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COMPARISON OF SOME BIOAGENTS AND APPLICATION TECHNIQUES FOR BIOCONTROL OF SOME SUGAR BEET DISEASES

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ABSTRACT

Two Tricoderma species (*Trichoderma harzianum* and *T. album*) as fungal biocontrol agent and four bacterial strains (*Pseudomonas cepacia, P. fluorescens, Bacillus subtilis* and *Azotobacter chroococcum*) were also tested for controlling some sugar beet diseases, such as Cercospora leaf spots and root-rot.

Different application techniques were used. Cell suspension of the bacterial biocontrol agent were tested as spraying on the foliar of sugar beet plants to control Cercospora leaf spots disease caused by *Cercospora beticola* in the greenhouse and field conditions. The fungal bioagents (*T. harzianum* and *T. album*) were also tested against root-rot disease. Sugar beet seeds was coated with the spores suspension of *Trichoderma spp.* by 24 hours before sowing. This technique was more effective than soil infestation with *Trichoderma spp.* Also, the bacterial bioagents were used as cell suspension with three concentrations (7×10^9, 7×10^8 and 7×10^7 cells/ml) against Cercospora leaf spots of sugar beet under field condition. While, the bacterial bioagents used as wheat-bran preparation in three treatments (100, 200 and 300 g/plot) under field conditions in two seasons (2000/2001 and 2001/2002) to control root-rot pathogens under greenhouse against root-rot and Cercospora leaf spot diseases. We used bioagent in three ratios 1:1, 2:1 and 1:2 (Bioagent : Pathogen). The control without bioagents

INTRODUCTION

Two strains of *Trichoderma spp* as fungal bioagents and four strains of bacterial bioagents (*Pseudomonas cepacia, P. fluorescens, Bacillus subtilis* and *Azotobacter chroococcum*) have been tested extensively as biocontrol agents of plant diseases. These strains have been particularly effective for controlling several soil borne pathogens (Kiewnick, 1998; Ventura, 1998 and Weslien, 1999).

Also, *Trichoderma spp.* were applied as bioagents against some soil borne pathogenic fungi (Lorito, 1998; Menendez, 1998 and Samasekhara, 1998). *Azotobacter spp.* especially *A. chroococcum* was used in controlling root and stalk rot diseases of maize (Al-Laithy, 1996).

Many researchers reported that the application of bacterial biocontrol agents (El-Sheshtawi and Dawood, 1988 and Ganonamanickam and Mew, 1992) as *Pseudomonas spp.* were very important to reduce the percentage of some sugar beet diseases (Mosa *et al.*, 1997). In this investigation, sugar beet plants, Oscar variety, sprayed with bacterial suspension (Leben, 1985 & 1995) to control Cerospora leaf spots disease caused by *Cercospora beticola* in the greenhouse and field conditions.

Cercospora leaf spots (CLS) has become more difficult to control because of the occurrence of fungicide resistance / tolerance in the pathogen population, and the potential loss of fungicides due to regulatory action of the environmental. The bacterial biocontrol agents can produce chitinase and gave direct antibiosis *in vitro* against *C. beticola* (Kiewnick, 1998).

MATERIALS AND METHODS

1. Source of *Pseudomonas spp.* isolates:

Sample of different soils were collected from different locations in Dakahlia governorate, Egypt. The soils sample were homogenized and serial dilution up to the concentration cell per ml (7×10^9) were prepared, in which 0.1 ml of each dilution was plated into 4 replicates on plate agar. This was made following the standard dilution plating technique. Different types of bacteria colonies were transferred to nutrient agar (NA) slant as pure culture for further studies.

2. Characterization and identification of *Pseudomonas* isolates under testing:

Gram strain reaction and cell morphology were observed on colony grown on nutrient agar slant for 24 hours at 28°C. The identification test was determined following the procedures given by Schaad (1980). Cells dimension were measured using the ocular micrometer attached to the eye piece of the microscope.

