J. Agric. Sci. Mansoura Univ., 29(9): 4865 - 4874, 2004

BIOLOGICAL CONTROL OF FLAX SEEDLING BLIGHT DISEASE

Ashour, A.Z.A.*; Aida H. Afify and ** M.E.A. Kineber***
*** Plant Pathology Research Institute, ARC, Giza, Egypt.**
**** Department of Microbiology, Fac. Agric., Mansoura Univ., Egypt.**
***** Field Crop Research Institute, ARC, Giza, Egypt.**

ABSTRACT

Antagonism between forty-two isolates of *Bacillus* spp. against two isolates of *Rhizoctonia solani* and *Fusarium oxysporum* the casuals of flax seedling blight were studied *in vitro*. Nine strains from bacteria consistently showed high level of *in vitro* antagonism against the fungal isolates. Other strains were either ineffective or showed low level of antagonism. *Bacillus* strains showed different levels of efficiency in increasing the surviving seedlings, yield and yield components and technological characters, however, two strains of *Bacillus* (No. 5 and 25) showed maximum efficiency under greenhouse and field conditions compared with the control. Fungicide Monceren significantly increased seedling survival and yield compared with *Bacillus* strains. A positive correlation was observed between seedling survival (stand) and each of straw yield and its components. Thus, these bacterial biocontrol agents could be recommended in combination with the chemical fungicide Monceren as integrated system for controlling *R. solani* and *F. oxysporum* in flax, and improving quality of straw yield.

Keywords: biological control, seedling blight, flax, *Bacillus* spp.

INTRODUCTION

Flax (*Linum usitatissimum L.*) is one of the most important sources of fiber and oil. Flax is susceptible to several fungal diseases such as seedling blight caused by *R. solani* Kühn and *F. oxysporum*. These fungi attack the plant at seedling stage causing great economic losses under favorable conditions. (Nyvall, 1981).

In the light of present day constraints on plant disease control by fungicides, biological control is increasingly capturing the attention of plant pathologists all over the world as a possible mean of controlling soil-borne pathogens. Among the bacterial antagonists against fungal diseases, *Bacillus* sp. showed a great inhibition effect of *Fusarium* spp. and produced antifungal antibiotics in culture (Katz and Demain, 1977). The gram-positive bacteria like *Bacillus* spp. have been studied as biocontorl agents less intensively than the gram negative bacteria (Silo-suh *et al.*, 1994). However, many *Bacillus* strains are known to suppress fungal growth *in vitro* (Katz and Demain, 1977) and *in vivo* (Baker *et al.*, 1983; Utkhede and Sholberg, 1986; Pusey *et al.*, 1986; Fravel, 1988) by the production of one or more antifungal antibiotics. *Bacillus subtilis* is among the widely used biocontol agents. *B. subtilis* RB_{14} produces the antibiotics iturin A and surfactin (Hiraoka *et al.*, 1992) Kado *et al.* (1987) isolated *B. polymyxa* 9A from the roots of field-grown potatoes highly infected with wilt. This strain suppressed the development of wilt in plants. Many strains of *B. cereus* produce zwittermicin a novel antibiotic that contributes to the antagonistic activity of *B. cereus* against certain plant pathogens (Raffel *et al.*, 1996).

Moreover, this antagonistic bacteria and fungi were repressed and decreased the disease incidence in plants (Afify and Ashour, 1995). Strains of *Bacillus* spp. were effective in increasing the percentage of surviving seedlings and the dry weight of cotton seedlings, also increasing stand and yield for plant (Ashour and Afify, 1999).

The aim of the present study, is to evaluate the possibilities of suppressing *R. solani* and *F. oxysporum* the incitants of flax seedling blight by bacterization with *Bacillus* spp.

MATERIALS AND METHODS

Isolation of aerobic spore-forming bacteria:

To isolate the aerobic endospore-forming bacteria, ten grams of soil samples collected from rhizosphere of flax seedlings were aseptically added to 90 ml of sterile tap water in Pyrex flasks. The sample was shaken for 10 min at room temperature. The soil suspension was placed in thermostatically controlled water bath at 80°C for 10 min., followed by cooling to 30°C. A serial dilution was prepared one ml of each dilution was pipetted and plated on nutrient agar. The plates from each dilution were incubated in duplicate at 30°C. Surface colonies from each plate were grouped on the basis of colony morphology, spore shape and the form of the sporangia. One isolate of each morphological group was selected for further characterization. Cultures were purified by re-streaking isolated colonies at least three times. The bacteria were maintained on slants of nutrient agar and stored at 4°C (Harry and Paul, 1989).

