RESEARCH ARTICLE

Mohamed E.A. Dawoud
Zienat K. Mahmoud
Hanaa E. Ahmed
Mohamed G. Farahat

GROWTH PROMOTION AND BIOCONTROL OF LEAF SPOT AND LEAF SPECK DISEASES IN TOMATO BY *PSEUDOMONAS SPP*.

ABSTRACT:

Thirty five endophytic fluorescent bacteria were isolated from root tissues of healthy tomato plants in Egypt (El-Giza, El-Quliobiya, El-Fayoum and El-Behira Governorates) and tested for their efficiency for inhibiting growth of tomato pathogen, *Alternaria solani* and *Pseudomonas syringae*. Two most effective isolates MG4 and MG18 against the three pathogens were selected and identified as *Pseudomonas putida* and *P. fluorescens*. Plant growth promoting activity of the two *Pseudomonas* spp. was evaluated. Both endophytic bacterial species produced siderophore, HCN, ammonia and IAA but IAA production was higher with *P. putida* which was also able to solubilize phosphate. Tomato roots were subjected to colonization after bacterization with two *Pseudomonas* species. Treatments with *P. putida* or *P. fluorescens* through seedling dip were highly effective in inhibiting leaf spot caused by *A. solani* and bacterial speck caused by *P. syringae* but *P. fluorescens* was more effective than *P. putida.* The potential of two *Pseudomonas* spp, for tomato growth promotion was evaluated in green house either individually or in mixtures. They significantly enhanced all growth parameters.

KEY WORDS:

Tomato (*Lycopersicon esculentum*), *Alternaria solani*, *Pseudomonas syringae, Pseudomonas putida, Pseudomonas fluorescens*, siderphores, HCN production chitinolytic activity, phosphate solubilization, Biological control.

CORRESPONDENCE:

Mohamed E. Dawoud

Botany Department, Faculty of Science, Cairo University, Giza 12613, Egypt

E-mail: mez_dawoud@yohoo

Zienat Kamel Mahmoud

Hanaa E. Ahmed

Mohamed G. Farahat

Botany Department, Faculty of Science, Cairo University, Giza 12613, Egypt

ARTICLE CODE: 07.02.12

INTRODUCTION:

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria (*Bacilli*, fluorescent Pseudomonad & *actinomycetes*). It can improve the extent and quality of plant growth directly and or indirectly by biological control of diseases, (Lucy *et al.*, 2004). Also, it increase plant systemic resistance by the production of secondary metabolites as antibiotics, chitinase, β-1, 3-glucosidase, exoprotease enzymes, siderphores, phenazine-1-carboxylic acid, 2-hydroxyphenazine, 2,4-diacetyl-pyoluteorin, pyrrolnitrin, phloroglucinol which suppress fungal growth by producing iron-chelating siderophores and cyanide. Promotion of plant growth and induction of systemic resistance by Fluorescent pseudomonads was reported (Persello-Cartieaux *et al*., 2003; Klein *et al.*, 2004; HaiMing *et al.,* 2007; Ahmed *et al*., 2008; Werra *et al*., 2008). Cyanide can also be a ligand of hydrogenase in certain aerobic bacteria that are not described as cyanogenic. It has an ecological role and confers a selective advantage to the producer strains (Sarani *et al.,* 2008).

Root colonization by introducing a more efficient root colonizer such as fluorescent *Pseudomonas* (PGPR) has been shown to reduce the population of major and minor pathogens (Anjaiah *et al.*, 2003). In this concern it was reported that treatment of soyabean with *Pseudomonas aureofaciens* increased the emergence rate, shoot fresh weight, shoot dry weight and root fresh weight at 7 days after inoculation (Jung *et al.,* 2007). Umesha and Hariprasor (2010) stated that seed

bacterization with *Bacillus subtilis* strain GBO3 showed increased plant growth and reduced bacterial leaf spot in tomato. Seedling raised from seed bacterized with GB03 showed increased root length, shoot length, fresh and dry weight, the activity of phenylalanine ammonia lyase (PAL) and polyphenal oxidese in seeds, phenolic accumulation were reached their peak at 96 hours after pathogen inoculation.

Wangdi *et al.* (2010) and Ishiga *et al*. (2011) stated that , coronatine (COR) a non Jasmonate mimic host – specific virulent effector produced by *pseudomonas syringae* pv. tomato is required for full virulence of *P. syringae* in tomato . COR is shown to induce a range of physiological processes including chlorosis, root growth inhibition and anthocyanin accumulation. Rhizo- and saprophytic-bacteria that live in plant rhizosphere and colonize the root system have been studied as plant promoters for increasing yield production and as biocontrol agents against plant diseases (Lucy *et al*., 2004). Colonization of plant root system can led to reduced pathogen attack directly through production of antimicrobial substances.

MATERIAL AND METHODS:

Plant growth and treatment:

Tomato seedlings (*Lycopersicon esculentum* Mill. Cv. Castel rock) of 30 days old was kindly provided from Agriculture Research Center, Egypt. These seedlings were transplanted (one/pot). The plants were divided into three sets. Each set was divided into four groups; the first was bacterized with *Pseudomonas putida,* the second with *Pseudomonas fluorescens*, the third with mixture of the two PGPR and the fourth represented the positive control. The four groups of one set were infected with *Pseudomonas syringae* and the second with *Alternaria solani* the third set represented a control series. Each treatment was represented by three replicates. The experiment was carried out during the summer season from June to August of two successive growth seasons 2006 and 2007. The pots were arranged in a random manner under prevailing environmental conditions. The samples were collected after 30 and 60 days for analysis. The yield was estimated at the end of the experiment.

Pathogens preparation:

A. Solani and *P. syringae* were isolated from naturally infected tomato plants showing leaf spot and bacterial speck symptoms, respectively. Plants were collected from four governorates in Egypt (El-Giza, El-Quliobiya, El-Fayoum, and El-Behira).

Foliar application by pathogens:

Suspension of *A. solani* was applied by hand atomizer to tomato plant leaves one week after transplanting (Schilder and Bergstrom, 1990). The infected plants were covered with polyethylene bags for 48 hours to provide enough moisture for conidial germination; control plants were sprayed with sterile water. Disease assessment was carried out according to 0-4 scale: 0 = No infection, 1= 20% infection, 2= 20-40% infection, 3= 40-60% infection, 4= 60-80% infection.