3. Isolation and identification of *Azotobacter chroococcum*:

It were made by subculturing a representative number of *Azotobacter* isolates differing in their cultural characteristics as for as possible, from the characteristic positive tubes. A loop of *Azotobacter* growth (pellicle) was transferred to N-free slant agar. The obtained pure *Azotobacter* isolates were characterized according to the methodology described by Sherman (1967) and Norris & Chapman (1967). Then identified on the basis of Buchanan and Gibbons (1974) keys.

4. Isolation of *Trichoderma spp.*:

T. harzianum and *T. album* were isolated from sugar beet rhizosphere by dilution plate technique on pepion-dextrose rose-benegal agar (Martin, 1950).

5. Isolation, purification and identification of (*Cercospora beticola* Sacc.):

The infested leaves of sugar beet cultivars, i.e. Pamela, Top, Pleno, Oscar and Gloria were carefully washed with tap water and kept between two filter papers for 24 hours. Small pieces of infected leaves showing lesions were cut and surface sterilized in sodium hypochlorite solution 5% for 2-3 minutes then washed twice with sterile distilled water, dried between two sterilized filter papers and then transferred into Petri-dishes (6 leaf spots /

Petri-dish) containing sugar beet leaves extract amended with containing 1.0 mg streptomycin / lire or potato dextrose agar (PDA). The Petri dishes were incubated at 23±1°C and fungal growth was daily observed. The fungal growth was examined microscopically and purified using the single spore and/or hyphal tip techniques. Colony characteristics, spore morphology were described and identified by the Department of Fungal Taxonomy, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

6. Isolation of the causal organisms of sugar beet root-rot:

Four virulent fungi typically isolated from rotted sugar beet roots, i.e. *Fusarium oxysporum, Rhizoctonia solani, Sclerotium rolfsii* and *Pythium altimum* were grown on BSP medium (Abada, 1980).

7. *In vitro* test to isolates of the bacterial bioagents:

The bacterial isolates obtained from different samples of soil rhizosphere of sugar beet varieties were screened for their antagonistic of the two diseases under study. This test was done in the laboratory using dual agar culture as mentioned by Thompson and Burns (1989). Isolates of bacteria were individually tested for their abilities to inhibit the mycelial growth of the pathogenic fungi at 28°C. Antagonism between mycelial growth of pathogenic fungi and each bacterial isolated was assessed after seven days of inoculation by measuring the mycelial growth diameter of the pathogen in comparison of the diameter of the fungus alone served as control (Tables 1 and 2).

Oscar variety of sugar beet was used and two strains of *Pseudomonas* species (*P. cepacia* and *P. fluorescencs*). One specie of *B. subitilis* and one specie of *Azotobacter spp.* (*A. chrococcum*) were used in this study. The four bacterial biocontrol agents are considered to be the most promising pathogens antagonist, which inhibited mycelial growth of some important soil borne pathogenic fungi, *Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum* and *Pythium altimum*. The cells of bacteria bioagents were harvested by centrifugation (20000 g 4°C, 10 minutes) and transferred to special liquid media.

The tested pathogens were maintained on potato dextrose agar (PDA) 7 days at 25°C (Thompson and Burns (1989). After 10 days at 25°C mycelial growth were broken up. Plates were incubated on laboratory bench at 25°C temperature. After two weeks, a 5 mm mycelial plug taken from an activly growing colony of the pathogen was placed apposite the bacterial colony at the outside edge of each section of the Petri-dish. Colony growth measured from the outside edge of the Petri-dish to the leading edge of the colony of the pathogen.

8. Greenhouse experiments:

A pot experiment was conducted at the greenhouse of corn and Sugar Crops Research Dept., Agric. Res. Cent., Giza during season 2001. Seeds Oscar variety were sowing in pots of 30 cm diameter filled with sandy loam soil (10 kg/pot) at a rate of 4 seeds / pot thinned to one seedling / pot after 30

days. The single plants were sprayed with the cell suspension of the bacterial bioagents with the concentration (7 x 10^9 cell/ml) every 15 days.