***In vitro* interaction between spore forming bacteria and fungal pathogens:**

Antagonism between the isolated endospore-forming bacteria and *Rhizoctonia solani* and *Fusarium oxysporum* was studied using potato dextrose agar (PDA) medium at pH 6.8. Petri dishes containing 10 ml of PDA medium were inoculated with mycelial discs (4mm in diameter) of the tested fungi. Discs of the tested fungi (previously grown on the same medium for 8 days) were placed at the periphery of plates. For each tested bacterial strain a loopful of a two day-old suspension (previously grown on nutrient broth medium) was streaked at opposite sides of the plates periphery. In the control treatment, plates were streaked with sterile nutrient broth medium. Five replicates from each treatment, were used (Sivamani and Gnanama nickam, 1988).

The antagonistic effects of the tested organisms were determined by measuring the maximum inhibition zone after 5-8 days when the surface of the control plate was covered by the mycelium of the tested fungus.

Identification:

Forty-two endospore-forming, gram-positive, catalase positive, rod-shaped isolates were assumed to be members of the genus *Bacillus*. Only nine isolates were highly antagonistic to the tested pathogenic fungi and these were identified.

Bacterial seed treatment:

Each bacterial strain was grown for 48 h at 28°C on nutrient broth medium. For a single strain inoculation, 1.5 ml of a bacterial suspension and 5 g of flax seeds (Sakha 1 cv.) were mixed in a small plastic bag, and sown in greenhouse potted soil and/or field experiment (Mew and Rosales, 1986).

Seed-dressing fungicide:

Monceren was applied at the recommended dose (3 g/kg seeds). The treated seeds were planted in greenhouse and field experiments.

Preparation of fungal inoculum:

R. solani Kühn and *Fusarium oxysporum*, isolated from roots of blighted flax seedling were used throughout this study, isolation, purification, and identification of these fungi were carried out at plant pathology lab. Sakha Agric. Res. Station. ARC. The inoculum was prepared by growing *R. solani* and *F. oxysporum* in 500 ml bottles containing barley grain medium (100 g of barley grains + 50 ml water), then incubated at 20°C for 20 days. The inoculum was mixed throughout with soil at rate of 0.1 g/kg of soil weight.

Greenhouse study was conducted by using clay pots of 20 cm in diameter. In the field experiment, treatments were sown in 1.5x4 m plots. Seed rate was 85.7 gm/plot which are equivalent to 60 kg/feddan. The soil used in both experiments was naturally clay soil (pH 7.5 clay 62.1 percent, E.C. 1.4 mmhos/cm). The design of layout of both trials was randomized complete block design with four replications. Greenhouse and field experiments were carried out during 2001/2002 and 2002/2003 growing seasons at Sakha Agric. Res. Station, ARC.

30 flax seeds (Sakha1 cv.) treated with bacterial suspensions were planted in each pot one week after soil infestation. Percentage of surviving seedlings, were recorded 40 day from sowing in both greenhouse and field experiments. Yield and yield components and technological characters were recorded at the end growth seasons in the field trials.

Statistical analysis:

Percentage data of greenhouse and field experiments were transformed into arc sine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. ANOVA was performed by the soft ware MSTAT (A Microcomputer program for the Design, Management and Analysis of Agronomic Research Experiments Michigan State Univ., USA).

RESULTS AND DISCUSSION

Of the forty-two *Bacillus* isolates tested for *in vitro* antagonism against isolates of *R. solani* and *F. oxysporum*, 9 strains numbers(3, 8, 12, 17, 24, 25, 30, 36 and 38) consistently showed high level of *in vitro* antagonism against the two tested fungy. Other isolates were either in effective or showed low level of antagonism (Table, 1). These results are in agreement with the previous reports which indicated that *Bacillus* isolates suppressed fungal growth *in vitro* (Ashour and Afify, 1999) by the production of one or more antifungal antibiotics (Katz and Demain, 1977; Leifert *et al.*, 1995).

Table 1: Screening of different *Bacillus* spp. for *in vitro* antagonism against two isolates of *R. solani* and *F. oxysporum*.

