

EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH AND YIELD OF FINGER MILLET

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B.Sc. (Ag.)

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(AGRICULTURAL MICROBIOLOGY)**

CHAIRPERSON: Dr. A. VIJAYA GOPAL



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2017

DECLARATION

I, **SAISREE CHALLA**, hereby declare that the thesis entitled, **“EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH AND YIELD OF FINGER MILLET”** submitted to the **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** in the major field of Agricultural Microbiology, is the result of original research work done by me. I also declare that no material contained in the thesis has not been published earlier in any manner.

Place: Guntur

Date:

(SAISREE CHALLA)

I.D. No. GAM/2015-09

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Place: Guntur

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ABSTRACT

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Phosphate solubilizing bacteria (PSB) plays an important role in dissolving both of fertilizer phosphorus (P) and bound P in the soil that is environmental friendly and sustainable. Phosphate solubilization is mainly through the reaction between organic acids excreted with phosphate binders such as Al, Fe, and Ca, or Mg to form stable organic chelates to free the bound phosphate ion. Phosphorus deficiency is widespread and phosphorus fertilizers are required to maintain crop production. Reductions in the supply of P fertilizers could severely diminish crop yields. One solution for mitigating the threat of this diminishing resource is to develop sustainable technologies to improve P use efficiently for plant uptake. Since chemical fertilizers are frequently used with finger millet crop, use of PSB, as biofertilizers would help in improving better crop stand and crop yields.

Sixteen PSB isolates were isolated from finger millet rhizospheric soils of twelve villages in Andhra Pradesh. All the 16 PSB isolates were able to solubilize phosphate qualitatively and quantitatively. The PSI for 16 PSB isolates were in the range of 6.61 to 2.62 qualitatively and all 16 PSB isolates were able to solubilize phosphate quantitatively in Pikovskaya's broth containing TCP in the range of 189.5 to 41.3 mg l⁻¹. The isolates were purified and they were culturally, morphologically and biochemically characterized. Biochemical characterization revealed that 9 isolates were positive for Voges Proskauer's test and motility test, 10 isolates were positive for nitrate reduction and catalase test, 11 isolates were positive for citrate utilization and oxidase test, 8 isolates were positive for starch hydrolysis and methyl red test, 6 isolates were positive for gas and acid production and 13 PSB isolates were positive for indole

production. These isolates were screened *in vitro* for plant growth promoting attributes like phosphate solubilization, siderophore, IAA, ammonia and HCN production. Results revealed that all 16 isolates solubilized phosphorous and 87.5% isolates produced ammonia, 75% isolates produced HCN, 56% isolates produced siderophores and 81% isolates produced IAA.

All the isolates were further screened *in vitro* for antagonistic activity against the fungal pathogen *Rhizoctonia solani* and found that all isolates inhibited the fungal pathogen and highest inhibition was found with the isolate BKP2 (51.49%). The best 3 efficient PSB strains i.e., PSB1, PSB2 and PSB3 were selected by estimating the phosphorus solubilizing ability of isolates qualitatively as well as quantitatively and by determining the morphological, biochemical and plant growth promoting rhizobacterial properties of isolates. Therefore, the best 3 PSB strains were identified as *Pseudomonas* sp. (isolate name, BKP2) *Bacillus* sp. (isolate name, SSP1) and *Enterobacter* sp. (isolate name, SBP1).

Pot culture experiment was conducted for evaluating the effect of PSB on growth and yield of finger millet. Results revealed that the highest microbial population (Bacteria, PSB, Fungi and Actinobacteria) was recorded at flowering stage compared to other growth stages while microbial population at flowering stage was more in the treatment (T₅) with 75% RDP+PSB1+PSB2+PSB3. Root length (23.1 cm), no. of fingers plant⁻¹ (10.0), phosphorus content in plant sample (0.183%), total dry matter (39.4 g plant⁻¹) and yield parameters like grain yield (4031.9 kg ha⁻¹) and straw yield (4691.5 kg ha⁻¹) were recorded highest in the treatment (T₅) with 75% RDP+PSB1+PSB2+PSB. The enzyme activities (acid phosphatase, alkaline phosphatase, dehydrogenase and urease) and soil physico-chemical properties (pH, EC, available N, P, K and Organic carbon) recorded highest at various stages of crop growth in the treatment (T₅) with 75% RDP+PSB1+PSB2+PSB3. The results of this study clearly shows that finger millet growth and yield can be enhanced by inoculating soil with PSB biofertilizer along with recommended dose of FYM and by varying RDF in different treatments. Further, from this study it is also concluded that, soil fertility and soil health can be improved with the inoculation of soil with PSB biofertilizer.

APPENDIX - I

Composition of different growth media/reagents/indicators used

1. Glucose Broth

Peptone	5.00 g
Beef extract	3.00 g
Glucose	5.00 g
Distilled water	1000 ml
Bromo Cresol Purple (BCP) Solution	15 ml
pH	7.0 ± 0.2

2. Glucose Peptone Agar

Glucose	5.00 g
Peptone	10.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.0 ± 0.2

3. Kuster's Agar

Starch	10.00 g
Sodium chloride	2.00 g
Calcium carbonate	0.20 g
Casein	0.30 g
Dipotassium hydrogen phosphate	2.00 g
Ferrous sulphate	0.10 g
Potassium nitrate	2.00 g
Magnesium sulphate	0.50 g
Agar	20.00 g
pH	7.1-7.8

4. MR – VP broth

Buffered peptone	7.00 g
Dextrose	5.00 g
Dipotassium phosphate	5.00 g
Distilled water	1000 ml
pH	6.9 ± 0.2

5. Nutrient Agar

Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 - 7.2

6. SIM Agar

Peptone	30.0 g
Beef extract	3.0 g
Ferrous ammonium sulphate	0.2 g
Sodium thiosulphate	0.025 g
Agar	3.0 g
Distilled water	1000 ml
pH	7.3

7. Pikovskaya's Agar

Yeast extract	0.50 g
Dextrose	10.00 g
Calcium phosphate	5.00 g
Ammonium sulphate	0.50 g
Potassium chloride	0.20 g
Manganese sulphate	0.0001 g
Magnesium Sulphate	0.10 g
Ferrous sulphate	0.0001 g
Agar	20.00 g
Distilled water	1000 ml
pH	7.0

8. Nutrient Gelatin Agar

Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
pH	6.8 – 7.0

9. Potato Dextrose Agar

Potato infusion	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	5.6 ± 0.2

10. Simmons Citrate Agar

Magnesium Sulphate	0.20 g
Ammonium dihydrogen phosphate	1.00 g
Dipotassium phosphate	1.00 g
Sodium citrate	2.00 g
Sodium chloride	5.00 g
Bromothymol blue	0.08 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8 ± 0.1

11. Starch Agar

Starch (soluble)	20.0 g
Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.0

12. Nitrate Broth

Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	5.0 g
Water	1000 ml
pH	6.8-7.0

13. King's B Agar

Peptone	16.0 g
K ₂ HPO ₄	1.6 g
MgSO ₄	1.6 g
Glycerol	10.0 g
Agar	18.00 g
Distilled water	1000 ml

14. Nutrient Broth

Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml
pH	7.0

15. Trypticase Soy Agar

Trypticase	15.0 g
Phytone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.3

16. Peptone Water

Peptic digest of animal tissue	10.0 g
NaCl	5.0 g
Distilled water	1000 ml

17. Spore Stain**a) Malachite green (5%)**

Malachite green	5.0 g
Distilled water	100.0 ml

b) Safranin	
Safranin (2.5% solution in 95% ethanol)	10.0 ml
Distilled water	100.0 ml

18. Gram Stain Solutions

a) Crystal violet solution	
Crystal violet	10.0 g
Ammonium oxalate	4.0 g
Ethanol	100 ml
Distilled water	1000 ml
b) Gram's Iodine solution	
Iodine	1.0 g
Potassium iodide	2.0 g
Ethanol	25 ml
Distilled water	100 ml
c) Counter stain	
2.5% safranin in ethanol	10 ml
Distilled water	100 ml
d) Ethyl alcohol (Decolouriser)	
Ethanol	95 ml
Distilled water	5 ml

19. Kovac's Reagent

P- Dimethylamino benzaldehyde	5.0 g
Amyl alcohol	75 ml
HCl (conc.)	25 ml

20. Nessler's Reagent

Potassium iodide	50.0 g
Distilled water	25 ml
Mercuric chloride (saturated)	35 ml
KOH (50% aqueous)	400 ml

21. Peptone Broth

Peptone	10.0 g
Carbohydrate	5.0 g
Sodium chloride	15.0 g
Phenol red	0.018 g
Distilled water	1000 ml
pH	7.3

CERTIFICATE

This is to certify that the thesis entitled **“EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH AND YIELD OF FINGER MILLET”** submitted in partial fulfilment of the requirements for the degree of **‘Master of Science in Agriculture’** of the Acharya N. G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Ms. SAISREE CHALLA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

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CERTIFICATE

Ms. **SAISREE CHALLA** has satisfactorily prosecuted the course of research and that thesis entitled **“EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH AND YIELD OF FINGER MILLET”** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date:

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Chapter I

INTRODUCTION

Phosphate solubilizing bacteria (PSB) is an environment friendly and cost effective approach as an alternative strategy to reduce chemical fertilizers usage. Phosphate solubilizing bacteria with multiple beneficial traits are important to increase the growth and yield of plants (Singh and Goel, 2015).

Finger millet (*Eleusine coracana* L. Gaertn) is an important small millet crop grown in India and has the pride of place in having highest productivity among millets. It is also known as ragi, African millet and bird's foot millet. It is an important staple food crop in certain parts of eastern countries, Central Africa and India.

It is grown both for grain and fodder purpose. In India, it is cultivated in Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Jharkhand, Uttaranchal, Maharashtra, and Gujarat (AICSMIP, 2002). It is a fast growing cereal crop that reaches maturity within three to six months and occasionally in only 45 days. It is commonly found at altitudes between 1000 and 2000 in eastern and southern Africa, and up to 2500-3000 m in the Himalayas. It grows best at an average temperature of 23°C but can withstand cooler and hotter conditions (FAO, 2012). An annual rainfall ranging from 500 to 1000 mm is suitable, provided it is well distributed across the growing season. It is adapted to a wide range of soil conditions though it prefers fertile, well-drained sandy to sandy loam soils with a pH ranging from 5 to 7. However, it grows well in lateritic or heavy black vertisols and has some tolerance to alkaline and moderately saline soils (Dida and Devos, 2006).

Phosphate solubilizing bacteria (PSB) plays an important role in dissolving both of fertilizer phosphorus (P) and bound P in the soil that is environmental friendly and sustainable (Khan *et al.*, 2007). Phosphate solubilization is mainly due to the reaction between organic acids excreted with phosphate binders such as Al, Fe, and Ca, or Mg to form stable organic chelates to free the bound phosphate ion (Arcand and Schneider, 2006; Gupta *et al.*, 2012). Exploration of phosphate solubilizing bacteria has been conducted by many researchers from soils (Chen *et al.*, 2006; Widawati *et al.*, 2008; Gupta *et al.*, 2012), mangrove (Vazquez *et al.*, 2000; Holguin *et al.*, 2001), and

rhizosphere (Chung *et al.*, 2005; Poonguzhali *et al.*, 2008; Oliveira *et al.*, 2009). From such explorations, various types of phosphate solubilizing bacteria have been successfully identified.

Microorganisms are involved in a wide range of processes that helps in the transformation of soil phosphorus and are an integral part of the soil P cycle (Chen *et al.*, 2006). Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants (Sagervanshi *et al.*, 2012). Reductions in the supply of P fertilizers could severely diminish crop yields. One solution for mitigating the threat is to develop sustainable technologies to improve P use efficiently for plant uptake. An equally perplexing challenge facing agriculture production is finding solutions to efficiently deliver P to plants (MacDonald *et al.*, 2011).

Phosphorus deficiency is widespread and phosphatic fertilizers are required to maintain crop production. When it is added to the soil as phosphatic fertilizer, only a small portion is utilized by plants (Padmavathi and Usha, 2012). Application of biological fertilizers such as biological phosphate fertilizers improves soil fertility (Abdol *et al.*, 2011). The degree of fixation and precipitation of phosphorus in soil is highly dependent upon the soil conditions such as pH, moisture content, temperature and the minerals already present in the soil (Walpola and Yoon, 2012). Inoculation of plants with PSMs generally results in improved plant growth and yield, in particular, under glasshouse conditions (Khan *et al.*, 2010). More importantly, investigations conducted under field level using wheat and maize plants have revealed that PSMs could drastically reduce the usage of chemical or organic fertilizers (Singh and Reddy, 2011).

Current agriculture management practices commonly experience low plant nutrient use efficiency due to natural chemical absorption and transformations when fertilizer is applied to soils. Up to 90% of P fertilizer applied to soil is made unavailable to plants because it gets bind to Ca, Al and Fe bearing soil mineral surfaces or is lost from the ecosystem by leaching (Doolette and Smernik, 2011; Randriamanantsoa *et al.*, 2013). Soils with a large capacity to bind P are especially concerning because P delivery to plants requires more inputs relative to the P outputs in harvested crops

(MacDonald *et al.*, 2011) resulting in extremely low agricultural phosphorus use efficiency. Soil bacteria can strongly influence the amount of soil P that is plant-available by solubilizing the mineral-associated P (Malboobi *et al.*, 2009; Osorio and Habte, 2014; Tawaraya *et al.*, 2006) and increasing plant P uptake by stimulating plant root growth (Bal *et al.*, 2013; Penrose and Glick, 2003; Rashid *et al.*, 2012). They achieve this by releasing metabolites and high-affinity iron chelating siderophores which solubilize mineral-bound P (Richardson *et al.*, 2009; Shropshire and Bordenstein, 2016) and by exuding plant hormones such as auxin (Spaepen, 2015).

Since there are multiple mechanisms by which microbes can solubilize P, microbial consortia (multiple species) may be more effective than single species isolates because no single strain is likely to be optimized for various mechanisms that drive this process. The use of microbial biostimulants offers the promise of improved microorganism activities to enhance plant growth (Richardson and Simpson, 2011).

Hence the use of chemical fertilizers and pesticides caused an incredible harm to the environment. These agents are both hazardous and may persist and accumulate in natural ecosystems. An answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term. Since chemical fertilizers are frequently used with finger millet crop, use of PSB, as biofertilizers would help in improving better crop stand and crop yields. Since little information is available on these aspects with regard to PSB effect on finger millet and keeping in view the importance of the crop, the present study is planned with the following objectives.

Objectives of investigation:

1. Enumeration and isolation of phosphate solubilizing bacteria from collected soil samples.
2. Characterization of phosphorus solubilizing bacterial isolates.
3. To assess the effective isolates of PSB on growth and yield of finger millet.

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LIST OF SYMBOLS AND ABBREVIATIONS

nm	:	Nanometre
cm	:	Centimetre
m	:	Metre
g	:	Gram
kg	:	Kilogram
t	:	Tonne
ml	:	Millilitre
ha	:	Hectare
kg ha ⁻¹	:	Kilogram per hectare
t ha ⁻¹	:	Tonne per hectare
dSm ⁻¹	:	Decisiemen per metre
%	:	Per cent
<i>et al.</i>	:	And others
CD	:	Critical Difference
CRD	:	Complete Randomized Design
RDF	:	Recommended Dose of Fertilizer
PSB	:	Phosphate Solubilizing Bacteria
PSM	:	Phosphate Solubilizing Microorganisms
PGPR	:	Plant Growth Promoting Rhizobacteria
SEm	:	Standard Error of mean
FAO	:	Food and Agriculture Organisation
mm	:	Millimetre
°C	:	Degree Celsius
P	:	Phosphorus
Al	:	Aluminium
Fe	:	Iron
Ca	:	Calcium
Mg	:	Magnesium
i.e.,	:	That is
ARS	:	Agriculture Research Station
h	:	Hour
min	:	Minute

ppm	: Parts per million
HCN	: Hydrogen Cyanide
IAA	: Indole Acetic Acid
TCP	: Tri Calcium Phosphate
PSI	: Phosphate Solubilization Index
KOH	: Potassium hydroxide
NaOH	: Sodium hydroxide
BOD	: Biochemical Oxygen Demand
EC	: Electrical Conductivity
OC	: Organic Carbon
MUB	: Modified Universal Buffer
ILA	: Indole-3-Lactic acid
IMA	: Indole-3-Acetamide
GA	: Gibberellic Acid
CAS	: Chrome Azurol S Agar
PCH	: Pyochelin
SIM	: Sulfide Indole Motility
H ₂ O ₂	: Hydrogen Peroxide
RDP	: Recommended Dose of Phosphorus
RDN	: Recommended Dose of Nitrogen
RDK	: Recommended Dose of Potassium
SSP	: Single Super Phosphate
MOP	: Muriate of Potash
rpm	: Revolutions per minute
PVD I	: Pyoverdine I
DAS	: Days After Sowing
RP	: Rock Phosphate
DAP	: Di Ammonium Phosphate
PDA	: Potato Dextrose Agar
PSE	: Phosphate Solubilizing Efficiency
DNA	: Deoxyribo Nucleic Acid
SA	: Salicylic acid
MPS	: Mineral Phosphate Solubilizing
PGP	: Plant Growth Promoting
Ca ₃ (Po ₄) ₂	: Calcium Phosphate

NBRIP	:	National Botanical Research Institute Phosphate
PKV	:	Pikovskaya
ERP	:	Egyptian Rock Phosphate
OB meal	:	OLD Bone meal
HPTLC	:	High Performance Thin Layer Chromatography
IBA	:	Indole-3-Butyrate
AlPO ₄	:	Aluminium Phosphate
FePO ₄	:	Iron Phosphate
FYM	:	Farm Yard Manure
CFU	:	Colony Forming Units
@	:	At the rate of
µg ml ⁻¹	:	Microgram per Millilitre
µm	:	Micrometre
Hcl	:	Hydrochloric acid
etc.	:	And so on
µl	:	Microlitre
µg	:	Microgram
CaCl ₂	:	Calcium chloride
CaCO ₃	:	Calcium carbonate
TPF	:	Tri Phenyl Formazon
N	:	Nitrogen
U.V	:	Ultraviolet
lb psi	:	Pounds per square inch
Conc. H ₂ SO ₄	:	Concentrated Sulphuric acid
<i>viz.</i> ,	:	Namely
K ₂ Cr ₂ O ₇	:	Potassium dichromate
KMnO ₄	:	Potassium permanganate
NH ₄ OAC	:	Ammonium Acetate
NH ₄ ⁺	:	Ammonium
KCL-PMA	:	Potassium chloride – Phenyl Mercuric Acetate
spp.	:	Species
Fig.	:	Figure
No.	:	Number
K	:	Potassium
pNP	:	para- Nitrophenol

mg l ⁻¹	:	Milligram per litre
CaHCO ₃	:	Calcium hydrogen carbonate
Na ₂ CO ₃	:	Sodium carbonate
STCR	:	Soil Test Crop Response
P ₂ O ₅	:	Phosphorus
K ₂ O	:	Potassium
wt.	:	Weight
PSMOS	:	Phosphate Solubilizing Microorganisms
GPS	:	Global Positioning System
Zn	:	Zinc
Cu	:	Copper
Mn	:	Manganese
TTC	:	Tri phenyl Tetrazolium Chloride
TSA	:	Trypticase Soy Agar

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Note: The literature is cited as per the “Thesis Guide Lines” prescribed by Acharya N. G. Ranga Agricultural University, Guntur.

Chapter III

MATERIAL AND METHODS

The present study on “Effect of phosphate solubilizing bacteria on growth and yield of finger millet” was carried out at Agricultural Research Station (ARS), Amaravathi. Attempts were made to isolate phosphate solubilizing bacteria from soil samples that were collected from finger millet growing areas of Andhra Pradesh and the isolates were screened and characterized. The efficient phosphate solubilizing bacterial isolates were selected and their influence on finger millet growth and yield was determined. The material used and methods employed in the investigation are outlined below.

The general laboratory techniques followed in the present study were those described by Cappuccino and Sherman (1992), Nene and Thapliyal (1993) and Aneja (2001) for the preparation of media, sterilization, isolation and maintenance of bacterial cultures, with slight modifications wherever necessary.

3.1 GLASSWARE

Petri plates, test tubes, microscopic slides, conical flasks of different capacities *i.e.*, 1000, 500, 250 and 100 ml, pipettes of 0.1, 1 and 10 ml beakers and measuring cylinders of 50, 100, 500 and 1000 ml. Volumetric flasks of 25, 100, 250, 500 and 1000 ml were used. All the glassware used were of Borosil make.

3.1.1 Cleaning of Glassware

Glassware was first washed with a detergent, then cleaned with tap water and finally placed in the Chromic acid solution prepared with following composition:

Potassium dichromate	:	60 g
Conc. H ₂ SO ₄	:	60 ml
Distilled water	:	1000 ml

The glassware were kept in the cleaning solution for 24 h and then thoroughly washed with running tap water before its final cleaning with distilled water and dried.

3.1.2 Sterilization of Glassware

Glassware was sterilized in hot air oven at 160⁰C for 2 h before use. Media, distilled water, etc., were sterilized in an autoclave at 15 lb psi (121⁰C) for 15 min.

3.1.3 Precaution to Avoid Contamination

The inoculation work of microbial cultures were carried out in laminar air flow. The laminar bench and air flow was disinfected using U.V lamp prior to commencement of work.

3.2 EQUIPMENT AND APPARATUS USED

Hot air oven and autoclaves were used for sterilization of glassware and media respectively. BOD (Biological Oxygen Demand) incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator. The pH was measured by using digital pH meter. Cyclomixer was used for homogenization during serial dilution. Samples were weighed using an electronic balance. Compound microscope was used to observe the morphology of bacterial cultures. Distillation unit was used for sterilization of water. Flame photometer was used for the estimation of potassium content in soil samples. Kjeldahl nitrogen analyzer was used for the estimation of nitrogen content in soil samples. Spectrophotometer was used to observe the activity of the enzymes by measuring the optical density. Laminar air flow was used to carry out microbiological inoculation work.

3.3 CHEMICALS USED

The chemicals used in the present investigation were of analytical and laboratory grade. The pH of the media was adjusted to the required level using 0.1N NaOH, 0.1N HCl and 0.1N KOH. Ethanol was used to sterilize the laminar air flow chamber and incubators for disinfection.

3.4 ISOLATION OF MICROBIAL POPULATION FROM SOIL SAMPLES

3.4.1 Soil Sample Collection

Samples were collected from rhizospheric soils of different finger millet growing areas of Andhra Pradesh. Twelve (12) rhizospheric soil samples of finger millet crop plants up to a depth of 10 to 15 cm were collected. The soil intimately adhering to the roots was collected and mixed to provide a composite soil sample. The GPS data (Latitude °N and Longitude °E) were collected at each sampling site.

3.4.2 Enumeration of Micro-organisms

Ten g of soil from each soil sample was taken in 250 ml conical flask to which 90 ml of sterile saline was added aseptically. The sample was agitated for 15 minutes on a cyclomixer and serial dilutions of soil suspensions were prepared. 0.1 ml of respective dilutions were spread on the surface of sterile agar medium (Nutrient agar medium for bacteria, PDA medium for fungi, Kuster's agar medium for actinomycetes, Pikovskaya's agar medium for PSB) contained in sterile petriplates. Then the petriplates were incubated at room temperatures ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 24-72 h in a bacteriological incubator. After incubation colonies formed were counted using digital colony counter and the population was expressed as CFU $\times$ dilution factor g^{-1} of soil. Media composition is presented in Appendix I.

3.5 IDENTIFICATION OF BACTERIAL ISOLATES

3.5.1 Morphological Characterization

All the 16 isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and Gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

3.5.2 Gram's Staining

A loopful of inoculum from young culture was taken and mixed with a drop of sterile distilled water placed in the center of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the lower side of the slide three to four times over the flame. Then each smear was covered with crystal violet for 30 seconds

followed by washing each slide with distilled water for few seconds. Then each slide was covered with Gram's iodine solution for 60 seconds, then washed with 95% ethyl alcohol followed with distilled water and drained. Safranin was then applied for 30 seconds and was washed with distilled water and blot dried. The bacteria that appeared purple were referred to as Gram-positive and those that were pink as Gram-negative (Aneja, 2003).

3.5.2.1 Endospore staining: Smears of organisms to be tested for endospores were prepared and were heat fixed. Smear was covered with a piece of absorbent paper cut to fit the slide and was placed on a wire gauze on a ring stand. Paper was saturated with malachite green and slide was heated until steam was seen rising from the surface. Reheating was done as needed to keep the slide steaming for about three minutes. As the paper began to dry malachite green was added drop by drop to keep it moist, so that the temperature is not appreciably reduced. Later the paper was removed with tweezers and the slide was thoroughly rinsed with tap water. Further the slide was drained and counterstain with 0.5% safranin for 45 seconds and was washed with tap water, blot dried and examined. The vegetative cells will appear red and the spores will appear green (Aneja, 2003).

3.5.3 Colony Morphology

Morphological characteristics of the colony of each isolate were examined on Nutrient agar and specialized medium and incubated as per the requirement of isolate. Cultural characterization of isolates was done by recording different characteristics of colonies such as shape, size, elevation, surface, margin, color, pigmentation etc.

3.5.4 Qualitative Measurement of Phosphate Solubilization

Bacterial isolates were screened for their tri-calcium phosphate (TCP) solubilizing activity on Pikovskaya's agar plates. Isolates were spot inoculated on the centre of agar plate aseptically. All the plates were incubated at $28 \pm 2^\circ\text{C}$ for 5 days. A clear zone around a growing colony indicated phosphate solubilization and was measured as phosphate solubilization index (PSI). PSI was calculated as the ratio of the total diameter (colony + halo zone) to the colony diameter (Premono *et al.*, 1996). All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily be identified as PSB's (Gyaneshwar *et al.*, 1999; Sundara-Rao and Sinha, 1963).

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

3.5.5 Quantitative Measurement of Phosphate Solubilization

The bacteria, found to be positive for TCP solubilization were further analyzed for their ability to solubilize it in liquid medium. Bacterial isolates were inoculated in Pikovskaya's broth (100 ml) in 250 ml of Erlenmeyer flasks and incubated at $28 \pm 2^\circ\text{C}$ for 5 days with interval shaking at 100 rpm. Triplicates were maintained for each treatment. After incubation the bacterial cultures were filtered through Whatmann No.1 filter paper and were clarified by centrifugation at 8,000 rpm for 20 minutes. Uninoculated broth served as control. The soluble phosphorus was determined in clear filtrate using standard procedures (Rao, 1982). The intensity of green colour was measured in Spectrophotometer at 430 nm. The pH of filtrate was recorded at the end of the experiment. A standard graph was then prepared from which phosphorus values for experimental samples were calculated.

3.6 BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION

Different biochemical tests were performed as per the procedure described by Aneja (2001). The protocols followed are briefly outlined below.

3.6.1 Indole Production

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at $28 \pm 2^\circ\text{C}$. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

3.6.2 Catalase Test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of pure isolates were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

3.6.3 Oxidase Test

The overnight cultures of the test isolates were spotted on plates poured with sterile Trypticase Soy Agar (TSA) and the plates were incubated for 24 h at $28 \pm 2^\circ\text{C}$. After incubation, 2-3 drops of N, N, N', N'- tetramethyl-p-phenylenediamine dihydrochloride (Wurster's reagent) was added on to the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

3.6.4 Gelatin Liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at $28 \pm 2^\circ\text{C}$. Then the tubes were kept in the refrigerator for 30 minutes at 4°C . The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test.

3.6.5 Methyl Red Test

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at $28 \pm 2^\circ\text{C}$ for 48 h. After incubation five drops of Methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

3.6.6 Voges Proskauer's Test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37°C for 48 h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of ten drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

3.6.7 Citrate Utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at $28 \pm 2^\circ\text{C}$ for 24 h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

3.6.8 Starch Hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at $28 \pm 2^{\circ}\text{C}$ for 24-48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

3.6.9 Hydrogen Sulfide Test

Sterilized Hydrogen Sulfide- Indole-Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at $28 \pm 2^{\circ}\text{C}$. Visualization of black colour along the line of inoculation indicated a positive reaction for the test.

3.6.10 Gas and Acid Production

PSB isolates were tested for their acid production behaviour by inoculating 5 ml of sterile glucose broth with bromo cresol purple as pH indicator in test tubes. The tubes were incubated for 7 days at 30°C . Change in colour of medium to yellow was taken as positive for acid production (Seeley and Vandemark, 1970).

3.6.11 Carbohydrate Utilization Test

All the pure bacterial isolates were screened for the carbohydrate fermentation abilities using four different carbohydrates (lactose, glucose and maltose) in peptone broth medium. Bacterial isolates were inoculated in broth containing specific carbohydrate. The change in colour of peptone broth was observed for utilization of particular carbohydrate present in broth.

3.6.12 Motility Test

This test was done using the hanging drop method. A drop of the test organisms in a saline suspension was placed on a cover slip. The cover slip was inverted and placed on cavity slide, this was viewed under the microscope.

3.6.12 Denitrification Test

Sterilized nitrate broth tubes inserted with Durham's tube in inverted position were inoculated with overnight grown cultures of the test organisms and incubated at 25°C for 10-15 days. After incubation, the isolates which showed accumulation of gas in the Durham's tubes were scored as positive for denitrification.

3.7 IN VITRO SCREENING OF PSB ISOLATES FOR VARIOUS PLANT GROWTH PROMOTING ATTRIBUTES

Pure isolates were isolated by streaking isolates on respective media plates and screened for following plant growth promotion properties.

3.7.1 Phosphate Solubilization

Sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petri plates and incubated for 24 h, after solidification. After incubation the Pikovskaya's plates were spot inoculated with 16 isolates of PSB, incubated at $28 \pm 2^\circ\text{C}$ for 4-5 days. Formation of a clear zone around the colonies was considered as positive result for phosphate solubilization.

3.7.2 Ammonia Production

The isolates were tested for ammonia production by inoculating the isolates in to 10 ml of pre-sterilized peptone water in the test tubes. The tubes were incubated for 48-72 h at $36 \pm 2^\circ\text{C}$. Nessler's reagent (0.5 ml) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as positive test for ammonia production.

3.7.3 Indole Acetic Acid Production

Indole acetic acid production was tested according to Gorden and Weber (1951). The active culture of each test isolate was raised in 5 ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of Ortho - phosphoric acid was added to 2 ml of supernatant and incubated for 30 min to develop the color. Development of pink color considered as positive for IAA production.

