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**Original Research Article**

# *In Vitro* Studies of Plant Growth Promoting Activities of Rhizospheric *Bacillus* sp in Chilli Seedlings Variety LCA344

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**A B S T R A C T**

Plant growth promoting rhizobacteria (PGPR) can enhance the growth and productivity by exerting beneficial effects through direct and indirect mechanisms. The effect of PGPR on the growth and yield of chilli under field conditions has to date, not been substantiated. In this study, 4 rhizo bacteria bacteria P1, P2, P3 and P4 isolated from chilli field and their morphological, biochemical, plant growth promoting, and biocontrol characteristics were elucidated. Plant growth and yield attributes were studied by seed bacterization of selected bacteria as individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4). Efficacy of individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4) on seed germination plant growth attribute were analysed under greenhouse conditions. 16s r RNA analysis revealed P1, P2 P3 and P4 as *Bacillus sp AVP5, B.subtilis AVP6, B.amyloliquifacience AVP8 and B.B.endophyticus AVP9.* Remarkable increase in growth characteristics such as seed germination, root length, shoot height, no of leaves and fresh weight was recorded in plants with combined inoculation under field conditions. The results clearly demonstrate the rhizo competence and plant growth enhancing efficacy of these strains. It can be surmised that the isolated strains have strong potential to be successful biofertilizers and bio-enhacers.

**Keywords**

*Bacillus* sp. Chilli seedlings, plant growth

## Introduction

Beneficial free-living soil bacteria are usually referred as plant growth promoting Rhizobacteria or PGPR (Kloepper *et al.*, 1989)[1]. PGPR is a group that includes. *Azotobacter, Azospirillum, Pseudomonas, Acetobacter, Herbaspirllum, Burkholderia and Bacillus* (Glick, 1995). Some Rhizobacteria can promote plant growth indirectly by modifying nodule formation and biological nitrogen fixation (Cattelan *et al.*, 1998). Specific bacteria introduction into rhizosphere in order to promote plant growth has been the subject of an intensive research during the last decades, and this practice has become accepted in agriculture because pollution by nutrients causes many problems on the environment (Bashan, 1998).One of the main sources of these problems are fertilizers used in agriculture. Industrial production and the usage of fertilizers have led to a sharp increase in food production that has been accompanied by the population growth in almost all countries around the world. Usage of Rhizobacteria as biofertilizers is one of the most promising biotechnologies for growing the primary

production with less quantity of fertilizers (Bashan, 1998). Moreover, usage of bacteria isolated from crop plants rhizosphere for productivity increase may be an eco-friendly alternative to organic nutrients (Compant *et al.,* 2005).

Surrounding plant roots there is an extremely important and active area for root activity and metabolism which is known as rhizosphere (Briskot *et al.,* 1986). Bacteria in habiting the rhizophere and beneficial to plants are termed plant growth promoting Rhizobacteria-PGPR (Cappuccino and Sherman, 1992. A rhizobacteria is qualified as PGPR when it is able to produce a positive effect on the plant upon inoculation (Demutskaya and Kalinichenko, 2010). These bacteria significantly affect plant growth by: providing the host plant with fixed atmospheric nitrogen (Gaur, 1990), solubilization of soil phosphorus compounds (Glick, 1995), producing biologically active substances such as auxins and other plant hormones (Gupta *et al.,* 2000), suppressing pathogens by producing antibiotics and siderophores. However, the mechanisms used by these bacteria to produce the effects mentioned are not enough understood (He, *et al.,* 1997).

Many environmental stresses affects crop productivity and impair electron transport system leading to the formation of activated oxygen, such as H2O2, O2, and OH, which may accumulate and damage the photosyenthetic apparatus (Holguin and Glick, 2001). In plants, the highly energetic reactions of photosynthesis and an abundant oxygen supply make the choloroplast a particularly rich source of reactive oxygen intermediates (Illmer and Schinner 1992).To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide dismutase, peroxidase and catalase as well as non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol (Joseph *et al.*,1998).

Chilli is a crop with major economic value. The present study was aimed to evaluate the plant growth promoting effect of Bacillus strains [p1, p2, p3 andp4] isolated from chilli rhizosphere as both independently and in combination on growth; physiological activites in low input systems.

## Materials and Methods

### Isolation from the chilli rhizospheric soil sample

One gram of rhizospheric soil sample was taken and added to 9ml sterile water and used as stock solution. 0.1ml of solution was taken from stock solution and diluted with 9.9 ml sterile water. The dilution was marked as 10-2.Further it was serially diluted to 10-3, 10-4 and 10-5 dilutions.0.1ml of each the dilution was taken and added to sterilized petriplates containing Nutrient Agar Medium (NA).The plates were incubated at 30±20ºC for 48hours.

### Green House studies of Rhizobacteria

### Preparation of Bacterial cell suspension

Bacterial cell suspension was prepared by harvesting cells from optimized medium grown at 28ºC±1ºC for 48hours followed by centrifugation at 6000rpm for 15minutes.The inoculum was resuspended in sterile distilled water and concentration was adjusted to OD 1.0(Thompson, 1996).

### Seed treatment and nursery experiment

Seeds of chilli were treated with the 48hrs old culture (approximately 108 CFU/ml) of the selected isolate for 30 minutes and

shade-dried at 28ºC for 1hr.Treated seeds were sown in pots containing coco peat and grown under greenhouse. Root length, Shoot height, Number of leaves and fresh weight of the seedlings were measured. Observations were recorded at two weeks interval by removing 10 seedlings from each replication (Amrutha V Audipudi *et al.*, 2016).The experiment was done in triplicates.

