Evaluation of some growth promoting bacterial strains exist on Eggplant root *Solanumm elongena* L. against *Rhizoctonia solani*

تقييم السلالات البكتيرية المحفزة لنمو النبات ضد الفطر *Rhizoctonia solani* على نبات الباذنجان *Solanumm elongena* L.

Waheed A. Q. **H. R. Hassan** **B. A. Abbas** **H. H. Nawar**
Ministry of Science and Technology
حيدر حميد نوار **بلاسم احمد عباس** **حيدر رشيد حسن** **أياد قحطان وحيد**
وزارة العلوم والتكنولوجيا

Abstract

In this study, six plant growth promoting bacterial strains were tested against eggplant root rot disease caused by *Rhizoctonia solani*. The bacterial strains were evaluated for their ability to promote growth and control *R. solani* in eggplant under greenhouse conditions. The results of antagonistic activity of the bacterial strains against *R. solani* showed that the tested strains controlled the radial growth of *R. solani* ranging from 24.66 to 40.33 mm, of these, *Bacillus subtilis* was the most promising strains which recorded 24.66 mm. Results of the treatment eggplant seeds with the bacterial suspension of the six strains showed that all tested strains significantly increased the percentage of seeds germination as compared to control treatment, *B. subtilis* strain was the best which recorded 92.16% as compared to 69.56% for control treatment. The greenhouse experiment revealed that the plants treated with *B. subtilis* recorded maximum (Shoot length, root length, fresh and dry weight of plant, rate of fruits weight, plant productivity). All these parameters were increased by 93.83 cm, 26.50 cm, 589.30 g/ plant, 163.03 g/ plant, 101.63 g, 1180 g/ plant respectively, also the results showed *B. subtilis* significantly decreased disease incidence and severity of eggplant infected by *R. solani* which recorded 34.06, 0.23 respectively as compared to both positive without pathogen and negative with pathogen control treatment (4.4 %, 77.33), (0.07 %, 0.71) respectively.

Key words: eggplant, promoting bacterial

المستخلص

في هذه الدراسة ، أختبرت ست سلالات بكتيرية محفزة لنمو النبات ضد مرض تعفن جذور نبات الباذنجان جرى تقييم السلالات البكتيرية لمعرفة قابليتها على تحفيز نمو النبات ومكافحة الفطر الممرض *Rhizoctonia solani* في نبات الباذنجان . أظهرت نتائج الفعالية التضادية للسلالات البكتيرية ضد الفطر الممرض *Rhizoctonia solani* بأن السلالات البكتيرية المختبرة ثبطت معنويا" النمو القطري للفطر الممرض بنسب تراوحت (24.66 الى 40.33) ملم . كانت البكتريا *Bacillus subtilis* هي السلالة المشجعة التي سجلت 24.66 ملم . بينت نتائج معاملة بذور نبات الباذنجان بالراشح البكتيري للسلالات الست بأن كل المعاملات المختبرة زادت معنويا " النسبة المئوية لانبات البذور بالمقارنة مع معاملة السيطرة ، كانت البكتريا *B. subtilis* هي الافضل حيث سجلت 92.16 % بالمقارنة مع 69.56 % لمعاملة المقارنة . كشفت تجربة البيت المحمي بأن النباتات المعاملة بالبكتريا *B. subtilis* سجلت اعلى القيم ، طول المجموع الخضري ، طول المجموع الجذري ، الوزن الطري والوزن الجاف للنبات ، معدل وزن الثمار ، إنتاجية النبات ، زادت جميع معايير النمو 93.83 سم، 26.50 سم، 589.3 غم/ نبات ، 163.03 غم/ نبات 101.63 غم/ نبات ، 118غم/ نبات وعلى التوالي ، أيضا" أظهرت النتائج بأن البكتريا *B. subtilis* خفضت معنويا" نسبة المرض وشدته لنباتات الباذنجان المصابة بالفطر *R. solani* حيث سجلت 34.06% ، 0.23 وعلى التوالي بالمقارنة مع معاملتي السيطرة الايجابية بدون المسبب المرضي والسيطرة السلبية بوجود المسبب المرضي حيث سجلتا (4.4، 77.33) %، (0.07 ، 0.71) وعلى التوالي .