3.7.4 Siderophore Production

Siderophore production was estimated qualitatively. 0.5% of cell free culture supernatant was added to 0.5 ml of 0.2% aqueous Ferric chloride solution. Appearance of orange or reddish brown color indicated the presence of siderophore (Yeole and Dube 2000).

3.7.5 Hydrogen Cyanide Production

The HCN production was tested by the method of Castric and Castric (1983). Medium plates i.e., Pikovskaya medium were prepared separately and incubated for 24 h. One ml of culture of each test isolate was inoculated on respective media plates separately. A disc of Whatman filter paper No.1 of the diameter equal to the petriplate size, impregnated with alkaline Picric acid solution (0.5% Picric acid (w/v) in 1% Sodium carbonate) was placed in the upper lid of the inoculated petriplates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at $28 \pm 2^{\circ}\text{C}$ for 48-72 h. Change in color from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

3.7.6 Antagonistic Activity

Pure isolate of the pathogen, *Rhizoctonia solani* was obtained from the Department of Plant Pathology, Lam farm, Guntur.

Antagonistic activity was verified by following dual culture plate technique (Skidmore and Dickinson, 1976). First, the bacterial isolates were streaked on respective media plates and incubated at respective temperature and time. Loopful of each bacterial isolate was streaked on the potato dextrose agar plate at one end, which was inoculated with 5 days old, 5 mm mycelial disc of test pathogen at the other end. Control plate was maintained by placing only pathogen mycelial disc on the plate without bacteria. The assay plates were incubated at $28 \pm 1^{\circ}\text{C}$ for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate three replications were maintained with suitable controls.

Per cent Inhibition =

$$\frac{\text{Growth of pathogen in control (mm)} - \text{growth of pathogen in treatment (mm)}}{\text{Growth of pathogen in control (mm)}} \times 100$$

Note: The per cent inhibition in control is taken as zero per cent

3.8 ESTIMATION OF DIFFERENT ENZYME ACTIVITIES

Enzymes like dehydrogenase, phosphatase, urease was estimated at different stages of crop growth (i.e., at initial, at flowering and at harvesting stage).

3.8.1 Phosphatase Activity

The procedure followed was of Tabatabai and Bremner (1969) (Acid phosphatases) and Eivazi and Tabatabai (1977) (Alkaline phosphatases). The principle in the estimation of phosphatase enzyme activity is that, 1 g of soil extract was allowed to react with para nitro phenol, which was estimated colorimetrically.

Phosphatase activity was estimated by taking 1g of soil sample and mixed it with 0.2 ml of toluene, 4 ml of MUB (Modified Universal Buffer) and 1ml of disodium para nitrophenol solution. This solution was kept in an incubator at 37°C for 1 h. Later 1 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH were mixed properly and the intensity of the yellow color of filtrate was read on spectrophotometer at 420 nm.

Calculation: Phosphatase activity is expressed as $\mu\text{g p-nitrophenol g}^{-1} \text{ soil h}^{-1}$.

1 ml of the sample liberates = $x \mu\text{g p-nitrophenol}$

10 ml of the sample liberates = $10 \times x \mu\text{g p-nitrophenol}$

→ liberated by 1g soil

3.8.2 Dehydrogenase Activity

Method was described by Casida *et al.* (1964). The enzyme activity in 1g soil was determined by adding 0.05 g of CaCO₃, 2.5 ml of distilled water and by using 1 ml of 3 % TTC (Try phenyl Tetrazolium Chloride) where it is reduced to light pink TPF (Tri Phenyl Formazon) on the incubation for 24 h. Later it was dissolved in 10 ml of methanol and finally made up to 25 ml. The intensity of the red color was measured on a spectrophotometer at 485 nm.

Calculation: Dehydrogenase activity is expressed as $\mu\text{g of TPF produced g}^{-1} \text{ soil day}^{-1}$.

1 ml of the extract gives = $x \mu\text{g of TPF}$

25 ml of the extract gives = $25 \times x \mu\text{g p-nitrophenol}$

→ for 1g soil

3.8.3 Urease Activity

Described by Tabatabai and Bremner (1972). Urease activity in soil can be assayed by quantifying the ratio of release of NH₄⁺ from the hydrolysis of urea. To estimate the urease activity 5 g of soil was taken and 5 ml of 0.2 M urea solution was

added and incubated at 37°C for 5 h. Then 50 ml of 2 M KCl-PMA solution was added and shaken in orbital shaker for 1 h. Later transferred 1 ml of the supernatant solution into a 50 ml volumetric flask and 10 ml of 2 M KCl-PMA solution, 30 ml of color reagent was added and placed in boiling water for 30 min, cooled for 15 min and made volume to 50ml with water. Measured the intensity of red color produced in spectrophotometer at 636 nm.

Calculation: Urease activity is expressed as μg of urea hydrolyzed g^{-1} soil 4h^{-1} .

1 ml of the sample contains = x μg of urea hydrolyzed

25 ml of the sample contains = $25 \times x$ μg of urea hydrolyzed

→ Urease activity of 5g soil

The urease activity of 1 g soil = $5 \times x$ μg of urea hydrolyzed

3.9 PHYSICO CHEMICAL PROPERTIES OF SOIL SAMPLES

3.9.1 Soil pH (Piper,1966)

pH of the soils were determined by taking (1:2) soil and water suspension. Ten g of soil was weighed in a beaker, 20 ml of distilled water was added and stirred the contents with glass rod intermittently, the suspension was allowed to stand for half an hour. The pH was measured by using pH meter.

3.9.2 Electrical Conductivity (Piper,1966)

EC of the soils were determined by taking (1:2) soil and water suspension. Twenty g of soil was weighed in a beaker, 40 ml of distilled water was added and, swirled frequently for some time over electric shaker. The suspension was allowed to stand for 30 min. The EC of the soil suspension was measured by using EC meter. EC is expressed in dSm^{-1} .

3.9.3 Organic Carbon

Soil organic-carbon was estimated following the method described by Walkley and Black (1934). One g of soil was placed in a 500 ml conical flask. 10 ml of 1N Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution was pipetted into the conical flask. 20 ml of concentrated sulphuric acid was added and the mixture was allowed to stand for 30 minutes. A blank was also run in the same manner. The contents were then diluted to 200 ml with distilled water. Further 10 ml of 85% Ortho Phosphoric acid, and 1ml of

Diphenyl amine indicator was added to each flask. The solution was titrated with 0.5 N Ferrous sulphate till a turbid blue colour changed to brilliant green. Organic carbon is expressed in per cent.

$$\% \text{ of carbon} = \frac{V_1 - V_2 \times 0.003 \times 100}{2 \times W}$$

Where, V_1 = Volume of $K_2Cr_2O_7$

V_2 = Volume of ferrous ammonium sulphate (Burette reading)

W = Weight of the soil

3.9.4 Available Nitrogen (N)

The alkaline potassium permanganate method of Subbaiah and Asija (1960) was followed for the estimation of available N content in soil. Twenty g of soil was taken in 800 ml dry Kjeldahl flask and 20 ml of distilled water was added. Then 100ml of each of 0.32% $KMnO_4$ and 2.5% NaOH solutions were added. The froth formation during boiling was prevented by adding liquid paraffin (1ml) and bumping by adding a few glassbeads. The contents were distilled in Kjeldahl assembly at a steady rate and liberated ammonia was collected in a conical flask (250ml) containing 20 ml of boric acid solution (with mixed indicator). The pink colour of the boric acid solution turns to green with the absorption of ammonia. 150 ml of distillate collected in about 30 minutes was titrated with 0.02 N H_2SO_4 till the original shade (pinkish) was obtained. Blank was performed without soil.

3.9.5 Available Phosphorus (P)

Available P in soil was determined by Olsen's method (Olsen *et al.*, 1954). 2.5g air dried soil was placed into a 250 ml conical flask add a pinch of Darco charcoal and 50 ml of Sodium bicarbonate extractant (0.5 M) was added, shake for 30 minutes and filtered through Whatman No 1 filter paper. Transfer 5 ml of clear and colourless filtrate into a 25 ml volumetric flask, add 2-3 drops of p-nitrophenol, it develops yellow colour. Add known quantity of 5N H_2SO_4 drop by drop, yellow colour will disappear, note the volume, add five ml of Olsen's extract solution, dilute up to 20 ml with distilled water then add 4 ml of ascorbic acid and makeup with volume to 25 ml and shake well. Wait for 5 minutes and measure the intensity of the blue colour developed at 660 nm. Run blank without soil, a standard curve of P was plotted. Available P expressed in $kg\ ha^{-1}$.

3.9.6 Available Potassium (K)

Available potassium content from soil was extracted by using 1N NH_4OAC as described by Jackson (1973). The concentration of potassium in the extractant was determined by flame photometer.

3.10 POT CULTURE STUDY

The pot experiment was carried out to study the effect of inoculation of efficient PSB strains on growth and yield of finger millet at ARS, Amaravathi during 2016-17.

3.10.1 Polyhouse Growth Conditions

The pots were arranged on the polyhouse benches in a Completely Randomized Design (CRD). Daylight temperature was 30°C, and night temperature was 25°C. The pots were irrigated alternate days and received % of RDF according to the treatment.

3.10.2 Preparation of Pots

Pots used in this experiment were sterilized by sterile deionized water. Minute holes were made at the bottom of each pot to allow water drainage. The collected field soil was placed in each pot i.e., 6 kg of soil per each pot. The pots were placed on glass house benches.

3.10.3 Maintenance of Nursery

The seeds were sown in pots for raising a ragi nursery.

3.10.4 Transplantation of Seedlings

Ragi seedlings after attaining 20-25 days of maturity from nursery were used for transplanting. Three seedlings per pot were transplanted and each treatment was replicated thrice.

3.10.5 Details of the Treatments Used in the Pot Experiment

Crop	:	Finger millet
Variety	:	VR-708 (Champavathi)
Season	:	<i>Kharif</i> -2016
No. of treatments	:	10
No. of replications	:	3
Pot size	:	24 × 24 cm
Experimental design	:	CRD
PSB inoculum load	:	6x10 ⁹ cells/ml
used in treatments		
Carrier used	:	Lignite
Mode of treatment	:	Soil application
RDF	:	60: 30: 30 NPK kg ha ⁻¹

T₁ -100% RDF

T₂ - 75% RDP + PSB₁

T₃ - 75% RDP + PSB₂

T₄ - 75% RDP + PSB₃

T₅ - 75% RDP + PSB₁ + PSB₂ + PSB₃

T₆ - 50% RDP + PSB₁

T₇ - 50% RDP + PSB₂

T₈ - 50% RDP + PSB₃

T₉ - 50% RDP + PSB₁ + PSB₂ + PSB₃

T₁₀- Control

Note: (100% RDN and RDK - T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP - Recommended dose of Phosphorus, RDK- Recommended dose of Potassium)

3.10.6 Fertilizer Application

PSB inoculum load used in treatments was 6x10⁹ cells/ml. It is carrier based. Carrier used is Lignite. 3 milligrams of PSB biofertilizer was applied per pot. The mode of treatment was soil application (biofertilizer is mixed with carrier lignite in 1:3 ratio and then directly applied to the soil). The fertilizers were applied as per the treatments in various combinations. The crop was supplied with recommended dose of fertilizers.

Nitrogen- 60 Kg ha⁻¹, Phosphorous- 30 kg ha⁻¹ and K- 30 kg ha⁻¹. Calculated quantities of N in the form of urea, P in the form of single super phosphate (SSP) and K in the form of muriate of potash (MOP) were applied with recommended (60:30:30 N: P₂O₅: K₂O kg ha⁻¹) and reduced levels as per treatment.

3.10.7 Irrigation

Immediately after sowing, the pots were irrigated. The irrigations were scheduled at regular intervals.

3.10.8 Weed Management

To check the weed growth during the cropping period, hand weeding was done in experimental pots.

3.11 GROWTH AND YIELD CHARACTERS

3.11.1 Number of Fingers per Plant

In each pot the number of fingers were counted on each plant at the time of flowering stage and expressed as total number of fingers per plant.

3.11.2 Root Length

Root length was recorded at harvest by uprooting plants carefully and measuring the root length from tip of longest root to the neck region and expressed in cm.

3.11.3 Grain Yield

The harvested plants from pots were threshed manually and each pot yield was separately oven dried, grains were cleaned and weighed. Grain yield was computed and expressed in kg ha⁻¹.

3.11.4 Straw Yield

The straw from pots after harvesting was dried in sun, weighed and converted into kg ha⁻¹.

3.11.5 Phosphorus Content in Plant Sample (%)

Plant samples collected at harvest stage were oven dried at 60⁰C. The dried samples were powdered and analyzed for total P content by adopting the standard procedure.

The di-acid digested plant samples were analyzed for phosphorus content by Vanado - molybdo phosphoric acid (Jackson, 1967). The intensity of yellow color developed was measured by using spectrophotometer at 420 nm.

3.11.6 Total Dry Matter

Plant samples collected at harvest stage were sun-dried. Total dry matter production of plant samples were determined by weighing the leaf, stem and earhead dry weights individually and as a sum of leaf, stem and earhead dry weights, the total dry matter content of plant samples were analyzed.

3.12 STATISTICAL ANALYSIS

The data obtained in different experimental components was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967) and Panse and Sukhatme (1985).

Chapter IV

RESULTS AND DISCUSSION

Phosphate solubilizing bacterial strains were isolated from different rhizospheric soils of finger millet growing areas of Andhra Pradesh. The isolates were characterized, identified based on morphological and biochemical characteristics and studied for their functional plant growth promoting activities. The best three efficient PSB strains were selected and used for inoculation in pot experiment. The results obtained on these aspects are presented under the appropriate headings.

- 4.1 Isolation of total microbial population from finger millet rhizosphere.
- 4.2 Qualitative and quantitative estimation of Phosphate Solubilizing Bacterial (PSB) isolates
- 4.3 Morphological and biochemical characterization of PSB isolates.
- 4.4 Screening of pure isolates for PGPR properties
- 4.5 Pot culture experiment to study the effect of PSB on growth and yield attributes of finger millet.
- 4.6 Response of PSB isolates on enzyme activities and soil health parameters.

4.1 ISOLATION OF TOTAL MICROBIAL POPULATION FROM FINGER MILLET RHIZOSPHERIC SOILS OF ANDHRA PRADESH

4.1.1 Collection of Soil Samples by Using GPS

Soil samples were collected from 12 sites in finger millet growing districts of Andhra Pradesh with their location details, latitude and longitude (Table 4.1)

4.1.2 Isolation and Enumeration of Bacteria

Rhizospheric soils were collected from twelve finger millet growing villages of Andhra Pradesh and were used for the isolation and enumeration of bacteria (Table 4.3). The bacterial population in the soils collected from different places ranged

between $6.1\text{--}8.9 \times 10^8$ CFU g⁻¹ soil (Fig. 4.1). Maximum bacterial population was found in the rhizospheric red soils of Boyanapalle village (8.9×10^8 CFU g⁻¹ soil), followed by rhizospheric red soils of Venkateswarapuram village (8.7×10^8 CFU g⁻¹ soil), rhizospheric black soils of Neelakantapuram village (8.6×10^8 CFU g⁻¹ soil) and least bacterial population was found in the rhizospheric red soils of Karlagatta village (6.1×10^8 CFU g⁻¹ soil).

Juottonen *et al.* (2005) reported that environmental conditions and farm management practices had a considerable impact on soil microorganisms. The number of microorganisms found in each soil sample were different because the composition and function of the microbial community was shown to vary according to land-use type, plant species, temperature, nutrients, oxygen availability, pollutants and other environmental variables.

Bacteria actively participate in organic matter decomposition and liberating nutrients and further in turn enhance plant growth. Microorganism numbers varied between different soil types and conditions, with bacteria being the most numerous. Bacterial counts in different soils ranged from 4×10^6 to 2×10^9 CFU g⁻¹ of dry soil (Whitman *et al.*, 1998)

4.1.3 Isolation and Enumeration of Phosphate Solubilizing Bacteria

Rhizospheric soils were obtained from twelve different finger millet growing villages of Andhra Pradesh and were used for isolating PSB on Pikovskaya's agar medium. The PSB population in the soils collected from different places ranged between $3.2\text{--}5.6 \times 10^7$ CFU g⁻¹ soil and maximum PSB population was found in the rhizospheric black soils of Sariki village (5.6×10^7 CFU g⁻¹ soil), followed by rhizospheric black soils of Konapalli village (5.3×10^7 CFU g⁻¹ soil), rhizospheric red soils of Venkateswarapuram village (5.1×10^7 CFU g⁻¹ soil) and least PSB population was found in the rhizospheric black soils of Voppangi village (3.2×10^7 CFU g⁻¹ soil) (Table 4.3 and Fig. 4.2).

Reyes *et al.* (2006) found a significantly higher PSB population in the rhizosphere. Pradhan and Sukla (2005) stated PSB as important components of soil and directly or indirectly influence the soil's health through their useful activities.

Rhizospheric microorganisms mediate soil processes like decomposition, nutrient mineralization and nitrogen fixation. Phosphate solubilizing micro-organisms were reported to range between 3.5 and 19.63×10^4 CFU g⁻¹ of soil in India (Haque and Dave, 2005).

4.1.4 Isolation and Enumeration of Fungi

Twelve rhizospheric soils were obtained from different villages of finger millet growing areas of Andhra Pradesh and isolated fungi on potato dextrose agar medium. The fungal population in the soils collected from different places ranged between $3.2\text{--}5.7 \times 10^3$ CFU g⁻¹ soil (Table 4.3 and Fig. 4.3). Maximum fungal population was found in the rhizospheric red soils of Hanuvayapalle village (5.7×10^3 CFU g⁻¹ soil), followed by rhizospheric red soils of Venkateswarapuram village (5.6×10^3 CFU g⁻¹ soil), rhizospheric black soils of Konapalli village (5.4×10^3 CFU g⁻¹ soil) and least fungal population was found in rhizospheric black soils of Singarapalle village (3.2×10^3 CFU g⁻¹ soil).

The dominant fungal species are dependent on the nature of substrate and temporal region that favors the colonization, growth and substrate possession of the fungi (Rani and Paneerselvam, 2010). Fungi are an important component of soil microbiota more in abundance than bacteria, depending on soil depth and nutrient conditions (Anisworth and Sussman, 1968). They play key role in essential processes like organic matter decomposition and mineralization (Christensen, 1989).

4.1.5 Isolation and Enumeration of Actinobacteria

Rhizospheric soils were obtained from twelve different finger millet growing villages of Andhra Pradesh and used for the isolation of actinobacteria on kuster's agar medium. The actinobacteria population in the soils collected from different places ranged between $3.3\text{--}5.6 \times 10^5$ CFU g⁻¹ soil (Table 4.3). Maximum actinobacteria population was found in the rhizospheric black soils of Sariki village (5.6×10^5 CFU g⁻¹ soil), followed by rhizospheric black soils of Konapalli village (5.4×10^5 CFU g⁻¹ soil), rhizospheric red soils of Barlakunta village (5.2×10^5 CFU g⁻¹ soil) and least actinobacteria population was found in rhizospheric red soils of Hanuvayapalle village (3.2×10^5 CFU g⁻¹ soil) (Fig. 4.4).

Rhizospheric soil organisms include spore-forming and Gram-negative bacteria, actinobacteria and fungi, but only a small percentage (1%) are culturable (Bakken, 1997). The actinobacteria counts showed small variations in different soil types ranging from 79.6 to 88.0 $\times 10^6$ CFU g⁻¹ of dry soil and higher numbers of actinobacteria were found when incubated at 30°C rather than 25°C (Vieira and Nahas, 2005).

4.2 QUALITATIVE AND QUANTITATIVE ESTIMATION OF PHOSPHORUS SOLUBILIZING BACTERIAL (PSB) ISOLATES

4.2.1 Qualitative Estimation of PSB Isolates

A total of 16 PSB isolates were isolated. Among 16 PSB isolates all the isolates were able to solubilize phosphate on Pikovskaya's media containing tri calcium phosphate (TCP) and solubilization ranged from 5.3 mm to 27.3 mm (Table 4.4 and Fig. 4.5). Among the isolates, SSP1 was found with the highest solubilization zone (27.3 mm), followed by SKP1 (22.3 mm) and least was observed in KVP1 (5.3 mm) (Plate 4.1).

The phosphate solubilization index (PSI) for the 16 PSB isolates ranged from 2.62 to 6.61. Isolate BKP2 had the highest PSI (6.61) followed by SSP1 (5.87), SBP1 (5.60) and the least PSI was observed in the isolate BHP1 (2.62).

Dipak and Sankar (2016) reported that PSB was effective with PSI of 2.85 in Pikovskaya's agar plates and a high soluble phosphate production of $219.64 \pm 0.33 \mu\text{g m l}^{-1}$ in liquid medium. Karpagam and Nagalakshmi (2014) isolated 37 microbial isolates. Out of 37 microbial isolates, eight isolates were with highest PSI that ranged from 1.13-3.0. Among these eight potent isolates, three strains showed maximum PSI of psm1, psm2 and psm6 in agar plates with highest phosphate production of 0.37 mg l^{-1} , 0.30 mg l^{-1} and 0.28 mg l^{-1} in broth culture.

Tripti *et al.* (2012) isolated 50 PSB colonies on Pikovskaya's (PKV) agar medium, containing insoluble TCP. Out of 50 bacterial isolates, 10 isolates showed highest PSI ranged from 1.4-3.0. The PSI of rice isolates varied from 1.2 to 6.7. The isolate BR-25 exhibited the highest PSI (6.7) followed by BR-15 (4.8) when calcium phosphate was used as P source. (Thakuria *et al.*, 2004).

Baig *et al.* (2010) showed that the most efficient P-solubilizing in the NBRIP plates had PSI of 2.88. Our results were in accordance with those of Yang *et al.* (2012) who reported increased halo zone with the increase in colony diameter.

4.2.2 Quantitative Estimation of PSB Isolates

All the isolates were able to solubilize phosphate quantitatively in Pikovskaya's broth containing TCP and ranged from 189.5 to 41.3 mg l⁻¹ (Table 4.5 and Fig. 4.6). Among 16 PSB isolates, BKP2 recorded the highest phosphate solubilization quantitatively (189.5 mg l⁻¹), followed by SSP1 (174.6 mg l⁻¹), SBP1 (146.2 mg l⁻¹) and least was observed by BBP1 (41.3 mg l⁻¹).

Manouchehr *et al.* (2016) reported maximum P solubilization to occur in broth. They reported a positive effect on total P solubilization as solubilizing efficiency increased by 373 mg l⁻¹. Panda *et al.* (2013) isolated 26 best PSB based upon their solubilization on solid media from 104 rhizospheric samples collected from three agroclimatic zones. Phosphate solubilization of these isolates varied from 46 to 160% and 30.2 to 203.7 mg l⁻¹ in solid and liquid Pikovskaya's medium respectively.

Parani and Saha (2012) evaluated *Pseudomonas fluorescens* K-34 that solubilized TCP and produced substantial amount of soluble phosphorus (968.5 mg l⁻¹) in PVK broth. Chatli *et al.* (2008) reported that SBC5 (*Bacillus* sp.) and SBC7 (*Bacillus* sp.) bacterial isolates exhibited maximum P solubilisation of 40 and 33 µg ml⁻¹ respectively. Joshi and Bohra (2011) isolated 72 rhizobacterial isolates from mungbean rhizospheric soil on King's B medium, from various locations near Jodhpur. Four isolates solubilized phosphate ranging from 42.69 µg TCP ml⁻¹ to 90.10 µg TCP ml⁻¹. Park *et al.* (2011) resulted that PSB isolated from P amended soils solubilized 217-479 mg l⁻¹ of P.

4.3 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF PSB ISOLATES

4.3.1 Morphological Characterization of PSB Isolates

A total of sixteen PSB isolates were isolated, culturally and morphologically characterized. The size of the isolates varied from very small to medium, with smooth, glistening colonies except the isolates SBP1 and SCP1 having only smooth

surface with no shiny appearance. The PSB isolates took about 48-72 h incubation to establish their growth on Pikovskaya's agar medium. Out of 16 isolates 13 isolates (BBP1, BSP1, SKP1, BVP1, BVP2, SSP1, BHP1, BHP2, KNP1, BKP2, KGP1, BKP1 and SBP1) were rods while three isolates (SCP1, KVP1 and BBP2) were cocci in their shape (Table 4.6).

The isolates colour varied from creamy white, white, dull white and yellow with convex elevation. Among 16 isolates, 11 isolates (BBP1, BBP2, SKP1, BVP1, BVP2, BHP1, BHP2, KGP1, BKP1, BKP2 and SBP1) showed Gram negative reaction and five isolates (SSP1, BSP1, KVP1, KNP1 and SCP1) showed Gram positive reaction. Out of 16 isolates, 13 isolates were non sporulating (BBP1, BBP2, SKP1, BVP1, BVP2, KVP1, BHP1, BHP2, BKP2, KGP1, BKP1, SCP1 and SBP1) and three isolates (BSP1, SSP1 and KNP1) were sporulating.

Gaur *et al.* (1973) studied the bacterial morphological, cultural and physiological and biochemical characteristics using the manual of microbiological methods and identified the organism *Bacillus* sp. using *Bergey's Manual of Determinative Bacteriology*. The morphological and biochemical characters of phosphobacteria were found to be Gram negative with rod shaped and non motile characteristics (Amutha *et al.*, 2014).

Sharma (2011) observed the morphological features (shape, size, elevation, surface form, margins and surface texture, color) of the isolated PSBs for their characterization. The isolates having the morphological characters like colorless non-pigmented colonies and Gram negative, rod shaped, based on the biochemical reactions was identified as *Pseudomonas* sp. The isolate showing slimy, white colonies with irregular margins, having Gram positive character and on the basis of biochemical reactions the isolate was identified as *Bacillus megaterium*.

Cappuccino and Sherman (1992) characterized 35 rhizobacterial isolates biochemically and found them to be positive for oxidase test, catalase, citrate utilization and nitrate reduction but negative for Methyl Red and Voges-Proskauer tests. He reported that 28.6% belonged to *Pseudomonas* sp.

Preeti *et al.* (2011) isolated 28 bacterial cultures from Korea and characterized. Four isolates were identified to be *Pseudomonas* sp. and others as *Bacillus subtilis*.

4.3.2 Biochemical Characterization of PSB Isolates

With the morphological and cultural characteristics, the isolates were further characterized with different biochemical tests viz., IMVIC test, nitrate reduction, motility test, citrate utilization, oxidase test, catalase test, carbohydrate utilization test, H₂S production, gas and acid production, starch hydrolysis, gelatin liquefaction (Plate 4.2 and Plate 4.3).

Results presented in table 4.7 reveal that 13 PSB isolates were positive for indole production (BSP1, SKP1, BVP1, BVP2, SSP1, KVP1, BHP1, BHP2, KNP1, BKP1, BKP2, SBP1 and SCP1). To methyl red test, eight isolates were positive (BSP1, BVP1, BVP2, KVP1, BHP2, KNP1, BKP1 and SCP1) while nine isolates were positive to Voges Proskauer's test (BBP2, SKP1, BVP1, SSP1, BHP1, KNP1, BKP1, SBP1 and SCP1). Ten isolates could reduce nitrate (BBP1, BSP1, BVP1, SSP1, BHP1, KNP1, KGP1, BKP2, SBP1 and SCP1). To motility test, nine isolates (BSP1, SKP1, BVP2, SSP1, KVP1, BHP2, BKP1, BKP2 and SBP) were positive. In citrate utilization 11 isolates (BBP1, BBP2, BSP1, BVP1, SSP1, KVP1, BHP2, KGP1, BKP2, SBP1 and SCP1) were positive.

In catalase test 10 isolates (BBP1, BBP2, SKP1, BVP1, SSP1, KVP1, BHP2, KGP1, BKP2 and SBP1) were found positive. In oxidase test 11 isolates (BBP1, BBP2, SKP1, BVP1, SSP1, KVP1, BHP2, KNP1, KGP1, BKP2 and SCP1) were positive. During starch hydrolysis 8 isolates (BBP1, BBP2, BVP1, SSP1, KVP1, BHP2, KGP1 and SBP1) were found to hydrolyze starch. For gas and acid production test six isolates (BBP2, BVP2, KVP1, KGP1, BKP2 and SBP1) were positive.

In carbohydrate utilization test glucose, maltose and lactose sugars were used and results were recorded. Glucose was utilized by 11 isolates (BBP1, BBP2, BSP1, SKP1, BVP1, SSP1, KVP1, BHP2, BKP1, SBP1 and SCP1). Maltose was utilized by seven isolates (BBP2, BSP1, BVP1, SSP1, BHP1, KNP1 and SBP1). Lactose was utilized by nine isolates (BBP1, SKP1, BVP2, SSP1, BHP1, KNP1, KGP1, BKP1 and SCP1). In gelatin liquefaction eight isolates (BBP2, BSP1, SSP1, BHP1, KNP1, BKP1, BKP2 and SCP1) were positive. To H₂S production six isolates (SKP1, BVP2, KVP1, BHP2, KGP1 and BKP2) were positive.

BKP2, SSP1 and SBP1 isolates were found superior over other isolates regarding phosphorous solubilizing ability and these isolates were identified upto genus level based on these biochemical tests.

Patil *et al.* (2001) stated a marked difference in the carbohydrate utilization between the species of PSB. Jayamma *et al.* (2013) isolated 57 bacterial isolates belonging to *Bacillus* and *Pseudomonas* from different rhizospheric soils of southern Telangana zone. These isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits. Dave (1999) reported glucose as best carbon source followed by galactose and sucrose for TCP solubilization for *Pseudomonas fluorescens*. Gaur (1990) reported glucose as best source followed by sucrose and galactose in phosphate solubilization by *P. straita*.

Yasmin and Bano (2011) isolated PSB from rhizosphere soil of various plant species. The morphology of the bacteria formed on the PVK agar plates was observed after 24 h.

Kumari *et al.* (2013) isolated 42 rhizobacterial isolates and identified them as *Pseudomonas* (19), *Bacillus* (22) and *Serratia* (1). Anitha and Kumudini (2014) isolated *Pseudomonas* from 15 rhizospheric samples from the field's of rice, chilly, ragi, beans and garden soils from different regions of India and characterized them morphologically and biochemically and concluded as genus *Pseudomonas*. Similar results were reported by Wasi *et al.* (2010) and characterized them on the basis of morphological, cultural and biochemical properties.

4.4 SCREENING OF PURE ISOLATES FOR PGPR PROPERTIES

Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. They are reported to influence the growth, yield and nutrient uptake both by direct and indirect mechanisms. Bacteria are most abundant among the PGPR. The use of PGPR offers an attractive way to replace chemical fertilizers, pesticides. PGPR also modify root functioning, improve plant nutrition and influence the physiology of the whole plant. Results presented in table 4.8 reveal the PGPR properties of pure isolates (Plate 4.4).

