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Evaluation of endophytic *Bacillus spp.* of tea *(Camellia sinensis* L.*)* for their plant growth promoting activities

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Abstract: **The present study aims to evaluate the in-vitro and in-vivo (pot culture) plant growth promoting activities of four different endophytic Bacillus species i.e. B. pumilus, B. cereus, B. flexus RN 6, and B. flexus RN 11 isolated from tea (Camellia sinensis L.) shrubs collected from different tea gardens of Jorhat district of Assam, India. All four endophytic Bacillus species were found to be efficient plant growth promoter in both in-vitro and pot culture experiment. Among the four test isolates, B. cereus was most efficient with a maximum indole acetic acid (IAA) production (16.22± 0.17µg/ml), phosphate (174.3 ±2.2 µg/ml) and zinc (45.06 ± 1.22µg/ml) solubilization in-vitro. B. pumilus was identified as highest K solubilizer among the test isolates with a solubilization index of 200 ± 2.8%. A perfect positive correlation was noticed between increases of mineral solubilization and acidity of the culture supernatant. Soil inoculation of the test isolates in to pot culture experiment revealed a significant enhancement in plant growth to the bean seedlings.**

Keywords: **Endophytes, Bacillus sp. phosphate solubilization, zinc solubilization, IAA production.**

I. INTRODUCTION

Microorganisms, a major group of biotic factors of an ecosystem, are ubiquitous in any environment of the world. They play a crucial role in nutrient cycling by the process of decomposition. Plants are constantly involved in interactions with a wide range of microbes including bacteria and fungi. The plant associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and inside of the plant tissue (endophytes). The word "endophyte" means "inside the plant" (derived from the Greek "endon" = "within", "phyton" = "plant"). Although there are diverse meanings for the term, endophytes are most commonly defined as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal" [1]. Though endophytes are ubiquitous in occurrence, their relationship with host plants is still not well known. Benefits of harboring the endophytic microorganisms for the plant itself are in various dimensions. From the support of a large number of experimental evidences it is revealed that endophytic microorganisms support the plant growth, development and yielding by synthesizing different plant hormones as well as solubilization and mobilization of insoluble mineral [2-4].

Different species of *Bacillus* are among the most commonly occurring rhizospheric as well as endophytic bacteria. *Bacillus subtilis*, a common bacterium, is well known for its positive influence on plant growth, vitality, and the ability of the plant to cope with pathogens often resulting in higher yield. *B. mucilaginous* has been observed for its capability in solubilizing potassium [5] zinc and phosphate [6]. Manipulation of soil micro-flora by inoculation of *Azotobacter* and *Bacillus* sp. increase the yield of wheat and other cereal crops remarkably. This is because *Azotobacter* spp. provides nitrogen and *Bacillus* sp are responsible for production of plant growth hormones as well as their capability to solubilize and mobilize different insoluble mineral salts. [7]. Furthermore, experiments of Woitke et al. [8] revealed the ability of *B. subtilis* to help hydroponically grown tomato plants to withstand salinity stress also.

Phosphorus is an important element for plant growth and development. Though soil contains more than 0.5% phosphorus, most of them are in insoluble form and hence cannot be absorbed by plants. To yield better, farmers apply excess amount of phosphate fertilizers. However, a major part of these phosphate fertilizers transform into insoluble forms according to soil pH [9]. The insoluble inorganic compounds of phosphorus can be converted by some bacteria into available phosphates for plants. Bacterial genera including *Pseudomonas, Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium* and *Sinorhizobium* have the ability to convert insoluble phosphates into soluble form by the process of acidification [10-12].

Similarly, potassium and zinc are two impotent major and minor nutrients with remarkable contribution to plant growth and development. Zinc acts as metal co factor for many vital enzymes in biological system. Some common fungi like *Aspergillus* and *Penicillium* can solubilize a major amount of zinc from insoluble inorganic zinc salts. Though, most soil bacteria are unable to act on insoluble zinc salts, however, some *Bacillus spp.* are well equipped for zinc solubilization [13]. Potassium is the most abundantly absorbed cation in higher plants. It activates enzymes, maintains cell turgor, enhances photosynthesis and energy production, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis too. In addition to plant metabolism, potassium improves crop quality in terms of grain filling and kernel weight strengthens straw, increases disease resistance and helps the plant better to withstand stress. Many soil bacteria like *Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, B. circulans and Paenibacillus sp*. are well studied for their potassium, silicon and aluminium solubilizing activities.

Though Assam produces one of the finest teas, tea cultivation in Assam is highly dependent on agrochemicals. Over application of chemical fertilizers is one of the major causes of soil and water pollution in Assam. Sometimes, foreign markets use to reject Assam tea because of its high chemical residues. Therefore, production of organic tea will be one of the best ways to improve tea quality in Assam. Though ,soil is one of the most common sources for isolation of biofertilizers, adverse effects of soil microbes to plant health is not well known. As endophytes are generally harmless to the plant and have better plant growth promoting activities, manipulation of soil microflora by plant growth promoting endophytes may open a new area in the field organic tea production.

II. MATERIALS AND METHODS

Collection of root samples:

Three years old tea (*Camellia sinensis)* roots were collected from different tea gardens of upper Assam. Healthy and disease free young roots were collected in ice box, packed immediately in poly bags and stored at 4^0 c for further use.

Isolation of root endophytic bacteria:

To remove the debris, root samples were washed properly with tap water followed by distilled water for five times. 70% ethanol (for 5 min) and 0.1% $HgCl_2$ (for 1minute) were used as surface sterilizing agents. Samples were then washed thoroughly (ten times) with sterilized distilled water to remove the surface sterilizing agents [14]. Five grams of the samples were homogenized in 50 ml of sterilized distilled water to prepare stock solution of tissue homogenate. Appropriately diluted tissue homogenates were inoculated in tryptic soya agar (TSA) and nutrient agar plates, incubated at 30^0C for 48-72 hrs for microbial growth. From these plates pure colonies were isolated by the process of streaking [15]. Bacterial isolates were classified according to their morphology.

