Occurrence and Distribution of Potato Brown Rot and its Biological Control in Egypt

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Received: 4/5/2011

Abstract: Brown rot of potato caused by *Ralstonia solanacearum* race 3 biovar 2 is among the most serious disease of potato worldwide. *R.solanacearum* was higher detected in potato tuber samples than rhizosphere samples. The disease distributed in most Egypt governorates i.e El-Monufia,El-Beheira, Ismailia and El-Sharkia. Potato varieties were more susceptible than tomato plants.Five antagonistic microorganisms (*Bacillus subtilis* (BS1), *Bacillus subtilis* (BS2), *Streptomyces* grisus, *Trichodema harzianum* and *T. viridi* were tested on King's B (KB) and Glucose nutrient agar (GNA) media for the inhibition of *R. solanacearum* growth. *Trichodema harzianum* and *T. viridi* were the most inhibitor to *R. solanacearum* in the two media (KB and GNA).Streptomycin was the most effective antibiotic in controlling potato bacterial brown rot disease compared with Ampicillin and Pencillin.Also extract of garlic,camphor and neem reduced the percentage of brown rot disease incidence.

Keywords: *Ralstonia solanacearum*, Biological control

INTRODUCTION

Potato *(Solanum tuberosum* L.) is considered one of the major vegetable crop in Egypt. Egypt is the largest potato producer in Africa, its annual cultivated area reached about 250.000 feddans, which produce more than two million tons of potato tubers. Egyptian potatoes were exported to European Union and Arabian countries (Balabel, 2006). Shehata,(2007) mentioned that potato brown rot caused by *Ralstonia solanacearum* is a major disease in Egypt, where consumption potatoes are grown for the European market. Potatoes are Egypt's largest horticultural export crop Yet, the total value of Egyptian potato exports fell from a peak value of US$ 102.12 million in 1995 to US$ 7.7 million in 2000 mainly due to quarantine restrictions on the potato brown rot imposed by the European Union (EU) which used to account for about 70-90% of Egyptian potato exports. *Ralstonia solanacearum* is a soilborne bacterial pathogen that is a major limiting factor in the production of many crop plants around the world, and cause a problem in developing countries, where control measures are inadequate (Van Broekhuizen *et al.*, 2002). Hsu (1991) reported that brown rot is one of the most destructive and widespread bacterial disease of plants, affecting numerous crops including tomato, tobacco, potato, pepper, eggplant, groundnut, streltiza, strawberry and seseme. and chilli (capsicum) seedling , all showed typical symptoms of brown rot.

Yingdong and Liyuanm (1995) applied some species of *Bacillus* to control bacterial wilt in potato and tomato. They reported that, isolates of *Bacillus* sp. in use had obvious control effect on tomatoes and significantly increased tuber yield up to 40%in potato compared with untreated potato plants. Laboratory studies showed that the use of antagonistic bacterial (*Bacillus subtilis* (BI), *Pseudomonas* spp. and *Pseudomonas cepacia* (*Burkholderia cepacia*) had highly inhibitor effect against *R. solanacearum* on culture medium. Signification reduction in the number of the wilted plants was achieved in green house tests when the antagonistic bacteria were applied to tomato seedlings, which were then infested with *R. solanacearum* .Similary, Mahmoud,(2007) reported that the bioagents *Pseudomonas fluorescens*, *Pseudomonas putida* and *Bacillus subtilis* were effective in controlling brown rot disease of potato when its used separately and *Pseudomonas putida* was the most efficient. Farag *et al.*, (1986) found that *P. solanacearum* is not sensitive to Chloramphenicol or Penicillin. Both virulent and a virulent form of the pathogen were sensitive to Streptomycin and Dihydrostreptomycin *in vitro* studies. However, contol trials with Streptomycin were made on susceptible early maturing cv.King Edward and tolerant late maturing cv. Alpa increase wilt incidence when antibiotic was added at 400 ppm. A major difficulty with the use of *Bacillus* spp. is that the control provided is often variable, with different results in different locations and indicated that Chloramphenicol at 50 ppm completely inhibited rot development. On the other hand, Ernestina, (2005) mentioned that Camphor is a crystallized substances (aromatic terpene ketone).Similar compounds come from Borneo Camphor *(Dryobalonops aromatica)* and Ngai Camphor *(Blumea balsamifera)* can be found in cavities (wounds) in the trunks of trees. A crystalline extract is made from the wood and leaves [zhang nao] by steam distillation followed by further heating to extract the oil. The aim of this research was to survey and detect of *R.solanacearum* in some different governorates and varietal reaction as well as biological control.