Disease severity index (DSI). DSI was calculated according to the following equation

$$DSI = \frac{\Sigma n \times v \times 100}{N \times S}$$ where:

Σn= numerical value of each category, v= number of leaves in each category, S= the highest number in the scale, N= total number of leaves in the sample.

The second set of plants was challenged with cell suspension of *P. syringae* (Romeiro, 2001). Disease severity was evaluated visually when the typical symptoms became evident and scored using a disease index with a range of 0 to 3 (0 signifies a healthy-looking plant; 1 signifies 2 to 5 specks together or spread over each leaf; 2 signifies 6 to 10 specks; and 3 signifies more than 10 specks), as described by Yunis *et al*. (1980).

Isolation of endophytic Fluorescent pseudomonads:

Endophytic fluorescent pseudomonads were isolated according to (Mathiyazhagan *et al*., 2004) with some modifications using King's B medium.

The two used PGPR, *P. putida isolate MG4 and P. fluorescens* isolate MG18 were previously isolated from healthy tomato root plants collected from different agriculture areas in Egypt and showed significant antagonistic activity against fungal and bacterial plant pathogens (Ahmed *et al.*, 2011). Bacteria were grown on King's B plates for 24 h at 28°C and the cells were harvested by centrifuging at 10.000 g the inoculums of *A. solani* was prepared according to Beshir (1990). The suspension of mycelial fragments was adjusted to 10^5 CFU/ml. Inoculums of *P. syringae* was prepared according to Romeiro (2001).

In vitro screening of fluorescent Pseudomonads isolates for antagonistic activity (on PDA medium):

The antagonistic effect of bacterial isolates against *Alternaria solani* and *P. Syringae* was measured by dual culture plate assay as described by Vidhyasekaran *et al.* (1997).

Identification of the most active antagonistic isolates:

They were identified according to standard physiological and biochemical identification tests as described in Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994). Further biochemical tests were performed using API 20NE kits (Biomerieux Co., France), commercially available kits;

consist of rows of micro tubes impregnated with various freeze-dried test substrates. They are rehydrated by inoculation with bacterial suspensions, and after incubation period, the results are recorded as color changes. The kit was used according to the instructions of the manufacturer.

Biochemical activities of microbial bioagents:

a- Antifungal assay (King's B and sabaroud dextrose media):

The agar well diffusion method as adopted earlier (Mehmood *et al*., 1999) was used.

b. Detection of siderophore production: Bacterial isolates were assayed for siderophores production on the Chrome Azurol S agar medium (CAS medium) described by Schwyn and Neilands (1987):

c. Detection of HCN production (using KBA): Production of HCN by bacterial isolates was observed according to the method of Lorck (1948).

d. Chitinolytic activity

i- Preparation of colloidal chitin: Colloidal chitin was prepared as described by Hsu and Lockwood (1975) from crab shell chitin (Sigma Co.).

ii- Detection of chitinase production:

To induce chitinolytic activity, bacteria were grown on synthetic medium with 1% colloidal chitin as described by Kamil *et al*. (2007).

e. Phosphate Solubilization(plating on Pikovaskya (PKV) agar):After incubation at 28±1ºC for 5 days, the formation of a clear zone around bacterial growth indicated phosphate solubilization capacity as described by Gaur (1990).

f. NH_3 production (on peptone water broth):

Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

g. Determination of indole acetic acid (IAA) production (using KB broth with tryptophane 1, 2, & 5 mg/ml): Quantitative analysis of IAA was performed using the method of Loper and Scroth (1986). Development of pink color of filterate + reagents indicates IAA production. Optical density was measured at 530nm using Jasco spectrophotometer. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA.

Evaluation of root colonization and plant growth promotion in response to PGPR:

a. Inoculum preparation of PGPR and application of biocontrol organisms to tomato roots: The roots of 30 days old tomato seedling were washed several times with sterilized distilled water and dipped in cell suspension (10^{10} CFU ml^{-1}/10 ml $MgSO_4$) of *P. putida, P. fluorescens* individually and in a mixture of the two isolates for one minute as separate treatments. Seedlings dipped in sterilized distilled water were used as controls.

b. Root colonization assay: Total and internal root population count were determined according to Press *et al*. (2001).

Evaluation of plant growth characteristics:

Plant growth criteria were measured (Black *et al*., 1965; Tang *et al*., 2005).

Statistical analysis:

The experiment followed a completely randomized design. The measured data were subjected to the analysis of variance (ANOVA) appropriate to the design (Snedcor and Cochran, 1980). The significant differences between treatments were compared with the critical difference at 5 % level of probability by the Duncan's test using SPSS for windows version 12.0 software (Walter and Duncan, 1969).

RESULTS:

Isolation and screening of antagonistic fluorescent pseudomonds:

Thirty five isolates of fluorescent pseudomonds (with various inhibitory activities against phytopathogenic fungi) were isolated from root tissues of healthy tomato plants. Some of 35 fluorescent Pseudomonads isolates showed highly inhibitory activity while others exhibited limited activity (Fig. 1).

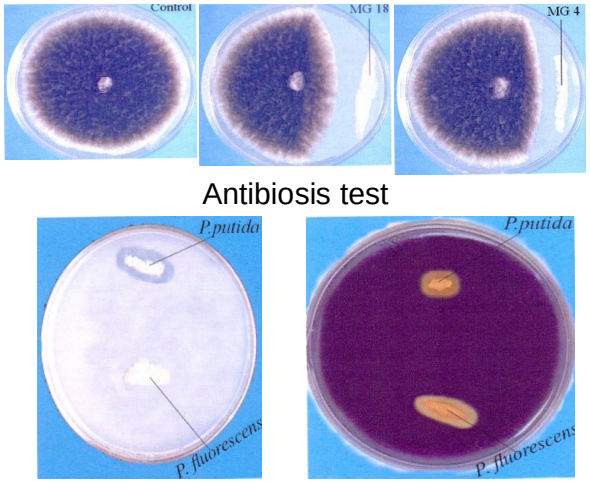

Fig. 1. Two isolates of *Pseudomonas* designated MG4 and MG18, originated from roots. They show an apparent effect against the phytopathogenic fungi.

Identification of selected PGPR:

Isolate MG4 was grouped under *Pseudomonas putida*, whereas isolate MG18 was classified as *P. fluorescens*. Identification was

confirmed by the standardized API 20NE strip System (Biomerieux Co., Marcy, Etoile, France).