IAA production:

Indole acetic acid production activity of bacterial isolates was assayed colorimetrically using ferric chloride perchloric acid ($FeCl_3$- $HCLO_4$) reagent [16]. This method estimated the quantities of IAA produced by bacteria in medium containing L- tryptophan as precursor.

Four *Bacillus* isolates were grown separately in 100 ml conical flaks containing 50 ml nutrient broths for 24 hrs at 30^0C in an incubator shaker (140 rpm). After overnight incubation, 1ml of 0.6 OD cultures from each flasks were transferred in to 250 ml conical flasks containing 100 ml of L-tryptophan amended (1000 µg/ml) minimal salt (MS) medium followed by an incubation period up to 4 days at 30^0C in an incubator shaker (150 rpm). Periodic estimation of IAA in the culture supernatants was done in two, four, six, and eight days. The MS medium contained (in 1000 ml) 1.36 g KH_2PO_4, 2.13 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$, and trace elements. L-tryptophan solution was prepared as stock solution containing (in 100 ml distilled water) 10 g glucose, 1 g L-tryptophan and 0.1 g yeast extract. Stock solution was filtered by syringe filter.

After incubation, 5 ml of cultures from each flask were centrifuged at 7,000 rpm for 12 minutes. For measuring the amount of IAA produced, 1ml of culture supernatant was pipetted into test tubes and mixed with 2 ml of $FeCl_3$-perchloric acid reagent (50 ml 35% perchloric acid + 1 ml 0.5 M $FeCl_3$ solution) [16] and 2 drops of *ortho*-phosphoric acid ([17]. After 25 mins, development of pink colour was measured at 530 nm wave lengths by UV-VIS spectrophotometer (CECIL CE 7250).

Phosphate solubilization:

Screening of *Bacillus* isolates for their phosphate solubilizing activity was done in Pikovskya's agar (glucose,10 g; $Ca_3(PO_4)$ 2, 5 g; $(NH_4)_2$ SO_4, 0.5 g; NaCl, 0.2 g; $MgSO_4.7H_2$ O, 0.1 g; KCl, 0.2 g; yeast extract, 0.5 g; $MnSO_4H_2O$, 0.002 g; and $FeSO_4.7H_2O$, 0.002 g, agar 15 g) medium plates. Isolates showing clear zones around the growing colonies after 72 hrs of incubation at 30^0 C were considered as positive for P solubilization [18].

Quantitative estimation of phosphate solubilization in broth culture was performed according to the procedure of Jackson (1973). All the bacterial isolates were grown seperately in 150 ml conical flasks containing 50 ml nutrient broth for 24 hrs at 30^0 C in incubator shaker with 150 rpm. One ml of 0.6 OD bacterial cultures from each flask were inoculated to 100 ml of Pikovskya's broth in 250 ml conical flasks. . One uninoculated flask was used as control. Flasks were incubated at 30^0 C for ten days in a shaking incubator at 250 rpm. Five ml of two, four, six, eight and ten day's old cultures were centrifuged at 10,000 rpm for 10 mins. One ml of supernatant from each flask was mixed with 10 ml of chloromolybdic acid and the volume was adjusted to 40 ml with distilled water. To this mixture, 1 ml of chlorostannous acid was added. Final volume was made 50 ml by adding distilled water. Absorbance of the developing blue colour was measured at 600 nm wave length with UV- VIS spectrophotometer (CECIL CE 7250). The amount of soluble phosphate was calculated from standard curve of $KH2PO_4$. Periodic estimation of culture pH was performed by using digital pH meter (EUTECH pc 510).

Zinc solubilization:

Screening of bacterial isolates for their zinc solubilization activity was done by using halo zone formation method in Basal medium (glucose-10.0 g; ammonium sulphate-1.0 g; potassium chloride-0.2 g; dipotassium hydrogen phosphate-0.1 g; magnesium sulphate-0.2 g; distilled water -1000 ml, pH 7.0) plates containing 0.2% insoluble ZnO. After 72 hrs of incubation, colonies showing clear zones around them were considered as zinc solubilizers.

Bacterial isolates were grown separately in 100 ml Erlenmeyer flasks containing 50 ml nutrient broth. One ml from each 24 hrs old cultures with 0.6OD was inoculated into separate 250 ml Erlenmeyer flasks containing 100 ml liquid basal medium amended with 0.1% ZnO. Flasks were incubated at 30^0C in an incubator shaker for ten days. One uninoculated flask was used as control. The samples were withdrawn at 2, 4, 6, 8 and 10 days intervals, centrifuged at 10,000 rpm for 7 mins to remove the debris and cells. Ten ml of this solution was fed to Atomic Absorption Spectrophotometry (AAS) to determination of the available zinc content[19]. Periodic estimation of culture pH was performed by using digital pH meter (EUTECH pc 510).

Potassium solubilization:

Screening of isolates for their potassium solubilizing activity was done by halo zone forming method in Aleksandrov agar medium (1% glucose, 0.5% Yeast extract, 0.05% $MgSo_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPo_4$ and 3 % agar) [20] containing o.2% insoluble potassium bearing mineral (mica). The plates were incubated at room temperature (30±1°C) for 3 days and the colonies exhibiting clear zones around them were considered as positive for potassium solubilization activity. Potassium solubilization index was calculated with the help of following formula.

Potassium Solubilization Index= A/B [A= total diameter (colony+ halo zone), B= diameter of colony]

Amplification of 16srDNA and sequencing:

Bacterial isolates were grown in nutrient broth at 30^0C for overnight. 2 ml of 0.6OD cultures were taken into eppendrof tubes and centrifuged at 10,000 rpm for five minutes to yield the cell pallet. Bacterial cells were lysed with the help lysis buffer and genomic DNA was isolated by conventional phenol-chloroform method. Amplification of 16s rDNA was carried out in 25 µl PCR reaction mixture [Taq buffer, 2.5 µl; $MgCl_2$, 2.0 µl, dNTP mix (2.5 mM), 2.5 µl, forward and reverse primer 1 µl of each, water 13 µl, template 2 µl, taq polymerase 1 µl] using universal 16s primer sequences (US16F8/20 5'AGAGTTTGATCCTGGCTGAG3' and US16R154/20 5'AAGGAGGTGATCCAGCCGCA3'). DNA was amplified over 35 cycles of PCR profile with 94^0C for 1 min, 94^0C for 3 min, 60^0C for 1 min, 72^0C for 1 min, 72^0C for 10 min 4^0C for α. Amplification was confirmed by gel electrophoresis and sequenced in ABS 3700 sequencer system.