Inoculum of *T. harzianum* and *T. album* was prepared on sterilized wheat bran in 250 ml glass bottles, while inocula of the pathogenic fungi were prepared individually on barley grain. Fifteen-day-old cultures of the antagonist and of each pathogen were used for the infestation of sterilized soil 7 days before sowing. Inoculum of each pathogen was applied at the rate of 2% of soil weight. While, the inoculum ratio of the bioagent : pathogen were 1:1, 2:1, 1:2 (w/w). Soil uninfested with the biocontrol was used as control treatments. The root-rot incidence was recorded. Disease severity index (DSI) was based on the scale described by Grainger (1949) was used.

9. Field experiment:

The effect of *Trichoderma spp.* under test as wheat bran preparation on root-rot of sugar beet were studied under field conditions with mature plants in infested soil during two successive seasons (2000/2001 and 2001/2002) at the farm of Tag El-Ezz Research Station, Dakahlia governorate, Egypt.

The trial was laid out in a randomized block design with three replicates and a plot size of 1/400 feddan (10.5 m^2). Inoculum of *Trichoderma spp.* under study was prepared as described before. The inoculum of *Trichoderma spp.* was applied on the day of sowing Oscar seed variety were sown and covered with wet soil. Inoculum was 100, 200 and 300 g/plot. All normal cultural practices were followed. At harvest, (180 days after sowing) plants were uprooted and examined for disease symptoms. Percentage of root-rot incidence and disease severity were recorded.

10. Statistical analysis:

Data were statistically analyzed according to the methods described by Gomez and Gomez (1984) and Snedecor and Cochran(1988). Treatments mean were compared by using LSD test at 0.05 level of probability.

RESULTS

1. *In vitro* inhibition of the main pathogens by the bacterial biocontrol agents:

All species of *Psedomonas, B. subtilis* and *A. chrococcum* under study inhibited the growth of the causal organisms *in vitro* compared with control. *P. fluorescens* was effective for controlling the causal organisms of root-rot disease more than *P. cepacia* when the density of cell suspension was (7 x 10^9 cell/ml). *B. subtilis* gave the best effective compared with others bioagents and control (plats without bacteria) or untreated with bioagents (Tables, 1 and 2). *A. chrococcum* was the latest in inhibition of all pathogenic growth.

Trichoderma spp. used as a fungal biocontrol agents had been grown faster than each of all pathogenic fungi. *T. harzianum* especially gave the highest effect against *F. oxysporum*, while the lowest effect was against *P. altimum* (Table 1 and 1a).

Table 1. Antagonistic effect between biocontrol agents and sugar beet root-rot pathogenic fungi under laboratory conditions.

Biocontrol agents / Pathogenic fungi	*T. harzianum*		*T. album*		*P. fluorescencs*		*P. cepacia*		*B subtilis*		*A. chroococcum*		Control***	
	Pathogen radial growth in cm and inhibition %												CRG****	Inh.** %
	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %		
P. altimum	5.0	66.66	5.7	62.00	7.6	49.33	7.0	53.33	6.0	60.00	10.3	31.33	15.0	0.0
F. oxysporum	7.6	49.33	8.0	46.66	8.3	44,66	7.5	50.00	7.0	53.33	11.2	32.00	15.0	0.0
R. solani	9.9	34.00	8.3	44.66	8.7	42.00	9.0	40.00	7.3	58.00	10.6	29.33	15.0	0.0
S. rolfii	10.3	31.33	9.6	36.00	9.2	38.66	9.5	30.00	9.7	35.33	11.4	24.00	15.0	0.0
LSD at 0.05	**7.3**	**30.6**	**6.0**	**32.40**	**7.8**	**35.60**	**6.7**	**7.80**	**7.6**	**33.70**	**6.6**	**32.3**	**0.0**	**0.0**

* PRG = Pathogen radial growth.

** Inh. = Inhibition in pathogen growth caused by the antagonist compared with control %.

*** Control = Treatment without bioagent.

**** CRG = Control radial growth.

2. Greenhouse experiments:

In general, the fungal and bacterial biocontrol agents reduce the incidence of root-rot and cercospora leaf spots diseases caused by the pathogens tested under study. Data of these diseases and disease severity were recorded in Table (3 and 4).

