J. Agric. Sci. Mansoura Univ., 27 (12): 8279 - 8288, 2002

Pseudomonas Spp. AS BIOCONTROL AGENT AGAINST SOME SUGAR BEET DISEASES.

Al-Laithy, B.E.A.
Plant Pathology Res. Inst., Agric. Res. Center, Giza, Egypt.

Four isolates of *Pseudomonas* species showed inhibitory effect against four important soil borne pathogenic fungi (*Fusarium oxysporum, Rhizoctonia solani, Seleratium rolfsii* and *Pythium altimum*), the causal organisms of damping-off and root rot of sugar beet diseases and against one of the important foliar diseases (Cercospora leaf spots) caused by *Cercospora beticola.*

The bacterial biocontrol agents (*P. fluorescens, P. cepacia, P. aureofaciens* and *P. putida*) showed the highest mycelial reduction from 60 to 20% in laboratory experiments. In greenhouse tests, all isolates were effective comparing with control (without bacteria). Application of *Pseudomonas spp.* as seed dressing and infestation of soil were used and also spraying as suspension with these bacterial bioagents on the foliar of sugar beet plant to control the causal organism of cercospora leaf spots disease. A density of 7 x 10^9 cells per ml reduced damping-off percentage when seeds were sown in artificially infested soil with one of the pathogens.

The objective of this research were to study the effect of using the bacterial biocontrol agent against damping-off, root rot and cercospora leaf spot diseases of sugar beet (*Beta vulgaris*, L.) and to study the effect of spraying with the bacterial suspension on the foliar to reduce or control cercospora leaf spots disease especially.

Key words: Biological control, *Pseudomonas* species, sugar beet diseases.

إستخدام أنواع من بكتريا الزيدوموناس فى المقاومة الحيوية لبعض أمراض بنجر السكر ٠

بهاء الكردى أحمد الليثى
مركز البحوث الزراعية – معهد بحوث أمراض النبات – الجيزة – مصر

أبدث أربعة عزلات من بكتريا الزيدوموناس فعلاً مثبطاً لفطريات التربة الممرضة٠ وقد تم إستخدم أربعة أنواع من هذه البكتريا كمقاوم حيوى هى:-

١- زيدوموناس فلوريسنسز ٢- زيدوموناس ايروفاشنسز
٣- زيدوموناس سيباسيا ٤- زيدوموناس بيوتيدا٠

وفطريات التربة الممرضة التى إستخدمت فى هذا البحث هى: سكليروشيم رولفسياى – رايزوكتونيا سولاتنى – فيوزاريوم إوكسيسبورام – بيثيوم ألتيمم٠ وهى تحدث أمراضاً خطيرة لنبات بنجر السكر خصوصاً مرضى (موت البادرات وعفن الجذور)٠ كما تم إستخدام نفس أنواع البكتريا السابق ذكرها فى مقاومة مرض تبقع الأوراق السركوسيوزى الذى يحدثه فطر (سركوسيورا بيتيكولا)٠ لقد أبدت البكتريا فعلاً مثبطاً لهذه الفطريات الممرضة فى المعمل حيث إختزلت تمو ميسيليوم الفطريات الممرضة من ٦٠ إلى ٢٠% وفى الصوبة أبدت كل الأنواع تثبيطاً لكنه متفاوت من نوع لآخر وحسب نوع الفطر الممرض مقارنة بالكنترول والتى لم يتم تلقيح البكتريا فيها٠

ولقد عوملت بذرة بنجر السكر من الصنف أوسكار بالمعلق البكتيرى المحتوى على تركيز عالى من خلايا الزيدوموناس (٧ × ١٠ ^{٩}) خلية فى سم^{٣} قبل الزراعة ـ ٢٤ ساعة أو أضيف المعلق بمعدل ٢٥ سم^{٣} / قصرية وتخلط مع التربة٠ وبخصوص إستخدام نفس معلق البكتريا بمفرده أو مختلط فقد زادت كفاءة إستخدام التثبيط عندما تم معاملة البذرة أو خلط التربة بنوعين مختلفين٠ نفس النيجة حصلنا عليها فى حالة إستخدام المعلق البكتيرى بالتركيز السابق فى رش المجموع الخضرى لبنجر السكر لمقاومة المسبب المرضى سركوسبورا بيتيكولا الذى يسبب مرض تبقع الأوراق السركوسبورى قبيل نهاية الموسم الزراعى٠