Bacillus No.	Fungi tested	
	R. solani	*F. oxysporum*
1	-	-
2	-	-
3	++	++
4	+	-
5	-	-
6	+	-
7	-	+
8	++	++
9	-	+
10	-	-
11	-	-
12	++	++
13	-	-
14	+	-
15	+	-
16	-	+
17	++	++
18	-	+
19	-	-
20	+	-
21	-	-
22	-	+
23	-	+
24	++	++
25	++	++
26	-	-
27	-	+
28	-	-
29	-	-
30	++	++
31	-	-
32	-	+
33	+	-
34	-	±
35	+	+
36	++	++
37	-	-
38	++	++
39	-	-
40	-	-
41	-	-
42	±	-

++Inhibition of pathogen by over growth.
+ Inhibition of pathogen.
- No inhibition.
± Inhibition of the potential antagonist by pathogen.

As shown in Table (2)the nine isolates (No. 3, 8, 12, 17, 24, 25, 30, 36 and 38) were long rods shape, endospore forming, gram positive motile and catalase producers and by biochemical characteristics they were aerobic or facultative anaerobic, positive with gelatin and variable reactions with others testes and maximum temperature (40 to 55). Bergey's Manual of Determinative Bacteriology 9th ed. (Holt *et al.*, 1994) used to identify these nine strains, it was found that they were belonging to *B. cereus* (strains No. 3 and 24); *B. subtilis* (No. 12 and 17); *B. pumilus* (No. 30); *B. polymyxa* (No. 36); and *Bacillus* sp. (No. 8, 25 and 38) (Table, 3).

Table 2: The morphological, physiological and biochemical characters of the antagonistic bacteria.

Character	Isolates No.								
	3	8	12	17	24	25	30	36	38
Cell diameter (µm)	3.0x1.0	3.0x1.4	5.0x1.2	2.0x7.5	3.0x7.0	5.0x0.8	4.0x0.7	5.5x3.0	3.5x1.5
Shape	R+C	CR	CR	R+C	R+C	CR	CR	CR	R+C
Sporulation	EC	EC	EC	ET	EC	ET	ET	EC	ET
Motility	+	+	+	+	+	+	+	+	+
Gram stain	+	+	+	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+	+	+	+
Degradation of:									
Gelatin	+	+	+	+	+	+	+	+	+
Casein	+	+	-	+	-	+	-	-	+
Starch	+	+	-	+	-	+	+	-	+
Aerobiosis	Aerobic or facultative anaerobic								
Anaerobic growth	+	+	+	-	-	-	-	-	+
V.P.test	+	+	+	-	+	-	-	+	-
Indole production	-	-	-	-	-	-	-	-	-
Tolerance of 5% NaCl	±	+	+	±	-	-	+	±	+
Tolerance of 7% NaCl	+	-	-	-	+	+	+	-	+
Production of acid from:									
Glucose	+	+	-	+	+	+	-	+	+
Mannose	-	+	+	-	-	+	+	-	+
Mannitole	+	-	-	-	+	+	-	-	-
Max. temp. for growth (°C):	50	55	40	50	40	55	50	55	40

Spore: (E = Ellipsoidal, C = Central, T = Terminal).
CR = Chains rods, R+C = Rods with capsules.
± = variable reactions + = Positive reaction - = Negative reaction.

Table 3: Scientific name of isolated strains.

No. of strain	Scientific name	Code name
3	*Bacillus cereus*	Bc-3
8	*Bacillus* sp.	Bsp-8
12	*Bacillus subtilis*	Bs-12
17	*Bacillus subtilis*	Bs-17
24	*Bacillus cereus*	Bc-24
25	*Bacillus* sp.	Bsp-25
30	*Bacillus pumilus*	Bp-30
36	*Bacillus polymyxa*	Bp-36
38	*Bacillus* sp.	Bsp-38

Data in Tables (4) and (5) showed that all *Bacillus* strains were effective in increasing the surviving seedlings whether they were applied under greenhouse or field conditions however their efficiency was always much higher in case of two strains of *Bacillus* (Bsp-8 and Bsp-25) under greenhouse and field conditions. The efficiency of *Bacillus* strains was comparable to that of Monceren. *Bacillus* strains were much more effective against *F. oxysporum* than *R. solani* and significantly increased percentage of surviving seedlings compared to the untreated control. The interaction between biocontrol agents and fungi failed to express any significant effect of the percentage of surviving seedlings. Monceren significantly increased surviving seedlings as compared with *Bacillus* strains. On the other hand *Bacillus* strains were more effective in reducing disease incidence that

control. Bsp-3 and Bsp-25 strains gave the maximum percentage of surviving seedling under greenhouse and field conditions as compared with the control.

