Exogenous Inoculation of Seed with Plant Growth-promoting Rhizobacteria Effectively Enhanced Growth of Cucumber in a Greenhouse

A. R. Oloyede[1*], A. E. Ojo[1] and W. R. Ajijola[2]

[1] *Department of Microbiology, College of Biosciences, Federal University of Agriculture, P.M.B. 2240,110001, Abeokuta, Ogun State, Nigeria*

[2] *Vegetable Research Department, National Horticultural Research Institute, P.M.B. 5432, Idi-Ishin, Jericho, Ibadan, Oyo State, Nigeria*

*Corresponding Author: *oloyedear@funaab.edu.ng*

Abstract

Cucumber (*Cucumis sativus* L.) production in most countries remains unprofitable due to poor growth and low yields resulting from low soil fertility. Concern over environmental contamination and human health hazards posed by increased use of chemical fertilizers requires the development of an alternative strategy to increase food production. The study was conducted to assess the effectiveness, and consistency, of plant growth-promoting rhizobacteria (PGPR) strains in enhancing growth promotion of cucumber plants in a greenhouse. The in-vitro germination bioassay was conducted on eight potential PGPR strains. The effectiveness of the two most efficacious PGPR strains (*Pseudomonas aeruginosa* OG04 and *Bacillus subtilis* MO07) on cucumber plants was repeatedly evaluated in the greenhouse for three trials using seed bacterization and soil inoculation methods. Data on plant heights, leaf lengths, leaf numbers, number of branches, root weights and shoot weights were collected and analysed. The seed germination bioassay showed that cucumber seeds inoculated with potential PGPR strains had significantly higher percentage germination rate (16.20% - 53.30%) and vigour index (18.30% - 114.70%) than un-inoculated seeds. Inoculation of cucumber with *P. aeruginosa* and *B. subtilis* significantly ($p \leq 0.05$) increased the plant heights (4.40 – 28.70%), leaf numbers (9.10 – 33.30%), leaf lengths (5.60 – 42.50%), fresh root weights (20.90 – 62.40%), dry root weights (29.50 – 113.30%), fresh shoot weights (13.20 – 54.30%) and dry shoot weights (46.40 – 100.0%) over un-inoculated plants in all trials. *Pseudomonas aeruginosa* OG04 and *Bacillus subtillis* MO07 could be exogenously applied as microbial inoculants to promote growth of cucumber under greenhouse conditions.

Keywords: *Cucumis sativus*,PGPR, microbial inoculants, growth promotion

Introduction

Synthetic fertilizers and pesticides are used in conventional agriculture to increase food production (Majeed et al., 2015; Sharma and Singhvi, 2017). Large amounts of synthetic fertilizers and pesticides applied to soils can result in increased levels of heavy metals in soil, particularly cadmium, lead and arsenic (Atafar et al., 2010). Excessive use of chemical fertilizers may also pollute below ground water with nitrate which is hazardous to humans or livestock (Delshadi et al., 2017; Sharma and Singhvi, 2017). Due to growing public concern about human health and environmental hazards posed by chemical fertilizers, coupled with their high cost, there is a need to develop safe, bio-rationale approaches for improving soil quality and agricultural output. Use of microbial inoculants, particularly plant growth-promoting rhizobacteria (PGPR), has been suggested to support sustainable agriculture in most parts of the world.

The PGPR are a heterogeneous group of bacteria found in the rhizosphere, at root surfaces and in association with roots. They stimulate plant growth by direct and/or indirect methods. The PGPR aggressively

colonize root surfaces and the closely adhering soil interface, the rhizosphere (Compant et al., 2005b). Some PGPR can enter roots and establish endophytic populations (Compant et al., 2005a,b; Gray and Smith, 2005). These bacteria can improve plant growth by enhancing nitrogen fixation, production of plant growth regulators or phytohormones, solubilize phosphorus, and have antagonistic effects against phytopathogenic microorganisms by production of siderophores, antibiotics, hydrolytic enzymes and fungicidal compounds, and through competition with pathogenic microorganisms for space and nutrients (Giongo et al., 2010; Marques et al., 2010; Patel et al., 2012; Majeed et al., 2015; Pande et al., 2017). These microorganisms play roles in nutrient transformation and element cycling, and influence availability of nutrients for plant uptake (Giongo et al., 2010). The bacteria that have been reported to promote plant growth include strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Acetobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Paenibacillus*, *Rhizobium* and *Serratia* (Patel et al., 2012; Ahmed et al., 2014; Dinesh et al., 2015; Majeed et al., 2015; Oloyede et al., 2017). The degree to which PGPR inoculation influences plant growth could depend on location in which the PGPR strains are applied, cultural conditions, PGPR strains and methods of inoculation (Kumar et al., 2015; Mangmang et al., 2015; Akinrinlola et al., 2018).

Cucumber (*Cucumis sativus* L.) is a major vegetable crop in the family *Cucubitaceae* that is widely grown all over the world (Eifediyi and Remison, 2010; Sanni et al., 2015). Though cucumber is naturally low in calories, saturated fats, carbohydrates, sodium and cholesterol, it is an excellent source of dietary fiber that helps to reduce constipation and protects the body against colon cancer. Cucumber fruit also contains 95% water and small amounts of potassium, some essential vitamins and antioxidants which are effective on human health. Cucumber could be cultivated under field or greenhouse conditions (Eifediyi and Remison, 2009). Poor growth and low yields due to low soil fertility can make cucumber production unprofitable.

Chemical fertilizers have been relied on to increase plant growth and yields. These chemical fertilizers could be expensive in small-scale agriculture. The study was therefore conducted to assess effectiveness and consistency of exogenously applied strains of PGPR on growth of cucumber plants in a greenhouse.

