

UTILIZATION OF CERTAIN BIOCONTROL AGENTS AGAINST SUGAR BEET DAMPING-OFF AND ROOT ROT DISEASES

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ABSTRACT

Some fungal and bacterial bioagents as well as an actinomycete isolate were screened for their antagonistic effects against *Sclerotium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Fusarium solani* *in vitro*. *Trichoderma hamatum*, *T. harzianum*, and *T. pseudokoningii*, as well as certain isolates of *Bacillus subtilis* and one isolate of *Pseudomonas fluorescens* were the most effective bioagents in suppressing the radial growth of the four pathogens. Yet, they were less effective in retarding growth of *Fusarium* spp. as compared with the other pathogens under study.

T. hamatum, *T. harzianum*, *P. fluorescens* and *B. subtilis* under greenhouse (*S. rolfsii*-infested soil) and field (natural infection) conditions may be utilized to biological control of sugar beet damping-off and root rot diseases.

Three different formulae, suspension, powder, and granules were prepared from *T. hamatum*, *B. subtilis* and *Actinomyces* isolate, to be utilized in field application. The most effective bioagent for controlling the seedling blight was Rhizo-N followed by the suspension of *B. subtilis* which exhibited the least percentage of seedling blight. While the least effective one was Plantguard the granules of *Actinomyces*, and *B. subtilis*. For controlling the root-rot diseases, the most effective bioagent was *T. hamatum* (powder), while *B. subtilis* (suspension or granules) and *Actinomyces* (granules) were the least effective bioagents in this respect. These treatments also caused an increase in root yield per plot.

Keywords: Biocontrol agents, damping-off, sugar beet, and root rot diseases.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is the second important sugar crop after sugar cane in terms of acreage, total production and cash value in the Arab Republic of Egypt. It is cultivated mainly for sugar extraction in approximated 135,000 feddans mainly in the Northern Delta of the river Nile in the A. R. of Egypt with a yield of over 21 tons/feddan (El-Kholi, 2000).

This crop is subjected to attacked by many soil-borne pathogens at all growth stages causing pre- and post-emergence damping-off, as well as various degrees of root-rots. *Sclerotium rolfsii* and *Rhizoctonia solani* were reported to cause serious root-rot diseases affecting yield crop (Awad, 1995 and El-Kazzaz *et al.*, 1987 and 1999).

The objective of the present work was to study the effect of some indigenous rhizosphere-biocontrol agents (from healthy sugar beet plants) against seedling damping-off and root rot diseases of sugar beet plants.

areas of the A. R. of Egypt. Ten grams of soil were added to 90 ml sterilized distilled water in conical flask (250 ml) and thoroughly shaken for 10 min. Dilution series up to (10^{-6} CFU/ml) was prepared. Portions of 0.1 ml from serial dilutions of the obtained suspensions were spread on the surface of Petri dishes containing media. Fungal antagonists were isolated on peptone dextrose agar plus rosebengal and streptomycin (Martin, 1950). The antagonistic bacteria were isolated on soil extract agar (Johanson *et al.*, 1960), King's medium B agar, for *P. fluorescens* (King *et al.*, 1959) and actinomycete isolates were isolated on Jensen's agar medium (Jensen 1930). Plates were incubated at 30°C for sufficient period and examined daily for the fungal bacterial and actinomycetal growth.

Identification of the bioagent isolates:

The selected isolated microorganisms were identified according to their cultural morphological and physiological characters (Waksman and Henrici, 1943) and key developed by Rifai (1969) and Bergey's Manual of Determinative Bacteriology (1984). Identification was confirmed through both the Department of Mycology and Plant Diseases Survey, and the Department of Bacterial diseases, Plant Pathology Research Institute, ARC, Giza. The total number of the isolated microorganism (fungi, bacteria and actinomycetes) from soil were divided each into groups or types according to shape, rate of growth ... etc. One isolate from each type was chosen for studying its antagonistic effect. Accordingly, 15 fungal isolates, 8 bacterial isolates and one actinomycetal isolate were selected for further study.