- وترشدنا نتائج هذا البحث إلى إمكانية تطبيق هذه الأنواع من البكتريا فى الصوبة والحقل وأن إستخدام أنواع بكتريا الجنس الواحد مختلطة أفضل من إستخدامها بمفردها٠
- أن تكرار رش المجموع الخضرى لنبات بنجر السكر على فترات منتظمة إدى إلى تقليل نسبة المرض وشدته٠
- معاملة البذرة (نقعها) فى المعلق البكتيرى لمدة ٢٤ ساعة قبل الزراعة أفاد عن خلط المعلق فى التربة٠
- ظاهرة التضاد تجلت فى أوضح صورة فى المعمل وكانت القدرة التثبيطية للأربعة أنواع من بكتريا الزيدوموناس تحت الدراسة مرتبة كالآتى:-
  - زيدوموناس فلوريسنسز - زيدوموناس ايروفاشنسز
  - زيدوموناس سيباسيا - زيدوموناس بيوتيدا٠
- التركيز ٧ × ١٠ ^{٩} أعطى نتيجة واضحة وقوية فى التثبيط لمسببات الأمراض المختارة فى المعمل كما قللت بكفاءة النسبة المئوية للمرض فى الصوبة والحقل٠

INTRODUCTION

The soil borne phytopathogenic fungi which cause seed rots and seedling damping-off of many crops including sugar beet and often resulting substantial economic loss. The fat of synthetic pesticides in the environment and the development of fungicide-resistance in pathogenic strains. Methods of disease control are under investigation. One of the best methods is biological control by introduction of selected microorganisms to the soil. This has been attributed to the failure of biological control agents either to survive or to express *in vitro* the antagonistic properties. These two characteristics are influenced by a number of soil a biotic factor, such as indigenous soil microbiota.(Acea *et al*., 1988). The use of carriers such as peat and clay has been shown to increase the survival of bacteria after introduction into soil (Delucca *et al*., 1990 and Trevors *et al*., 1993).

Sugar beet (*Beta vulgaris*, L.) is susceptible to damping-off within the first few days of planting as reported by Osburn *et al*. (1989), when the number of microorganisms may be still high enough to inhibit the growth of the pathogen as found by Weller (1988), Mew (1990), Lemanceau (1991) and Gnanamaanikan *et al*. (1992).

Many researchers reported that the application of a bacterial biocontrol agents (El-Sheshtawi *et al*., 1988 and Gananamaanickan and Mew, 1992) as *Pseudomonas spp.* very important to reduce damping-off disease percentage of sugar beet (Mosa *et al*., 1997; Paulitz *et al*., 1992; Cartwright *et al*., 1993 and Cartwright, 1994). In this investigation, seeds coated with the bacterial suspension were used. The same suspension as spraying on the foliar of sugar beet (Leben, 1985 & Leben *et al.,* 1995) was used to control *Cercospora beticola*, which causes cercospora leaf spots disease (Kiewnich, 1998).

MATERIALS AND METHODS

1. Source of *Pseudomonas spp.* isolates:

Samples of different soil were collected from different locations in Dakahlia governorate, Egypt in the 2000 season. Soil samples were homogenized, serial dilutions up to 7 x 10^9 were prepared, in which 0.1 ml of each dilution was plated into 4 replicates of plate agar. This was made following the standard dilatation plating technique. Different types of bacterial colonies were transferred to nutrient agar (NA) slant as pure cultures for further studies.

2. Characterization and identification of the isolates under testing:

Gram stain reaction and cell morphology were observed on colony grown on nutrient agar slant for 24 hours at 28°C. The identification tests were 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. In vitro test of bacterial isolates:

Bacterial isolates obtained from different samples of soil rhizosphere of sugar beet varieties were screened for their antagonistic activities against four pathogenic fungi.

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 mycelial growth of the pathogens at 28°C.

