BIOCONTROL OF *FUSARIUM* DAMPING-OFF OF PEA BY CERTAIN BACTERIAL ANTAGONISTS

BY

El-kot, G. A. N*. and E. B. A. Belal**

Dept. of Agric. Botany, Fac. of Agric. Kafr El-sheikh, Tanta Univ.

* Plant pathology ** Agricultural Microbiology

ABSTRACT

Pea (*Pisum sativum* L.) root rots caused by *Fusarium semitectum*, *F. solani* and *F. moniliforme* are a major yield-limiting factor for field pea production. Different bacterial antagonistic isolates were isolated from rhizosphere-soil samples of healthy pea plants. These antagonistic isolates exhibited antifungal activity against the tested fungal pathogens. The predominant antifungal bacterial isolates belonged to bacilli and pseudomonads. They were identified as *Bacillus subtilis* (isolates No. 9, 10 and 6), *B. pumilus* (No. 1E) and *Pseudomonas* sp. (No. p).

Bioassays were conducted under greenhouse conditions at the experimental greenhouse of Dept. of Agric Botany, Fac. of Agric., Kafr El-Sheihk, Tanta Univ. during the two successive seasons 2004/2005 and 2005/2006 to test the efficacy of antagonists applied to pea seeds for protection against seed rot and seedling damping-off in a field soil artificially infested with the tested pathogens. Seed coating with bacterial cell suspension of (9, 10 and 6) *Bacillus subtilis*, and *B. pumilus* (1E) and *Pseudomonas* sp. (p) (10^8 cfu/ml) significantly decreased pre-, and post- emergence damping-off and increased survival plants, stem length, number of flowers, number of branches as well as dry weight of shoots compared with the untreated seeds grown in pathogen-infested soil (control). Seed treatment with the bacterial isolates *B. subtilis* (No. 6 and 10) was more efficient than the other tested bacterial antagonistics in the most estimated parameters. The effects were similar in more cases to those of Benlate fungicide, which increased germination, emergence and reduced root rot severity. Results of this study suggest that *B. subtilis* is an effective bioagent in controlling damping – off and root rots caused by the tested pathogenic fungi and could be considered as promising alternative to existing chemical products.

Keywords: Bioagents, *Pisum sativum*, *Fusarium* spp., rhizosphere, seed treatment, Biological control

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important winter vegetables in Egypt. It grows well all over the country provinces and is cultivated for green vegetable pods and dry seeds (Hussein, 1973). As production increases and the crop is grown with increasing frequency in the same fields, there has been a greater problem with soil-borne diseases. These diseases, are caused by single or combinations of pathogens such as *Aphanomyces euteiches*, *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Belal *et al.*, 1996) and Xue (2003). For decades, fungicide seed treatments have been the only means to combat soil-borne diseases. However, fungicidal seed treatment is not desirable for disease control due to some adverse effects on the environment and ecosystem, such as harm to nontarget organisms especially beneficial microorganisms such as bioagents, animals, and plants; residues in soil and contamination of the water and food chains. Fungicides also may induce pathogen resistance. making their effects variable and short lived (Cook and Baker 1983, Wilson *et al.*, 1992, Wilson *et al.*, 1993, Baird *et al.*, 1994, Mathre and Johnson 1995 and Belal *et.al.*, 1996, Sesan and Oprea 1998a, 1998b and 1998c, Xue 2001 and El-kazzaz *et al.*, 2002).. In addition, fungicides are expensive in comparison with the relatively low commodity price of field pea. Hence, there is a need for an improved soil-borne management system with reduced fungicide use.

