Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* in reducing cucumber seed rot and seedlings death caused by *Rhizoctonia solani* and *Fusarium solaniin* green house conditions

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Abstract

The study was conducted to evaluate the efficacy of two bacterial biological control agents controlling the pathogenic fungi that cause seed rot and seedlings death on cucumber growth in green house in Abbasiya, Al-Huraira, Al-Haidari and Qizwiniya towns in Najaf province. *Bacillus subtilis* and *Pseudomonas fluorescens* were isolated from soils of the above areas and biochemically identified at ministry of science and technology. The pathogenic fungi *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber seeds that have been grown in gerrn house and diagnosed morphologically and molecularly using the PCR technique. The results showed two bacterial isolates were highly antagonistic to *R.solani* and *F.solani*. Results of the pot experiment showed the ability of both bacterial isolates introspecting cucumber plants from infection by *R. solani* and *F. solani*, in addition to their high efficiency in increasing the growth indicators (germination, total vegetation and root length, soft and dry plant weight and leg diameter) after 14 and 28 days post planting.

The field experiment in green house when testing the effect of two bacteria isplutes was showed increasing of the total chlorophyll content of leaves of cucumber plants at 28 days, especially when treated with *P. fluorescens* was significant differed from the rest of treatments with chlorophyll content of 1027.3 mg. 1. in addition that some bacteria was significantly increased the rate of plant contact of the major elements (total nitrogen ,total Phosphor, total potassium) NPK after 14 and 28 days of planting 2.9 and 3.55%. The best treatment of*B. subtilis + P. fluorescens*. was significantly increased the total cucumber yield 6.185 kg / plant for 20 harvests, compared with the rest of treatments.

Keywords: cucumber, P. *flourecens, B. subtilis, F. solani, R. solani*

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Introduction

Cucumber is one of the most important vegetable crop plant under greenhouse conditions and fields. The total cultivated area in Iraq was estimated 47060.5 ha in 2010 with total production reach to 192525 Tons^{-1} and productivity of 2294.2 kg. dunum^{-1}(14). Cucumber root rot, seed decay and seedling death cause by *Pythium* spp, *Phytophthora* spp, *Rhizoctonia solani Fusarium solani* are serious problems associated with cucumber cultivation worldwide(2). Soil-borne plant pathogenic fungi are among the most dangerous and damaging actors, affecting the quantity and quality of agricultural production(13). That is have a wide host range, resistance to adverse environmental conditions and different ways of survival in the soil and/or infected plants remains, in addition to various ways of infection in the field. Fungi that cause seed rot, seedling death and root rot leading to high yield losses and increase the cost of production (2).

Soil-born fungi are among the most destructive plant pathogens. They are highly competitive because of its ability to survive on various feed resources (facultative parasites), possessing developed enzymatic system and their tolerance to toxins and antibiotics produced by other soil microorganisms, as well as their rapid growth and ability to produce large quantities of infective units that are able to survive at severe conditions and spread through various mechanisms (2).

Using biological agents and antagonistic microbes for plant disease management is rapidly increased during last year's, mainly to reduce the use of highly toxic fungicides and their environmental effects (21)

The Biological control of soil-borne pathogens is given wide attention by many researchers, Al-Hitar(3)Use of bacterial species including *Psedomonas flourescens* and *Bacillus subtilis* as biomass, leach ate or both in biological control against plant pathogens is substantially effective in reducing infection and raising plant health and yield .*P. flourescens* and *B. subtilis* were found to be effective in contraling fungal diseases due to their ability to produce antibiotics and stimulate plant systemic resistance through production of phytoalxines, in addition to their ability as plant growth promoters (6)

Objectine of study was to evaluate the efficacy of *P. flourescens* and *B. subtilis*that have been isolated from cucumber roots in green house from several areas of the Najaf province to control seed rot disease and seedlings death of cucumber caused by *Rhizoctoniasolani* and *Fusarium solani* in pot and field experiments.