Antagonistic and some biochemical activities of the two selected PGPR *Pseudomonas* isolates:

The two selected isolates were tested for their plant growth promotion activities by assaying phosphate solubilization, HCN, siderophore, ammonia and IAA production (Table 1&2).

Table 1. Biochemical activities of bacterial antagonists

Characteristics	Bacterial isolates	
	Pseudomonas fluorescens	*Pseudomonas fluorescens*
Antifungal activity, diameter of inhibition of *A. solani* (mm)	33	48
Siderophore production	+	+
HCN production	+	+
Chitinolytic activity	-	-
Phosphate solubilization	+	-
NH_3 production	+	+

Table 2. Production of IAA by the two biocontrol Plant growth promoting rhizobacteria

Tryptophan concentration (mg/ml)	Production of IAA (µg/ml)	
	Pseudomonas putida	*Pseudomonas fluorescens*
0	6.15	1.88
1	21.65	13.92
2	38.62	29.25
5	67.65	46.7

Root colonization:

Total population of *P. putida* and *P. fluorescens* on roots did not differ significantly but were higher than control while internal population of the *P. fluorescens* isolate were significantly lower than those of the *P. putida* 30 days after treatment. After 60 days total and internal population of *P. fluorescens* decrease sharply while *P. putida* populations were significantly higher than control (Table 3).

Table 3. Total and internal population count of the two Plant growth promoting rhizobacteria 30 and 60 days after root treatment

Bacterial isolate	Internal bacterial population (log CFU/g fresh weight root)		Total bacterial population (log CFU/g fresh weight root)	
	30 DAT	60 DAT	30 DAT	60 DAT
P. putida	8.77 [(a)]	6.85 [(a)]	7.64 [(a)]	5.33 [(a)]
P. fluorescens	8.61 [(a)]	3.38 [(b)]	6.71 [(b)]	1.58 [(b)]
Control	2.63 [(b)]	1.56 [(c)]	1.95 [(c)]	1.22 [(b)]

DAT= days after root treatment

Within rows, data followed by the same letter do not differ significantly according to Duncan's multiple range tests ($p < 0.05$).

Plant growth promotion activity of two rhizobacterial species:

The data in tables 4 a, b & c reveal the following results: In general, microbial inoculated plants with individual and mixed bacterial isolates showed significant improvement in the biocontrol of the plant diseases and in all growth criteria compared to control, but the mixture of *P. putida* and *P. fluorescens* exhibited the maximum increase in the measured plant growth criteria number of leaves, flowers and fruits, chlorophyll content, yield fresh weight at the two stages of growth. This was followed by plants inoculated with *P. putida* while plants inoculated with *P. fluorescens* recorded the least effect and did not show any significant difference compared to the untreated control.

Table 4a. Effect of single Plant growth promoting rhizobacteria (*Pp* or *Pf*) and their mixed inoculation on different growth criteria of uninfected tomato plants shoot and root

Plant organ / Bioagent and date of Application after transplanting		Shoot system						Root system					
		Height (cm)		fresh weight (g)		Dry weight (g)		Length (cm)		Fresh weight (g)		Dry weight (g)	
		Mean	%	Mean	%	M	%	Mean	%	Mean	%	M	%
Pseudomonas putida (*Pp*)	30d	35.52 ±1.733	122.22 ±6.22	35.99 ±2.73	127.26 ±0.70	4.84 ±0.21	123.47 ±6.9	9.15 ±0.60	115.82 ±6.2	5.14 ±0.18	134.20 ±6.9	0.78 ±0.70	
	60d	51.62 ±3.40	128.22 ±6.50	65.42 ±4.16	124.61 ±6.9	9.59 ±0.42	133.01 ±7.0	13.61 ±1.01	119.91 ±6.7	7.44 ±0.44	130.99 ±6.2	1.30 ±0.10	
Pseudomonas fluorecense (*Pf*)	30d	30.1 ±1.05	103.58 ±5.6	29.98 ±2.21	106.01 ±5.8	4.1 ±0.22	104.59 ±5.8	7.96 ±0.41	100.76 ±5.9	4.24 ±0.18	110.70 ±5.11	0.68 ±0.05	
	60d	42.01 ±1.49	104.35 ±5.6	55.17 ±2.54	103.09 ±5.2	8.15 ±0.41	113.04 ±6.1	11.38 ±0.89	100.26 ±5.2	6.92 ±0.42	121.83 ±6.0	1.21 ±0.13	
Pseudomonas putida + Pseudomonas fluorecense (*Pp*+*Pf*)	30d	47.07 ±2.62	161.97 ±7.9	40.56 ±1.98	143.42 ±7.9	5.72 ±0.25	145.92 ±7.2	12.51 ±0.62	158.35 ±8.1	5.77 ±0.19	150.65 ±7.9	0.89 ±0.07	
	60d	66.91 ±3.68	166.19 ±8.0	76.83 ±4.75	146.34 ±7.5	10.9 ±0.35	151.18 ±7.5	19.91 ±1.52	175.42 ±9.2	9.01 ±0.5	158.63 ±6.11	1.63 ±0.10	
Control	30d	29.06 ±1.03	100.0 ±5.01	28.28 ±3.12	100.0 ±5.2	3.92 ±0.29	100.0 ±5.2	7.9 ±0.45	100.0 ±5.11	3.83 ±0.16	100.0 ±6.0	0.63 ±0.06	
	60d	40.26 ±2.03	100.0 ±4.90	52.50 ±2.77	100 ±4.8	7.21 ±0.39	100.0 ±5.9	11.35 ±0.64	100.0 ±6.12	5.68 ±0.4	100.0 ±6.22	0.96 ±0.13	

Table 4b. Effect of single Plant growth promoting rhizobacteria (*Pp* or *Pf*) and their mixed inoculation on tomato plant number of leaves and chlorophyll content.