Antagonism between mycelial growth of the pathogenic fungi and each bacterial isolates was assessed after seven days of inoculation by measuring the mycelial growth diameter of the pathogen towards the line of potential bacterial antagonists. Plates of the fungus alone served as control. Oscar variety of sugar beet was used after sowing and four strains of *Pseudomonas* species (*Pseudomonas cepacia, P. fluorescens, P. aureofaciens* and *P. putida*) were used in this study.

The four bacterial biocontrol agents are considered to be the most promising pathogens antagonist, which inhibited mycelial growth of four soil-borne pathogenic fungi are *Sclerotium relfsii, Rhizoctonia solani, Fusarium oxysporum* and *Pythium altimum*.

The cells of *Pseudomonas spp.* were harvested by centrifugation (20.000 g, 4°C, 10 minutes) and transferred to especial liquid media.

The tested pathogens were maintained on (P.D.A.) Potato Dextrose Agar after 7 days at 25°C. Cultures of the pathogens growing on (PDA) (Thompson and Burns, 1989) after 10 days at 25°C mycelial mats were broken up using flame sterilized forceps 10 release spores. A clay loam soil (43% clay, 35% silt) was used in all experiments under greenhouse and field conditions.

In all experiments, five sugar beet seeds were planted per each pot of 25 cm diameters at a depth of 30 mm. Planted pots were incubated under standard condition in plant growth room (20°C 12 h light / 12 h dark) for 14 days and watered daily to maintain a constant metric potential. Total damping-off was expressed as the sum of pre-emergence damping-off (i.e. seeds rotted in soil or seedling failed to emerge) and post emergence damping-off (seedlings died within 14 days after planting).

4. Greenhouse experiment:

Virulent isolates of the tested were transferred into especial medium (banana medium) slant for sporulation. Fungal inoculum was prepared by adding 5 ml of sterile water per tube of pure culture of each pathogens under study, and shaken to obtain free spores. To prepare the bacterial suspension, bacterial isolates were grown on NA plates for 24 hours at 28°C was prepared by adding 20 ml of sterile distilled water per plate and scraping the growth with a wire loop. The concentration of bacteria suspension was adjusted to 7×10^9 forming units per ml.

Seed treatment:

Seed of sugar beet (Oscar cv.) were surface sterile by immersion in 2.5% calcium hypochorite solution for three minutes and then rinsed in three changes of sterile distilled water. The seeds were soaked for 24 hours into the previously prepared bacteria suspension and sown in 25 cm diameter pots (5 seeds /.pot). Each pot contains 5 kg autoclaved loamy soil.
Pseudomonas spp. suspension were also used as soil treatment by adding 25 cm^3 from suspension to every pot and mixed with the soil 3 days before sowing. Then 25 cm^3 from the liquid media of each pathogens was added separately.

After 7-10 days from planting, the percentages of damping-off pre- and post-emergence were recorded. Pots without bacterial suspension considered as control and planted with same sugar beet variety (Oscar). Severity of root-rot disease was recorded after 100-120 days from sowing.

Field experiments:

Field experiments were carried out in clay loamy soil at the winter season 2000 in two locations. These experiments were done to study the effect of seed inoculation with single or mixture of four *Pseudomonas spp.* in controlling damping-off and root-rot diseases of sugar beet. Oscar seeds of sugar beet variety were soaked for 24 hours with 7×10^9 cells per ml 3 days before sowing. Untreated seeds were used as control in the same area. The same concentration of suspension from the bacteria was used as spraying on foliar every 15 days to control *Cercospora beticola*, which causes cercospora leaf spots disease.

Statistical analysis and experimental design:

A randomize block design was used for all experiments, four replicates for each treatment. Data collected from both seasons were statistically analyzed according to Snedecor and Cochran (1988). Treatments mean were compared by using LSD test at 0.05% level of probability (Waller and Duncan, 1969).

RESULTS

Using the bacterial biocontrol agent namely *Pseudomonas spp.* gave promising results in protection of sugar beet (*Beta vulgaris*, L.) from the soil-born pathogenic fungi, which cause damping-off and root-rot diseases through production of the antifungal secondary metabolite 2, 4-diacetyl phlorglucinol and extracellular proteolytic activity. The four biocontrol agents were combined in the inhibition the growth of the four soil borne pathogenic fungi.