## Bacterial Strain and Inoculant preparation

Six bacterial strains (P1, P2, P3, P4, P1+P3, P1+P2+P3+P4) were selected to assess their potential to enhance the physiological activities and yield of in a low- input organic system. The strains were. The six Rhizobacteria were cultivated from slant material in Nutrient Agar Medium and incubated in 2000ml flasks on an orbital shaker at 210rpm at 27ºC.After five days the strains were sub cultured under the same conditions as described above. The cell densities were adjusted to approximately 1.5x109 CFU/ml. The strains were further used to inoculate runner bean seeds just prior to sowing.

## Field Experiments

Experiments were carried out with runner bean plants using a local population (C2) and sited at the experimental farm of the local climate is characterized by an average annual temperature of 9.6ºC(49.3ºF)and a total average rainfall 521mm-year -1. In terms of the morphological and systematic soil conditions, the soil is classified as chernozem(Cz), with an average supply of nutritive elements, 3.8% organic matter and a PH of 5.8.Bacterial application of P1, P2, P3, P4 strains, as well as their combinations( P1+P3, P1+P2+P3+P4), was performed using the seed coating method.Runner bean seeds were surface sterilized in sodium hypo chlorite (2%solution containing 4ml/l Tween 20)and rinsed five times with distilled water. Seeds were coated for a total time of 1minute with the inoculum prior to sowing. The control seeds were coated with sterilized tap water.

## Seed nutritive value estimation

Total soluble proteins were extracted using cold phosphate buffer saline(PBS)PH 7.4 in 1g seed /10Ml buffer ratio. The homogenate was further centrifuged at 14000rpm for 30 minutes. The protein amount in the supernatant was assayed by the dye –binding micro method of Bradford, using the Roti-Quant reagent from the Roth(Karlsruhe, Germany).Total soluble protein content was expressed as mg bovine serum albumin (BSA)per g harvested seeds.

## Statistical analysis

The experimental data concerning physiological measurements were statistically processed using ANOVA: two-factor with replication [26]followed by a post-hoc analysis using Duncan's multiple range test. All results are expressed as means ±S.E.M.F values for which $p<0.05$ were considered significant. Experimental data regarding the biochemical assays and grain yield were statistically processed using Student (t) test.

## Results and Discussion

The ability of bacteria to solubilise phosphate is important for agriculture as it may enhance the availability of phosphorus for plants (Beneduzi *et al.*, 2008). Whereas F.F Araujo *et al.*, (2005) reported bacillus isolates 2B, 4B and 6B produced more than 10µg $ml^{-1}$.In particular, the isolate 5A was the most efficient IAA producer, with

21.310µg $ml^{-1}$.. IAA production by PGPR can vary among different species and isolates, and it is also influenced by culture condition, growth stage and substrate availability. In addition to IAA production these three isolates are positive to the production of Ammonia, Siderophore and HCN.. The ability bacteria to produce ammonia are also a significant trait for agriculture as it enhances the availability of nitrogen.

These three isolates are positive to siderophore and HCN production may be linked to inhibition of soil borne plant pathogens (Gupta *et al.,* 2000).This activity is important for biological control of plant pathogens in rhizosphere and indirectly promotes plant growth. individual(P1, P2, P3, P4) and mixed(P1+P3, P1+P2+P3+P4) rhizobacterial isolates are considered as multiple potential PGPR bacteria and characterized further for invitro analysis of plant growth promotion, physiological characterization under greenhouse conditions.

Results of present study encompasses selection of inoculum on the basis of turbidity and compatibility of PGPR strains, percentage(%) seed germination, in vitro growth analysis in them of root length, shoot length, no of leaves, fresh weight and physiological parameters like chlorophyll& proteins late were analyzed in treated seedling at an interval of 2weeks.

## Inoculum preparation

Combination of P1+P3 and P1+P2+P3+P4 were used as coinoculum in the study individual inoculum and coinoculum was shown in Plate1. Four morphologically distinct Rhizobacterial strains P1, P2, P3, P4 were selected as individual inoculum.

## Turbidity and Compatibility

Turbidity of 7 different PGPR strains were analyzed at O.D570 nm and O.D 600 nm in order to standardize the cell count of inoculum suitable for seed bacterization. Turbidity was measured P1, P2, P3andP4 isolates and also in combination isolates of P1+P3 and P1+P2+P3+P4. At an interval of 12 hrs, 24hrs, 42hrs and 72hrs. t 570nm, isolates P1, P2, P3, P4, P1+P3, P1+P2+P3+P4 showed maximum turbidity at 48 hrs of incubation.[, table:1]. At 600nm, all six isolates attained maximum turbidity [O.D 1.0] at 24hrs of incubation [ table:2]

## In vitro greenhouse studies

PGPR treated seeds were sowed in 96 well nursery beds with 5 seeds in each well. A total of 480 seedlings were analyzed for each treatment and experiments were carried out in triplicates. Untreated seedlings were taken as control.

## Effect of bioformulations on% seed germination and seed vigor

% seed germination was analyzed 7days after inoculation [DAI] and measured the seed germination every day from 8th day onwards. The values are statistically analyzed in one way ANOVA 12.5% of seed germination was reported in control on 22 DAI, whereas the same ( 12.5%) % of seed germination was recorded after 12DAI in P1, 17DAI in P2, 14 DAI in P3, 17 DAI in P4, 114 DAI in[ P1+p 3 ]and[ P1+P2+P3+P4] treated seedling.[Table:3] Results revealed that % seed germination triggered early in treated seedling beds than control. Among 7 PGPR treated beds 12.5% germinates was attained very early in P1[12DAI] followed by p5, P1+P3 and P1+P2+P3+P4 [14DAI] indication that bio stimulation induced by PGPR resulted in

early % seed germination.. It is also observed that % seed germination was initialy very slow and later triggered fast in mixed inoculum indication the compatability among P1+P3 and P1+P2+P3+P4 as well. It also revealed mixed inoculum showed synergetic impact on % seed germination.