Screening for antagonism and biological control:

***In vitro* experiment:**

The selected isolated microorganisms were subjected to the test under laboratory conditions to evaluate their antagonistic effect against the root-infecting fungi. Petri-dishes (9.0 cm in diameter) containing 15 ml of gliotoxin fermentation medium (GFM) developed by Brain and Hemming (1945) were used to study antagonism between the isolated fungi and the pathogenic fungi. To study the effect of either bacterial or actinomycetal isolate on the pathogenic fungi, nutrient glucose agar was used as recommended by Dowson (1957).

Plates were inoculated with 3-7 days old culture discs (6 mm in diameter) of the phytopathogenic isolates at the peripheral of the plate surface. The antagonistic organism was inoculated (6 mm disc) at the opposite side of the pathogenic fungus and plates were incubated at 27°C and periodically examined at 24 h. intervals. Three replicates were used. After complete growth of check plates, percentage of reduction in the mycelial growth was calculated according to the following formula adopted by Ferreira *et al.*, 1991 as follows: $R = (A-B)/A \times 100$.

Where:

R = percentage of growth reduction, A = The distance of mycelial growth of the pathogenic fungus and B = The distance of mycelial growth of the pathogenic fungus towards the antagonistic fungus.

The relative power of antibiosis (RPA) of each isolate was estimated through the ratio as described by Ibrahim *et al.*, (1987) as follows:

R.P.A. = Z/C.

Where: Z = diameter of inhibition zone and C = diameter of spotted antagonistic isolate.

Pot experiments:

For soil infestation, 500 ml glass bottles containing 190 gm. clean moistened sand and 10 gm corn meal were autoclaved for 30 minutes at 1.5 atm., then inoculated with the tested fungus and incubated at 28-30 °C for 15 days. Sterilized-35cm diameter pots were used in this experiment. Pot soil was infested with the fungal inoculum at the rate of 2% of the soil weight. Infested soil was mixed thoroughly and moistened with water every other day for one week before planting to ensure the distribution and uniformity of the pathogen. Infested potted-soil prepared was used.

Diluted suspension (10^6 , 10^8 , 10^7 /ml from culture broth of fungi, bacteria, and actinomycete, respectively) were added to soil at the rate of 30 & 20 ml/kg soil for *Trichoderma* & *Gleocladium* respectively. Whereas, bacteria and actinomycete were added at the rate of 10 ml/kg soil. Fifteen seeds were planted in each pot in three replicates. Pre- and post-emergence damping-off was recorded after 15 & 45 days of planting, respectively. Plants thereafter, were thinned to 2 plants per pot and root rot was estimated and recorded as infection percent. Disease severity was estimated, 150 days post sowing, according to the 1-10 grades of Grainger scale (Grainger, 1949).

The fungicide, Rhizolex T50 was used as a reference for these treatments at the recommended dose (3gm /kg seeds). Moreover, the commercial bioagents, Plantguard (*T. harzianum* 0.4% dilution) and Rhizo-N (*B. subtilis*) were used as seed dressing at the recommended dose (4 ml/L. and 4 gm/L., respectively). These are products of El-Naser co., for Fertilizers & Biological treatments.

Formulae of the bio-control agents used in the field experiment:

An aqueous suspension at the concentrations of 10^6 , 10^8 & 10^7 / ml were prepared from *T. hamatum*, *B. subtilis* and an actinomycete isolate, respectively. The three antagonists were used in a field trial each in three different formulae, suspension, powder or granules.

The aqueous suspensions were seed dressed at the rate of 20 ml/kg. Powder form was prepared by mixing the suspension of each bioagent with talc powder (1:1 v/w) and left to dry. It was applied to soil at the rate of 150 kg/feddan before sowing. The granules form was prepared by thoroughly mixing 1 L of the aqueous suspension with 0.5 kg wheat bran and 7gm sodium alginate. Calcium chloride (3%) was added drop by drop to the mixture until granule formation and left to dry. It was applied to soil at the rate of 150kg/feddan before sowing.