4.4.1 Phosphate Solubilization

Phosphate solubilization was observed by all the isolates of which strong (+++) phosphate solubilization was recorded by 12 isolates (SKP1, SSP1, KGP1, BKP2, KVP1, BHP1, BSP1, KNP1, BKP1, BHP2, SBP1 and SCP1). Moderate (++) phosphate solubilization was noticed by four isolates (BBP1, BBP2, BVP1 and BVP2).

Verma *et al.* (2010) reported phosphate solubilization by PGPR spp. from chickpea rhizosphere whereas largest halo zone produced by *Pseudomonas* sp. was documented by Minaxi *et al.* (2011) in cowpea rhizosphere. Song *et al.* (2008) stated that the PSE depends on other factors such as substrate, medium, temperature, time, and other mechanisms in addition to organic acid production. However, the acidification of culture supernatants seemed to be generally the main mechanism for phosphate solubilization. Six PSB were isolated from paddy fields of Eastern Uttar Pradesh, India and identified as members of *Enterobacter* and *Exiguobacterium* genera. Out of the six isolates, *Enterobacter* sp. exhibited a high level of phosphate solubilization in the liquid medium (Kumar *et al.*, 2010).

Alemu (2013) isolated twelve phosphate solubilizing bacterial species from rhizospheric soils of faba bean and tested for phosphate solubilization. All tested isolates have a potential of phosphate solubilization on Pikovskaya's media. He summarized that all phosphate solubilizing bacterial species can be used as bio-fertilizers for soil fertility improvement. Roy *et al.* (2013) isolated 31 *Pseudomonas* sp. from rhizospheric soil of rice. Out of 31, nine isolates were able to solubilize $\text{Ca}_3(\text{PO}_4)_2$ on Pikovskaya's agar medium. Isolate MGR38 was identified as *P. fluorescens* which showed 83.33% efficiency in phosphate solubilization and the other isolates MGR31, MGR37, MGR39, MGR40, MGR41, MGR42, MGR43 and MGR44 showed 33.33, 57.14, 50.00, 50.00, 16.16, 40.00, 60.00 and 80.00% efficiency of phosphate solubilization respectively.

4.4.2 Siderophore Production

Strong (+++) siderophore production was noticed by two isolates (SSP1, SBP1), moderate (++) siderophore production was recorded by three isolates (BSP1, KGP1 and BKP2), weak (+) siderophore production was observed by four isolates (SKP1, BVP2, BHP1 and BKP1) and siderophore production was not noticed (–) by seven isolates (BBP1, BBP2, BVP1, KVP1, BHP2, KNP1 and SCP1).

Sayyed *et al.* (2005) reported that siderophores are known to chelate with iron and other metals and contribute to disease resistance by limiting the supply of essential trace minerals in natural habitats. Siderophores may directly stimulate the biosynthesis of other antimicrobial compounds by increasing the availability of these minerals to bacteria and can play an important role in systematic host resistance.

Siderophores are the agents having high affinity for iron and are usually secreted by almost all the microorganisms in response to the iron deficiency. Therefore, under iron limitations, the siderophores serves as the solubilizing agents for iron from mineral or organic complexes. Nearly 500 known siderophores are being exclusively employed by the microbial species and the strains which produce them. The excretion of the siderophores has also been found in the P solubilizing microorganisms (Vassilev *et al.*, 2006). Hamdali *et al.* (2008) isolated phosphate solubilizing strains from Moroccan phosphate mine and showed that the isolated strains produce siderophores but not organic acids.

Kannahi and Kowsalya (2013) evaluated on efficiency of PGPR for the enhancement of *Vigna mungo* growth. They concluded that the isolates of *pseudomonas* species were strong siderophore production while few isolates of *Bacillus* were able to produce siderophore. Souza *et al.* (2013) isolated 336 bacterial strains belonging to the genera *Agrobacterium*, *Burkholderia*, *Enterobacter*, and *Pseudomonas* spp. Siderophore and indole compound producers were widely found among isolates, but one hundred and one isolates were able to solubilize phosphate.

4.4.3 Ammonia Production

The results revealed strong (+++) ammonia production by two isolates (BVP2, BKP2), moderate (++) ammonia production was noticed by four isolates (SSP1, BHP1, KNP1 and SBP1), weak (+) ammonia production was shown by eight isolates (BBP1, BBP2, SKP1, BVP1, KVP1, BHP2, KGP1 and SCP1) and ammonia production was not noticed by two isolates (BSP1 and BKP1).

Prasad *et al.* (2013) isolated and identified 26 *Pseudomonas* sp. and all *Pseudomonas* isolates were reported to be positive for IAA production, 76.9% were phosphate solubilizing, 92.3% could produce ammonia and 88.46% in siderophores. Production of ammonia is an important attribute of PGPR that influences plant growth indirectly (Wani *et al.* 2007).

Joseph *et al.* (2007) revealed the production of ammonia commonly detected in the isolates of *Bacillus* (95%) followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%). Chacko *et al.* (2009) isolated the *P. putida* from the rhizosphere of *Pisum sativum* that exhibited a battery of PGPR characteristics found to produce ammonia. Pavlica *et al.* (1978) concluded that ammonia is the only gas present in sufficient concentration in soil to inhibit soil fungus.

4.4.4 Hydrogen Cyanide (HCN) Production

Moderate (++) HCN production was noticed by five isolates (BBP2, BVP2, SSP1, BKP2 and SBP1), weak (+) HCN production was observed by seven isolates (BBP1, SKP1, BVP1, KVP1, BHP2, KNP1 and BKP1) and HCN production was not noticed by four isolates (BSP1, BHP1, KGP1 and SCP1).

Siddiqui and Shakeel (2009) reported HCN production by 21 *Pseudomonas* isolates from pigeonpea rhizosphere, out of which 11 were moderate producers but three of them were potent producers of HCN. Selvakumar *et al.* (2009) gave evidence in support of our results where *P. fragi* CS11RH1, a psychotolerant bacterium produced HCN and seed bacterization with the isolate significantly increased the percent germination and rate of germination, plant biomass and nutrient uptake of wheat seedlings.

Bakker and Schippers (1987) observed that nearly 50% of the Pseudomonads from potato and wheat rhizospheres produce HCN which has a primary mechanism in suppression of root fungal pathogens. Karmel *et al.* (2014) isolated, purified indigenous *Pseudomonas* spp. and *Bacillus* spp. and evaluated its ability to produce HCN. Results showed that among these isolates on qualitative analysis of HCN indicated a strong production of HCN in Pf1 and CPf5. Ahmadzadeh and Sharirif-tehrani (2009) identified the production of HCN by six isolates out of the 41 selected Pseudomonads.

HCN is a secondary metabolite implicated in plant protection. Thus the ability to produce HCN is a desired quality of plant growth promoting rhizobacteria. By synthesizing HCN some rhizobacteria inhibit plant disease development thus strengthening the host's disease resistance mechanism (Schippers *et al.*, 1990). The

presence of HCN in the soil can also act as an efficient biological weed control measure by inhibiting seed germination and seedling vigor. The strain TRSB16 was found to be a strong HCN producer.

4.4.5 Indole Acetic Acid (IAA) Production

Moderate (++) IAA production was observed by five isolates (BVP1, SSP1, BHP1, BKP2 and SBP1), weak (+) IAA production was noticed by eight isolates (BSP1, SKP1, BVP2, KVP1, BHP2, KNP1, BKP1 and SCP1) and IAA production was noticed by three isolates (BBP1, KGP1 and BBP2).

Hossain *et al.* (2016) isolated ten isolates of bacteria designated as SS01, SS02, SS03, SS04, SS05, SS06, SS07, SS08, SS09 and SS10 from rhizosphere of chickpea. All the isolates produced IAA in various ranges. On the contrary, SS01 was found to be a weak IAA producer in comparison to the minimum IAA producer isolates viz. SS02, SS03, SS04, SS05 and SS09. SS06 and SS07 produced the medium quantity IAA producer. On the other hand, only SS10 produced the highest IAA among the isolates.

Sanjani *et al.* (2011) reported that PSB (*Bacillus* sp., *Pseudomonas* sp.) were positive reaction to IAA production. Sagervanshi *et al.* (2012) studied on five strains of PSB and reported that two isolates were Gram negative, round shape and the two isolates were positive reaction to IAA production. Rhizobacterial IAA production plays a significant role in the host plant's growth. IAA production in microbial has been investigated by several researchers (Gulati *et al.*, 2009).

Tien *et al.* (1979) stated that PSB isolated from rhizosphere soil of pearl millet produced IAA which enhanced the plant metabolism. However, production of IAA varied greatly among different species of bacteria and mainly influenced by culture conditions, growth stage and availability of substrate (Vijila, 2000) which is in agreement with the results of the present studies.

4.4.6 Antagonistic Activity of PSB Isolates Against *Rhizoctonia solani* by Dual Culture Method

All the sixteen isolates inhibited *Rhizoctonia solani*. Maximum per cent inhibition was observed in the isolate BKP2 (51.49%), followed by isolate SSP1 (44.34%), SBP1 (42.71%) and the least per cent inhibition was recorded in isolate BHP2 (25.68%) (Table 4.9 and Fig. 4.7).

Mezeal (2014) studied the antagonistic microorganisms against the pathogens by dual culture technique. *P. fluorescens* isolate was found to show 81.3% and 77.4% of growth inhibition against *R. solani* and *Fusarium oxysporum* respectively while *B. subtilis* was found to show 77.4% and 73.2% of growth inhibition against test pathogens respectively. Soltani *et al.* (2012) reported three isolates of pseudomonads (PA24, PA1, PA18) to have antifungal activity against *R. solani* under *in vitro* conditions. Results showed that Pseudomonads had the potential to be used for promotion of plant growth and suppression of soil borne plant pathogens. Ahmadzadeh and Sharirifi-tehrani (2009) detected the production of HCN by six isolates out of the 41 selected fluorescent Pseudomonads having good antagonistic activity against *R. solani* under *in vitro* conditions.

4.5 POT CULTURE EXPERIMENT FOR THE STUDY OF EFFECT OF PSB ON GROWTH AND YIELD ATTRIBUTES OF FINGER MILLET

The three efficient PSB strains *i.e.*, PSB1, PSB2 and PSB3 were selected based on their phosphorus solubilizing ability of isolates qualitatively as well as quantitatively and by determining the morphological, biochemical and plant growth promoting rhizobacterial properties of isolates. They were identified as *Pseudomonas* sp. (isolate name, BKP2) *Bacillus* sp. (isolate name, SSP1) and *Enterobacter* sp. (isolate name, SBP1).

The experiment was conducted during *kharif*, 2015-16 with Completely Randomized Design, in the green house at Agriculture Research Station, Amaravathi, Guntur district (Plate 4.5) and the results are presented and discussed here. Experimental data was statistically analyzed, further it is furnished in tables and illustrated through figures wherever necessary.

4.5.1 Influence of PSB Isolates on Total Microbial Poulation at Various Stages of Finger millet Crop Growth

4.5.1.1 Enumeration of bacteria (CFU $\times 10^9$ g⁻¹ of soil): Bacterial population count at initial, 50% flowering and harvesting stages of crop growth is given in table 4.10 and fig. 4.8. Bacterial population at initial stage was found significantly highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 1.24 $\times 10^9$ CFU g⁻¹ soil followed by 1.18 $\times 10^9$ CFU g⁻¹ soil with T₂ (75% RDP+PSB₁), T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 1.17 $\times 10^9$ CFU g⁻¹ soil and least was found with T₁₀ (control) 1.0 $\times 10^9$ CFU g⁻¹ soil.

Bacterial population count at 50% flowering stage of crop growth was highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 2.91×10⁹ CFU g⁻¹ soil and T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 2.91×10⁹ CFU g⁻¹ soil, followed by T₂ (75% RDP+PSB₁) 2.83 ×10⁹ CFU g⁻¹ soil, T₃ (75% RDP+PSB₂) 2.81×10⁹ CFU g⁻¹ soil and least was found with T₁₀ (control) 2.43×10⁹ CFU g⁻¹ soil.

Bacterial population at harvesting stage of crop growth was significantly highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 2.74×10⁹ CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 2.65×10⁹ CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 2.63 CFU×10⁹ g⁻¹ soil and least was found with T₁₀ (control) 2.07×10⁹ CFU g⁻¹ soil.

Bacterial population significantly reduced at harvesting stage of crop growth because of reduction in available nutrients and there was no much root exudates from plants as compared to flowering stage. The results showed that bacterial biomass is highly correlated with soil organic matter content, availability of root exudates and nutrients in soil. These results were in accordance to Pankhurst *et al.* (1995), who reported that flowering stage increases the bacterial population in rhizosphere.

The combined application of biofertilizers and inorganic fertilizers having higher bacterial population as compared to inorganic fertilizers alone. This may be due to the rhizospheric effect, which provides a rich source of energy and nutrients to the bacteria resulting in higher bacterial populations. It also enhances availability of root exudates and growth promoting substances during growth of crop. The similar results were observed by Gray and Smith (2005).

The present study revealed that there was an increasing trend regarding total bacterial population till the flowering stage of crop and decreased thereafter, irrespective of treatment combinations. This may be due to enhanced availability of root exudates and promoting substances during flowering growth stage of crop (Chu *et al.*, 2007). High soil microbial population during flowering stage may also be attributed to sufficient moisture availability due to proper irrigation. The result is in consistence with earlier researcher (Singh *et al.*, 1989). The similar results were observed by Zhong and Cai (2007).

4.5.1.2 Enumeration of PSB (CFU×10⁸ g⁻¹ of soil): PSB population count at initial, 50% flowering and harvesting stages of crop growth is given in table 4.11 and fig. 4.9. PSB population at initial stage was found significantly highest in the treatment T₅

(75% RDP+PSB₁+PSB₂+PSB₃) 1.54×10^8 CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 1.49×10^8 CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 1.47×10^8 CFU g⁻¹ soil and least was found with T₁₀ (control) 1.06×10^8 CFU g⁻¹ soil.

PSB population count at 50% flowering stage of crop growth was highest in the treatments T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 3.54×10^8 CFU g⁻¹ soil followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 3.44×10^8 CFU g⁻¹ soil, T₂ (75% RDP+PSB₁) 3.24×10^8 CFU g⁻¹ soil and least was found with T₁₀ (control) 2.73×10^8 CFU g⁻¹ soil.

PSB population at harvesting stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 3.11×10^8 CFU g⁻¹ soil followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 2.96×10^8 CFU g⁻¹ soil, T₂ (75% RDP+PSB₁) 2.92×10^8 CFU g⁻¹ soil and least was found with T₁₀ (control) 2.52×10^8 CFU g⁻¹ soil.

Ponmurugan and Gopi (2006) reported that the population density of phosphobacteria in the rhizosphere soils of brinjal, chilly, cotton, green gram, groundnut, maize, paddy, ragi, sorghum and turmeric using Ketznelson and Bose medium following dilution plate technique. The result showed that the population levels of phosphobacteria were higher in the rhizosphere soil of groundnut plant (14.90×10^5 CFU g⁻¹ soil dry wt.) and in ragi (07.33×10^5 CFU g⁻¹ soil dry wt.). This variation in the population of PSB might be attributed to many soil factors such as soil nutrients, pH, moisture contents, organic matter and some soil enzyme activities. Chand *et al.* (2006) reported that the PSB population was directly related with phosphorus content in soil and higher doses of biofertilizers significantly increased the PSB population.

Kucey (1983) reported the occurrence of phosphate solubilizing bacteria in Alberta soils that ranged between 1.4×10^4 to 11.5×10^4 CFU g⁻¹ of soil in five districts of Marathwada region respectively. Further, population studies on phosphate-solubilizing bacteria revealed that the population gradually increased from 30 days and reached maximum level at 60 days in most cases which is in agreement with the reports of Bilolikar *et al.* (1996). PSB populations lesser than 1×10^2 g⁻¹ of soil have also been reported in soil samples from northern Spain (Peix *et al.*, 2001). Thus many reports on population densities of PSMOS in different soils have been studied which is in close agreement with the population densities of PSB observed in the present study.

4.5.1.3 Enumeration of fungi (CFU×10³ g⁻¹ of soil): Fungal population count at initial, 50% flowering and harvesting stages of crop growth is given in the table 4.12 and fig. 4.10. Fungal population at initial stage was found significantly highest in the treatment T₅ (75% RDP+PSB₁ +PSB₂+PSB₃) 2.65×10³ CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 2.63×10³ CFU g⁻¹ soil, T₃ (75% RDP+PSB₂) 2.35×10³ CFU g⁻¹ soil which is equal to T₆ (50% RDP+PSB₁) 2.35×10³ CFU g⁻¹ soil and least was found with T₁₀ (control) 1.33×10³ CFU g⁻¹ soil.

Fungal population count at 50% flowering stage of crop growth was found highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 5.33×10³ CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 5.10×10³ CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 4.66×10³ CFU g⁻¹ soil and least was found with T₁₀ (control) 2.63×10³ CFU g⁻¹ soil.

Fungal population at harvesting stage of crop growth was found highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 4.35×10³ CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 3.65×10³ CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 3.36×10³ CFU g⁻¹ soil and least was found with T₁₀ (control) 1.66×10³ CFU g⁻¹ soil.

In soil, P-solubilizing fungi constitute about 0.1-0.5% of total fungal populations (Kucey, 1983). Moreover, P-solubilizing fungi do not lose the P dissolving activity upon repeated sub culturing under laboratory conditions as occurs with the P-solubilizing bacteria (Sperber, 1958). Nakhro and Dkhar (2010) concluded that fungal population has significantly decreased at harvest stage of crop growth when compared to other time intervals. The lower fungal biomass was reported due to the insufficient availability of nutrients at the time of harvest stage of the crop. They further stated that soil fungal population to be highly correlated with soil organic matter, available root exudates and N fertilizer.

Yevdokimov *et al.* (2008) found that the addition of increasing amounts of N fertilizer increase soil fungal biomass significantly. This phenomenon explained the increase in fungal biomass with the increase in recommended dose of N fertilizer (Zhong *et al.*, 2010).

4.5.1.4 Enumeration of actinobacteria (CFU×10⁵ g⁻¹ of soil): Actinobacteria population count at initial, 50% flowering and harvesting stages of crop growth is given in the table 4.13 and fig. 4.11. Actinobacteria population at initial stage was found significantly highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃)

3.64×10^5 CFU g⁻¹ soil followed by T₂ (75% RDP+PSB₁) 3.63×10^5 CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 3.35×10^5 CFU g⁻¹ soil and least was found with T₁₀ (control) 1.65×10^5 CFU g⁻¹ soil.

Actinobacteria population count at 50% flowering stage of crop growth was highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 5.65×10^5 CFU g⁻¹ soil followed by T₈ (50% RDP+PSB₃) 5.63×10^5 CFU g⁻¹ soil, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 5.33×10^5 CFU g⁻¹ soil and least was found with T₁₀ (control) 4.09×10^5 CFU g⁻¹ soil.

Actinobacteria population at harvesting stage of crop growth was significantly highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 4.66×10^5 CFU g⁻¹ soil and T₈ (50% RDP+PSB₃) 4.66×10^5 CFU g⁻¹ soil followed by T₃ (75% RDP+PSB₂) 4.64×10^5 CFU g⁻¹ soil and least was found with T₁₀ (control) 2.63×10^5 CFU g⁻¹ soil.

Increase of competition between slow growing actinobacteria and other microbes caused decrease in population of actinobacteria at harvesting stage of crop growth as compared to flowering stage (Zhong *et al.*, 2010). Hamdali *et al.* (2008) has indicated that approximately 20% of actinobacteria can solubilize P, including those in the common genera *Streptomyces* and *Micromonospora*. Actinobacteria population decreased with biofertilizer inoculation. This may be due to production of antibiotics such as 2,4-diacetylphloroglucinol (antifungal properties) by inoculated PGPR which inhibits the growth of actinobacteria. Hence antifungals and antibiotics, when produced in higher quantities suppress the bacterial metabolism that results in the decrease of actinobacterial population. The result was in accordance with Roesti *et al.* (2006) who showed that certain PGPR strains exhibit antifungal properties.

4.5.2. Influence of PSB Isolates on Growth and Yield Attributes of Finger millet Crop Growth

4.5.2.1 Root length (cm): Highest root length was found with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 23.1cm which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 21.8 cm, T₂ (75% RDP+PSB₁) 21.4 cm and least was found with T₁₀ (control) 17.3 cm (Table 4.14 and Fig. 4.12) Plate (4.7).

Srivastava and Chaudhary (1993) reported an increase of 31.10, 25.02, 24.37 and 22.99% in root length of sorghum plants due to inoculation with PSBV-14, PSBV-4, PSBV-11 and PSBV-8 respectively over RP control and are comparable with the results of Hoeflich and Ruppel (1994). Aipova *et al.* (2010) reported the inoculation of PSB enhanced the radicle and root length of wheat as compared to individuals. This could be because of higher amount of growth promoting substances released by inoculants.

The inoculation of PSB in ragi and pearl millet proved the potential to increase plant height, numbers of tillers, root length and shoot length. This report is similar to the earlier report of Sankaralingam *et al.* (2014) who stated that the inoculation of PSB on *Sesbania grandiflora* and *Moringa oleifera* showed excellent growth, plant height, numbers of tillers, root length and shoot length. Gomaa and kholas (1999) reported that the root length in mungbean was increased with the combined effect of organic manure, chemical fertilizers and biofertilizers. Hariprasad and Niranjana (2009) isolated 16 phosphate solubilizing rhizobacterial isolates, the strain PSRB19 showed significant increase in shoot length (14.0 cm), root length (18.0 cm), fresh mass (0.762 g seedling⁻¹) and dry mass (0.110 g seedling⁻¹) followed by isolate PSRB8 as compared to control.

4.5.2.2 No.of fingers per plant: Number of fingers per plant was found highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 10.0 which was significant over other treatments followed by T₉ (50% RDP+PSB₁ +PSB₂+PSB₃) 9.6 and the least was found with T₁₀ (control) 6.6 that is depicted in table 4.14 and fig. 4.13 and Plate 4.6.

Kumar *et al.* (2003) stated that application of RDF recorded significantly lower and significantly higher fingers per earhead than FYM @ 5 t ha⁻¹ plus RDF and application of 10 t of FYM ha⁻¹ along with biofertilizers. Significantly maximum number of fingers per earhead (8.1) was obtained in finger millet with conjunctive use of recommended RDF (100:50:50 kg N, P₂O₅, K₂O ha⁻¹) + 7.5 t FYM ha⁻¹ compared to recommended RDF (4.7) alone (Kumara *et al.*, 2007).

4.5.2.3 Grain yield (kg ha⁻¹): Highest grain yield was found in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 4031.9 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 3720.8 kg ha⁻¹, T₂ (75% RDP+PSB₁) 3484.4 kg ha⁻¹ and least was found with T₁₀ (control) 2812.4 kg ha⁻¹ (Table 4.14 and Fig. 4.14).

Thilakarathna *et al.* (2015) found that in finger millet, application of 50% of the recommended inorganic fertilizers, gliricidia green leaf manure and biofertilizers (*Azotobacter* and PSB) produced highest grain yield (3.95 t ha^{-1}) compared to farmers traditional practice (1.76 t ha^{-1}). Afsal and Bano (2008) stated that 30-40% more efficiency of PSB strains as biofertilizer increased the growth and grain yield of wheat (*Triticum aestivum*).

Apporva *et al.* (2010) stated the highest grain yield in finger millet (3740 kg ha^{-1}) was recorded with the application of fertilizers and FYM on STCR basis along with bio fertilizers which differed significantly over control (2649 kg ha^{-1}). The results indicate that higher yield target may be achieved through integrated supply of nutrients from different sources. Hameeda *et al.* (2006) reported that inoculation of maize seed with PSB under greenhouse and field conditions increased yield and yield components of maize. These results were in accordance with Yazdani *et al.* (2009).

Balochgharayi (2011) stated that application of beneficial microbes (*Pseudomonas*) significantly increased maize yield over control. Increase in maize yield with PSB inoculation may be due to the promotion of root growth which in turn enhancing nutrients and water uptake from the soil (Lin *et al.*, 1983).

4.5.2.4 Straw yield (kg ha^{-1}): Straw yield was found to be significantly highest in the treatment T_5 (75% RDP+PSB₁ +PSB₂+PSB₃) $4691.5 \text{ kg ha}^{-1}$ followed by T_9 (50% RDP+PSB₁+PSB₂+PSB₃) $4567.1 \text{ kg ha}^{-1}$, T_2 (75% RDP+PSB₁) $4417.7 \text{ kg ha}^{-1}$ and the least was found with T_{10} (control) $3646.2 \text{ kg ha}^{-1}$ (Table 4.14 and Fig. 4.14).

Govindappa *et al.* (2009) reported that the combined application of organic and inorganic manures significantly increased the growth and yield of finger millet. Application of 100% RDF+ FYM @ 7.5 t ha^{-1} (T_8) produced significantly higher grain yield (3660 kg^{-1}) and straw yield (6610 kg^{-1}) over rest of the treatments. This higher yield levels may be attributed to superiority of nutrient status and better response of finger millet to poultry manure compared to other organic manures.

Grain and straw yields of wheat were also significantly influenced with the interaction between P levels and PSB inoculants with all soils. Saad and Hammad (1998) reported that the greatest grain and straw yields of wheat was found with inoculation of bacteria and application of calcium superphosphate. These results were also in conformity with the findings of Dwivedi *et al.* (2004).

4.5.2.5 Phosphorus content in plant sample (%): Phosphorus content in plant sample was found to be the highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.183% which was significant over other treatments followed by T₉(50% RDP+PSB₁+PSB₂+PSB₃) 0.176%, T₂ (75% RDP+PSB₁) 0.173% and least was found with T₁₀(control) 0.131% (Table 4.15 and Fig. 4.15).

Inoculation of biofertilizers increased the phosphorus content of pea plants. The increase in phosphorus concentration can be partially attributed to the production of a variety of organic acids by the inoculated PGPR which decrease the soil pH, leading to the conversion of non available form of phosphorus into the available phosphorus. These results were reported by Osman *et al.* (2010).

Afzal and Bano (2008) demonstrated that PSM's in combination with phosphatic fertilizer and organic manure significantly enhanced phosphorus content in plant, tillers per m², grain and biological yield of wheat.

Dey *et al.* (2004) showed that inoculation of phosphate solubilizing *Pseudomonas fluorescens* has been reported to enhance growth, yield, nitrogen and phosphorus concentrations in the shoots and seeds of peanut. The PGPR strains such as *Pseudomonas* and *Bacillus* when seed treated increased the P content in different crop plants such as rice, wheat, sorghum, maize, sugarcane, banana, forage crops and medicinal plants. The increase in plant P content might be due to high availability and better translocation in the plants. The findings are in conformity with that of Joshi *et al.* (2013).

4.5.3 Influence of PSB Isolates on Total Dry Matter Production (g plant⁻¹) of Finger millet

4.5.3.1 Leaf dry weight: The highest leaf dry weight was found in the treatment T₅ (75% RDP+PSB₁ +PSB₂+PSB₃) 5.7 g plant⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 5.5 g plant⁻¹, T₂ (75% RDP+PSB₁) 5.4 g plant⁻¹ and least was found with T₁₀ (control) 4.2 g plant⁻¹ (Table 4.16 and Fig. 4.16).

4.5.3.2 Stem dry weight: Significantly high stem dry weight was observed in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 15.3 g plant⁻¹ when compared over other treatments, followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 14.6 g plant⁻¹, T₂ (75% RDP+PSB₁) 12.9 g plant⁻¹ and the least was found with T₁₀ (control) 11.8 g plant⁻¹ (Table 4.16 and Fig. 4.16).

4.5.3.3 Earhead dry weight: Earhead dry weight was found highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 18.9 g plant⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 18.2 g plant⁻¹, T₂ (75% RDP+PSB₁) 17.8 g plant⁻¹ and the least was found with T₁₀ (control) 15.8 g plant⁻¹ (Table 4.16 and Fig. 4.16).

4.5.3.4 Total dry matter: Total dry matter was found highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 39.4 g plant⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 38.4 g plant⁻¹, T₂ (75% RDP+PSB₁) 35.6 g plant⁻¹ and least was found with T₁₀ (control) 31.3 g plant⁻¹ (Table 4.16 and Fig. 4.16).

The total dry matter content of sorghum plants due to inoculation of PSBV-10, PSBV-3, PSBV-15, PSBV-9 and PSBV-2 increased upto 50.34, 49.99, 47.75, 45.51 and 43.85% respectively over RP control. These results are in agreement with those of Alagawadi and Gaur (1992) who obtained 30% increase in dry matter content of sorghum plants due to inoculation of *Pseudomonas striata*. Similar results on enhanced dry matter content of paddy, sorghum, maize and groundnut due to inoculation with PSB are also reported earlier (Pandey *et al.* 2006).

Alagawadi and Gour (1992) reported that co-inoculation of *B. polymyxa* and *P. striata* strains showing phosphate-solubilizing ability resulted in significant improvement of grain and dry matter yield compared with individual inoculations in sorghum.

Higher total dry matter production was noticed in T₈ (100% RDF+ FYM @ 7.5 t ha⁻¹) 41.80 g plant⁻¹. This higher total dry matter production was attributed to better plant growth, which resulted in higher dry matter accumulation in leaves and stem at earlier growth stages and better translocation to ear head during later stages. Higher dry matter production in leaf (5.5 g plant⁻¹), stem (16.20 g plant⁻¹) and at ear head (25.10 g plant⁻¹) were noticed due to application of 100% RDF + FYM. Results of the current study are in conformity with earlier results of Arunachalam *et al.* (1995).

4.6 RESPONSE OF PSB ISOLATES ON ENZYME ACTIVITIES AND SOIL HEALTH PARAMETERS OF FINGER MILLET

4.6.1 Enzyme Activities

4.6.1.1 Acid phosphatase: Acid phosphatase enzyme activity at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.17 and fig. 4.17. Acid phosphatase enzyme activity at initial stage was highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 61.6 µg pNP g⁻¹ of soil h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 58.6 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 53.3 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 40.4 µg pNP g⁻¹ of soil h⁻¹.

Highest acid phosphatase enzyme activity at 50% flowering stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 86.3 µg pNP g⁻¹ of soil h⁻¹ which was significant with over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 80.6 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 77.5 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 58.8 µg pNP g⁻¹ of soil h⁻¹.