Table 4: Effect of *Bacillus* spp. on seedling survival of flax under greenhouse conditions.

Treatments	Fungi involved in flax seedling disease						Mean	
	R. solani		*F. oxysporum*		Non infested soil[c]			
B. cereus (Bc-3)	55.7[a]	(48.31)[b]	67.2	(55.06)	67.2	(55.06)	61.8	(51.84)
Bacillus sp. (Bsp-8)	65.2	(53.85)	78.0	(62.05)	78.0	(62.05)	69.6	(56 56)
B. subtilis (Bs-12)	58.6	(49.97)	72.2	(58.19)	72.2	(58.19)	64.3	(53.28)
B. subtilis (Bs-17)	47.9	(43.77)	65.8	(54.24)	85.8	(54.24)	53.7	(47.12)
B. cereus (Bc-24)	45.0	(42.11)	63.0	(52.53)	63.0	(52.53)	52.2	(46.27)
Bacillus sp. Bsp-25	68.2	(55.56)	74.5	(59.68)	74.5	(59.68)	69 9	56 77
B. pumilus Bp-30	44.9	(42.09)	66.8	(54.69)	54.9	(47.81)	58.0	(49.59)
B. polymyxa Bp-36	54.3	(47.48)	66.5	(54.63)	66.5	(54.63)	58.0	(49.59)
Bacillus sp. Bsp-38	52.9	(46.68)	67.2	(55.07)	67.2	(55.07)	58.7	(50.03)
Nutrient broth	27.7	(31.76)	52.9	(46.67)	52.9	(46.67)	37.7	(37.87)
Monceren	71.0	(57.42)	92.6	(74.28)	92.6	(74.28)	81.8	(64.72)
Control	24.9	(29.96)	52.2	(46.27)	52.2	(46.27)	33.8	(35.55)
Mean	51.4	(45.75)	68.2	(55.67)	68.2	(55.67)	58.0	(49.60)

[a] Percentage of surviving seedlings.
[b] Arc sine-transformed data.
[c] Soil non infested either *R. solani* or *F.oxysporum*
L.S.D. for bacterial agents = 3.71 (p = 0.05) or 4.91 (p = 0.01).
for fungal treatments = 1.86 (p = 0.05) or 2.45 (p = 0.01).

Table 5: Effect of *Bacillus* spp. on seedling survival of flax under field conditions.

Treatments	Seedling survival %			
	2001/2002		2002/2003	
B. cereus (Bc-3)	56.5[a]	(48.73)[b]	62.3	(52.09)
Bacillus sp. (Bsp-8)	69.5	(56.47)	68.1	(55.60)
B. subtilis (Bs-12)	56.6	(48.77)	64.3	(53.33)
B. subtilis (Bs-17)	61.5	(51.64)	60.8	(51.22)
B. cereus (Bc-24)	61.5	(51.64)	62.2	(52.08)
Bacillus sp. Bsp-25	73.0	(58.71)	73.0	(58.66)
B. pumilus Bp-30	61.5	(51.64)	60.1	(50.63)
B. polymyxa Bp-36	56.5	(48.73)	57.2	(49.13)
Bacillus sp. Bsp-38	59.5	(50.38)	61.7	(51.79)
Nutrient broth	49.9	(44.98)	48.6	(44.18)
Monceren	78.9	(62.85)	76.7	(61 13)
Control	43.5	(41.29)	42.8	(40.83)
Mean	60.9	(51.30)	61.7	(51.74)
L.S.D. (p = 05)		5.60		5.66
(p = 01)		7.54		7.62

[a] Surviving seedlings, was calculated as percentage of the planted seeds in a random row
[b] Arc sine-transformed data.

Data in Table 6 show that Bacillus strains significantly increased straw yield per feddan as compared with untreated control. Bsp-8 and Bsp-25 strains were effective in increasing straw yield per feddan and its components. On the other hand, fungicide Monceren treatment gave the maximum straw yield per feddan, while the lowest value obtained from the untreated treatment. Data in Table 7 indicated that Bsp-25 strain significantly increased fiber percentage, fiber length (cms), fiber yield per feddan (kgs) and fiber fineness.