MATERIALS AND METHODS

I-Survey, Isolation and Identification of the Causal Pathogen

Potato tubers was collected from potato fields at various localities of El – Beheira (South Tahrir, Village Om – Saber), El- Menufia (Village Meet – Khalaf) , El-Sharkia (El–Salhia) and Ismalia (Eltal El-Kaber) governorates. All samples were randomly collected and

transferred to laboratory. To detect the occurrence of *R.solanacearum* in the latently infected potato tubers were used in isolation trials.Each 200 tuber/feddan was applied as a sample.Tubers were washed in running tap water, surface sterilized by flaming. Cores of 5-10 mm in diameter and 5 in length, containing main vascular and cortical tissues were macerated in 1.0ml sterile phosphate buffer in sterile plastic bags. Incubation was made at 28öö°C for 48 hours. Cultural and morphological characteristics of the isolated bacteria were studied by re-inoculating them on nutrient agar (NA), King'B (KB) and triphenyl tetrazolium chloride (TZC) (King *et al.*, 1954). Shape of bacterial cells, sporulaton and reaction to gram stain were recorded.

Potato tubers showed symptoms of brown rot disease were used in isolation of *R. solanacearum*. The inoculated bacterium was re-isolated from plants, developing showing wilt syndromes typical of brown rot disease. Thin sections taken from potato stems were placed in 1ml of phosphate buffer saline (PBS) 0.01M,for 30 minutes and streaked on Yeast Peptone Glucose Agar (YPGA) medium. Incubation was made at 28 °C / 3 days. The cultures were subjected to complete morphological and biochemical identification tests according to Krieg and Holt (1984).

Slightly raised irregular, white or white with red centers colonies, typical for the described virulent *R.solanacearum* were selected and propagated on glucose nutrient agar (GNA) medium(Dowson, 1957) for 48 hours. The selected cultures were serologically examined using immunofluorescent antibody staining (IFAS) to confirm *R. solanacearum* identity.Positive isolates were tested for pathogenic potential via inculation into potato seedlings (Wenneker *et al.*, 1999).

2-Pathogenisty Test

Potato (*Solanum tuberosum*, L., cv.Sponta and cv. Diamont) and Tomato *(Lycopersicon esculentum*, Mill, cv. Peto 86 and cv.Casl rock), were used in this study and were kindly provided by the Potato Brown Rot Project (PBRP) and Horticulture Res. Inst., Agric. Res. Center, Giza, Egypt. The tested plants were used to determine the virulence of *R.solanacearum* isolates. Germinated tubers were planted in sterilized pots (30 cm diameter) containing sterilized sand /clay soil (v:v). Each pot was separately inoculated with isolate suspention (10^8 CFU/ml) of *R.solanacearum* with 250 ml. inoculum/pot (Michel and Mew, 1998). Inoculum was prepared by growing each bacterial isolate for 48hrs.on (GNA) medium. Healthy potato tubers from different cultivars, were surface sterilized and three plants of potatoes and others of tomato were planted in each pot and three pots were used for each treatment. Control treatment was prepared by applying few drops of sterile water instead of bacteria. All treated and control treatment were evaluated after 70 days from inculation.

Pathogenicity of *R. solanacearum* isolates recovered from potato tubers and rhizosphere area were confirmed by inoculating tomato plants (3 leaves/seedling), grown in pots under greenhouse conditions (Janse,*et al* 1998). Injection was made at the leaf axis by a needle with bacteria. The inoculated plants were covered with polyethylene bags for 3 days at 30°C, then bags were removed and pots were irrigated dialy.

Disease severity was recorded according to scale developed by Marin and El-Nashaar (1992), where 1= No visible symptoms, 2= 1-25% of the plant is wilting, 3= 26-50 wilt, 4= 51-75% wilt and 5= more than 75% wilt. Virulence of each isolate was rated according to its average of performance on 10 plants randomly chosen from two hosts, the used scale was consist of 3 levels, they were avirulent = 1.0, low virulence = 1,1 -2.5, medium virulence = 2.6-4.0 and highly virulence =4.1-5.0 (Kempe and Sequeira, 1983).

3- Biological Control

I- *In vitro*

Isolates of *R. solanacearum* were tested in the laboratory for their ability to grow on Glucose Nutrient Agar (GNA) and Kingś B (KB) media. *R. solanacearum* of (24 hrs.-old) was streaked at the center of plates containing any of the media used and incubated at 28 °C. After 48hrs, the pathogen (24 hrs.-old) was streaked vertically (Hartman *et al.*, 1992).

Isolates of *Bacillus subtilis* (BS_1) and (BS_2), *Streptomyces griseus*, *Trichoderma harzianum* and *Trichoderma virdi* were kindly provided by Biological Control Dept.Plant Pathology Research Inst., Agric.Res.Center, Giza, Egypt.These isolates were tested against *R. solanacearum*. Length of inhibition was measured after 48 hrs from inoculation. Five selected non-pathogenic microorganisms (*Bacillus subtilis* (Bs1), *B. subtilis* (Bs2), *Trichoderma harzianum*, *T. virdi* and *Streptomyces griseus* were subjected to *in vitro* studies to evaluate their antagonistic capacity against pathogenic bacterium *R. solanacearum*. Evaluation was carried out using inhibition zone in growth of pathogenic bacterium as a parameter media on the efficiency of the antagonist.

To determine the effect of selected microorganisms on pathogenic brown rot bacterium, the pathogens and antagonist were inoculated on same plate containing Glucose nutrient agar (GNA) medium also, on same plate containing Kingś B (KB) medium.