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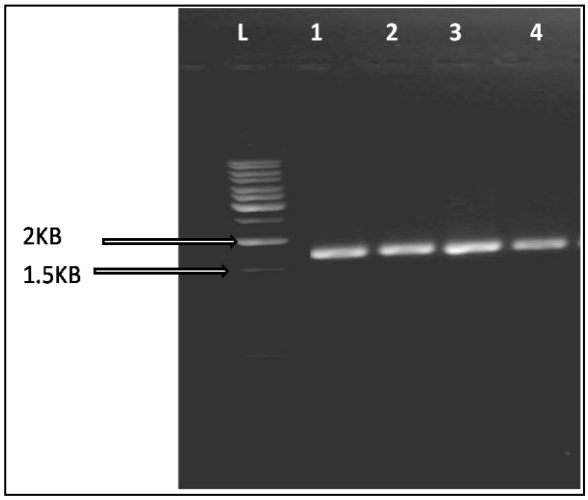

Fig 1: PCR amplification of 16s rDNA. L-ladder, 1-*B. pumilus*, 2- *B. cereus*, 3- *B. flexus* RN6, 4- *B. flexus* RN 11

Application of the isolates into pot culture:

To evaluate the efficacy of the isolates, they were applied into bean seedling pot cultures. Four different sets of bean seedling pot cultures (with ten replications of each) were conducted to reveal the ability of test isolates for their plant growth promotion (table 1). Enhancement in plant growth was measured in terms of leaves and branch production as well as increase of plantlet height.

TABLE 1: Different soil treatments in pot culture

Exptl. set	Soil used	Treatments
Control	Normal soil	No other ingredients(10 replications)
Treatment I	Normal soil	100 g vermi. (10 replications)
Treatment II	Normal soil	NPK normal dose(10 replications)
Treatment III	Normal soil	50g vermi.+ 10 gm activated charcoal with10^7/gm test isolates (10 replications)

III. RESULTS

Biochemical characterization:

Table2 strands for the biochemical characteristics of the isolates for their identification purpose. All the isolates were gram positive with their rod like appearance. Most of the isolates produced catalase, gelatinase, protease and amylase *in vitro*. Only one isolate i.e. *Bacillus flexus* RN6 was found to be cellulase producer and *B. pumilus* was able to produce pectinase.

TABLE 2: Biochemical characteristics of the isolates

Isolates	Gene bank acc. No	Gram reaction	Catalase	Gelatinase	Citrate utilization	Casein hydrolysis	Starch hydrolysis	Cellulase	Pectinase
Bacillus pumilus	KF225783	+ rod	+	-	-	+	+	-	+
Bacillus cereus	KF225785	+ rod	+	+	+	+	+	-	-
Bacillus flexus RN6	KF225786	+ rod	+	+	+	+	+	+	-
Bacillus flexus RN11	KF225789	+ rod	+	+	+	+	+	-	-

In vitro plant growth promoting activities:

❖ ***IAA production:***

The present study focuses on *in-vitro* IAA production and mineral solubilization activities of the test isolates along with their evaluation for plant growth promotion in tea plantlet pot cultures. Periodic estimation of IAA in the culture supernatant revealed a range between 4.62±0.10µg/ml and16.22± 0.17µg/ml. It was observed that the ability of IAA production increased gradually up to day six with a fall of pH of the medium followed by decrease of IAA production with a gradual rise of pH. Among the four test isolates, *B. cereus* was found to be most efficient with a maximum IAA production of 16.22± 0.17µg/ml after six days incubation followed by *B. flexus* RN6 (15.87± 0.19µg/ml) and *Bacillus flexus* RN11 (14.88± 0.24 µg/ml, table 3).

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TABLE 3: Periodic estimation of *in- vitro* IAA production

Isolates	Amount of IAA (in µg/ml) in the medium			
	24 hrs	48 hrs	72 hrs	96 hrs
Bacillus pumilus	6.05 ± 0.21	10.06 ± 0.24	14.22± 0.26	11.42± 0.14
Bacillus cereus	5.80± 0.11	11.52 ± 0.22	16.22± 0.17	13.66± 0.17
***Bacillus flexus* RN6**	4.62± 0.10	8.72 ± 0.20	15.87± 0.19	10.04± 0.19
***Bacillus flexus* RN11**	5.02± 0.22	9.06 ± 0.21	14.88± 0.24	12.62± 0.21

