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Research Paper

Effect Of Co-Inoculation of Bacterial Cultures of Plant Growth Promoting Rhizobacteria on White Beans (*Phaseolus vulgaris*) Seedlings Development

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ABSTRACT
The effect of co-inoculating bacterial cultures of plant growth-promoting rhizobacteria (PGPR) on the development of white bean seedlings (Phaseolus vulgaris) and their germination potential were analyzed. Five PGPR were isolated from the rhizosphere of Okra plants and were initially assessed for their Indole acetic acid (IAA) producing ability. The IAA concentrations ranged from 17.48 mg/l to 27.43 mg/l for Serratia sp. having the highest concentration with 27.43mg/l and Bacillus sp. the least with 17.48mg/l. The bacteria species were co-inoculated under five treatments to assess germination potential. The treatments included BS + Klebsiella sp. + Micrococcus sp., Micrococcus sp. + Serratia sp., Staphylococcus sp. + Bacillus sp., Serratia sp.+ Staphylococcus sp. and Klebsiella sp. + Bacillus sp. Four treatments peaked at 50% germination potential except Klebsiella sp. + Bacillus sp. which recorded germination potential of 33.3%. The effect of these PGPR treatments on the root and shoot elongation were studied hydroponically for 7 days. Significant increases ($P<0.05$) in root and shoot elongation were observed. The highest seedling root length of 18.5cm was recorded in treatment Serratia sp. + Staphylococcus sp. while the highest seedling shoot length of 13.5cm was recorded in treatment Staphylococcus sp. + Bacillus sp. the use of mixed PGPR as bio-inoculants can be a contemporary sustainable practice to facilitate nutrient supply to white bean seedling.
Keywords: *Plant Growth-promoting Rhizobacteria (PGPR), White Bean (Phaseolusvulgaris), Co-inoculation.*

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I. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are soil bacteria that aggressively colonize the rhizosphere and enhance the growth and yield of plants when applied to seeds or crops [1]. Plant growth promoting rhizobacteria are an important cluster of beneficial root colonizing bacteria thriving in the plant rhizosphere and bulk soil. They exhibit synergistic and antagonistic interactions with the soil microbiota and engage in an array of activities of ecological significance. They promote plant growth by facilitating biotic and abiotic stress tolerance and support the nutrition of plants [2]. Reported PGPRs include members of the genera *Acinobacter, Agrobacterium, Arthrobacter, Azospirillium, Enterobacter, Klebsiella, Pseudomonas, Rhizobium, Serratia, Streptomyces* etc [3, 4, 5]. PGPR are known for their ability to influence plant growth judging by their essential attributes of populating the root, surviving and building up in microhabitats associated with root surface in competition with other micro organisms within that environment, at least for the particular period needed to demonstrate their growth promotion/shielding activities [3].They form symbiotic, associative or neutral association with plants and have a significant influence on crop growth and development and can directly cause enhanced plant growth, early seed emergence or improvement in crop yields by supplying biologically fixed nitrogen and increasing minerals uptake, producing and secreting plant growth regulators and other beneficial substances [6]. Also, PGPR indirectly influences the plant growth promotion by suppressing of pest and diseases in vegetables.

Seeing that chemical fertilizers and their debris can greatly foul the environment and equally endanger the well being of humans and animals [7], PGPR is an eco-friendly alternative [1]. These rhizosphere bacteria enhance plant growth by different mechanisms such as solubilisation of phosphate and production of plant hormones such as Indole-3-acetic acid [8].

The use of efficient PGPR as bio-fertilizers and biological control agents is deliberated as a suitable substitute for minimizing the use of synthetic agrochemicals in crop production [9]. During the past couple of decades, the use of plant growth promoting rhizobacteria for sustainable agriculture has increased tremendously in various parts of the world and they are the trend for the future. [8].

With increasing awareness about the chemical fertilizer based agricultural practices, it is important to search for region specific microbial strains which can be used as growth promoting and enhancing inocula to achieve desired crop production [10].

