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The Activities of Rhizosphere Bacteria in Improving the Growth of Habanero Pepper (*Capsicum chinense* Jacq.) and Soil Fertility

Isabella Avwerosuoghene Onoharigho[1*], Adeniyi Adewale Ogunjobi[2]

[1,2]Dept. of Microbiology, Environmental Microbiology and Biotechnology Unit, University of Ibadan, Ibadan, Oyo State, Nigeria

**Corresponding Author: isabella.onoharigho@gmail.com, Tel:+2348060201654*

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Abstract—Plant growth-promoting rhizobacteria (PGPR) enhances plant growth by direct and indirect mechanisms, making them a good alternative to chemical fertilizers. Rhizosphere soil were collected and bacteria were isolated from them. The bacterial isolates obtained were screened for their ability to enhance the growth of plants. Those with the best activity were used to inoculate the seeds of the pepper plant before planting in an experimental plot. The plant growth parameters and the physico-chemical properties of the soil used were monitored before and after the experiment. Sixteen bacterial isolates belonging to *Azospirillum* spp., *Pseudomonas* spp., *Aeromonas* spp., and *Bacillus* spp. were obtained from the rhizosphere of habanero pepper plant. In all the bacterial isolates, *Azospirillum brasilense* C1 had the highest nitrogen fixed (0.002%), *Pseudomonas putida* P4 and *Bacillus subtilis* A2 had the largest zones of clearance of 4mm and 8mm, respectively. Plants inoculated with isolates had the highest growth, biomass, yield, and mineral nutrient content. T4 (*Azospirillum brasilense* C1 + *Pseudomonas putida* P4) had the highest values in root length, leaf length, leaf width, shoot length, number of fruit, and plant dry weight of 13.86cm, 8.69cm, 5.69cm, 18.43cm, 2.67 and 0.29g, respectively, over uninoculated controls. Soil treated with T7 (*Azospirillum brasilense* C1 + *Pseudomonas putida* P4 + *Bacillus subtilis* A2) had the highest mineral content compared to soil treated with chemicals. Thus, combined rhizobacteria inoculants inoculated into habanero pepper seeds are suitable for excellent growth and performance of habanero pepper plant and also for improving soil fertility.

Keywords—PGPR, Habanero pepper, Soil fertility, Growth, Mineral uptake

I. INTRODUCTION

The habanero chili pepper (*Capsicum chinense* Jacq.), is popular to be very aromatic and also the strongest pepper in terms of hotness which is largely distributed worldwide compared to other *Capsiciums* [1]. The habanero pepper is from the Solanaceae family, the genus is *Capsicum*, native to Mexico but presently cultivated in Africa, Asia, and other countries along the Mediterranean Sea [2]. It grows to form small firm perennial bushes of about 0.5m in height. It has glossy green leaves; small white flowers with five petals; ripe fruit measuring 2.5-4cm long by 2.5-3.5cm wide which change from green to colors ranging from scarlet red, orange, and yellow; flat yellow seeds; and a taproot with numerous lateral roots [3].

Due to the increase in demand of habanero pepper by the multiplying world population, inorganic fertilizers have been used to increase crop yield. The application of chemical fertilizers in agriculture makes a country self-dependent in food production, however, this results in the deterioration of the environment and causes harmful impact on soil, plants, and human health [4]. The unselective use of artificial soil stimulants has led to the pollution and contamination of the soil and destruction of soil microorganisms, thereby making the crops more prone to diseases, increasing cost of fertilizers, and decreasing soil fertility due to the inadequate uptake of these inorganic fertilizers by plants which spread to water bodies through flood, triggering agar bloom in water bodies and affecting living beings including the growth of residing microorganisms [5]. For the enhancement of growth, higher harvest of agricultural crops, and soil fertility, (PGPR) has turned out to be a vital alternative concept of bio-fertilizers which serves as a decent replacement of soil chemical stimulants [4].

PGPR is an association of favorable plant microbes, potentially valuable for the stimulation of plant development and potentiation of crop yields [6]. PGPR is responsible for the decomposition of organic matters, manufacture of phytohormones, fixation of nitrogen, and solubilization of insoluble phosphate and potassium in the soil for plant utilization [7]. It builds up lost soil microflora, increases nutrients without any antagonistic effect on the environment, and importantly alters plant growth parameters such as plant and shoot height, root length, number of roots, number of branches, and dry accumulated matter in plants [6],[7],[8].

