens by many earlier investigators including Waksman (1952), Abdou et al. (1970) and Lang (1975). Recently, Trichoderma sp. isolates were applied successfully in the control of soil-borne diseases (Abd El-Moity and Shatla, 1978; Chet et al., 1978 and Henis et al., 1979).

2- Seed inoculation with the antagonist Streptomyces sp.isolate:

Seed inoculation with the antagonist Streptomyces sp. isolate decreased the pre- and post-emergence damping-off and increased survival plants percentages, as compared to their respective control, for both broad bean varieties sown in sterilized and unsterilized soil infested with F.solani, R.solani or Scl.rolfsii.

Seed inoculation of Giza 2 variety with the antagonist Streptomyces sp. then sowing in natural soil infested with the three pathogens (F.solani + R.solani + Scl.rolfsii) reduced the pre-emergence damping-off from 23.33% (control) to 20% and post-emergence damping-off from 20% (control) to 13.33% and increased survival plants from 56.67%(control) to 66.67%. In case of Rebaya 40, the pre-emergence damping-off decreased from 33.33% (control) to 26.67% and post-emergence damping-off from 23.33% (control) to 16.67% and increased survival plants percentages from 43.33% (control) to 56.66%.

In sterile soil, the antagonist showed the same trend as in non-sterile soil, but the effect of the antagonist was more obvious. In the absence of soil microflora, the antagonist was given a better chance for antagonizing the pathogens. Members of the genus Streptomyces are known as antibiotics producers (Waksman, 1952). Kurylowicz (1972) reported that more than 50% of the known antibiotics are produced by actinomycetes, and 98% of these are produced by members of the genus Streptomyces. Streptomyces sp. isolates were found to antagonize and control soil-borne pathogens by many earlier investigators including Stevenson (1956); Shklyar and Mansurova (1968) broadbent and Baker (1969) and Broadbent and Waterworth (1971).

Seed inoculation with the antagonist Bacillus sp. isolate:

The inoculation of the antagonist Bacillus sp. isolate to seeds of both broad bean varieties then sowing in sterilized and unsterilized soil infested with F.solani, R. solani or Scl.rolfsii; almost decreased the damping off and increased survival plants percentages.

Seed inoculation of Giza 2 variety with the antagonist *Bacillus* sp., then sowing in unsterilized (natural) soil infested with the three pathogens (*F.solani* + *R.solani* + *Scl. rolfsii*), decreased the pre-emergence damping-off from 23.33% (control) to 16.67% and post-emergence damping-off from 20% (control) to 13.33% and increased survival plants from 56.67% (control) to 70%. In case of Rebaya 40 the pre-emergence damping-off decreased from 33.33% (control) to 23.33% and post-emergence from 23.33% (control) to 16.67% and increased survival plants from 43.33% (control) to 60%.

Sterilized soil showed the same trend of the unsterilized soil. These results are in agreement with the results of Shklyar and Mansurova (1968), Broadbent and Baker (1969), Broadbent and Waterworth (1971) and Mahmoud *et al*. (1980) which indicate that *Bacillus* isolates from soil antagonized the soil-borne pathogens. Moreover, members of the genus *Bacillus* were reported as antibiotic producing organisms in soil (Olsen, 1965 and Roa and Roa, 1968).

Seed inoculation with the three antagonists, *Trichoderma* sp., *Streptomyces* sp. and *Bacillus* sp. isolates:

Seed inoculation with the three antagonists (*Trichoderma* sp. + *Streptomyces* sp. + *Bacillus* sp. isolates), then sowing in sterile and non-sterile soil infested with *F.solani*, *R.solani*, *Scl.rolfsii* or all of the three pathogens, almost decreased the pre-and post-emergence damping-off and increased survival plants percentages as compared to the respective values when any of the antagonists was inoculated solely.

Seed inoculation with multiple antagonists is better than appling single antagonist, because it contains various organisms which give different antagonistic secretions, each had its mode of action in antagonizing the pathogen.

<u>Effect of seed inoculation with the antagonists on the growth of survival plants in infested soil</u>:

The infestation of soil with the pathogens <u>F.solani</u> , <u>R.solani</u>, <u>Scl.rolfsii</u> or all the three pathogens almost reduced the length, fresh and dry weight of root and shoot system of survival plants for both broad bean varieties Giza 2 and Rebaya 40. This result is in agreement with those of earlier investigators including Ali (1967) and Sirry <u>et al</u>. (1970). Slight infections, not sufficient to cause the damping-off, decreased the growth of survival plants.

Seed inoculation with the antagonists decreased the deleterious effect of the pathogens <u>F.solani</u>, <u>R.solani</u> and <u>Scl.roflsii</u> on the growth of survival plants. However, seed inoculation with all of the three antagonists was the most efficient treatment, since it almost decreased the deleterious effect of the pathogens on the growth of survival plants than when any of the antagonists was inoculated alone.

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المقاومة الحيوية للذبول فى صنفى الفول جيزه ٢ ورباية ٤٠

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تم تلقيح البذرة لصنفى الفول جيزه ٢ ورباية ٤٠ بعزلات ميكروبية تنتمى لاجناس الباسيلس والاستربتوميسس والتريكودرما ، والتى أثبتت فى تجارب معملية سابقة كفاءتها العالية فى تضاد فطريات الفيوزاريوم سولانى والريزوكتونيا سولانى وسكليروشيم رولفزياى . وقد أدت معاملات تلقيح البذرة بأى من الميكروبات المضادة الى نقص النسبة المئوية للذبول وزيادة النسبة المئوية للنباتات المقاومة ونقص التأثير الضار للفطريات المرضية على نمو النباتات المقاومة وذلك بالمقارنة بالكنترول (البذور غير الملقحة) .

وأدى تلقيح البذرة بمخلوط العزلات الثلاثة ذات الكفاءة العالية فى التضاد الى أحسن النتائج فى الحد من الاصابة بالفطريات المرضية المذكورة . وقد أعطت هذه المعاملة أقل نسبة للذبول وأعلا نسبة للنباتات المقاومة وأقل تأثير ضار للفطريات المرضية على نمو النباتات المقاومة وذلك بالمقارنة بمعاملات تلقيح أى من هذه الميكروبات المضادة بمفرده .

وقد وجدت هذه النتائج فى كل من صنفى الفول جيزه ٢ ورباية ٤٠ المنزرعة فى تربة معقمة أو تربة عادية كلاهما معدى بالفطريات فيوزاريوم سولانى أو ريزوكتونيا سولانى أو سكليروشيم رولفزياى أو بالثلاثة فطريات مجتمعة .