RESULTS AND DISCUSSION

Biological control of sugar beet root pathogens:

In vitro experiments:

Experiments were conducted to study the effect of certain fungal, bacterial and actinomycetal antagonists isolated from rhizosphere of healthy sugar beet plants against some serious isolated pathogens of sugar beet. A

number of 15 fungal isolates belonging to *Trichoderma hamatum*, *T. harzianum*, *T. viride*, *T. pseudokoningii*, & *Gliocladium virens* were screened in this respect. In addition, 8 bacterial isolates identified as *Bacillus subtilis*, *Pseudomonas fluorescense* and one *Actinomyces sp.* were also used in this evaluation.

Table 1: Effect of the antagonistic fungal isolates on the radial growth (cm) on some phytopathogenic fungi of sugar beet.

Antagonists	Phytopathogenic fungi							
	<i>Sclerotium rolfisii</i>		<i>Rhizoctonia solani</i>		<i>Macrophomina Phaseolina</i>		<i>Fusarium oxysporum</i>	
	L.G.	R.	L.G.	R.	L.G.	R.	L.G.	R.
<i>Trichoderma harzianum</i>	1.47 b	80.03 ab	1.73 a	78.43 a	1.77 a	78.88 a	2.83 b	48.37 d
<i>Trichoderma hamatum</i>	1.17 a	82.59 a	1.83 a	75.57 b	1.97 b	75.87 b	2.50 a	65.30 a
<i>Trichoderma viride</i>	2.67 d	59.88 cd	3.40 b	53.24 c	3.57 d	51.73 d	3.60 b	48.43 c
<i>Trichoderma Pseudokoningii</i>	1.93 c	70.89 d	3.93 bc	45.25 d	2.27 c	71.34 c	3.82 c	51.37 b
<i>Gliocladium virens</i>	4.43 e	33.18 e	4.77 c	40.72 e	4.00 e	45.25 e	4.30 e	24.45 e
Control	6.63 f	0.00	6.93 d	0.00	7.00 f	0.00	5.03 d	0.00

R. = % reduction in colony diameter. & L.G. = Fungal liner growth (cm).

Means followed by the same letter (in the same column) are not significantly different at 5% level by DMRT.

Table 2: Relative power of antibiosis (R.P.A.) by bacterial and actinomycetal antagonistes against the tested phytopathogenic fungi.

Antagonists	Relative power antibiosis (R.P.A.) against			
	<i>Sclerotium rolfisii</i>	<i>Rhizoctonia Solani</i>	<i>Macrophomina Phaseolina</i>	<i>Fusarium oxysporum</i>
<i>Bacillus subtilis</i>	2.85 a	2.40 b	2.55 a	1.30 b
<i>Pseudomonas fluorcenes</i>	2.75 b	2.75 a	2.65 a	1.40 ab
<i>Actinomyceete isolate</i>	2.50 c	1.10 c	2.10 b	1.50 a
Control	-	-	-	-

R.P.A. = diameter of inhibition zone / diameter of spotted antagonistic isolate.

Mean followed by the same letter in the same column are not significantly different at the 5% level by DMRT.

Results shown in Table (1&2) indicated that the majority of these bioagents had antagonistic effect against the phytopathogenic fungi under study in general. *Trichoderma spp.* (Table 1) were found to be the most bioagent that could affect drastically the growth of all the sugar beet pathogens followed by some isolates of *Bacillus subtilis* & *Pseudomonas fluorescens* (Table 2). While, *Gliocladium virens* showed the least effect against all tested pathogens. Data in Table (1) also indicated that *S. rolfisii* was obviously affected by 4 isolates of *Trichoderma spp.*, i.e. *T. hamatum*, *T. harzianum*, *T. pseudokoningii* and *T. viride*. Whereas, *R. solani* was affected by 3 isolates of *Trichoderma spp.* Its growth was retarded significantly by the antagonism with isolates as *S. rolfisii*. *Trichoderma hamatum* was as effective on the growth of *M. phaseolina* as on the two mentioned pathogens. *B. subtilis* as well as *P. fluorescense* affected significantly the radial growth of *S. rolfisii*, *R. solani* & *M. phasiolina*. *F. oxysporum* was slightly affected by all antagonists, but *T. hamatum* was the most effective one in inhibiting the radial growth of *F. oxysporum* comparable to the other antagonists.