Acid phosphatase enzyme activity at harvesting stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 78.5 µg pNP g⁻¹ of soil h⁻¹ over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 70.5 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 69.5 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 48.5 µg pNP g⁻¹ of soil h⁻¹.

4.6.1.2 Alkaline phosphatase: Alkaline phosphatase enzyme activity at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.18 and fig. 4.18. Alkaline phosphatase enzyme activity at initial stage was highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 47.4 µg pNP g⁻¹ of soil h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 40.7 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 39.4 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 21.6 µg pNP g⁻¹ of soil h⁻¹.

Alkaline phosphatase enzyme activity at 50% flowering stage of crop growth was significantly highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 68.4 µg pNP g⁻¹ of soil h⁻¹, followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 60.4 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 58.3 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 40.5 µg pNP g⁻¹ of soil h⁻¹.

Alkaline phosphatase enzyme activity at harvesting stage of crop growth was found to be highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 57.5 µg pNP g⁻¹ of soil h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁ +PSB₂+PSB₃) 50.4 µg pNP g⁻¹ of soil h⁻¹, T₂ (75% RDP+PSB₁) 47.2 µg pNP g⁻¹ of soil h⁻¹ and least was found with T₁₀ (control) 29.5 µg pNP g⁻¹ of soil h⁻¹.

Phosphatases are the group of enzymes which help in releasing inorganic phosphorus from nucleotides and sugar phosphates and thus can be regarded as organic phospho-ester scavengers. These enzymes have been used in a number of biotechnological applications over the recent past (Rodriguez *et al.*, 2006).

Acidic phosphatases from certain PSB have been reported to work very efficiently as far as mobilization of phosphorus is considered (Rossolini *et al.*, 1998). Barik and Purushothaman (1998) showed that the strain GP12 that was isolated from groundnut soil had higher activity (36.87µ moles⁻¹g⁻¹h) followed by the strain isolated from sorghum soil (32.38µ moles⁻¹g⁻¹h). He stated that there was a positive correlation between phosphate solubilizing capacity and phosphatase activity which might be due to availability of higher amount of phosphorus in the medium and the ability of the strains.

Phosphatase enzyme catalyzes the hydrolysis of a wide variety of phosphomono esters and catalyzes trans phosphorylation reaction by transferring phosphoryl group to alcohol in the presence of certain phosphate acceptors (Coleman, 1992). The provision of organic carbon and stimulation of microbial growth under the combined treatments (biofertilizers + inorganic fertilizers) could have promoted the synthesis of phosphatase enzymes. These results were supported by Saha *et al.* (2008) who showed that organic amendments have positive effects on biochemical properties of soil.

Balakrishnan *et al.* (2007) found that the application of FYM in combination with PSB significantly increased soil microflora such as bacteria, fungi and actinobacteria and soil enzyme activities such as phosphatase and dehydrogenase. Mandal *et al.* (2007) studied the long term effect of manure and phosphorus levels on soil enzyme activities during different crop developmental stages of wheat. They found that application of organic manure in addition to inorganic fertilizers improved the alkaline phosphatase activity status of soil.

4.6.1.3 Dehydrogenase: Dehydrogenase enzyme activity at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.19 and fig. 4.19. Dehydrogenase enzyme activity at initial stage was highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 137.0 µg TPF g⁻¹ of soil day⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 128.0 µg TPF g⁻¹ of soil day⁻¹, T₂ (75% RDP+PSB₁) 120.3 µg TPF g⁻¹ of soil day⁻¹ and least was found with T₁₀ (control) 77.3 µg TPF g⁻¹ of soil day⁻¹.

Dehydrogenase enzyme activity at 50% flowering stage of crop growth was found to be highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 194.3 µg TPF g⁻¹ of soil day⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 187.3 µg TPF g⁻¹ of soil day⁻¹, T₂ (75% RDP+PSB₁) 181.0 µg TPF g⁻¹ of soil day⁻¹ and least was found with T₁₀ (control) 115.6 µg TPF g⁻¹ of soil day⁻¹.

Dehydrogenase enzyme activity at harvesting stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 163.3 µg TPF g⁻¹ of soil day⁻¹ over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 151.3 µg TPF g⁻¹ of soil day⁻¹, T₂ (75% RDP+PSB₁) 142.0 µg TPF g⁻¹ of soil day⁻¹ and least was found with T₁₀ (control) 97.0 µg TPF g⁻¹ of soil day⁻¹.

Dehydrogenase activity was reported in viable cells and was considered to reflect the total range of oxidative activity of soil microflora. Hence dehydrogenase activity was considered to be a good indicator of microbial activity (Nannipieri *et al.*, 1990). Kumar *et al.* (2013) concluded that dehydrogenase activity as measure for microbial oxidative activity in soil. The enzyme activities in the soil was reported to be closely related to the organic matter content.

Similar results were reported by Beyer *et al.* (1993) where enzyme activities in the soil corresponded with organic matter content. Significant increase in dehydrogenase activity with biofertilizer inoculation was reported due to the enhancement of colonization of rhizospheric bacteria with plant roots that lead to accumulation of available nutrients stimulating the growth of microorganisms in rhizosphere which supplement to the soil dehydrogenase pool. Similar trend of results was observed by Garcia *et al.* (2000).

Balanced fertilization (organic and inorganic combined) resulted in higher microbial metabolic activity than sole inorganic fertilization. Results were in accordance with findings of Chu *et al.* (2007) who studied soil dehydrogenase activity in response to long-term fertilizer management. They found that dehydrogenase activity significantly increased with addition of organic and inorganic fertilizers over uninoculated control.

4.6.1.4 Urease: Urease enzyme activity at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.20 and fig. 4.20. Urease enzyme activity at initial stage was highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 1476.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 1406.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹, T₂ (75% RDP+PSB₁) 1393.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ and least was found with T₁₀ (control) 1006.6 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹.

Highest urease enzyme activity at 50% flowering stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 1991.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 1961.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹, T₂ (75% RDP+PSB₁) 1934.0 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ and the least was found with T₁₀ (control) 1724.1 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹.

Urease enzyme activity at harvesting stage of crop growth was found to be highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 1323.6 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 1605.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹, T₂ (75% RDP+PSB₁) 1582.0 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹ and least was found with T₁₀ (control) 1094.6 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹.

Frankenberger and Dick (1983) documented that urease enzyme activities are highly related to soil organic matter content and microbial activity of soils. The urease activity was observed to be positively correlated with bacterial population and available nitrogen in soil. This might be due to the reason that urease enzyme is responsible for the hydrolysis of urea fertilizer thus its activity increases with increase in the level of N-fertilizer in the form of urea.

The results at 60 days time interval showed that application of biofertilizers, organic fertilizers and inorganic fertilizers had significant effect on soil urease activity. When compared with control, there was a significant increase in urease activity in all other treatments. This may be due to the fact that urease activity is dependent on soil organic matter content as well as nitrogen levels. All the treatments had 100% nitrogen levels either from inorganic sources or from organic sources or from both 50 inorganic and organic combined. The results were supported by findings of Nannipieri *et al.* (1983) and Hendrickson (1991) who showed that urease activity increased with addition of organic manure, promoting microbial growth.

There are certain evidences that plant materials may directly contribute to urease enzyme in soil. Microorganisms associated with the organic materials may also add to the urease in the soil enzyme pool. The results were supported by findings of Dharmakeerthi and Thenabadu (1996) who found that urease activity is affected by certain factors including various soil organic amendments.

4.6.2 Soil Health Parameters

4.6.2.1 pH: pH at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.21 and fig. 4.21. pH activity at initial stage was highest with the treatment T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 7.7 which was significant over other treatments followed by T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 7.6, T₂ (75% RDP+PSB₁) 7.5 which is equal to T₆ (50% RDP+PSB₁) 7.5 and least was found with T₁₀ (control) 7.0.

Highest pH activity at 50% flowering stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 8.1 which was significant over other treatments followed by T₆ (50% RDP+PSB₁) 8.0 which is equal to T₂ (75% RDP+PSB₁) and T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 8.0 and least was found with T₁ (100% RDF) 7.3 which is equal to T₁₀ (control) 7.3.

pH activity at harvesting stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 8.0 followed by T₂ (75% RDP+PSB₁) 7.9, T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 7.8 and the least was found with T₁₀ (control) 7.1.

Addition of microbial inoculants may increase the content of organic acids through their secretions thus lowering the soil pH. The results were in accordance with findings of Gopinath *et al.* (2009) and Jaipaul *et al.* (2011) who studied the effect of biofertilizers and inorganic fertilizers on soil properties. They found that soil pH decreased significantly with the addition of farm yard manure along with biofertilizers.

4.6.2.2 Electrical Conductivity (EC): EC at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.22 and fig. 4.22. EC activity at initial stage was highest with the treatment T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 0.249 dSm⁻¹ and T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.249 dSm⁻¹ which was significant over other treatments followed by T₂ (75% RDP+PSB₁) 0.237 dSm⁻¹, T₃ (75% RDP+PSB₂) 0.235 dSm⁻¹ and least was found with T₁₀ (control) 0.217 dSm⁻¹.

EC at 50% flowering stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁ +PSB₂+PSB₃) 0.259 dSm⁻¹ followed by T₉ (50% RDP+ PSB₁+PSB₂+PSB₃) 0.254 dSm⁻¹, T₂ (75% RDP+PSB₁) 0.247 dSm⁻¹ and least was found with T₁₀ (control) 0.228 dSm⁻¹.

Highest EC at harvesting stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.255 dSm⁻¹ which was significant over other treatments followed by T₉ (50% RDP+ PSB₁+ PSB₂+ PSB₃) 0.251 dSm⁻¹, T₂ (75% RDP+PSB₁) 0.246 dSm⁻¹ and least was found with T₁₀ (control) 0.225 dSm⁻¹.

It can be concluded that EC of soil is directly related to the ions present in it. It may be due to the production of acids or acid forming compounds through the decomposition of organic materials, that reacted with the sparingly soluble salts already present in the soil or either converted them into soluble salts there by increasing their solubility. The results were in accordance with the findings of Bulluck *et al.* (2002) who reported that addition of alternate fertility amendments increased soil ions exchange capacity and thus improved soil quality.

EC of a soil can be increased by adding CaCO₃ that may be converted to CaHCO₃ or even to Na₂CO₃ which are more soluble forms. The similar results have been reported by Sarwar *et al.* (2003).

4.6.2.3 Available nitrogen in soil: Available nitrogen at initial, 50% flowering and harvesting stages of crop growth was given in the table 4.23 and fig. 4.23. Available nitrogen at initial stage was highest with the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 286.3 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 244.3 kg ha⁻¹, T₂ (75% RDP+PSB₁) 226.3 kg ha⁻¹ and least was found with T₁₀ (control) 146.6 kg ha⁻¹.

Available nitrogen at 50% flowering stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 306.0 kg ha⁻¹ with other followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 292.6 kg ha⁻¹, T₂ (75% RDP+PSB₁) 284.3 kg ha⁻¹ and least was found with T₁₀ (control) 205.0 kg ha⁻¹.

Highest available nitrogen at harvesting stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 293.6 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 273.6 kg ha⁻¹, T₂ (75% RDP+PSB₁) 263.0 kg ha⁻¹ and least was found with T₁₀ (control) 196.0 kg ha⁻¹.

The increase of nitrogen and phosphorus is due to the influence of PGPR in the rhizosphere. PGPR enhanced nutrient uptake from soil solution at a faster rate which increased the accumulation of NPK and thereby dry matter content (Sarig *et al.*, 1984). The inoculation of PGPR consortium increased N, P and K contents of *Catharanthus roseus* and in soil which indicated the positive effect of consortium in augmenting the availability of nitrogen and phosphorus to plants (Sivamurugan *et al.*, 2000).

Anwar *et al.* (2005) who studied the effect of biofertilizers and inorganic fertilizers on soil physico-chemical properties. He found that soils treated with biofertilizers and inorganic fertilizers had higher content of available nitrogen than soils having inorganic fertilizers alone. Microbial fertilizers like *Rhizobium* and phosphate-solubilizing bacteria (PSB) are highly beneficial in enhancing nitrogen (N) and phosphorus (P) content due to nitrogen fixation and solubilization of native P making the two essential nutrients available to the plant (Singh *et al.*, 2011)

4.6.2.4 Available phosphorus in soil: Available phosphorus at initial, 50% flowering and harvesting stages of crop growth was given in table 4.24 and fig. 4.24. Available phosphorus at initial stage was highest in the treatment T₅ (75%

RDP+PSB₁+PSB₂+PSB₃) 38.6 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 34.6 kg ha⁻¹, T₂ (75% RDP+PSB₁) 30.6 kg ha⁻¹ and least was found with T₁₀ (control) 19.3 kg ha⁻¹.

Available phosphorus at 50% flowering stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 51.4 kg ha⁻¹ over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 49.2 kg ha⁻¹, T₂ (75% RDP+PSB₁) 45.2 kg ha⁻¹ and least was found with T₁₀ (control) 26.6 kg ha⁻¹.

Highest available phosphorus at harvesting stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 45.4 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 41.3 kg ha⁻¹, T₂ (75% RDP+PSB₁) 39.3 kg ha⁻¹ and least was found with T₁₀ (control) 23.3 kg ha⁻¹.

Higher available phosphorus was due to inoculation and solubilization of insoluble phosphorus in soil by microbes. Similar views were expressed by Yazdani *et al.* (2009) who demonstrated that use of growth stimulating bacteria in combination with chemical fertilizer was able to reduce phosphorus fertilization without reduction in corn yield. Kucey *et al.* (1989) measured increased levels of available P in soils that had been amended with farmyard manure, rock phosphate and PSM isolates of *Bacillus* and *Streptomyces*.

Rajeswar and Khan (2010) reported that the phosphorus availability was increased with the application of biofertilizers in *kharif* rice. Presence of PSB in the consortium biofertilizer which was effective in increasing the availability of phosphorus in soil by solubilization of native soil phosphorus (Jeon *et al.*, 2003).

Richardson and Simpson (2011) stated that the inoculation with PS-12 (*Bacillus* sp.) resulted in maximum increase in soil available P which were 38 and 62% higher than respective uninoculated control, respectively. The increase in soil available P might have resulted due to greater microbial activities in response to inoculation with selected strains which dissolved the fixed forms of soil phosphorus (inorganic P) or they might have mineralized organic matter to release organic phosphorus.

Sundara (2002) reported that application of PSB resulted in about 25% of reduction in inorganic P fertilizer requirement and increased the available P in soil.

The effect of addition of FYM could also be seen in increasing the amount of available phosphorus in soil, as it helps in sustaining higher population of several bacteria and fungi, which are capable of solubilizing soil phosphorus. These results were supported by findings of Singh *et al.* (2014) who showed that available phosphorus content of soil increased with the application of organic manure (pressmud) with recommended dose of nitrogen and phosphorus.

The available P range 51-100 ppm was the most suitable for the occurrence of PSMs. PSMs appear to be highly efficient in mobilizing the natural reservoir of the elemental P (Alexander, 1977). Biofertilizer enhance soil fertility by solubilizing unavailable sources of bound phosphate into available forms in order to facilitate the plant to absorb them (Singh and Yadav, 2008). Chen *et al.* (2006) stated that available phosphorus in soil can be increased by low-molecular-mass organic acids produced by PSB. The results of the study demonstrated the production of various types of organic acids by PSB.

4.6.2.5 Available potassium in soil: Available potassium at initial, 50% flowering and harvesting stages of crop growth was given in table 4.25 and fig. 4.25. Available potassium at initial stage was highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 356.0 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 332.3.0 kg ha⁻¹, T₂ (75% RDP+PSB₁) 304.6 kg ha⁻¹ and least was found with T₁₀(control) 217.0 kg ha⁻¹.

Highest available potassium at 50% flowering stage of crop growth was in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 394.3 kg ha⁻¹ which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 374.6 kg ha⁻¹, T₂ (75% RDP+PSB₁) 367.0 kg ha⁻¹ and least was found with T₁₀(control) 243.6 kg ha⁻¹.

Available potassium at harvesting stage of crop growth was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 364.0 kg ha⁻¹ followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 356.6 kg ha⁻¹, T₂ (75% RDP+PSB₁) 352.0 kg ha⁻¹ and the least was found with T₁₀(control) 231.6 kg ha⁻¹.

Potassium (K) plays essential roles in plants. It is an activator of many enzymes responsible for plant processes such as energy metabolism, starch synthesis, nitrate reduction and sugar degradation. Release of K from organic compounds and

minerals present in soil *via* decomposition as well as solubilization by soil microorganism thus facilitating release of organically bound K. Bear (1976) reported similar results regarding the release of K from organic compounds present in soil. Tolanur and Badanur (2003) observed positive changes in available nutrient status of the soil with the addition of organic fertilizers in legumes.

Yaduvanshi (2001) stated that available K was increased in both organic and inorganic treated plots when compared with initial value. The higher K status in soil might be due to the organic manures on decomposition, released the organic acids which might have mobilised the native or non-exchangeable forms of K and charge the soil solution with K ions, so that it may be readily available.

4.6.2.6 Organic carbon in soil: Organic carbon at initial, 50% flowering and harvesting stages of crop growth was given in table 4.26 and fig. 4.26. Organic carbon at initial stage of crop growth was highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.843% which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 0.823%, T₂ (75% RDP+PSB₁) 0.794% and least was found with T₁₀ (control) 0.616%.

Organic carbon at 50% flowering stage was significantly highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.910% followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 0.882%, T₂ (75% RDP+PSB₁) 0.821% and least was found with T₁₀ (control) 0.652%.

Organic carbon at harvesting stage of crop growth was highest in the treatment T₅ (75% RDP+PSB₁+PSB₂+PSB₃) 0.896% which was significant over other treatments followed by T₉ (50% RDP+PSB₁+PSB₂+PSB₃) 0.863%, T₂ (75% RDP+PSB₁) 0.814% and the least was found with T₁₀ (control) 0.623%.

Bhandari *et al.* (2002) reported that the organic carbon per cent increased with application of FYM. They reported that buildup of OC could be attributed to the manures and subsequent addition of leaf residue and debris of plants. Further they stated that limited presence of PSMs in soil samples with organic matter less than 1% indicate the importance of organic matter for the abundance of PSMs. Similar results were reported by Yahya and Al-Azawi (1989) in Iraqi soils.

Albaladejo *et al.* (1994) suggested that the increase in soil OC to be faster during early stage of crop development and gradually declined later. The build-up of OC in surface layers was reported due to the addition of sloughed roots and the

incorporation of leaf litter with the increasing age of crop. Accumulation of organic matter has proved to be very important to soil improvement; increase in soil OC may be accompanied by improvement in soil structure, a decrease in bulk density and an increase of hydraulic conductivity.

High microbial biomass production and high rhizodeposits of carbonaceous materials through root exudates may be one of the reasons for higher OC in soils (Franzluebbers *et al.*, 1995). Kumar and Sharma (2015) has indicated the need for the presence of surplus amount of organic matter for the abundance of PSM's.

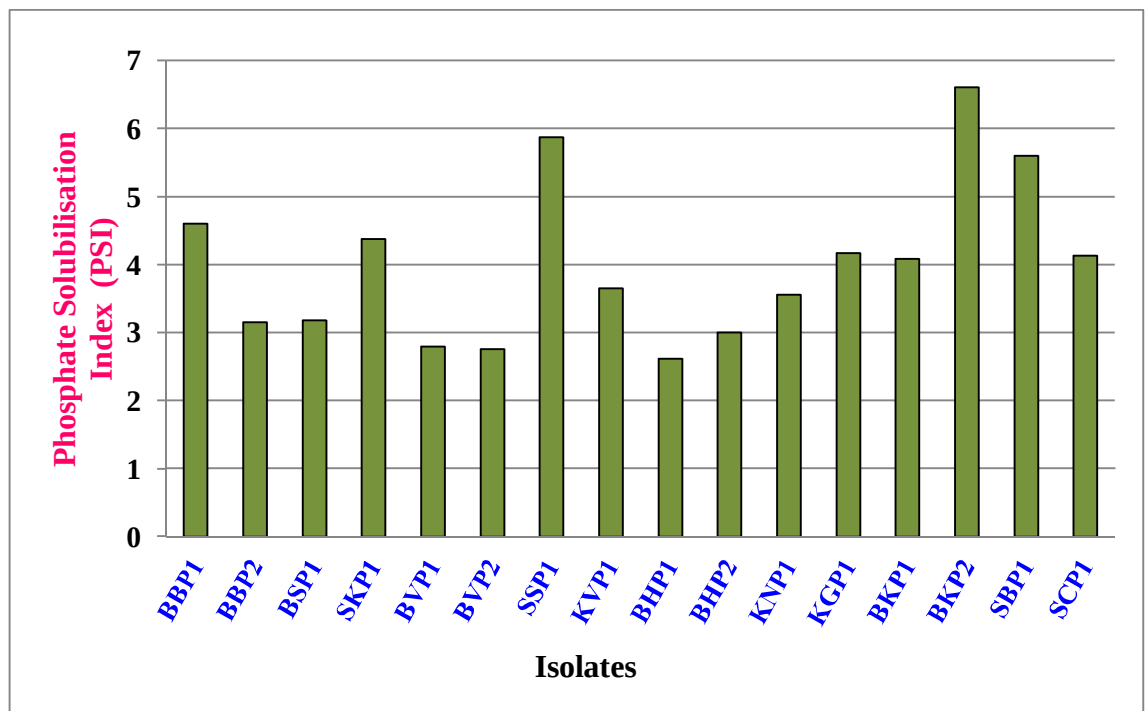


Fig. 4.5 Estimation of phosphate solubilisation qualitatively for the screening of PSB isolates

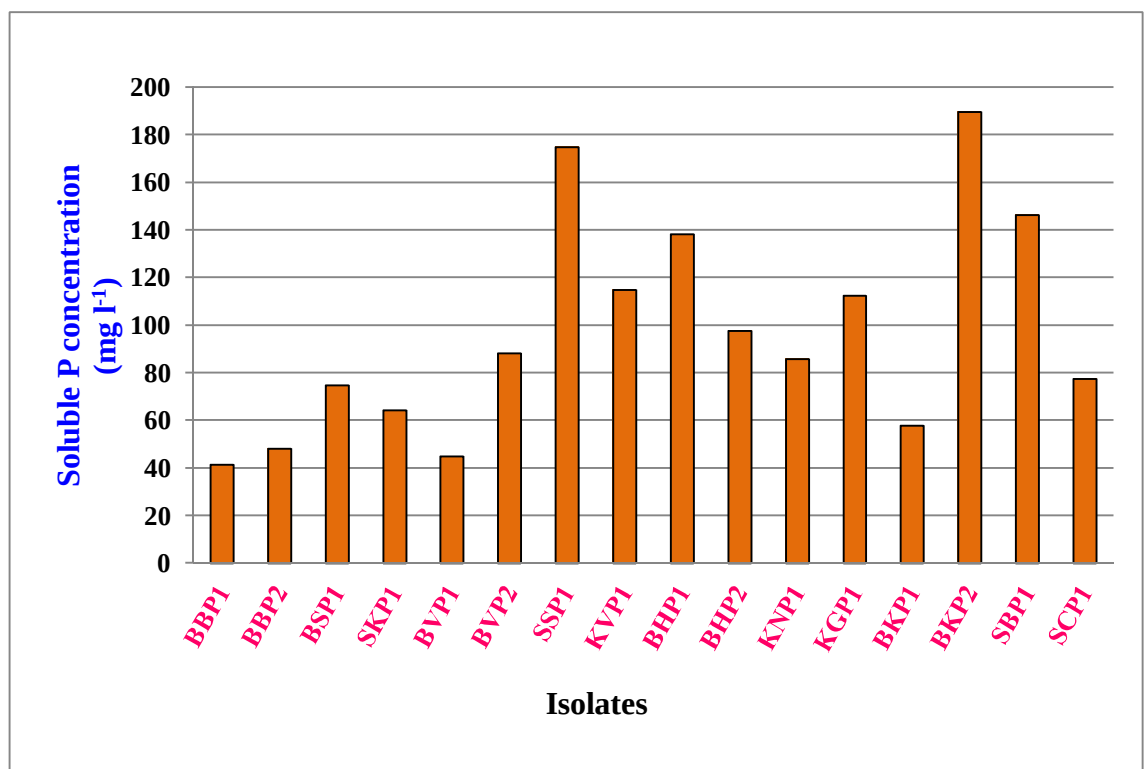


Fig 4.6 Estimation of phosphate solubilization quantitatively for the screening of PSB isolates

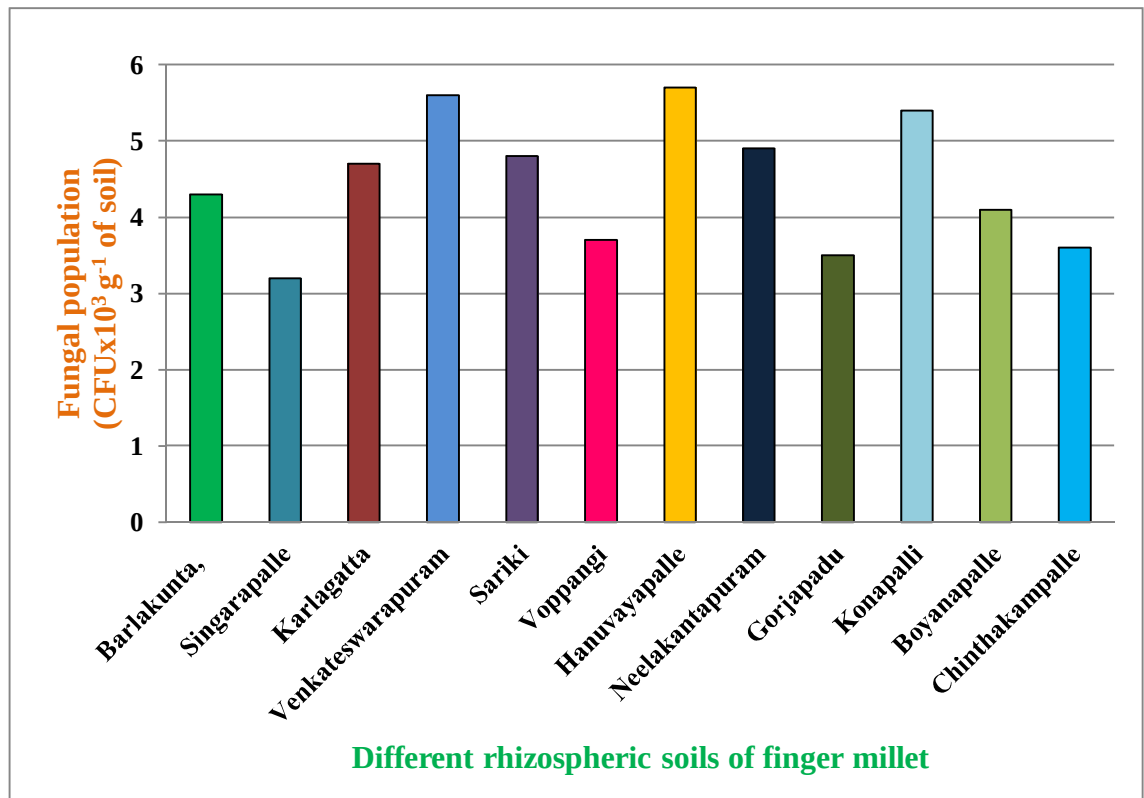


Fig. 4.3 Fungal population count from different rhizospheric soils of finger millet in Andhra Pradesh

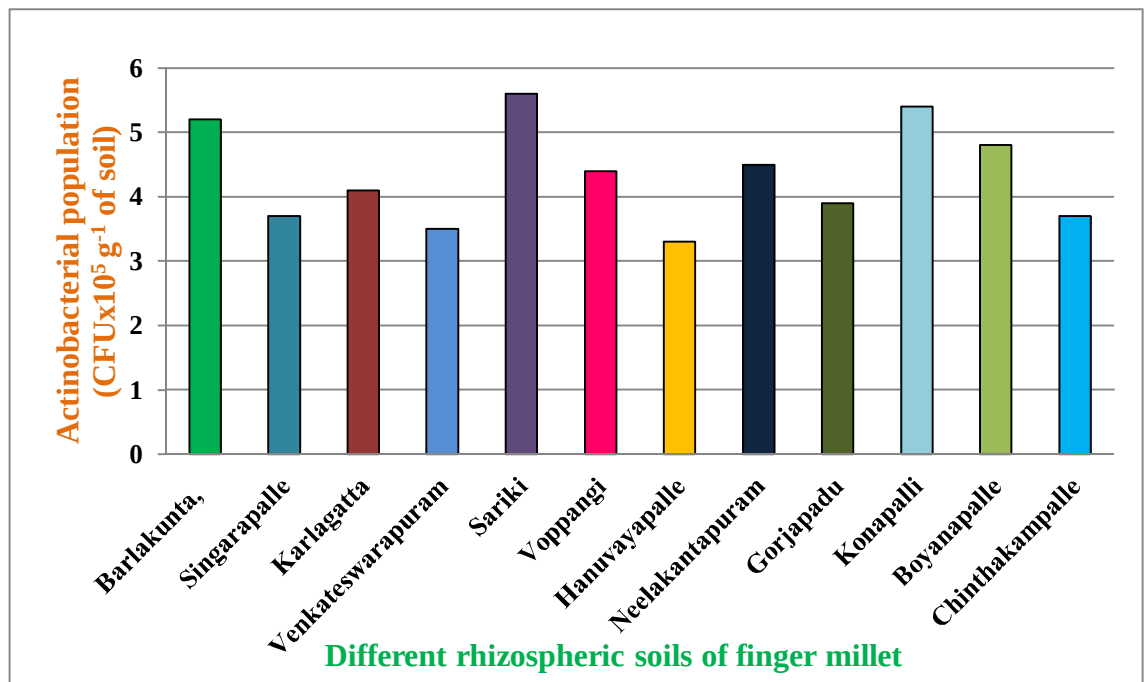


Fig. 4.4 Actinobacterial population count from different rhizospheric soils of finger millet in Andhra Pradesh