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Naim 1966, Cook and Baker 1983 and Osman et al., 1986). Accordingly, it is desirable to identify a microorganism that displays antagonistic effects against target pathogens and is capable of survival and propagation in target locations. Several fungi and bacteria have been reported to have antagonistic effects to soil-borne pathogens. Specifically, Windels and Kommedahl (1978) reported the potential use of *Penicillium oxalicum* as a seed protectant against seedling blight of pea. Harman *et al.*, (1980) and Nelson *et al.*, (1988) reported the use of *Trichoderma hamatum* for the control of *Pythium* seed rot and Rhizoctonia root rot in pea. Parke *et al.*, (1991) and Xi et al. (1996) noted that *Pseudomonas cepacia, P. fluorescens* and *Bacillus subtilis* were effective against the different pathogenic fungi such as *Pythium*, *Aphanomyces*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Fusarium oxysporum f. spp.* when applied as seed treatment (Papavizas *et al.*, 1984, Callan *et al.*, 1990, lewis *et al.*, 1991 Parke *et al.*, 1991, Hebbar *et al.*, 1992, Bowers and Parke, 1993 Baird *et al.*, 1994, Jackson and Schisler, 1995, lewis *et al.*, 1995, Lumsden *et al.*, 1995, Mathre and Johnson, 1995, Belal *et al.*, 1996, Farvel *et al.*, 1996 El-kazzaz *et al.*, 2002, and Tah-Nagllaa, 2004). Therefore, the present investigation was designed to investigate the potential of seed treatment with the biocontrol agents to reduce damping-off caused by *Fusarium solani, F. moniliforme* and *F. semitectum.*

MATERIAL AND METHODS

Pathogens

Samples of pea plants showed typical damping-off and root rotted were collected from different locations at kafr El-Sheikh governorate, Egypt. Small pieces of the diseased samples were kept in a damp chamber for 48h. After that, hyphal tip of each isolate from the surface growing fungus was transferred onto Potato Dextrose Agar medium (PDA). After 2-3days of incubation at 25-28°C, the isolates were purified by the hyphal-tip method according to Booth (1977) and the pure cultures were transferred on PDA. Cultural, morphological, microscopical and phytopathlogical properties were considered to identify the isolated pathogens according to Burgess *et al.*, (1994). Pathogenicty tests were performed on cv. Master B and reisolated pathogenic fungi were maintained on PDA slants at 4°C for further experiments according to the method described by Khalifa (1991).

Antagonists

Screening and isolation

Antagonists were isolated from rhizosphere soil samples of healthy pea plants by the methods described by Belal *et al.* (1996). The relative power of antibiosis (RPA) was estimated for each isolate as described by Ibrahim *et al.*, (1987). where: RPA = Z/C, Z = diameter of inhibition zone, C = diameter of spotted antagonistic isolate.

Identification

Isolates having broad antagonistic spectra with the highest RPA were identified according to Parry *et al.*, (1983) and Bergy's manual of systematic bacteriology (1984).

In Vitro, Effect of different pesticides on growth of bacterial antagonists

100µl (72 hour - old culture) for each isolate (10^8cfu/ml) were spreaded on nutrient agar plates by using driagisky triangle. After that, 50µl from each pesticide (Silicron and malathion Insecticides), Punch as fungicides, Stomb as herbicide) with recommended dose were putted in wells (5mm in diameter) in nutrient agar medium and then the plates were incubated at 28-30°C and examined daily. The inhibition zone was recorded between the isolates after 5days as incubation periods. Three replicates were used for each isolate.

Effect of different pH on growth of bacterial antagonists

100ml from fructose mineral medium with different pH4, 7 and 8 were used to determine the optimum pH for the antagonistic isolates according to DSMZ, 2004-catalogue medium No.55 with replacing fructose by glucose supplemented with 10gm glucose/L for bacterial isolates. The total microbial count in all treatments was determined after 72h using dilution series on fructose mineral medium.

Control of the tested pathogens on pea plants

Antagonistic, exhibiting broad spectra and high relative power of antagonism were used to control the tested pathogens in pot experiments. Pots (15cm in diameter were used in the season 2004/2005 and 30cm diameter were used in the second season 2005/2006) were filled with clay soil and Master B pea cultivar seeds were planted (5seeds/pot). Pathogens were added to soil one week before planting. Inocula of pathogens were prepared by growing on corn meal sand medium at 28-30°C for 15 days. Soil infestation was carried out one week before planting at the rate 3% (Khalifa 1991 and Belal *et al.,* 1996) Infested soil was mixed thoroughly and moistened every other day. Antagonists were applied at the time of planting as seed treatment. Seeds were immersed in each bacterial suspension (10^8cfu/ml) for 30min. and then dried. Seeds were then sown in each pot (10 seeds/pot). Benlate, the most used fungicide for controlling soil-borne diseases of plants (Khalifa 1987), was applied for comparison as seed treatment at the recommended dose (1g 50%wp benomyl/kg seed). Each treatment was represented by 4 replicates. Pots were kept in greenhouse and watered when needed. Degrees of disease incidence were recorded as percentages of pre- and post- emergence damping-off, and percentage of survival plants were also recoded as well as stem length, number of flowers, number of branches and dry weight of shoots (Hussein, 1973).