Table(2): Antagonistic effect between biocontrol agents and cercospora leaf spots of sugar beet plants under laboratory conditions.

Biocontrol agents	Biocontrol agents / pathogen ratio	Radial growth in Petri dish (cm)**	Inhibition (%)
T. harzianum	1 : 0	0.00	100.0
	1 : 1	5.00	66.66
	2 : 1	3.00	80.00
Control*	0 : 1	12.00	20.00
LSD at 0.05		4.60	50.60
T. album	1 : 0	0.00	100.0
	1 : 1	5.00	60.00
	2 : 1	3.50	76.66
Control	0 : 1	14.00	6.66
LSD at 0.05		5.3	40.60
P. fluorescens	1 : 0	0.00	100
	1 : 1	5.00	66.66
	2 : 1	2.90	80.00
Control	0 : 1	13.30	11.33
LSD at 0.05		5.70	50.00
P. cepacia	1 : 0	0.00	100.0
	1 : 1	6.70	49.33
	2 : 1	2.80	81.33
Control	0 : 1	14.20	5.33
LSD at 0.05		4.80	60.70
B. subtilis	1 : 0	0.00	100.0
	1 : 1	7.00	53.33
	2 : 1	3.20	78.66
Control	0 : 1	13.60	9.33
LSD at 0.05		5.30	60.30
A. chroococum	1 : 0	0.00	100.0
	1 : 1	6.70	55.33
	2 : 1	3.30	78.00
Control	0 : 1	12.80	14.66
LSD at 0.05		3.80	60.90

* Control = Treatments without biocontrol agents.

** Radial growth of the causal organism (*Cercospora beticola*).

Table 3. Effect of biocontrol agents on sugar beet root-rot pathogens under greenhouse conditions.

Biocontrol agents	Sugar beet root-rot pathogen fungi								
	Bio.* Path.	*S. rolfsii*		R. solani		*F. oxysporum*		*P. altimum*	
	ratio	(%)	DSI	(%)	DSI	(%)	DSI	(%)	DSI
T. harzianum									
	1 : 1	45.0	3.1	38.66	2.7	35.2	2.8	40.6	2.8
	2 : 1	17.0	0.5	15.90	0.4	16.3	0.4	14.8	1.2
	1 : 2	60.0	4.7	55.17	3.3	50.0	3.8	57.9	4.4
Control*		88.7	7.2	92.0	8.0	90.0	7.3	94.0	8.1
LSD at 0.05		5.6	0.7	6.20	0.6	3.4	0.7	3.4	0.4
T. album									
	1 : 1	48.6	3.3	36.50	2.3	50.7	3.1	46.0	3.0
	2 : 1	19.0	0.7	17.33	4.9	18.8	0.7	18.0	0.6
	1 : 2	67.0	5.0	60.12	3.6	70.0	6.0	73.0	5.0
Control		90.5	7.5	93.0	7.4	89.0	7.3	90.0	7.5
LSD at 0.05		5.1	0.8	6.30	0.5	3.4	0.7	3.9	0.3
P. fluorescens									
	1 : 1	59.0	3.9	40.0	2.6	36.7	2.4	40.6	2.4
	2 : 1	18.0	0.7	12.0	1.4	17.8	0.6	20.3	0.8
	1 : 2	65.0	5.2	70.0	6.0	72.7	7.0	70.6	6.1
Control		97.0	8.3	90.0	7.0	95.0	7.6	90.0	7.0
LSD at 0.05		4.9	0.8	7.0	0.7	3.2	0.7	4.2	0.3
P. cepacia									
	1 : 1	55.0	3.2	60.0	4.0	40.8	2.3	48.0	3.2
	2 : 1	17.8	0.6	20.6	0.9	14.0	0.3	30.3	1.9
	1 : 2	68.0	5.0	70.3	6.1	75.6	7.0	77.9	5.4
Control		96.0	8.1	92.3	7.3	90.0	8.0	90.7	7.6
LSD at 0.05		5.9	0.8	7.1	0.6	4.0	6.7	4.2	0.4
B. subtilis									
	1 : 1	57.0	4.4	50.0	3.0	45.7	3.1	47.0	3.2
	2 : 1	17.0	0.6	14.0	0.3	15.3	0.3	18.0	0.6
	1 : 2	70.0	5.5	72.0	6.8	75.0	6.8	77.0	5.7
Control		95.0	82.0	90.7	7.3	95.0	8.3	90.0	7.3
LSD at 0.05		5.7	0.8	5.6	0.7	3.8	6.3	5.3	0.4
Azotobacter chroococum									
	1 : 1	76.0	5.2	70.6	6.1	60.0	3.0	70.3	6.1
	2 : 1	44.0	3.0	50.4	3.1	55.4	4.0	50.3	3.1
	1 : 2	75.0	0.2	60.3	3.6	70.0	5.4	60.6	3.7
Control		98.0	8.6	93.0	7.4	90.3	7.1	95.0	8.3
LSD at 0.05		6.2	0.8	4.7	0.7	4.6	0.6	3.3	0.4