II-Greenhouse Experiments

1-Antagonists Microorganisms

Antagonists were applied singly or in combination *B. subtilis* (BS_1), (BS_2), *T. harzianum,T. virdi* + *T. harzianum, Streptomyces griseus, S. griseus*+ *T. harzianum* (soil treatment). All previously mentioned antagonists microorganisms were grown on Yeast extract Peptones Glucose Agar (YPGA) medium for 48hrs. At 28 °C the bacterial cells were suspended in distilled water and centrifuged at 3000 rpm for 30 min. The precipitation was suspended in distilled water to concentration of 10^8 cfu /ml as determined from a standard curve based on absorbance at 620 nm (Shekhawat *et al.*, 1993). Potato tubers (cv.sponta) were soaked in suspension of *R. solanacearum* (R6 isolate) bacterial cells and methyl cellulose (0.1%) (v.v) plus 0.1m magnesium sulfate ($MgSO_4$), for 15 min. before planting. Tubers which used in control were soaked in water as control treatment. The reduction of wilt disease was observed after 70 days of infection.

2-Antibiotics

Three antibiotics, i.e Streptomycin, Ampicillin and Penicillin were used in different doses i.e, 25, 50 and 75mg/L. *R.solanacearum* was prepared as suspension and added to the soil before planting. Potato tubers were dipped into the solution of each dose of the antibiotics for 2 minutes before sowing. Treated tubers were planted at the rate of 3 tubers/replicate. Control pots were only inoculated with 10^8 cfu /ml *R.solanacearum*. All the treated pots and control were evaluated after 70 days from inoculation, as percentage of wilt.

3-Plant Extract

Applying of certain extracts i.e. Garlic cloves (*Allium sativum*), Neem leaves (*Azadirachta indica*) and Camphor leaves (*Dryobalonops aromatica*) on controlling brown rot disease on potato wilt. Crude extracts were prepared by mixing 100 gm of frozen materials of selected plants with 100 ml of water using electric blender for 5 minutes. Extracts were filtrated through double layer of filter paper then filtrates were centrifuged at 3000 rpm for 10 mintues. The supernatants were sterilized using sterilizer membrane (0.2 µm Millipore's filter) and then diluted to 1:10 (100 ml of crude extract : 1000 ml water). Potato tubers were dipped in each sterilized plant extract for 10 minutes directly. After 70 days from planting, percentage of infection with wilt was calculated.

RESULTS AND DISCUSSION

I-Survey and Detection of *R. solanacearum* in Different Samples Collected from Different Governorates.

Infected potato plants showed a sudden wilting of leaves,occurred without yellowing. Typical symptoms of potato brown rot disease on tuber, where distinct brown vascular discoloration and grayish white slime ooze. Results in Table (1) show that *R. solanacearum* was highly detected in potato tuber samples. Percentage of positive samples was 25.7, 21.3,17.3 and 11.7% in El- Menufia, El–Beheira, Ismailia and El- Sharkia governorates, respectively. Meantime, this bacterium was less detected in rhizophere samples, where percentage of positive samples were 15.1, 12.4, 9.2 and 4.6 % in El- Menufia, El-Beheira, El-Ismailia and El-Sharkia governorates, respectively Potato brown rot, caused by *Ps. solanacearum (R. solanacearum)*, has created a lot of quarantine problems during the course of exportation of potatoes to Europe (Farag, 2000).

Date in Table (2) indicate that,the pathogenic isolates of *R. solanacearum* were selected to their severe reaction and morphology on Triphenyl Tetrazolium Chloride (TZC) medium and incubated at 28°C for 3 days.The cultures were subjected to complete morphological and biochemical identification tests.

Table 1. Survey of *R. solanacearum* in different samples collected from different governorates.

Governorates	Site	Different Sources: Potato tubers No.of samples	Potato tubers No.of Infected samples	Potato tubers (%) Infection	Mean	Rhizosphere No.of samples	Rhizosphere No.of Infected samples	Rhizosphere Infection%	Mean
El–Beheira	1	30	7	23.3		10	1	10.0	
	2	20	3	15.0		6	1	16.7	
	3	36	7	19.4	21.3	10	1	10.0	12.4
	4	18	5	27.8		7	1	14.3	
	5	24	5	20.8		9	1	11.1	
El-Menufia	1	25	7	28.0		11	2	18.2	
	2	29	6	20.1		12	2	16.7	
	3	17	4	23.5	25.7	9	1	11.1	15.1
	4	24	6	25.0		13	2	15.4	
	5	22	7	31.8		7	1	14.3	
El - Sharkia	1	23	3	13.0		11	-	-	
	2	16	2	12.5		7	-	-	
	3	41	5	12.9	11.7	21	2	9.5	4.6
	4	27	2	7.4		13	1	7.7	
	5	32	4	12.5		18	1	5.6	
El - Ismailia	1	22	4	18.2		13	-	-	
	2	35	5	14.3		18	2	11.1	
	3	31	6	19.4	17.3	15	2	13.3	9.2
	4	39	7	17.9		17	2	11.8	
	5	18	3	16.7		10	1	10.0	
LSD 0.05					4.61				5.45

Table 2. Morphological and Biological characteristics of *R. solanacearum* isolates in Egypt.