Statistical analysis:

The obtained data were subjected to the proper statistical procedures for analysis of variance according to Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Isolation and identification of the causal organisms

Isolation trials, carried out on damped-off, root-rotted pea plants, collected from different locations at Kafr El-Sheikh Governorate, Egypt, resulted in the isolation of seven fungal isolates belonging to the genus *Fusarium* as shown by preliminary microscopic examination. The isolated *Fusarium* isolates identified as *Fusarium solani, F. moniliforme and F. semitectum.*

Screening and identification of bacterial antagonists

The initial screening of more than 100 bacterial originated from different rhizosphere-soil samples of healthy pea plants, resulted in the isolation of 20 isolates exhibiting obvious antagonistic action on plates against one or more of the tested pathogens of pea. Only five isolates could antagonize all the tested pathogens of pea. Results of identification (Table 1 and 2) showed 3 isolates (9, 10 and 6) as *Bacillus subtilis*, and one (1E) as *B. pumilus* and (p) as *Pseudomonas* sp.. The anatagonistic activity of *Bacillus* and *Pseudomonas* was recognized by many investigators. Many species of genus are known to be potent producers of many antibiotics against bateria and fungi

Many biocontrol species of *pseudomonads* produce extracellular secondary metabolites that inhibit the growth of fungal pathogens such as Pyrrolnitrin, pyoluteonin and Pyoverdine (Howell and Stipovonich, 1979 and 1980 and Kraus and Loper, 1992)

Table (1) Morphological characterstics and biochemical activities of the antagonistic tested isolates (No 9, 10, 6 and 1E)

Test	(Isolate No. 9 and 10, 6)	Isolate No. 1E
Shape of cell	Rods	Rods
Sporulation, Spore shape	+	+
Motility	Motile	Motile
Gram reaction	+	+
Anaerobic growth	-	-
Lecithinase reaction (Lv reaction)	-	-
Citrate utilization	+	+
V.P. reaction	+	+
Nitrate reduction	+	-
Indole production	-	-
Growth in 7%NaCl	+	+
Starch hydrolysis	+	-
Casein hydrolysis	+	+
Gelatin hydrolysis	+	+
Catalase reaction	+	+
Urease activity	+	-

+ positive - negative

Table (2) Morphological characteristics and biochemical activities of the antagonistic tested isolate (No.p)

Test	Isolate No. p
Shape of cell	Short rods
Sporulation	Non – Sporeformer
Motility	Motile
Gram reaction	-
Anaerobic growth	-
Pigment production	+
Oxidase test	+