❖ Values are mean ± SD of five replic

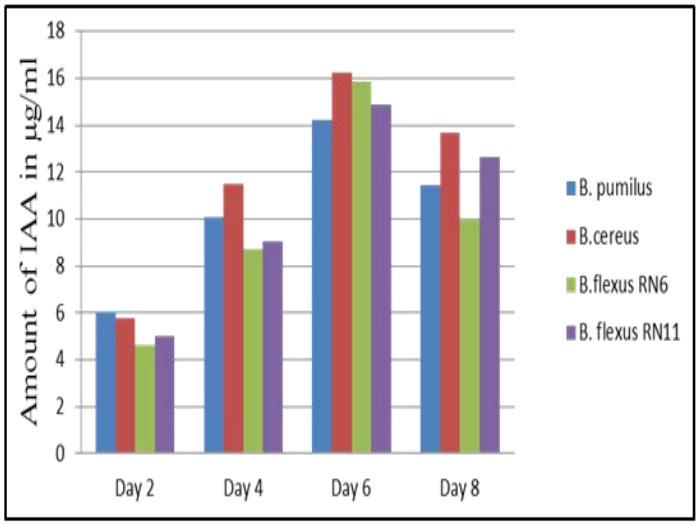

Fig 2: Periodic estimation of IAA production

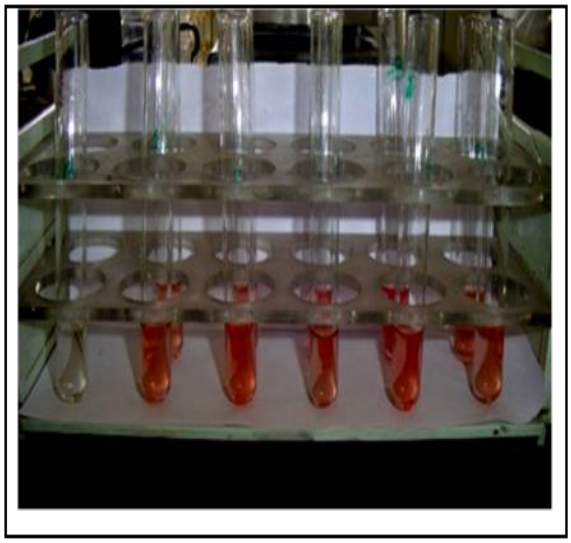

Fig 3: IAA producing activity of the isolates

❖ ***Phosphate solubilization:***

Periodic estimation soluble phosphates in the culture media revealed a remarkable ability of the isolates to solubilize insoluble tricalcium phosphate *in vitro*. As, organic acid production is the main mechanism of mineral phosphate solubilization, a drop of pH was observed with a rise of soluble phosphate level in the medium. Soluble P level in the culture supernatant increased gradually up to day eight with a gradual decrease of pH of the medium. Amount of soluble P in the culture supernatant ranged between 70.2 ±1.0µg/ml and 174.3 ±2.2µg/ml. Maximum phosphate solubilization ability was observed in *B. cereus* (174.3 ±2.2 µg/ml) followed by *B. flexus* RN11 (166 .02±1.7µg/ml) and *B. pumilus*(160.2 ±1.6µg/ml). Drop of pH of the medium observed up to 4.6±0.2 with maximum level soluble P in the medium. (table 4)

TABLE 4: Periodic estimation of *in- vitro* phosphate solubilizing activity of the isolates

Incubation period in days	Amount of soluble P in µg/ml and pH of the medium							
	Bacillus pumilus		*Bacillus cereus*		*Bacillus flexus* RN6		*Bacillus flexus* RN11	
	P conc.	pH	P conc.	pH	P conc.	pH	P conc.	pH
2	70.2±1.0	6.2±0.3	79.3±1.7	6.4±0.4	66.2±1.4	6.6±0.1	72.6±1.8	6.4±0.1
4	110.1±1.2	5.8±0.2	122.4±1.2	5.4±0.7	78.7±1.7	6.2±0.7	86.7±1.3	6.0±0.2
6	140.5±1.0	5.3±0.7	156.5±1.8	5.0±0.3	120.6±2.4	5.8±0.2	1116.0±1.0	5.2±0.4
8	160.2±1.6	5.0±0.5	174.3±2.2	4.6±0.2	146.2±2.3	5.2±0.5	166 .02±1.7	4.8±0.1
10	152.4±1.4	5.2±0.2	158.0±1.0	5.2±0.4	140.7±1.9	5.3±0.8	152.2±2.0	5.0± 0.2

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❖ Values are mean ± SD of five replicas:

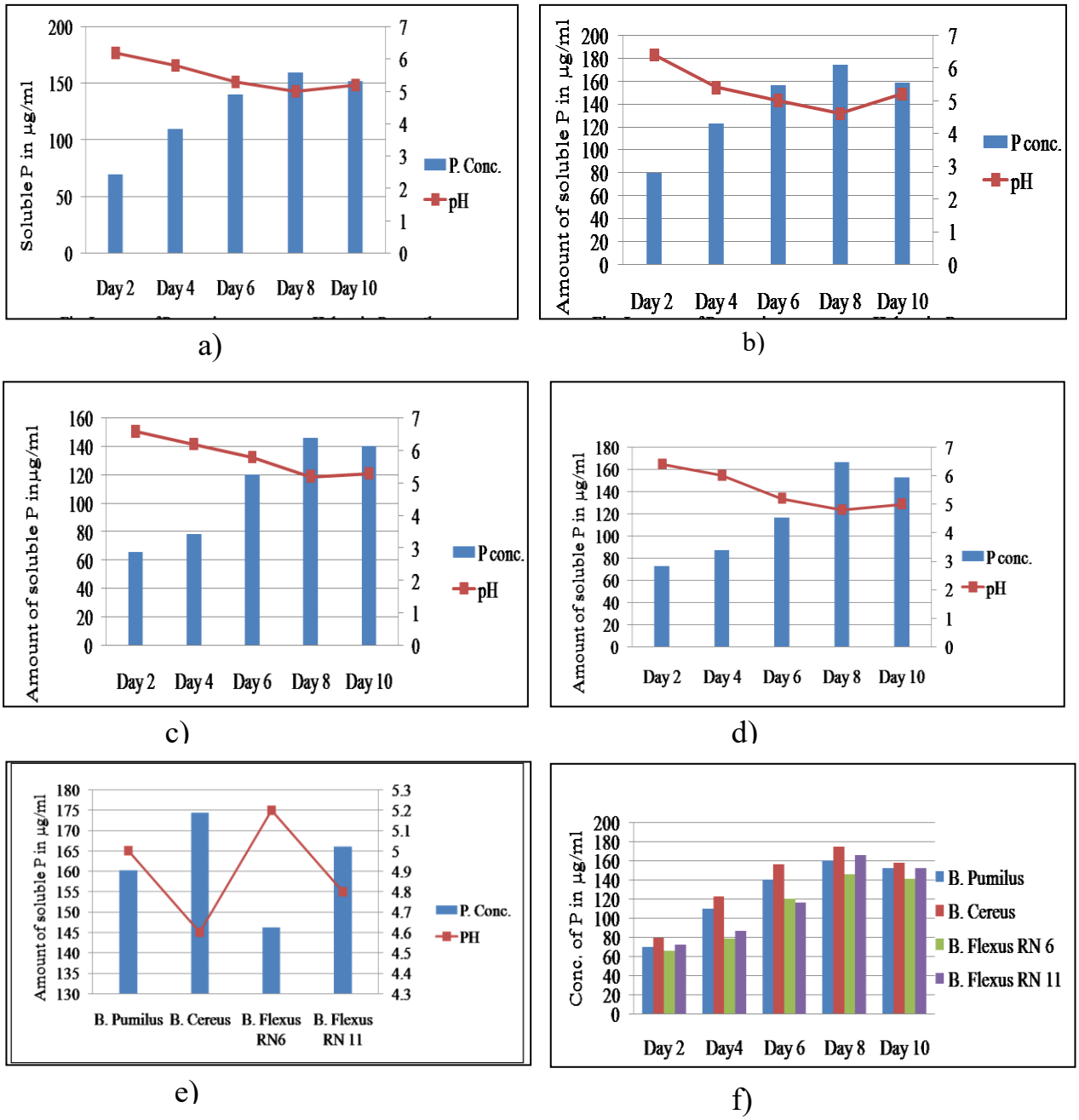

Fig 4: Periodic estimation of P solubilization in response to pH drop by the isolates a) *B. pumilus, b) B. cereus, c) B. flexus RN 6, d) B. flexus RN 11*. e) Maximum P solubilization activity of the isolates in day 8 with drop of pH. f) Comparison of periodic P solubilization activity of the isolates.

❖ ***Zinc solubilization:***

Zinc is a crucial trace element for the growth and development of all kinds of plants in the world. To observe the zinc oxide solubilization ability of the isolates, periodic estimation of soluble zinc in the culture medium was carried out up to ten days. Maximum amount of soluble zinc in the culture medium was observed after eight days of incubation. Further incubation and estimation revealed a gradual decrease of soluble Zn concentration in the culture supernatant. Among the four isolates, maximum zinc solubilization activity was observed in *B. cereus* (45.06 ± 1.22µg/ml) followed by *B. flexus* RN11 (40.2± 1.32 µg/ml) and *B. flexus* RN11 (40.2± 1.32 µg/ml) with a fall of pH 4.2± 0.12, 40.2± 1.32 and 5.0± 0.15 respectively. (table 5)

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TABLE 5: Periodic estimation of *in- vitro* zinc solubilizing activity of the isolates

Incubation period in days	Amount of soluble Zn in µg/ml and pH of the medium							
	Bacillus pumilus		*Bacillus cereus*		*Bacillus flexus* RN6		*Bacillus flexus* RN11	
	Zn conc.	pH	Zn conc.	pH	Zn conc.	pH	Zn conc.	pH
2	8.32±1.02	6.3±0.18	11.6 ±1.20	6.2± 0.20	8.26±1.00	6.5± 0.16	10.2±1.02	6.4±0 .22
4	21.45±2.03	5.7±0.20	28.06 ±2.22	5.4± 0.17	18.42±1.52	6.2± 0.23	14.06±1.62	5.0± 0.24
6	30.07±1.16	5.3±0.22	36.22 ±1.74	4.6± 0.14	32.50±1.8	5.6± 0.18	28.67±3.2	5.2± 0.20
8	33.04±2.12	5.0± 0.12	45.06 ± 1.22	4.2± 0.12	38.22± 1.41	5.0± 0.15	40.2± 1.32	4.8± 0.10
10	28.76±1.32	5.2± 0.24	42.17 ±2.50	5.3± 0.21	34.07±2.6	5.1± 0.25	36.02±2.8	5.0± 0.23

❖ Values are mean ± SD of five replica:

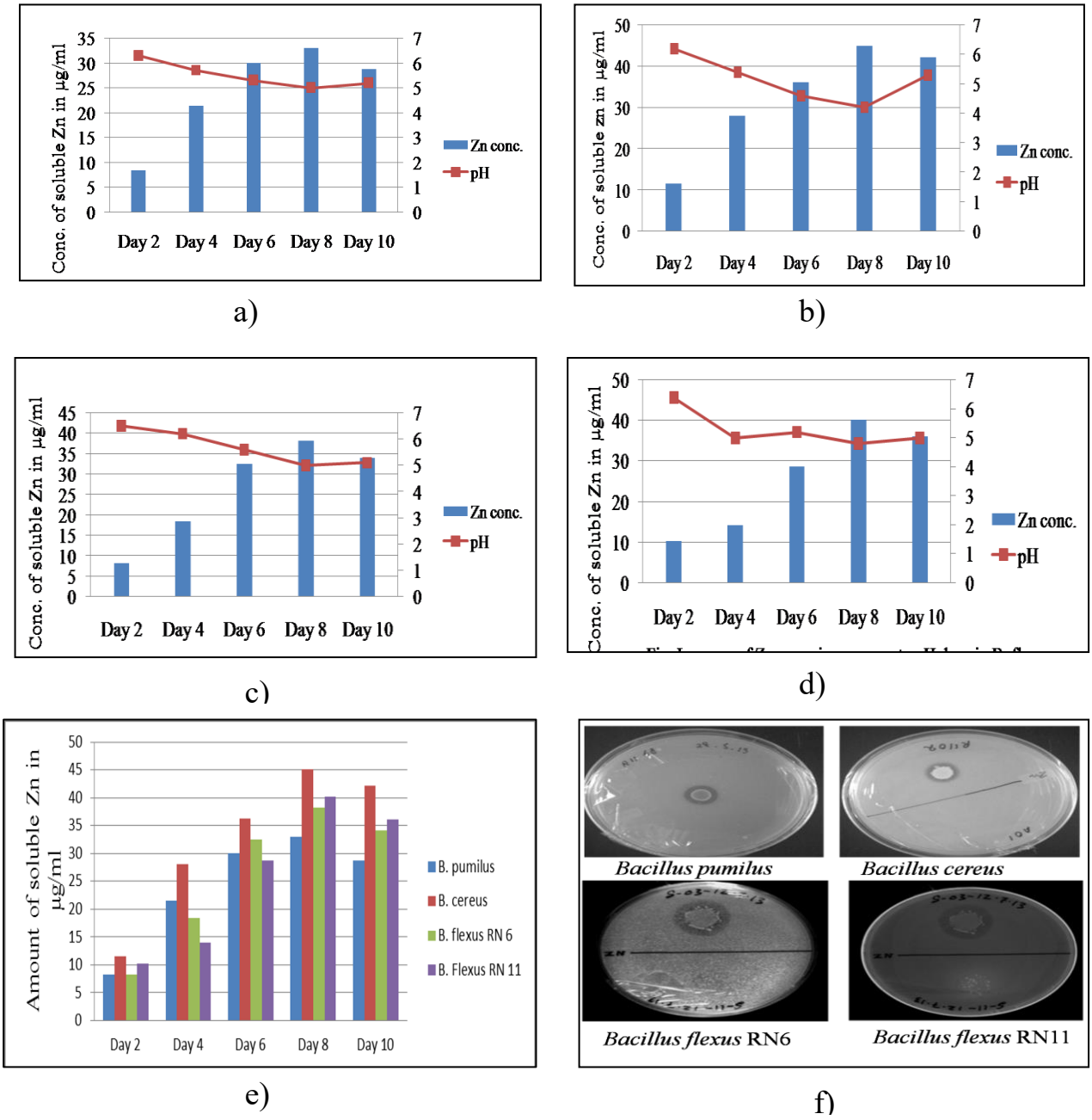

Fig 5: Periodic estimation of Zn solubilization in response to pH drop by the isolates a) *B. pumilus, b) B. cereus, c) B. flexus RN 6, d) B. flexus RN 11.* e). Comparison of periodic P solubilization activity of the isolates f). clear zone formation by the growing isolates in response to Zn solubilization on solid medium.

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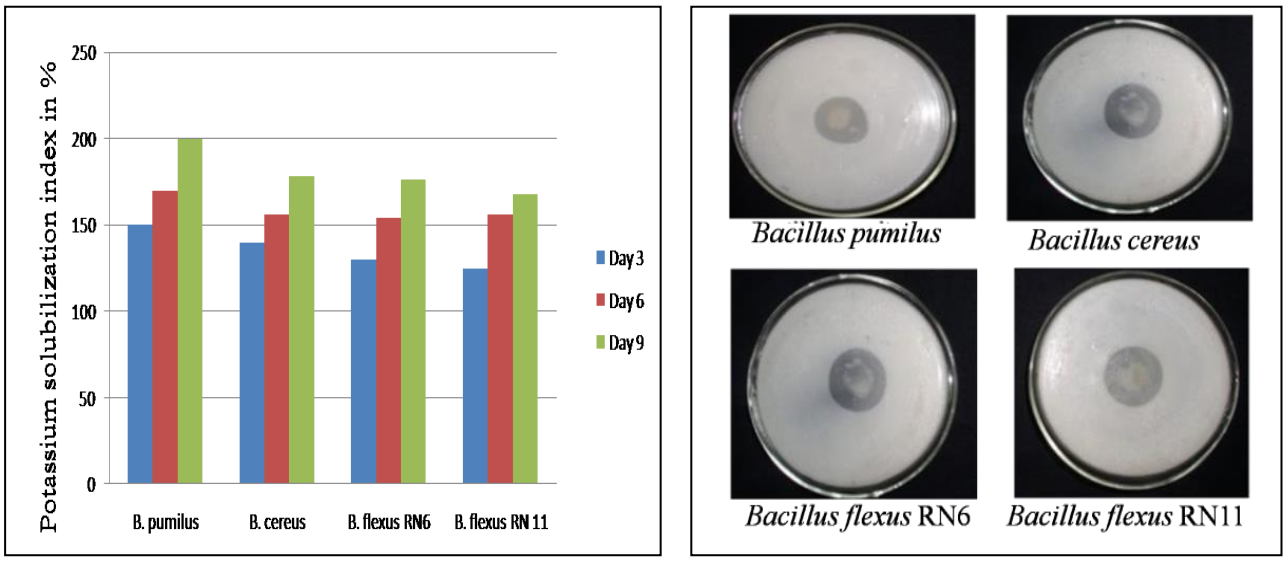
❖ ***Potassium solubilization:***

Potassium is one of the major nutrients for plant growth and development. Without adequate potassium, the plants will have poorly developed roots, grow slowly, produce small seeds and have lower yields. Periodic estimation of potassium solubilization was measured in terms of clear zone formation index in solid medium. In vitro potassium solubilization ranged between 125 ± 5.0% and 200 ± 2.8%. *B. pumilus* was identified as highest K solubilizer among the test isolates with a solubilization index of 200 ± 2.8. Increase of solubilization index was noticed in due course of time. (table 6)

TABLE 6: Potassium solubilization index

Isolates	**Potassium solubilization index in %**		
	Day 3	Day 6	Day 9
Bacillus pumilus	150 ± 3.0	170 ±3.4	200 ± 2.8
Bacillus cereus	140 ± 2.5	156 ± 4.1	178 ± 3.2
***Bacillus flexus* RN6**	130 ± 1.6	154 ± 2.2	176 ±2.6
***Bacillus flexus* RN11**	125 ± 5.0	156 ± 4.2	168 ±3.5

❖ Values are mean ± SD of five replica:



a) b)

Fig 6: a) Comparison of K solubilization index of the isolates. b) Formation of clear zones around the colonies in response to K solubilization.

❖ ***Phylogenetic analyses:***

Sequence similarities were determined using the BLAST program against the database of type strains with validly published prokaryotic names at the EzTaxon 2.1 server. Molecular Evolutionary Genetics Analysis software (MEGA version 4.0) was used for phylogenetic analyses. The sequences of identified phylogenetic neighbors were aligned with the sequences of representative strains, using Clustal W inbuilt with MEGA 4. Neighbor-Joining method was employed to construct the phylogenetic tree with 1000 bootstrap replications to assess nodal support in the tree. Bootstrap values of 80% or greater were used to define well-supported clusters of the nucleotide sequences in the tree.