Pesticide celest is a fungal pesticide used in the Ministry of Agriculture and Public Health in Iraq, the common name Fludioxonil and the commercial name is Celest used to against *R. solani* fungi and *F. solani* on cucumber and potatoes which is lead to increase crop activity and good performance. The pesticide contains the active substance Fludixonil and extracted from soil bacteria in a form that makes them stick to the seeds in a good working way, they stick to the seeds in a good working way, making them effective in the immune-acquired diseases of other pesticides, which gives more ease of application and higher efficiency of results,

this pesticide registered trademark for the Syngenta Company.(32)

Materials and methods

General experimental procedure two experiments (pot and micro-plot) were carried out in a green house belonged to Department of Plant Production\Directorate of Agriculture in Najaf Governorate in 10/2/2018 using 2 Liter pots (25 cm diameter, 17 cm long, width 45 cm). The soil used ethanol sterilized, covered with polyethylene for two days and then re-covered and exposed to aeration for two days. The soil was mixed with sterilized peat moss by 1:2 ratio soil: peat moss (V/V). The soil was distributed into the plastic cans or plastic micro-plots to be used according treatments. Treatments included pathogenic *R. solani* and *F. solani*. The fungus was isolated from the affected cucumbers in the field, they were diagnosed with the reaction of PCR in the laboratories of the Faculty of Agriculture of Karbala University.

biological control bacteria *P. flourescens* and *B. subtillis*, fungicide Celest ST and all their interactions as well as the untreated control treatment.

R. solani and *F. solani* were selected as they caused in the lowest *in vitro* germination rates of cucumber seeds. Each pot or micro-plot was inoculated with the entire fungal biomass of each fungus after being grown in Petri dishes on standard P.D.A \incubated for 72hours. The combined treatment of both fungi was performed by mixing content of a half dish of each fungus. Fungal biomass was shallow incorporated to the pot's soil. Bacteria from the surrounding soil isolated the seeds of the healthy cucumber plant and were diagnosed with bio-biochemical tests at the Ministry of Science and Technology-Baghdad ,*P. flourescens* and *B. subtillis* were propagated on the liquid medium of nutrition broth (N.B) and incubated for 48h before being used. After Three days soil incorporation with fungi, the bacterial inoculums of each *P. flourescens* and *B. subtillis* was added by 100ml/2L pot or 200ml/4L micro-plot at concentration of 1×10^9CFU. All fungal and bacterial treatments and its combination and interactions were applied to the pot soil two days pre-planting. Untreated pots (soil was incorporated with sterile medium only) served as controls. Cucumber seeds variety 'Hybrid' were superficial sterilized with 5% sodium hypochlorite NaOCl for 2 minutes, washed with distilled water and then dried with sterilized filter paper. And put two seeds in each plot.

1 - Effect of *P. flourescens* and *B. subtilis* on *R. solani* and *F.solani* infection severity and growth of cucumber pot plants under plastic house conditions

Pots of all treatments were maintained in the green house at 35C° and irrigated as needed. The experiment was RCBD with 3 replicates and 12 plants in each treatment. Seeds germination rates were recorded at 14 days. while plant growth parameters including shoot and root length (cm), fresh and dry weight (g) and stem diameter (cm) were recorded at 14 and 28 day post planting.

2 - Efficacy of micro-plot soil treatment with *P. flourescens* and *B. subtilis* on *R. solani* and *F. solani* infection, growth and

yield of cucumber under greenhouse conditions

This experiment was conducted in the same location(greenhouse at Najaf cultures) using the same soil mix and treatment combinations and interaction as in the pot experiment. It was micro-plot experiment using 4 Liter polythene bags. The bags were filled with the prepared sterile soil and buried to be even to the ground of the plastic house in 1 meter wide furrows. Furrows were 50 cm apart while the distance between plots was 30cm. Double-side drip-lines were performed and irrigation was applied as required. Plants were maintained in the green house at 35C° and irrigated as needed. The experiment was adopted into Randomized Complete Block Design (R.C.B.D)(4) with 3 replicates and 12 plants in each treatment. Plant growth parameters including shoot and root length (cm), fresh and dray weight (g) and stem diameter(cm) were recorded at 28 day after planting after three months. The total chlorophyll content was evaluated in cucumber leaves using UV (Spectrophotometer) at 28 days post planting. The plant content of plant content of the major elements total nitrogen, total phosphorus and total potassium(NPK) was measured after 14 and 28 days post planting. Plant content of nitrogen was estimated using Kjeldahl method (1), while the total amount of potassium was estimated using the photometric spectrometer(5). The total content of phosphorus in the cucumber leaves was also estimated using the spectrophotometer (5). The total collective yield per plant (kg) of 20 harvest times was calculated for each treatment.