Materials and Methods

Bacterial Strains and Growth Conditions

A total of 135 bacterial strains were isolated from the rhizosphere of *Moringa oleifera* Lam., *Azadirachta indica* A. Juss., *Vernonia amygdalina* Del., *Ocimum gratissimum* L. and *Mangifera indica* L. using standard microbiological methods (Ahmed et al., 2014). Each rhizospheric soil sample was serially diluted and inoculated on nutrient agar and King' B medium plates. The plates were incubated at 28°C for 48 h and pure bacterial isolates were obtained by series of sub-culturing. The isolates were screened for production of hydrolytic enzymes (α-amylase, pectinase and cellulase), hydrogen cyanide (HCN), indole acetic acid, ammonia and

phosphate solubilization following the methods of Oloyede et al. (2017). The potential PGPR strains: *Pseudomonas aeruginosa* (PGPR OG04), *P. aeruginosa* (PGPR VA01), *P. aeruginosa* (PGPR AI05), *Acinetobacter* sp. (PGPR AI03), *Bacillus subtilis* (PGPR OG06), *B. subtilis* (PGPR MO07), *B. subtilis* (PGPR MI02) and *B. megaterium* (PGPR VA04) were selected for further studies.

Planting Materials

Raw seed of cucumber (*Cucumis sativus* L.), cv.Ashley, obtained from the Department of Horticulture, Federal University of Agriculture, Abeokuta, Nigeria, were subjected to a viability test using the paper towel method with little modification.

Seed Germination Bioassay

The inocula were prepared by culturing each PGPR strain in sterile nutrient broth and incubated at 30°C for 48 h under constant shaking at 120 rpm. Each culture was centrifuged (Eppendorf centrifuge 4515C, Hamburg, Germany) at 5,000 ×g for 10 min at 4°C to pellet the cells. After centrifugation, the supernatant was discarded, the cells were washed twice with sterile distilled water and suspended in a sterile 0.1 M phosphate buffer (pH 7.0). Each bacterial suspension was made to 1.0×10^{8}cfu·mL^{-1}.

Seeds of cucumber were surface-sterilized with 5% sodium hypochlorite solution for 2 mins, washed three times in sterile distilled water and air dried at 25±2 °C. Dry seeds were immersed in each bacterial suspension and stirred frequently for 1hr. Treated seeds were air-dried overnight at 25±2 °C. Seeds used as control were treated with sterile distilled water.

Ten cucumber seeds treated with each PGPR strain were placed in 10 Petri dishes (9.0 cm dia) lined with moistened Whatman filter paper No. 1 and incubated for 7 days at 25±2°C. Seeds treated with sterile distilled water were the control. Germinated seeds were counted on the 7th day and germination percent determined. The average radicle and plumule lengths were measured.

Influence of PGPR Treatments on Growth Performance of Cucumber Plants

Pot experiments were conducted in the greenhouse of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria. The experiments were conducted between November and December 2017, 2018 and 2019 using the efficacious PGPR strains: *B. subtilis* MO07 and *P. aeruginosa* OG04 with seed bacterization and soil inoculation. Each experimental trial was conducted in a completely randomized design with five treatments and five replicates. Each replicate consisted of a single pot with one plant per pot. The treatments were: T_1 = control (cucumber plants without PGPR inoculation), T_2 = cucumber plants inoculated with B. subtilis MO07 by seed bacterization, T_3 = cucumber plants inoculated with B. subtilis MO07 by soil inoculation, T4 = cucumber plants inoculated with P. aeruginosa OG04 by seed bacterization, and T5 = cucumber plants inoculated with P. aeruginosa OG04 by soil inoculation.

For seed bacterization, dry surface-sterilized seeds were treated with PGPR strains by immersing in 1.0×10^{8} cfu·mL^{-1} of each bacterial suspension at a rate of 0.1 mL per seed for 2 h with constant agitation. Treated seeds were air-dried over night at 25±2°C. Seeds used for the control were treated with sterile distilled water. For soil inoculation,

25.0 mL of each PGPR suspension was inoculated into sterilized sandy loamy soil (61.6% sand, 17.4% silt, 21% clay and pH 7.5) in pots (18.0 cm dia) one day before sowing seed and the pot soil inoculated with 25.0 mL of sterile distilled water served as the control. Each pot was irrigated with 25.0 mL of sterile distilled water one day before sowing and all pots were covered with polyethylene bags for 24 h to maintain high humidity. Each pot was sown with two inoculated or un-inoculated seeds, and thinned to one plant per pot of comparable height 7 days after sowing. All pots were maintained in a greenhouse, at a 12 h photoperiod and 25 to 30°C. The plants were watered regularly, no fertilizer was applied. Each trial lasted for 4 weeks.

Plant height, leaf length, number of leaves and number of branches were collected at 4 weeks after sowing. Plants were carefully uprooted and the soil washed off roots. Shoots and roots were separated, and weighed to determine fresh weights. Shoots and roots were cut into small pieces and dried in a forced air oven at 60°C for 3 days. Shoot and root dry weights were determined. Percent growth increase (PGI), the amount to which a PGPR strain increased a growth parameter over the control in a trial, was calculated using the method of Akinrinlola et al. (2018).