الكلمات المفتاحية : الباذنجان ، البكتريا المحفزة

Introduction

Eggplant *Solanumm elongena* L. is one of the most popular and important commercial vegetable crops grow throughout the world. Eggplant is affected by a number of fungi diseases causing substantial losses in yields including root rot and vascular wilt and damping-off diseases, which inflict heavy losses in its production [1]. Microorganisms such as bacteria are important to control phytopathogenic fungi and to promote the circulation of plant nutrients and reduce the need of chemical fertilizers, which are costly and create environmental problems for warranting high yield and quality [2]. Hence, there has recently been a resurgence of interest in environmentally friendly, sustainable and organic agricultural practices [3]. The use of beneficial microorganisms is positively known to affect on disease and plant growth [4,5]. Number of inoculated bacterial species mostly associated with the plant

rhizosphere have been tested and found to be beneficial for plant growth, yield and crop quality [2]. They have been called plant growth promoting bacteria (PGPB), including the strains in the genera *Azotobacter*, *Bacillus*, *Azosperilium*, *Rhizobium*, *Pseudomonas* [6,7].These bacteria were previously reported as plant growth promoting bacteria and had potential biocontrol agents against a wide range of fungal pathogens [8,9,10].

The objective of this study was to determine the effects of inoculation bacteria (*Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas fluorescence*, *Azotobactre chroococcum*1, *Azotobactre chroococcum*2, *Bacillus sp.*) in control root rot disease caused by *Rhizoctonia solani* and on yield and growth of eggplant vegetable crop in greenhouse conditions.

Materials and methods

Bacterial strains and culture conditions

Strains of plant growth promoting bacteria, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas fluorescence* were obtained from Organic Culture Center/ Ministry of Agriculture, whereas, bacterial strains, *Azotobactre chroococcum*1, *Bacillus sp.*, *Azotobacter chroococcum*2 were isolated from bean, okra rhizosphere and garden soil Table (1), identified according to characteristic features of colony and bacterial cells. Bacterial strains were grown on Nutrient Agar (Difco Laboratories, USA) for routine use; a single colony was transferred to 250ml flasks containing Nutrient Broth and grown aerobically in flasks on a rotations shaker 95 rpm for 48hr at 28˚C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 CFU/ml, and the resulting suspensions were used to treat seeds and seedlings eggplant plants.

Table (1): Source of Bacterial Isolates used in this study

Bacterial isolates	Source	Location
***Azotobacter chroococcum*1**	**Bean rhizosphere *(Phaseolus vulgaris)***	**Field in Al-Tweeth town / south east of Baghdad**
***Azotobacter chroococcum*2**	**Garden soil**	**Al-Zafarania city / Baghdad**
Bacillus sp.	**Okra rhizosphere *(Hibiscus esculentus)***	**= = =**
Bacillus subtilis	**MinistryofAgriculture/ Organic Culture Center**	
Bacillus pumilus	**= = =**	
Pseudomonas fluorescence	**= = =**	

Fungal pathogen (*R. solani*)

One isolate of fungal pathogen *R. solani* was obtained from Department of Biocontrol for Plant Diseases at Agricultural Researcher Office/ Ministry of Science and Technology was used in this study.

Bacterial antagonistic activity evaluation

The method described by [10] was used to determine antagonistic activity of the six strains of plant growth promoting bacteria *Azotobacte rchroococcum*1, *Azotobacter chroococcum*2, *Bacillus sp.*, *Bacillus subtilis*, *Bacillus pumilus* and *Pseudomonas fluorescence* against fungal pathogen *R.solani*. One 5mm disk of pure culture of the pathogen was placed at the center of Petri dish 10cm diameter containing PSA potato sucrose agar media. Circular line, made of a 5cm diameter Petri dish dipped in a bacterial suspension of one of the six strains of bacteria was placed surrounding the fungal pathogen. Plates were incubated for 72hr. at 25˚c and growth diameter of the pathogen was measured and compared to control growth, where the bacterial suspension was replaced by sterile distilled water.

Results were expressed as the means of the percentage of the growth inhibition in the presence of any of the bacterial strains.

Inhibition percentage was calculated using the following formula:

%Inhibition =[1-fungal growth / control growth] × 100[11]