Results and discussion

1 - Effect of *P. flourescens* and *B. subtilis* on *R. solani* and *F.solani* infection severity and growth of cucumber pot plants under plastic house condition

Percentage of cucumber seed germination and seedlings death 14 days post planting.

The results of Table (1) showed that the isolates of *F. solani* and *R. solani* caused decrease in seed germination rate and an increase in the percentage of plant death under the green house conditions. *R. solani* resulted in a reduction of germination rate of 19.44% with a significant difference, compared to all treatments except for the treatment of *F. solani*, which resulted in germination rate of 24.99%. The table(1) also showed high rates of seed germination (80.55-100%) in the treatment (*B. subtilis* and *P. fluoresens*) and its interactions with the pathogenic fungi. These results are in agreement with the results of other studies which have shown that fungal pathogens differ in their pathogenicity on their host species (12,14). These differences in seeds germination may related to differences in the ability of fungal isolates in toxic secretion, secondary metabolic compounds and or enzymes that break down the plant contents such as pectin and cellulose in the first stage of infection that play an important role in pathogenicity to inhibit plant's defenses (19). As was showed that both fungi isolates increased the percentage of seedlings death by 75.5 and 80.55% *R. solani* and *F. solani* respectively compared to (0.0%) in control treatment, control treatment was also showed a significant different in a rate of seed death (0%)compared with other treatments, while the percentage of

seedling death was reduce in case of biological control agents *B. subtilis* and *P. fluoresens* and their interactions with no significant differences between these agents.

Table1. Effect of different treatments by biological control bacteria and pathogenic fungi on percentage of cucumber seed germination and seedlings death in pot experiment 14 days post planting

Treatments	% germination	% seedlings death
Control	100	*0.00
Fusariumsolani	24.99	75.00
Rhizoctoniasolani	19.44	80.55
Fusariumsolani+Rhizoctoniasolani	30.55	69.44
Pseudomonas +(F. solani)	80.55	19.44
Pseudomonas+(R. solani)	80.55	19.44
Bacillus+(F. solani)	100.00	0.00
Bacillus+(R. solani)	100.00	0.00
Pseudomanas+(F. solani+R. solani)	100.00	0.00
Bacillus +(F. solani+R. solani)	100.00	0.00
Pseudomonas+ Bacillus +(F. solani)	100.00	0.00
Pseudomonas+ Bacillus +(R. solani)	94.44	5.56
Pseudomonas+ Bacillus +(F. solani+ R.solani)	96.66	3.33
Celest	94.44	5.56
Bacillus	100.00	0.00
Pseudomonas	100.00	0.00
Pseudomonas+ Bacillus	100.00	0.00
LSD ($P \leq 0.05$)	7.806	7.806

*Data were means of six replicates

A - Effect of different treatments on cucumber plants growth parameters 14- and 28-days post planting

The results of Table (2) showed that the highest shoot length of the cucumber 14 days after planting was recorded in Celest treatment. This treatment significantly differed from most of the experimental parameters except (*P. flourescens + F. solani+B. subtilis + R. solani*) It was 8.06, 6.19 and 6.22 cm respectively, while the lowest length was 0.83 cm in the treatment of *R. solani*. The highest shoot length 28 days post planting (14.32 cm) was in the treatment of *P. fluorescens + B. subtilis* which was significantly different from all treatments except in the control and *B. subtilis* treatments with shoot length of 12.74 and 13.50 cm. respectively .The lowest shoot length was 1.59 cm in *R. solani* treatment. As for the root length at 14 days post planting, the highest root length (4.877cm) was in treatment of *P. flourescens + B. subtilis*, while the lowest root length recorded in the treatment of which was *R. solani* 0.497 cm. There was no significant difference between most of the treatments, while the root length of 28 days was 9.59 cm in *B. subtilis* treatment which was significantly higher, compared to *R. solani* treatment(1.29cm). The fresh plant weight after 14 days was (0.860g) in *P. flourescen + B. subtitis* treatment and the lowest value was recorded in the treatment of *R .solani*