Seed or soil inoculation with Phosphate Solubilizing Bacteria is known to improve solubilisation of fixed soil P and applied phosphates, resulting in higher crop yields [11]. Application of plant growth promoting rhizobacteria (PGPR) has become widely acceptable due to its advantages in crop production and environmental protection like increment in productivity, the diminutive in the use of chemical fertilizers and toxic compounds. These merits male beneficial bacteria formulations the better option in sustainable crop production [12]. The general effect of PGPR is an increased growth and productivity of plants. Their contribution can be exerted through different mechanisms including root system, architecture modulation and increased shoot growth by production of phytochemical such as auxins and cytokinins. PGPR can change growth pattern and result in bigger and branched roots with a greater surface area. As a result, plants are able to access more nutrients from the soil. The utilization of microbial growth enhancers can boost the agriculture of developing countries because they can be produced and marketed inexpensively. This study was primarily aimed at hydroponically studying the effect mixed bacterial cultures of selected plant growth promoting rhizobacteria on root and shoot of white beans seedlings.

II. MATERIALS AND METHODS

Location of study site

This study was carried out at the research farmland of the School of Agriculture and Agricultural Technology (SAAT) of the Federal University of Technology, Owerri, Imo-State, Nigeria.

Soil Sampling

Soil samples from the rhizosphere of mature okra (*Abelmoschus esculentus)* growing in the research farmland of the Federal University of Technology, Owerri Imo-State Nigeria, were used. The rhizospheric soil samples were collected with sterile spatula at the root hairs at a depth of 0-30cm. The collected samples were homogenized and transferred to the laboratory in sterile polythene bags within 1hr of collection for analysis.

Microbiological analysis

The heterotrophic bacterial count was done by serial dilution using physiological saline as the diluents. 0.1ml of the various dilutions was plated out on Nutrient agar using the Spread plate method. Plates were incubated in an incubator at 28± 2^oC for 24hrs. Discrete colonies were counted after 24 hours. The colonies were purified by sub-culturing on Luria-Bertani (LB) agar.

Estimation of Indole Acetic Acid (IAA) production

The isolates were screened for ability to produce indole-3-acetic acid (IAA) using the method described by [13]. One ml of each of the inoculum was added separately into 2 ml of sterile Jeon's medium contained in 15 ml test tubes. The test tubes were incubated for 3 days at room temperature after which they were centrifuged at 3000 rpm for 15 min. One ml of each was pipetted into 15 ml test tubes and 2 ml freshly prepared Salkowski reagent (2% 0.5M $FeCl_3$ in 35% per chloric acid) was added to each tube. This set up was incubated in a dark cupboard for 30mins. The tubes which showed red discoloration with Salkowki's reagent, indicating IAA presence, were quantified for IAA amount spectroscopically at 530nm. The Isolates were identified using microbiological methods as outlined in [14].

Preparation and Standardization of Inoculums

A loop-full of each bacterial isolate was picked from the slants and inoculated differently into a 200 ml conical flask containing 30 ml sterile tryptone soy broth. The cultures were incubated on a rotary shaker at 150 rpm at room temperature (28 ± 2^0C) for 24 hours. Thereafter, the cultures were centrifuged at 3000 rpm for 15 minutes, washed in physiological saline and re-suspended in sterile distilled water. The optical density (O.D) of the suspension was adjusted to 0.6 at a wavelength of 600 nm. One milliliter (1 ml) of each concentration of IAA was mixed with 2 ml of Salkowski's reagent contained in a 10ml test tube. The mixture was allowed to set for 30 min at room temperature (28±2^oC) in the dark and IAA concentration was measured spectroscopically at 530 nm and quantified in IAA standard curve.

Collection of White Bean Seedling and Seedling Bioassay

Whole white beans seeds (*Phaseolus vulgaris*) free from weevil infestation and other deformities were sorted from those bought in the open market. The seeds were disinfected by soaking them in 1% sodium hypo chloride for 5 minutes. Thereafter the seeds were washed three times with sterile distilled water to remove any trace of sodium hypochloride on them. To estimate the effect of inoculants on bean seed germination, a set of 6

disinfected seeds per plate were placed in 7 sterile Petri dishes containing sterile cotton wool. The seeds were watered with 1ml of distilled water in order to moisten the sterile cotton wool to stimulate germination and incubated on a laboratory bench at room temperature (28 ± 2^{o}C). A total of five bacterial preparations and one control were set up. The sprouted seeds were inoculated with 100 *u*l of microbial inoculants using sterile 1ml micropipette. The seeds were allowed to germinate.

Estimation of the effects of inoculation of white Beans seeds with mixed cultures of bacterial isolates was done by monitoring germination and root and shoot elongation.