1-Morphology	Isolates	
	Potato tubers	**Rhizosphere**
-1 Character		
· Cell shape	Short – rod	Short – rod
· Motility	+	+
· Gram stain	G-	G-
· Sporulation	-	-
-2 Colonies on solid media		
· Form	irregular – round	irregular – round
· Elevation	Convex	Convex
· Surface	Smooth	Smooth
· Margin	Entire	Entire
· Density	Transluscent	Transluscent
-3 Colour on media		
· NA	Yellowish – Brown	Yellowish – Brown
· KB	Whitish – Gray	Whitish – Gray
· TZC	Fluidal white colony with pink center	Fluidal white colony with pink center
II – Biochemical		
· Catalase		+
· Starch hydrolysis	+	-
· Oxidase reaction	-	+
· KOH 3%	+	-
· Arginine dihydrolase	-	-
· Levan formation	-	+
· b - hydroxyl butrate	+	+
· Gelatine Liquefaction	+	-
· Nitrate reduction	-	+
· Indol production	+	-
· H_2S	-	-
· Biolog system	-	+
· IF	+	+
	+	

The results showed that all isolates (tubers and rhizosphere) were similar in their morphological and physiological reaction and no strain variation could be recognized. Isolates showed short rod cells, non–spore formation, weak satiability negative with gram method and weak motility. Their colonies on nutrient agar (NA) medium were irregularly round, convex with smooth surface, entire margin translucent and yellowish brown in colour. Meantime, these colonies were whitish gray in colour on King's (KB) medium and fluidal white pink center on tetrazolium chloride (TZC) medium.

Isolates of *R. solanacearum* were showed oxidative metabolism of glucose, positive oxidase and catalase reactions. *R. solanacearum* isolates were positive for leaven formation, □ - hydroxyl butrate and mitrate reduction. On the other hand, isolates gave negative reaction with starch hydrolysis, KOH%, arginine dihydrolase, gelatin liquefaction, indol production and H_2S production EU (1998),

2-Pathogenicity Test

Twenty isolates were tested for their pathogenicity on potato cultivars (cv.Sponta and cv.Diamont) and tomato cultivars (cv. Casl rock and cv. Peto 86) , under artificial inoculation conditions. Results in Table (3) demonstrate that potato plants were more susceptible than tomato plants, where percentage of infection at El–Menufia isolates was 67.7 % for cv. Sponta and 56.5 % for cv. Diamont and 43.1 % for Casl rock and 35.1 % for Peto 86 , followed by El – Beheira, El-Sharkia, and Ismailia isolates .

However, tubers isolates were highly virulence, where the disease severity was ranged from 3–4 and 2–3 for potato and tomato cultivars respectively. Meanwhile rhizosphere samples expressed low virulence where disease severity ranged from 1 -2. Pathogenicity test showed typical symptoms of brown rot where the inoculated plants showed wilting, stunting and yellowing of foliage compared with non – inoculated plants as control treatment. *Ralstonia solanacearum* was the most frequently detected in samples of El-Menufia isolate and less detected in samples of El- Sharkia isolate and that result was agree with Balbel, (2006) and Zayed,(2004). All isolates were identified depending on cultural, morphological and biochemical characters as previously mentioned gave positive reaction with indirect immunfluorescent technique (IF) when bacterial cells gave green fluorescent ,that were in agreement with Janse,(1988) and Zayed,(2004).

In this regard, *R. solanacearum* is shown to have two growth phases in nature i.e, the soil phase which is essentially saprophytic in general, and the plant phase which is parasitic in particular. The infection takes place via the underground parts of the plants and spreads

through the vascular elements to different plant organs. The plant is then succumb to infection followed by wilting, dying of the foliage, and exist of the pathogen again to soil (Katznelson, 1965).

3-Biological Control

A- Laboratory Experiments

Size of inhibition zones was used as indicator on the efficacy of antagonist. Data presented in Table (4) reveale that isolates of *Trichoderma harzianum,* and *Trichoderma virdi* showed the most antagonistic effect against tested pathogenic brown rot bacterium, with (8.0) on KB medium,(6.0) on GNA medium in diameter and 6.8 on KB, 4.3 on GNA medium for *T. harzianum* and *T. virdi* respectively.*Bacillus subtilis* occupied the second rank after *Trichoderma i*solates in antagonistic effect against tested pathogenic brown rot with (5.4) on GNA medium, (2.2) on KB medium and (5.6) on GNA,(1.9) on KB medium in diameter for *B. subtilis* (Bs1) and *B. subtilis* (Bs2) respectively. *Streptomyces griseus* showed lowest antagonistic effect compared with other bacteria.

Also, the table showed that, the KB medium was the best medium for inducing the inhibition reaction between *T. harzianum ,T. virdi* and *S. griseus* and potato brown rot bacterium. Whereas GNA medium was the best medium for inducing the inhibition reaction between *B. subtilis* (BS1) and *B. subtilis* (BS2) and potato brown rot bacterium.