## Seed vigor

Seed vigor was analyzed in control and PGPR treated seedling in 8th week after inoculation by using the formula

Seed vigor = Seedling length X % germination the results were shown in Table: 4

## Growth analysis of chilli seedlings

Chilli seedlings treated with P1, P2, P3, P4, P1+P3, P1+P2+P3+P4 showed variations in root length, shoot length, no: of leaves, fresh weight, protein content and chlorophyll content from 2nd week to 8th week. The results were analyzed using one way ANOVA. [Plate2]

## Root length

In the 2nd week % increase in root length of seedlings was observes in response to P1(22%), P2 (21.3%), P3(-30%), P4 (19.3%), P1+P3 (70.6%), P1+ P2+ P3+P4 (95.3%). When compared to control [Table 5].

In the 4th week % increase in root length of seedlings was observed in response to P1(21.5%), P2(19.9%), P3(-6.9%), P4(3.0%), P1+P3(80%), P1+P2+P3+P4(129.9%) when compared to control. In the 6th week % increase in root length of seedlings was observed in response to P1(-24.4%), P2(-23.6%), P3(-31.5%), P4(18.9%), P1+P3(-30.7%), P1+P2+P3+P4(-20.2%) compared to control

In the 8th week % increase in root length was observed in P1 treated seedlings and decreased in response to P1, P1+P3 (15.7, -30.4%) and P1+P2+P3+P4. When compared to control. No significant variation was observed in % increase of root length in P3, P4 when compared to control. Results revealed a gradual increase in root length was observed in seedlings treated with P1, P2, P3, P4 isolates from 2nd week to 8th week Whereas the no significant increase in root length was observed in seedlings treated with combinational treatment (P1+3 andP1+4)

## Shoot height

In the 2nd week % increase in shoot height was observed very high in P3(149%) followed by P1+P2+P3+P4(112.7%), P2(96.07%), P1(70.5%), P4(69.6%), P1+P3(66.6%). In the 4thweek % increase in shoot height was observed very high in P3(71.4%) followed by P1+P2+P3+P4(57.4%), P2(52.1%), P1(44.4%), P4(39.1%), P1+P3(28.0%).
In the 6thweek % increase in shoot height was observed very high in control compared to treated seedlings. In the 8thweek % increase in shoot height was observed very high in P3(18.1%) followed byP1(16.5%), P1+P3(8.48%), P2(6.65%), P4(-2.5%) and P1+P2+P3+P4(-6.65%) as shown in table 6

## No of leaves

In the 2nd week % increase in no of leaves was observed in response to P1(50%), P2(50%), P3(100%), P4(50%), P1+P3(100%), P1+P2+P3+P4(100%). In the 4th week % increase in no of leaves was observed in response to P1(0%), P2(33.3%), P3(33.3%), P4(33.3%), P1+P3(33.3%), P1+P2+P3+P4(33.3%). In the 6th week % increase in no of leaves was observed in response to P1(0%), P2(0%), P3(-20%), P4(0%), P1+P3(20%), P1+P2+P3+P4(0%).

In the 8th week % increase in no of leaves was observed in response to P1(0%), P2(0%), P3(0%), P4(0%), P1+P3(20%), P1+P2+P3+P4(20%) as shown in table 7.

## Fresh weight

In the 2nd week % increase in fresh weight was observed in response to P1(28.2%), P2(53.8%), P3(66.6%), P4(53.8%), P1+P3(66.6%), P1+P2+P3+P4(15.3%).In the 4th week % increase in fresh weight was observed in response to P1(30.6%), P2(55.1%) 123, P3(73.4%), P4(71.4%), P1+P3(55.1%), P1+P2+P3+P4(6.12%). In the 6th week % increase in fresh weight was observed in response to P1(440%), P2(600%), P3(830%), P4(450%), P1+P3(710%), P1+P2+P3+P4(1060%). In the 8th week % increase in fresh weight was observed in response to P1(3.1%), P2(17.7%), P3(12.7%), P4(-20.5%), P1+P3 (5.5%), P1+P2+P3+P4(-16.6%) as shown in table 8.

## TPC

In the 2nd week % increase in Protein content was observed in response to P1 (66.6%) P2 (33.3%), P3 (33.3%), P4 (66.6%), P1+P3(100%), P1+ P2+P3+ P4 (222.2%).

In the 4thweek % increase in Protein content was observed in response to P1(31.5%) P2(23.6%), P3(31.5%), P4(21.0%), P1+P3(21.0%) P1+P2+P3+P4 (36.8%).In the 6th week % increase in Protein content was observed in response to P1(10%), P2(4%), P3(10%), P4(4%), P1+P3(10%), P1+P2+P3+P4(20%). In the 8th week % increase in Protein content was observed in response to P1(9.5%), P2(4.7%), P3(17.4%), P4(4.7%), P1+P3(9.5%), P1+P2+ P3+P4(22.2%). Total protein content in chilli seedling treated with PGPR was observed at an interval of two weeks [2nd, 3rd, 4th, and 8th]

In the 2nd week significantly highly protein content was observed in coinoculation treatment compared to p4 inoculations and control. Recorded differences were not significant in p1, p2, p3, p4, treated chilli seedling.[Table:9]As the vegetative growth progressed, total protein content of seedlings was significantly enhanced in 3rd, 4th, and 8th in all treatment. However the difference of TPC in individual p1, p2, p3, p4, inoculation was milder when compared to coinoculations.

## Chlorophyll content

The % increase in chlorophyll-a was observed in response to P1 (0%), P2(100%), P3(100%), P4(100%), P1+P3(100%), P1+P2+P3+P4(199.9%). The % increase in chlorophyll-b was observed in response to P1(0%), P2(100%), P3(100%), P4(199.9%), P1+P3(300%), P1+P2+P3+P4(300%).The % increase in chlorophyll-a/b was observed in response to P1(1.13%), P2(-1.1%), P3(-7.9%), P4(26.1%), P1+P3(-32.9%), P1+P2+P3+P4(-27.2%). The % increase in total chlorophyll was observed in response to P1(0%), P2(33.3%), P3(33.3%), P4(66.6%), P1+P3(100%), P1+P2+P3+P4(166.6%).