Hence, Percentage germination was calculated according to [15]:

$$\frac{\text{Number of germinated seeds x 100}}{\text{Total number of seeds planted x 1}}$$

EFFECTS OF MIXED BACTERIAL INOCULATION ON SEEDLING ELONGATION

The influence of bacterial inoculants on root and shoot elongation of white beans was determined daily for a period of 7 days. This was done hydroponically by growing the germinated seeds in the absence of soil as support [16]. The best sprouted seeds were suspended via a sterile Millipore net over a 40ml beaker containing sterile water in such a manner as to allow the root gravitate towards the water (hydrotropism) and the emerging shoot gravitates towards natural sunlight (Lithotropism). This set up made for easy measurement of the root and shoot length without interrupting the growth process. This set up was placed on a laboratory bench at the temperature of 37^{o}C. The primary root and shoot elongations were measured in centimeter.

The data were subjected to two-way analysis of variance (two-way ANOVA) using SPSS 16.0 statistical program followed by post hoc testing. Mean values were separated using the Duncan and Student-Newman Keuls method at P=0.05 respectively.

III. RESULT

Five PGPR were isolated namely *Klebsiella* sp., *Micrococcus* sp., *Serratia* sp., *Staphylococcus* sp. and *Bacillus* sp. The Isolates were mixed given *Klebsiella* sp. + *Micrococcus* sp., *Micrococcus* sp. + *Serratia* sp., *Staphylococcus* sp. + *Bacillus* sp., *Serratia* sp. + *Staphylococcus* sp. and *Klebsiella* sp. + *Bacillus* sp.

Estimation of Indole Acetic Acid (IAA)

The maximum IAA production was by *Serratia species* recorded as 27.43mg/l^{-1}while the least amount of IAA was produced by *Bacillus* sp. recorded as17.48mg/l^{-1} as shown in Table 1 below. The ranking order of IAA production was *Serratia* sp. > *Staphylococcus* sp. > *Micrococcus* sp. > *Klebsiella* sp. > *Bacillus* sp.

Table 1: Estimation of IAA production of isolates

Isolate	IAA (mg/l^{-1})
Klebsiella species	17.52
Micrococcus species	17.57
Staphylococcus species	17.90
Serratia species	27.43
Bacillus species	17.48

Effect of Co-inoculation on seed germination

Germination was monitored for 2-3 days. Results obtained are shown in Table 2 below.

Table 2: **Percentage germination of seedling treatments**

	Mixed inoculants seedling Treatment	% Germination
I.	BS + *Klebsiella* sp. + *Micrococcus* sp.	50
II.	BS + *Micrococcus* sp. + *Serratia* sp.	50
III.	BS + *Staphylococcus* sp. + *Bacillus* sp.	50
IV.	BS + *Serratia* sp. + *Staphylococcus* sp.	50
V.	BS + *Klebsiella* sp. + *Bacillus* sp.	33.3
B.	**Control**	
I.	BS + Water	50

KEY: BS= Beans Seed

Effect of mixed inoculants on seedling Roots and Shoots elongation:
Effect of mixed bacterial inoculants on Roots and Shoots of White bean seedlings was monitored over a 7 day period on a hydroponic set up. The results are as shown below in Table 3.

Table 3: **Effect of mixed inoculants on seedling Root elongation**:

	DAY	CONTROL	*Klebsiella* sp. +*Micrococcus* sp.	*Micrococcus* sp.+ *Serratia* sp.	*Staphylococcus* sp.+ *Bacillus* sp.	*Serratia* sp. +*Staphylococcus* sp.	*Klebsiella* sp. +*Bacillus* sp.
	1	0.5	4.5	3	2	2	3
ROOT	2	1.5	6	3.8	3.5	3.5	4.3
LENGTH	3	3	6.5	6.8	5.8	5.8	6.8
(cm)	4	4	7	7.9	7.9	8	7.5
	5	4.5	7.2	11	11.5	11.5	9
	6	6.5	11	13	13	14	11.4
	7	8	14.5	15	15	18.5	13

Table 4: **Effect of mixed inoculants on seedling Shoot elongation**:

	DAY	CONTROL	*Klebsiella* sp. +*Micrococcus* sp.	*Micrococcus* sp.+ *Serratia* sp.	*Staphylococcus* sp.+ *Bacillus* sp.	*Serratia* sp. +*Staphylococcus* sp.	*Klebsiella* sp. +*Bacillus* sp.
	1	0	0	0	0	0	0
SHOOT	2	0	1	2	0.5	1.8	0.4
LENGTH	3	2	2.4	4	3	2.8	1.8
(cm)	4	3	3.5	5.9	4.5	4.1	3.8
	5	4.5	6	7	7.8	6.2	5
	6	7.5	7	9	9	7.9	7
	7	9.2	11.8	12	13.5	11.1	10.3

Table 5: **7th Day response of mixed inoculation treatments of plant growth rhizobacteria isolates on white beans seedling root and shoot lengths:**
In each column, means which share same letters do not differ significantly.