Bioagents and date of application transplanting	Plant organs	No. of leave/plant		No. of Chlorophyll content (mg/g fresh wt.)	
		Mean	%	Mean	%
Pseudomonas putida (*Pp*)	30d	13.3 ±0.95	175.0 ±9.9	4.79 ±0.12	120.96 ±7.9
	60d	30.2 ±1.87	155.27 ±8.11	5.64 ±0.30	120.77 ±7.1
Pseudomonas fluorecense (*Pf*)	30d	8.0 ±0.67	105.26 ±6.6	3.49 ±0.11	100.76 ±6.2
	60d	23.0 ±2.16	118.25 ±7.0	4.55 ±0.40	105.32 ±6.2
Pseudomonas putida + *Pseudomonas fluorecense* (*Pp*+*Pf*)	30d	14.4 ±0.52	189.47 ±9.8	5.57 ±0.17	140.66 ±8.11
	60d	31.9 ±2.13	164.01 ±9.22	6.69 ±0.39	154.86 ±8.11
Control	30d	7.6 ±0.70	100.0 ±6.90	3.96 ±0.11	100.0 ±7.0
	60d	19.4 ±1.71	100.0 ±5.12	4.32 ±0.20	100.0 ±7.8

Table 4c. Effect of single (*Pp* or *Pf*) and mixed inoculation of Plant growth promoting rhizobacteria on number of flowers and their development into tomato fruits (Yield, g/plant)

Plant yield / Bioagents and date of application	No. of flowers / plant		No. of fruits/plant		Fruits (fresh wt./plant)	
	M	%	M	%	M	%
Pseudomonas putida (*Pp*) + *Pseudomonas fluorecense* (*Pf*)	16^{A}	133.33^{A}	12^{B}	109.09^{B}	31^{C}	88.57^{C}
	18^{A}	150.0^{A}	16^{AB}	145.45^{A}_{B}	36^{B}	106.86^{B}
	21.0^{A}	175.0^{A}	18^{A}	163.64^{A}	42.0^{A}	120.0^{A}
Pseudomonas putida (*Pp*) + *Pseudomonas fluorecense* (*Pf*)	10^{B}	83.33^{B}	8^{B}	72.72^{B}	28^{C}	80.00^{C}
	12^{B}	100.0^{B}	10^{B}	90.90^{B}	30^{C}	85.71^{C}
	14.9^{B}	124.17^{B}	12^{B}	190.09^{B}	36.0^{B}	102.86^{B}
Pseudomonas putida (*Pp*)	18^{A}	150.0^{A}	15^{A}	163.63^{A}	30^{C}	85.71^{C}
	20^{A}	166.67^{A}	17^{A}	154.55^{A}	36^{B}	106.86^{B}
	22.5^{A}	187.5^{A}	19^{A}	172.73^{A}	42.5^{A}	121.43^{A}
Control	12.0^{D}	100.0^{D}	11^{B}	100^{B}	35.0^{B}	100.0^{B}

Data with similar letter, at the same columns, are insignificant.

1: Fungal infected-tomato plant

2: Bacterial infected- tomato plant

3: Uninfected plant (Positive control)

In general, the bacterization of tomato with mixture of the two PGPR showed the maximum enhancement effect than individual isolate. *P. putida* was found to be superior in enhancing growth parameters than *P. fluorescens* which showed no significant changes (Figs 2 & 3).

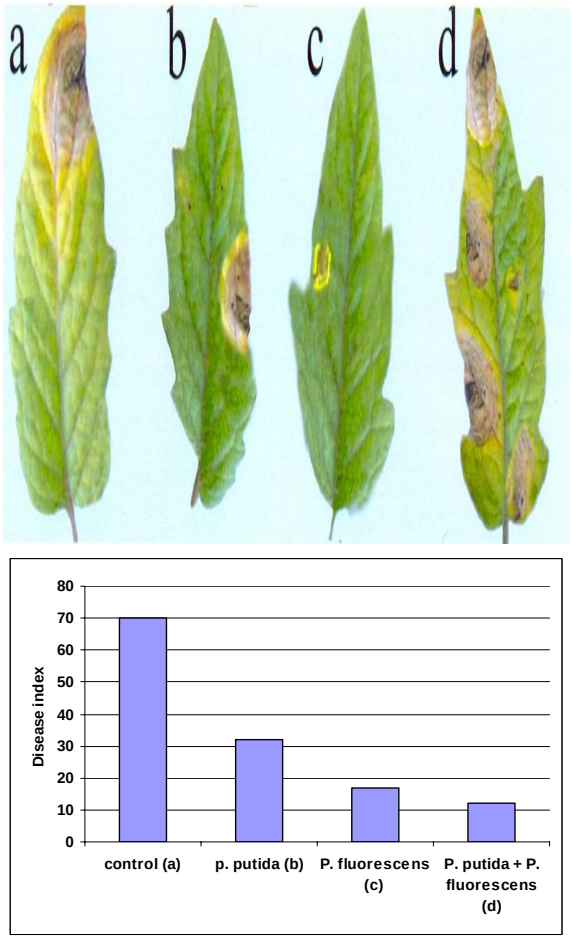

Fig. 2. Effect of different tomato roots-treatment with Plant growth promoting rhizobacteria either single or mixed on leaf spot disease incidence after infection with *A. solani*

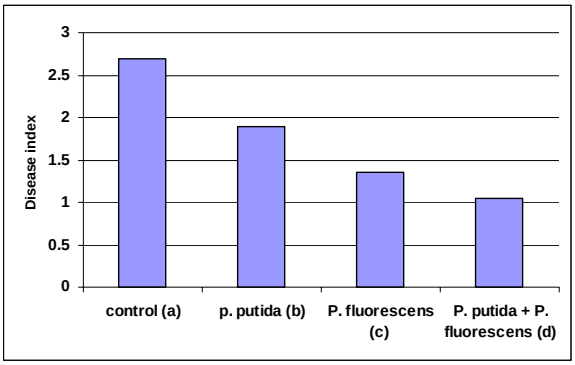

Fig. 3. Effect of different tomato roots-treatment with Plant growth promoting rhizobacteria either single or mixed on bacterial speck disease index after infection with *P. Syringae*

DISCUSSION:

Several strains of *Pseudomonas spp.* have been shown to produce wide range of antifungal compounds including phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), 2,4-diacetylphloroglucinol (Phl) and pyoluteorin (Plt) as reported by Chin *et al*. (2003). Bolwerk *et al*. (2003) have shown the dramatic effect of PCN on the *invitro* growth of *F. oxysporum f. sp. radicis-lycopersici* that was expressed by several hyphal growth abnormalities and acceleration of the aging process. Deora *et al*. (2008) documented that *Pseudomonas jessenii* isolate EC-S101, an antagonistic rhizobacterium, induced morphological abnormalities such as topical swelling and excessive lateral branching in phytopathogenic *Pythium aphanidermatum* hyphae as a result of radial growth inhibition in a dual culture assay. The rhizobacteria *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365 reduced growth, sporulation, and conidial germinability of *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose (Bardas *et al*., 2009).