1. Laboratory experiment:

Inhibition the growth of the four soil borne pathogenic fungi by using four species of *Pseudomonas spp.* was studied. There are many differences between the bacterial species in affecting on the pathogens. For example, *Pseudomonas fluorescens* was the first in controlling the causal organisms of damping-off disease. The optimum density which gave the best control was 7 x

10^9 cells/ml. Percentage of reduction of mycelial growth was recorded (Tables 1 and 2).

Table 1. Antagonistic effect between pathogenic fungi and one of *Pseudomonas spp.* as bacterial biocontrol agents under laboratory conditions.

Biocontrol agent / Pathgenic fungi	*P. fluorescens*		*P. cepacia*		*P. aureofaciens*		*P. putida*		Control***	
	Pathogen radial growth in cm and inhibition %									
	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %	PRG*	Inh.** %	CRG****	Inh.** %
Sclerotium rolfsii	7.0	53.33	8.4	44.0	8.0	46.66	6.0	60.0	15.0	0.0
Rhizoctonia solani	8.5	43.33	9.7	35.33	9.9	34.00	7.6	49.33	15.0	0.0
Fusarium	10.0	33.33	10.6	29.33	10.0	33.33	8.9	40.66	15.0	0.0
oxysporum	13.0	13.33	8.7	42.0	9.5	36.66	9.5	36.66	15.0	0.0
Pythium altimum										
LSD at 0.05	1.5	2.60	1.1	3.60	1.0	1.00	1.2	3.40	0.0	0.0

*** PRG = Pathogen radial growth. ** Inh. % = Inhibition in pathogen growth caused by the antagonist compared with control %**
***** Control = without bioagent. **** CRG = Control radial growth.**

Table 2: Antagonistic effect between pathogenic fungi and mixture of *Pseudomonas spp.* as bacterial biocontrol agents under laboratory conditions.

Biocontrol agent / Pathgenic fungi	*P. fluorescens* + *P. cepacia*		*P. aureofaciens* + *P. utida*		Control***	
	Pathogen radial growth in cm and inhibition %					
	PRG*	Inh.** %	PRG*	Inh.** %	CRG****	Inh.** %
Sclerotium rolfsii	5.5	63.3	6.7	53.33	15.0	0.0
Rhizoctonia solani	7.3	51.33	8.7	42.00	15.0	0.0
Fusarium oxysporum	9.6	36.00	9.5	36.66	15.0	0.0
Pythium altimum	11.5	30.00	12.0	20.00	15.0	0.0
LSD at 0.05	1.3	2.20	1.7	3.40	0.0	0.0

*** PRG = Pathogen radial growth. ** Inh. % = Inhibition in pathogen growth caused by the antagonist compared with control %**
***** Control = without bioagent. **** CRG = Control radial growth.**

2. Greenhouse experiment:

All treatments with the 7 x 10^9 cells per ml were significantly reduced the incidence of damping-off disease compared with the non-treated control. The density that give optimum disease control was 7 x 10^9 when percentage of damping-off was reduced from 80 to 20%. *Pseudomonas fluorescens* gave the highest effect in controlling the soil borne pathogenic fungi under study. While, *P. aureofaciens* was lowest with the concentration of 7 x 10^9 cells / ml.
Incidence of damping-off disease in sugar beet seedlings reduced to 20, 27, 30 and 33% according to the type of biocontrol agent (Tables 3 and 4).
Using of bio-control agents *Pseudomonas spp.* in controlling some diseases were single (one specie) or mixture (two species). The effect of mixture was faster and higher than the single case (Tables 4 and 6).

3. Field experiment:

Pseudomonas spp. are considered beneficial as biocontrol agents against soil borne pathogenic fungi. These bacteria have abilities to produce growth inhibitory compounds.

In this study, all species of tested *Pseudomonas spp.* inhibited *Rhizoctonia solani*, *Fusarium oxysporum, Pythium sp.* and *Sclerotium rolfsii*.

The level of inhibition differed among species. The importance of antibiotic production has been demonstrated in some cases.