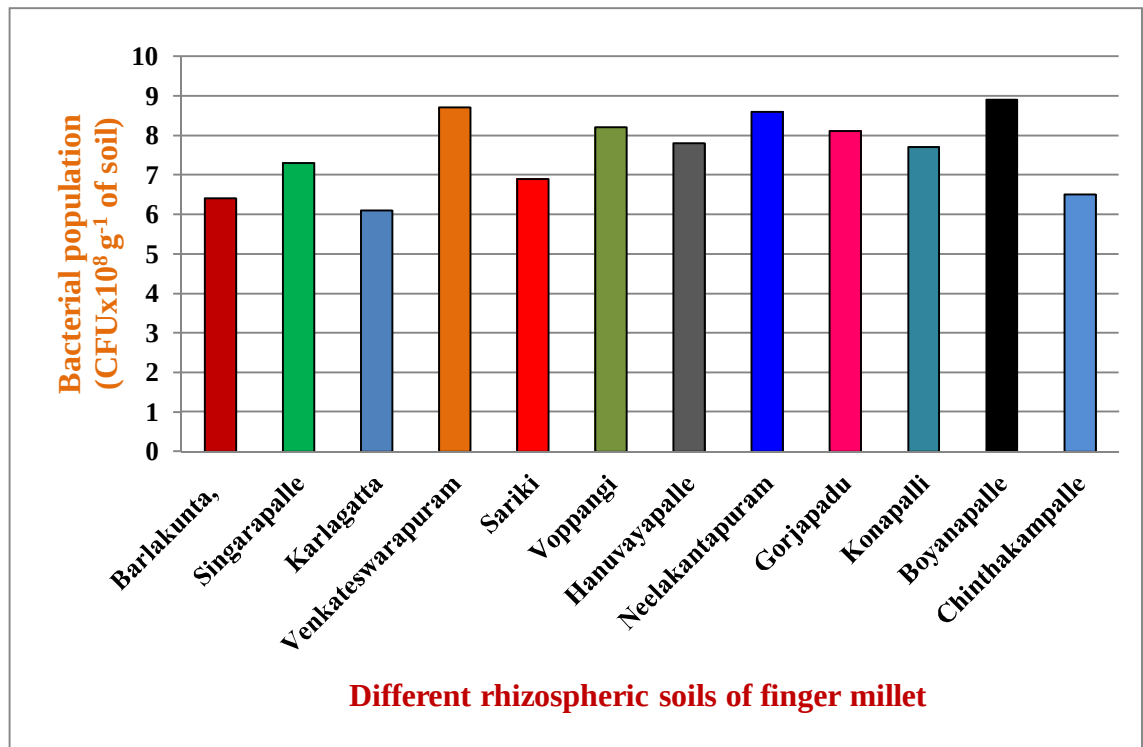


Fig. 4.1 Bacterial population count from different rhizospheric soils of finger millet in Andhra Pradesh

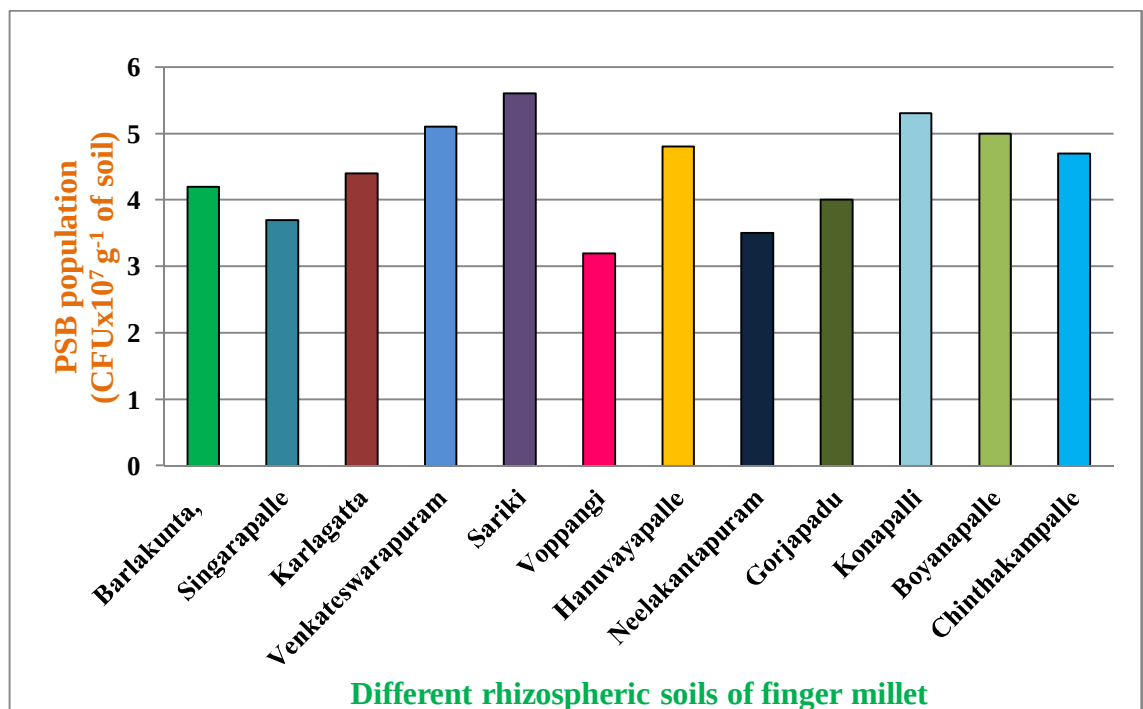


Fig. 4.2 PSB population count from different rhizospheric soils of finger millet in Andhra Pradesh

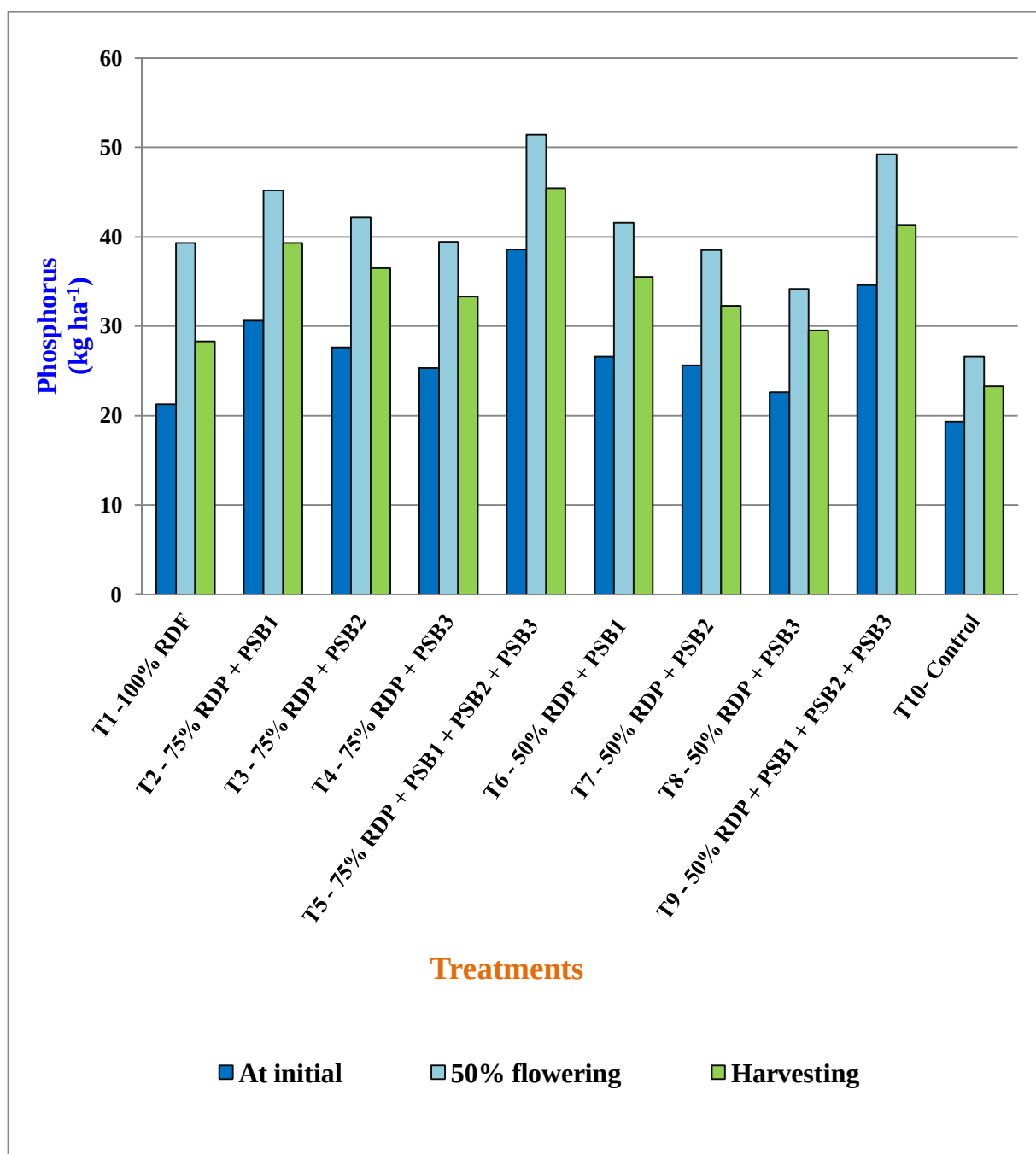


Fig. 4.24 Influence of PSB isolates on available phosphorus in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

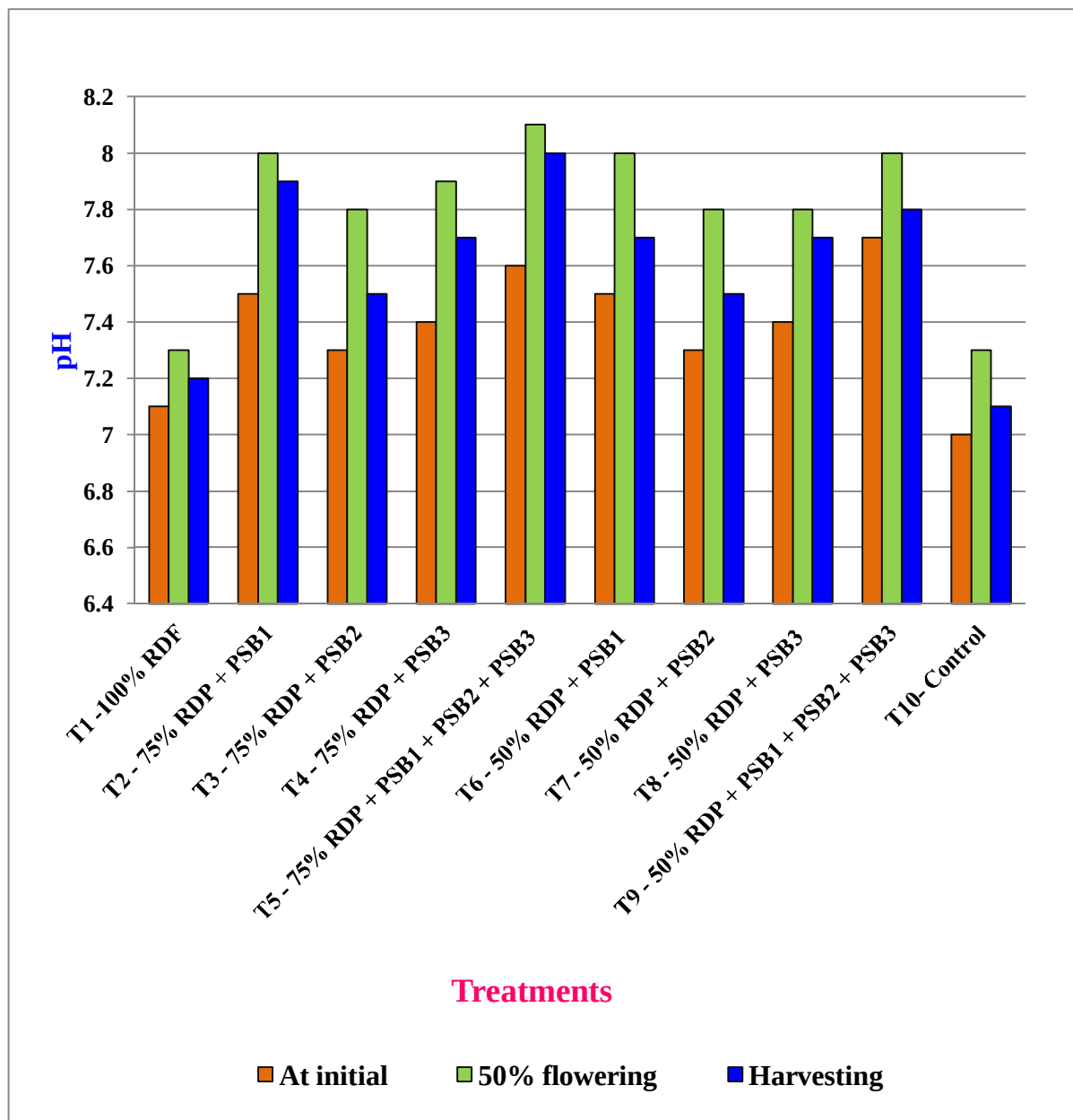


Fig. 4.21 Influence of PSB isolates on pH in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

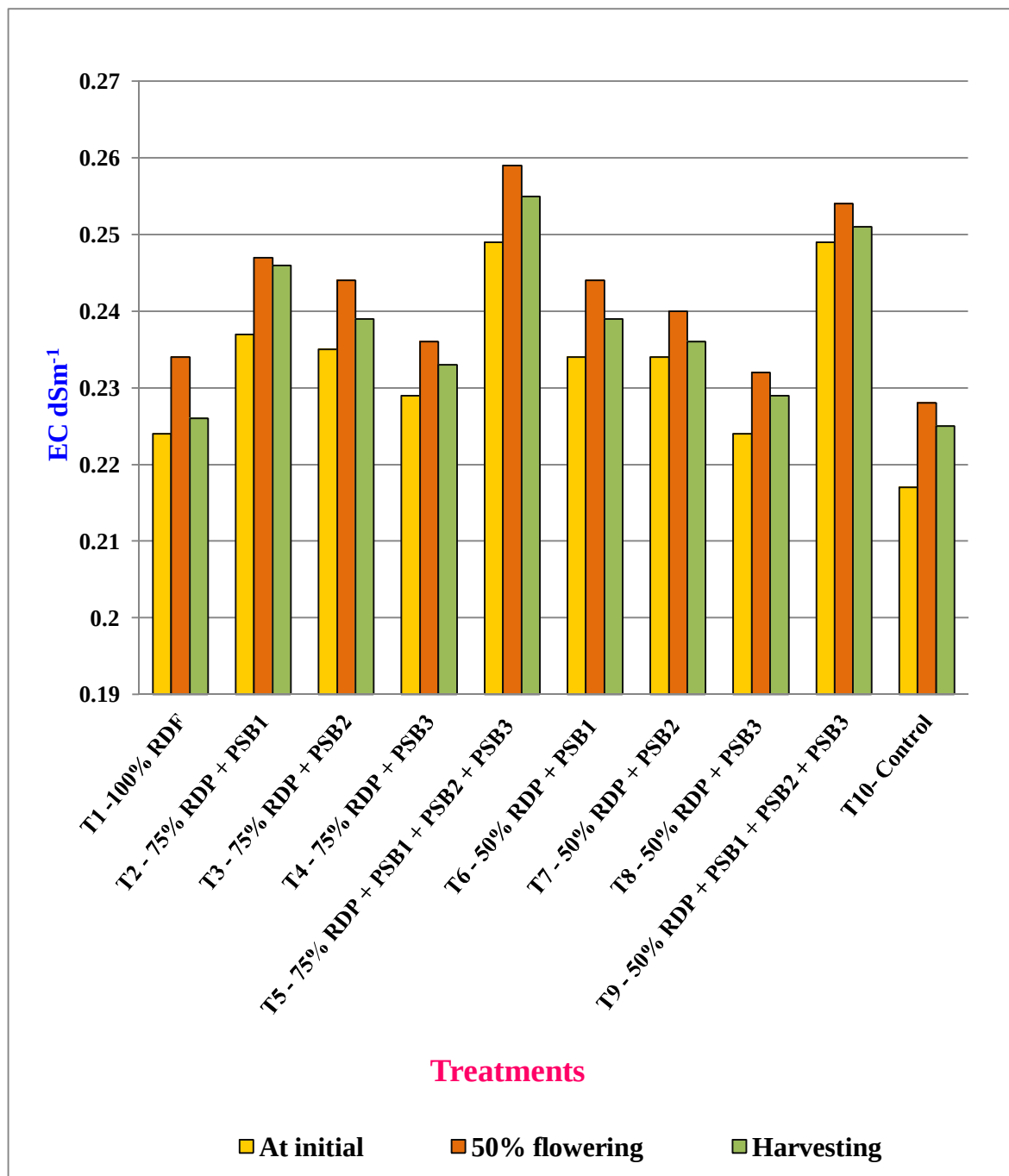


Fig. 4.22 Influence of PSB isolates on EC in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

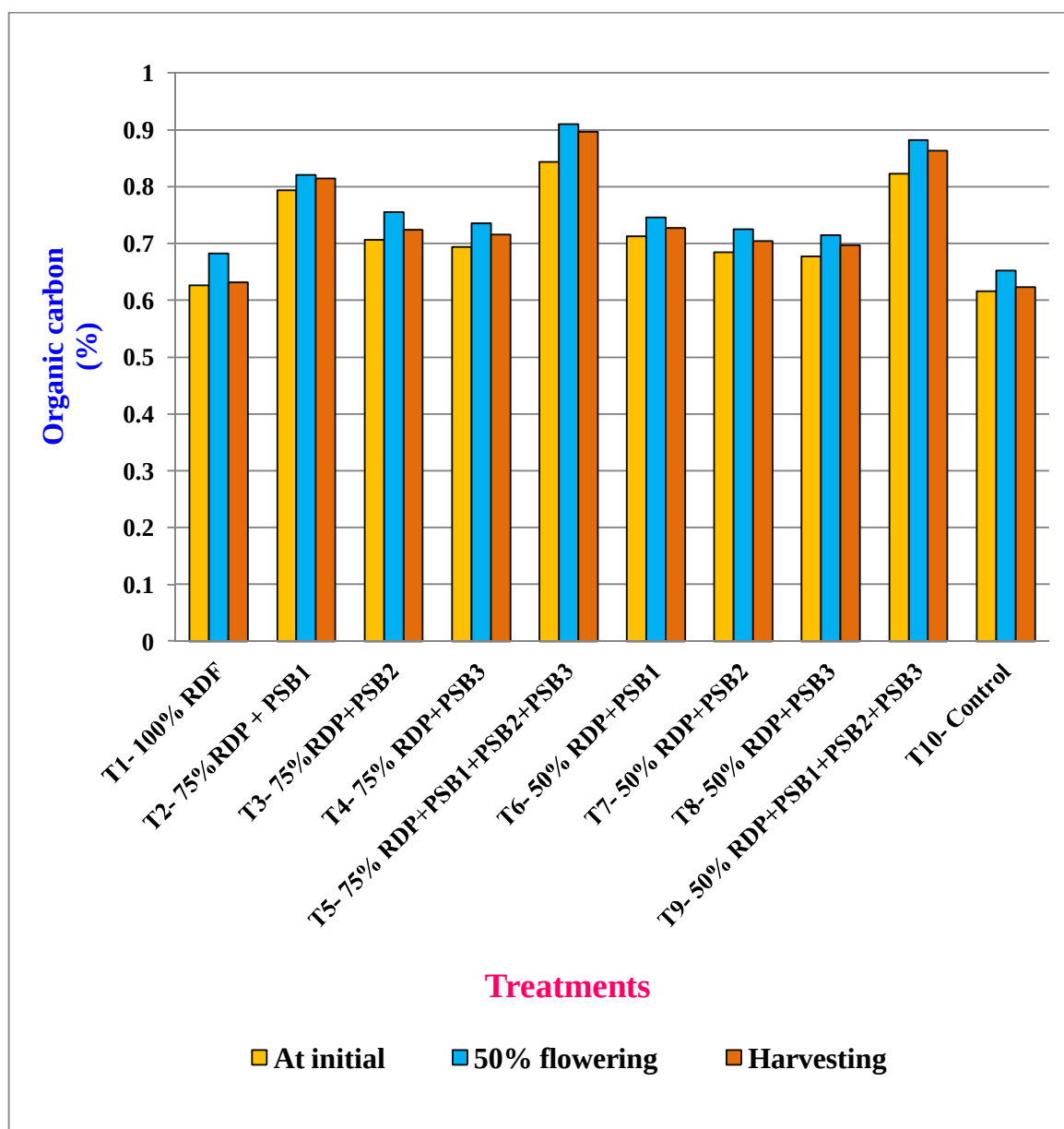


Fig. 4.26 Influence of PSB isolates on organic carbon in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

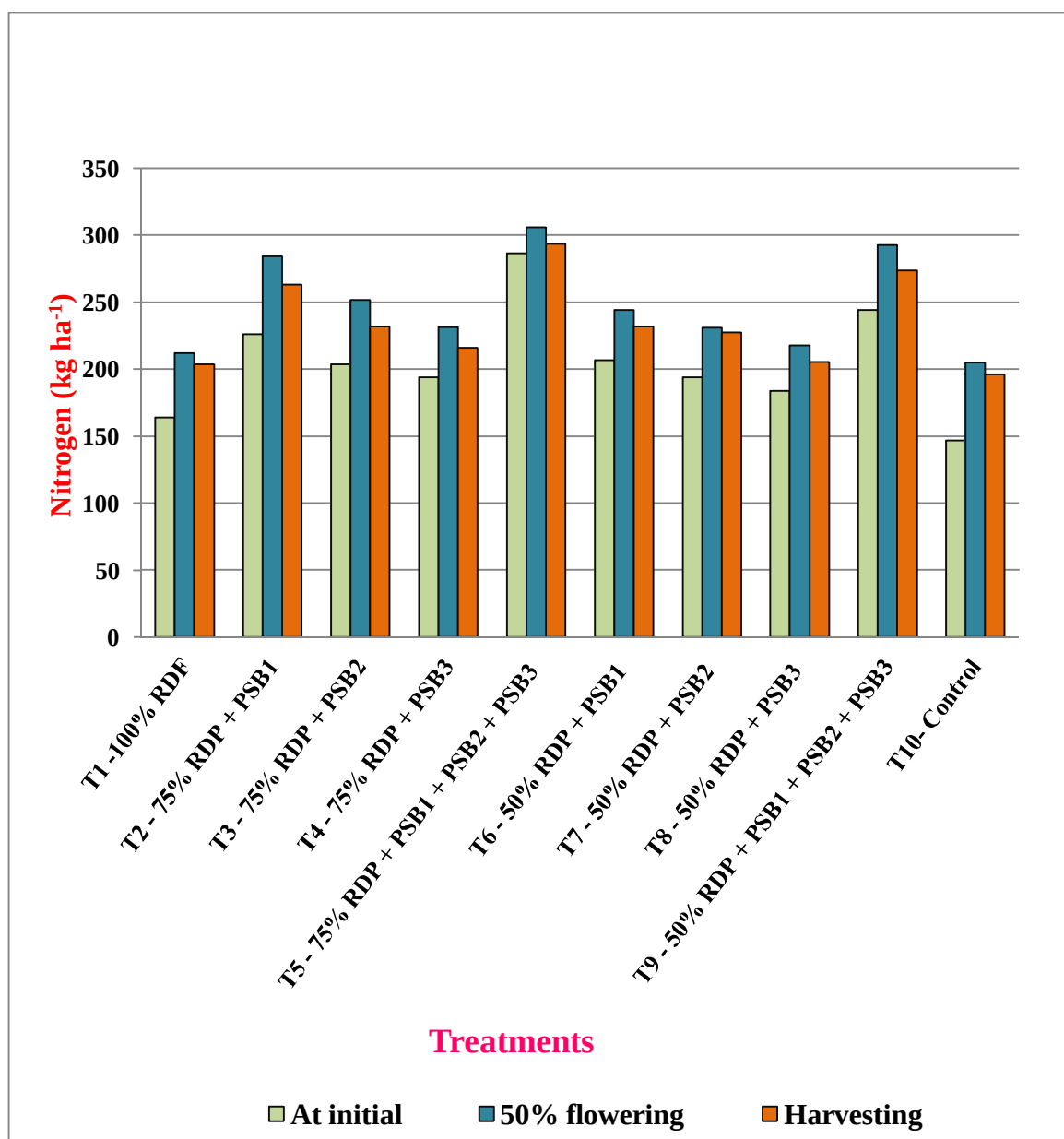


Fig. 4.23 Influence of PSB isolates on available nitrogen in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

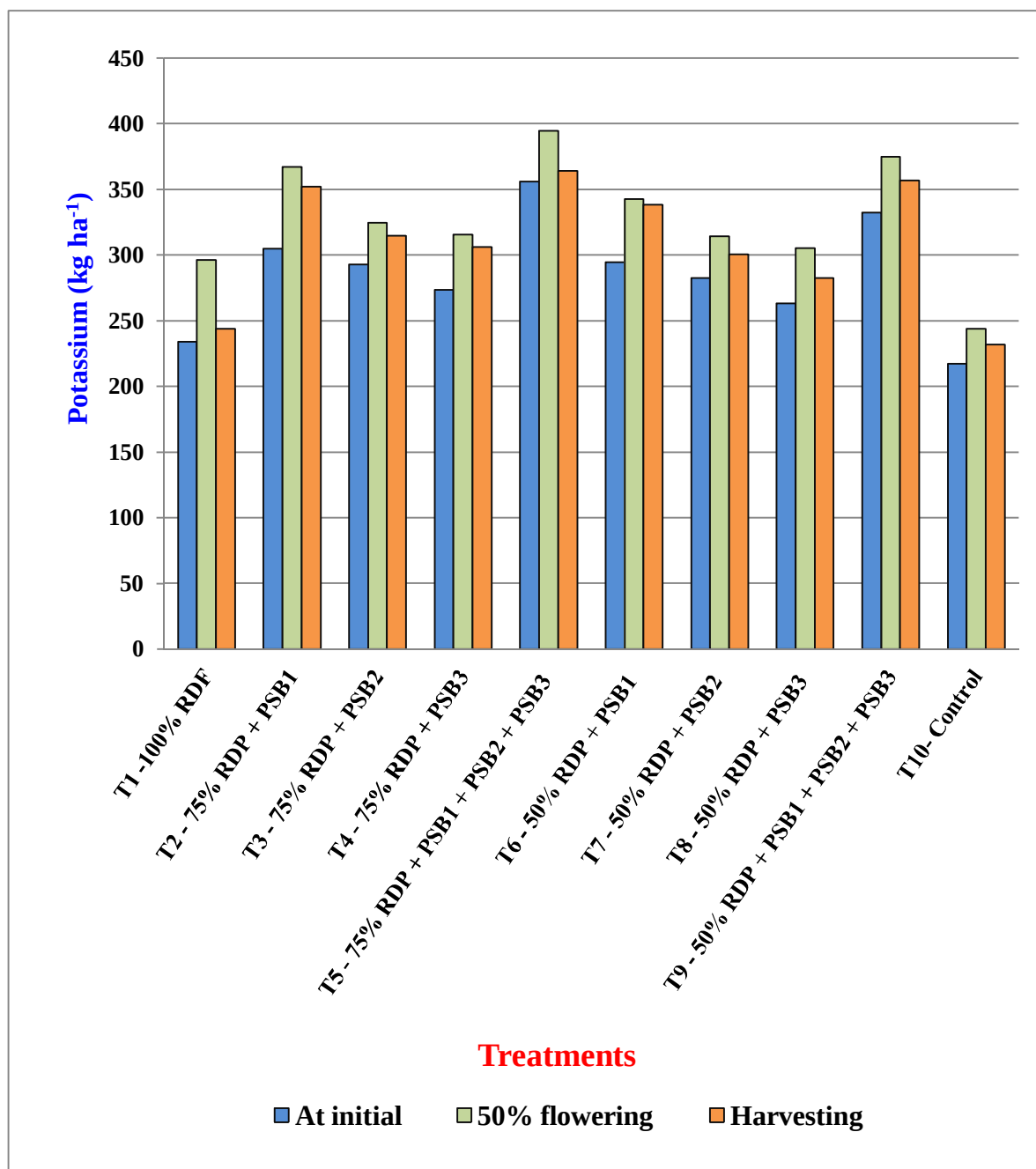


Fig. 4.25 Influence of PSB isolates on available potassium in soils at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

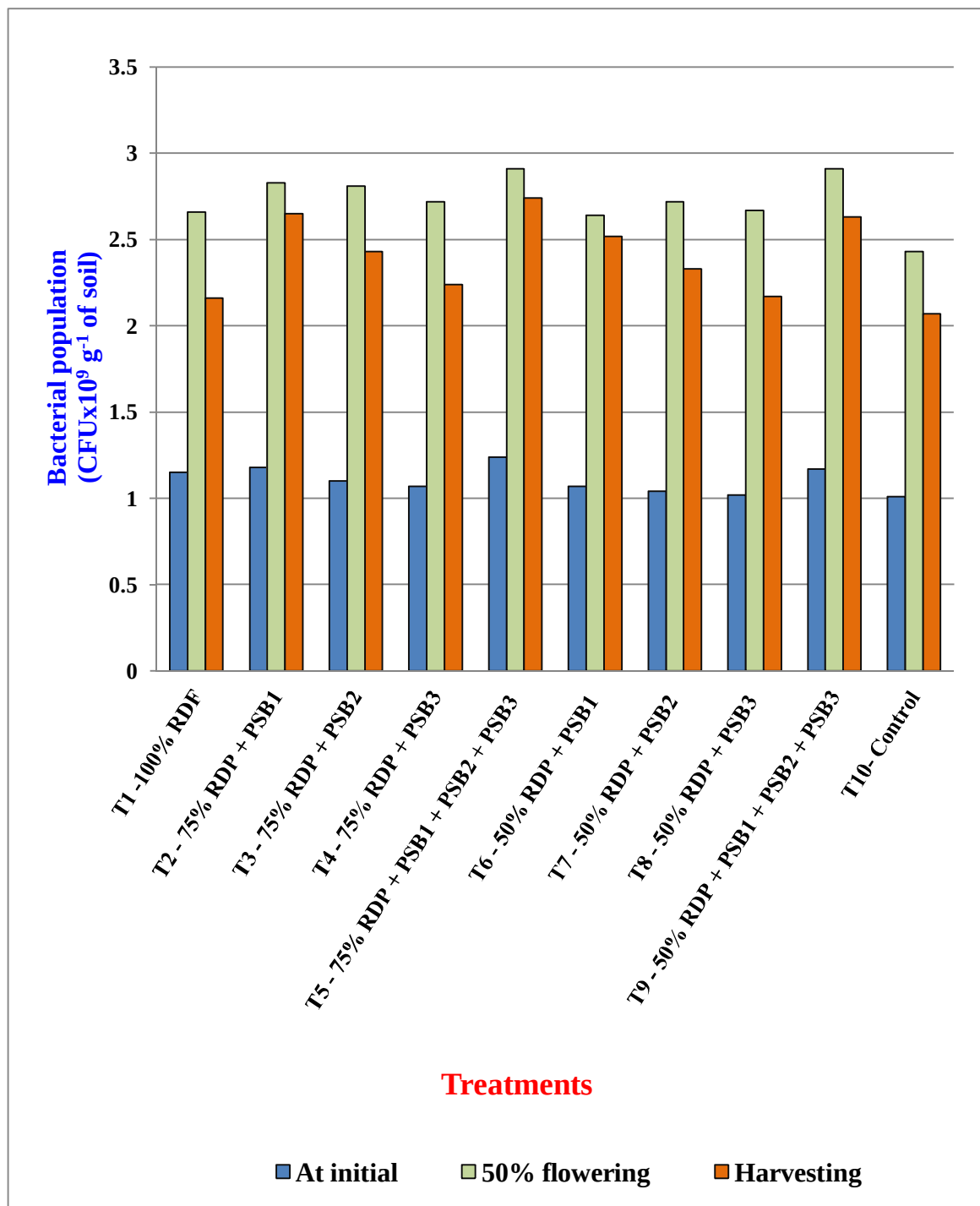


Fig. 4.8 Influence of PSB isolates on enumeration of bacterial population at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