\+ positive - negative

Efficacy of antagonists against soilborne fungal pathogens

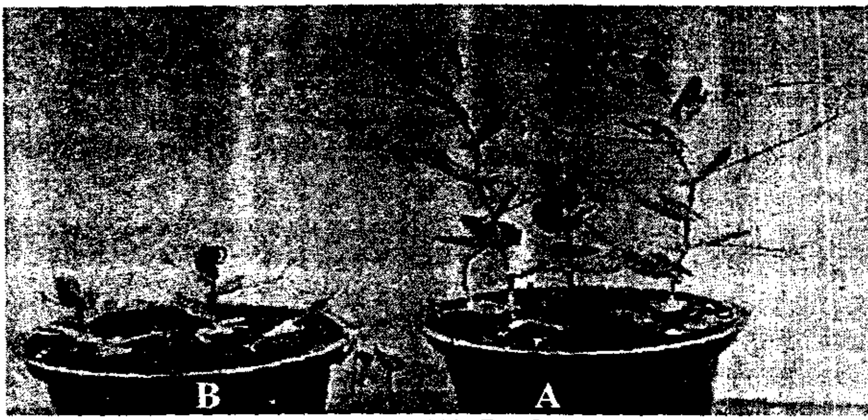
Under greenhouse conditions, the selected antagonists exhibited their efficacy to control the damping – off and root rot disease on pea during growth season 2004/2005 (Fig.1). Treatment of pea seeds with the bacterial antagonists increased emergence of pea seedlings grown in soil infested with each of *F. solani*, *F. moniliforme* and *F. semitectum.* These effects were significant for most pathogens and were similar to or less than those obtained with Benlate Fungicide. Although the bioagent appeared to reduce severity of root rot caused by most of the tested pathogens, the effect was statistically significant only for seed treatment with two *Bacillus subtilis* isolates (No. 6 and 10) and *Pseudomonas* sp. (p) but the isolate *B. subtilis* (9) did not significantly reduce pre-emergence damping - off caused by *F. moniliforme*. Also, the same isolate completely prevented the incidence of post-emergence damping – off caused by the tested pathogenic fungi (Table 3). The efficient antagonists in controlling damping – off of pea plants were *B. subtilis* (No. 6 and 10) and *Pseudomonas* sp. (p). Data showed also that seed inoculation of pea at the time of planting significantly increased the % of survival plants from 31 – 75, 33 – 92 and 17 -75 in soil infested with *F. solani*, *F. moniliforme* and *F. semitectum,* respectively.

Table (3) Effect of seed treatment with different antagonists on disease incidence by the tested pathogenic fungi during 2004/2005 season.

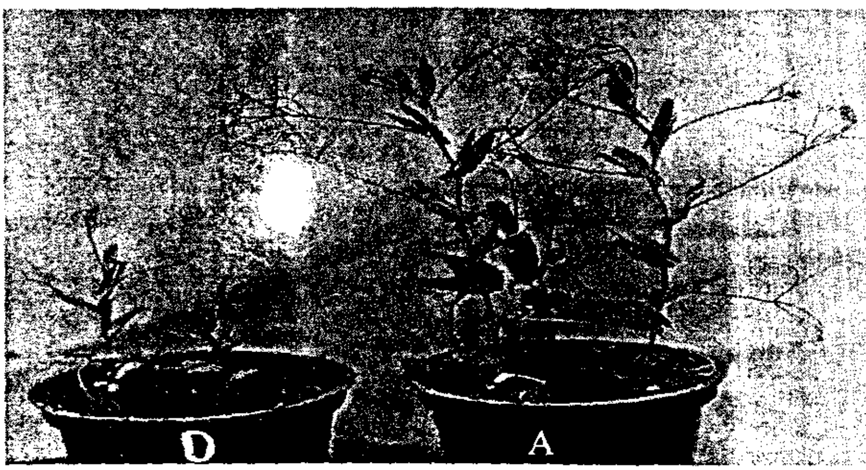
Treatments	**% Disease expressions**								
	Fusarim solani			***F. moniliforme***			***F. semitectum***		
	Pre- *	Post- **	Surv. ***	Pre- *	post **	Surv. ***	Pre- *	Post **	Surv. ***
F. solani	50e	18c	31a	33d	34d	33a	25b	58e	17a
B. pumilus(1E)	42d	0a	58c	42e	11c	47b	34c	11b	55c
B. subtilis (9)	33c	0a	67e	33d	0a	67c	33c	0a	67d
B.subtilis(10)	33c	11b	55b	25c	0a	75d	25b	0a	75e
B. subtilis (6)	25b	0a	75f	17b	0a	83e	25b	0a	75e
Psedomonas sp. (p)	25b	11b	64d	8a	0a	92f	33c	34d	33b
Benlate	10a	12b	78g	7a	9b	84e	11a	15c	74e

Values having the same alphabetical letters within column are not significantly different ($P < 0.05$).
*Pre-emeregence damping –off, **post-emergence damping-off
***Survival plants