In the field condition, *Pseudomonas spp.* tested in these experiments to control damping-off and root-rot diseases (by inhibition the main causal organisms. *P. fluorscens* gave the highest effect, while *P. putida* gave the lowest value (Tables 5 and 6). Also, it can be seen that *Pseudomonas spp.* at all concentration tested when each *Pseudomonas spp.* was used single or mixture in reducing or controlling damping-off and root-rot diseases were effectively controlled when the seed were soaked for 24 hours into the previously prepared bacterial suspension 3 days before sowing or addition 1000 cm^3/feddan from the same suspension. Untreated seeds were used as control. To control or reduce the foliar disease cercospora leaf spot, the same suspension was used 7 x 10^9 cells / ml as spraying every 15 days on sugar beet plants. 0.0 concentration of the bacterial suspension cells / ml used as control at all treatments.

Table 3: Effect of biocontrol agents on soil borne pathogenic fungi of sugar beet under greenhouse conditions.

Biocontrol agent	Bioagent/ Pathogen ratio	Soil borne pathogenic fungi							
		S. rolfsii		*R. solani*		*F. oxysporum*		*P. altimum*	
		Pre %	Post %	Pre %	Post %	Pre %	Post %	Pre %	Post %
P. fluorescens	1 : 1	50.00	15.56	36.10	5.66	35.80	3.70	15.33	2.60
	2 : 1	18.00	3.66	6.33	2.80	8.60	2.90	3.16	0.00
	3 : 1	3.70	0.17	2.70	0.00	5.15	1.30	0.00	0.00
	1 : 2	58.16	4.70	48.66	4.12	44.66	8.12	8.00	2.10
	1 : 3	66.00	20.30	55.10	5.16	50.12	9.20	16.32	3.00
Control		77.11	25.66	56.33	8.10	52.66	7.00	22.00	6.00
LSD at 0.05		5.00	3.70	4.00	3.60	3.80	3.20	2.70	2.00
P. cepacia	1 : 1	58.00	12.00	33.22	5.60	39.10	5.00	16.80	4.00
	2 : 1	8.12	7.00	7.70	4.33	9.00	3.60	3.60	0.00
	3 : 1	3.60	1.00	3.60	0.10	6.70	1.66	0.00	0.00
	1 : 2	48.66	9.12	45.10	5.00	40.10	8.44	7.10	3.20
	1 : 3	66.00	18.00	55.00	6.00	52.15	8.80	20.00	6.24
Control		69.00	25.12	62.00	8.30	58.00	8.00	29.00	8.66
LSD at 0.05		5.00	3.70	4.70	3.20	3.30	3.10	3.20	2.6
P. aureofaciens	1 : 1	56.00	17.10	33.10	5.10	36.16	3.10	18.66	4.60
	2 : 1	8.44	6.10	6.16	3.16	9.60	8.12	4.22	0.00
	3 : 1	3.16	0.0	3.42	1.00	6.30	1.20	1.20	1.00
	1 : 2	55.00	9.2	49.00	3.33	45.00	9.10	9.16	3.60
	1 : 3	59.00	18.6	59.16	6.70	7.00	10.22	20.10	4.10
Control		72.10	27.30	58.16	9.70	70.00	9.50	24.60	9.16
LSD at 0.05		5.10	3.60	3.80	4.30	3.60	3.30	2.90	3.00
P. putida	1 : 1	50.00	16.70	36.18	3.75	35.15	3.60	18.10	3.18
	2 : 1	9.00	8.60	5.70	3.12	10.60	4.00	3.70	0.00
	3 : 1	4.80	1.30	3.15	1.60	8.14	1.10	0.00	0.00
	1 : 2	60.00	9.00	48.12	3.40	48.11	10.70	82.00	3.10
	1 : 3	70.00	20.00	58.10	5.00	60.10	9.60	19.60	3.80
Control		44.00	30.12	60.00	7.80	57.70	8.90	25.12	5.90
LSD at 0.05		4.50	3.20	3.30	4.80	4.60	3.30	2.50	3.20

Table 4. Antagonistic effect of mixture of *Pseudomonas spp.* against some soil borne pathogenic fungi of sugar beet under greenhouse conditions.