Greenhouse experiment

The experiment was carried out in greenhouse at Department of Biocontrol for Plant Diseases/Agricultural and Food Technology Researches Center in Al-Zafrania City from 22/3/2011-4/7/2011 to evaluated the interaction between six strains of plant growth promoting bacteria and the pathogen *R. solani* for their potential to stimulate eggplant resistance against root rot disease. One isolate from the pathogen was used as causal agent of eggplant root rot disease at rate of 1ml of fungal suspension 105spore/ml of the 7 days old culture on PSA Potato sucrose agar medium per 1 ml of soil before planting. Eggplant seeds were soaked in bacterial suspension 10^8 CFU/ml for 10 min and air

dried before seeded at rate of 5 seeds/ pot. Eggplant seeds were sown in trays 30,50,10cm deep containing autoclave coarse clay sand 1:1 v/v and watered twice a week. After 20 days, similar healthy seedlings 10cm length were uprooted and treated with bacterial suspension of the six strains 106 CFU/ ml and planted in holes at rate 2 seedlings/ hole. Treatments were distributed in greenhouse according to Randomized Complete Blocks Design RCBD in three replicates each replicate 10 plants to evaluate the following treatments:

1- Control.
2- Pathogen only.
3- *Azotobactre chroococcum* 1+ pathogen.
4- *Azotobactre chroococcum* 2 + pathogen.
5- *Bacillus sp.* + pathogen.
6- *Bacillus subtilis* + pathogen.
7- *Bacillus pumilus* + pathogen.
8- *Pseudomonas fluorescence* + pathogen.

Plants were harvested at the end of the experiment, growth parameters were recorded beside disease incidence and severity as following:

1-Mean length of shoot and root of plants.
2-Fresh and dry weight of plants.
3-Seeds germination and the first day of germination.
4-Fruits weight and plant productivity.
5-Disease incidence and severity.

Results and Discussion

Antagonistic activity of bacterial strains against *R. solani*

The results showed that the tested strains varied in their ability in reducing radial growth rate of the pathogen and the percentage of pathogen growth inhibition Table (2). *Bacillus subtilis* strain showed significant reduction in radial growth of *R. solani* 24.66mm as compared to other strains *A. chroococcum*1, *A. chroococcum*2 and *Bacillus sp*. Which were recorded 37.66,40.33,35.33mm respectively. Also *B. subtilis* appeared significant increment in percentage of pathogen growth inhibition 46.26% as compared to *A. chroococcum*1, *A. chroococcum*2 and *Bacillus sp*. 17.96, 12.16,23.06% respectively. These results are due to the antagonistic metabolites secreted by bacterial strains and suggests that the mode of action exerted and the type of antifungal metabolites produced by the six strains was varied. Reduction of fungal growth of *R. solani* and formation of inhibition zones were presumably due to the antifungal substances and/ or cell wall degrading enzymes released by bacterial strains into the culture media [12,13,14] .

Table (2): Antagonistic Activity of Bacterial Isolates against *R. solani*

Bacterial isolate	*R. solani*	
	Radial growth rate (mm)	% Inhibition
***A. chroococcum*1**	**37.66**	**17.96**
***A. chroococcum*2**	**40.33**	**12.16**
Bacillus sp.	**35.33**	**23.06**
B. subtilis	**24.66**	**46.26**
B. pumilus	**27.66**	**39.83**
P. fluorescence	**30.66**	**28.36**
Control	**46.0**	**-**
LSD (P= 0.05)	**1.75**	**5.05**

Treatment of eggplant seeds with bacterial suspension of PGPB strains and their effect on some eggplant growth parameters under greenhouse conditions

Treatment of eggplant seeds with PGPB strains showed that all the strains significantly increased seeds germination, *B. subtilis* showed 92.16 % as compared to control treatment 69.56% Table (3). Also, the results revealed that most of bacterial strains significantly increased eggplant growth parameters shoot and root length, fresh and dry weight of plant, fruit weight, and plant productivity Tables (4,5). *B. subtilis* was the superior which recorded 93.83,26.5 cm , 589.3,163.03g/ plant, 101.63g, 1180.0)g/ plant respectively as compared to both positive and negative control treatment which recorded 78.33,

47.66cm, 16.5, 12.83cm, 305.3, 148.3g / plant, 88.03, 39.46g/ plant, 62.63, 34.43g , 642.33, 141.0g/ plant respectively.

Table (3): Treatment of Eggplant Seeds with Bacterial Suspension and Their Effect on Seed Germination under Greenhouse Conditions

Treatment	%Seed germination	1st Germination
*A. chroococcum*1	80.16	12
*A. chroococcum*2	82.16	12
Bacillus sp.	71.50	14
C. subtilis	92.16	11
A. pumilus	84.83	12
P. fluorescence	89.66	14
Control	69.56	15
LSD (P= 0.05)	1.50	-

Table (4): Treatment of Eggplant Seeds and Seedlings With Bacterial Suspension and Their Effect on Some Plant Growth Parameters Infected by *R. solani* Under Greenhouse Conditions