Genotypic Characterization of PGPR Strains

Molecular characterization was performed to confirm the strains PGPR MO07 and PGPR OG04 using the 16S rRNA gene sequencing method. Extraction of total genomic DNA of the PGPR isolates was carried out using Cetyl Trimethyl Ammonium bromide (CTAB) as described by Chen et al. (2006) and Sridhar et al. (2010) with little modification. Three-hundred µL of an overnight broth culture of each strain was centrifuged (Eppendorf) at 13,100 ×g at 25°C for 2 min to pellet cells. The supernatant was carefully discarded, and pellets re-suspended in 400 µL of pre-warmed CTAB buffer. Exactly 75 µL of 10% SDS was added to the suspension which was gently vortexed. The suspension was heated in a water bath at 65°C for 30 min, then allowed to cool. Exactly 10 µL of 20 $mg \cdot mL^{-1}$ proteinase K solution was added to the suspension which was incubated at 37°C for 30 min. After incubation, 500 µL of chloroform was added and mixed thoroughly. The cell suspension was centrifuged at 6,700 ×g for 10 min and the supernatant carefully collected into a fresh micro-centrifuge tube. One-µL of RNAse solution was added to the supernatant and incubated at 37°C for 30 min. After this, 500 µL of isopropanol was added to the mix and kept at -20°C for 1 h. The suspension was centrifuged at 6,700 ×g for 10 min and the supernatant carefully discarded. The pellet was rinsed with 500 µL of 70% ethanol, mixed thoroughly and centrifuged at 6,700 ×g for 10 min to remove residual contaminants. The supernatant was discarded, and the DNA pellet air-dried at 25±2°C for 1 h. Finally, the DNA pellet was re-suspended in 200 µL of nuclease-free water. The purified genomic DNA was stored at -20°C for further analysis. The concentration and purity of DNA extracted from each isolate were determined using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

The 16S rDNA primers designated as 27F (5'- AGA GTT TGA TCC TGG CTC AG-3') for forward and 1492R (ACG GCT ACC TTG TTA CGA CTT-3') for reverse (Jiang et al., 2006) were used for amplification of 16S rRNA genes of PGPR strains. The PCR was performed in a 20.0 µL reaction volume containing 2.0 µL of template DNA (1 µg),

10.0 µL of 2×PCR master mix (Norgen Biotek Corporation, Ontario, Canada), 1.0 µL of forward primer (2.5 µM), 1.0 µL of reverse primer (2.5 µM) and 6.0 µL of nuclease-free water. The PCR amplification was performed using initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 2 min and final extension of 72°C for 10 min. The PCR fragments were analysed in a 1.0% (w/v) agarose gel electrophoresis in 1× TAE buffer at 100V for 1 h, purified and sequenced. Gene sequences of isolates were identified using the BLASTn program of GenBank database of National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov.)

Statistical Analysis

The data were subjected to two-way analysis of variance. If an interaction was significant, it was used to explain the results. If an interaction was not significant, means were separated using Duncan Multiple Range Test at 5%.

Results

In vitro Plant Growth Promotion Traits of Potential PGPR Strains

The results of the plant growth-promoting traits of potential PGPR strains isolated from the rhizosphere of selected medicinal plants are shown in Table 1. Four of the isolates (AI05, MI02, OG04 and MO07) were found to be potent phosphate solubilizers giving clear halo zones around their colonies. Similarly, the results obtained from IAA production assay revealed that the IAA producers exhibited a pink-red colour with a little variation in intensity. The amounts of IAA produced by the isolates ranged from 1.2916 g/ml to 2.2942 g/ml (Table 1). The highest quantity of IAA was produced by PGPR OG04 (*Pseudomonas aeruginosa*) isolated from the rhizosphere of *Ocimum gratissimum*). The results also showed that four of isolates were positive for pectinase activity on pectin agar medium, six isolates were positive for cellulase, five exhibited the potential to produce hydrogen

TABLE 1

In vitro plant growth promotion traits of potential PGPR strains isolated from rhizosphere of selected medicinal plants

Bacterialisolates	PO_4	NH_3	IAA (g/ml)	HCN	Hydrolytic enzymes		
					Cellulase	Pectinase	α-amylase
OG04	+++	+++	2.2942	++	+	+	+
VA01	−	−	0.00	+	+	−	+
AI05	+	+	0.00	−	+	+	+
AI03	−	+	0.00	+	−	−	+
OG06	−	+	0.00	−	+	−	+
MO07	+++	++	2.2920	++	+	+	++
MI02	+	+	1.2916	−	−	−	+
VA04	−	+	0.00	+	+	+	+

Keys:

PO_4: Phosphate solubilization; IAA: Indole acetic acid; HCN: Hydrogen cyanide;

NH_3: Ammonia production; +: Low, ++: Medium, +++: High

cyanide while all eight isolates produced α-amylase enzyme (Table 1).

Seed Germination Assay of Potential PGPR Strains

The seed germination and vigour index indicated that treatment of cucumber seeds with PGPR strains (OG04, VA01, AI05, AI03, OG06, MO07, MI02 and VA04) displayed higher levels of germination and seedling vigour compared to untreated seed. The PGPR strains significantly enhanced radicle and plumule lengths of seedlings *in vitro* resulting in higher vigour index. Germination rate and vigour index of cucumber seeds treated with PGPR strains increased by 16.20 - 53.30% and 18.30 - 114.70% respectively (Figure 1). The greatest increase in germination rate and vigour index was observed in seed treated with *Bacillus subtilis* MO07 and *Pseudomonas aeruginosa* OG04 and were considered the most efficacious strains in increasing germination rate and vigour index of cucumber seedlings.

Growth of Cucumber Plants to PGPR Inoculation in a Greenhouse

The results of the greenhouse trials on cucumber plants inoculated with test PGPR strains indicated that irrespective of application method, *Bacillus subtilis* MO04 and *Pseudomonas aeruginosa* OG04 significantly enhanced growth of cucumber plants under green house conditions compared to non-inoculated plants. Interaction effects between trials, PGPR strains and inoculation methods indicated that, with the exception of root dry and fresh weights, a significant relationship existed between trial and all agronomic traits examined (Table 2). Inoculation methods influenced all agronomic traits except number of branches (Table 2).