Mixed inoculation Treatment	N	Root Length (cm)	Shoot Length (cm)
Klebsiella sp. + Micrococcus sp.	3	10.8333^{b}	11.9333^{cd}
Micrococcus sp. + Serratia sp.	3	13.1667^{c}	10.8333^{bc}
Staphylococcus sp. +Bacillus sp.	3	13.6667^{c}	12.6667^{d}
Serratia sp. + Stapylococcus sp.	3	13.8333^{c}	10.2000^{ab}
Klebsiella sp. + Bacillus sp.	3	11.6000^{b}	9.1000^{a}
Control	3	7.5000^{a}	9.2333^{a}

N =Harmonic Mean Sample Size

Root length

The PGPR isolates increased the root length of beans seedlings after a 7 day period (Table 5). The effect of combinations *Staphylococcus species + Bacillus species* and that of *Serratia species + Staphylococcus species* were statistically similar and produced highest root lengths of 13.67 cm and 13.83 cm respectively in comparison to other combinations, *Klebsiella species + Micrococcus species* and *Klebsiella species +Bacillus species* which showed reduced effect (10.83 cm and 11.60 cm respectively).

Shoot length

A significant variation in shoot length was observed in response to mixed inoculation of PGPR isolates (Table 4). Results show that the isolates increased shoot length of beans seedlings. The highest effect was recorded in combination of *Klebsiella species + Micrococcus species* and *Staphylococcus species + Bacillus species* (11.93 cm and 12.67 cm respectively), been statistically similar, and followed by *Serratia species + Staphylococcus species* (10.20cm).

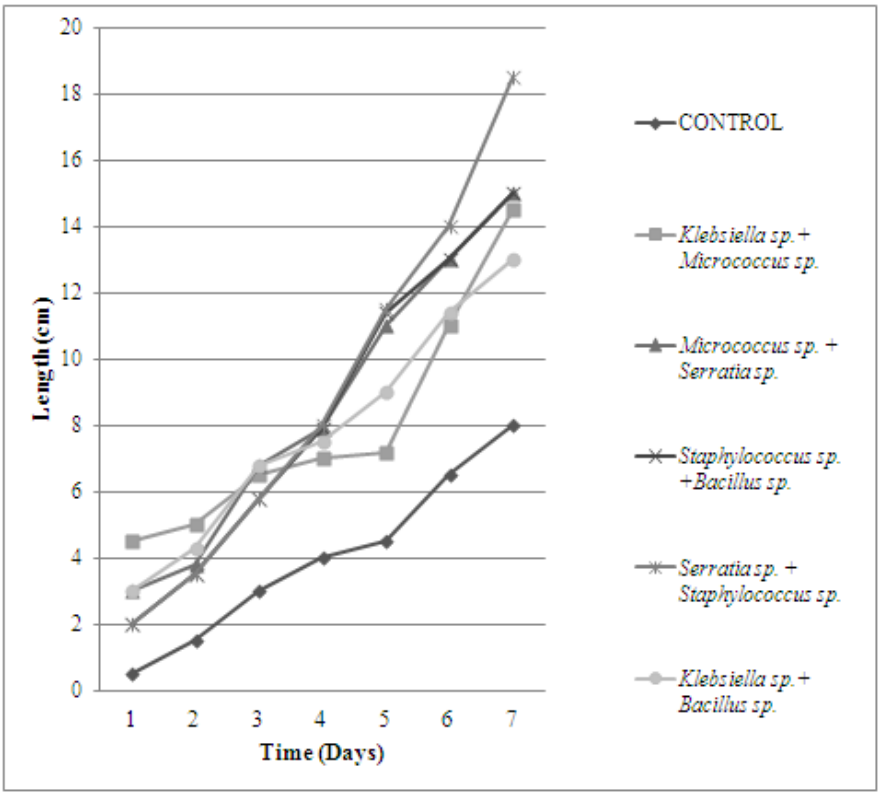

FIGURE 1: ROOT ELONGATION OF BEANS SEED INOCULATED WITH MIXED BACTERIAL ISOLATES.