Mixture of Biocontrol agents	Bioagent/ Pathogen ratio	Soil borne pathogenic fungi							
		S. rolfsii		*R. solani*		*F. oxysporum*		*P. altimum*	
		*Pre %	**Post %	Pre %	Post %	Pre %	Post %	Pre %	Post %
P. fluorescens + *P. cepacia*	1 : 1	33.0	10.00	30.00	8.00	25.33	22.24	16.50	9.00
	2 : 1	9.00	5.00	4.00	0.00	4.00	0.00	0.00	0.00
	3 : 1	4.00	0.00	3.60	0.00	2.60	0.00	0.00	0.00
	1 : 2	36.20	8.00	30.00	5.00	3.70	3.00	1.00	0.70
	: 3	60.00	17.50	45.00	16.00	12.80	18.40	24.60	19.70
Control	0 : 1	75.30	22.00	70.00	30.00	79.60	36.60	70.00	25.60
LSD at 0.05		5.50	3.00	4.00	3.10	3.70	3.60	3.00	2.70
P. fluorescens + *P. aureofaciens*	1 : 1	48.60	16.66	45.33	10.00	34.12	22.33	20.00	6.00
	2 : 1	7.30	6.60	5.16	0.00	3.00	3.40	2.00	1.00
	3 : 1	4.40	0.00	0.00	0.00	3.60	2.70	3.00	1.00
	1 : 2	44.33	10.66	35.16	4.00	27.60	25.00	23.00	21.00
	1 : 3	56.36	19.00	52.32	12.60	44.00	43.00	44.00	40.00
Control	0 : 1	70.00	25.00	75.66	35.10	75.60	27.00	82.00	17.60
LSD at 0.05		5.00	3.70	4.20	3.20	3.30	3.70	3.20	2.40
P. fluorescens + *P. putida*	1 : 1	45.00	16.00	50.00	12.00	32.00	11.66	18.70	6.00
	2 : 1	10.00	8.00	5.70	0.00	6.70	2.44	6.30	0.00
	3 : 1	7.20	5.60	7.70	0.00	8.60	2.10	6.00	0.00
	1 : 2	46.30	12.00	47.00	4.00	44.00	12.00	34.00	6.00
	1 : 3	55.00	16.00	57.00	16.00	56.66	15.00	55.00	35.0
Control	0 : 1	75.60	24.00	79.00	36.30	75.00	16.00	81.00	3.00
LSD at 0.05		5.00	3.20	3.20	4.30	3.30	2.80		

*** Pre = Pre-emergence damping-off.**

**** Ppst = Post-emergence damping-off.**

Table 5: Effect of *Pseudomonas spp.* on damping-off, root rot and cercospora leaf spots under field conditions during winter 1999/2000 season.

Biocontrol agent	Conc. of bacterial Biocontrol Agent cells	Soil and foliar diseases					
		Damping-off		Root rot		Cercospora leaf spots	
		Pre %	Post %	Pre %	Post %	Pre %	Post %
P. fluorescens	7 X 10^9 cells / cm^3	7.00	3.00	2.30	1.0	8.40	1.00
	6 X 10^9 cells / cm^3	10.00	5.40	4.30	1.0	10.50	2.70
	5 X 10^9 cells / cm^3	12.00	5.70	4.00	1.0	10.00	2.60
	4 X 10^9 cells / cm^3	22.00	8.60	6.00	1.0	12.60	2.60
Control	Zero	40.00	7.00	18.00	3.0	25.33	13.30
LSD at 0.05		2.80	3.00	2.30	3.0	3.00	2.00
P. cepacia	7 X 10^9 cells / cm^3	9.33	5.00	3.60	1.30	10.00	2.60
	6 X 10^9 cells / cm^3	13.00	6.20	5.60	1.60	12.00	4.00
	5 X 10^9 cells / cm^3	16.00	6.00	5.80	1.00	12.66	4.00
	4 X 10^9 cells / cm^3	27.00	10.00	9.00	2.00	13.50	3.65
Control	Zero	44.00	29.00	29.00	3.00	25.75	11.12
LSD at 0.05		2.50	2.00	2.50	3.20	3.40	2.00
P. aureofaciens	7 X 10^9 cells / cm^3	9.70	4.60	4.20	1.00	10.00	1.00
	6 X 10^9 cells / cm^3	16.00	7.80	6.33	1.00	13.00	4.00
	5 X 10^9 cells / cm^3	16.00	6.30	6.00	1.00	13.00	4.00
	4 X 10^9 cells / cm^3	25.12	9.60	12/00	2.00	14.00	4.00
Control	Zero	50.66	28.00	40.75	3.00	18.66	14.00
LSD at 0.05		1.80	2.00	2.60	3.30	3.00	2.00
P. putida	7 X 10^9 cells / cm^3	7.30	3.60	7.66	6.00	9.00	3.00
	6 X 10^9 cells / cm^3	17.80	8.00	8.33	8.00	15.00	3.00
	5 X 10^9 cells / cm^3	18.00	9.00	8.66	6.00	15.00	2.20
	4 X 10^9 cells / cm^3	29.33	10.60	10.00	9.00	16.00	6.33
Control	Zero	50.00	20.00	33.33	7.00	23.12	18.16
LSD at 0.05		1.80	2.00	2.50	3.10	3.10	2.00