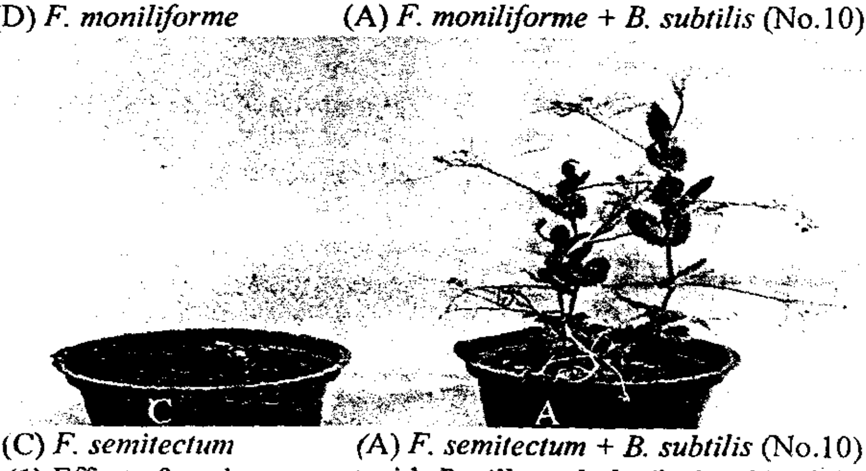
Data presented in Tables 4, 5 and 6 and Fig. 2 reassure the efficiency of the *Bacillus pumilus* (1E), *B. subtilis* (9, 10 and 6) and *Pseudomonas* sp. (p) (10^8cfu/ml) against the soil-borne fungal pathogens when applied as seed inoculation during growth season 2005/2006. The % of survival plants were increased from 38.4 – 76, 35.4 – 87.4 and 17.1 – 79 in soil infested with *F. solani*, *F. moniliforme* and *F. semitectum*, respectively. Data concerning growth parameters showed that the values of growth parameters such as stem length (cm), number of flowers, number of branches and dry weight of shoot (g)/plant increased significantly in inoculated plants with the antagonists compared with non-inoculated control. Inoculation with bacterial antagonists significantly increased stem length of plant from 36.6 – 71.6, 41.6 – 68.3 and 50 – 66.6cm in soil infested with *F. solani*, *F. moniliforme* and *F. semitectum*, respectively, indicating that these antagonists may produce plant growth promoting substances. On the other hand, insignificant differences of stem length values were recorded between some antagonists.



(B) *Fusarium solani* (A) *F.solani* + *B. subtilis* (No.10)



(D) *F. moniliforme* (A) *F. moniliforme* + *B. subtilis* (No.10)



(C) *F. semitectum* (A) *F. semitectum* + *B. subtilis* (No.10)

Fig. (1) Effect of seed treatment with *Bacillus subti'is* (isolate No. 10) on disease incidence by the tested *Fusarium* spp..

From the foregoing results, it can be concluded that the % disease expression representing in pre- and post- emergence damping - off was reduced by the application of the isolated antagonists at the time of planting as seed treatment. Moreover, the growth parameters of pea plants were also significantly increased by inoculation with these antagonistic bacterial isolates.

The efficiency of the antifungal isolates *Bacillus pumilus* (1E), *B. subtilis* (9, 10, 6) and *Pseudomonas* sp. (p) to protect pea plants against damping – off and root rot has been clearly proved throughout the present study. Also, Attempts had been successfully carried out using antagonists to control soil – borne fungal pathogens on pea (Harman *et al.*, 1991, Belal *et al.*, 1996, Mao *et al.*, 1997, Xue, 2001 and 2003).

The obtained results of this study suggest that the tested bacterial isolates proved to be an effective bioagents in controlling the tested pathogenic fungi and could be considered an alternative to existing chemical products and hence it can reduce the environmental pollution resulting of using fungicide in controlling plant diseases.

Table (4) Effect of seed treatment with the antagonists on disease incidence by *Fusarium solani* during 2005/2006 season.