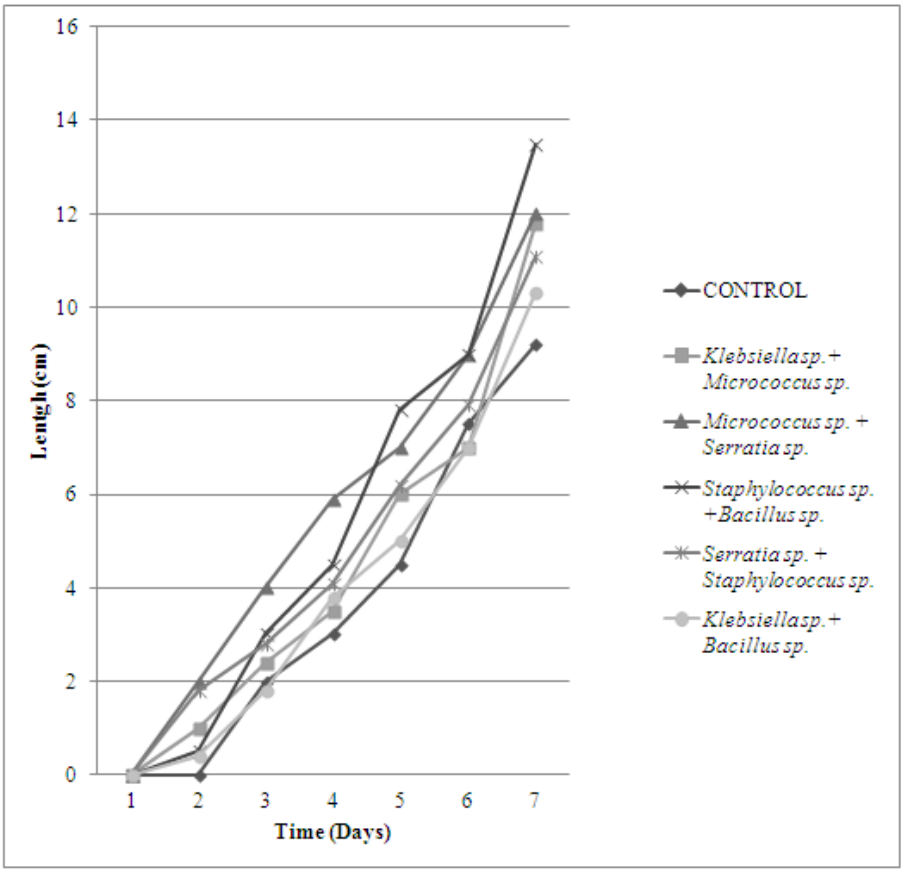

FIGURE 2: SHOOT ELONGATION OF BEANS SEED INOCULATED WITH MIXED BACTERIAL ISOLATES

IV. DISCUSSION

In this study, plant growth promoting rhizobacteria, *Serratia sp., Klebsiella sp., Micrococcus sp., Bacillus sp.* and *Staphylococcus sp*. were isolated from the rhizospheric soil of okra and applied in combination, showing positive effects on shoot and root prominence of white beans seedling and germination of *Phaseolus vulgaris*. Based on the hydroponic culture results, *Serratia sp.* showed better influence on root prominence of *Phaseolus vulgaris.* As regarding shoot elongation, the dual effect of *Staphylococcus sp. + Bacillus sp.* and *Klebsiella sp. + Micrococcus sp.* showed synergistic and improved effect on lateral growth and shoot elongation over the uninoculated control. *Serratia sp. + Staphylococcus sp*. and *Staphylococcus sp. +Bacillus sp*. and *Klebsiella sp.+ Micrococcus sp*. showed synergistic and improved effect on lateral growth and root elongation over their uninoculated control. These out-weighed the effects of other treatments showing very significant effect. Seed germination increased slightly when seeds were pretreated with mixed PGPR isolates. Minaxi *et al*., 2012 [17] noted that the higher germination observed in certain inoculated treatments could be due to plant growth. Their combinations positively initiated effects on the shoot and root. This shows that certain bacterial inoculants fair better when applied in combination with others hence possessing additive tendencies to boost plant growth promoting ability of others. When multiple factors co occur their combined impact on plant growth is often greater than that based on single factor studies, having synergistic effects [18].