The results of the present study revealed that the two isolates (*P. fluorescens* and *P. putida*) inhibited *A. solani* growth efficiently. *P. fluorescens* (inhibition zone diameter, 48mm) was more efficient than *P. putida* (inhibition zone diameter, 33mm) as antifungal agent. Several isolates of *Pseudomonas spp.* are recorded by other workers to be effective antagonistic agents (Deora *et al*., 2008).

In the current study, the two bacterial isolates *Pseudomonas putida* and *P. fluorescens* were found to produce siderophores under iron-limiting conditions and form ferric-siderophore complex and make it unavailable to other organisms causing iron starvation, the same result was stated by O'Sullivan and O'Gara (1992). Previous studies indicated that iron starvation can cause growth inhibition, decrease in nucleic acid synthesis, inhibition of sporulation and change in cell morphology of pathogenic fungi and bacteria in the rhizosphere (Muleta *et al*., 2007).

Concerning the interrelation between siderphores and plant growth, Bakker *et al*. (1986) found that, siderophore- negative mutant of *P. fluorescens* strain WCS358 lost its ability to promote the growth of potato. It has been suggested that siderophores cause induction of systemic resistance in plants (Leeman *et al*. 1996). The present study showed that HCN was produced by *P. putida* and *P. fluorescens.* The cyanide ion derived from HCN is a potent inhibitor of many metalloenzymes, especially copper containing cytochrome *c* oxidases (Blumer and Haas, 2000). Voisard *et al*. (1989) found that a HCN-mutant of *P. fluorescens* strain CHAO obtained by insertional inactivation, lost its ability to suppress black root rot of tobacco caused by *Thielaviopsis basicola* and suggested that HCN produced by *P. fluorescens* was contributed in suppression of black root rot. It has been reported that transfer of hcn biosynthetic gene clusters to non-HCN producing *Pseudomonas* strains increased their biocontrol activity against black root rot, as well as against the foliar pathogens *Septoria tritici* and *Puccinia recondite f.sp. tritici* in wheat (Flaishman *et al*., 1996).

In this study *P. putida* and *P. fluorescens* were negative for chitinolytic activity, these results indicated that the antimicrobial activity of these two isolates depends on other mechanisms as production of siderophore and HCN or synergistic interaction of these two or with other inhibitory secondary metabolites rather than cell wall degrading enzymes, These results concur with those of Ahmed *et al*. (2008). Also, the present study indicated that *P. putida* has the ability to solubilize phosphate whereas *P. fluorescens* showed negative results (Fig. 1). Phosphate solubilizing bacteria increase availability of phosphorus (biofertilizer) to plants (Rodriguez *et al*., 2004). Many reports recorded plant yield increases due to phosphate solubilizing bacteria in different crops as maize and other cereals (Afzal *et al.,* 2005). Seed inoculation of maize (*Zea mays* L. SC. 704) by phosphate solubilizing *Glomus intraradices* (AM) and *Pseudomonas fluorescens* (Pf) enhanced plant growth, nutrient uptake, grain yield and yield components in maize plants (Ehteshami *et al*., 2007).

Moreover, the results in this study revealed that both *P. putida* and *P. fluorescens* were able to produce ammonia, the produced ammonia increases available nitrogen in soil as documented by Ahmed *et al*. (2008).

Furthermore, the present finding showed that both tested *Pseudomonas spp* has the ability to produce IAA, in presence or in absence of tryptophan. These results agreed with those reported by Naik and Sakthivel (2006). Tryptophan and indole-3-acetamides are the key intermediates in the IAA biosynthesis pathways (Patten and Glick, 2002). Auxin may increase plant growth through cell enlargement, cell division, root initiation, growth rate and apical dominance (Frankenberger and Arshad 1995). Mazumdar *et al*. (2007) reported that fluorescent *Pseudomonas* isolated from rhizosphere of tea plants produced IAA-like substances which enhanced the growth of tea seedlings and increased total shoot and root biomass of 1-year-old tea seedlings.

Endophytic bacteria colonize a broad spectrum of plant species and plant parts, it has been stated that all plants in nature harbor endophytic bacteria (Sturz *et al*., 2000). The viable bacterial cells of *P. putida* in tomato roots was significantly greater than that of *P. fluorescens* 60 days after treatment (DAT) but not after 30 days, while the control was less than both. These results suggested that the bacterial population of *P. putida* persisted more than *P. fluorescens* in the root system. In agreement of these results Ryu *et al*. (2007) found that the population dynamics of the two PGPR strains *Bacillus subtilis* GB03 and *B. amyloliquefaciens IN937a* on *Arabidopsis* roots did not statistically differ until four weeks after sowing, suggesting that initial bacterial colonization on the plant roots can play a critical role in sustaining the population.

Rhizosphere colonization is considered to be a crucial step in the application of microorganisms or beneficial purposes such as biofertilization, bicontrol and phytoremediation (Lugtenberg *et al*. 2001). Colonization of plant roots with certain rhizosphere bacteria promotes plant growth and induces long lasting systemic protection against a broad spectrum of plant pathogens (Rao, 2007).

The present results indicated that application of the bioagents significantly improved all measured growth parameters. The Maximum growth promotion was induced by application of the mixture of *P. putida* and *P. fluorescens* or *P. putida* alone which increased number of flowers and fruits significantly. The yield of tomato plants treated with a mixture of *P. putida* and *P. fluorescens* increased by about 30% over control while *P. fluorescens* did not induce significant increase compared to control. This might be due to that, *P. putida* was able to solubilize phosphate, produced higher levels of IAA, also *P. putida* was more efficient root colonizer. The present findings verify that these microorganisms can interact positively in promoting plant growth, these results agreed with those of Ehteshami *et al*. (2007). In agreement with the present results, Trivedi and Pandey (2007) reported that, addition of insoluble phosphate to the inoculated soil significantly increased the growth parameters in all the cases. Carlier *et al*. (2008) found that, inoculation of wheat (*Triticum sativum* L.) with the rhizobacterium *Pseudomonas chlororaphis subsp. aurantiaca* strain SR1 resulted in significant increase in plant height, root length, weight of grains, number of spikes per plant and number of grains per spike. *Pseudomonas fluorescens* strain increased plant numbers, head numbers and significantly improved plant establishment and harvest yield in winter wheat (Amein *et al*., 2008).