This study was aimed at investigating the influence of selected rhizobacteria isolates, which have the capacity to fix nitrogen and solubilize phosphate and potassium, on the growth, biomass, yield, and mineral uptake of *capsicum chinense* Jacq. and on soil fertility. This plant was chosen because it is used worldwide as a food, vegetable, and as an external remedy; and it is usually consumed either fresh or processed and employed as spices in the food industry [9]. The other aspect of this article is organized as follows, section II contains the related work of PGPR, sector III include methodology, sector IV explains the results and discussion, and section V concludes research work.

II. RELATED WORK

Various studies have revealed that the use of chemical fertilizers and pesticides have serious deleterious effects in the environment. These chemicals are not easily degradable, they can be detrimental to some beneficial microorganisms and also possess the tendency to by-accumulate in plants thereby posing danger to human health [10]. The application of PGPR as low-cost, eco-friendly biofertilizers has been identified to improve plant growth without causing harm to soil, plant, and human health [11]. Hence, there is need to identify PGPR that will be beneficial to our environment and health.

III. METHODOLOGY

Samples Collection

Rhizosphere soil and root were collected aseptically from habanero pepper plant of a well-established pepper farm along Obafemi Awolowo hostel, University of Ibadan, at the early hours of the morning at a depth of 5-10cm and were transported for analysis in the laboratory within one hour of collection.

Isolation and Characterization of Soil Rhizosphere Bacteria

The organisms were obtained from rhizospheric soil of *Capsicum chinense* Jacq. plant. Ten grams of soil was transferred into 90ml sterile distilled water to form a suspension which was serially diluted. One ml of each dilution of 10^{-4}, 10^{-6}, and 10^{-8} were inoculated into sterile petri dishes of Congo red medium, Pikovskaya's agar medium, and Alexandrov's agar medium by pour plate method [12],[13],[14]. The plates were kept at room temperature (28±2°C) for 48-72 hrs in an incubator. Pure bacterial isolates obtained were characterized by their cultural, morphological, and biochemical characteristics using standard methods [15].

***In-vitro* Screening of the Isolates for their Beneficial Traits**

The isolated bacteria from the rhizospheric soil were screened to obtain three most efficient bacteria to study their performance in enhancing the growth and biomass of habanero pepper plant. The bacterial isolates with the highest percentage of fixed nitrogen and isolates with highest zone of clearance on Pikoskaya's agar medium and Alexandrov's agar medium were inoculated into the Habanero pepper seeds for planting.

Screening the Isolates for the Ability to Fix Nitrogen

The amount of nitrogen fixed by *Azospirillum* isolates were estimated by the standard procedures. A loopful of 24 hrs old culture of each isolate was used to inoculate 5ml of semisolid N-free malate medium and incubated at 28±2°C for 4 days and checked for color change from greenish yellow to blue. One ml of this culture was incubated at 28±2°C for 15 days and 10ml of the culture was used for the estimation of nitrogen by micro kjeldhal using the method of [12].

Phosphate and Potassium Solubilization Ability

All isolates were examined for their capability to solubilize insoluble inorganic phosphate and potassium on Pikoskaya's and Alexandrov's agar plates, respectively, by spotting 18-24 hrs old cultures and incubating the plates at 30°C for 48-72 hrs. After 72 hrs, the colonies showing the zone of clearance around the colony were taken as phosphate and potassium solubilizers.

Evaluation of Efficient Bacteria for Growth, Biomass, Yield, and Mineral Up-Take in Habanero Pepper and Soil Fertility

A pot culture experiment was set-up using 3 most efficient bacteria to study their performance in enhancing the growth, biomass, yield, and the mineral up-take of pepper plant and soil fertility.

Experimental Treatments: The treatment pattern for pot culture experiment is shown in Table 1. A total of 9 treatments each with 3 replicates were designed.