that of 0.300 g. While, the fresh weight 28 days post planting was the highest (4.62 g) in the treatment of *P. fluorescens* + *B. subtilis*, compared to the lowest (0.57 g) resulted from *R .solani*. Relative to plant dry weight 14 days post planting, the highest weight was (0.653) gram in the treatment of *B. subtilis* and the lowest weight (0.196 g) in the treatment of *R. solani*. As for plant dry weight after 28 days, the highest weight was (0.850 g) in the treatment of *B. subtilis* + *P. flourescens* while the lowest value was(0.350g) in the treatment of *P. fluorescens* + *R. solani*. For stem diameter 14 days post planting, the maximum diameter was (1.970)cm in *B. subtilis* + *P. flourescens*, while the lowest was (0.207) cm in *R. solani*. stem diameter 28 days post planting, the maximum diameter was(3.38) cm in the treatment of *P. fluorescens* + *B. subtilis* + *F. solani* + *R. solani* while the lowest stem diameter (0.51 cm) resulted from *R. solani* which significantly differed from the highest.

The reason for increasing the plant fresh weight could be due to the accumulation of plant growth hormones and this was consistent with the study of Sakhabutdinova *et. al*.(23). The increase of plant growth indicators should be due to the presence of biological control agents. where bacteria *B. subtilis* increases nutrients availability that required by plant, in addition to its ability to induce plant resistance (31). *P. flourescens* was found to produce growth regulators, as well as Auxine, which help to increase the yield of the treated plants (17). These bacteria stimulate systemic resistance in the treated plants (30).

The decrease in the growth indicators was common in case of infected cucumber plants. The cultivation of cucumber had accompanied the emergence of many diseases that negatively affect it in particular seedling death and root rot, which were the most important soil borne diseases including diseases caused by pathogenic *F. solani* and *R. solani*(9). Various biological agents have been used against these pathogens including the bacteria *Pseudomonaes* and *Bacillus* (22). These two bacteria were reported to have ability to improve plant growth and increase production which attributed to their ability as growth promoter by producing active metabolic compounds and organic compounds, as well as the production of IAA and degrading enzymes of pathogen cell walls, antibodies and hormones that are believed to inhibit the pathogen (27). Activities of these bacteria not only induce plant resistance, but also improve the plant growth and production. The general increase in plant growth parameters by these bacteria could be related to their different mechanisms directly or indirectly that occurs separately or combined at different stages of plant growth. Several theories were developed to explain the growth and resistance of plants where exposing to these factors. The most common theory was the production of antibiotics, releasing compounds that highly competent chemical elements needed by the pathogen for development, production of plant growth regulators as well as the release of the operator genes by breaking the its linkage into the receptor protein (20)

*B. subtilis*was one of the most important biological agents against plant pathogens. Killan *et. al*. (18) noted that the use of these bacteria leads to systemic resistance in the treated plants. This could be

achieved by increasing promoter's activities, which regulates the effectiveness of the resistance gene and the speed of systemic signal transmission to induce resistance in the plant.

Table2. Effect of different treatments on cucumber growth parameters 14- and 28-days post planting (DPP) in pot experiment under plastic house condition