*** Bioagant pathogen ratio.**

Field experiment:

Data in Table (5 and 6) indicate that all inoculum levels of fungal or bacterial biocontrol agents tested gave significantly reduction to root-rot and cercospora leaf spot diseases. Disease incidence and severity were increased by increasing the inoculum dose. *Trichoderma spp.* were more effective in controlling root-rot when field soil was infested with wheat bran preparation (300g/plot), but the 200g/plot was the middle and 100g/plot was the latest (Tables 5, 6 and 7) in the two seasons.

Pseudomonas spp., B. subtilis and *A. chrococcum* were different in the effect according to concentration of cell suspension (Table 4) and total count of the cells per cm^3 or ml. The inoculum (7×10^9) cells per ml was the best and more effective in reduce the diseases or controlling diseases under study comparing with control.

Table 4. Effect biocontrol agents on (Cercospora leaf spots) caused by *C. beticola* of sugar beet plants under greenhouse conditions..

Biocontrol agents	Biocontrol agents / pathogen ratio	Disease incidence (%)**	Disease severity Index***
T. harzianum	1 : 0	00.00	0.0
	1 : 1	50.10	3.1
	2 : 1	11.60	1.4
Control*	0 : 1	75.90	5.6
LSD at 0.05		4.70	0.7
T. album	1 : 0	00.00	0.0
	1 : 1	53.70	3.6
	2 : 1	12.80	1.5
Control	0 : 1	80.44	5.6
LSD at 0.05		4.20	0.6
P. fluorescens	1 : 0	00.00	0.0
	1 : 1	55.40	3.4
	2 : 1	13.90	1.6
Control	0 : 1	95.00	8.4
LSD at 0.05		4.11	0.6
P. cepacia	1 : 0	00.00	0.0
	1 : 1	49.00	3.0
	2 : 1	13.00	1.6
Control	0 : 1	93.40	7.4
LSD at 0.05		3.80	0.7
B. subtilis	1 : 0	00.00	0.0
	1 : 1	57.12	3.6
	2 : 1	12.27	1.5
Control	0 : 1	92.10	7.0
LSD at 0.05		3.14	0.7
A. chroococum	1 : 0	00.00	0.0
	1 : 1	60.16	3.7
	2 : 1	6.70	0.1
Control	0 : 1	95.10	8.4
LSD at 0.05		4.50	0.6

* Control = Treatments without biocontrol agents.
** Disease incidence % of the causal organism (*Cercospora beticola*).
*** Disease severity of the causal organism (C.b.)

Table 5. Effect of biocontrol agents on sugar beet root-rot pathogens under field conditions during 2000/2001 season.