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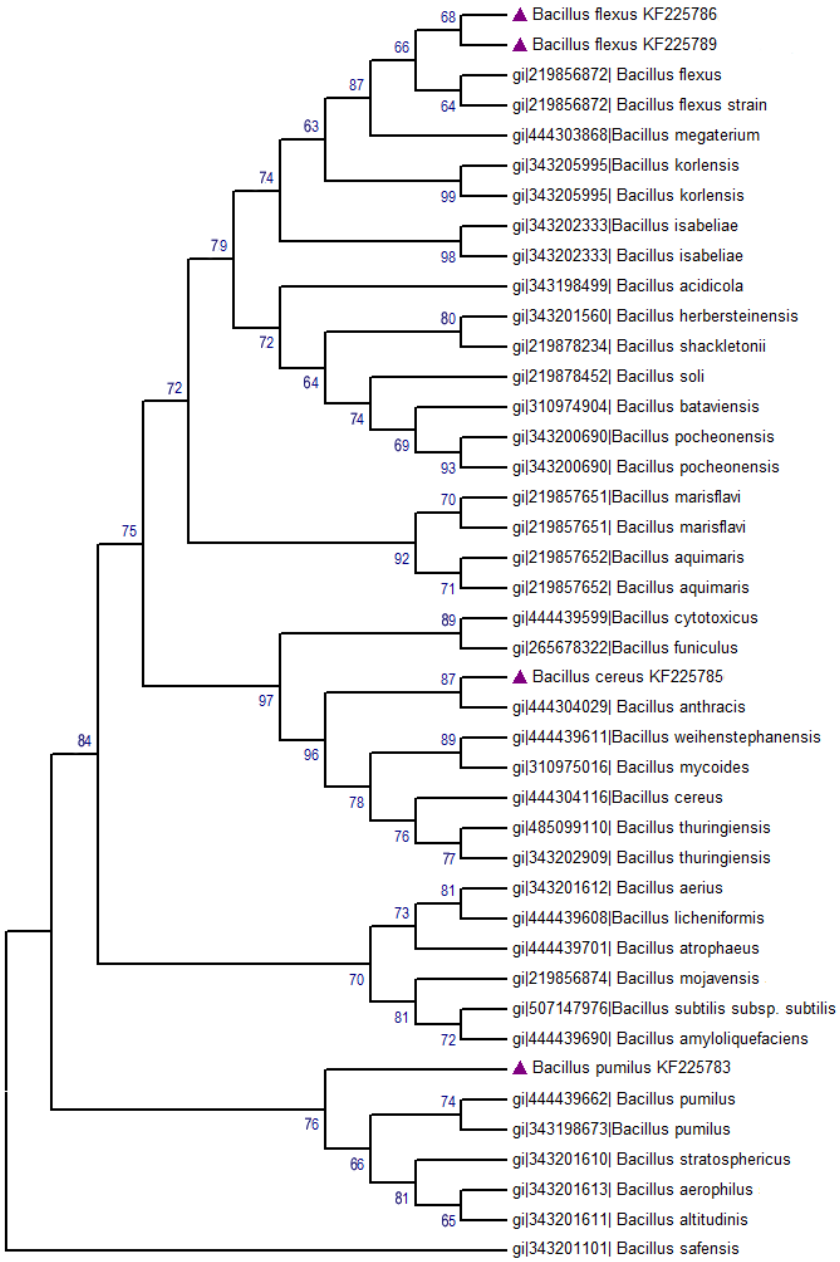

Fig 7: Phylogenetic tree of the isolates to show their evolutionary relationship.

❖ ***Plant growth promotion of the isolates in pot culture experiment:***

In the pot culture experiment, efficiency of the test isolates was measured in terms of production of leaves and branches as well as the increase of height of the plantlets. It was observed that test isolates significantly enhanced the growth and development of the bean plantlets as shown in table no. 7.

TABLE 7: Plant growth promotion of the test isolates in pot culture experiment (after 30 days).

Exptl. set		No. of leaves	Stem height in cm	No. of Branches
Control		11±1.6	40.29±1.3	4±1.1
Treatment I		15±2.4	52.17±2.8	5±1.3
Treatment II		17±1.4	57±2.6	7±2.0
Treatment III		18±2.1	60±2.4	7±2.2

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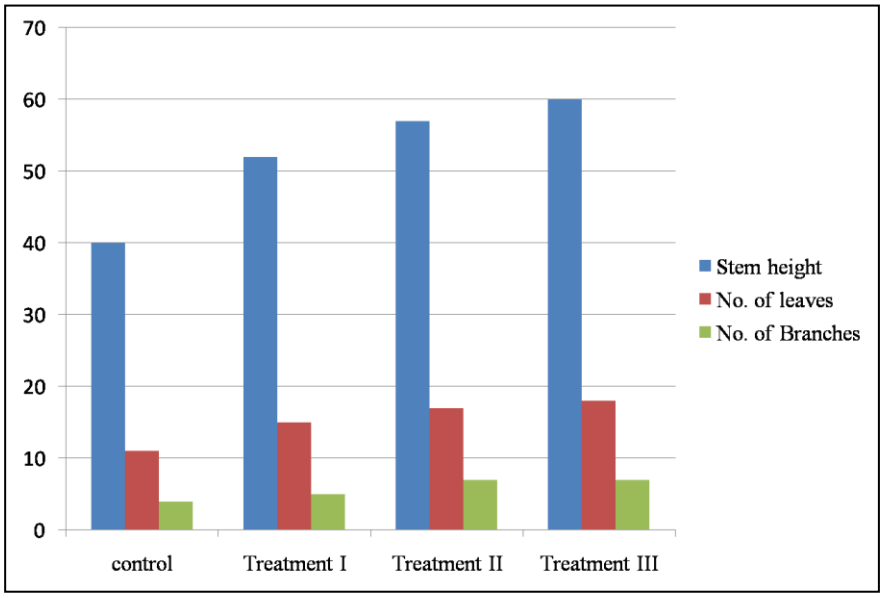

Fig: Graphical representation of plant growth promotion by the test isolates in pot culture experiment (after 30 days).