PGPR play a vital role in management of various fungal and bacterial diseases. The results obtained from greenhouse experiment demonstrated significant suppression of leaf spot and bacterial speck diseases in tomato plants previously treated with the two selected isolates either individually or in mixture. For leaf spot disease, the mixture of *P. putida* and P. fluorescens reduced disease severity to 15% where it was 70% for control. Application of mixture of *P. putida* and *P. fluorescens* caused the highest significantly suppression of both diseases compared to control. Similarly, Bashan and De-Bashan (2002) reported significant protection against bacterial speck disease in tomato after application of *Azospirillum brasilense.* da Silva and Almeida (2008) evaluated five rhizobacterial strains for biological control of multiple pathogens causing foliar diseases in tomato plants including *P. syringae* and *A. solani* and observed reduced disease intensity in plants microbiolized with rhizobacteria. Wilson *et al*. (2002) reported that the non pathogenic bacteria (*P. syringae* strains TLP2 and Cit7, *P. fluorescens* strain A506 and *P. syringae* pv. Tomato DC3000 hrp mutants) were found to reduce foliar bacterial speck disease severity in tomato. The current results proved that, bacterization of tomato by mixture of *P. putida* and *fluorescens* gave the highest disease suppression compared to single treatments, which was similar to data, reported in previous studies of Meziane *et al*. (2005). The main mode of action includes combining biological control agents with antagonistic properties with those inducing systemic resistance in addition to the production of siderophores which contributed to suppression of pathogens the same was conducted by Bargabus *et al*. (2004) and Mathiyazhagan *et al*. (2004).

In this study, treatment of tomato roots with rhizobacteria induced significant protection against the phylospheric pathogens *A. solani and P. syringae* the causal agents of leaf spot and bacterial speck diseases,

respectively that support the suggestion of systemic resistance and excluding the possibility of direct antagonism because of the spatial separation between rhizobacteria in the rhizosphere and pathogens in the phyloplane.

REFERENCES:

Afzal A, Ashraf M, Asad SA, Farooq M. 2005. Effect of phosphate solubilizing microorganisms on phosphorus uptake, yield and yield traits of wheat (*Triticum aestivum* L.) in rainfed area. Int. J. Agric. Biol. 7(2): 207–209.

Ahmed E, Mohamed Zk, Dawoud MEA, Farahat MG. 2011. Induced systemic protection against tomato leaf spot (Early leaf blight) and Bacterial speck by rhizobacterial isolate. Egypt. J. Exp. Biol. (Bot), 7(1): 49-57.

Ahmed F, Ahmad I, Khan MS. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res., 163(2): 173-181.

Ahmed NE, osman NA, Elhussein SA, Mohomed W, Adam YS. 2009. Antibacterial activity of *Prosopis juliflora* against tomato bacterial leaf spot disease agent *Xanthomonas campestris* Pv *vesicatoria*. Acta Hortic., 808: 333-338.

Amein T, Omer Z, Welch C. 2008. Application and evaluation of *Pseudomonas* strains for biocontrol of wheat seedling blight. Phytopathology, 27(3-5): 532–536.

Anjaiah V, Cornalis P, Koedam N. 2003. Effect of genotype and root colonization in biological control of *Fusarium* wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. Can. J. Microbiol., 49(2): 85-91.

Bakker PAHM, Lamers JG, Bakker AW, Marugg JD, Weisbeek PJ, Schippers B. 1986. The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in a short rotation of potato. Neth. J. Plant Pathol., 92(6): 249–256.

Bardas GA, Lagopodi AL, Kadoglidou K, Tzavella-Klonari K. 2009. Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. Biol. Control, 49(2): 139–145.

Bargabus RL, Zidack NK, Sherwood JW, Jacobsen BJ. 2004. Screening for the identification of potential biological control agents that induce systemic acquired resistance in sugar beet. Biol. Control, 30(2): 342-350.

Bashan Y, De-Bashan LE. 2002. Protection of tomato seedlings against infection by *Pseudomonas syringae pv. tomato* by using the plant growth-promoting bacterium *Azospirillum brasilense*. Appl. Environ. Microbiol., 68(6): 2635–2643.

Beshir Ma. 1990. Pathological and physiological studies on the effect of certain growth regulators on plant disease resistance. Ph.D. thesis, Fac. Agric., Al-Azhar Univ., Cairo Egypt.

Black CA, Evans DD, Ensminger LE, White JL, Clarck FE. 1965. Methods of soil analysis. Part 1. Chap. 18. Water capacity by Klute A, ASA Monogr. Ser., No. 9, Am. Soc. Agron. Madison, Wisconsin, USA.

Blumer C, Haas D. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch. Microbiol., 173(3): 170–177.

Bolwerk A, Lagopodi AL, Wijfjes AH, Lamers GE, Chin-A-Woeng TF, Lugtenberg BJ, Bloemberg GV. 2003. Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum f. sp. radicis-lycopersici*. Mol. Plant Microbe Interact., 16(11): 983–993.

Cappuccino JC, Sherman N. 1992. In: "Microbiology: A Laboratory Manual. (3rd Ed.). Benjamin/cummings Pub. Co., New York, pp. 125–179.

Carlier E, Rovera M, Jaume AR, Rosas SB. 2008. Improvement of growth, under field conditions, of wheat inoculated with *Pseudomonas chlororaphis subsp. aurantiaca* SR1. World J. Microb. Biot., 24(11): 2653–2658.

Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ. 2003. Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol., 157(3): 503–523.

da Silva GA, Almeida EA. 2006. Production of Yellow-Green Fluorescent Pigment by *Pseudomonas fluorescens*. Braz. Arch. Biol. Technol., 49: 411-419.

Deora A, Hashidoko Y, Tahara S. 2008. Antagonistic effects of *Pseudomonas jessenii* against *Pythium aphanidermatum*: morphological, ultrastructural and cytochemical aspects. J. Basic Microb., 48(2): 71–81.

Ehteshami SMR, Aghaalikhani M, Khavazi K, Chaichi MR. 2007. Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (*Zea mays* L.) under water deficit stress. Pakistan J. Biol. Sci., 10(20): 3585-3591.

Flaishman MA, Eyal Z, Zilberstein A, Voisard C, Haas D. 1996. Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide producing strains of *Pseudomonas putida*. Mol. Plant Microbe Interact., 9: 642–645.