The values of chlorophyll content [chl-a, chl-b and total chlorophylls] was higher in treated seedlings when compared to control. Chlorophyll content was significantly increased by the utilization of p1+p3 and p1+p2+p3+p4.Followed by individual treatements of p1, p2, p3 and p4.However the effect of rhizobacterial inoculation on photosynthesis was not consistent across treatment and growth stages.Inoculation with p1and p2 resulted higher chl a/b ratio compared to p1+p3 and p1+p2+ p3+p4.

Coinoculation (p1+p3) and (p1+p2+p3+p4) significantly increase photosynthetic activity using control and individual inoculations.

The response of mixed(P1+P3, P1+P2+P3+P4) rhizobacterial isolates are more significant on growth of chilli seedlings when compared to individual(P1, P2, P3, P4) isolates and control.Significant increase in the root length of seedlings were observed from second week onwards. Whereas a gradual enhancement in shoot length was recorded in response to individual(P1, P2, P3, P4) and mixed(P1+P3, P1+P2+P3+P4) rhizobacterial isolates. No significant increase in shoot dry mass and plant height but increases in root growth by application of Bacillus isolates 2B and 4B as PGPR are reported as an exception (Holguin; Glick, 2001; Silva *et al.,* 2007)..Results suggested that PGP traits of bacterial isolates were highly specific and application of mixed inoculation of bacterial isolates with varied specificity can influence growth and ISR more efficiently than application of individual PGP traits.

Plant growth promoting bacteria(PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Glick 1995).Given the negative environmental impact of chemical fertilizers and their increasing costs, the use of PGPB as natural fertilizers is advantageous for the development of sustainable agriculture.

The use of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture has been increased in the Last decades in several regions of the world. Various bacteria genus are included in PGPR group, such as *Pseudomonas, Azospirillum, Bacillus, Enterobacter, Xanthomonas* and *Serratia* (Khalid *et al.,* 2004).The mechanisms of action of PGPR may initially linked to inhibition of soil plant pathogens and there by stimulate plant growth indirectly (Gupta *et al.,* 2000).It is often difficult to recognize the mechanisms and relate directly to promotion of plant growth, since more than one mechanism produced by bacteria (Araujo *et al.,* 2005).

During the last two decades knowledge on phosphate solubilising microorganisms increased significantly (Richardson 2001; Rodriguez and Fraga 1999).Several strains of bacterial and fungal species have been described and investigated in detail for their phosphate solubilising capabilities (Glick 1995; He *et al.,* 1997). Typically such microorganisms have been isolated using cultural procedures with species of pseudomonas and bacillus bacteria (Illmer and Schinner 1992) and *Aspergillus* and *Penicillium* fungi being predominant(Wakelin *et al.,* 2004).These microorganisms are ubiquitous but vary in density and mineral phosphate solubilising bacteria constitute 1-50% of the total respective population. They are generally isolated from rhizosphere and non-rhizosphere soils, rhizoplane, phyllosphere, and rock P deposit area soil and even from stressed soils using serial plate dilution method or by enrichment culture technique (Zaidi *et al.,* 2009).It is very important to find novel strains of bacteria and to know their potential for producing substances that improve plant growth. However there are few studies about evaluation of phosphate solubilising bacteria strains and their efficiency in plant growth promotion in sub-tropical soils. The aims of this paper were to (1) find novel phosphate solubilising bacteria strains isolated from chilli fields (2) to evaluate their plant growth promoting activities and (3) to assess their efficacy on plant growth promotion and ISR.

Bacillus subtilis is a ubiquitous, saprophytic soil bacterium which is thought to contribute

to nutrient cycling due to its ability to produce a wide variety of enzymes. it has been used for industrial production of proteases, amylases, antibiotics and chemicals. B.subtilis strain QST713 has natural fungicidal activity, and is employed as a bio control agent. Bacillus subtilis has shown antagonistic activity towards Fusarium solani in vitro(Richardson A E 2001). Rodriguez and Fraga R (1999)) isolated a B.subtilis strain whose metabolites are able to induce systemic resistance against powdery mildew on Barley.Similarly, Bacillus spp.Have been tested on awide variety of plant species for their ability to control diseases (Silva, 2007)]. *Bacillus* spp are able to form endospores that allow them to survive for extended periods of time under adverse environmental conditions. Some members of the group are diazotrophs, and B.subtilis was isolated from the rhizosphere of a range of plant species at a concentration as high as 107 per gram of rhizosphere soil(Sudhir *et al.,* 2015). Bacillus subtilis also synthesizes an antifungal antibiotic inhibiting Fusarium oxysporum f.sp.ciceris, the agent of fusarial wilt in chickpea (Zaid *et al.,* 2009).

In present investigation After 56 days of P1+P3 and P1+P2+P3+P4 treatments resulted an a significant increase in plant height compared to control plants by 9.5% and 22.2% respectively. These significant increases were also seen in terms of root and shoot dry weight with treatment.

Application of individual inoculums P1, P2, P3&P4 and co inoculums of P1+P3 and P1+P2+P3+P4 resulted in significant increases in chilli plant height, root length, number of leaves and biomass. Effects of Bacillus rhoizobacteria on growth parameters of other tropical crops have been reported (Kloepper*et al.,* 2008). Biological activity of Phosphate dissolving bacteria and their effects on same genotypes of Barley production(Compant, *et al.,* 2005).