Based on percent growth increase, the two strains increased all growth parameters across the three trials. Inoculation of cucumber plants with *Bacillus subtilis* and *Pseudomonas aeruginosa* by seed bacterization and soil inoculation enhanced plant height of cucumber in all three trials with percent growth increase

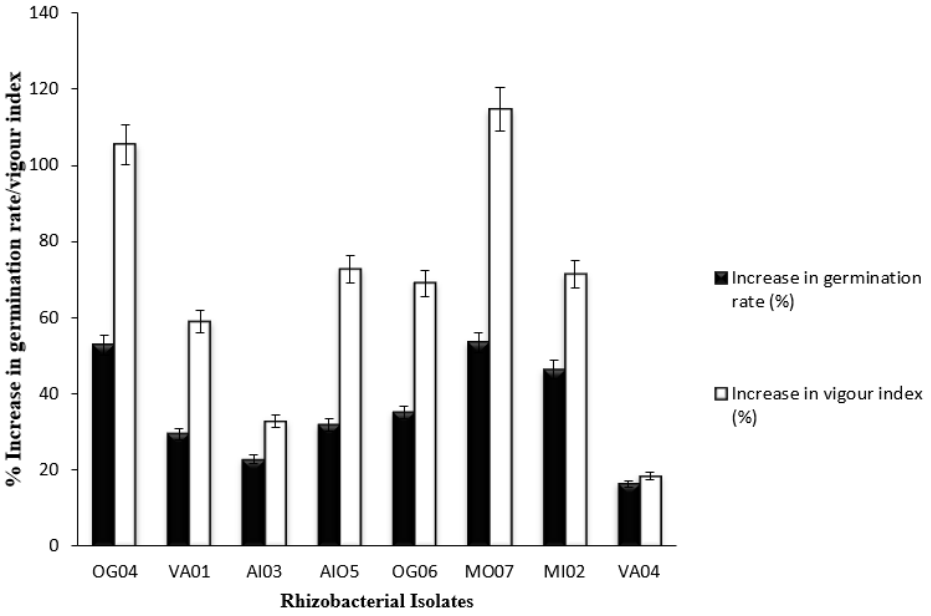

Figure 1 Effect of selected plant growth-promoting rhizobacteria isolates on seed germination and vigor index of cucumber plants. Error bars indicate ± standard errors of means. Isolates are: OG04 = *Pseudomonas aeruginosa*, VA01 = *Pseudomonas aeruginosa*, AI05 = *Pseudomonas aeruginosa*, AI03 = *Acinetobacter baumanni*, OG06 = *Bacillus subtilis*, MO07 = *Bacillus subtilis*, MI02 = *Bacillus subtilis* and VA04 = *Bacillus megaterium*

TABLE 2

Interaction effects between experimental trials, PGPR strains and inoculation method on agronomic traits of cucumber seedlings in a greenhouse

Exp. Trials	PGPR strains	Inoculation methods	Height (cm)	Leaf length (cm)	Leaf No.	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	No. of branches
Trial 1	Control	Control	24.86	7.10	5.40	4.62	1.62	2.74	0.88	0.80
	Bacillus subtilis	seed treatment	29.72	8.28	7.20	6.38	3.16	4.42	1.78	1.40
		soil inoculation	25.96	7.70	6.00	6.04	3.00	3.84	1.44	1.00
	Pseudomonas aeruginosa	seed treatment	31.28	9.00	7.20	6.16	3.24	4.40	1.66	1.20
		soil inoculation	26.56	7.50	6.20	6.00	3.16	3.54	1.14	1.00
Trial 2	Control	Control	20.86	7.70	6.40	4.33	1.72	2.66	0.90	0.80
	Bacillus subtilis	seed treatment	26.04	8.46	8.20	6.68	3.18	4.28	1.92	1.20
		soil inoculation	23.54	8.30	7.80	6.10	2.70	3.38	1.56	0.80
	Pseudomonas aeruginosa	seed treatment	26.22	10.12	8.20	6.20	3.02	4.32	1.61	1.40
		soil inoculation	23.10	8.94	7.80	6.16	3.10	3.48	1.48	1.00
Trial 3	Control	Control	24.22	7.10	6.60	4.24	1.66	2.78	0.84	1.20
	Bacillus subtilis	seed treatment	28.48	9.38	8.20	6.19	3.06	4.36	1.60	2.20
		soil inoculation	26.46	8.70	7.40	4.80	2.46	3.36	1.20	1.80
	Pseudomonas aeruginosa	seed treatment	31.18	10.12	8.00	5.58	2.98	4.22	1.54	1.80
		soil inoculation	27.14	8.98	7.20	5.00	2.43	3.48	1.50	1.40
Source of variation										
Trials (T)			0.000*	0.001*	0.000*	0.000*	0.035*	0.412	0.100	0.009*
PGPR strains (P)			0.111	0.002*	0.905	0.183	0.521	0.760	0.125	0.602
Inoculation methods (I)			0.000*	0.000*	0.008*	0.000*	0.003*	0.000*	0.000*	0.060
T × P			0.386	0.184	0.905	0.964	0.691	0.683	0.042*	0.444
T × I			0.517	0.748	0.592	0.045*	0.125	0.810	0.316	0.970
P × I			0.274	0.041*	0.905	0.063	0.234	0.951	0.268	0.862
T × P × I			0.861	0.832	0.986	0.635	0.478	0.594	0.179	0.970
Adjusted R^2			0.620	0.559	0.311	0.674	0.688	0.662	0.630	0.094