Pot experiment:

An experiment was designed to evaluate a number of 6 out of the 24 bioagents against pre- and post-emergence damping-off and root rot caused by *S. rolfsii* in a greenhouse. This experiment was carried out in 1998-1999 and 1999-2000 growing seasons. *S. rolfsii* -infested potted-soil prepared as mentioned (in the materials and methods) was used. Bioagents were added to soil before sowing and disease readings were taken and recorded as infection percent. To compare the efficacy of the bioagents with the recommended fungicide, Rhizolex T.50 was used as seed dresser. *S. rolfsii*-free soil served as control.

Data presented in Table (3) revealed that most of the screened bioagents were effective in reducing damping-off of sugar beet expressed as the survived seedlings after 30 days of planting. Came after the effect of the fungicidal treatment on pre- and post-emergence damping-off, *T. hamatum* followed by *T. harzianum* and *B. subtilis* which were highly effective in controlling the disease compared with the untreated control. Concerning root rot caused by *S. rolfsii*, the majority of bioagents successfully reduced the disease incidence and disease severity. The most effective agents on root rot after Rhizolex T 50 were *P. fluorescense*, *T. hamatum*, *B. subtilis*. This is true over the two seasons of experimentation, i.e. 1998-1999 & 1999-2000.

Table 3: Effect of some bioagents on the damping-off and root-rot diseases caused by *S. rolfsii* in a greenhouse, during 1998-1999 and 1999- 2000 seasons.

Bioagent	1998-1999 season			1999-2000 season		
	Damping-off	Root rot		Damping-off	Root rot	
	Surviving plants %	Disease incidence %	Disease severity	Surviving plants %	Disease incidence %	Disease severity
<i>Trichoderma harzianum</i>	68.87 c	33.33 cd	2.33 b	71.22 d	22.22 c	3.00 c
<i>Trichoderma hamatum</i>	73.33 d	22.22 b	1.67 ab	75.55 de	11.11 b	1.67 b
<i>Gliocladium virens</i>	55.55 b	41.11 de	5.00 d	55.55 b	55.56 f	4.50 d
<i>Actinomycte isolate</i>	60.00 bc	32.33 cd	1.33 ab	62.22 cd	44.44 e	5.33 d
<i>Bacillus subtilis</i>	60.00 bc	44.44 e	3.33 c	60.00 c	55.56 f	5.33 d
<i>Pseudomonas fluorescense</i>	55.56 b	66.67 f	4.33 f	51.11 b	55.56 f	6.67 e
Rhizolex T. 50	95.55 e	11.11 a	0.33 a	97.78 g	0.00 a	0.00 a
Control	11.11 a	88.89 g	9.10 e	4.45 a	100.00 g	0.00 a

Mean followed by the same letter in the same column are not significantly different at the 5% level by DMRT.

Control of seedling blight and root rot by different formulae of biocontrol agents:

This experiment was performed at Sakha Farm A.R.C. during 1998-1999 and 1999-2000 seasons. Results in Table (4) show that all bioagents used were capable to control the diseases in any of the experimented formulae when compared with the untreated control. It was found that although Rhizolex T50 was superior in controlling the diseases, Rhizo N (commercial) followed by *B. subtilis* (suspension), *Trichoderma hamatum* & the *actinomycte* (powder) gave good results in reducing the seedling blight compared to the untreated ones. Granules of the *Actinomyces*, *Bacillus* &

Trichoderma and the commercial substance, plant guard, on the other hand gave the least effect on seedling blight.