There is a limited information on the response of chilli plant to Bacillus rhizobacteria under greenhouse conditions reported increased chlorophyll content and protein content associated with root length, shoot length and biomass Bacillus species inhabiting to rhizosphere of crop plants stimulated vegetative growth in the early stages and promoted physiological activities and reproductive growth in the early stages of crop development and suggested that increased biomass was due to increased physiological activity seedlings of chilli. We observed that increased root length, shoot height, number of leaves and biomass of co inoculums treatment was significantly very high compared to individual inoculums treatment, which suggests that there would have been compatability among the isolates present in co inoculums in the partitionary of carbon in form of plant growth.Similar results were found in using Bacillus strains in chilli found 68% increase in biomass of treated chilli seedlings.

Enhanced plant growth following application of P1, P2, P3, P4, P1+P3 and P1+P2+P3+P4 can be due to biostimulation. We also found a significant increase in plant soluble protein and chlorophyll content in the seedlings treated with Bacillus rhizobacteria. Treatment with co inoculum[P1+P3] and P1+P2+P3+P4 showed 100% and 166.6%enhancement in chlorophyll content when compared to individual inoculums. of P1 inoculum in chlorophyll contents who very low when compared to other P2, P3 and P4.Similarly increase in total protein was al;so recorded in P1+P2+P3+P4 is 22.2% higher than control.

**Table.1** Turbidity optimization of individual (P1, P2, P3, P4) and mixed (P1+P3, P1+P2+P3+P4) rhizobacterial isolates in NAM broth at 570nm

| Isolates | Turbidity or OD values | | | |
|---|---|---|---|---|
| | 12 Hrs | 24Hrs | 36Hrs | 48Hrs |
| Control | 0 | 0 | 0 | 0 |
| P1 | 0.04 | 0.07 | 0.07 | 0.06 |
| P2 | 0.04 | 0.06 | 0.06 | 0.05 |
| P3 | 0.04 | 0.06 | 0.06 | 0.05 |
| P4 | 0.05 | 0.07 | 0.07 | 0.06 |
| P1+P3 | 0.05 | 0.08 | 0.08 | 0.06 |
| P1+P2+P3+P4 | 0.07 | 0.11 | 0.11 | 0.09 |

**Table.2** Turbidity optimization of individual (P1, P2, P3, P4) and mixed (P1+P3, P1+P2+P3+P4) rhizobacterial isolates in NAM broth at 600nm

| Isolates | Turbidity or OD values | | | |
|---|---|---|---|---|
| | 12 Hrs | 24Hrs | 36Hrs | 48Hrs |
| Control | 0 | 0 | 0 | 0 |
| P1 | 0.05 | 0.07 | 0.09 | 0.07 |
| P2 | 0.08 | 0.09 | 0.06 | 0.05 |
| P3 | 0.04 | 0.06 | 0.08 | 0.06 |
| P4 | 0.05 | 0.08 | 0.09 | 0.08 |
| P1+P3 | 0.03 | 0.05 | 0.1 | 0.09 |
| P1+P2+P3+P4 | 0.07 | 0.11 | 0.11 | 0.09 |

**Table.3** % of Seed Germination of Chilli seeds treated with individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4)

| Age of seedling (days) | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | control | P1 | P2 | P3 | P4 | P1+P3 | P1+P2+P3+P4 |
| 8 | 18 | 24(33.3) | 20(11.1) | 16(-11.1) | 17(-5.5) | 20(11.1) | 18(0) |
| 9 | 25 | 33(32) | 26(4) | 22(-12) | 25(0) | 28(12) | 29(16) |
| 10 | 33 | 40(21.2) | 32(-3) | 27(-18.1) | 30(-9) | 37(12.1) | 33(0) |
| 11 | 33 | 47(42.4) | 52(57.5) | 38(15.1) | 48(45.4) | 46(39.3) | 50(51.5) |
| 12 | 38 | 55(44.7) | 53(39.4) | 49(28.9) | 49(28.9) | 49(28.9) | 53(39.4) |
| 13 | 42 | 65(54.7) | 55(30.9) | 53(26.1) | 52(23.8) | 56(33.3) | 57(35.7) |
| 14 | 46 | 66(43.4) | 56(21.7) | 62(34.7) | 52(13) | 58(26) | 59(28.2) |
| 15 | 49 | 66(34.6) | 56(14.2) | 62(26.5) | 52(6.1) | 58(18.3) | 59(20.4) |
| 16 | 52 | 65(25) | 57(9.6) | 63(21.1) | 57(9.6) | 59(13.4) | 61(17.3) |
| 17 | 55 | 65(18.1) | 59(7.2) | 63(14.5) | 60(9) | 60(9) | 63(14.5) |
| 18 | 56 | 67(19.6) | 59(5.3) | 65(16) | 61(8.92) | 63(12.5) | 65(16) |
| 19 | 56 | 67(19.6) | 65(16) | 65(16) | 65(16) | 65(16) | 66(17.8) |
| 20 | 56 | 69(23.2) | 67(19.6) | 67(19.6) | 65(16) | 68(21.4) | 69(23.2) |
| 21 | 56 | 69(23.2) | 67(196) | 69(23.2) | 66(17.8) | 68(21.4) | 72(28.5) |
| 22 | 58 | 73(25.8) | 71(22.4) | 71(22.4) | 70(20.6) | 73(25.8) | 74(27.5) |
| 23 | 59 | 75(27.1) | 75(27.1) | 74(25.4) | 72(22) | 74(25.4) | 76(28.8) |
| 24 | 63 | 76(20.6) | 76(20.6) | 75(19) | 73(15.8) | 76(20.6) | 78(23.8) |
| 25 | 65 | 79(21.5) | 78(20) | 77(18.4) | 76(16.9) | 79(21.5) | 78(20) |
| 26 | 68 | 81(19.1) | 79(16.1) | 79(16.1) | 78(14.7) | 82(20.5) | 80(17.6) |

*Values in parameters are % increase or decrease of treated seedlings compared to control Values are mean of three replicates significance of variance is (P: 0.00, F: 176.80)