Table 6: Mean values of yield and yield components of flax from combined analysis over seasons

Characters / Treatments		Technical stem length (cm)	Stem diameter (mm)	Straw yield/plant (gm)	Straw yield/fed. (tons)	Fruiting zone length (cms)	Number of capsules/plant	Number of seeds/plant	Seed yield/plant (gm)	Seed index (weight 1000) seeds)	Seed yield/fed. tons
B. cereus (Bc-3)		78.77	2.10	2.23	3.286	16.78	23.87	196.69	1.821	9.16	0.784
Bacillus sp. (Bsp-8)		90.81	2.02	2.78	3.728	10.03	14.28	117.81	1.148	9.12	0.584
B. subtilis (Bs-12)		76.78	2.11	2.17	3.187	18.51	25.86	213.35	2.021	9.16	0.786
B. subtilis (Bs-17)		85.67	2.06	2.68	3.689	11.03	17.58	145.04	1.363	9.13	0.654
B. cereus (Bc-24)		91.28	2.05	2.72	3.712	10.89	16.36	134.97	1.296	9.12	0.644
Bacillus sp. Bsp-25		92.83	2.01	2.88	3.890	9.87	12.79	106.27	1.038	9.11	0.591
B. polymyxa Bp-30		83.96	2.07	2.69	3.607	12.81	19.76	163.19	1.533	9.14	0.674
B. polymyxa Bp-36		74.81	2.12	2.09	3.071	19.38	29.74	245.36	2.286	9.18	0.863
Bacillus sp. Bsp-38		80.81	2.09	2.31	3.367	14.96	21.81	179.93	1.663	9.15	0.711
Nutrient broth		72.78	2.13	2.00	2.986	17.22	38.78	319.94	2.979	9.21	0.987
Monceren		96.78	1.99	2.96	4.071	8.96	10.78	88.94	0.840	9.10	0.521
Control		82.78	2.08	2.49	2.662	13.07	20.07	165.58	1.599	9.14	0.682
L.S.D.	0.05	2.81	NS	0.09	0.622	2.85	0.21	0.02	0.209	0.12	0.123
	0.01	3.77	NS	0.12	0.836	3.83	0.32	1.37	0.324	0.16	0.189

Table 7: Mean values of technological characters of flax from combined analysis over seasons

Characters / Treatments		Fiber percentage	Fiber length (cms)	Fiber yield/fed. (kgs)	Fiber fineness
B. cereus (Bc-3)		10.33	71.51	339.44	230.81
Bacillus sp. (Bsp-8)		11.76	81.50	443.28	260.78
B. subtilis (Bs-12)		10.58	74.52	356.23	242.71
B. subtilis (Bs-17)		11.23	78.39	414.28	258.81
B. cereus (Bc-24)		11.89	83.53	443.26	260.42
Bacillus sp. Bsp-25		12.78	85.55	485.14	271.82
B. polymyxa Bp-30		10.98	76.68	396.05	251.71
B. polymyxa Bp-36		10.18	69.50	324.44	225.81
Bacillus sp. Bsp-38		10.73	75.50	366.11	248.11
Nutrient broth		10.12	67.53	310.78	220.81
Monceren		13.10	89.50	5433.30	282.18
Control		9.98	65.50	298.00	217.18
L.S.D.	0.05	0.20	1.06	11.52	4.44
	0.01	0.27	1.43	15.48	5.96

These results are in agreement with previously reported results by other workers (Wolk and Sarkar, 1993; Fukui *et al.*, 1994; Pierson *et al.*1995; Sedra and Maslouhy, 1995 and Yeom-Ju Rip et *al.*, 1995). A positive correlated was observed between seedling survival (stand) and each of straw yield and its components. On the other hand, the correlation was negative between stand and each of seed yield and its components (Table 8). These correlations are in agreement with the results of El-Kassaby *et al.* (1999), Abul-Dahab (2002) and Kineber (2003). These results imply that the increase in seedling stand by *Bacillus* spp. led to quantitative increase in straw yield and qualitative improvement in its technological traits.

Table 8: Correlation coefficient (r) between flax seedling survival and each of yield and yield components and technological characters under field conditions.