Table 4: Biological control of seedling blight and root rot of sugar beet by seed dressing with different bioagents formula in the field at Sakha during, 1998-1999 and 1999-2000 seasons.

Treatment	Formulae	1998-1999 season				1999-2000 season			
		Seedling blight %	Root rot %	Disease Severity	Yield/Plot (kg)	Seedling blight %	Root rot %	Disease Severity	Yield/Plot (kg)
<i>Trichoderma Hamatum</i>	Suspension	8.00 cd	6.67 cde	2.33 bc	56.40 c	3.67 cd	3.67 ef	1.17 ab	56.67 c
	Powder	6.33 bc	3.67 b	1.33 ab	68.33 a	2.00 b	1.00 a	1.33 ab	71.17 a
	Granules	9.00 d	5.67 bc	1.67 b	62.83 ab	5.67 f	3.00 cde	1.33 ab	60.83 bc
<i>Bacillus Subtilis</i>	Suspension	5.33 b	9.67 f	2.67 bc	43.83 d	4.33 de	4.33 fg	1.83 bcd	42.00 d
	Powder	9.33 d	6.33 cd	1.33 ab	59.83 bc	5.33 ef	2.33 bc	1.33 ab	67.33 ab
	Granules	11.67 e	8.33 def	2.33 bc	45.17 d	7.00 g	3.33 de	1.67 bc	42.77 d
<i>Actinomycece Isolate</i>	Suspension	8.33 b	7.33 cde	2.33 bc	49.17 d	6.67 g	4.67 g	1.67 bc	55.17 c
	Powder	6.33 bc	5.33 bc	2.67 bc	62.33 abc	4.67 def	2.00 b	1.33 ab	63.07 abc
	Granules	12.33 e	8.67 ef	3.33 c	43.50 d	7.67 g	5.00 g	2.33 bcd	45.27 d
Plantguard	Suspension	12.67 e	13.67 g	3.33 c	45.17 d	6.67 g	8.33 h	3.00 d	40.67 d
Rhizo-N	Powder	5.00 b	5.33 bc	1.67 b	65.00 ab	2.83 bc	2.67 bcd	2.00 bcd	66.00 ab
Rhizolex T50	Powder	0.67 a	1.33 a	0.33 a	66.83 a	0.01 a	0.67 a	0.33 a	66.93 ab
Control		19.33 f	23.33 h	4.67 d	43.00 e	15.33 h	10.33 i	2.67 cd	40.83 d

Mean followed by the same letter in the same column are not significantly different at the 5% level by DMRT.

As regards to root rot and disease severity, *Trichoderma* powder followed by each of Rhizo N and the *Actinomyces* (powder) gave the best effect all over the experimented materials during the two seasons. Treatments increased yield per plot compared with the control. The yield per plot, however, was significantly affected by the powder natures of these materials more than the other forms.

The obtained results agrees, to a great extent with the findings of El-Kazzaz et al., (2000) who reported that isolate of *B. subtilis* followed by an *Actinomycece* and *T. harzianum* isolate could inhibit the growth of both of *R. solani* and *S. rolfsii* *in vitro*. Upadhyay & Mukhopadhyay, 1986 and Esh, 2000 reported that *T. harzianum* is known to have the ability to produce some extracellular lytic enzymes that are involved in the process of antagonism against a variety of pathogenic organisms. *T. hamatum* was very effective in retarding the growth of *S. rolfsii* & *R. solani* in Petri dishes and explained the positive effect of this fungus against the tested fungi by the hyperparasitism. Also, Asaka and Shoda, (1998) suggested that the antagonistic activity of *B. subtilis* against several host fungi *in vitro* may be referred to the production of the antibiotics such as Iturin A and Surfactin. Gurusiddaiah et al., (1986) was reported that *P. fluorescens* produces phenazine-1-carboxylic acid (a product of the shikimic acid pathway) which is one of the most thoroughly studied biocontrol antibiotic. This product has an activity against a broad spectrum of fungal pathogens including *R. solani*. Suppressive effect of this antibiotic against sugar beet root rot fungi is suggested to be utilized in biocontrol of sugar beet root rot diseases.