Data obtained from Table (4) show also, no clear differences, due to the different media was noticed in case of *T. harzianum ,T. virdi* and *S. griseus.* On the other hand, *B. subtilis* (Bs1) and *B. subtilis* (Bs2) were more effective on GNA medium than KB medium. King's B medium gave the highest number of colony – forming units on King's B as well Zayed,(2004).

Table 3. Pathogenicity test on potato and tomato cultivars using twenty isolates of *R. solanacearum* under artificial inoculation conditions.

Isolates	Isolates code	Source	Potato Cultivars											Tomato Cultivars
			Sponta			Diamont			Casl rock					Peto 86
			D.S	Wilt %	Mean	D.S	Wilt %	Mean	D.S	Wilt %	Mean	D.S	Wilt %	Mean
El–Beheira	Rs 1*	Tu.	4	66.7		4			3	54.5		3	44.4	
	Rs 2	Tu.	4			3	71.1		3	33.3		2	22.2	
	Rs 3	Tu.	4		58.2	4	46.7	58.0	3	47.8	36.5	3	38.9	26.7
	Rs 4	Tu.	3	61.1		3	62.2		2	30.0		2	22.2	
	Rs 5	Rhi	2	82.2		2	44.3		2	16.7		1	5.6	
	Contro	.		55.6			18.9			0.0			0.0	
El Menufia	Rs 6*	Tu.	4	94.4		4	82.2		3	60.0		3	53.4	
	Rs 7	Tu.	4	82.2		4	76.6		3	55.6		3	46.7	
	Rs 8	Tu.	3	50.0	67.7	3	31.3	56.5	2	23.3	43.1	2	16.7	35.1
	Rs 9	Tu.	4	83.3		4	70.0		3	60.0		3	46.7	
	Rs 10	Rhi	2	28.9		2	22.2		2	16.7		2	12.2	
	Contro			0.0			0.0			0.0			0.0	
El - Sharkia	Rs 11	Tu.	3	44.5		2	24.4		2	16.7		2	11.1	
	Rs 12*	Tu.	4	88.9		4	74.4		3	53.1		4	61.1	
	Rs 13	Tu.	4	75.6	54.9	4	66.7	41.3	3	50.0	30.8	3	41.1	28.2
	Rs 14	Tu.	3	38.9		2	24.4		2	22.2		2	16.7	
	Rs 15	Rhi	2	25.0		2	16.7		2	12.2		2	11.1	
	Contro			0.0			0.0			0.0			0.0	
El - Ismailia	Rs 16	Tu.	4	66.7		3	46.7		3	38.9		2	28.9	
	Rs 17*	Tu.	4	83.3		3	60.0		3	53.3		3	42.2	
	Rs 18	Tu.	3	44.4	53.9	2	24.5	37.3	2	23.3	30.2	2	11.1	21.1
	Rs 19	Tu.	3	46.4		3	38.5		2	28.9		2	17.8	
	Rs 20	Rhi	2	28.9		2	16.7		1	6.7		1	5.6	
	Contro l			0.0			0.0			0.0			0.0	

Tu= Tuber
Rhi= Rhizosphere

Disease serivity (D.S)
Avirulent = 1.0
low virulence = 1,1 -2.5,
Medium virulence = 2.6-4.0
Highly virulence = 4.1-5.0

Table 4. Antagonistic reaction between antagonistic organisms and *R. solanacearum* isolates on different media *in vitro*.

Antagonistic organism	Isolates No.	Inhibition Zone (mm)			
		KB medium	Mean	GNA medium	Mean
Bacillus subtilis (Bs1)	RS 1	2.3	2.2	6.1	5.4
	RS 6	1.3		5.8	
	RS 12	3.0		5.7	
	RS 17	2.1		6.2	
Bacillus subtilis (Bs2)	RS 1	1.4	1.9	5.9	5.6
	RS 6	2.6		5.2	
	RS 12	2.0		6.0	
	RS 17	1.7		4.4	
Streptomyces griseus.	RS 1	2.1	2.4	0.0	1.0
	RS 6	2.8		1.0	
	RS 12	2.3		1.1	
	RS 17	2.2		1.0	
Trichoderma harzianum	RS 1	8.7	8.0	6.4	6.0
	RS 6	7.2		6.0	
	RS 12	7.7		5.9	
	RS 17	8.5		5.8	
Trichoderma virdi	RS 1	7.0	6.8	4.6	4.3
	RS 6	6.4		4.3	
	RS 12	7.1		3.6	
	RS 17	6.6		4.7	
LSD 0.05			0.6		0.8

Table 5. Effect of using antagonistic microorganisms separately or in combination on severity of potato wilt under artificial inoculation *in vivo*.

Antagonistic microorganisms	Diseases incidence%	Diseases reduction%
Bacilus subtilis(BS1)	61.1	19.2
B.subtilis(BS2)	66.7	12.17
Trichoderma harzianum	55.6	26.46
T.virdi	58.6	22.49
B.subtilis (BS1) +*T.harzianum*	50.0	33.86
S.griseus +*T.harzianum*	64.4	14.81
Streptomyces griseus	69.9	7.54
Control	75.6	

Table 6. Effect of some antibiotics at different doses on severity of potato wilt under artificial inoculation conditions.