Frankenberger WT, Arshad M. 1995. Auxins, In: Phytohormones in soils: microbial production and function, (Frankenberger WT, Arshad M. Ed.)". Marcel Dekker, Inc., New York, pp. 17–136.

Gaur AC. 1990. Physiological functions of phosphate solubilizing micro-organisms. In: "Phosphate Solubilizing Micro-organisms as Biofertilizers, (Gaur AC. Ed.)". Omega Scientific Publishers, New Delhi, pp. 16–72.

HaiMing L, YanJing H, HaiXia J, HuaSong P, XianQing H, XueHong Z, Thomashow LS, YuQuan X. 2007. Characterization of a phenazine-producing strain *Pseudomonas chlororaphis* GP72 with broad-spectrum antifungal activity from green pepper rhizosphere. Curr. Microbiol.. 54: 302-306.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. Bergey's manual of determinative bacteriology, 9th Ed. London: Williams and Wilkins Press. pp: 150-157.

Hsu SC, Lockwood JL. 1975. Powdered chitin agar as a selective medium for enumeration of Actinomycetes in water and soil. Appl. Microbiol., 29: 422-426.

Ishiga y, Uppalapati SR, Ishiga T, Mysore KS. 2011. Involvement of SGT1 in COR mediated signals transduction pathway leading to disease symptom development. Plant signal. Behav., 6(7): 1072-1073.

Jung WJ, Mabood F, Kim TH, Smith DL. 2007. Induction of pathogenesis-related proteins during biocontrol of *Rhizoctonia solani* with *Pseudomonas aureofaciens* in soybean (*Glycine max* L. Merr.) plants. Biocontrol, 52(6): 895-904.

Kamil Z, Rizk M, Saleh M, Moustafa S. 2007. Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol. Global J. Mol. Sci., 2(2): 57-66.

Klein S, Veselova M, Mayatskaya A, Khmel I, Chet I, Chernin L. 2004. Biocontrol activity of phenazine-producing rhizobacterium *Pseudomonas chlororaphis* 44. B. OILB/SROP. 27: 145-150.

Leeman M, Den Ouden FM, Van Pelt JA, Dirkx FPM, Steijl H, Bakker PAHM, Schippers B. 1996. Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. Phytopathology 86(2):149-155.

Loper JE, Scroth, MN. 1986. Influence of bacterial sources on indole-3 acetic acid on root elongation of sugarbeet. Phytopathology, 76: 386–389.

Lorck H. 1948. Production of hydrocyanic acid by bacteria. Physiol. Plantarum, 1(2): 142–146

Lucy M, Reed E, Glick BR. 2004. Applications of free living plant growth-promoting rhizobacteria. Anton. Leeuw., 86(1): 1-25.

Lugtenberg BJJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu. Rev. Phytopathol., 39: 461-490.

Mathiyazhagan S, Kavitha K, Nakkeeran S, Chandrasekar G, Manian K, Renukadevi P, Krishnamoorthy AS, Fernando WGD. 2004. PGPR mediated management of stem blight of *Phyllantuus amarus* (Schum and Thonn) caused by *Corynespora cassiicola* (Berk and Curt) WEI. Arch. Phytopathol. Plant Prot., 37: 183-199.

Mathiyazhagan S, Kavitha K, Nakkeeran S, Chandrasekar G, Manian K, Renukadevi P, Krishnamoorthy AS, Fernando WGD. 2004. PGPR mediated management of stem blight of *Phyllantuus amarus* (Schum and Thonn) caused by *Corynespora cassiicola* (Berk and Curt) WEI. Arch. Phytopathol. Plant Prot., 37: 183 – 199.

Mazumdar T, Goswami C, Talukdar N C. 2007. Characterization and screening of beneficial bacteria obtained on King's B agar from tea rhizosphere. Indian J. Biotechnol., 6: 490-494.

Mehmood Z, Ahmad I, Mohammad F, Ahmad S. 1999. Indian medicinal plants: a potential source of anticandidal drug. Pharm. Biol. 37(3): 237–242.

Meziane H, Van der Sluis I, Van Loon LC, Höfte M, Bakker PAHM. 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol. Plant Pathol., 6(2): 177-185.

Muleta D, Assefa F, Granhall U. 2007. In vitro antagonism of rhizobacteria isolated from *Coffea arabica* L. against emerging fungal coffee pathogens. Eng. Life Sci., 7(6): 577-586.

Naik PR, Sakthivel N. 2006. Functional characterization of a novel hydrocarbonoclastic *Pseudomonas sp*. strain PUP6 with plant-growth-promoting traits and antifungal potential. Res. Microbiol., 157(6): 538-546.

O'Sullivan DJ, O'Gara F. 1992. Traits of fluorescent *Pseudomonas spp*. involved in suppression of plant root pathogens. Microbiol. Rev., 56(4): 662–676.

Patten CL, Glick BR. 2002. Role of *Pseudomonas putida* indole-acetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68(8): 3795–3801.

Persello-Cartieaux F, Nussaume L, Robaglia C. 2003. Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ., 26(2): 189–199.

Press CM, Loper JE, Kloepper JW. 2001. Role of iron in rhizobacteria mediated induced systemic resistance of cucumber. Phytopathology, 91(6): 593-598.

Rao MS. 2007. Papaya seedlings colonized by the bio-agents *Trichoderma harzianum* and *Pseudomonas fluorescens* to control root-knot nematodes. Nematol. Medit., 35: 199-203.

Rodriguez H, Gonzalez T, Goire I, Bashan Y. 2004. Gluconic acid production and phosphate solubilization by the plant growth promoting bacterium *Azospirillum spp*. Naturwissenschaften, 91(11): 552–555.

Romeiro RS. 2001. Métodos en bacteriologia de plantas. Vicosa: UFV. P. 279.

Ryu Choong-Min, John FM, Reddy MS, Joseph WK. 2007. A two-strain mixture of rhizobacteria elicits induction of systemic resistance against *Pseudomonas syringae* and *Cucumber mosaic* virus coupled to promotion of plant growth on *Arabidopsis thaliana*. J. Microbiol. Biotechnol., 17(2): 280–286.

Sarani SA, Tehrani AS, Ahmadzadeh M, Nikkhah MJ. 2008. Application of fluorescent pseudomonads in biological control of *Rhizoctonia solani*, causal agent of colza. J. Sci. Technol. Agric. Nat. Resour., 11(42): 261-271.