Treatments	Length (cm)				Plant weight (g)				Stem diameter cm	
	Shoot		Root		Fresh		Dry		14DPP	28 DPP
	14DPP	28 DPP	14DPP	28 DPP	14DPP	28 DPP	14DPP	28 DPP		
Control	*6.44	12.74	4.507	8.26	0.833	3.09	0.653	0.830	1.423	2.47
Fusarium solani	3.29	4.36	1.463	2.28	0.350	1.01	0.336	0.530	0.430	1.06
Rhizoctoniasolani	0.83	1.59	0.497	1.29	0.300	0.57	0.196	0.530	0.207	0.51
Fusarium solani +Rhizoctoniasolani	2.89	3.81	1.160	2.59	0.427	0.92	0.383	0.350	0.340	0.59
Pseudomonas + (F. solani)	6.19	11.21	3.963	6.83	0.690	1.87	0.587	0.450	1.153	2.21
Pseudomonas + (R. solani)	5.80	11.17	4.287	6.53	0.673	1.52	0.523	0.610	0.897	1.04
Bacillus + (F. solani)	5.44	11.16	3.380	6.35	0.587	1.61	0.577	0.540	0.967	1.43
Bacillus + (R. solani)	6.22	9.17	4.677	6.14	0.630	1.66	0.557	0.597	0.857	1.71
Pseudomonas + (F. solani + R. solani)	5.28	10.94	3.750	8.98	0.590	2.98	0.457	0.583	0.813	2.34
Bacillus + (F.solani + R. solani)	5.64	11.05	3.827	8.03	0.643	2.18	0.473	0.480	0.667	2.35
Pseudomonas + Bacillus + (F. solani)	5.55	11.78	4.083	7.89	0.647	2.51	0.570	0.517	0.850	2.12
Pseudomonas + Bacillus + (R. solani)	5.52	11.22	3.80	8.33	0.657	2.07	0.593	0.623	0.960	1.73
Pseudomonas + Bacillus + (F. solani+R. solani)	5.50	11. 08	3.94	9.12	0.65	3.07	0.58	0.600	0.90	3.38
Celest	8.06	10.50	4.697	9.22	0.720	2.51	0.603	0.537	1.027	2.07
Pseudomonas	5.41	9.87	4.827	8.36	0.707	2.25	0.560	0.473	0.863	2.17
Bacillus	5.87	13.50	4.187	9.59	0.767	3.52	0.653	0.683	1.163	2.79
Pseudomonas + Bacillus	5.95	14.32	4.877	8.06	0.860	4.62	0.593	0.850	1.970	3.17
LSD (P≤0.05)	2.839	2.047	1.183	2.562	0.1682	1.545	0.180	0.1255	0.583	1.286

*Values were average of three replicates

Effect of different treatments on cucumber plants of NPK contect on 14 and 28 days post planting in micro-plots grown under plastic house condition

The results in Table (3) showed that the highest nitrogen content in cucumber leaves 14 days post planting was 2.913% in the treatment of *P. fluorescens* which significantly differed from all the other treatments while the lowest amount of nitrogen content was 0.001% in the *F. solani* treatment. *P. fluorescens* had also the highest percentage of nitrogen in leaves of cucumber 28 days post planting which was 3.550% with significant difference of all treatments except treatments of Celest

and*P. fluorescens* + *B.subtilis*, which resulted in nitrogen content of 3.490 and 3.483, respectively. As for leaf content of phosphorus 14 days post planting, *B. subtilis* was significantly higher among all the treatments resulted in 2.790%, however, *F. solani* resulted in the lowest amount of phosphorus that of 0.003%. In the case of 28 days post planting, the highest amount of phosphorus was 3.780% in the combined treatment of *F. solani*+*B. subtilis*, which was significantly higher than all the other treatments except that of *P. fluorescens*+ *B. subtilis* (3.510%). Regarding the amount of potassium in the leaves of cucumber 14 days post planting, the treatment of *P.fluorescens* was significantly the highest resulted in potassium content of 2.296 %, while the lowest amount of potassium was 0.001% in the treatment of *F. solani*. Potassium content 28 days post planting were significantly (P <0.001) higher in *P. fluorescens* + *B. subtilis* compared to other treatments.

It is clear from the results that *F. solani* reduced the amount of nutrients available to cucumber plants. This was common as this fungus was the most important wilt causing fungi, and affect many economic crops (28) including cucurbits especially cucumber plants(8). *B. subtilis* was used to reduce the damage of this fungus by producing bacterial antibiotic compounds such as Zwittenicin A, Cerexine and bacillin as well as the enzyme chitinase (16). The lowest amount of NPK in cucumber leaves was always associated with infection by *F. solani* and *R. solani* while much higher amount of these elements was detected in the presence of biological control agents (10). Researchers showed that *B. subtilis*was able to inhibit endemic soil borne pathogenic fungi and stimulate plant growth (29). *P. fluorescens* also plays an important role in increasing plant production indices, as it belong to PGPR (Plant Growth Promotion Rhizobacteria). Khakipour *et.al.*(17) also pointed out that was through several mechanisms that produce clavicle compounds for iron, thus depriving pathogens from it, it also helps the plant to increase nutrient uptake required and improve the availability of essential elements including phosphorus and nitrogen (7). The increase in the potassium and nitrogen in the plant's rhizosphare area could be attributed to the presence of *P. fluoresens*.