Experimental Soil:

About 135kg of soil was collected from the vegetation plantation along Awolowo hall, University of Ibadan. The soil sample was sieved through 2mm and 0.5mm meshes after air-dried and crushed for the determination of pH, (H_2O), (soil, water 1:2.5), organic carbon, available nitrogen (N), phosphorus (P) and potassium (K) from the soil before planting using the method described by Oyeyiola *et al.* [16]. One hundred and twenty kilogram of the soil was subjected to dry heat sterilization in the oven for about 2-3 hrs at about 120°C. Pots were filled with 5kg of sterile soil each (for inoculated plants positive control) and unsterile soil (for the negative control). The experimental pots were also surface disinfected with absolute ethanol and sodium hypochlorite. Each pot was filled up to 5cm before the brim to avoid unwanted washing away of the topsoil particles and fertilizers during watering or when it rains. Five holes were cautiously made at the bottom of each pot using hot-red 4 inches nail, before filling the pots, and perforations made were plugged with cotton wool to regulate and encourage proper drainage and aeration.

Seed Treatment: The *Capsicum chinense* Jacq. seeds used in this work were collected from National Horticultural

Research Institute (NIHORT), Ibadan, Oyo State. The pepper seeds were inoculated by employing the method of Ashrafuzzaman *et al.* [17]. The habanero pepper seeds were disinfected in 70% ethanol for 2 minutes as well as 0.2% sodium hypochlorite for 10 minutes and then rinsed 10 times in sterile water. Pure culture of the selected isolates was grown in nutrient broth at 28°C and diluted using organism free saline solution to a final concentration of 10^8cfu/ml. The seeds were then treated by immersing them in the bacterial suspensions (10^8cfu/ml) for 45 minutes on a rotary shaker at 120rpm. After this, seeds were air-dried and sow immediately [18].

Sowing and Maintenance

The bacteria inoculated seeds were sown in 8 diameter size pots. Three seeds were planted in each 3 holes per pot in 3 replicates. After germination, one plant per pot was removed for periodic examination and analysis. The pots were watered morning and evening with distilled water to maintain optimum moisture necessary and weeded manually with hand to remove emerging weed seedlings from the pots weekly.

Plant Growth Measurement

The plant growth measurements were carried out for a period of 2 months. Parameters were examined and recorded at 20th, 40th and 60th days after planting. Parameters included: length of shoot, root, leaf; number of roots, leaves, fruit; leaf width; fruit wet weight; fruit dry weight; plant wet weight; and plant dry weight.

Estimation of Mineral Content in Habanero Plant

At day 60, the plants were uprooted and the adhering soil particles were removed. Macronutrient (NPK) contents of habanero pepper plants were determined. The total nitrogen content in the plant sample was estimated following the Micro kjeldahl method [12]. Phosphorus and potassium contents of the plant were estimated as described by [16],[19].

Physico-Chemical Properties of the Soil after the Experiment

After planting, soil A (soil from plant treated with 3 combined inoculants) and soil B (soil from plant treated with chemical fertilizer) were analyzed for pH (H_2O), (soil, water 1:2.5), organic carbon, nitrogen (N), phosphorus (P), and potassium (K) from the soil using the method described by Oyeyiola *et al.* [16].

Statistical Analysis

The experimental design used was the complete randomized design as described by Kuntal *et al*. [20]. Analysis of variance (ANOVA) was use to analyze the data and means were compared using Duncan's multiple-range test at the P = 0.05 level of significance. Interpretation of the data was carried out as described by Kuntal *et al*. [20].

Table 1. Detail of the treatment pattern used for pot experiments.

T	Description	Soil status
T1	*A. brasilense* C1	Sterilized
T2	*P. putida* P4	‘’
T3	*B. subtilis* A2	‘’
T4	*A. brasilense* C1+ *P. putida P4*	Sterilized
T5	*P. putida* P4 + *B. subtilis* A2	‘’
T6	*A. brasilense* C1 + *B. subtilis* A2	‘’
T7	*A. brasilense* C1+ *P. putida* P4 + *B. subtilis* A2	‘’
T8	Positive control (chemical fertilizer)	Sterilized
T9	Negative control(uninoculated seeds)	Unsterilized

Key: Design- Complete randomized design, Number of each replicates 3, Total number of Treatments- 27, Quantity of soil- 5kg, T-treatment

IV. RESULTS AND DISCUSSION

Results

Isolation and Identification of Rhizosphere Bacteria from Habanero Pepper

A total of 16 rhizosphere bacteria were obtained from the habanero pepper in a well-established farm along Awolowo hall of residence, University of Ibadan, Oyo state. From the 16 bacteria isolates, 6 were nitrogen fixers (*Azospirillum* spp.), 5 were phosphorus solubilizing bacteria, and the last 5 were potassium solubilizing bacteria isolated using Congo red medium, Pikovskaya's medium, and Alexandrov's medium, respectively.