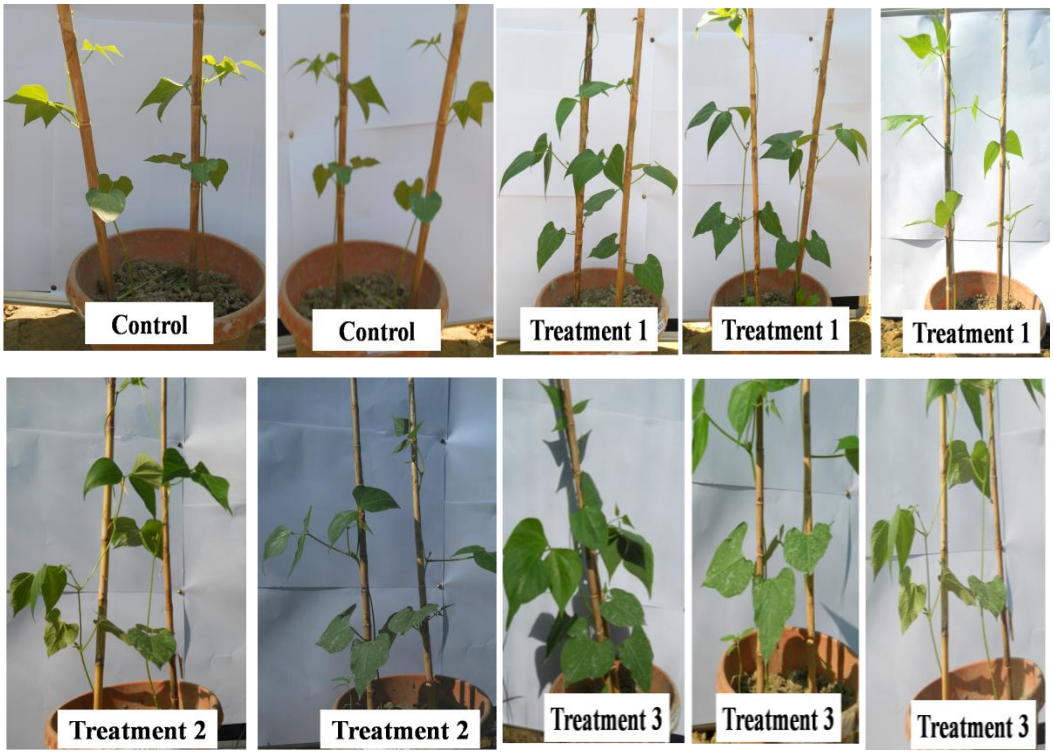

Fig: Photographs showing the pot culture experiments to evaluate plant growth promotion by the bacterial isolates. a) control- normal soil only b) treatment 1-treated with vermicompost c) treatment 2- treated with chemical fertilizers d) treatment 3- treated with vermicompost and bacterial isolates.

IV. DISCUSSION

Today's world is very much concerned to the health hazards by agrochemical residues present in different food and beverages. As tea is one of the most popular and widely consumed beverages, amount of agrochemical residues is a major interest in maintaining tea quality. Similarly, improper use of chemical fertilizers is the only cause of declining soil quality as well as a major fact for water pollution and eutrophication. Excess use of chemical fertilizers imbalances the soil ecology and thereby disrupts the normal and swift natural way of nutrient cycling. To minimize the above mentioned health as well as environmental hazards, organic cultivation is considered to be the best way. Organic cultivation may be

carried out by the use of different kinds of organic manures. However, organic manures alone are not enough to enhance the growth and development of crops and hence yield low. This problem can be overcome by the use biofertilizers. Though, soil is a very good source for isolation of different kinds of biofertilizers, however, present agriculture needs more sources for isolation of microbes having high biofertilizer activities. Consequently, adverse effects of soil microbes to the crop health are not well known. As endophytes are native to the plant itself and have better plant growth promoting activities, they may be a good source for isolation of crop special biofertilizers.

In our present study, all four endophytic *Bacillus* isolates were very much efficient in most of the plant growth promoting activities. All the isolates were found to be good IAA producers, phosphate, zinc and potassium solubilizers, and none of them inhibited the growth of other three isolates. Hence, they were found to be suitable for co inoculation to manipulate soil microflora. Several earlier studies have revealed that *Bacillus* is one of the most abundant genera with PGP activities. There are a number of metabolites secreted by different *Bacilli* which strongly affect the soil environment by increasing nutrient availability to the plants [21, 22]. *B. subtilis* is able to maintain stable contact with higher plants and hence, not only promote their growth but helps in the process of acclimatization too. Colonization of rhizosphere by *Bacillus licheniformis* enhances the growth of tomato and pepper without any adverse effects to the native rhizobacterial community [23]. Jaizme-Vega et al. [24] evaluated the plant growth promoting activities of a consortium of *Bacillus sp.* in micropropagated bananas and concluded that this bacterial consortium can be used as a biofertilizers for the crop. *Bacillus* is also found to have potential to increase the yield, growth and nutrition of organically cultivated raspberry [25]. *Bacillus megatorium* is well known for its positive effects on root parameters like rooting performance, root length and dry matter content etc. in different crop plants [26]. *Bacillus megaterium* and *Bacillus mucilaginosus* are well known for their mineral solubilizing activities and co inoculation of both the species in nutrient limited soil showed that they were able to release a considerable amount of phosphate, zinc and potassium from insoluble soil salts and increased mineral availability, uptake and plant growth of pepper and cucumber [27, 28]. The *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia [29]. From the results of the present study as well as the earlier supports it is well understood that the endophytic *Bacillus spp*. may be a good biofertilizers consortium for different crops.

Pot culture experiment for verification of the isolates for their biofertilizers activity in field condition revealed that the experimental treatment III inoculated with the four endophytic *Bacillus* isolates have the maximum growth in all the aspects taken in to consideration. From the ANOVA analysis it was found that at 5% probability level the growth of bean plants in treatment III i.e. inoculated with the test isolates is significant in comparison to the control set i.e. without any treatment and treatment I i.e. treated with vermicoposts. Hence the four endophytic *Bacillus* i.e. *B. pumilus, B. cereus, B. flexus RN 6, and B. flexus RN 11* may be a good consortium for production of organic crops.

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