Antibiotics (A)	Dose (Mg)	Diseases incidence %	Diseases reduction %
Streptomycin	25	76.7	6.7
	50	64.4	21.7
	75	57.8	29.7
Ampicillin	25	77.8	5.4
	50	72.2	12.2
	75	71.1	13.5
Penicillin	25	75.6	8.0
	50	76.7	6.7
	75	80.3	2.3
Contol		82.2	
LSD 0.05			
Antibiotic (A)		2.4	
Dose (D)		0.7	
A&D		2.9	

Table 7. Effect of some plant extracts on control of brown rot disease.

Treatment	cv. Diamond incidence %	Efficinecy%	cv. Sponta incidence%	Efficinecy%
Garlic extract	6.03	73.6	8.53	67.6
Neem extract	7.51	67.1	8.07	69.3
Camphor Extract	7.42	67.5	8.22	68.7
Control	22.80		26.30	
LSD 0.05	1.3		1.7	

b- Greenhouse Experiments

1-Effect of Using Antagonistic Microorganisms Separately or in Combination

Data presented in table (5) indicate that all treatments were significantly reduced the disease compared with control treatment. Different antagonistic organisms varied in their effect on the total percentage of disease incidence. Mixture of *B. subtilis* (Bs1) and *T. harzianum* was the most effective antagonists in controlling potato bacterial brown rot disease with 50% infection, followed by (55.6 and 61.1% infection), for *T. harzianum* and *B. subtilis* BS1 separately and respectively. *Streptomyces griseus* showed highest percentage with infection 69.9%. Antagonists (*B. subtilis* (Bs1)., *S. griseus* and *T. harzianum*) were effective biocontrol agents against *R.solanacearum*. When tubers dipped in bacterial suspensions of *Bacillus* spp. before planting. It found that all *Bacillus* treatment significantly inhibited wilt incidence. The efficacy of *S. griseus* and *B. subtilis* on *R. solanacearum*. isolates significantly inhibited the growth of *R. solanacearum*. *S. griseus* and *B. subtilis* isolates were the most effective (Farag, *et al* 1980).

2-Effect of Some Antibiotics on Severity of Potato Brown Rot

To proof efficiency of some antibiotics on controlling brown rot bacterium was carried out at Seed Pathology Research Department, Plant Pathology Research Institute. Percentage of disease was used as parameters to compare effect of different antibiotics.

Data obtained in Table (6) indicate that all antibiotics were used at different doses i.e 25,50 and 75 mg. Different antibiotics and doses varied in their effect on the total percentage of disease incidence and decrease infection. Streptomycin was the most effective antibiotic in controlling potato bacterial brown rot disease compared with Ampicillin and Pencillin. Streptomycin at 75 mg dose was the best concentration used which, give 57.8 % infection, followed by 50 mg dose with, 64.4 % infection. On the contrary, Pencillin at different doses showed no significant differences when used compared to control. Disease incidence was evaluated after planting spraying at 4 -7 days intervals gave the highest degree of *R. solanacearum* control. The bacterial growth inhibition was observed on the treatment containing 25mg of Streptomycin or 75 mg of Penicillin. Streptomycin was superior over the antibiotics against the *R. solanacearum* pathogen while, Ampicilin particial inhibit the brown rot at some of the tested concentration Farag, *et al* (1986).

3-Effect of Plant Extracts for the Control on Plant Pathogenic Bacteria

Extract from different plants (Garlic, Camphor and Neem) were tested for their effects on percentage of infection on potao wilt (Table 7). Data indicated that the different extracts significantly reduced the percentage of brown rot disease incidence. Moreover the lowest disease incidence was recorded with garlic extract. Garlic extract reduced the percentage of brown rot infection on cv. sponta as 8.53%, while in camphor extract the percentage of infection was reduced as 8.22%. Diamont cv. was more resistance than Sponta as susceptible cultivar. The lowest percentage was recorded when garlic extract was applied and the highest disease incidence occurred when neem extract was applied.

More recently, biological control is consider as one of the most important measures for the control of soil borne diseases. Considerable attention has been given to the control of plant pathogenic fungi with antagonisitic organisms, some Rhizobacteria or fungi.Antagonism can be describe, injury or inhibition the growth of one microorganisms by anther without indicating the possible mechanisms involved.

Five antagonistic microorganisms (*Bacillus subtilis* (BS1), *Bacillus subtilis* (BS2), *Streptomyces* grisus, *Trichodema harzianum* and *T. viridi* were tested on King's B (KB) and Glucose nutrient agar (GNA) media for the inhibition of *R. solanacearum* growth.The investigation obtained that *T. harzianum* and *T. viridi* were the most inhibitor to *R. solanacearum* in the two media (KB and GNA), This results were inagreement with Arora (2006) which mentioned that one isolate of each *T. harzianum* and *T. viridi* were effective against soil and tuber- borne disease *R. solanacearum*.

Bacillus subtilis occupied the second rank after *Trichodema* isolates in antagonistic effect against *R. solanacearum* on both media (KB and GNA). That was inagreement with Arora (2006) which mentioned that mass production of the most promising antagonists (*B.subtilis*) for using against brown rot bacterium *R. solanacearum*. Also Dhanbir and Rana (2000) performed that all *B.subtilis* treatments significantly improved yield and inhibited wilt incidence.