These results are consistent with those obtained by other investigators who got significant results in reducing the seedling damping-off as well as the root rots of sugar beet by *T. harzianum* & *B. subtilis* in the greenhouse and field (Ruppel et al., 1983 and El-Kazzaz et al., 2000). Similar

effects of these bioagents were obtained by many other investigators (Ciccarese *et al.*, 1990; Khalifa *et al.*, 1995 and Asaka & Shoda, 1998).

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استخدام بعض الكائنات المضادة في مكافحة أمراض موت البادرات وأعفان الجذور في نباتات بنجر السكر

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تم عزل بعض الكائنات الحية الدقيقة من منطقة حول الجذور (ريزوسفير) لنباتات سليمة من بنجر السكر، لاستخدامها في مكافحة الجيوبية لأمراض موت البادرات و أعفان الجذور في بنجر السكر. وقد أوضحت الدراسة أن الكائنات ذات القدرة التضادية من هذه العزلات عبارة عن ١٥ عزلة فطرية وعرفت أنها أربعة أنواع تابعة للجنس تريكوثيرما وهي: (هاماتام، نرايكوديرما هاريزيانام، فيردى و سيدوكوتنجاي) وكذا الفطر جليوكلاثيوم فيرنس. كذلك تم تعريف ٩ عزلات بكتيرية على أنها باسيلس سابيتلس، سيدوموناس فلوريسنس، وكذلك عزله من الأكتينومييسينات.

أظهرت التجارب المعملية أن هذه الكائنات ذات اختلافات في قدرتها كمضادات على تقليل النمو الفطري للمسيبات المرضية لبنجر السكر وهي: سكليروشيام رولفزاي، ريزوكتونيا سولاني، ماكرو فومينا فاسبولينا، فيوزاريوم أوغيسبوروم و فيوزاريوم سولاني. وقد أوضحت النتائج أن الكائنات المضادة من الفطريات تريكوثيرما هاماتام، نرايكوديرما هاريزيانام و تريكوثيرما سيدوكوتنجاي وبعض عزلات من بكتيريا باسيلس سابيتلس و عزلة واحدة من بكتيريا سيدوموناس فلوريسنس كانت ذات كفاءة عالية في تقليل النمو الفطري للمسيبات المرضية المذكورة آنفا فيما عدا نوعي الجنس فيوزاريوم، حيث لم يتأثرا إلا قليلا.

أظهرت نتائج الدراسة في الصوبة باستخدام تربة معديبة اصطناعيا بالمسبب المرضي سكليروشيام رولفزاي أن تريكوثيرما هاماتام، نرايكوديرما هاريزيانام، سيدوموناس فلوريسنس و باسيلس سابيتلس يمكن استخدامها بكفاءة في مكافحة الجيوبية للمرض.

تم تجهيز صور مختلفة (معلق و مسحوق و محبات) من كل من الفطر تريكوثيرما هاماتام و بكتيريا باسيلس سابيتلس وكذا عزلة الأكتينومييسين، لاستخدامها في تجارب الحقل، وقد أظهرت النتائج أن كل الصور كانت ذات تأثير فعال سواء في مكافحة أمراض موت البادرات أو أعفان الجذور وكذا في زيادة المحصول. بينت النتائج أن أكثر الكائنات المضادة المعزولة تأثيرا على مرض موت البادرات كانت معلق الباسيلس سابيتلس حيث أظهرت أقل نسبة مئوية للإصابة بمرض موت البادرات، بينما كانت محبات كل من الأكتينومييسين وكذلك الباسيلس سابيتلس أقلها تأثيرا على مرض موت البادرات. أعطى مسحوق تريكوثيرما هاماتام أعلى فاعلية في مكافحة أعفان جذور بنجر السكر، بينما محبات كل من الباسيلس سابيتلس و الأكتينومييسين كانت أقلها فاعلية على أعفان الجذور.