Treatments	% Disease expressions			Growth Parameters after 70 days			
	Pre-*	Post **	Surv ***	Stem length (cm)	No. of flowers/ plant	No. of branches/ plant	Dry weight of shoot/ plant (g)
F. solani	43.3f	18.3f	38.4a	36.6a	13a	1.6a	8.2a
B. pumilus(1E) +*F. solani*	38e	2.3c	76d	63.3c	21c	3.6d	8.9b
B. subtilis(9)+ *F. solani)*	31d	2b	67c	43.3b	20.3c	2.6b	9.3c
B. subtilis(10) +*F. solani*	31d	9.3d	59.7b	71.6f	24.3e	5f	9.9e
B. subtilis(6) +*F. solani*	2ce	0a	76d	70.3e	20.3c	3.3c	10.3f
Psedomonas sp. (p) + *F. solani*	21b	12e	67c	43.3b	18.6b	4.6e	9.6d
Benlate + *F. solani*	a⁹	12e	79e	64.5d	22.4d	3.2c	9.7d

Values having the same alphabetical letters within column are not significantly different ($P < 0.05$).

*Pre-emeregence damping –off, **post-emergence damping-off

***Survival plants

Table (5) Effect of seed treatment with the antagonists on disease incidence by *Fusarium moniliforme* during 2005/2006 season.

Treatments	% Disease expressions			Growth Parameters after 70 days			
	Pre-*	Post **	Surv ***	Stem length (cm)	No. of flowers/ plant	No. of branches/plant	Dry weight of shoot/plant (g)
F. moniliforme	34f	30.6 g	35.4a	41.6a	15.6a	1.6a	5.4a
B. pumilus(1E) +*F. monil.*	32e	9.6f	58.4b	66.6f	18b	2.6b	9.7d
B. subtilis(9) + *F. monil.*	27d	1.3b	71.7c	68.3g	23.3d	3.6c	7.1c
B.subtilis(10) + *F. monil.*	20c	3d	77d	58.3e	20c	3.6c	9.6d
B. subtilis(6) + *F. monil.*	12.6 b	0a	87.4g	53.3b	18b	2.6b	6.9b
Psedomonas sp. (p) + *F. monil.*	11.3 b	2.6c	86.1fc	56.6c	20.3c	3.6c	12.6f
Benlate +*F. monil.*	6.9a	7.8e	85.3e	58.1d	19.3c	2.6b	10.1e

Table (6) Effect of seed treatment with the antagonists on disease incidence by *Fusarium semitectum* during 2005/2006 season.

Treatments	% Disease expressions			Growth Parameters after 70 days			
	Pre-*	Post **	Surv ***	Stem length (cm)	No. of flowers/ plant	No. of branches/plant	Dry weight of shoot/plant (g)
F. solani	30.6f	52.3f	17.1a	50a	17a	1.6a	6.9a
B. pumilus (1E) +*F. semitectum*	28.3e	9c	62.7c	66.6f	19.3c	2.6b	10d
B. subtilis (9) +*F. semitectum)*	30.3f	1.6a	68.1d	56.6b	19.6c	3.6c	10.9f
B. subtilis (10) +*F. semitectum*	23c	2.3b	74.7g	63.3e	19.6c	2.6b	9.9c
B. subtilis (6) +*F. semitectum*	20b	1a	79f	56.6b	18b	2.6d	11.9g
Psedomonas sp. (p) +*F. semitectum*	26.6d	24.6 e	48.8b	63.3d	19.6c	1.6a	9b
Benlate +*F. semitectum*	13.2a	16.1 d	70.7e	58.4c	19.5c	2.6b	10.2e

Values having the same alphabetical latter within column are not significantly different ($P < 0.05$).

*Pre-emeregence damping –off, **post-emergence damping-off

***Survival plants

Fig. (2) Effect of seed treatment with *B. subtilis* (No. 10) on pea plant parameters., where A: *Bacillus subtilis* (No. 10) + *F. solani*, B: *F. solani*.

Effect of some environmental factors on activity of antagonists

Disease suppression depends on the prevailing environmental conditions such as pH in the soil and biological components, including all root-colonizing plant-beneficial bacteria and fungi. The influence of pH on growth of the bacterial antagonistics is shown in Fig (3). Generally, pH7 was the optimal for all isolates. The maximum total microbial count as cfu/ml was recorded at pH7. Most of the bacterial strains are known to prefer the neutral pH. The measured pH of the used soil in this work was pH7.5. Therefore, it can be deduced from the results that the pH is considered an important environmental factor in the rhizosphere affect on the efficiency of the bioagents.