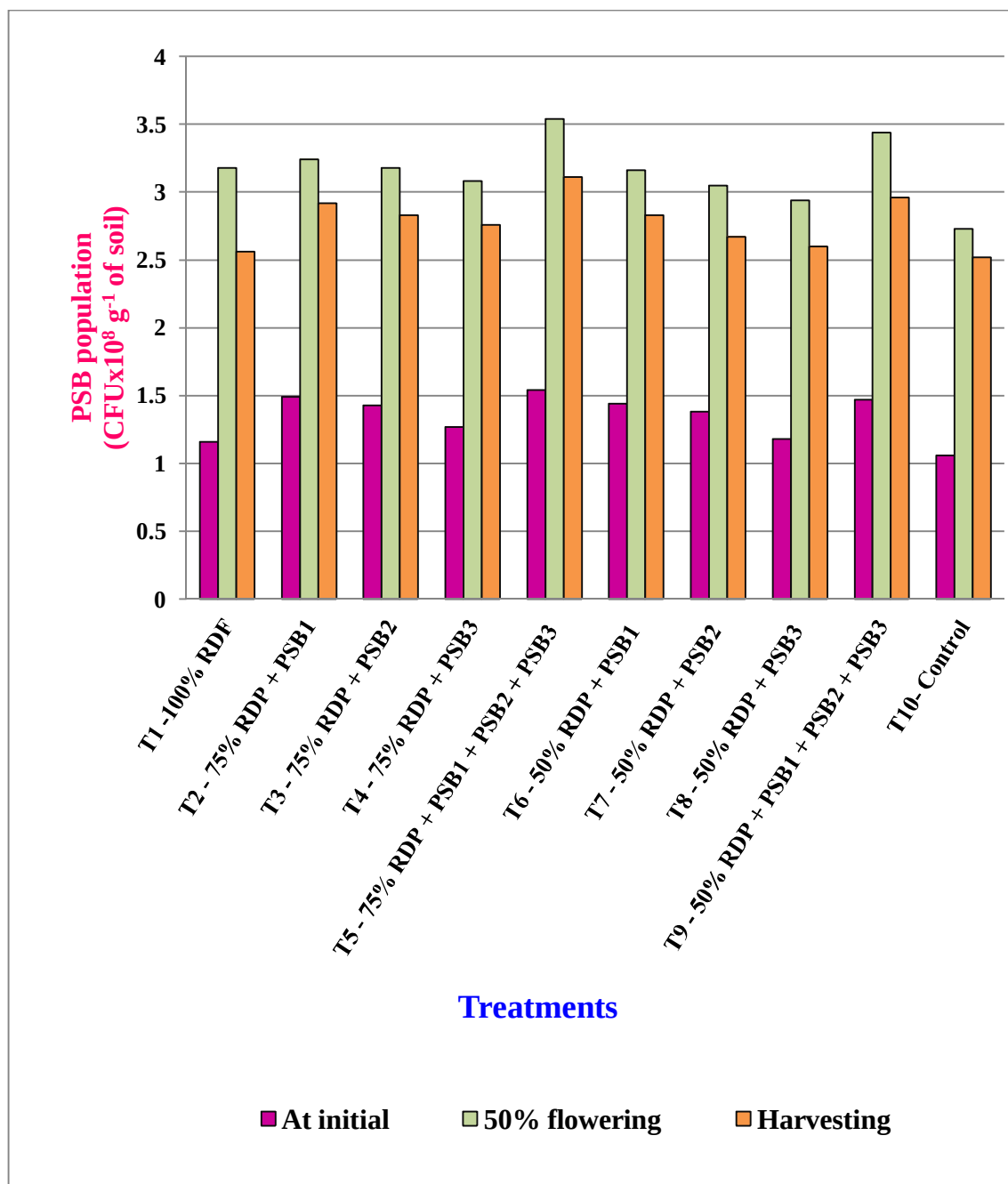


Fig. 4.9 Influence of PSB isolates on enumeration of PSB population at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

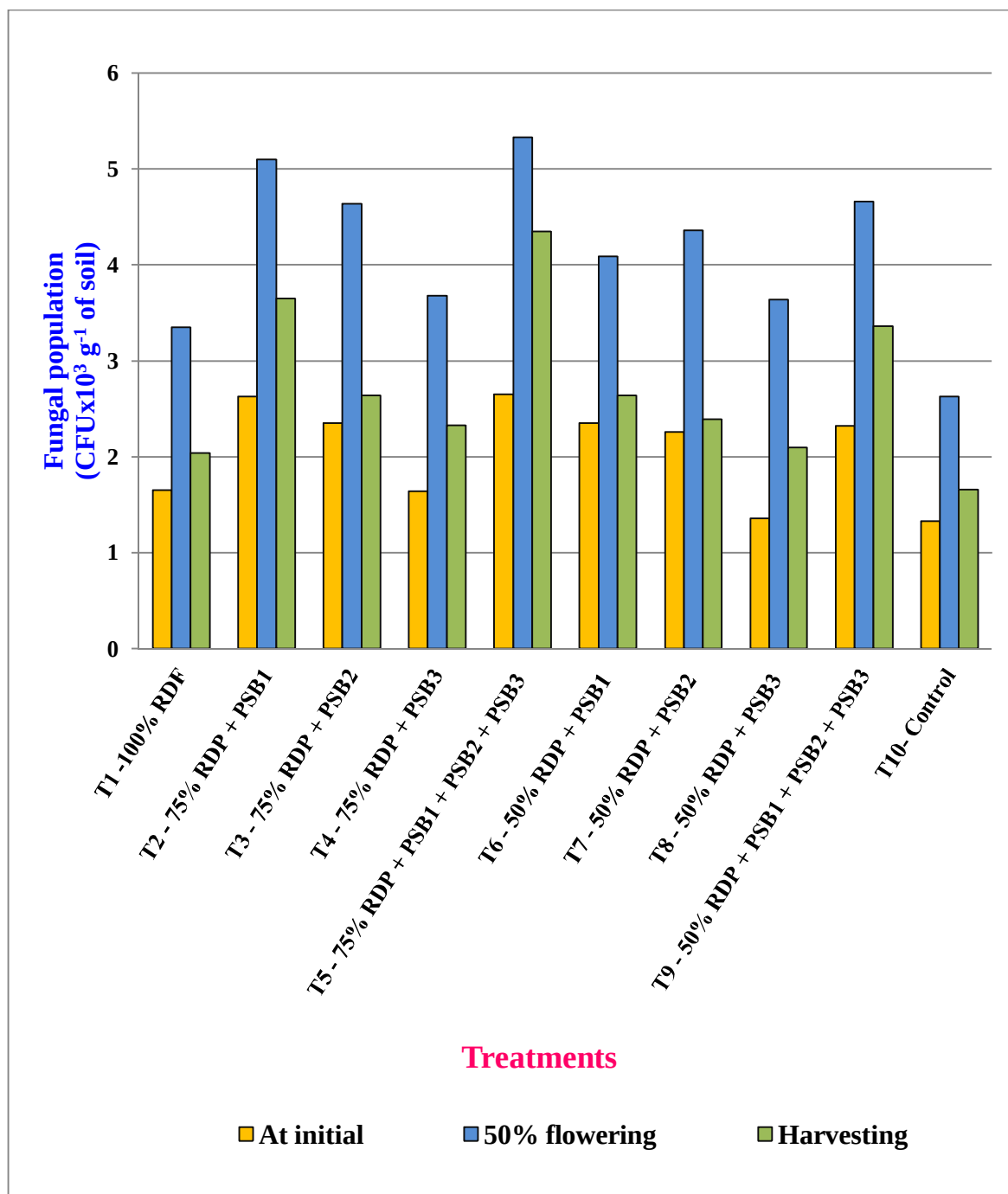


Fig. 4.10 Influence of PSB isolates on enumeration of fungal population at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

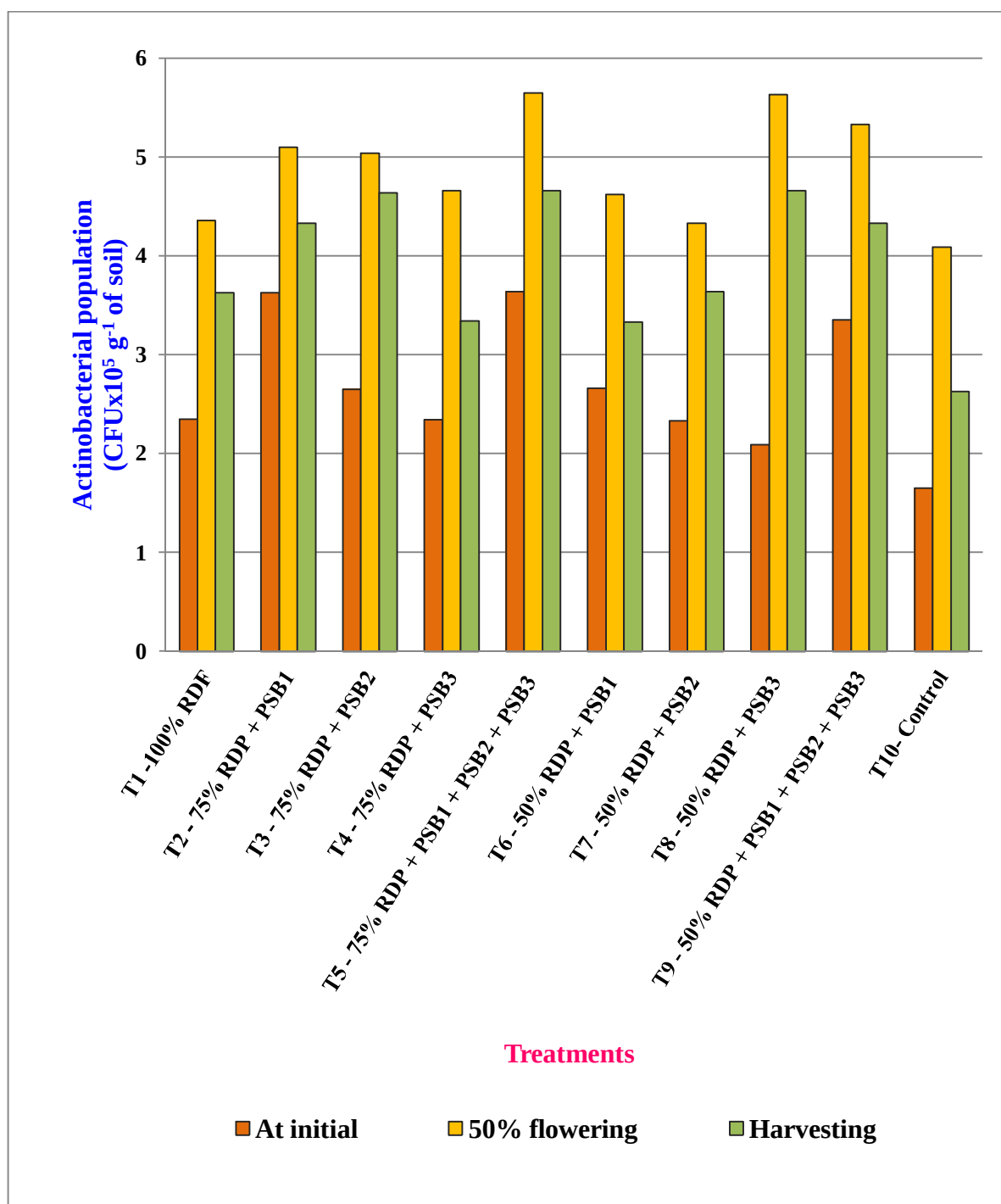


Fig 4.11 Influence of PSB isolates on enumeration of actinobacterial population at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

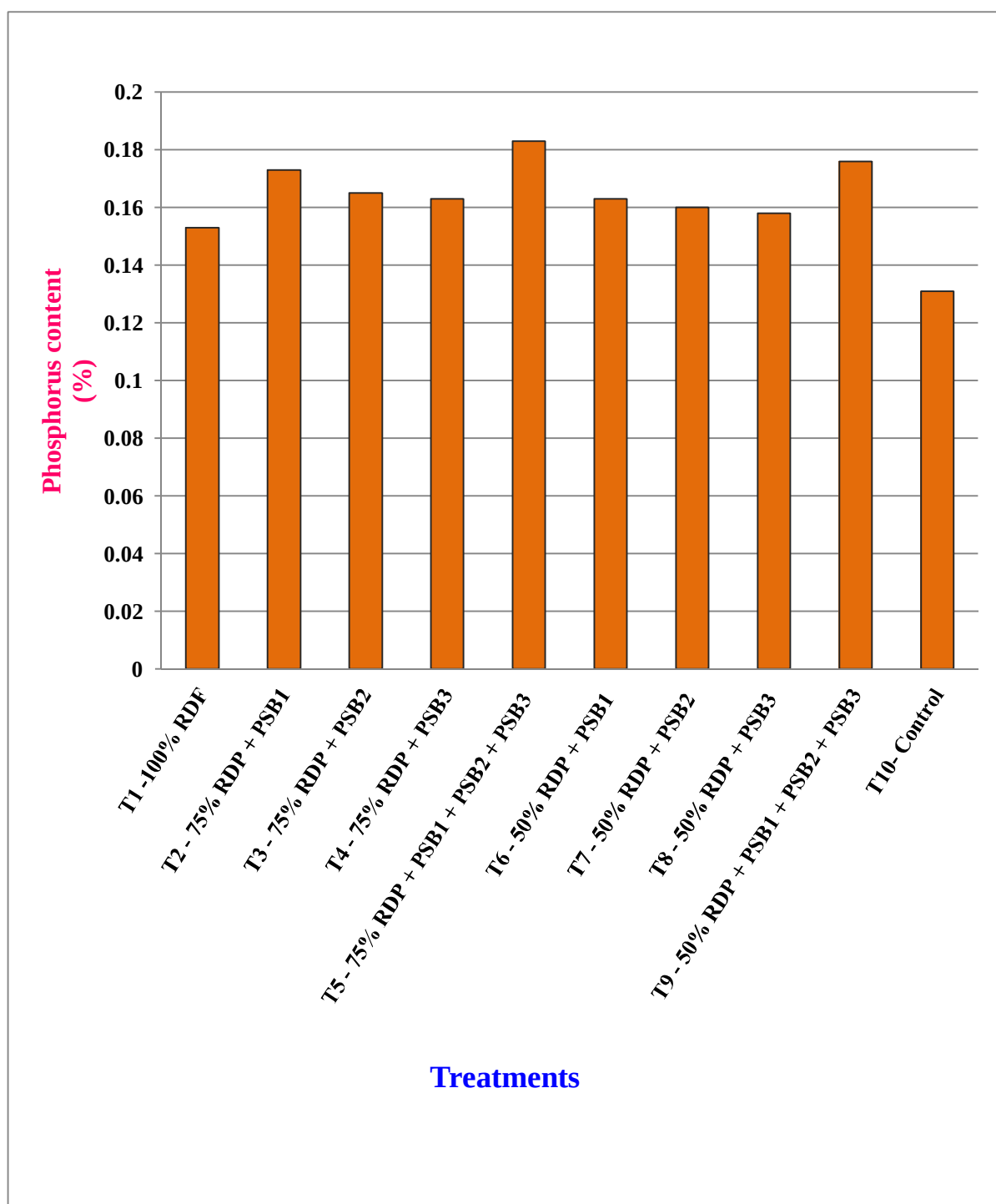


Fig 4.15 Influence of PSB isolates on phosphorus content present in plant sample at various treatments

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

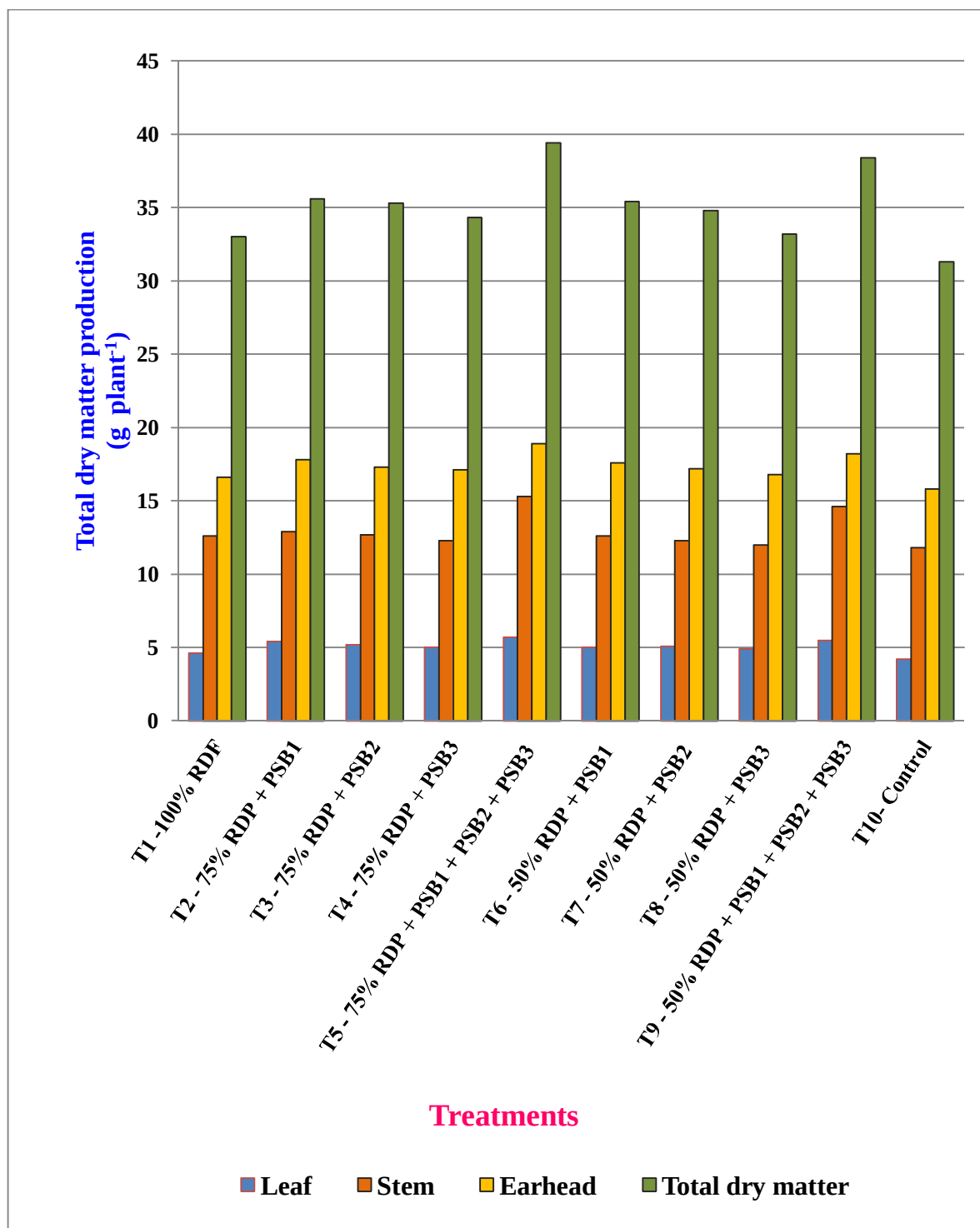


Fig 4.16 Influence of PSB isolates on total dry matter production of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

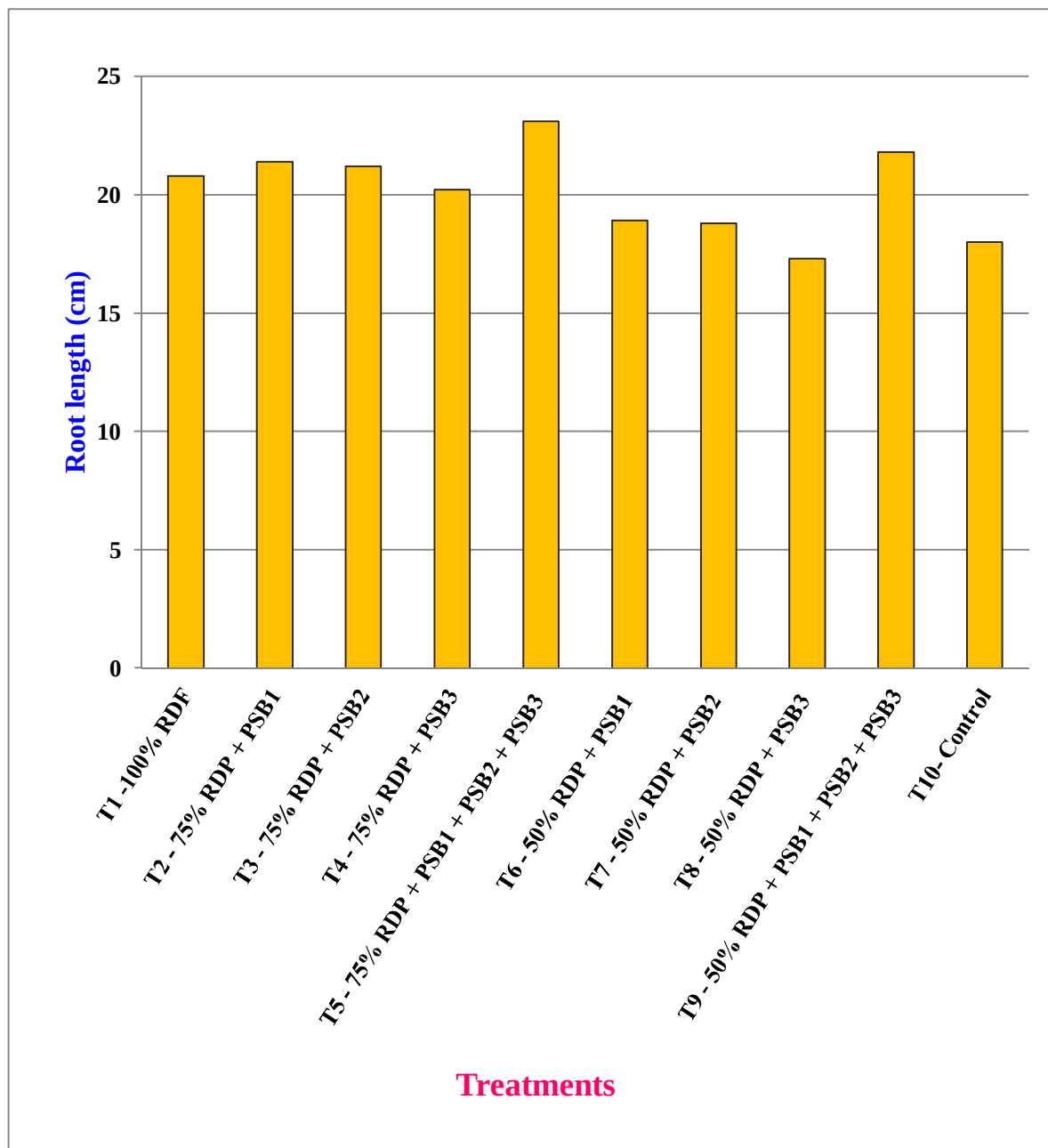


Fig. 4.12 Influence of PSB isolates on root length of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

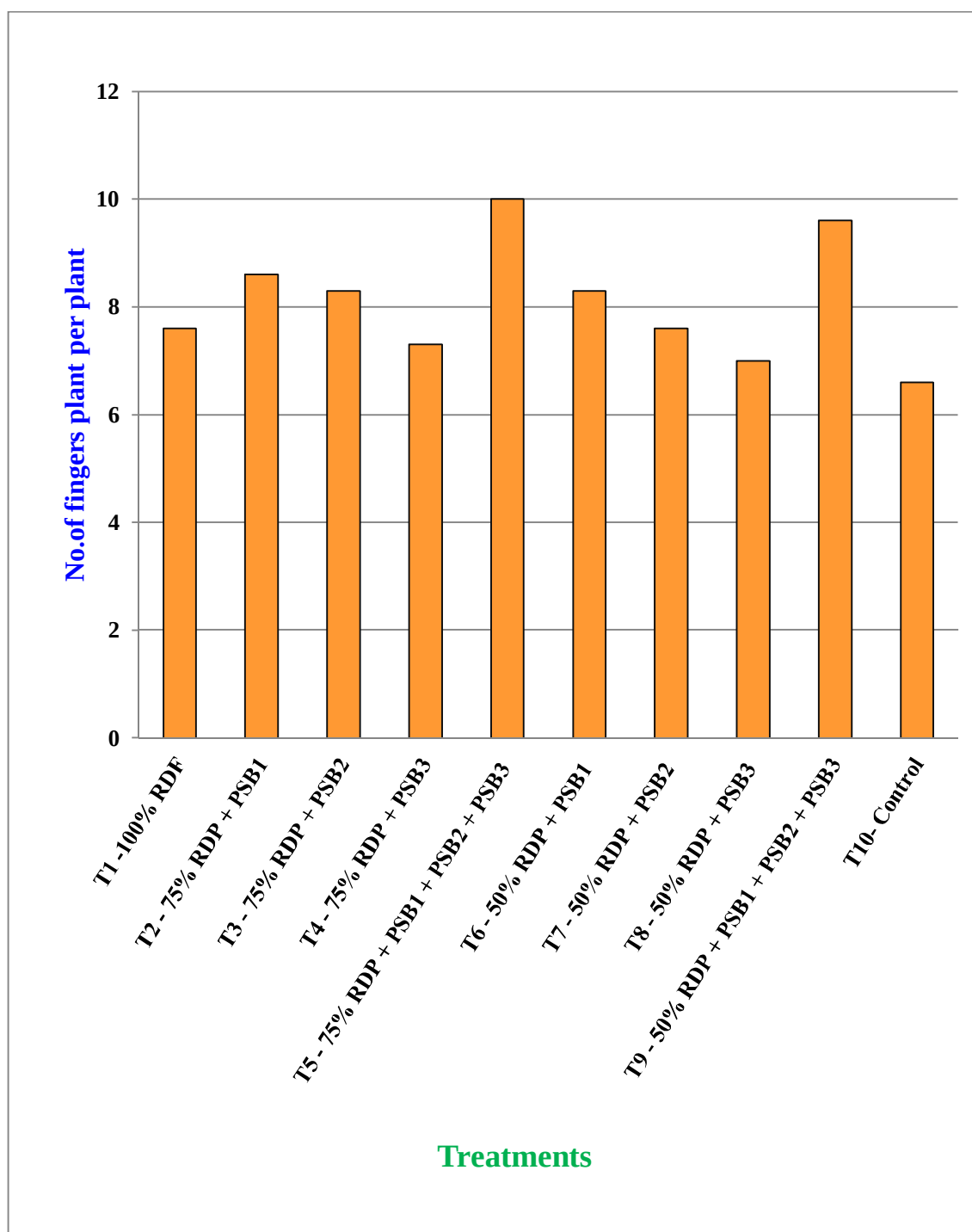


Fig. 4.13 Influence of PSB isolates on no. of fingers per plant in finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

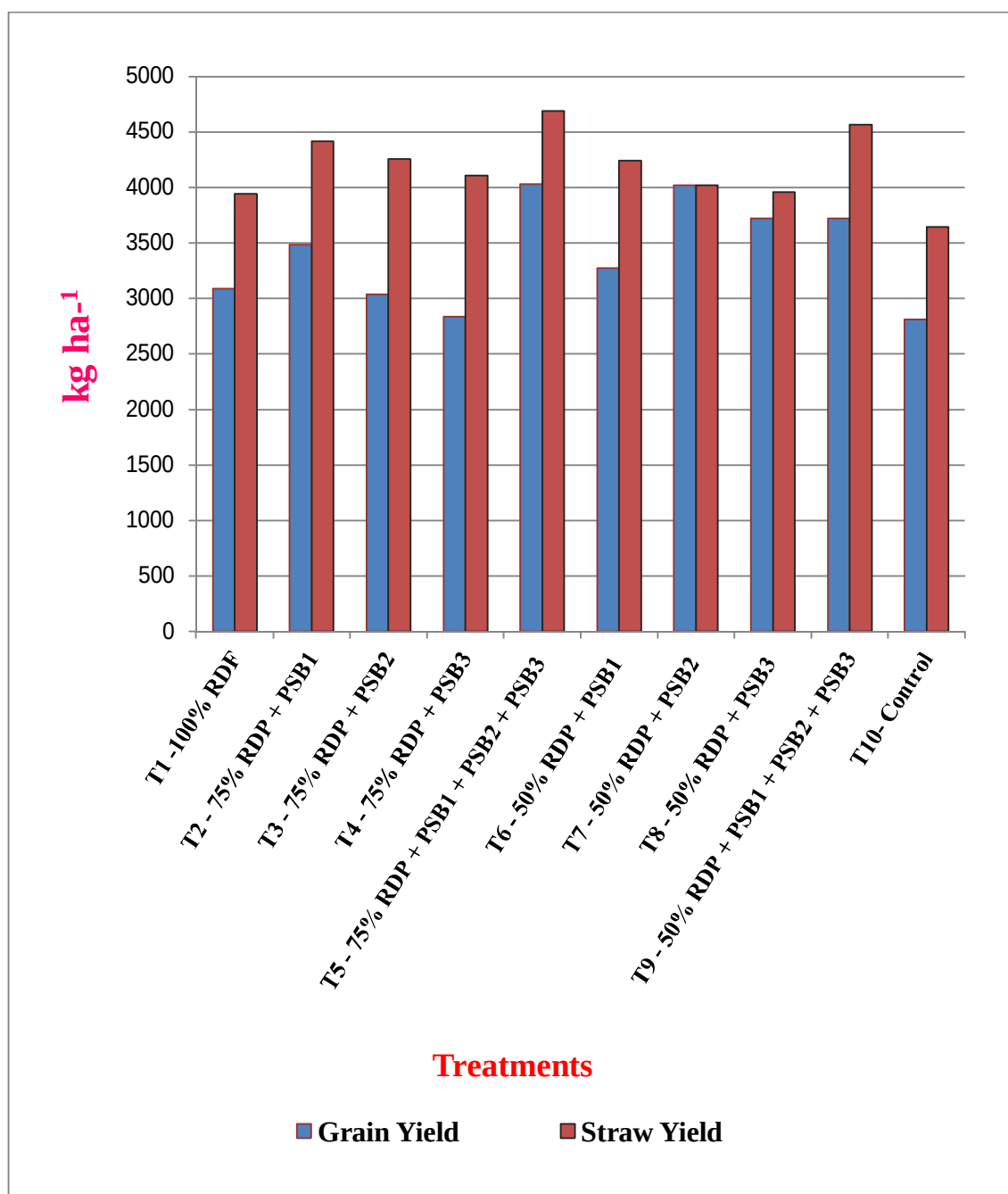


Fig. 4.14 Influence of PSB isolates on grain and straw yield of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