* significant at $p \leq 0.05$, ANOVA

(PGI) ranging from 4.40 to 25.80%, 10.70 to 25.70% and 9.20 to 28.70% for first, second and third trials respectively (Table 3). The PGPR inoculation by seed bacterization produced taller plants than the soil inoculation method. There was improvement in leaf length of cucumber seedlings inoculated with the PGPR strains over un-inoculated controls. The strains increased leaf lengths by 5.60 to 26.80%, 7.80 to 31.40% and 22.50 to 42.50% over un-inoculated control plants in the first, second and third trials respectively (Table 4). While seed bacterization with the PGPR improved leaf length in the first and third trials more than did soil inoculation, *Pseudomonas aeruginosa* inoculated by seed bacterization

TABLE 3

Influence of plant growth-promoting rhizobacteria (PGPR) on height of cucumber seedlings in a greenhouse

	Plant height (cm/plant)		
Treatment	Trial 1	Trial 2	Trial 3
T_1	24.86b	20.86b	24.22c
T_2	29.72a (19.50%)	26.04a (24.80%)	28.48b (17.60%)
T_3	25.96b (4.40%)	23.54ab (12.80%)	26.46b (9.20%)
T_4	31.28a (25.80%)	26.22a (25.70%)	31.18a (28.70%)
T_5	26.56b (6.80%)	23.10ab (10.70%)	27.14b (12.10%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.

values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.

values in parentheses are Percent growth increase

TABLE 4
Influence of plant growth-promoting rhizobacteria (PGPR) on leaf lengths of cucumber seedlings in a greenhouse

	Leaf lengths (cm/plant)		
Treatment	Trial 1	Trial 2	Trial 3
T_1	7.10c	7.70c	7.10c
T_2	8.28ab (16.60%)	8.46bc (9.90%)	9.38ab (32.10%)
T_3	7.70bc (8.50%)	8.30bc (7.80%)	8.70b (22.50%)
T_4	9.00a (26.80%)	10.12a (31.40%)	10.12a (42.50%)
T_5	7.50bc (5.60%)	8.94b (16.10%)	8.98ab (26.50%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.

values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.
values in parentheses are Percent growth increase

and soil inoculation were more effective in the second trial (Table 4).

The PGPR strains increased leaf number over un-inoculated controls across all trials. *Bacillus subtilis* and *Pseudomonas aeruginosa* inoculated by seed bacterization had similar effects in the first and second trials, and reduced responses in the third trial. Their PGIs reduced from 33.30% in the first trial to 28.10% in the second trial, and then to 24.20% and 21.20% for *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively in the third trial (Table 5). While PGPR inoculation by seed bacterization increased leaf numbers than did soil inoculation, no significant difference occurred between numbers of leaves in plants inoculated by seed bacterization (Table 5). Number of branches were not significantly different between PGPR-inoculated and un-inoculated plants. The PGPR strains slightly increased number of branches of cucumber seedlings under green house conditions over control plants in all trials except treatment 3 in the second trial where *B. subtilis* inoculated by soil inoculation performed at par to the control, and then improved in the third trial

TABLE 5
Influence of plant growth-promoting rhizobacteria (PGPR) on number of leaves of cucumber seedlings in a greenhouse

	Leaf Number/plant		
Treatment	Trial 1	Trial 2	Trial 3
T_1	5.40b	6.40b	6.60b
T_2	7.20a (33.30%)	8.20a (28.10%)	8.20a (24.20%)
T_3	6.00ab (11.10%)	7.80ab (21.90%)	7.40ab (12.10%)
T_4	7.20a (33.30%)	8.20a (28.10%)	8.00ab (21.20%)
T_5	6.20ab (14.80%)	7.80ab (21.90%)	7.20ab (9.10%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.
values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.
values in parentheses are Percent growth increase

TABLE 6
Influence of plant growth-promoting rhizobacteria (PGPR) on number of leaves of cucumber seedlings in a greenhouse

Treatment	Number of branches/plant		
	Trial 1	Trial 2	Trial 3
T_1	0.80a	0.80a	1.20a
T_2	1.40a (75.0%)	1.20a (50.0%)	2.20a (83.30%)
T_3	1.00a (25.0%)	0.80a (0.0%)	1.80a (50.0%)
T_4	1.20a (50.0%)	1.40a (75.0%)	1.80a (50.0%)
T_5	1.00a (25.0%)	1.00a (25.0%)	1.40a (16.7%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.
values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.
values in parentheses are Percent growth increase

(Table 6).

During greenhouse trials, effects of PGPR inoculation by seed bacterization and soil inoculation increased root and shoot weights over un-inoculated controls in all trials. There were significant differences between mean weights of PGPR-inoculated and un-inoculated plants. Inoculation of cucumber plants with *Bacillus subtilis* and *Pseudomonas aeruginosa* by seed bacterization enhanced root fresh weights over control plants in the first trial by 61.30% and 60.60% respectively, 60.90% and 62.40%, respectively, in the second trial, and 56.80% and 51.08%, respectively, in the third trial, soil inoculation enhanced root fresh weights by 40.10% and 29.20%, 27.10% and 30.80%, and 20.90% and 25.20% in the first, second and third trials, respectively (Table 7).The PGPR inoculation resulted in higher root dry weights in all trials (Table 7). The PGPR strains consistently enhanced root fresh and dry weights with no significant differences observed between the strains applied to plants with the same inoculation methods except in few cases. Shoot fresh and dry weights of the PGPR-inoculated plants in the first and

TABLE 7
Influence of plant growth-promoting rhizobacteria (PGPR) on root growth of cucumber seedlings in a greenhouse