Schilder AMC, Bergstrom. 1990. Variation in virulence within the population of *pyrenophora tritici-repentis* in New York. Phytopathology, 80: 84-90.

Schwyn B, Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160(1): 47–56.

Snedcor GW, Cochran WG. 1980. Statistical Methods, 7th ed. Iowa state Univ. press Iowa, USA.

Sturz AV, Christie BR, Nowak J. 2000. Bacterial endophytes: potential role in developing

sustainable systems of crop production. Crit. Rev. Plant Sci., 19(1): 1-30.

Tang D, Qian H, Zhao L, Huang D, Tang K. 2005. Transgenic tobacco plants expressing BoRS1 gene from *Brassica oleracea* var. *acephala* show enhanced tolerance to water stress. J. Biosci., 30(5): 647-655.

Trivedi P, Pandey A. 2007. Low temperature phosphate solubilization and plant growth promotion by psychrotrophic bacteria, isolated from Indian Himalayan region. Res. J. Microbiol., 2: 454-461.

Umesha S, Hariprasor P. 2010. Plant health improvement by *Bacillus subtilis* strain GB03 in tomato against bacterial spot disease. Indian phytopthol., 63(2): 127-130.

Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, Vasumathi K. 1997. Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol., 46(3): 291–297.

Voisard C, Keel C, Hass D, Defago G. 1989. Cyanide production by *Pseudomonas fluorescens* suppress black root rot of tobacco under gnotobiotic conditions. EMBO J., 8(2): 351-358.

Walter A, Duncan DB. 1969. Multiple range and multiple test. Biometrics, 11: 1-24.

Wangdi T, Uppalapati SR, Nagaraj S, Ryu C, Bender CL, Kirankumar S Mysore KS. 2010. A role for chloroplast-localized *thylakoid formation 1* (*THF1*) in bacterial speck disease development. Plant Singl. Behav., 5(4): 425-427.

Werra PD, Baehler E, Huser A, Keel C, Maurhofer M. 2008. Detection of plant-modulated alterations in antifungal gene expression in *Pseudomonas fluorescens* CHA0 on roots by flow cytometry. Appl. Environ. Microbiol., 74(5): 1339-1349.

Wilson M, Campbell HL, Ji P, Jones JB, Cuppels, DA. 2002. Biological control of bacterial speck of tomato under field conditions at several locations in North America. Phytopathology, 92(12): 1284-1292.

Yunis H, Bashan Y, Okon Y, Henis Y. 1980. Two sources of resistance to bacterial speck of tomato caused by *Pseudomonas tomato.* Plant Dis., 64: 851-852.

تعضيد نمو نباتات الطماطم والمقاومة الحيوية لأمراض تبقع الأوراق وتخرقها بواسطة أنواع بكتريا السودوموناس

محمد عز الدين عبد الحليم داود، زينات كامل محمد، هناء البدوى أحمد عامر، محمد جمال فرحات

كلية العلوم، جامعة القاهرة، قسم النبات

تم عزل 35 عزله من بكتريا السودوموناس الداخل جزريه من انسجة جذور نباتات الطماطم المصريه. تم اختبار هذه العزلات لمعرفة مقدرتها على تثبيط نمو الفطريات والبكتريا الممرضه لهذه النباتات (الألترناريا سولانى والفيوزاريوم أوكسى سبورم). بناء على هذه الاختبارات تم اختيار وتعريف العزلتان الاكثر نشاطاً وأثراً (سودوموناس بوتدا م ج 4 ، سودوموناس فلورسنس م ج 18) ضد الفطريات الممرضه. تم تقييم نشاط هذه العزلات المعضده لنمو نباتات الطماطم . أوضحت هذه النتائج ان كل من العزلاتان أنتجت مركبات سيدرفور ، حمض الهيدروسيانيك ، الأمونيا وهرمون أندول حمض الخليك وهذا الهرمون تم انتاجه بكميات كبيرة بواسطة بكتريا السودوموناس بوتدا والتى كانت أيضاً قادرة على إذابة مركبات الفوسفات بكثرة . تم بكترة جذور نباتات الطماطم بعزلتين السودوموناس وذلك لتكوين مستعمرات جذر بكتيريه. وكان تقدير العدد الكلى لبكتريا السودوموناس على الجذور أكبر من تقديرها على جذور نباتات الكنترول بعد 60 يوم. ولكن تقدير العدد الكلى لبكتريا السودوموناس بوتيدا معنويا أكثر من تلك على نباتات الكونترول (المقارنة) بعد 30 يوم . لقد تناقص العدد الكلى والداخلى لبكتريا سودوموناس فلورسنس بحدة ولكن كان عدد سودوموناس بوتيدا معنويا أكثر من نباتات المقارنه. معاملة نباتات الطماطم ببكتريا سودوموناس فلورسنس وسودوموناس بوتيدا بطريقة غمر جذر البادرة كانت أكثر أثرا في تثبيط مرض تبرقع الاوراق المتسبب بفطرة الترناريا سولانى وتحرق الأوراق المسبب ببكتريا سودوموناس سيرنجى ولكن كانت أثار بكتيريا سودوموناس فلورسنس أقل من سودوموناس بوتيدا. خلط نوعى بكتيريا السودوموناس أعطى فرصة أكبر للحماية ضد كلا المرضين. حقن بكتريا السودوموناس بوتيدا وفلورسنس منفصلة أو مجتمعة زادت تكوين كمية اللجنين زيادة معنوية في أوراق نباتات الطماطم مقارنة بالنباتات غير المحقونه. تم تقييم أثر كلا من السودوموناس في تعضيد نمو نباتات الطماطم في الصوبات المحمية وأوضحت النتائج أن كلا من البكتريا منفصلة أو مختلطة عضدت وزادت مقايس النمو المختلفة لنباتات الطماطم متمثلة قي طول الساق والوزن الغض والجاف وعدد الأوراق ومحتوى اليخضور وطول الجذر ووزنه الغض والجاف وانتاج الثمار الا أن الخليط من سودوموناس بوتيدا وسودوموناس فلورسنس أعطت أقصى تعضيد لنمو نباتات الطماطم .

المحكمون:

أ.د. سعاد أبو السعود	قسم النبات، علوم طنطا
أ.د. إلهام مسعد الرفاعي	قسم النبات، علوم طنطا