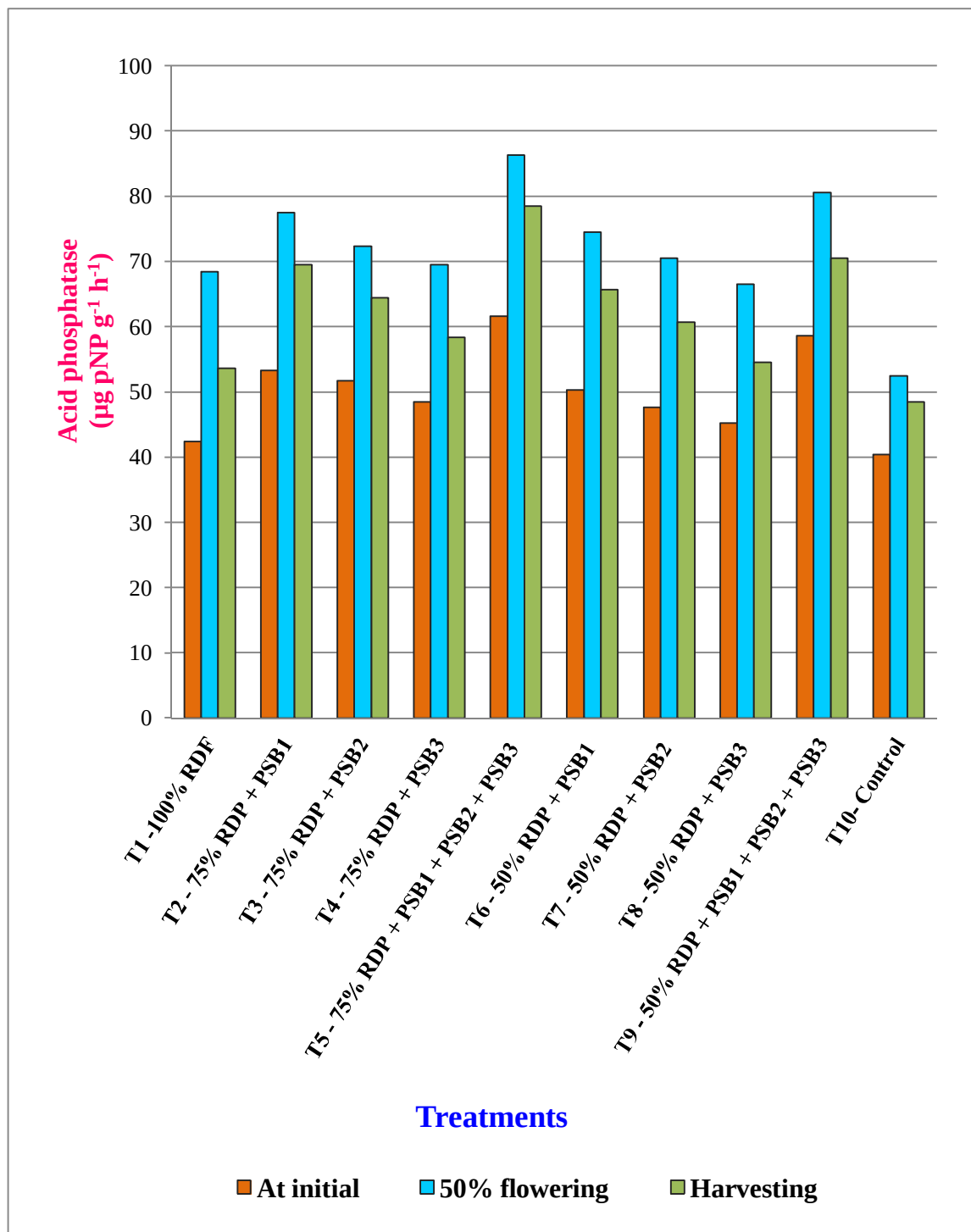


Fig. 4.17 Influence of PSB isolates on acid phosphatase enzyme activity in soil at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

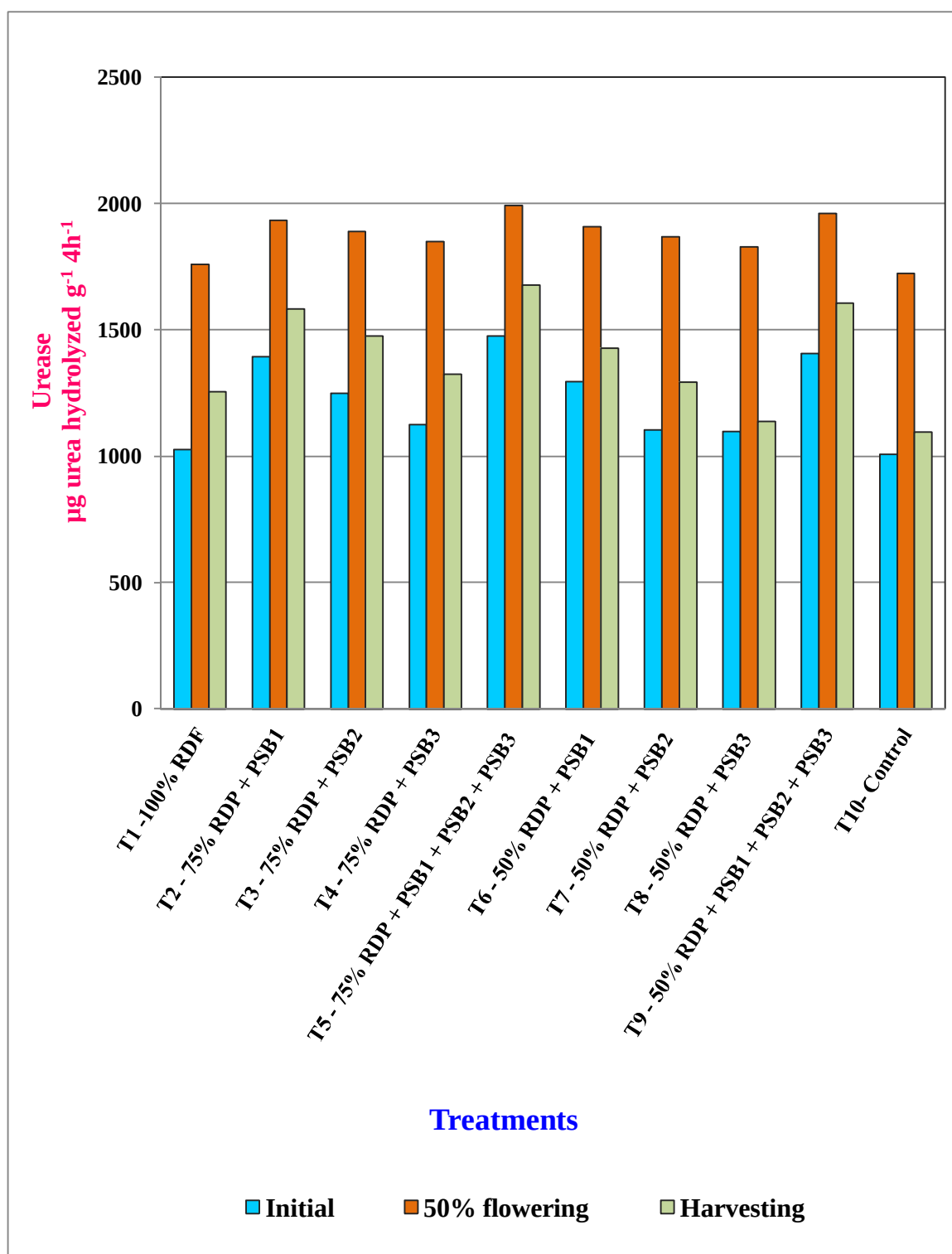


Fig 4.20 Influence of PSB isolates on urease enzyme activity in soil at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

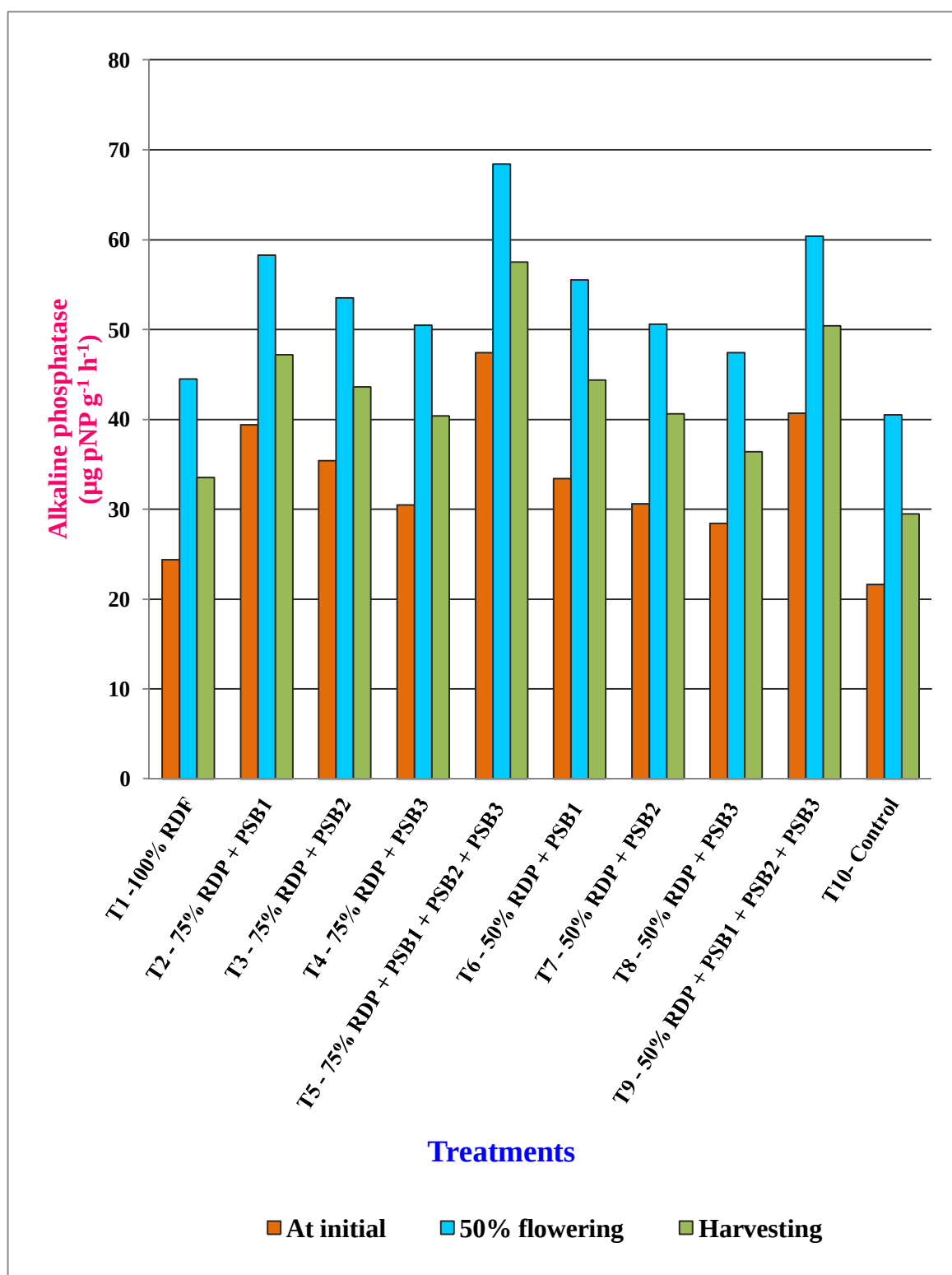


Fig 4.18 Influence of PSB isolates on alkaline phosphatase enzyme activity in soil at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

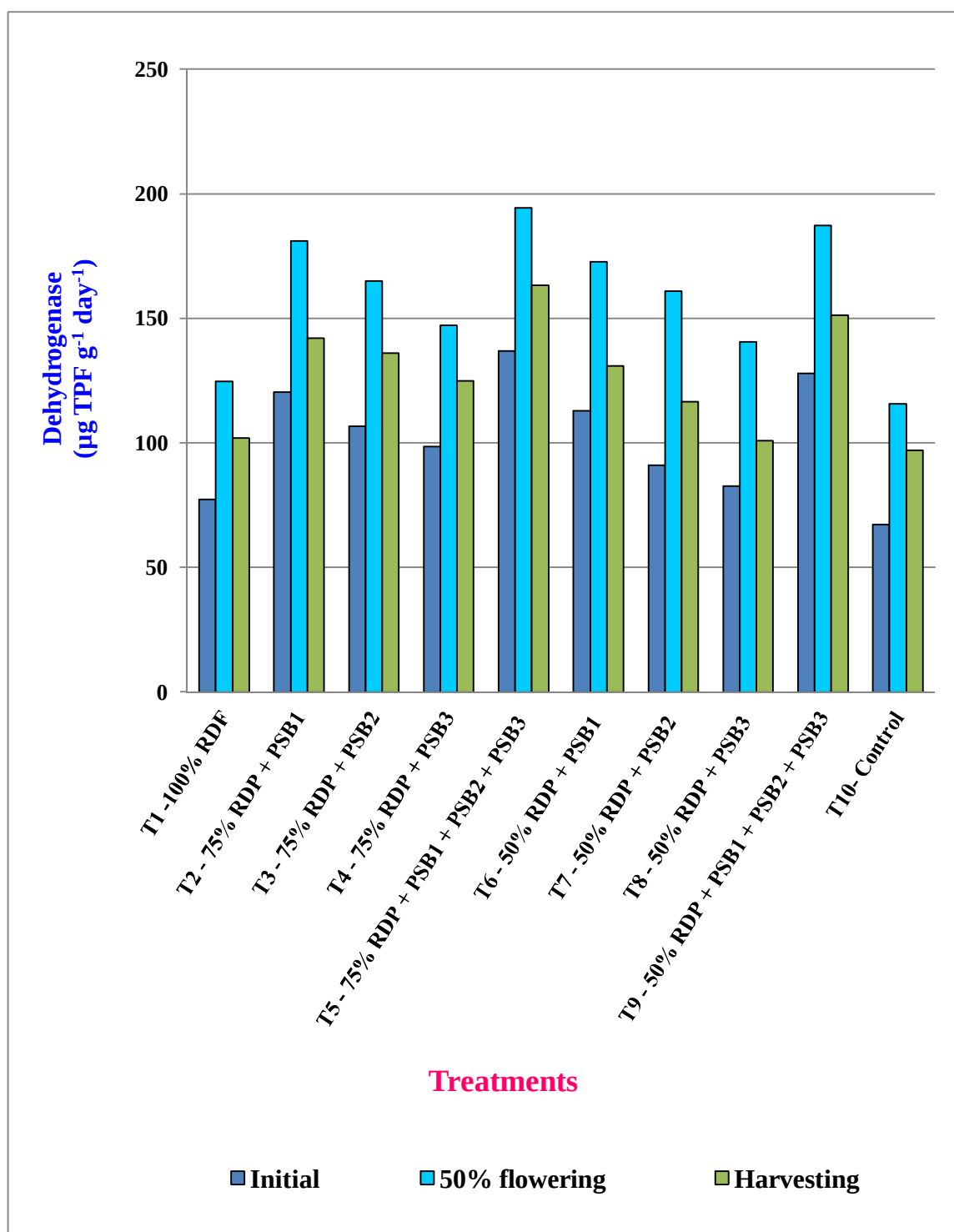


Fig 4.19 Influence of PSB isolates on dehydrogenase enzyme activity in soil at various stages of finger millet crop growth

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

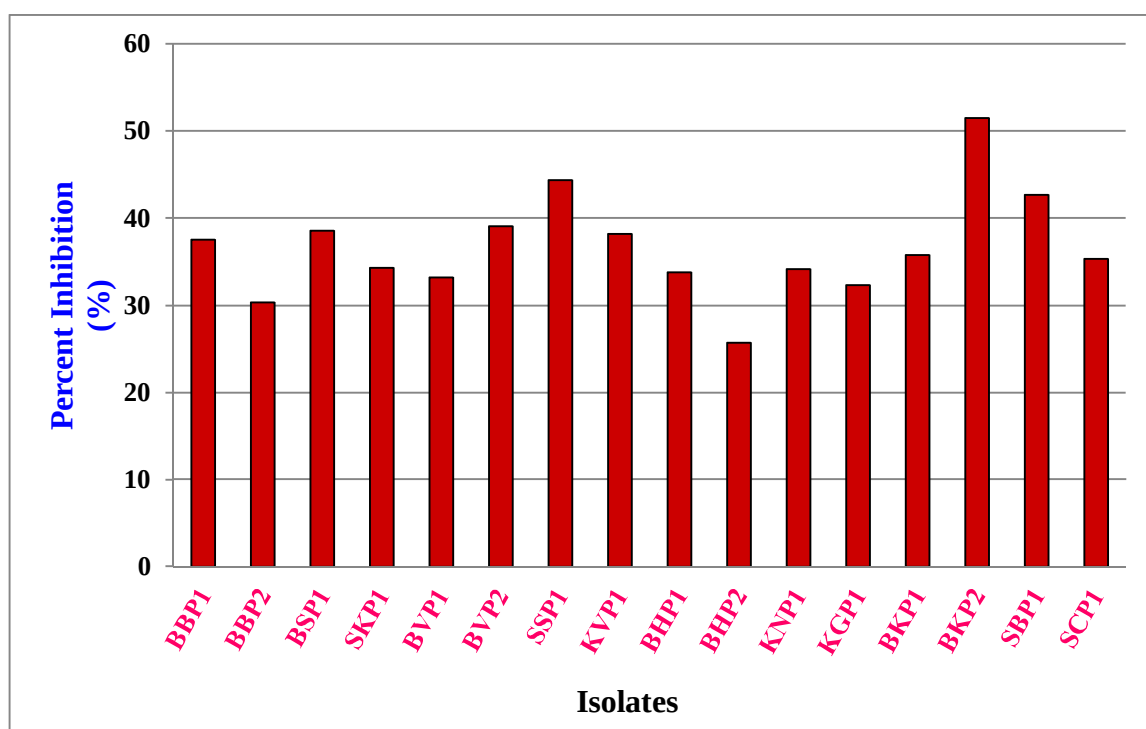
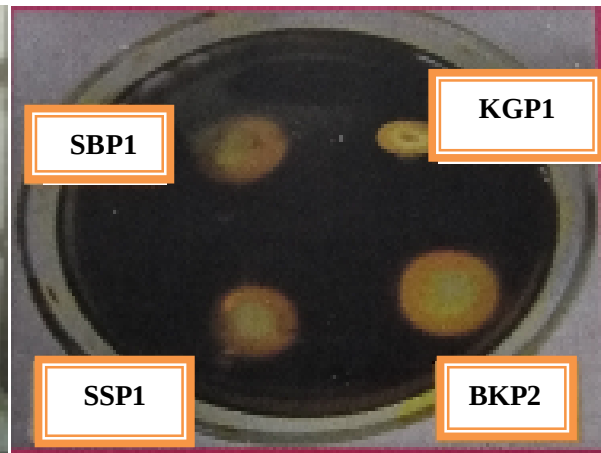


Fig. 4.7. Antagonistic activity of PSB isolates against *Rhizoctonia solani* by dual culture method



Ammonia Production



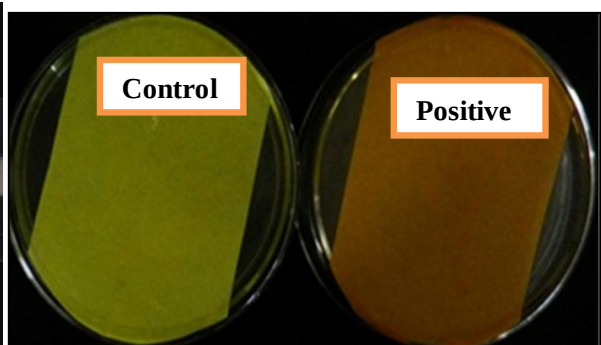
Siderophore production



IAA Production

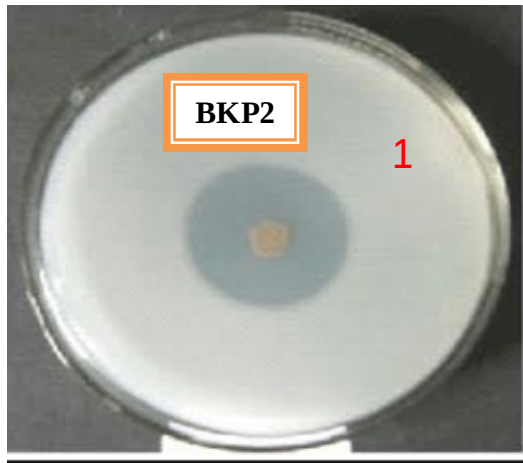


Antagonistic activity

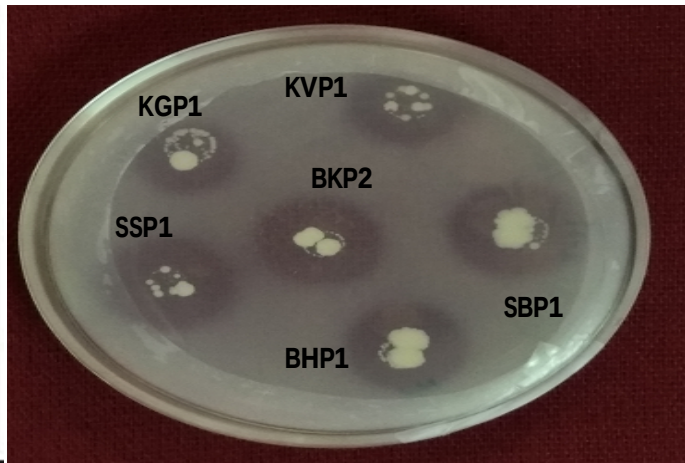


HCN Production

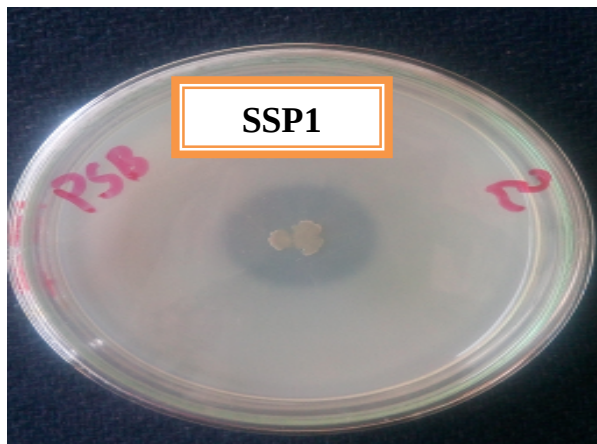
Plate 4.4 Plant growth promoting attributes of PSB isolates



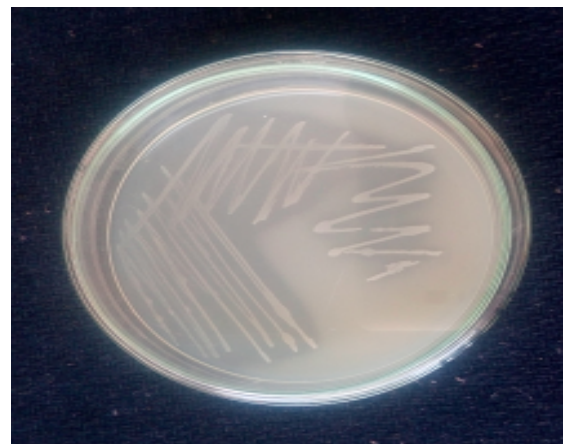
(a)



(b)



(c)

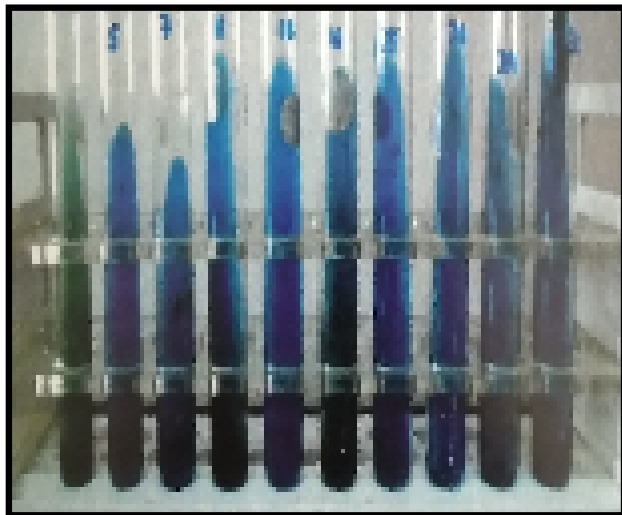


(d)



(e)

Plate 4.1 Phosphate solubilization by the PSB isolates



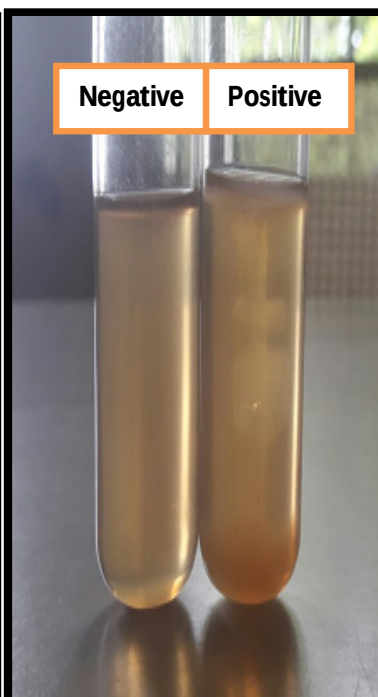
a) Citrate Utilization



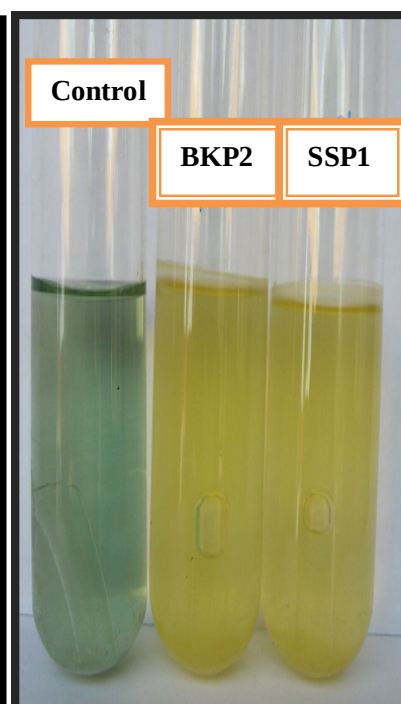
b) Oxidase test



c) Methyl red test

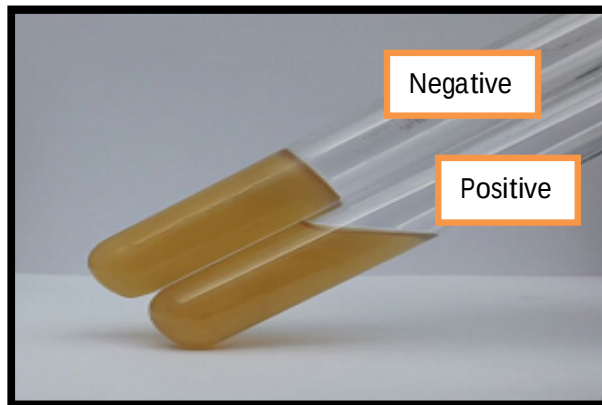


d) Motility test

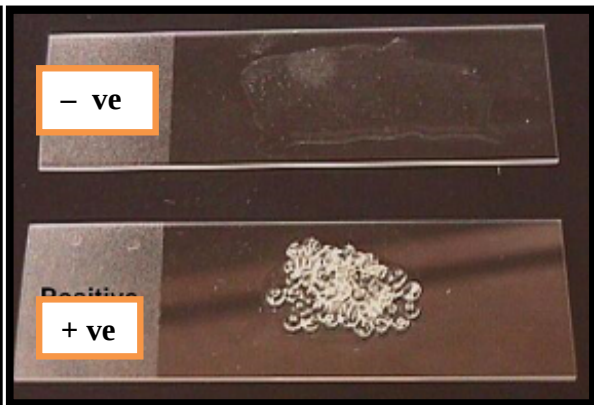


e) Gas and Acid production

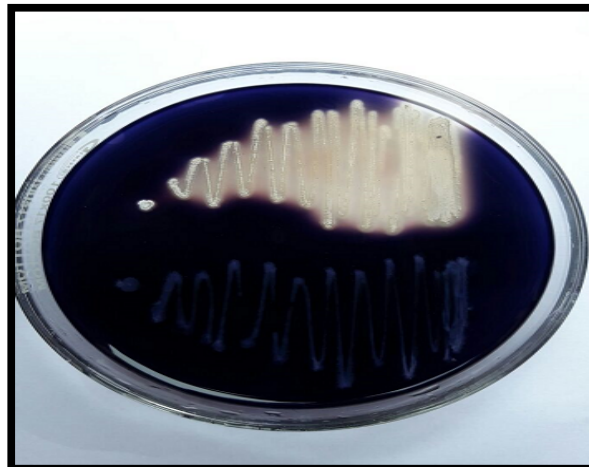
Plate 4.2 Biochemical characterization (a, b, c, d and e) of PSB isolates



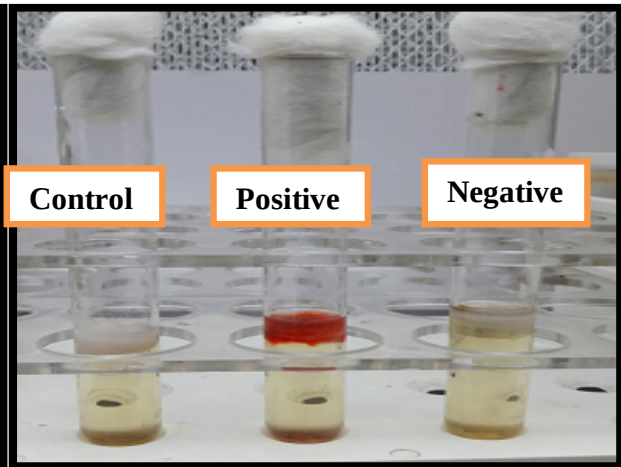
f) Gelatin liquefaction



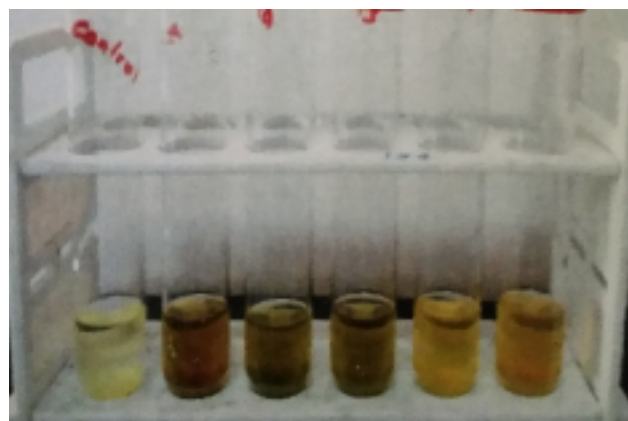
g) Catalase test



h) Starch hydrolysis



i) Voges proskauer's test

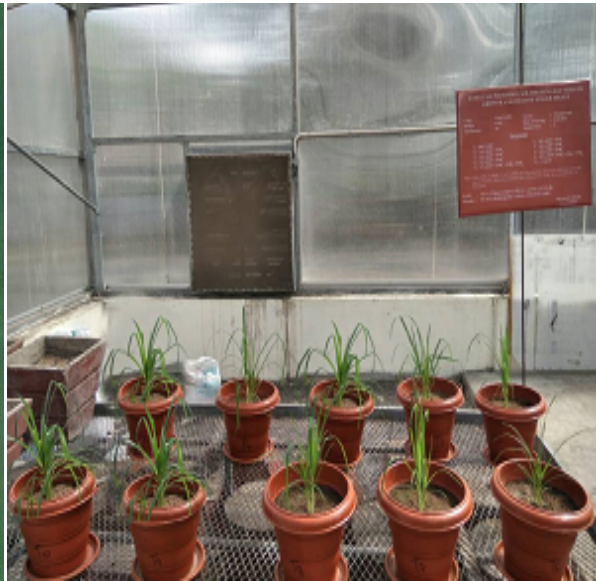


j) H₂S Production

Plate 4.3 Biochemical characterization (f, g, h, i and j) of PSB isolates



a) Ragi nursery



b) Transplanted ragi seedlings



c) Crop growth stage at 60 days after sowing

Plate 4.5 Pot culture study of PSB isolates on growth of finger millet



Plate 4.6 Influence of PSB isolates on no. of fingers per plant



Plate 4.7 Influence of PSB isolates on root length

Chapter II

REVIEW OF LITERATURE

The present investigation was carried out by screening and characterization of sixteen phosphate solubilizing bacterial isolates from different soils of finger millet growing areas in Andhra Pradesh, India and their influence on finger millet crop. The literature pertaining to these aspects is reviewed and presented below under appropriate headings, which will provide an overview of the current status of research work.