Treatment	Root fresh weight (g/plant)			Root dry weight (g/plant)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
T_1	2.74c	2.66c	2.78c	0.88d	0.90c	0.84c
T_2	4.42a(61.30%)	4.28a(60.90%)	4.36a(56.80%)	1.78a(102.30%)	1.92a(113.30%)	1.60a(90.50%)
T_3	3.84b(40.10%)	3.38b(27.10%)	3.36bc(20.90%)	1.44bc(63.60%)	1.56b(73.30%)	1.20b(42.90%)
T_4	4.40a(60.60%)	4.32a(62.40%)	4.22a(51.80%)	1.66ab(88.60%)	1.61b(78.90%)	1.54a(83.30%)
T_5	3.54b(29.20%)	3.48b(30.80%)	3.48b(25.20%)	1.14cd(29.50%)	1.48b(64.40%)	1.50ab(78.60%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.
values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.
values in parentheses are Percent growth increase

TABLE 8
Influence of plant growth-promoting rhizobacteria (PGPR) on shoot growth of cucumber seedlings in a greenhouse

Treatment	Shoot fresh weight (g/plant)			Shoot dry weight (g/plant)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
T_1	4.62b	4.33b	4.24c	1.62b	1.72b	1.66c
T_2	6.38a (38.10%)	6.68a(54.30%)	6.19a (46.0%)	3.16a(95.10%)	3.18a(84.90%)	3.06a(84.30%)
T_3	6.04a (30.70%)	6.10a(40.90%)	4.80bc(13.20%)	3.00a(85.20%)	2.70a(57.0%)	2.46ab(48.20%)
T_4	6.16a (33.30%)	6.20a(43.20%)	5.58ab(31.60%)	3.24a(100%)	3.02a(75.60%)	2.98ab(79.50%)
T_5	6.00a (29.90%)	6.16a(42.30%)	5.00bc(17.90%)	3.16a(95.10%)	3.10a(80.20%)	2.43b(46.40%)

T_1 = Control (cucumber plants without PGPR inoculation); T_2 = cucumber plants inoculated with *Bacillus subtilis* MO07 by seed bacterization; T_3 = cucumber plants inoculated with *Bacillus subtilis* MO07 by soil inoculation; T_4 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by seed bacterization, T_5 = cucumber plants inoculated with *Pseudomonas aeruginosa* OG04 by soil inoculation.
values in columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan Multiple Range Test; means of 5 plants per trial.
values in parentheses are Percent growth increase

second trials were not significantly different. Differences between PGPR-inoculated and un-inoculated controls occurred in shoot fresh and dry weights in all trials. The PGPR strains enhanced shoot fresh and dry weights by 13.20 - 54.30% and 46.4 - 100.0%, respectively, over un-inoculated controls across the three trials (Table 8).

Genotypic Characterization of PGPR Strains
The amplification of 16S rRNA genes of the two most efficacious PGPR strains resulted in a DNA amplification of approximately 1,500bp. The sequences of PGPR OG04 showed 98% similarity with *Pseudomonas aeruginosa* while PGPR MO07 showed 95% sequence homology with *Bacillus subtilis*. There was confirmation that PGPR OG04 and PGPR MO07 were *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively.

Discussion

Reduction of chemical fertilizers by use of biological fertilizers based on bacteria involved in promoting plant growth is effective in sustainable agriculture. Plant growth-promoting rhizobacteria play important roles that directly, or indirectly, influence plant growth and development (Gerhardt et al., 2009; Islam et al., 2016). The effectiveness of PGPR strains isolated from another crops depends on crop species, growth conditions, inoculation methods, colonization potentials of PGPR strains and composition of root exudates (Kumar et al., 2015). In this study, treatment of cucumber seed with exogenous strains of PGPR improved seedling emergence and vigour index with *Pseudomonas aeruginosa* OG04 and *Bacillus subtilis* MO07 displaying maximum levels of germination and vigour index which agrees with the previous reports of Islam et al. (2016) on the effects of PGPR strains on seed germination and vigour index of cucumber. The potential of these PGPR strains to enhance the cucumber seed germination and vigour index could be due to their ability to produce some hydrolytic enzymes such as α-amylase. The α-amylase hydrolyzes starch into metabolizable sugars, which provide energy for growth of roots and shoots in germinating seedlings (Akazawa and Nishimura, 2011; Islam et al., 2016). Also, the

PGPR strains could indirectly promote seed germination and vigour index by reducing the incidence of seed microflora which could negatively affect plant growth.

Furthermore, the PGPR strains (*Pseudomonas aeruginosa* OG04 and *Bacillus subtilis* MO07) applied by either seed bacterization or soil inoculation exhibited potential to increase growth of cucumber plants under greenhouse conditions. Plant heights, leaf length, leaf number, and shoot and root biomass of PGPR-treated cucumber seedlings were increased compared to un-inoculated plants in all trials. Growth promotion of cucumber plants by exogenous strains of *Bacillus subtilis* and *Pseudomonas aeruginosa* agrees with previous studies that demonstrated native strains of PGPR being effective for growth promotion on a variety of crop plants, including cucumber (Kidoglu et al., 2008); tomato and pepper (Almaghrabi et al., 2013). Other study conducted by Gul et al. (2013) also showed that PGPR improved growth and yields of cucumber under greenhouse conditions. Application of *Bacillus pumilis* and *Alcaligenes piechaudii* strains as seed and/or drench treatments also increased seedling height, leaf number, leaf area, shoot and root weights of cucumber plants compared with control (Yildirim et al., 2015). Inoculation with *Acetobacter pasteurianus* and *Stenotrophomonas* species increased shoot and root lengths, shoot and root dry weights, and nitrogen content of wheat seedlings when compared with un-inoculated seedlings (Majeed et al., 2015). Similarly, inoculation of tomato plants with *Pseudomonas aeruginosa*, *P. syringae* and *P. fluorescens* improved growth of plants, and provided some levels of reduction in tomato bacterial wilt disease incidence (Mohammed et al., 2020). Similar study conducted by Oloyede et al. (2021) also reported that non-native strains of *Alcaligenes faecalis* and *Acinetobacter* sp. significantly increased the agronomic traits of canker-infected tomato plants under greenhouse conditions.