**Table.4** Analysis of Seed vigor in Chilli seeds treated with individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4) at an interval of 2 weeks

| Seed Vigor | | | | |
|---|---|---|---|---|
| Treatment | 2nd week | 4th week | 6th week | 8th week |
| Control | 164.7 | 333.2 | 671 | 701.8 |
| P1 | 280.23 | 480.83 | 577.27 | 816.34 |
| P2 | 321.82 | 506.4 | 593.3 | 749.1 |
| P3 | 407.45 | 569.86 | 557.8 | 828.16 |
| P4 | 278.59 | 462.86 | 414.12 | 684.8 |
| P1+P3 | 274.56 | 427.6 | 642.63 | 759.72 |
| P1+P2+P3+P4 | 350.13 | 526.2 | 644.63 | 655 |

**Table.5** Analysis of Root length in Chilli seedling var LCA 344 treated with individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4) at an interval of 2 weeks

| Root length | | | | |
|---|---|---|---|---|
| Treatment | 2 week | 4 week | 6 week | 8 week |
| Control | 1.5 | 2 | 3.8 | 4.2 |
| P1 | 1.83(22) | 2.43(21.5) | 2.87(-24.4) | 3.54(-15.7) |
| P2 | 1.82(21.3) | 2.4(19.9) | 2.9(-23.6) | 5.1(21.4) |
| P3 | 1.05(-30) | 1.86(-6.9) | 2.6(-31.5) | 4.16(-0.95) |
| P4 | 1.79(19.3) | 2.06(3.0) | 4.52(18.9) | 4.18(-0.47) |
| P1+P3 | 2.56(70.6) | 3.6(80) | 2.63(-30.7) | 2.92(-30.4) |
| P1+P2+P3+P4 | 2.93(95.3) | 4.6(129.9) | 3.03(-20.2) | 3.8(-9.5) |

*Values in parameters are % increase or decrease of treated seedlings compared to control. Values are mean of three replicates significance of variance is (P: 0.004, F: 3.09)

**Table.6** Analysis of Shoot height in Chilliseedling var LCA 344 treated with individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4) at an interval of 2 weeks

| Shoot height | | | | |
|---|---|---|---|---|
| Treatment | 2 week | 4 week | 6 week | 8 week |
| Control | 2.04 | 4.14 | 8.34 | 8.72 |
| P1 | 3.48(70.5) | 5.98(44.4) | 7.18(-13.9) | 10.16(16.5) |
| P2 | 4(96.07) | 6.3(52.1) | 7.38(-11.5) | 9.3(6.65) |
| P3 | 5.08(149) | 7.1(71.4) | 6.94(-16.7) | 10.3(18.1) |
| P4 | 3.46(69.6) | 5.76(39.1) | 5.12(-38.6) | 8.5(-2.5) |
| P1+P3 | 3.4(66.6) | 5.3(28.0) | 8(-4.0) | 9.46(8.48) |
| P1+P2+P3+P4 | 4.34(112.7) | 6.52(57.4) | 8.02(-3.8) | 8.14(-6.65) |

*Values in parameters are % increase or decrease of treated seedlings compared to control. Values are mean of three replicates significance of variance is (P: 00 F: 3.09)

**Table.7** Analysis of Foliage in Chilli seedling var LCA 344 treated with individual inoculum (P1, P2, P3, P4) and mixed inoculum (P1+P3, P1+P2+P3+P4) at an interval of 2 weeks

| Foliage | | | | |
|---|---|---|---|---|
| Treatment | 2 week | 4 week | 6 week | 8 week |
| Control | 2 | 3 | 5 | 5 |
| P1 | 3(50) | 3(0) | 5(0) | 5(0) |
| P2 | 3(50) | 4(33.3) | 5(0) | 5(0) |
| P3 | 4(100) | 4(33.3) | 4(-20) | 5(0) |
| P4 | 3(50) | 4(33.3) | 5(0) | 5(0) |
| P1+P3 | 4(100) | 4(33.3) | 6(20) | 6(20) |
| P1+P2+P3+P4 | 4(100) | 4(33.3) | 5(0) | 6(20) |

*Values in parameters are % increase or decrease of treated seedlings compared to control. Values are mean of three replicates

**Table.8** Analysis of total Biomass yield(mg/ml) in old chilli seedlings treated with individual inoculum (P1, P2, P3, P4) and co inoculum (P1+P3; P1P2=P3+P4) at an interval of 2 weeks

| **Biomass yield** | | | | |
|---|---|---|---|---|
| Treatment | 2nd week | 4th week | 6th week | 8th week |
| Control | 78 | 98 | 100 | 1800 |
| P1 | 100(28.2) | 128(30.6) | 540(440) | 2360(3.1) |
| P2 | 120(53.8) | 152(55.1) | 700(600) | 2120(17.7) |
| P3 | 130(66.6) | 170(73.4) | 930(830) | 2030(12.7) |
| P4 | 120(53.8) | 168(71.4) | 550(450) | 1430(-20.5) |
| P1+P3 | 130(66.6) | 152(55.1) | 810(710) | 1900(5.5) |
| P1+P2+P3+P4 | 90(15.3) | 104(6.12) | 1160(1060) | 1500(-16.6) |

*Values in parameters are % increase or decrease of treated seedlings compared to control. Values are mean of three replicates

**Table.9** Analysis of total protein content (ug/ml) in old chilli seedlings treated with individual inoculum (P1, P2, P3, P4) and co inoculum (P1+P3; P1+P2+P3+P4) at an interval of 2 weeks

| Total protein content | | | | |
|---|---|---|---|---|
| Treatment | 2nd week | 4th week | 6th week | 8th week |
| Control | 9 | 38 | 50 | 63 |
| P1 | 15(66.6) | 50(31.5) | 55(10) | 69(9.5) |
| P2 | 12(33.3) | 47(23.6) | 52(4) | 66(4.7) |
| P3 | 12(33.3) | 50(31.5) | 55(10) | 74(17.4) |
| P4 | 15(66.6) | 46(21.0) | 52(4) | 66(4.7) |
| P1+P3 | 18(100) | 46(21.0) | 55(10) | 69(9.5) |
| P1+P2+P3+P4 | 29(222.2) | 52(36.8) | 60(20) | 77(22.2) |