Seedling survival %	r
Characters	
Yield and yield components:	
1. Technical stem length (cms)	0.810 **
2. Stem diameter (mm)	- 0.790 **
3. Straw yield per plant (gm)	0.682 *
4. Straw yield per feddan (tons)	0.929 **
5. Fruiting zone length (cms)	- 0.635 *
6. Number of capsules per plant	- 0.762 **
7. Number of seeds per plant	- 0.761 **
8. Seed yield per plant (gm)	- 0.771 **
9. Seed index weight (1000 seeds)	- 0.701 **
10. Seed yield per feddan (tons)	- 0.716 **
Technological characters:	
1. Fiber percentage	0.899 **
2. Fiber length (cms)	0.415 NS
3. Fiber yield per feddan (kgs)	0.922 **
4. Fiber fineness	0.924 **

- Linear correlation coefficient (r) is significant at $p < 0.01$ (**) or $p < 0.05$ (*).

REFERENCES

Abul-Dahab, A.A. 2002. Effect of seedling rate on yield of some flax cultivars. J. Agric. Sci., Mansoura Univ. 27(4) 2005-2017.

Afify, Aida H. and A.Z.A. Ashour. 1995. Biological and chemical control of *Rhizoctonia solani* of cotton. J. Agric. Sci., Mansoura Univ. 20: 1441-1452.

Ashour, A.Z.A. and Aida H. Afify. 1999. Use of *Bacillus* strains mixture to improve biological control of cotton seedling disease. The second International Conference on Fungi. The regional center for mycology and biotechnology. The African Journal of Mycology and Biotechnology. 7(3): 109-120.

Baker, C.J.; J.R. Stavely and N. Mock. 1983. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. Plant Disease 69: 770-772.

El-Kassaby, A.T.; M.H. Ghoneima; M. El-Farouk and A.S. Mostafa. 1999. Response of some flax genotypes to planting dates and seedling rates. J. Agric. Sci., Mansoura Univ. 24(4): 1541-1548.

Fravel, D.R. 1988. Role of antibiosis in the biocontrol of plant diseases. Annual Review of Phytopathology. 26: 75-91.

Fukui, R.; M.N. Schroth; M. Hendson and J.G. Hancock. 1994. Interaction between strains of Pseudomonads in sugar beet spermospheres and their relationship to pericarp colonization by *Pythium ultimum*. Phytopathology 84: 1322-1330.

Harry, W.; Jr. Seeley and J. Paul Van Denmark. 1989. Microbes in action a laboratory manual of microbiology 3rd ed. First Published in the Unites States b W.H. Freeman and Company New York and Oxford.

Hiraoka, H.; O. Asaka; T. Ano and M. Shoda. 1992. Characteristics of *Bacillus subtilis* RB 14 Coproducer of peptide antibiotics iturin A and surfactin. J. Gen. Appl. Microbiol. 38: 635-640.

Holt, J.G.; N.R. Krieg; P.H.A. Sneath; J.T. Staley and S.T. Williams eds. 1994. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore.

Kado Clarens, I.; W.C. Schnathort and R. Azad Hamid. 1987. Method of using *Bacillus polymyxa* 9A to protect against Verticillum wilt. The regents of the University of California. Patent USA No. 589606. 14-03-84.

Katz, E. and A.L. Demain. 1977. The peptide antibiotics of *Bacillus*: chemistry biogenesis and possible functions. Bacteriological Review 41, 449-474.

Kineber M.E.A. 2003. Flax plants performance as influenced by planting methods and seedling rate. J. Agric. Res. Tanta Univ. 29(1): 64-73.

Leifert, C.; H. Li; Siripun Chidburee; S. Hampson; Susanne Workman; D. Sigee; H.A.S. Epton and Agnes Harbour. 1995. Antibiotics production and biocontrol activity by *Bacillus subtilis* CL 27 and *Bacillus pumilus* CL 45. J. of Appl. Bact. 78: 97-108.

Mew, T.W. and A.M. Rosales. 1986. Bacterization of rice plants for control of sheat blight caused by *Rhizoctonia solani*. Phytopathology, 76(11): 1260-1264.

Nyvall, R.F. 1981. "Field Crop Diseases Handbook". Avi Publishing Company, INC Connecticut. pp: 436.