In addition, the present study revealed that inoculation of exogenous strains of PGPR by seed bacterization was generally more effective in enhancing cucumber growth than soil inoculation method which agrees with Mangmang et al. (2015) who reported that strains of *Azospirillum* species enhanced more growth of tomato, lettuce and cucumber seedlings when inoculated by seed soaking than the drenching method.

The results of the experimental trials indicated consistency of growth promotion effects of the exogenously applied PGPR strains and inoculation methods, but this consistency contrasts with Cakmakci et al. (2006) and Akinrinlola et al. (2018). The consistency in the responses of cucumber plants to PGPR inoculation could be due to pot experiments being conducted using the same planting medium under the same environmental/ greenhouse conditions. The PGPR strains inoculated by seed bacterization were more consistent in growth promotion of cucumber seedlings across the three trials than those inoculated by soil inoculation method.

The effectiveness of exogenous *Pseudomonas aeruginosa* OG04 and *Bacillus subtillis* MO07 in stimulating growth of cucumber plants could be due to combinations of some direct and indirect growth promotion traits associated with the two strains, suggesting the mechanisms by which these strains could enhance the growth of cucumber. The direct modes of action of the PGPR strains include production of indole acetic acid (IAA),

activation of phosphate solubilization, and ammonia production. Indole acetic acid is involved in root initiation, cell division, cell enlargement and increasing root surface area (Gray and Smith, 2005; Majeed et al., 2015), and it promotes root development and uptake of nutrients, resulting in improved plant growth. Similarly, the two PGPR strains, being able to solubilize phosphate, might provide available forms of phosphorus to cucumber plants during growth and they could serve as substitutes to chemical phosphatic fertilizers. Besides providing phosphorus to cucumber plants, the PGPR strains could also augment growth of plants by enhancing availability of other trace elements (Ahemad and Kibret, 2014). The indirect mechanisms exhibited by these PGPR strains include antifungal antagonism, hydrogen cyanide (HCN) production and synthesis of hydrolytic enzymes (α-amylase, protease, cellulase and pectinase).

Conclusion and Recommendations

Inoculation of exogenously applied *Pseudomonas aeruginosa* OG04 and *Bacillus subtillis* MO07 by seed bacterization effectively, and consistently, promoted growth of cucumber plants in a greenhouse and these strains may be applied as bio-rationale bio-inoculants to reduce chemical fertilizer use in cucumber under greenhouse conditions. However, further studies need to be carried out to ascertain the effectiveness of these microbial strains in enhancing nutrient availability and stimulating growth of cucumber under field conditions. Recommended application rates, processes influencing the establishment success and persistence of these strains in the soil under field conditions also need to be identified. Finally, efforts should be made to make these cost-effective microbial strains readily available to cucumber growers as coated seeds, powdery or solution forms.

Acknowledgements

The authors are grateful to the Microbiology Department, Biotechnology Centre and Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria, for making their facilities available to carry out this research.

Declaration of Interest

The authors hereby declare that they have no conflict of interest.

References

Ahemad, M. and Kibret, M. 2014. Mechanisms and applications of plant growth-promoting rhizobacteria: Current perspective. *Journal of King Saud University – Science*. **26**: 1-20.

Ahmed, E.A., Hassan, E.A., El Tobgy, K.M.K. and Ramadan, E.M. 2014. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. *Annals of Agricultural Science*. **59(2)**: 273-280.

Akazawa, T. and Nishimura, H. 2011. Topographical aspects of biosynthesis, extracellular secretion and intracellular storage of proteins in plant cells. *Annual Review of Plant Physiology*.**36**: 441-472.

Akinrinlola, R.J., Yuen, G.Y., Drijber, R.A. and Adesemoye, A.O. 2018. Evaluation of *Bacillus* strains for plant growth promotion and predictability of efficacy by *in vitro* physiological traits. *International Journal of Microbiology*. **2018**: 1-11.

Almaghrabi, O.A., Massoud, S.I. and Abdelmoneim, T.S. 2013. Influence of inoculation with plant growth-promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under green house conditions. *Saudi Journal of Biological Sciences*. **20**:57-61.

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M. and Mahvi, A.H. 2010. Effect of fertilizer application on soil heavy metal concentration. *Environmental Monitoring and Assessment.* **160(1-4)**: 83-89.

Cakcakci, R., Donmez, F., Aydin, A. and Sahin, F. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under green house and two different field soil conditions. *Soil Biology and Biochemistry*.**38(6)**: 1482-1487.

Chen, H., Hopper, S.L., Li, X., Ljungdahl, L.G. and Cerniglia, C.E. 2006. Isolation of extremely AT-rich genomic DNA and analysis of genes encoding carbohydrate-degrading enzymes from *Orpinomyces* sp. strain PC-2. *Current Microbiology*. **53**:396-400.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clement, C. and AitBarka, E. 2005a. Endophytic colonization of *Vitis vinifera* L. by a plant growth-promoting bacterium, *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology*.**71**:1685-1693.

Compant, S., Duffy, B., Nowak, J., Clement, C. and AitBarka, E. 2005b. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. *Applied and Environmental Microbiology*.**71(9)**: 4951-4959.

Delshadi, S., Elbrahimi, M. and Shirmohammadi, E. 2017. Influence of plant growth-promoting bacteria on germination, growth and nutrients'uptake of *Onobrychis sativa* L. under drought stress. *Journal of Plant Interactions*.**12(1)**: 200-208.