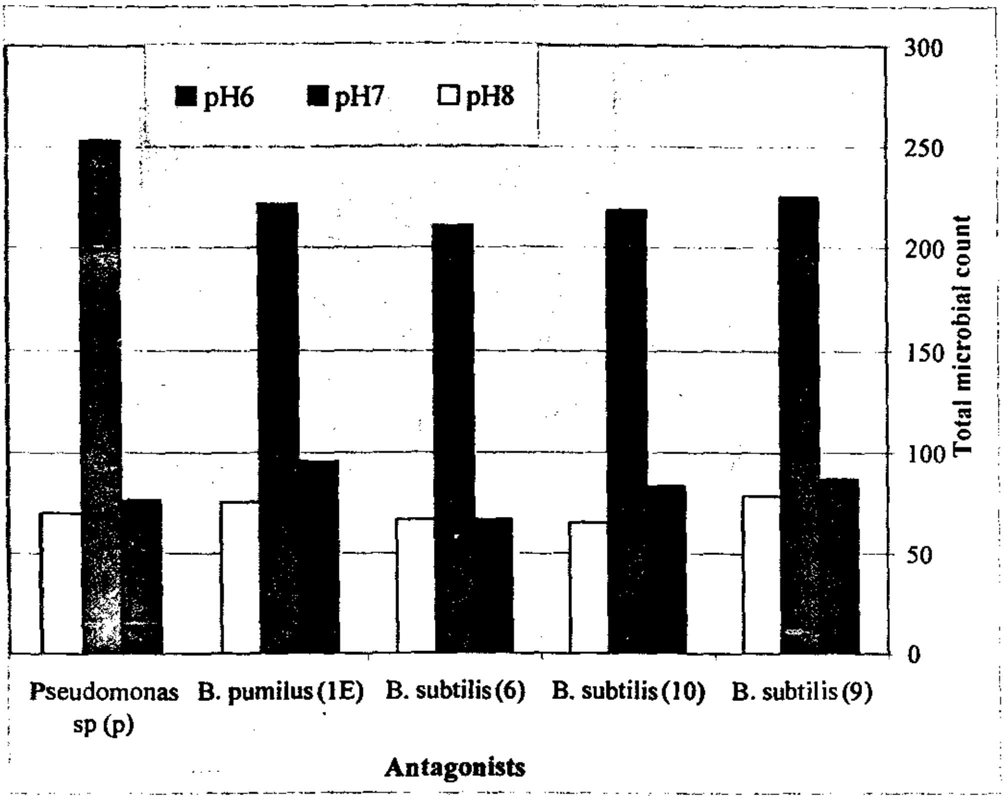


Fig. (3): Effect of pH on growth of bacterial antagonistics (counts 10^6 cfu/ml).

Effect of some pesticides on growth of bacterial antagonists

Application of pesticides has a pronounced harmful effect on many beneficial organisms such as antagonists. Results in Table 7 and Fig 4 showed the effect of some pesticides such as Insecticide (Silicron and Malathion), fungicide (Punch) and herbicide (Stomb) applied at recommended doses on growth of the bacterial antagonists grown on nutrient agar plates by recording of inhibition zone (mm). Generally, growth of all the tested bacterial antagonists was inhibited by the most tested pesticides. *B. subtilis* or *B. pumilus* were inhibited by all the tested pesticides, whereas *Pseudomonas* sp. was inhibited by only the two tested insecticides, i.e Silicron and Malathion was tolerant to Punch and Stomb and probably degrade them.

Table (7) Inhibition of bacterial antagonistic bacterial by some pesticides

Antagonists	Inhibition zone (mm)			
	Silicoron	Malathion	Punch	Stomb
Bacillus subtilis (9)	14 a	7.5 a	7.5 b	8.5 a
B. subtilis (10)	11 bc	5.5 b	10 a	5.5 b
B. subtilis (6)	12.5 ab	7.5 a	6.5 b	7.5 a
B. pumilus (1E)	9.5 c	5.5 b	6 b	5.5 b
Pseudomonas sp.(p)	10 c	6.5 ab	0 c	6.5 ab