These results are in agreement with those reported by King *et al*.,(1954) and also they stated that KBA medium improve the production of antibiotics or toxic substances by different antagonists. On the other hand, *B. subtilis* (Bs1) and *B. subtilis* (Bs2) were more effective on GNA medium than on KB medium. These results were in agreement with those reported by Moura

and Romeiro (2000). *Bacillus subtilis* was used by Farag *et al.*, (1980) and Phae *et al.*, (1992) against *R. solanacearum*, *B.subtilis* is well known as producer of an deterimental antibiotics which possibly responsible for this antagonistic effect. Schober (1984), stated that, *B. subtilis* produce at least 66 different antibiotic compound.

Streptomyces spp. applied by Tu (1988) and Liu *et al.,(*1995) as a biocontrol measure against many diseases and stated that, many strains *Streptomyces* spp, produce antibacterial metabolites or antibiotics were active against several plant pathogens.

Trichoderma spp used by Elad *et al.*,(1986), which stated that, *Trichoderma* spp produce antifungal and antibacterial compound i.e, Viridin , antibiotics, dermadin active against gram negative and gram positive bacteria and wide range of fungi. Also, they stated that, *Trichoderma* spp had a synergistic effect in controlling many diseases.

The effect of antagonists may be sensivity of *R. solanacearum* to an antibiotic complex containing bicilysin and fengymycin (fingycin) produced by *B. subtilis* (Reddy *et al.*, 1994).

Three antibiotics (Streptomycin, Ampicilin and Pencillin) were tested for controlling *R. solanacearum* bacterium. Streptomycin was the most effective antibiotics in controlling *R. solanacearum* followed by Ampicilin and Pencillin. This results were in agreement with (Farag *et al.*, 1986).

Sensitivity of *R. solanacearum* to antibiotics was tested by the disc diffusion method. Amipicilin, Pencillin and streptomycin antibiotics under investigation showed considerable variation against *R. solanacearum*, and low sensitivity to Amipicilin. No sensitivity could be detected to pencillin at any concentration tested. However, Streptomycin had a highly determental effect. The mode of action of the antibiotics on protein synthesis and cell wall formation was considered. This result were inagreement with Gunawan , 1989 and Farag *et al.*, 1986 .Actinomycetes with a positive growth promotion effect were effective for biocontrol of bacterial wilt of tomato (Moura and Romeiro, 2000).

Effect of plant extracts (Garlic, Camphor and Neem) were tested on controlling of *R. solanacearum*. Organic matter i.e, garlic cloves and camphor used in controlling virulent and avirulent isolates of *R. solanacearum* causing brown rot in potato cv. Diamount was determined under green- house conditions (ShenghHua, *et al.*, 2009).

REFRENCES

Arora, R.K;, I.D. Garg; and S.M.P Khurana (2006). Achievements in biological control of diseases of potato with antagonistic organisms. Proceedings of the group meeting on antagonistic organisms in plant disease-management held at Project Directorate of Biological Control, Bangalore, India on 10-11th,July-2003. 2006; 236-243.

Balabel, and Naglaa M (2006). Persistance of *Ralstonia solanacearum* (syn. *Pseudomonas solanacearum*) in different habitats. (Ph.D.thesis) Fac.of Agric., Ain Shams University.

Dhanbir, S. and S.K. Rana (2000). Biocontrol of Bacterial wilt / brown rot *R.solanacearum* of potato.J.Mycol.and Plant Pathol.30:420 – 421.

Dowson, W.J. (1957).Plant disease due to bacteria 2nd ed. Cambridge at the university press,pp.232.

Elad, Y.; Y. Zvieli and I. Chet (1986). Biological control of *Macrophomina Phaseolina* (Tassi) Goid by *Trichoderma harzinaum* Crop protection, 5:288-292

Ernestina,P.Ch (2005).Herbdatabase. Herb library.4pp.

EU (1998),Council Directive 98/57/EC of 20 July 1998 on the control of *Ralstonia solanacearum*. Annex II-test scheme for the diagnosis, detection and identification of *Ralstonia solanacearum*. Official Journal of the European Communities, no. L235, 8–39.

Farag, N.S. (2000).Spotlights on potato brown rot in Egypt Proc.9 th. congress of the Egypt. Phytopathol.S.C.May , 405 – 408.

Farag,N.S.; F. Bishay; S.A.Z Mohammed; A.M. Abd El–Hafez and M.El–Sawy, (1980). Bacterial wilt of potato in relation to antagonistic and rhizosphere microflora Agric. Res .Rev. 58:185-192.

Farag, N.S; Faiza, G. Fawzi; S.I.A. El–Said and M.S. Mikhail (1986). Streptomycin in relation to potato brown rot control. Acta Phytopathology et Entomologica Hungarica, 21(1-2): 115 – 122.

Gunawan, O.S. (1989). Control of bacterial with *R. solanacearum*.E.F. Smith, with Agrimycin 15/ 1.5 wp on tomato in Dago Bandung.Buletin - Penelition . Hortikultura, 1989, 17 (3):41 -44.