Bioagents	Wheat bran (g/plot)*	Sugar beet root-rot pathogen fungi							
		S. rolfsii		*R. solani*		*F. oxysporum*		*P. altimum*	
		Perc. (%)	DSI**	Perc. (%)	DSI	Perc. (%)	DSI	Perc. (%)	DSI
T. harzianum	100	7.30	1.70	7.50	1.30	5.70	0.98	4.00	1.25
	200	3.30	0.85	3.90	0.80	1.77	0.76	1.39	0.27
	300	1.20	0.14	1.60	0.40	0.20	0.30	0.80	0.16
Control*		13.60	3.70	11.00	3.40	8.72	1.80	8.16	2.40
LSD at 0.05		4.30	0.70	5.30	0.60	5.30	0.50	6.00	0.30
T. album	100	8.60	1.90	8.60	1.70	6.60	1.33	5.60	0.97
	200	4.00	0.95	3.41	0.80	2.75	0.67	2.50	0.66
	300	1.80	0.21	1.70	0.70	1.90	1.69	1.77	0.76
Control		13.0	3.71	9.30	1.66	13.40	3.72	13.10	3.70
LSD at 0.05		3.80	0.60	5.20	0.50	6.10	0.60	5.00	0.30
P. fluorescens	100	8.40	1.75	7.11	1.20	6.70	1.60	5.24	0.95
	200	3.60	0.92	3.00	0.81	2.91	0.74	2.30	0.62
Control	300	2.20	0.75	2.11	0.20	2.30	0.76	2.00	0.21
LSD at 0.05		13.00	3.20	13.60	3.22	12.60	2.91	13.90	3.79
		3.90	0.60	5.30	0.40	6.10	0.70	6.00	0.40
P. cepacia	100	9.33	2.00	8.33	1.72	7.30	1.26	6.12	1.22
	200	4.00	0.96	4.60	0.95	4.75	0.90	4.00	0.60
	300	3.00	0.72	3.60	0.77	3.00	0.70	3.70	0.81
Control		13.60	3.78	12.00	3.30	12.94	3.00	12.00	2.93
LSD at 0.05		3.00	0.70	6.00	0.40	6.00	0.80	7.30	0.40
B. subtilis									
	100	9.72	1.77	9.33	2.10	8.30	1.75	7.60	1.30
	200	4.44	0.91	4.33	0.89	4.11	0.65	4.00	0.60
	300	3.11	0.85	2.99	0.73	2.80	0.79	2.60	0.76
Control		12.16	2.98	13.14	3.70	14.65	3.90	13.22	3.72
LSD at 0.05		4.80	0.80	7.00	0.70	7.00	0.70	8.10	0.30
A. chroococum									
	100	10.00	1.90	9.72	2.30	8.00	1.60	7.60	1.32
	200	6.00	0.95	4.16	0.99	3.92	0.99	3.16	0.80
Control	300	5.66	0.92	2.00	0.73	2.11	0.20	2.00	0.21
LSD at 0.05		12.60	2.95	13.00	3.20	13.70	12.96	12.96	2.95
		5.00	0.30	6.30	0.60	7.10	7.30	7.30	0.40

* Wheat bran Preparation of bioagents (g/plot), Plot = 1/400 feddan = 10.5 m^2

** DSI = Disease severity index according Grainger (1949).

Table 6. Effect of biocontrol agents on sugar beet root-rot pathogens under field conditions during 2001/2002 season.