2 - Efficacy of micro-plot soil treatment with *P. flourescens* and *B. subtilis* on *R. solani* and *F. solani* infection, growth and yield of cucumber under plastic house condition.

Effect of different treatments on total chlorophyll content in cucumber leaves 28 days post planting:

The results of (Figure. 1) showed that the highest chlorophyll content in cucumber leaves was 1027.3 mg / m1 in the treatment of *P. flouresens* after 28 days of planting with significant difference from the lowest chlorophyll content ($0.0mg.g^{-1}$) in treatments of *F. solani* and *R. solani*, while there was no significant difference among other treatments. The results of high chlorophyll content in the leaves were noted in the presence of biological control agents *P. flouresens* and *B. subtilis* (15). The amount of chlorophyll in the treatment of pathogenic *R. solani* was 1.148 mg.

Table3. Effect of different treatments on cucumber plant's content of NPK 14 and 28 days post planting (DPP) in micro-plots under plastic house condition

Treatment	%					
	N		P		K	
	14DPP	28DPP	14DPP	28DPP	14DPP	28DPP
Control	*0.476	2.400	1.610	1.526	0.900	1.476
Fusariumsolani	0.001	0.000	0.003	0.000	0.001	0.000
Rhizoctoniasolani	0.003	0.000	0.004	0.000	0.030	0.000
Fusarium solani+Rhizoctoniasolani	0.001	0.016	0.026	0.002	0.051	0.003
Pseudomonas +(F. solani)	0.573	1.790	0.340	1.550	1.103	2.803
Pseudomonas+(R. solani)	0.513	1.906	0.686	1.193	0.733	1.636
Bacillus+(F. solani)	0.493	1.516	0.433	3.780	0.546	2.806
Bacillus+(R. solani)	0.553	1.346	1.436	1.173	0.906	1.656
Pseudomanas+(F. solani+R. solani)	0.380	1.89	0.570	1.183	0.520	1.606
Bacillus +(F. solani+R. solani)	0.413	1.753	0.230	1.533	0.670	1.620
Pseudomonas+ Bacillus + (F. solani)	0.860	1.683	1.490	2.413	0.880	2.356
Pseudomonas+ Bacillus +(R. solani)	1.243	1.613	2.410	1.276	0.790	1.883
Pseudomonas+ Bacillus +(F. solani+ R.solani)	1.340	2.023	1.676	1.360	1.190	1.823
Celest	2.103	3.490	2.323	3.453	1.810	2.263
Bacillus	2.390	2.966	2.790	2.343	1.520	2.733
Pseudomonas	2.913	3.550	2.350	3.363	2.296	3.116
Pseudomonas+ Bacillus	2.483	3.483	2.350	3.510	0.843	3.250
LSD ($P \leq 0.05$)	0.2449	0.4980	0.2947	0.3098	0.2935	0.4394

*Values were average of three replicates

such as phosphorus, nitrogen and potassium. They also incrase availabilty of nutritional elemnts for the plant leading to increase plant growth, which was also reflected in increased chlorophyll (11). *B.subtilis*was also known for their antagonism to plant pathogens in addition to its potential to improve plant growth by increasing availabilty of certain menerals and nutritional elements (24).

100 g^{-1} of plant fresh weight while the ratio was increased to be 6.291 and 6.478 mg. 100 g^{-1} in the treatment of *B.subtilis* and *P.flouresens*, respectively, compared to 2.610 mg.100 g^{-1} from the control.