Combined inoculation of *Rhizobia* and *Rhizobacteria* showed positive effects on root nodulation and growth in legumes [19]. *Bacillus polymyxa* showed 110% increase in nodule number, 121% increase in nodule weight and 44% increase in grain yield of *Vigna radiata*. Combinations of *Rhizobium* and *Bacillus cereus* showed 382% increase in nodule number, 196% increase in nodule dry weight, 116% increase in Nitrogen fixation and 54% increase in grain yield in their study on *Cajanus cajan*[20].When *Bradyrhizobium, Serratia marcescens* and *Trichoderma harzianum* were used in combination on *Arachis hypogaea* effects increased in nodule number, nodule dry weight and grain yield by 115%, 94% and 41% respectively [21].

Co-inoculation of PGPR and Rhizobia has a synergistic effect on bean growth. The use of PGPR improved effectiveness of *Rhizobium* biofertilizers for common bean production [22]. This corroborates this research where the effect of co-inoculation of *rhizobium* and PGPR, on nodulation and growth of common bean (*Phaseolus vulgaris* L.) was investigated using a low phosphorous soil under greenhouse conditions. *Paenibacillus polymyxa*, *Bacillus megaterium* and two rhizobia strains (IITA-PAU 987 and IITA-PAU 983) and one reference rhizobia strain (CIAT 899) were used in the co-inoculation study resulting in significant root and shoot dry weight. In the study of Elkoca *et al* 2010, bacterial inoculations significantly increased all the parameters investigated compared with the control treatment. Increases of seed yield under different inoculation treatments ranged between 6.6% and 12.2% over the control whereas N, P and NP applications corresponded to increases of 5.6%, 4.0% and 7.4%. All bacterial inoculations, especially triple inoculations significantly increased uptake of macronutrients and micronutrients by common beans [23].

V. CONCLUSION

The results of this work confirmed the capacity of certain plant growth promoting rhizobacterial species to enhance seedling development of white beans and act as potential biofertilizers. Growth promotion and enhancement using PGPR in combination significantly improved shoot and root length in white beans seedlings, proving its synergistic effect as a great source for sustainable development in agriculture.

It is therefore recommended that these rhizobacterial inoculants especially a combination of *Staphylococcus* sp. + *Bacillus* sp. and *Serratia* sp. + *Staphylococcus* sp. can be used in the production of bio-fertilizers and for crop yield improvement by farmers and researchers since they are non-toxic, environmentally-friendly and act as potential bio-tools for plant growth and yield promotion.

REFERENCES

[1]. Kumar V.K., Reddy M.S., Kloepper J.W., Lawrence K.S., Zhou X.G., Groth D.E., Zhang S., Sudhakara Rao R., Wang Q., Raju M.R.B, Krishnam R., DilanthaFernandoW.G.,Sudini H., Du B. and Miller M.E. (2011). Commercial potential of microbialinoculants for sheath blight management and yield enhancement of rice. In: Maheshwari D.K. (ed) bacteria in agrobiology: *Crop Ecosystems Springer*, Berlin, 237-264.

[2]. Basu, A., Parsad, P., Das, S.N., Kalam, S., Sayyed, R.Z., Reddy, M.S. and El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as Green bioinoculants: Recent Developments, Constraints and Prospects. *Sustainability*, 13: 1140.

[3]. Ahemed, M. and Mohammad, S.K. (2011). Functional aspects of plant growth promoting rhizobacteria: Recent Advancements. *International Microbiology*, **1**: 39-54.

[4]. Kalam, S., Basu, A. and Podile, A.R. (2020). Functional and Molecular characterization of PGP *Bacillus* isolates from tomato rhizosphere. *Heliyon*, 6.

[5]. Parray, J.A., Jan, S., Kamilo, A.N., Quadri, R.A., Egamberdieva, D. and Ahmad, P. (2016). Current perspective on PGPR. *Journal of plant growth regulation*, ***35***: 877-902.

[6]. Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R.K., Gowda, C.L. and Krishnamurthy, L. (2015). Plant growth promoting rhizobia: Challenges and opportunities. *Journal of Biotechnology*, 5:355-377.