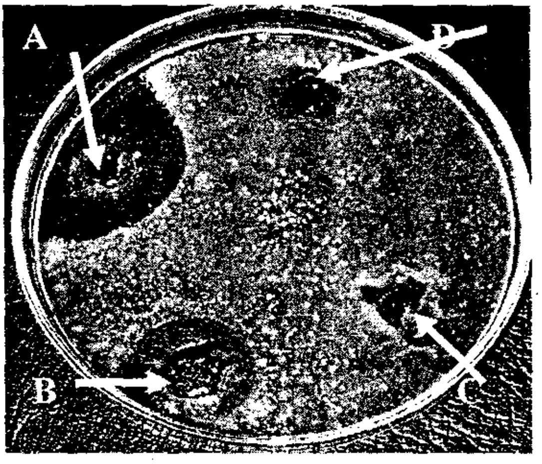


Fig. (4) Effect of some pesticides on *Pseudomonas* sp. on nurient agar medium, where A: Silicron, B: Malathion, C: Stomb, and D: Punch.

The obtained results indicate the necessity of applying such promising bioagents effective in suppressing the tested fungal soil pathogens and could be an alternative to chemical fungicides.

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الملخص العربي

المقاومة الحيوية لمـرض تسـاقط بادرات البسلة المتسببة عن أنواع من الفيوزاريوم باستخدام كائنات التضاد الحيوي البكتيرية

* جبر عبد الونيس نصر القط ** السيد بلال عبد المنطلب بلال
قسم النبات الزراعي- كلية الزراعة بكفر الشيخ- جامعة طنطا
* أمـــراض نبات ** ميكروبيولوجيا زراعية

يعتبر أعفان جذور البسلة المتسببة عن *Fusarium solani*، *F. semitectum*، *F. monliforme* عامل محدد لإنتاج البسلة. تم عزل عزلات مضادة بكتيرية مختلفة من عينات تربة الرايزوسفير لنباتات البسلة السليمة. هذه المضادات أظهرت قدرة تضادية ضد الفطريات المختبرة المسببة للأمراض. تنتمى هذه العزلات البكتيرية المضادة للفطريات إلى كل من مجموعتى الباسيلاى والبسيدومونات. وهذه العزلات تم تعريفها علـى أنها *Bacillus subtlies* (عـزلات أرقـام ٩، ١٠، ٦) و*B.pumilus* (رقـم 1E) و*Pseudomonas* sp. (رقم P).

أجريت الدراسة تحت ظروف الصوبة بقسم النبات الزراعى- بكلية الزراعة بكفر الشيخ جامعة طنطا أثناء موسم نمو ٢٠٠٤/ ٢٠٠٥، ٢٠٠٥/ ٢٠٠٦ لاختبار تأثير الكائنات المضادة للبكتيريا المعامل بها بذور البسلة للحماية من عفن البذرة وتساقط البادرات فى تربة حقل معداه صناعيا بالمسببات المرضية المختبرة. أدت معاملة تغليف البذور بكل من معلق الخلايا لتلك العزلات البكتيرية المضادة كل على حده بتركيز (١٠ ^ cfu/ مل) *Bacillus subtlies* (أرقام ٩، ١٠، ٦) و*B.pumilus* (رقم 1E) و*Pseudomonas* sp (رقم P) إلى تقليل تساقط البادرات وزيادة نسبة النباتات الباقية وذلك بالاضافة إلى زيادة المقاييس النباتية مثل طول الساق، عدد الأزهار، عدد الأفرع وأيضاً الوزن الجاف للمجموع الخضرى وذلك مقارنة بالمعاملات الغير معاملة (كنترول).

كانت معاملة البذور بواسطة *Bacillus subtilis* (أرقام ١٠، ٦) كانت الأكثر كفاءة وتأثيراً عن العزلات البكتيرية المضادة الأخرى فى كثير من المقاييس التى تم تقديرها.

التأثير كان متشابه إلى حد ما فى حالات كثيرة بالتأثير الذى أحدثه المبيد والذى أدى إلى تقليل الشدة المرضية المتسببة عن تلك الفطريات.

أظهرت النتائج المتحصل عليها من هذه الدراسة مدى تأثير العوامل الحيوية المختبرة على مقاومة تلك المسببات المرضية الفطرية والتى يجعل من استخدامها بديل للمواد الكيماوية.