P. flouresens increase the leaf content of chlorophyll. This was becaues of action of the bacteria to provide essential nutrients

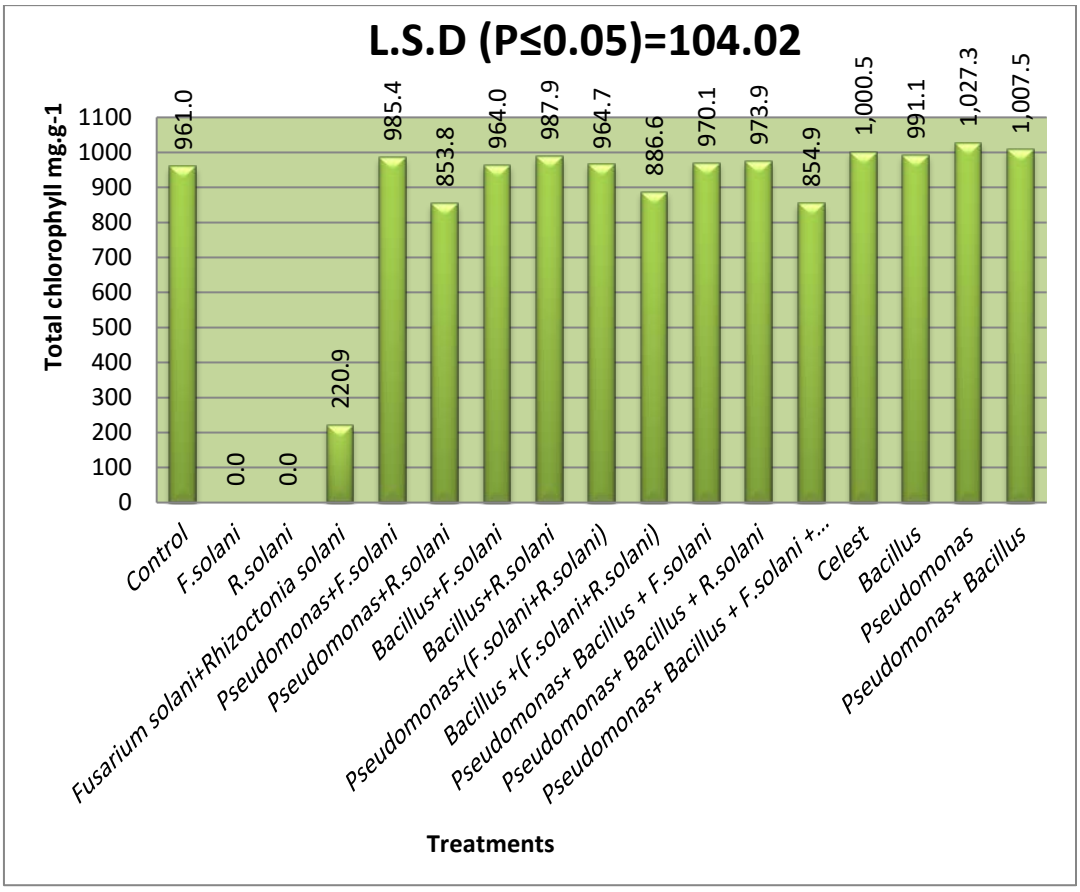

Figure1. Total chlorophyll content mg / g^{-1} in cucumber plants leaves 28 days post planting in micro-plots under green house conditions. Data are means of three replicates

A -Effect of different treatments on the total yield of 20 harvests within 3 months for micro-plot cucumber plants grown in the plastic house

The results of (Figure. 2) showed a significant differences among treatments in total accumulative cucumber yield per plant. The combined treatment of *P. fluorescens +B. subtilis* was significantly superior compared with all treatments resulting in highest total yield weight of 6.18 kg/plant, while the lowest was 0.0 kg/plant due to both pathogenic *R. solani* and *F. solani*. However, cucumber yield was also increased in *R. solani* infected plants in the presence of biological control bacteria. *R.solani* affected the aboveground plant parts such as pods, fruits, leaves, stems, bulbs and tubers (2). Negative effects of this fungus pathogen was not only became of reducing plant growth and yield but also due to its ability to produce toxic compounds increasing risks of human toxicity (2)

The results showed that *Pseudomonas* increase the plant leaf area, number of leaves and flowers per plant, and this was reflected positively on the plant fresh and dry weight, root weight and length, and consequently the yield (11). Ahmed-Sid *et al*. (25) found that seed treatment or root dipping in *B. subtilis* HS93 (6 10 cells / ml) suspension significantly reduced pepper plant rot caused by *R.solani* and increased plant growth indicators, which led to increased production in the glass house.