The six isolates of *Azospirillum* spp. formed sub-surface pellicle in the semisolid N-free medium (NFM) and small scarlet to pink colonies with lobate and entire edge on Congo red medium. Malic acid which is a carbon source supported the growth of all isolates, and based on the biochemical test, C1, C4, C5, and C6 were identified as *Azospirillum brasilense* and C2 and C3 were identified as *A. lipoferum*. The 5 isolates from Pikovskaya`s agar medium showed white to cream colonies with entire edge ranging from small to medium size. P1 and P5 were identified as *Pseudomonas aeruginosa*, P2 and P4 as *Pseudomonas putida*, and P3 as *Aeromonas hydrophila*. All 5 isolates from Alexandrov's agar medium showed light gray and small to medium colonies with entire edges and were identified as follows. Three of the bacteria (A1, A2, and A5) were *Bacillus subtilis*, A3 was *Bacillus circulans*, and A4 was *Bacillus megaterium*.

Screening the Isolates for their Beneficial Traits

All the 16 bacteria isolates were screened for their ability to promote plant growth by applying selective criteria. The number of selected isolates was reduced to 3 efficient bacteria using 3 selective media.

Screening of Isolates for their Nitrogen Fixing Ability

From the 6 *Azospirillum* isolated from Congo red medium, 4 *Azospirillum* were selected based on the initial color change from bluish green to blue in the N-Free Malate medium and they were further screened for the amount of nitrogen fixed using Micro kjedahl method. *A. brasilense* C1 showed the highest amount of nitrogen fixation 0.002% (Table 2).

Table 2. Percentage Nitrogen Fixed by *Azospirillum* Isolates.

S/N	ISOLATES	NITROGEN (%)
1	*Azospirillum brasilense* C1	0.002
2	*Azospirillum lipoferum* C2	0.000
3	*Azospirillum lipoferum* C3	0.001
4	*Azospirillum brasilense* C4	0.000
5	*Azospirillum brasilense* C5	0.000
6	*Azospirillum brasilense* C6	0.000

Screening of Phosphate Solubilizing Bacteria (PSB) and Potassium Solubilizing Bacteria (KSB)

Only 3 out of 5 bacteria were able to solubilize Tricalcium phosphate on Pikovskaya's agar medium by forming zones of clearance on the medium. Among the 3 isolates, *P. putida* P4 solubilized the highest clear zone of 4mm and this necessitated its choice for insoluble phosphate in the experiment. From the 5 KSB isolated, two solubilize mica powder on Alexandrov's agar medium by forming clear zone on the medium. Among the 2 isolates, *B. subtilis* A2 solubilized the highest amount of potassium salt at 8mm which necessitated its choice as the inoculants for the experiment (Table 3).

Table 3. Zones of Clearance by the Phosphate Solubilizing Bacteria and Potassium Solubilizing Bacteria

S/N	ISOLATES	Zone of clearance (mm)
1	*Pseudomonas aeruginosa* P1	0.90
2	*Pseudomonas putida* P2	0.00
3	*Aeromonas hydrophila* P3	1.00
4	*Pseudomonas putida* P4	4.00
5	*Pseudomonas aeruginosa* P5	0.00
6	*Bacillus megaterium* A1	4.00
7	*Bacillus subtilis* A3	8.00
8	*Bacillus circulans* A3	0.00
9	*Bacillus subtilis* A4	0.00
10	*Bacillus subtilis* A5	0.00

Physico-chemical Properties of the Soil before Planting

The physico-chemical properties of the soil sample were examined before planting. It was observed that the soil was alkaline with a pH of 7.28 with low mineral content, organic carbon, and moisture content (Table 4).

Table 4. Physiochemical Properties of the Soil before planting.