*Values in parameters are % increase or decrease of treated seedlings compared to control Values are mean of three replicates significance of variance is (P: 00 F: 3.04)

**Table.10** Analysis of chlorophyll in 12th week old chilli seedlings treated with individual inoculum (P1, P2, P3, P4) and co inoculum (P1+P3; P1P2=P3+P4)

| Chlorophyll | | | | |
|---|---|---|---|---|
| ***Treatment*** | ***Chl a*** | ***Chl b*** | ***Chl a/b*** | ***Total Chl*** |
| Control | 0.01 | 0.01 | 0.88 | 0.03 |
| P1 | 0.01(0) | 0.01(0) | 0.89(1.13) | 0.03(0) |
| P2 | 0.02(100) | 0.02(100) | 0.87(-1.1) | 0.04(33.3) |
| P3 | 0.02(100) | 0.02(100) | 0.81(7.9) | 0.04(33.3) |
| P4 | 0.02(100) | 0.03(199.9) | 0.65(-6.1) | 0.05(66.6) |
| P1+P3 | 0.02(100) | 0.04(300) | 0.59(-2.9) | 0.06(100) |
| P1+P2+P3+P4 | 0.03(199.9) | 0.04(300) | 0.64(-7.2) | 0.08(166.6 |

*Values in parameters are % increase or decrease of treated seedlings compared to control Values are mean of three replicates. significance of variance is (P:004 F:3.68)

**Plate.1** Isolates and their corresponding formulations with references to control

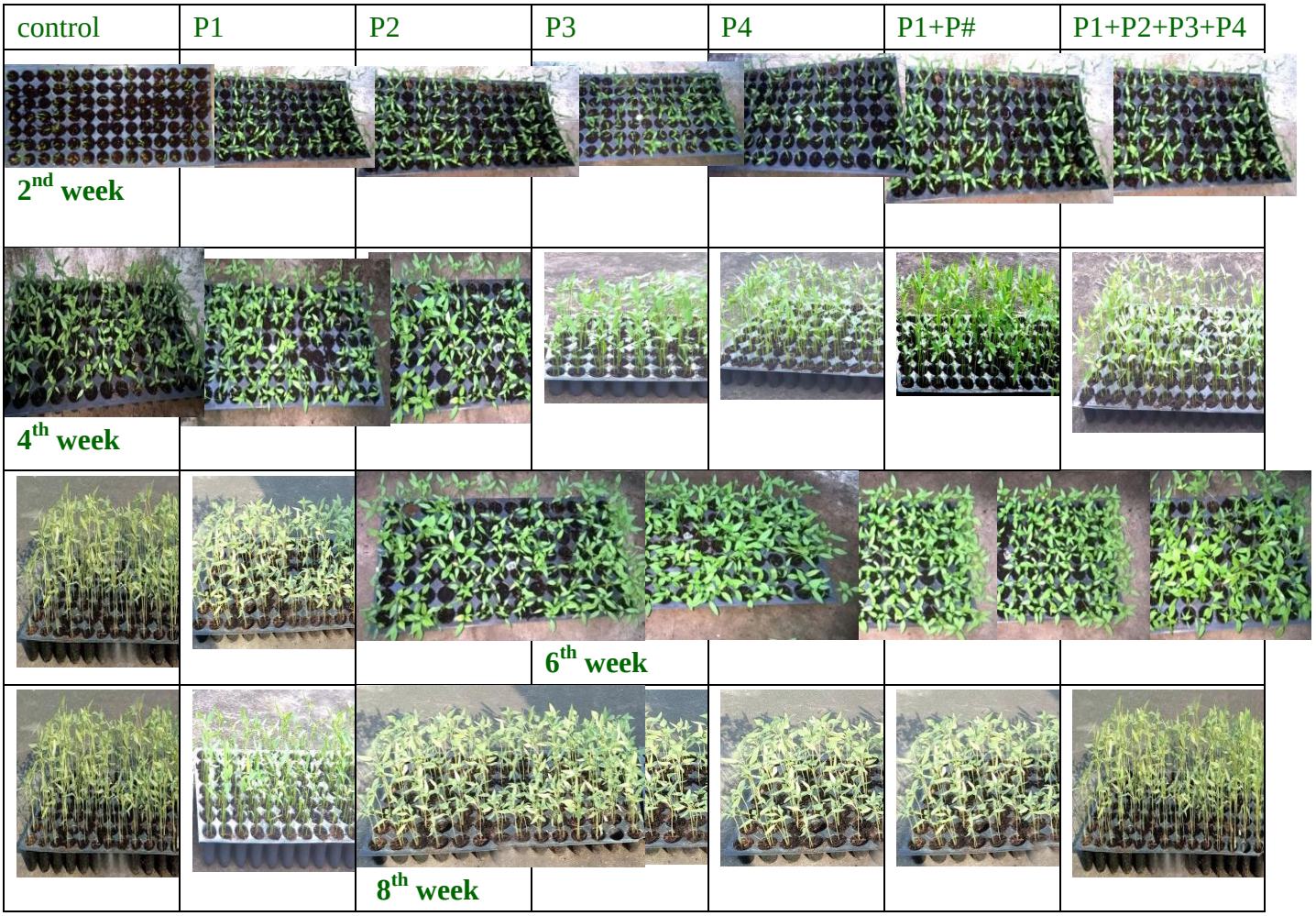
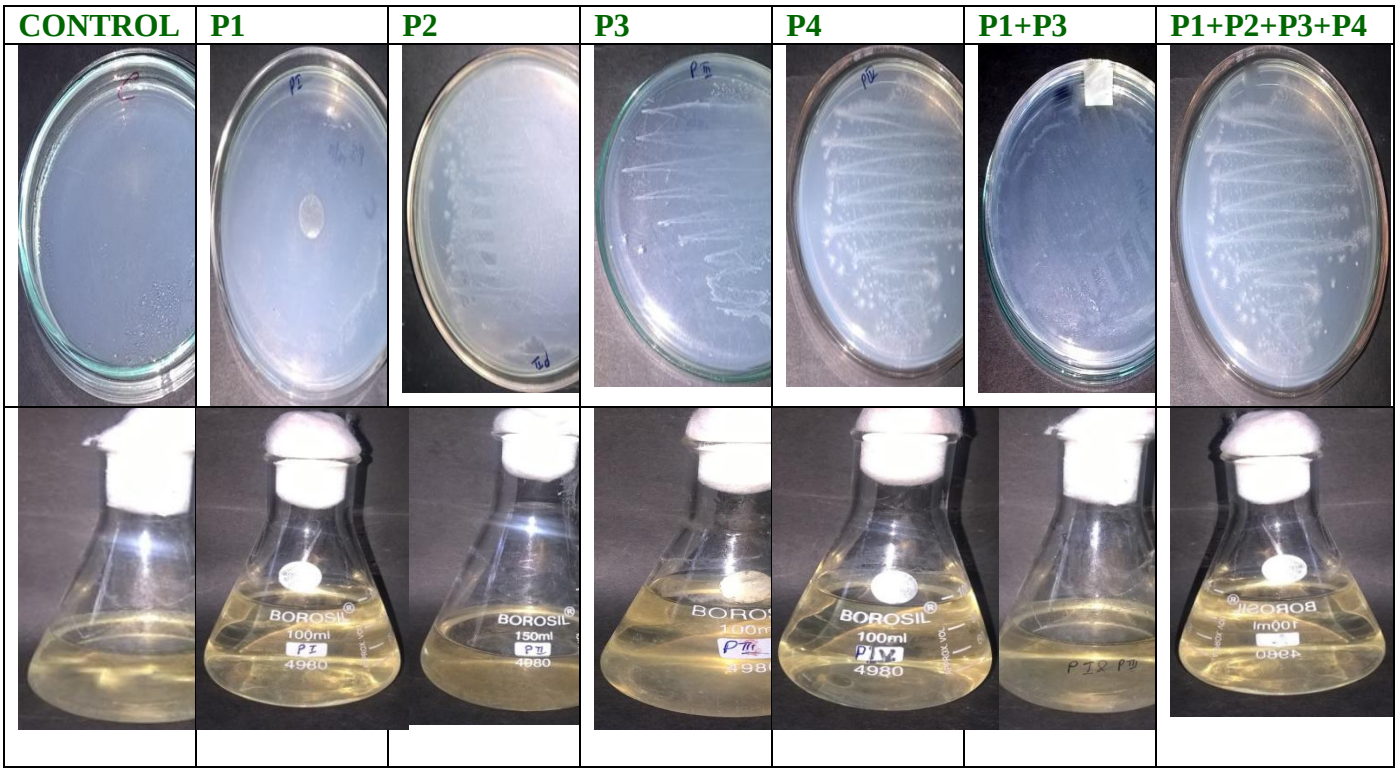