Treatment	Shoot length cm	Root length cm	Fresh plant weight (g/plant)	Dry plant weight (g/plant)
Control	78.33	16.5	305.3	88.03
Pathogen only	47.66	12.83	148.3	39.46
A.chro. 1+Path.	88.56	22.83	530.6	127.5
*A. chro.*2+Path.	83.40	21.5	520.3	120.4
Bacillus sp. +Path.	82.50	19.5	529.16	124.4
B.subtilis+Path.	93.83	26.5	589.3	163.03
B.pumilus+Path.	91.50	20.5	566.30	141.4
P. fluorescence+Path.	87.46	24.16	561.13	144.1
LSD (P= 0.05)	2.07	1.14	1.88	1.46

The results of Table (4) revealed that *B. subtilis* + pathogen treatment was the best in increment all the tested growth parameters (shoot and root length, fresh and dry weight of plant which recorded 93.83,26.5cm, 589.3,163.03/ plant respectively as compared to control treatment 78.33,16.5cm, 305.3,88.03g/ plant respectively. Also the results showed that *B. subtilis* recorded significant increment in fruits weight and plant productivity Table (5) which recorded 101.63g, 1180.0g/ plant while, in control treatment 62.63g, 642.33g/ plant respectively.

Plant growth promoting bacteria produced a variety of growth promoting substances like IAA, gibberellins, vitamins [15,16] and antifungal substances [17,18,19,14], so it improved seeds germination and increased plant growth parameters. The results obtained from Tables [17,18,20] may be to beneficial effects of bacterial strains on growth and eggplant seeds germination in multiple ways, which include, ability to produce vitamins and growth promoting substances that enhance seeds germination end eggplant growth.

Table (5): Rate of fruits weight and plant productivity of Eggplant plants under greenhouse conditions

Treatment	Fruit weight (g)	Plant productivity g/ plant
Control	62.63	642.33
Pathogen only	34.43	141.0
A.chro. 1+Path.	69.76	739.66
*A. chro.*2+Path.	71.96	743.66
Bacillus sp. +Path.	64.83	675.73
B.subtilis+Path.	101.63	1180.0
B.pumilus+Path.	88.23	1045.0
P. fluorescence+Path.	86.43	889.66
LSD (P= 0.05)	3.48	3.71

Results of Table (6) showed that all the tested treatment significantly decreased the disease incidence and severity of eggplant infected by the pathogen under greenhouse conditions as compared to both negative and positive control treatment. *B. subtilis* +pathogen treatment was the best which recorded 46.30%, 0.23 respectively as compared to control treatment 4.4,77.33%, 0.07,0.71 respectively.

Table (6): Disease incidence and severity of eggplant plants infected by *R. solani* under greenhouse conditions.

Treatment	Disease incidence %	Disease severity
Control	**4.4**	**0.07**
Pathogen only	**77.33**	**0.71**
***A.chro.* 1+Path.**	**51.93**	**0.35**
***A. chro.*2+Path.**	**47.33**	**0.33**
***Bacillus sp.* +Path.**	**46.30**	**0.29**
***B.subtilis*+Path.**	**34.06**	**0.23**
***B.pumilus*+Path.**	**38.16**	**0.28**
***P. fluorescence*+Path.**	**41.33**	**0.30**
LSD (P= 0.05)	**2.26**	**0.04**

PGPB can affect plant growth in two general ways, either directly or indirectly [20, 21]. Indirect promoting occurs when PGPB lessen or prevent the harmful effects of one or more deleterious microorganisms. This is chiefly attained through biocontrol, or the antagonisms of soil pathogens. Specifically, colonization or the biosyntheses of antibiotics and other secondary metabolites can prevent pathogen invasion and establishment. Direct promoting of plant growth by PGPB occurs when the plant is supplied with a compound that is synthesized by the bacteria, or when PGPB otherwise facilities plant uptake of soil nutrients, possibilities include nitrogen fixation, siderophore synthesis, phytohormone synthesis, and solubilization of minerals to make them available for plant uptake and use [20].