Hartman , G. l.; W.W.F. Hong, A. Hanudin and A.C. Hayward (1992). Potential of biological control and chemical control of bacterial wilt.ACIAR proceeding 45 : 322 – 326

Hsu, S.T. (1991).Ecology and control of *Pseudomonas solanacerum* in Taiwan. Plant protection Bulletin Taiwan . Plant protection Bulletin Taippi,33(1):72-79; 4/ref.

Janse, J.D.; F. Arulappan; J. Schans; M. Wenneker and W. Westerhuis (1998). Experiences with bacterial brown rot *Ralstonia solanacearum* biovar2, race 3 in The Netherlands. In: Prior P, Allen C and Elphinstone J (eds.) Bacterial Wilt Disease: Molecular and Ecological Aspects. Springer Publishing, Berlin, Germany, pp. 146-152.

Katznelson, H. (1965). Nature and importance of the rhizosphere. In Ecology of soil – borne plant pathogens, 187-207(Barker,K.f; w.c synder (Eds.), univ.California Press , Berkeley , Calif.571 pp.)

Kempe, J. and L. Sequeira (1983). Biological control of bacterial wilt of potatoes: Attempt to induce resistance by treating tubers with bacteria. Plant Dis. 67: 499-503.

King, E. O.; M. K. Ward and D. E. Raney(1954). Two simple media for the demonstratation of pyocyanim and fluorescin J.Lab.Clin.Med., 44: 301-307.

Krieg, N. R. and J. C. Holt (1984). *Pseudomonas solanacearum* Bergey's Manual of Systemic Bacterology, vol.1 Williams and Wikins , Baltimore, London.

Lui, L.; Kloepper, J. W. and S. Tuzun (1995). Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth promoting rhizobacteria Phytopthology 85: 843 – 847.

Mahmoud, S. M. (2007). Management of brown rot disease of potato. Arab-Universities-Journal-of-Agricultural-Sciences. 2007; 15(2): 457-463.: Faculty of Agriculture, Ain Shams University Cairo, Egypt.

Marin, J.E. and H.M. El-Nashaar.(1992). Pathogenicity of the new phenotypes of *Pseudomonas solanacearum* from Peru. ACIAR Proceeding 45:78-84.

Michel, V.V. and T.W. Mew (1998). Effect of soil amendment on the survival of *R. solanacearum* in different soils Phytopathology 88:300 -305.

Moura, A.B. and Romeiro (2000). Use of Actinomycetes preselected for control of *R. solanacearum* as tomato plant growth promoter. Revistaceres 47:613-626 (c.f. Rev. Plant Pathol. 80: 7178, 2001).

Phae, C.G.; M. Shoda; N. Kita; M. Nokano and K. Ushiyama(1992). Biological control of crown and root and bacterial wilt of tomato by *B. subtilis* NB_{22} Annals of phypathol.Soc.Jap.,58(3):329 – 339

Reddy, M.S.; R.K. Hynez and G. Lazarovits (1994). Relationship between *in vitro* growth inhibition of pathogens and suppression of pre-emergance damping-off and post-emergance root rot of white bean seedlings in greenhouse by bacteria. Can.Jaur of Microbiol. 40(2): 113-119.

Schober, B. (1984). Potato common scab, Information on integrated plant protection. Pflanzen schutzdient (1984), 36(6). 89.Biol.

Shehata, Nevein A.S. (2007). Disease suppression and biological control of brown potato rot in organically and conventionally managed soils. Biological Farming Systems Group (WUR). PhD Thesis.

Sheng Hua, S; Ning, L; QingYuan, C. and Xiaoli, Wang (2009). The inhibition effect of garlic extraction solution on *Pseudomonas solanacearum* and *Phytophthora parasitica* var *nicotianae*. Guizhou Agricultural Sciences 2009 No. 10 pp. 94-96

Tu, J.C. (1988). Antibiotics of *Streptomyces griseus* against *Colletoricum lindamuthianum* J. phytopathology. 121: 97 – 102.

Van Broekhuizen, W.; L. Kosrsten and P.S. Hammes (2002). Development of an alternative method for the detection of *R. solanacearum* in naturally infested soil.3rd International Bacterial wilt symposium, Feb.4-8 p.49.2002.

Wenneker, M.; M.S.W. Verdel; R.M.W. Groenereld; C. Kempeneaar; A.R. Van Beuningen and J.D. Janse (1999). *Ralstonia. solanacearum* race 3 (biovar II) in surface water and nature weed hosts: first report on stinging nettle (*Urtica dioica*). Europeab J. plant pathology 105:307 – 315.

Yindong, T. and Liyuanm He. (1995).A preliminary study on biocontrol of bacterial wilt on potato and tomato with beneficial bacteria. In: Integrated Management of Bacterial wilt .B.Hardly & E.R.French (ed.). pp.71-77 International Potato center (CIP).

Zayed, K.A.M. (2004). Studies on potato bacterial wilt disease. Ms.c Faculty of Agriculture, Ain Shams Univ. pp.