**Plate.2** Biweekly Plant growth responses of chilli seed lings against P1, P2, P3, P4, P1+P3 ans P1+P2+P3+P4 inoculations




Whereas P1+P3 has not shown significant enhancement. In the present study 4 rhizobacterial isolates and two combinations. Rhizobacterial isolates [P1, P2, P3, P4, P1+P3, and P1+P2+P3+P4] were analyzed to assess their plant growth promoting potential of chilli plants. At present scenaria of sustainable agriculture, a little is known about the PGPR'S interactions in the rhizosphere. The experiments were carried out using single strains P1, P2, P3, P4 and combination of strain (P1+P3) and (P1+P2+P3+P4) to establish highly suitable formulation for growth of chilli.

Based on data revealed in Fig 1, Fig 2, Table 1, Table 2, 24hrs old culture of individual isolates[P1, P2, P3, P4] and combination isolates[P1+P3] and [P1+P3] were identified as suitable inoculum for invitro greenhouse studies. It is also observed growth curve was triggered very high in combination inoculum, P1+P3 and P1+P2+P3+P4, when compared to individual inoculum [P1, P2, P3, P4].Results revealed that the isolates, used in combination inoculum are highly compatible with each other and hence the turbidity reached maximum at the earliest of 24hrs than 48hrs. Values are statistically analyze (n: 30) and significant variation was observed among the isolates ($p=0.02$ and $F=3.09$ at 95 CL)

The present study revealed that growth, physiological activities and soluble portent content were increased in the presences of PGPR strains. Effect was significantly high in coinoculation. There for we may assume that bacterial consortium can promote health of the crop better than individual consortium. Elevated chlorophyll content in treated seedlings. Could be correlated with increased stress tolerance. Similar results

were reported by other authors [Illmer and Schinner F 1992);Khalid *et al.*,2004).Previous studies reported that Bacillus pumilles R53 strain increased with 66% of seed soluble protein, probably due to stimulation of protein biosynthesis process in soya bean(Lorck H 1948).

The results of the present study suggests that p1, p2, p3, p4 strains alone or in combination have a great potential to increase Root length, Shoot height, No. of leaves, Fresh weight, Chlorophyll content and total protein content.

There PGPR strains can indirect enhance stress tolerance and improve the nutritive value of chilli seedlings. The response of inoculation was very high at 8th week seedling. Our study suggests that the 4 PGPR isolates may be useful as bio fertilizers either alone or in combination for vegetative production in sustainable and ecological agricultural systems.

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