Pierson, A.M.; L. Thomashow, R.J. Cook and T.W. Mew. 1995. Isoaltion and identification of antifungal metabolites produced by rice-associated antagonistic *Pseudomonas* spp. Phytopathology 85: 1028-1032.

Pusey, P.L.; C.L. Wilson, M.W. Hotchkiss and J.D. Franklin. 1986. Compatibility of *Bacillus subtilis* for post harvest control of peach brown rot with commercial fruit waxes dicloran and cold-storage conditions. Plant Disease 70: 587-590.

Raffel, S.J.; E.V. Stabb; J.L. Milner and J. Handelsman. 1996. Genotypic and phenotypic analysis of Zwittermicin A- producing strains of *Bacillus cereus*. Microbiology Rending 142:12, 3425-3436.

Sedra, M.H. and M A. Maslouhy. 1995. Fusarium wilt of date palm (bayoud). Inhibitory activity of filtrates of six antagonistic microorganisms isolated from Marrakech date palm grove soils towards *Fusarium oxysporum* f.sp. *albedinis*. Al-Awamia. No. 90, 1-8.

Silo-shu, L.A.; B.J. Lethbridge; S.J. Raffel; H. He; J. Clardy and J. Handelsman. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW 85. Appl. Environ. Microbiol. 60: 2023-2030.

Sivamani, E. and S.S. Gnanamanickam. 1988. Biological control of *Fusarium oxysporum* f.sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. Plants and Soil 107, 3-9.

Utkhede, R.S. and P.L. Sholberge. 1986. *In vitro* inhibition of plant pathogens: *Bacillus subtilis* and *Enterobacter aerogenes in vivo* control of two post harvest cherry disease. Canadian Journal of Microbiology 32: 963-967.

Wolk, M. and S. Sarkar. 1993. Antagonism *in vitro* of *Bacillus* spp. against *Rhizoctonia solani* and *Pythium* spp. Anzeiger-fur Schadingskunde, Pflanzenschutz, Umwelt schutz. 66: 121-125.

Yeom, Ju Rip; J.R. Park Chang Seuk, J.R. Yeom and C.S. Park. 1995. Enhancement of plant growth and suppression of damping-off of cucumber by low temperature growing *Pseudomonas fluroescens* isolates. Korean J. Plant Pathol., 11: 252-257.

المقاومة الحيوية لمرض لفحة بادرات الكتان

عبد الودود زكى عبد الله عاشور* عايدة حافظ عفيفى محمد السيد أحمد قنيبر*****

*** معهد بحوث امراض النباتات– مركز البحوث الزراعية– الجيزة – مصر.**

**** قسم الميكروبيولوجى – كلية الزراعة – جامعة المنصورة – المنصورة – مصر.**

***** معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية– الجيزة – مصر.**

درس التضاد بين ٤٢ عزلة من بكتريا *Bacillus* spp. وفطرى *Rhizoctonia solani* و *Fusarium oxysporum* المسببان لمرض لفحة البادرات فى الكتان وذلك تحت ظروف المعمل. تسع عزلات من تلك البكتريا أظهرت تضاد عالى ضد الفطريين الممرضين أما باقى العزلات فإنها لم تظهر تضاد أو أظهرته بمستوى منخفض. عند تقييم العزلات تحت ظروف الحقل والصوبة أظهرت مستويات مختلفة من الكفاءة فى زيادة النسبة المئوية للبادرات السليمة وكذلك المحصول ومكوناته والصفات التكنولوجية للكتان. أظهرت العزلتان *Bacillus* sp. Bsp-8 و *Bacillus* sp. Bsp-25 أفضل تأثير تحت ظروف الصوبة والحقل. استعمال المبيد الفطرى مونسرين أدى إلى زيادة معنوية للنسبة المئوية للنباتات السليمة والمحصول ومكوناته وكذلك الصفات التكنولوجية للكتان مقارنة بعزلات الـ *Bacillus* spp. لوحظ ارتباط موجب بين نسبة الإنبات وكل من محصول القش ومكوناته. تدل نتائج الدراسة على أنه يمكن استخدام البكتريا التابعة لجنس *Bacillus* للمقاومة الحيوية كبديل للمبيد مونسرين لمقاومة فطرى *F. oxysporum, R. solani* المسببان لمرض لفحة البادرات ولتحسين الخواص التكنولوجية للقش مع تجنب التأثيرات الجانبية الغير مرغوبة للمونسرين .