[7]. Kaymak, H.C. (2019). Potential of PGPR in improvement of environmental- friendly vegetable production. In: Maheshwari, D., Dheeman S. (eds) Field Crops: sustainable management by PGPR. Sustainable Development and Biodiversity. *Springer Cham*., 23.
[8]. Yasar E, Sezai E, Ayhan H and Ramazan C. (2010). Effects of Plant growth promoting rhizobacteriaon rooting and root growth of Kiwifruit (*Actinidia deliciosa*) stem cuttings. *Biology Resources*, 43(3): 1663-1668.
[9]. Atieno, M., Hermann, L., Nguyen, H.T., Phan, H.T., Nguyen, N.K., Srean, P., Than, M.M., Zhiyong, R., Tittabutr, P. and Shut-Srirung, A. (2020). Assessment of bio-fertilizer use for sustainable agriculture in the great Mekong Region. *Journal of Environmental management.*111300: 0301-4797.
[10]. Deepa, C.K., Dastager, S.G. and Pandy, A. (2010). Isolation and characterization of plant growth promoting rhizobacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (l.) walp.) seedling growth. *World Journal of Microbiology Technology*, 26:1233-1240.
[11]. Kapri, A. and Tewari, L. (2010). Phosphate solubilization potential and Phosphatase activity of rhizospheric *Trichoderma sp*. *Brazilian journal of Microbiology*, 1517-8382.
[12]. Morales-Gracia, Y.E., Baez, A. and Quintero-Hemandez, V. (2019). Bacterial mixtures, the future generation of inoculants for sustainable crop production. Fields crops: *Sustainable management by PGPR*, 11-14.
[13]. Jeon, J.S., Lee, S.S., Kim, S.Y., Ahn, T.S. and Song, H.G. (2003). Plant growth promotion in soil by some inoculated micro organism. *Journal of Microbiology*, 41:271-276.
[14]. Cheesbrough, M. (2002). *District Laboratory practice in tropical countries part two.* Low price edition, Cambridge university press. Uk. Pp. 117.
[15]. Laboriau. (1983). The germination of the Seeds. Organization of America States, regional program for scientific and technological development. Biology Series, Monograph.
[16]. Woitke, M., Junge, H. and Schnitzler, W.H. (2004). *B. subtilis* as a growth parameter in hydroponically grown tomatoes under saline conditions. *International society of Horticultural science* (ISHS), 659:363-369.
[17]. Minaxi, N. L., Yadav, R.C. and Saxena, J. (2012). Characterization of multifaceted *Bacillus sp*. RM-2 for its use as plant growth promoting bio- inoculants for crops grown in semi arid deserts. *Applied Journal of Soil Ecology*, 59:124-135.
[18]. Verma,J.P., Yadav, J., Tivan, K.N., Singh, V. and Singh, L. (2010). Impact of plant growth promoting rhizobacteria on crop production. *International Journal of agricultura*l research, 5:954-983.
[19]. Bansal, R.K. (2009). Synergistic effect of Rhizobium, phosphate solublizing bacteria and plant growth promoting rhizobacteria on nodulation and grain yield of mungbean. *Journal of Food Legume*, 22: 37-39.
[20]. Tilak, K.V.B.R., Ranganayaki, N. and Mancharachari, C. (2006). Synergistic effects of PGPR and Rhizobium on nodulation and Nitrogen fixation by Pigeonpea (*Cajanus cajan*). *European Journal of Soil Science*, 57:61-71.
[21]. Badawi, F.S.F., Biomy, A.M.M. and Desoky, A.H. (2011). Peanut plant growth and yield as influenced by co-inoculation with Bradyrhizobium and some rhizo microorganisms under sandy loam Soil conditions. *Journal of Soil Science*, 57:67-71.
[22]. Korir H, Mungai NW, Thuita M, Hamba Y, Masso C. (2017). Co-inoculation effect of Rhizobia and plant growth promoting Rhizobacteria on common bean growth in a low phosphorus soil. *Frontiers in Plant Science*, 7: 8-141.
[23]. Elkoca, E., Turan, M. and Donmez, M.F. (2010). Effects of single, dual, triple inoculation of *Bacillus subtilis*, *B. megaterium* and *Rhizobium leguminosarius* BV. Phaseoli on nodulation, nutrient uptake yield and yield parameters of common bean (*Phaseoli vulgaris* L. CV. Elkoca-05). *Journal of plant Nutrition*, 33(14): 2104-2119.