Dinesh, R., Anandaraj, M., Kumar, A., Bini, Y.K., Subila, K.P. and Aravind, R. 2015. Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger. *Microbiological Research*. **173**: 34-43.

Eifediyi, E.K. and Remison, S.U. 2009. Effect of time of planting on the growth and yield of five varieties of cucumber (*Cucumis sativus* L.). *Report and Opinion*. **1(5)**: 81-90.

Eifediyi, E.K. and Remison, S.U. 2010. Growth and yield of cucumber (*Cucumis sativus* L.) as influenced by farmyard manure and inorganic fertilizer. *Journal of Plant Breeding and Crop Science*. **2(7)**: 216-220.

Gerhardt, K.E., Huang, X.D., Glick, B.R. and Greenberg, B.M. 2009. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Science*. **176**:20-30.

Giongo, A., Beneduzi, A., Ambrosini, A., Vargas, L.K., Stroschein, M.R., Eltz, F.L., Bodanese-Zanettini, M.H. and Passaglia, L.M.P. 2010. Isolation and characterization of two plant growth-promoting bacteria from the rhizoplane of a legume (*Lupinus albescens*) in sandy soil. *Revista Brasileira de Ciencia do Solo*.**34**:361-369.

Gray, E.J. and Smith, D.L. 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant – bacterium signalling processes. *Soil Biology and Biochemistry*. **37**:395-412.

Gul, A., Ozaktan, H., Kıdoglu, F. and Tuzel, Y.2013. Rhizobacteria promoted yield of cucumber plants grown in perlite under Fusarium wilt stress. *Scientia Horticulturae*. **153**:22-25.

Islam, S., Akanda, A.M., Prova, A., Islam, M.T. and Hossain, M.M. 2016. Isolation and identification of plant growth – promoting rhizobacteria from Cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Frontiers in Microbiology*. **6**:1-12.

Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L.R. and Fields, M.W. 2006. Microbial diversity in water and sediment of Lake Chaka, an Athalassohaline Lake in northwestern China. *Applied and Environmental Microbiology*.**72(6)**:3832-3845.

Kidoglu, F., Gul, A., Ozaktan, H. and Tuzel, Y. 2008. Effect of rhizobacteria on plant growth of different vegetables. *Acta Horticulturae*.**801**:1471-1477.

Kumar, G.P., Desai, S., Amalraj, E.D. and Pinisetty, S. 2015. Impact of seed bacterization with PGPR on growth and nutrient uptake in different cultivable varieties of Green Gram. *Asian Journal of Agricultural Research*.**9(3)**:113-122.

Majeed, A., Abbasi, M.K., Hameed, S., Imran, A. and Rahim, N. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology*. **6(198)**:1-10.

Mangmang, J.S., Deaker, R. and Rogers, G. 2015. Early seedling growth response of lettuce, tomato and cucumber to *Azospirillumbrasilense* inoculated by soaking and drenching. *Horticultural Science* (Prague).**42(1)**:37-46.

Marques, A.P.G.C., Pires, C., Moreira, H., Rangel, A.O.S.S. and Castro, P.M.L. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biology and Biochemistry*. **42**:1229-1235.

Mohammed, A.F., Oloyede, A.R. and Odeseye, A.O. 2020. Biological control of bacterial wilt of tomato caused by *Ralstoniasolanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants. *Archives of Phytopathology and Plant Protection*. **53(1-2)**:1-16.

Oloyede, A., Ajijola, W. and Albert, O.2017. Characterization and evaluation of rhizobacteria isolated from selected medicinal plants for plant growth promoting and antagonism of *Fusarium oxysporum* f. sp *cucumerinum*. Proceedings of the Maiden Bioscience Annual Conference, 15-17 August 2017, Abeokuta, Nigeria. Pp 69 – 76.

Oloyede, A. R., Ogbuagor, C. J., Afolabi, C. G. and Akintokun, A. K. 2021. Biological control of bacterial canker of tomato (*Lycopersicon esculentum* Mill.) by use of non-native strains of plant growth-promoting rhizobacteria.*Archives of Phytopathology and Plant Protection*.**54(15 -16)**:1182-1203.

Pande, A., Pandey, P., Mehra, S., Singh, M. and Kaushik, S. 2017. Phenotypic and genotypic characterization of phosphate solubilising bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology*.**15**:379-391.

Patel, H.A., Patel, R.K., Khristi, S.M., Parikh, K. and Rajendran, G. 2012.

Isolation and characterization of bacterial endophytes from *Lycopersicon esculentum* plant and their plant growth- promoting characteristics. *Nepal Journal of Biotechnology*. **2(1)**:37-52.

Sanni, K.O., Ewulo, B.S., Godonu, K.G. and Animashaun, M.O. 2015. Effects of nutrient sources on the growth and yield of cucumber (*Cucumis sativus*) and on soil properties in Ikorodu agro-ecological zone. *Report and Opinion*. **7(4)**:24-32.

Sharma, N. and Singhvi, R. 2017. Effects of chemical fertilizers and pesticides on human health and environment: A review. *International Journal of Agriculture, Environment and Biotechnology*. **10(6)**:675-679.

Sridhar, M., Kumar, D., Anandan, S., Prasad, C.S. and Sampath, K.T. 2010. Morphological and molecular characterization of polycentric rumen fungi belonging to the genus *Orpinomyces* isolated from Indian cattle and buffaloes. *Research Journal of Microbiology*. **5:**581-594.

Yildirim, E., Ekinci, M., Dursun, A. and Karagoz, K. 2015. Plant growth-promoting rhizobacteria improved seedling growth and quality of cucumber (*Cucumis sativus* L.). Proceedings of International Conference on Chemical, Food and Environment Engineering. 11-12 January 2015. Dubai, U.A.E.