*** Pre = Pre-emergence damping-off.**

**** Ppst = Post-emergence damping-off.**

Table 6: Reducing effect of mixed of *Pseudomonas spp.* on damping-off, root rot and cercospora leaf spots under field conditions during winter 1999/2000 season.

Biocontrol agent	Conc. of bacterial Biocontrol Agent cells	Soil and foliar diseases					
		Damping-off		Root rot		Cercospora leaf spots	
		* Pre %	** Post %	Pre %	Post %	Pre %	Post %
P. fluorescens + *P. cepacia*	7 X 10^9 cells / cm^3	5.33	2.00	2.66	2.00	6.40	1.60
	6 X 10^9 cells / cm^3	10.66	9.00	3.10	3.00	9.10	2.30
	5 X 10^9 cells / cm^3	18.14	10.00	3.75	4.00	9.60	2.10
	4 X 10^9 cells / cm^3	20.00	18.66	5.00	2.10	10.00	2.10
Control	Zero	60.77	40.12	19.00	6.15	27.00	16.33
LSD at 0.05		2.70	2.20	2.00	3.10	3.00	2.00
P. fluorescens + *P. aureofaciens*	7 X 10^9 cells / cm^3	7.11	3.00	2.60	1.60	9.00	2.50
	6 X 10^9 cells / cm^3	12.66	8.50	4.30	2.00	10.00	3.70
	5 X 10^9 cells / cm^3	24.16	13.00	4.00	1.60	10.00	3.00
	4 X 10^9 cells / cm^3	38.33	20.60	8.00	2.00	12.33	3.60
Control	Zero	70.00	45.00	27.10	12.60	27.66	
LSD at 0.05		2.60		2.30	3.40	3.40	2.20
P. fluorescens + *P. putida*	7 X 10^9 cells / cm^3	7.50	2.00	3.00	2.00	9.66	1.00
	6 X 10^9 cells / cm^3	13.16	8.00	5.00	1.00	11.00	3.00
	5 X 10^9 cells / cm^3	20.22	12.00	5.00	3.00	12.00	3.33
	4 X 10^9 cells / cm^3	29.00	19.66	10.00	6.00	15.00	3.00
Control	Zero	75.00	33.33	35.00	15.00	17.00	22.60
LSD at 0.05		1.7	2.00	2.10	3.10	3.40	2.10

*** Pre = Pre-emergence damping-off.**
**** Ppst = Post-emergence damping-off.**

DISCUSSION

The widespread use of chemical crop protection is becoming increasingly unsatisfactory from both public health and environmental concerns, as well as in its role in the selection of more virulent plant pathogens. These limitations have stimulated interest in alternative means of disease suppression, such as biological control. Natural suspension of fungal diseases of plant roots in certain soils or in response to crop monoculture has been attributed to the role of diverse indigenous microorganisms, which display a variety of antifungal determinations (Cook *et al.*, 1983). The effect of this bacteria in protection of crop plants may involve antagonism as result of the production of secondary metabolites or extracellular lytic enzymes. Kiewnick *et al*. (2000) reported that the ability of controlling *Cercospora beticola* by *Pseudomonas spp.* and other bacteria may be due to production chitinase and 1, 3 glucanase and direct antibiosis against *Cercospora beticola*. All species of *Pseudomonas* were tested in greenhouse and field conditions against (CLS) Cercospora leaf spots disease of sugar beet (c.v. Oscar). Both species gave efficient from (65-85%) of disease control, which cell densities of the bacterial suspension were 7 x 10^9 cell/ml. The suspension was sprayed on the foliar of sugar beet plant every 15 days and data were recorded (Table 5).