References

1. Gupta, S K. and Third, T S. (2006). Disease problems in vegetable production. In: Disease of eggplant. Scientific Publishers – India. 409-429.
2. Dursun, A., Ekinci, M. and Domez, M F. (2010). Effects of foliar application of plant growth promoting bacteria on chemical contents, yield and growth of tomato (*Lycopersiconesculentum* L.) and cucumber (Cucumissativus L.). Pak. J. Bot. 42(5): 3349-3356.
3. Esitken, A., Pirlak, L., Turan, M. and Sahin, F. (2006). Effects of foliar application of *Bacillus subtilis* on the yield, growth and control of shot-hole disease (Coryneum *blight*) of apricot, Gartenbauwissenschaft. 67: 139-142.
4. Fatima, F., Saleemi, M., Zia, M., Sultani, T., Aslam, M., Rehman, R. and Chaudhary, M F. (2009). Antifungal activity of plant growth promoting rhizobacteria isolates against *Rhizoctoniasolani* in wheat. Afri. J. Biotechnol. 8(2): 219-225.
5. Gholami, A., Shahsavani, S., and Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Acad. Sci. Eng. & Technol. 49:19-25.
6. Narula, N., Remus, R., Deubel, A., Dudeja, S. and Bell, R. (2007). Comparison of the effectiveness of wheat roots colonization by *Azotobacter chroococcum* and *Pantoeaagglomerans* using serological techniques. Plant Soil Environ. 53 (4): 167- 176.
7. Siddiqui, I A. (2001). Effect of microbial antagonists on in vitro growth of *Pythium aphanidermatum* . J. Biol. Sci. 1(4):224-226.
8. Al-Azawy, A Q. (2010). Efficiency of interaction between *Azotobacter sp*. and *arbuscular mycorrhizal* fungi for their potential to stimulate tomato plant resistance to root rot disease. Ph.D. Thesis. Baghdad University/ College of Science.
9. Kamal, A M., Hashem, A. and Mohamed, M. (2009). Biological control of *Fusarium* wilts in tomato by yeasts and rhizobacteria. Plant Pathol. J. 25:199-204.
10. Montealegre, J R., Reyes, R., Perez, LM., Herrera, R., Silva, P. and Besoain, X. (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electron J. Biotechnol. 6(2): 115-127.
11. Mojica-Marin, V., Luna –Olvera, H A., Sandoval-Coronado, C F., Pereyra – Alferez, B., Morales–Ramos, L H., Hernandez-Luna, C E. and Alvarado-Gomez, O G. (2008). Antagonistic activity of selected strains of *Bacillus thuringinsis* against *Rhizoctonia solani* of chili pepper. Afric. J. Biotechnol. 7(9): 1271-1276.
12. Mali, G V. and Bodhanker, M G. (2009). Antifungal and phytohormone production potential of *Azotobacter chroococcum* isolates from Graundnut (Arachis *hypogeal* L.) rhizosphere. Asian J. Exper. Sci. 23 (1): 293-297.
13. Verma, S., Kumar, V., Narula, N. and Merbach, W. (2001 b). Studies on *in vitro* production of antimicrobial substances by *Azotobacter chroococcum* isolates/ mutants. (Abstract).

14. Todorova, S. and Kozhuharom, L. (2010). Characteristics and antimicrobial activity of *Bacillus subtilis* strains isolates from soil. World J. Microbiol & Biotechnol. 26:1207-1216.

15. Verma, S., Kukreja, K., Pathak, D V., Suneja, S. and Narula, N. (2001a) In *vitro* production of plant growth regulators by Azotobacter *chroococcum*. Ind. J. Microbiol. 41 (4): 305 – 307.

16. Ahmed, F., Ahmed, I. and Khan, M S. (2005). Indole acetic acid production by the indigenous isolates of *Azotobacter* and Florescent *Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol. 29: 29 – 34.

17. Cartwright, D K., Chilton, WS. and Benson, DM. (1995). Pyrrolnitrin and phenazine production by *Pseudomonas cepacia*, strain 5.5B, a biocontrol agent of *Rhizoctonia solani*. Appl. Microbiol. Biotechnol. 43:211-216.

18. Cartwright, D K. and Benson, D M. (1995). Comparison of Pseudomonas species and application techniques for biocontrol of *Rhizoctonia* stem rot Poinsettia. Plant Disease. 79(3): 309-313.

19. Ramyasruthi, S., Pallavi, O., Pallavi, S., Tilak, K. and Srividya, S. (2012). Chitinolytic and secondary metabolite producing *Pseudomonas fluorescence* isolated from *solanaceae rhizosphere* effective against broad spectrum fungal phytopathogns. Asian J. Plant Sci. & Res. 2(1): 16-24.

20. Barea, J., Pozo, M J., Azcon, R. and Azcon – Aguilar, C. (2005). Microbial co-operation in the rhizosphere. J. Exp. Bot. 56:1761-1778.

21. Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E A. (2005). Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. &Environ. Microbiol. 71:4951-4959.