Bioagents	Wheat bran (g/plot)*	Sugar beet root-rot pathogen fungi							
		S. rolfsii		*R. solani*		*F. oxysporum*		*P. altimum*	
		Perc. (%)	DSI**	Perc. (%)	DSI	Perc. (%)	DSI	Perc. (%)	DSI
T. harzianum									
	100	8.33	1.80	9.60	2.40	9.80	1.78	6.80	1.33
	200	4.60	0.93	4.20	0.91	3.90	0.80	3.00	0.72
	300	1.80	0.15	2.60	0.78	2.30	0.75	1.98	0.16
Control*		14.6	3.80	13.8	3.78	13.0	3.20	14.6	3.78
LSD at 0.05		5.20	0.60	7.00	0.50	5.00	0.60	6.00	0.40
T. album	100	8.77	1.70	9.80	1.78	7.32	1.26	7.35	1.27
	200	4.10	0.96	3.90	0.80	3.16	0.84	3.16	0.84
	300	2.30	0.75	2.60	0.76	2.00	0.20	2.15	0.74
Control		14.5	3.77	13.4	3.77	14.0	3.72	14.6	3.78
LSD at 0.05		4.80	0.60	6.30	0.50	4.80	0.50	5.00	0.30
P. fluorescens									
	100	8.00	1.60	7.50	1.30	6.00	1.30	6.30	1.20
	200	2.95	0.93	2.00	0.20	3.22	0.86	3.12	0.83
	300	2.50	0.77	3.00	0.72	1.99	0.16	1.86	0.15
Control		14.6	3.75	14.0	3.72	13.8	3.78	14.6	3.75
LSD at 0.05		4.00	0.50	4.60	0.40	4.60	0.50	6.30	0.30
P. cepacia	100	9.30	1.77	8.16	1.78	7.30	1.48	7.10	1.20
	200	4.00	0.96	4.22	0.91	3.60	0.90	3.00	0.72
	300	3.60	0.92	3.55	0.91	3.40	0.90	2.00	0.20
Control		14.10	3.73	13.72	3.77	13.5	3.76	14.5	3.77
LSD at 0.05		5.00	0.50	5.90	0.50	4.30	0.60	6.00	0.40
B. subtilis									
	100	9.20	1.76	9.00	1.99	8.77	1.70	7.60	1.30
	200	4.10	0.96	4.10	0.96	5.60	0.91	4.60	0.92
	300	3.62	0.92	2.77	0.79	3.75	0.92	2.16	0.73
Control		13.6	3.78	13.33	3.75	13.22	3.71	13.0	3.20
LSD at 0.05		6.00	0.50	6.00	0.60	4.00	0.60	5.80	0.40
A. chroococum									
	100	10.20	1.93	9.00	1.75	10.66	1.95	9.30	1.77
	200	7.00	1.18	4.00	0.95	3.16	0.73	3.09	0.73
Control	300	5.00	0.90	2.60	0.76	2.27	0.74	2.10	0.74
LSD at 0.05		14.2	3.74	13.7	3.79	12.87	2.96	13.17	3.21
			0.40	6.00	0.50	4.60	0.60	5.80	0.50

* Wheat bran Preparation of bioagents (g/plot), Plot = 1/400 feddan = 10.5 m^2

** DSI = Disease severity index according Grainger (1949).

Table 7. Effect of biocontrol agents on sugar beet cercosporal spots caused by *C. baticola* of sugar beet (*Beta vulgaris*, L.) under field conditions during two seasons.

Biocontrol agent	Conc. of Spraying suspension	Cercospora leaf spots caused by *C. beticola*			
		1st season (2000/2001)		2nd season (2001/2002)	
		Perc. %	DSI	Perc. %	DSI
T. harzianum					
	1	25.00	2.70	23.30	2.10
	2	20.00	2.00	19.60	1.60
Control*	3	5.00	1.00	4.30	1.00
LSD at 0.05		38.00	3.00	36.00	3.00
		15.0	0.90	15.60	0.40
T. album	1	22.00	2.00	26.00	2.30
	2	18.00	1.70	17.60	1.60
	3	4.00	1.00	3.80	1.00
Control		35.00	3.00	38.00	3.00
LSD at 0.05		14.00	0.80	16.22	0.60
P. fluorescens					
	1	27.00	2.40	25.30	2.80
	2	19.60	1.30	18.00	1.70
Control	3	4.70	1.10	4.20	1.00
LSD at 0.05		33.00	3.00	4.20	3.00
		13.20	0.90	15.33	0.60
P. cepacia	1	23.00	2.00	26.10	2.60
	2	17.60	1.30	16.70	1.20
	3	3.30	1.00	4.20	1.00
Control		34.00	3.20	44.00	4.00
LSD at 0.05		11.20	0.90	16.10	0.50
B. subtilis	1	27.00	2.70	28.60	2.50
	2	20.00	2.00	14.70	1.30
	3	3.90	1.00	5.30	1.00
Control		35.33	3.10	32.30	3.00
LSD at 0.05		13.60	0.90	16.00	0.50
A. chroococum	1	32.00	3.10	33.16	3.10
	2	17.00	1.20	16.20	1.50
	3	3.60	1.00	3.90	1.00
Control		40.10	4.00	44.20	4.20
LSD at 0.05					

1 = 1 (7×10^7 cells/ml).
2 = 1 (7×10^6 cells/ml).
3 = 1 (7×10^5 cells/ml).
Control* = Spraying with water.