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إستخدام أنواع من بكتريا الزيدوموناس فى المقاومة الحيوية لبعض أمراض بنجر السكر ٠

بهاء الكردى أحمد الليثى

مركز البحوث الزراعية – معهد بحوث أمراض النبات – الجيزة – مصر

أبدث أربعة عزلات من بكتريا الزيدوموناس فعلاً مثبطاً لفطريات التربة الممرضة٠ وقد تم إستخدم أربعة أنواع من هذه البكتريا كمقاوم حيوى هى:-

١- زيدوموناس فلوريسنسز ٢- زيدوموناس ايروفاشنسز

٣- زيدوموناس سيباسيا ٤- زيدوموناس بيوتيدا٠

وفطريات التربة الممرضة التى إستخدمت فى هذا البحث هى: سكليروشيم رولفسياى – رايزوكتونيا سولاتنى – فيوزاريوم إوكسيسبورام – بيثيوم ألتيمم٠ وهى تحدث أمراضاً خطيرة لنبات بنجر السكر خصوصاً مرضى (موت البادرات وعفن الجذور)٠ كما تم إستخدام تفس أنواع البكتريا السابق ذكرها فى مقاومة مرض تبقع الأوراق السركوسيوزى الذى يحدثه فطر (سركوسبورا بيتيكولا)٠ لقد أبدت البكتريا فعلاً مثبطاً لهذه الفطريات الممرضة فى المعمل حيث إختزلت تمو ميسيليوم الفطريات الممرضة من ٦٠ إلى ٢٠% وفى الصوبة أبدت كل الأنواع تثبيطاً لكنه متفاوت من نوع لآخر وحسب نوع الفطر الممرض مقارنة بالكنترول والتى لم يتم تلقيح البكتريا فيها٠

ولقد عوملت بذرة بنجر السكر من الصنف أوسكار بالمعلق البكتيرى المحتوى على تركيز عالى من خلايا الزيدوموناس (٧ × ١٠ ^{٩}) خلية فى سم^{٣} قبل الزراعة ـ ٢٤ ساعة أو أضيف المعلق بمعدل ٢٥ سم^{٣} / قصرية وتخلط مع التربة٠ وبخصوص إستخدام نفس معلق البكتريا بمفرده أو مختلط فقد زادت كفاءة إستخدام التثبيط عندما تم معاملة البذرة أو خلط التربة بنوعين مختلفين٠ نفس النيجة حصلنا عليها فى حالة إستخدام المعلق البكتيرى بالتركيز السابق فى رش المجموع الخضرى لبنجر السكر لمقاومة المسبب المرضى سركوسبورا بيتيكولا الذى يسبب مرض تبقع الأوراق السركوسبورى قبيل نهاية الموسم الزراعى٠

- وترشدنا نتائج هذا البحث إلى إمكانية تطبيق هذه الأنواع من البكتريا فى الصوبة والحقل وأن إستخدام أنواع بكتريا الجنس الواحد مختلطة أفضل من إستخدامها بمفردها٠
- أن تكرار رش المجموع الخضرى لنبات بنجر السكر على فترات منتظمة إدى إلى تقليل نسبة المرض وشدته٠

- معاملة البذرة (نقعها) فى المعلق البكتيرى لمدة ٢٤ ساعة قبل الزراعة أفاد عن خلط المعلق فى التربة٠
- ظاهرة التضاد تجلت فى أوضح صورة فى المعمل وكانت القدرة التثبيطية للأربعة أنواع من بكتريا الزيدوموناس تحت الدراسة مرتبة كالآتى:-
- زيدوموناس فلوريسنسز - زيدوموناس ايروفاشنسز
- زيدوموناس سيباسيا - زيدوموناس بيوتيدا٠
- التركيز ٧ × ١٠ ^{٩} أعطى نتيجة واضحة وقوية فى التثبيط لمسببات الأمراض المختارة فى المعمل كما قللت بكفاءة النسبة المئوية للمرض فى الصوبة والحقل٠