O.C (g/kg)	P (mg/kg)	N (g/kg)	K (mol/kg)	%Moisture Content	pH (H2O)
19.61	6.67	2.03	0.460	11.73	7.28

KEY: O.C: Organic Carbon, P: Phosphorus, N: Nitrogen, K: Potassium

Effect of Inoculation of Selected Rhizobacteria on Growth, Biomass, and Yield of Habanero Pepper Plant

All inoculated plant had higher number of roots as compared with T8 (positive control) and T9 (negative control). T4 (*A. brasilense* C1 + *P. putida* P4) stimulated the highest length of shoot, root, leaf, leaf width, and number of fruit over uninoculated controls with the values of 18.43cm, 13.36cm, 8.69cm, 5.69cm and 2.67, respectively (Table 5). Plant inoculated with T1 (*A. brasilense* C1) had the maximum increase in number of roots, plant wet weight, wet weight of fruit and dry weight of fruit than the controls with values of 51.89, 4.94g, 5.90g and 0.77g, respectively. T7 (*A. brasilense* C1 + *P. putida* P4 + *B. subtilis* A2) had the highest number of leaves values 18.22cm and T9 (negative control) had the lowest number of leaves 11.67cm while plants inoculated with T3 (*B. subtilis* A2), T4 (*A. brasilense* C1 + *P. putida* P4), and T6 (*A. brasilense* C1 + *B. subtilis* A2) had higher plant dry weight compare to the controls as seen in Table 5.

Table 5. Mean Effect of treatment on Growth parameters of Habanero pepper plant.

Treatment	Shoot length (cm)	Root length (cm)	Number of roots	Number of leaves	Leaf length (cm)	Leaf width (cm)	Number of fruits
T1	17.48^{ab}	13.11^{ab}	51.89^{a}	17.67^{a}	8.61^{a}	4.62^{b}	1.75^{a}
T2	15.24^{bc}	10.98^{cd}	48.44^{a}	17.56^{a}	7.02^{bc}	3.98^{bc}	0.00^{a}
T3	14.28^{cd}	11.22^{bcd}	48.89^{a}	17.00^{a}	7.36^{abc}	4.13^{bc}	1.33^{a}
T4	18.43^{a}	13.86^{a}	51.22^{a}	16.67^{a}	8.69^{a}	5.69^{a}	2.67^{a}
T5	14.41^{cd}	11.12^{bcd}	51.22^{a}	16.56^{a}	7.23^{bc}	4.17^{bc}	1.33^{a}
T6	16.53^{abc}	12.43^{abc}	50.89^{a}	17.56^{a}	7.70^{ab}	4.57^{b}	1.00^{a}
T7	15.02^{bcd}	11.52^{bcd}	49.56^{a}	18.22^{a}	7.70^{ab}	4.34^{bc}	0.00^{a}
T8	13.72^{cd}	10.19^{ed}	34.22^{b}	13.88^{b}	6.67^{bc}	3.81^{c}	0.00^{a}
T9	12.12^{d}	8.74^{e}	29.33^{b}	11.67^{c}	6.10^{c}	3.97^{bc}	2.00^{a}
LSD	2.69	1.84	7.37	2.14	1.21	0.65	2.12

Means with the same letter within a column are not significantly different at $p < 0.05$.

Effect of Inoculation of Selected Rhizobacteria on Mineral Uptake of Habanero Pepper Plant

The effect of this bio-fertilizer on mineral uptake of habanero pepper plant is shown in Table 6. T7 (*A. brasilense* C1 + *P. putida* P4 + *B. subtilis* A2) had the highest in nitrogen content (5.19%), T2 (*P. putida* P4) in phosphorus content (0.51%) and T5 (*P. putida* P4 + *B. subtilis* A2) in potassium content (5.00%) when compared with the positive and negative control (Table 6).

Table 6. Mean Effect of treatment on the Growth Parameters and Mineral Content of Habanero pepper plant.