DISCUSSION

Application of biocontrol agents in controlling plant pathogens gave the most effective and safety, because the application of fungicides to control pathogenic fungi is expensive and causes environmental pollution. Therefore, effective and safe methods have been considered in the last few years (Mathur and Saebhoy, 1978; Upadhyay and Mukhopadhyay, 1986 and Grandona *et al.*, 1993) against soil borne pathogenic fungi especially *S. relfsii*. *Trichoderma spp.* use also against *R. solani* (Wu, 1980). Culture filtrate of *Trichoderma spp.* suppressed the mycelial growth of *F. solani* and *F. oxysporum* (Khalifa, 1993). *Trichoderma spp.* were able to parasite many soil-borne pathogens (Upadhyay and Mukhopadhyay, 1986), and able to produce inhibitory substances retarding the growth of pathogenic fungi (Dennis *et al.*, 1971 a and b, and Wu, 1980).

The effect of the bacterial biocontrol agents in protection of crop plants may involve antagonism as result of this production of secondary metalsolites or extra cellular lytic enzymes (Cook, 1963). Also, Kiewnick *et al.* (1998) reported that the ability of controlling Cercospora leaf spots caused by *Cercospora beticola* by *Pseudomonas spp.* and other bacteria may be due to production chitinase and 1, 3 glucanase and direct antibiosis against *Cercospora beticola*, which attack the foliar of sugar beet plant.

Azotobacter chrococcum was used as biocontrol agent (Al-Laithy, 1996) to control root and stalk rot disease of maize.

Bacillus subtilis was used against potato diseases (Schmiedeknecht, 1998) reported that *Rhizoctonia solani* was controlled by different strains of *Bacillus subtilis* in greenhouse and field conditions.

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مقارنة بعض المقاومات الحيوية وطرق التطبيق على مقاومة بعض أمراض بنجر السكر

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تم مقارنة ست كائنات حية دقيقة فى القدرة على مقاومة المسببات المرضية لمرض تعفن الجذور وكذلك تبقع الأوراق السركوسبورى على نبات بنجر السكر وهذه الكائنات إثنان منها وهى (ترايكودرما هارزيانم وترايكيودرما ألبم) تمثلان المقاومة الحيوية الفطرية وأربعة أخرى وهى (زيدوموناس فلوريسنسز وزيدوموناس سيباسيا وباسيلاس ساتلاس وأزوتوباكتر كروكوكم) تمثل المقاومة الحيوية البكتيرية.

وقد تم عمل اللقاحات والتركيزات وإستخدمت هذه الطرق كما ذكر فى الجداول المختلفة حيث أظهرت النتائج أن الفطر ترايكودرما هارزيانم وهو مقاوم حيوى فطرى كان أقوى هذه الكائنات المقاومة بالنسبة لمرض عفن الجذور. وأثر فى قدرته على المقاومة كمية اللقاح المستخدم. أما بخصوص المقاومات البكتيرية فقد أوضحت النتائج أن البكتريا زيدوموناس فلوريسنسز أقوى المقاومات الأربعة وكان أقلها تأثيرا بكتريا الأزوتوباكتر كروكوكم وظهر ذلك فى تجارب المعمل والصوبة والحقل وكان لتركيز اللقاح دور كبير فى المقاومة.

ويمكن الإستفادة من هذا البحث فى الجانب التطبيقى لمقاومةأمراض هامة تصيب بنجر السكر مثل عفن الجذور وموت البادرات والتبقع السركوسبورى للأوراق . كما أوضح البحث فإن إستخدام هذه الكائنات فى المعمل والصوبة والحقل فى مقاومة فطريات التربة الممرضة وكذلك فطريات تصيب المجموع الخضرى مما يعود فى النهاية على المحصول من حيث لكم والجودة ولا يؤثر على البيئة ولا يضر بالإنسان أو الحيوان أو الطيور والأسماك.