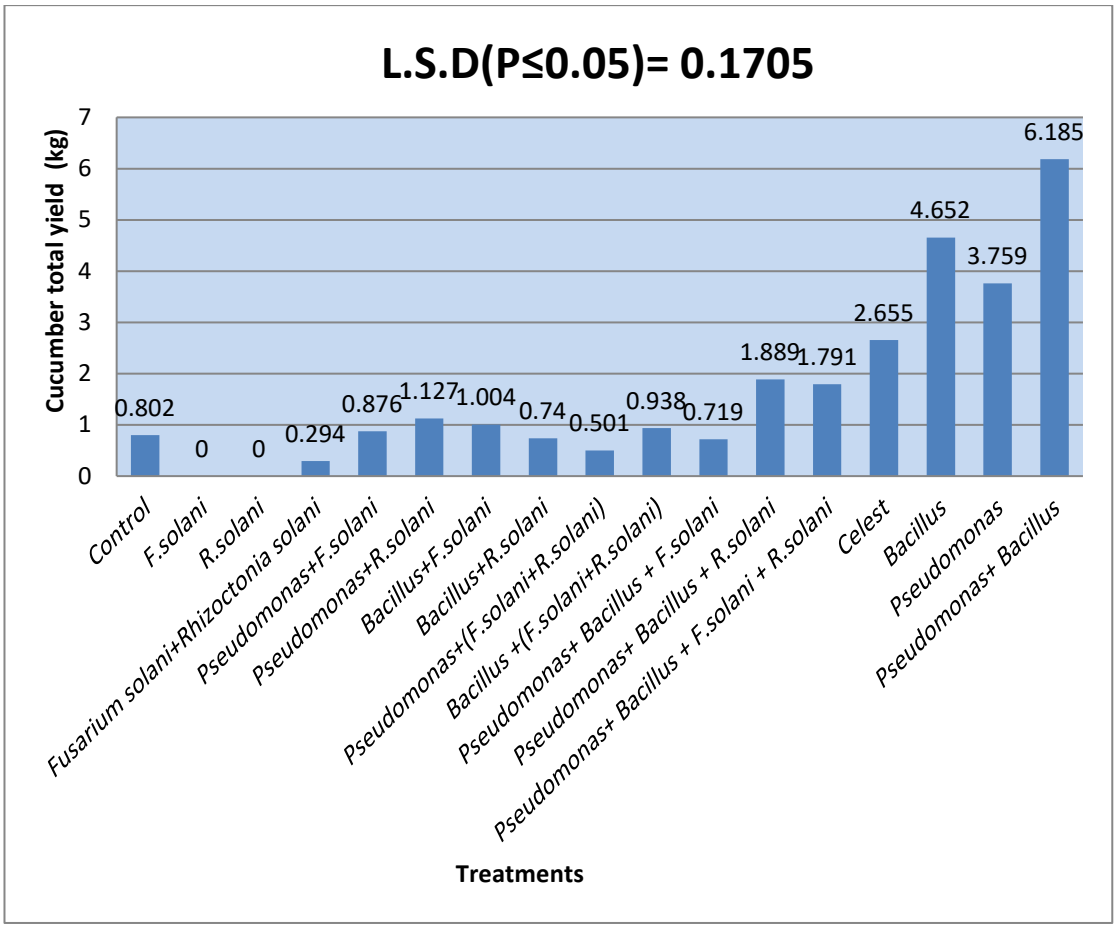

Figure2. Effects of different treatments on cucumber yield average of twenty harvests during three months of growing under green house condition.

Conclusion

Our findings of both pot and micro-plot experiments showed that soil application with bio-control bacteria *P. fluorescens*and *B. subtilis*have been reduced cucumber seed rot and seedlings death, while increasing the growth and yield of cucumber, compared to application of fungicide Celest. *R. solani* and *F. solani* were isolated from cucumber rotted seeds, roots and crown area of cucumber plants and confirmed to be responsible for seed rot disease, seedling death and high pathogenicity on cucumber plants. Introduction of bio-control agents used in this study as a part of the integrated management of cucumber production in greenhouses and ambient cultivation would be a preventive measure against pathogenic fungi affecting seeds and plants. On the other hand, it increased the yield by improving the soil properties and increasing the nutrient availability for the plant.

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