T	Fruit wet weight (g)	Fruit dry weight (g)	Plant wet weight (g)	Plant dry weight (g)	N content (g/kg)	P conent (mg/kg)	K content (Cmol/kg)
1	5.90[a]	0.77[a]	4.94[a]	0.28[a]	1.34[a]	0.31[a]	2.03[f]
2	0.00[a]	0.00[a]	4.71[a]	0.26[a]	1.38[a]	0.51[a]	2.54[e]
3	3.17[a]	0.42[a]	4.74[a]	0.29[a]	1.36[a]	0.18[a]	4.87[a]
4	5.30[a]	0.72[a]	4.93[a]	0.29[a]	1.42[a]	0.22[a]	3.59[b]
5	3.07[a]	0.36[a]	4.87[a]	0.28[a]	1.25[a]	0.31[a]	5.00[a]
6	2.33[a]	0.34[a]	4.84[a]	0.29[a]	1.36[a]	0.29[a]	3.04[c]
7	0.00[a]	0.00[a]	4.60[a]	0.28[a]	5.19[a]	0.28[a]	2.79[d]
8	0.00[a]	0.00[a]	4.12[b]	0.21[b]	1.31[a]	0.18[a]	2.74[d]
9	5.87[a]	0.76[a]	3.72[b]	0.20[b]	1.28[a]	0.08[a]	1.11[g]
LSD	0.00	0.00	0.42	0.02	0.00	0.00	0.00

Means with the same letter in column are not significantly different at $p < 0.05$. T-Treatment

Physico-chemical Properties of the Soil after Planting
The analysis of the soil was determined after planting and the results obtained are shown in Table 7. It was observed that the soil A (soil with the T7 [*A. brasilense* C1 + *P. putida* P4 + *B. subtilis* A2]) was higher in mineral content, moisture content and lower in pH when compared to soil B (soil with T9 [positive control]). It was also observed that soil A and soil B were higher in mineral and moisture content and lower in pH when compared to soil before planting (Table 7).

Table 7. Physico-chemical properties of Bacteria inoculated soil and Chemical fertilizer treated soil at Day 60.

S/N	I.D	O.C (g/kg)	P(mg/kg)	N (g/kg)	K (mol/kg)	%Moisture Content	pH
1	Soil A	20.05	116.48	2.44	0.39	11.75	6.06
2	Soil B	17.80	92.89	2.28	0.3	11.73	7.10

KEY: S/N: Serial number, I.D: Identity, Soil A: Inoculated Soil, Soil B: Soil chemical fertilize, O.C: Organic Carbon, P: Phosphorus, N: Nitrogen, K: Potassium.

Discussion
In the rhizosphere, there are numerous bacteria with plant growth promoting traits that boost plant growth, development and output. In search of rhizobacteria strains with efficient PGPR traits, sixteen bacteria belonging to the genera *Azospirillum* (6), *Pseudomonas* (4), *Bacillus* (5), and *Aeromonas* (I) were obtained. Results similar to this in which *Azospirillum* spp. and *Bacillus* spp. were isolated from the rhizosphere of wheat and barley plant have been reported [21]. Cakmakci *et al.* [22] also detected the presence of *Bacillus* spp. isolated from the rhizosphere of pepper and tomatoes plant.

The plants treated with rhizobacteria had the highest growth parameters such as length of shoot, root, leaf, leaf width, and number of fruit. This is due to the application of *Azospirillum* alone and combined inoculation of *Azospirillum* with phosphorus solubilizing bacteria (PSB) which enhance plant and possibly penetrating inside root tissues where it lives and produces phytohormones such as indole acetic acid, gibberellins, and cytokinins in the plant. Hormones also enhance the nitrogen fixation capacity of diazotrophs [23]. These lead to induction of morphological changes such as increasing root length, number of root, diameter and density of both lateral and adventitious root, number of leaves, length and width. Similar results have been documented on the increase of leaf area in different crop co-inoculated with *Pseudomonas*, *Azospirillum*, and *Azotobacter* strains [24],[25],[26],[27],[28].

In this study, the effect of the inoculation on the plant wet and dry weight, fruit wet and dry weight, and number of fruit showed that the inoculated plants had higher values than uninoculated controls. It has been stated that seeds primed with PGPR increased dry matter buildup and output. This result was due to the complementary uptake of nutrients in the root milieu and the possible valuable effect of hormones and enzymes on the plant development and yield [29]. Continuous and stable stream of mineral elements particularly nitrogen and phosphorus, increase flowering rate, fruit production and enhance reproductive growth in plant [29]. This result is in line with related studies which reported the surge in dry root weight of rice cultivar treated with *Azospirillum brasilense* [26],[30],[31]. Khorshidi *et al*. [32] also reported the use of *Pseudomonas fluorescence* and *Azospirillum lipoferum* for increased number of grains per panicles in rice.

In this present research, it was observed that plants treated with nitrogen fixers, phosphorus and potassium solubilizers, were significantly improved over the uninoculated controls. Nitrogen, phosphorus and potassium are vital nutrients essential for the development and growth of plant as well as microorganisms. Increasing the supply of primary nutrients is one of the foremost direct mechanisms of PGPR which benefit plants in healthy growth. *Pseudomonas* and *Bacillus* species have been reported as beneficial solubilizers owing to their production of inorganic acids, carboxylic acid such as citric acid, tartaric acid, hydroxylic acid, phenolic compounds, protons, gluconic and ketogluconic acid for phosphorus and potassium solubilization in soil; hence, this result correlates with the research of other findings where combination of *Azospirillum* and other rhizobacteria were used in increasing the amount of nitrogen in different crops [29]. Similarly, increase in macronutrients in wheat plant as a result of inoculation with rhizobacteria has been reported by other researchers who reported potassium solubilizing bacteria in the growth and nutrient absorption in tobacco plant and increase potassium content in soil [18],[19],[33],[34],[35].

Soil contain essential nutrients such as nitrogen and phosphorus which are necessary for plant development. The combined rhizobacteria are capable of accumulating accessible primary nutrient in the soil by converting non-available nutrient bound in form of inorganic compound into available nutrient through mineralization, production of phytohormones and also decomposition. This result is in agreement with other studies which reported the enhancement of nutrient uptake in different crops as resulted from inoculation of microsymbionts [36].

V. CONCLUSION

In these findings, a total of 16 plant growth-promoting rhizobacteria (PGPR) were isolated from rhizosphere of habanero pepper plant. Among the 16 isolates, 3 most efficient bacteria were selected based on their plant growth promoting traits such as nitrogen fixation and solubilization of insoluble phosphorus and potassium. *Azospirillum brasilense* C1 fixed atmospheric nitrogen at (0.002%), *Pseudomonas putida* P4 showed the highest zone of clearance (4mm) in phosphorus solubilization and *Bacillus subtilis* A2 showed the highest of zone of clearance (8mm) in potassium solubilization. With these, the 3 isolates were inoculated into the seeds of habanero pepper seeds. Soil treated with T7 (*Azospirillum brasilense* C1 + *Pseudomonas putida* P4 + *Bacillus subtilis* A2) had the highest mineral content compared to soil treated with chemical fertilizers. With these findings, co-combination of PGPR of T4 (*Azospirillum brasilense* C1 + *Pseudomonas putida* P4) and T7 (*Azospirillum brasilense* C1 + *Pseudomonas putida* P4 + *Bacillus subtilis* A2) showed great performance on the habanero pepper plant and soil due to their synergistic effect.

Application of PGPR as cheaper, eco-friendly biofertilizers are efficient and attractive alternative in replacing inorganic fertilizers and chemical pesticides for viable habanero pepper farming in Nigeria and other unindustrialized countries. It can be recommended to farmers for enriched soil, healthy plant growth and productivity for commercially grown plants and this can prevent environmental pollution. Undoubtedly, there is a prospective and potential of PGPR as biofertilizers in crop cultivation to achieve attractive yield in agriculture.

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CONFLICT OF INTEREST

There is no conflict of interest on this study known the authors.

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AUTHORS PROFILE

Isabella A. Onoharigho pursed her B.Sc and M.Sc in Microbiology and Environmental Microbiology at Delta State University and University of Ibadan, respectively. Her main research work focuses on the use of microorganisms in enhancing plant growth, soil fertility and restoration of degraded environments. She has 3 years teaching experience, over 5 years research experience and she holds memberships on different scientific societies.

Adeniyi A. Ogunjobi is a professor of Microbiology, in the Department of Microbiology, University of Ibadan, Nigeria. His areas of research are Environmental Microbiology and Biotechnology, with focus in Bioremediation of polluted environment, Biodegradation of waste, water sanitation, antibiotic resistant bacteria in the environment as well as the use microbes in soil fertility and plant growth improvement. He has more than 80 research publications in both local and international outfits in his areas of specialization. He is a member of several academic and professional associations. He is an Ex-officio of Science Association of Nigeria (SAN).