2.1 Isolation of Phosphate Solubilizing Bacteria (PSB) from rhizospheric soil samples.

2.2 Characterization of phosphorus solubilizing bacterial isolates.

2.3 Screening of pure isolates for PGPR properties

2.4 Effect of PSB on growth and yield of crops.

2.1 ISOLATION OF PHOSPHATE SOLUBILIZING BACTERIA (PSB) FROM RHIZOSPHERIC SOIL SAMPLES

Baliah and Begum (2015) isolated, identified and characterized phosphate solubilizing bacteria and biology of the selected strains was also studied. The study revealed that the population level of PSB was higher in the rhizosphere soils of cluster bean. The selected strains were identified as *Bacillus* and *Pseudomonas* sp.

Aarab *et al.* (2015) isolated three hundred and five bacteria from the rhizosphere of rice fields in Northwestern Morocco, of which one hundred and thirty six rhizobacteria were tricalcium phosphate solubilizers. Analysis of 16S rDNA partial sequencing demonstrated that these nine bacteria belong to three genera: *Aeromonas*, *Pseudomonas* and *Enterobacter*.

Anitha and Kumudini (2014) isolated *Pseudomonas* from fifteen rhizospheric samples from the field's of rice, chilly, ragi, beans and garden soils from different regions of India. They characterized morphologically and biochemically and concluded as genus *Pseudomonas*.

Rameshkumar *et al.* (2014) isolated and analyzed the genetic diversity of fluorescent Pseudomonads associated with the rice rhizosphere. Based on 16S rDNA sequence similarity the isolates were designated as *Pseudomonas plecoglossicida* (11), *P. monteilii* (3), *P. mosselii* (3), *P. libaniensis* (4) and *P. aeruginosa* (4).

Singh *et al.* (2013) had reported that most PGPR belong to gram negative genera, and the greatest numbers of strains are members of the fluorescent Pseudomonads and isolated *Pseudomonas* sp. from nine different rhizospheric soils of wheat and pigeon pea. Among the 21 *Pseudomonas* sp. four different *Pseudomonas* sp. isolates (YSY-13, YSY-15, YSY-17 and YSY-19) exhibited maximum plant growth promoting activity.

Jeyanthi and Ganesh (2013) isolated *Pseudomonas fluorescens* and identified as *Pseudomonas fluorescens* by 16S rRNA gene sequencing after following the conventional biochemical tests as per *Bergey's manual of Systematic Bacteriology*.

Mahantesh and Patil (2011) isolated PSB from the area around Bidar region and screened on the basis of their solubilization of inorganic TCP in liquid cultures. Ten strains that had higher solubilization potential were selected and characterized.

Castagno *et al.* (2011) isolated and characterized 50 phosphate solubilizing strains from a constrained environment such as the Salado river basin and assessed their phosphate solubilizing mechanisms. Among them they identified 17 non-redundant strains, *Pantoea*, *Erwinia*, *Pseudomonas* and *Enterobacter*. The results indicated that the isolates showed a significant plant growth promoting activity on *Lotus tenuis*.

Mishra *et al.* (2010) isolated two PGPR isolates from the rhizosphere soil of Pyrethrum (*Chrysanthemum cineræfolium*) designated as MA-2 and MA-4 and identified as *Bacillus subtilis* and *Pseudomonas fluorescens* and reported that they increased the productivity of *Pelargonium graveolens*.

Lin *et al.* (2006) reported phosphate-solubilizing bacterium that was isolated from soil in Pingdong and Taiwan and identified as *B. cepacia* based on its 16S ribosomal DNA (rDNA) sequence. The supernatant was filtered through a 0.22 µm Millipore filter and assessed for bacterial population, pH, organic acids, and phosphate solubilizing capability.

Fankem *et al.* (2006) collected seven rhizosphere soil samples from oil palm tree of Cameroon. The collected soil samples were air dried, crushed to pass through 2mm sieves and the PSB were isolated by using the soil samples. For the estimation of phosphate solubilization Reyes basal medium having calcium, aluminum and iron phosphates was used. At the end of incubation time, it appeared that, phosphate solubilization resulted from a combined effect of pH decrease of the media and organic acids production. Furthermore, each of the tested isolates was able to produce at least one of the most important organic acids such as citrate, malate and tartrate. Among the ten isolates tested, three were identified as *Pseudomonas fluorescens* and would be considered as potential biofertilizers.

Ponmurugan and Gopi (2006) reported that the population density of phosphobacteria in the rhizosphere soils of brinjal, chilly, cotton, green grain, groundnut, maize, paddy, ragi, sorghum and turmeric using Ketznelson and Bose medium following dilution plate technique. The result showed that the population levels of phosphobacteria were higher in the rhizosphere soil of groundnut plant (14.90×10^5 CFU⁻¹g soil dry wt.) and paddy (10.08×10^5 CFU⁻¹g soil dry wt.).

Chen *et al.* (2006) isolated, screened and characterized 36 strains of PSB from Central Taiwan. Mineral Phosphate Solubilizing (MPS) activities of all isolates were tested on TCP medium by analyzing the soluble phosphate content after 72 h of incubation at 30°C.

Chung *et al.* (2005) isolated and identified the bacterial groups that actively solubilized phosphates *in vitro* from rhizosphere soil of various crops from Korea. Out of several 100 colonies that grew on Pikovskaya's medium, 13 best isolates were selected based on the solubilization of insoluble phosphate in liquid culture, further characterized and clustered under the genera *Klebsiella*, *Pantoea*, *Enterobacter*.

2.2 CHARACTERIZATION OF PHOSPHORUS SOLUBILIZING BACTERIAL ISOLATES

Saikrithika *et al.* (2016) isolated a PSB from the vermicomposted soil in Chennai, which also had the ability to utilize TCP at different concentrations. The bacterium was studied for its biochemical characteristics. The 16S rRNA gene sequencing proved that the bacterial strain SK1 belongs to *Bacillus flexus*. The bacterial strain SK1 showed maximum growth when they were cultured at temperature 35°C and at pH 4. The bacterial strain was able to utilize up to 125 mg l⁻¹ of TCP in the medium.

Rai and Sharma (2015) isolated 21 rhizobacteria from rhizospheric soils of Uttarakhand, Tarai region. The isolated soil bacteria were screened for the production of IAA, phosphate solubilization and siderophore production. Out of 21 isolates, 3 isolates (P3, P9, and P19) were screened for IAA production that are Gram negative, catalase positive and starch hydrolysis positive. Isolate P19 was the best IAA producer strain ($50.25\mu\text{g ml}^{-1}$) while isolate P3 was lowest IAA producer ($15.25\mu\text{g ml}^{-1}$) comparatively. All three isolates showed siderophore production while phosphate solubilization was shown by only P19 isolate.

Nehra *et al.* (2014) isolated PGPR strains from the rhizospheric soil of the cotton plant from various places of Haryana, India. Isolate exhibiting maximum PGP traits was identified as *Pseudomonas fluorescens* based on the biochemical characterization, phenotypic microarray analysis and 16S rDNA sequencing. The isolate was screened *in vitro* for their plant growth promoting traits like production of IAA, ammonia production, HCN production, siderophore production, phosphate solubilization. The isolate exhibited positive results for ammonia and siderophore production and phosphate solubilization.

Ratnakar (2014) isolated seventy eight strains of *P. fluorescens* from the rhizosphere of banana. Eighteen strains were selected for their plant growth promoting activity. They were tested for *in vitro* plant growth promotion activities such as phosphate solubilization, siderophore production, IAA production and fungal cell wall degrading enzyme production. All the strains were positive for siderophore and IAA production. Ten strains were positive for phosphate solubilization. Six strains possess the ability to produce chitinase, cellulase and protease.

Bumunang and Babalola (2014) screened species of *Pseudomonas*, *Aeromonas*, *Sphingomonas*, *Burkholderia*, *Stenotrophomonas*, *Achromobacter*, *Ewingella* and *Bacillus* *in vitro* for PGP traits such as ammonia production, catalase activity, indole acetic acid production, Phosphate solubilization and antifungal activity. All the thirty two rhizobacterial strains tested in this study were positive for catalase activity, ammonia production and IAA production; 90.6% were positive for phosphate solubilization, 34.3% indicated antifungal activity.

Hongsheng *et al.* (2014) isolated a novel phenol-degrading bacterium SKDP-1 from crude oil contaminated soil, Guano oil field in the northeast Shandong Dongying, East China. The biochemical tests indicated that strain SKDP-1 was Gram-negative and utilized glucose, citrate and starch not gelatin. Both Voges-Proskauer and H_2O_2 tests were positive and the SKDP-1 strain identified as *Pseudomonas*.

Kumar *et al.* (2014) isolated and characterized PGPR from the rhizospheric soil of wheat from different areas of Uttar Pradesh, India. 20 isolates were isolated, biochemically characterized and screened for plant growth promoting traits like IAA production, ammonia production, siderophore production and phosphate solubilization. Out of 20 isolates 20, 6 and 10 produced ammonia, siderophore and solubilized phosphate on PVK's agar medium.

Jyothi and Reddy (2014) reported that 55 (JS-1.....JS-55) isolates of fluorescent *Pseudomonas* were isolated from the rhizosphere soil of groundnut fields in Rayalaseema region of Andhra Pradesh. Based on colony morphology and biochemical tests, out of 120 samples, only 55 isolates were identified as fluorescent *Pseudomonas*. These were tested for their ability to produce secondary metabolites such as HCN, Ammonia, Salicylic acid (SA), IAA etc. Maximum production of secondary metabolites (HCN-0.094 Abs 625; SA-4.68 mg ml⁻¹; IAA-22.5 µg ml⁻¹) was found with fluorescent *Pseudomonas*.

Shivalingaiah and Umesha (2013) isolated ten strains of fluorescent Pseudomonads from the rhizosphere soils of rice growing areas in Karnataka, India. All the ten tested isolates of Pseudomonads were positive for the production of siderophores, HCN, IAA, chitinase, β-1,3-glucanase, cellulase, SA production, and phosphate solubilization.

Deshwal *et al.* (2013) isolated 140 strains of *Pseudomonas* from potato rhizosphere. Strains were isolated on King's B medium and Cetrimide agar medium and various biochemical tests were done. Out of one hundred and forty isolated *Pseudomonas* strains, thirty seven strains were identified as *P. fluorescens*.

Jayamma *et al.* (2013) isolated 57 bacterial isolates belonging to *Bacillus* and *Pseudomonas* from different rhizospheric soils of southern Telangana zone. These isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like phosphate solubilization, production IAA, HCN and siderophore. Among the *Pseudomonas*, 65% produced HCN, 45% solubilized phosphate with highest solubilization zone of 17mm and only 25% produced IAA and siderophores.

Kumari *et al.* (2013) isolated forty two rhizobacterial isolates and the isolates were characterized bio-chemically and found belonging to genera *Pseudomonas* (19), *Bacillus* (22) and *Serratia* (1).

Saranraj *et al.* (2013) isolated and characterized *Pseudomonas fluorescens* by Gram staining, motility test, plating on King's B medium and bio-chemical tests. Ten *Pseudomonas fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of IAA production. The maximum IAA production was recorded by the isolate PF-8. The minimum production of IAA was found in PF-4 isolates. The isolate *Pseudomonas fluorescens* (PS-8) showed maximum siderophore production and the least siderophore production was showed by the *Pseudomonas fluorescens* isolate PS-4.

Paramageetham and Babu (2013) isolated fluorescent Pseudomonads from forest litter of Seshachalam hills the first ever biodiversity reserve. Among the 34 isolates four isolates were tentatively identified as *Pseudomonas fluorescens* based on morphological and biochemical characters.

Saravanan *et al.* (2013) studied biochemical characteristics of fluorescent *Pseudomonas* and showed that all ten isolates were positive to catalase, amylase, gelatinase and siderophore production, while three isolates (Pf5, Pf6 and Pf9) were oxidase positive, nine isolates (Pf1, Pf2, Pf3, Pf4, Pf6, Pf7, Pf8, Pf9, and Pf10) were tolerant to 6.5% NaCl.

Vishal and Punkuj (2013) studied on production of plant growth promoting substance by Pseudomonads (55), *Pseudomonas aeruginosa* (22), *P. putida* (26), *P. cepacia* and 37 *P. fluorescens* strains were screened for their plant growth promoting activity. *Pseudomonas aeruginosa* showed positive PGPR activity i.e., 44 (IAA), 34 (HCN), 35 (siderophore production), 43 (P solubilization). Similarly *Pseudomonas putida* confirmed positive PGPR activity i.e., 14 (IAA), 12 (HCN), 14 (Siderophore production), 14 (P-solubilization). *Pseudomonas cepacia* also produced positive results i.e., 19 (IAA), 16 (HCN), 15 (siderophore production), 15 (P - solubilization) and *Pseudomonas fluorescens* showed 22 (IAA), 19 (HCN), 21 (siderophore production), 23 (P - solubilization) positive PGPR activity. The study showed that *Pseudomonas* as an effective plant growth promoting bacterium.

Ghodsolavi *et al.* (2013) isolated 40 colonies of bacteria from the rhizosphere of Valerian. They produced siderophore, IAA, HCN, lipase and protease were tested *in vitro*. Results revealed that the effects of seven isolated bacteria (belong to *Pseudomonas* genus) showed a high potential of siderophore, IAA, HCN, lipase and protease production and the quantity of root extracts under greenhouse condition.

Belkar and Gade (2012) isolated fifteen isolates of *Pseudomonas fluorescens* from the acidic and alkaline rhizospheric soils of different field crops of Vidarbha and Konkan region. All the cultural and biochemical studies confirmed them to be *P. fluorescens*. The isolates showed positive response for siderophore production and phosphate solubilization, while negative for IAA and HCN production.

Parani and Saha (2012) evaluated *Pseudomonas fluorescens* K-34 a solubilized TCP and produced substantial amount of soluble phosphorus (968.5 mg l^{-1}) in PVK broth as compared to others and exhibited the production of IAA, siderophore, cell wall degrading enzyme activities and growth inhibition against fungal and bacterial pathogens.

Panhwar *et al.* (2012) isolated PSB from aerobic rice grown in Penang Malaysia and determined the biochemical properties of the isolates such as, organic acids, enzymes, indoleacetic acid (IAA), siderophore production and its antagonistic effect against pathogen *Rhizoctonia solani*. The highest P solubilizing activity (69.58%) was found in PSB9 strain grown in NBRIP plate.

Sarvani and Reddy (2012) isolated 30 *Bacillus* cultures from the rhizospheric soils of groundnut and red gram crops growing in Rangareddy district, India. These were screened for PGP attributes and antagonism against soil borne phytopathogens viz., *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani*. Results revealed that 50% (15/30 isolates) reacted positively for one or more PGP properties. A high prevalence of antagonists was found against the three fungal pathogens. Majority of the bacterial isolates (33%) displayed antagonism through the production of siderophores / HCN and the remaining isolates showed antagonism with negative results for siderophores and HCN indicating that, the rhizospheric soils are the rich sources of *Bacillus* fungal antagonists, which have a potential to be used in the future as PGP inoculants.

Mansoureh and Halimi (2012) isolated 20 strains of Pseudomonads from the rhizosphere soils of paddy areas in Malaysia and were screened for their plant growth promoting activity. All the 20 tested isolates of Pseudomonads were positive for the siderophores production and HCN, 15 strains (75%) showed positive for the plant growth-promoting hormone, IAA. All the twenty bacterial isolates (except DL21) inhibited the pathogen in the dual culture assay.

Showkat *et al.* (2012) collected rhizosphere soil from different wheat growing regions of Kashmir valley which were evaluated for presence of *Pseudomonas fluorescens* using King's B medium. Based on colony morphology, siderophore production and biochemical tests, out of 136 rhizosphere soil samples, only 52 isolates were identified as *Pseudomonas fluorescens*.

Noori and Saud (2012) isolated twenty Pseudomonad strains from the rhizosphere soils of paddy areas in Malaysia and were screened for their plant growth promoting activity. All the 20 tested isolates of Pseudomonads were positive for the production of siderophores and HCN. Out of 20 tested isolates, 15 strains (75%) showed positive for the production of plant growth promoting hormone, IAA. Among the 20 isolates, 18 isolates (90%) produced phosphate solubilization on NBRIP medium. Following biochemical identification kit, of the 20 isolates, 15 strains were identified as *Pseudomonas fluorescens*.

Negi *et al.* (2011) collected six hundred native cold-tolerant rhizospheric bacterial isolates from Uttarakhand Himalayas, of which three hundred and thirty six were confirmed as fluorescent *Pseudomonas* spp. On the basis of specific biochemical tests, these were characterized into three major groups: *P. fluorescens* (308), *P. aeruginosa* (20), and *P. putida* (8).

Mehnaz (2011) isolated *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Gluconacetobacter*, etc. from sugarcane and reported that use of PGPR can minimize the cost of fertilizer, environmental hazard and suppress the diseases as well. PGPR are very well known for their role in plant growth promotion mainly for biological nitrogen fixation, phytohormone production acting a biocontrol agent.

Preeti *et al.* (2011) isolated twenty eight bacterial cultures from Korea and they were characterized by various microscopic and cultural examinations. Out of which four isolates were identified as *Pseudomonas* sp. and others were *Bacillus subtilis*.

Anand and Kulothungan (2010) isolated *Pseudomonas fluorescens* from groundnut rhizosphere and tested for their ability to produce secondary metabolites such as HCN, Salicylic Acid (SA) and iron chelating siderophores using standard protocols. Maximum production of secondary metabolites (HCN - 0.08, SA - 6.14 mg ml⁻¹; Siderophores - 4.92 µ mol benzoic acid ml⁻¹) were found with *P. fluorescens*.

Maleki *et al.* (2010) reported that out of 144 bacteria from cucumber rhizosphere, eight isolates were identified as *Pseudomonas fluorescens*. He stated that these isolates were selected for root colonization and PGP properties. Among these CV-6 strain produced considerable amounts of siderophore, indole acetic acid and also shown positive reactions for HCN, catalase, protease and phosphatase.

Abbas *et al.* (2010) reported PGP activities of 40 different strains of *Pseudomonas fluorescens* and *Pseudomonas putida*, isolated from the rhizosphere of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.). Most of the bacterial isolates were able to produce IAA, HCN, siderophores and also solubilised phosphorus.

Rekha *et al.* (2010) isolated a proteobacterium from rhizosphere soil and it was identified using morphological, cultural and biochemical characteristics as *Pseudomonas fluorescens*.

Mehta *et al.* (2010) selected a highly efficient P-solubilizing strain, presumptively, identified as *Bacillus circulans* MTCC 8983 and screened for plant growth promoting traits. They reported that the strain solubilized TCP and produced substantial amount of soluble phosphorus (957.3 mg lit⁻¹) in PVK broth and exhibited the production of IAA (15.13 ug ml⁻¹), siderophores (57.80%) and growth inhibition against *Dermatophora necatrix* (46.57%).

Sachdev *et al.* (2009) investigated on the microbial biota associated with wheat rhizosphere during flowering-stage and their plant growth promoting traits. 2 *Acinetobacter* strains, 5 *Pseudomonas* strains and 4 *Bacillus* strains produced siderophores. Strains of *Pseudomonas* exhibited fungal growth inhibition. *Arthrobacter globiformis* Y2S3 exhibited IAA production, siderophore production and antifungal activity. They reported that PGP traits of these rhizobacteria indicated beneficial relationship between rhizobacteria and wheat plants.

Shahab and Ahmed (2008) studied phosphate solubilization efficiency of 10 soil bacteria for various parameters like carbon sources such as Glucose, Fructose, Sucrose and Lactose, variable concentration of NaCl and Glucose. Glucose was the most favorable carbon source for solubilization while lactose is the least favorable carbon source. *Acinetobacter lwoffii*, *Pseudomonas aeruginosa* and *Bacillus thuringiensis* were found to be the most promising isolates.

Megha *et al.* (2007) isolated 52 fluorescent *Pseudomonads* from soils of different forests in Western Ghats of Uttara Kannada District, Karnataka. 37 isolates were identified upto species level based on biochemical and physiological characters as *Pseudomonas fluorescens*.

2.3 SCREENING OF PURE ISOLATES FOR PGPR PROPERTIES

2.3.1 Phosphate Solubilization

Dipak and sankar (2016) found that the strain KUPSB12 was effective in phosphate solubilization with phosphate solubilization index of 2.85 in PVK agar plates along with very high soluble phosphate production of $219.64 \pm 0.33 \mu\text{g ml}^{-1}$ in liquid medium. The phosphate solubilizing bacterium was identified using physiological, morphological and biochemical characters. The phosphate solubilizing bacterium was identified as a strain of *Pseudomonas aeruginosa*.

Manouchehr *et al.* (2016) studied the phosphate solubilisation potential of inhabiting bacteria from rhizosphere of *Avicennia marina* plants. Among 13 isolates screened, four high PSB were selected based on the formation and dimension of clear halo zone around the colonies. Phylogenetic analysis of 16S rDNA of PSBs showed their close similarity to *Bacillus*, *Pseudomonas* and *Acinetobacter* species. Results showed that PSB1 and PSB3 were found as the most potent isolates with 3.5 and 2.6 Phosphate Solubilization Index (PSI) values, respectively. However, maximum P solubilization was found in broth for PSB10 (357 mg l^{-1}) and PSB3 (282 mg l^{-1}), respectively. The consortium experiment showed a positive effect on total P solubilized by these isolates as solubilizing efficiency increased by 373 mg l^{-1} . P solubilizing efficiency also achieved in a high percentage range for consortium culture (74-78%) and PSB10 (71-77%).

Tensingh *et al.* (2015) studied on isolation, identification and characterization of PSB isolated from economically important crop plants. The population level of PSB was higher in the rhizosphere soils of clusterbean. The selected strains were identified as *Bacillus* and *Pseudomonas* sp. The isolated strains were characterized under *in vitro* conditions. The selected strains were differed in P solubilization zone formation, pH change, phosphatase activity, organic acids production and P solubilization. PSB strains could grow well at the temperature ranged from 28°C to 35°C. All the strains differed in utilization of different carbon, nitrogen, amino acid, vitamin sources.

Karpagam and Nagalakshmi (2014) isolated a total of 37 phosphate solubilizing microbial colonies on PVK's agar medium, containing TCP from agricultural soil. The colonies showing clear halo zones around the microbial growth were considered as phosphate solubilization. Out of 37 microbial isolates, 8 isolates showed highest PSI ranged from 1.13-3.0 were selected for further study as qualitative as well as quantitative activities. Among these 8 potent isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates along with high soluble phosphate production of 0.37 mg l^{-1} , 0.30 mg l^{-1} and 0.28 mg l^{-1} in broth culture. These isolates psm1, psm2 and psm6 belongs to genus *Pseudomonas*, *Bacillus* and *Rhizobium* as identified by their morphological and biochemical properties respectively.

Panda *et al.* (2013) studied native population of PSB in the rhizosphere of maize, rice, ginger and large cardamom grown in different regions of Sikkim. A total of 26 best PSB were isolated based upon their solubilization on solid media from 104 rhizospheric samples collected from three agroclimatic zones. Phosphate solubilization of these isolates varied from 46 to 160% and 30.2 to 203.7 mg l^{-1} in solid and liquid Pikovskaya's medium respectively. All the isolates were able to solubilize aluminium and iron phosphate significantly. Among the three phosphate sources all the isolates solubilized $\text{Ca}_3(\text{PO}_4)_2$ to a greater extent than AlPO_4 and FePO_4 with AlPO_4 exhibiting poor solubilization. The highest PSB number and greatest variability were found in the rhizosphere of rice. Based on the morphological and biochemical analysis the isolates were clustered under the genera *Bacillus*, *Pseudomonas*, *Micrococcus* and *Delftia*. Among the four genera, *Bacillus* was the most predominant PSB found in all soils tested.

Jena and Rath (2013) reported that the production of acidity in the medium is directly correlated to the reduction in pH of the medium due to the production of variety of organic acids. In the present study, PMD-7 isolate recorded highest P-solubilization indicating its potential as an efficient P-solubilizer.

Roy *et al.* (2013) isolated thirty one *Pseudomonas* sp. from rhizospheric soil of rice. Out of thirty one, nine isolates were able to solubilize $\text{Ca}_3(\text{PO}_4)_2$ on Pikovskaya's agar medium. Isolate MGR38 was identified as *P. fluorescens* which showed 83.33% efficiency in phosphate solubilization and the other isolates MGR31, MGR37, MGR39, MGR40, MGR41, MGR42, MGR43 and MGR44 showed 33.33, 57.14, 50, 50, 16.16, 40, 60 and 80 % efficiency of phosphate solubilization respectively.

Alemu (2013) isolated twelve *Pseudomonas fluorescens* species from rhizospheric soils of faba bean and tested for phosphate solubilization. All tested isolates of *Pseudomonas fluorescens* species have a potential of phosphate solubilization on Pikovskaya's media. He summarized that all *Pseudomonas fluorescens* spp. can be used as bio-fertilizers for soil fertility improvement.

Worapon *et al.* (2013) studied PSB isolated from rhizosphere soil of chilli cultivated on organic and conventional farms. Characterization of organic acid and ability of mineral phosphate solubilization were also investigated. The effects of PSB on the growth of chilli were observed in a pot trial. The most PSB (over 68%) could be obtained from organically managed soil. Ten isolates of PSB were selected based on tricalcium phosphate solubilizing ability.

Tripti *et al.* (2012) isolated fifty PSB colonies on Pikovskaya's (PKV) agar medium, containing insoluble TCP, from repeatedly chemical pesticide used agriculture soil of Dhanbad area. The colonies showing clear halo zones around the bacterial growth were considered as phosphate solubilizers. Out of 50 bacterial isolates, 10 isolates showing highest PSI ranged from 1.4 - 3.0 were selected for the further study as qualitative as well as quantitative performance. Among these 10 potent isolates, two strains S2 and S30 showed the maximum PSI of 3.1 and 3.0 in agar plates along with high soluble phosphate production of 373.07 mg l⁻¹ and 368.58 mg l⁻¹ in broth culture, respectively. Isolates S2 and S30 belong to genus *Bacillus* sp. and *Pseudomonas* sp. as identified by their morphological and biochemical characteristics, respectively.

Maheshwar and Sathyavani (2012) isolated PSM's *Bacillus subtilis* and *Bacillus cereus* from the groundnut rhizosphere soil, showed maximum solubilization around 40°C. Screening of PSB was done in *Bacillus subtilis*, *Bacillus cereus* in both solid and liquid medium. *B. subtilis* is the most efficient for phosphate solubilizing activity at pH 7 with carbon sources (0.35±0.02), nitrogen sources (0.28±0.03) and potash sources (0.12±0.09).

Manivannan *et al.* (2011) reported six different phosphate solubilizing microorganisms (*Pseudomonas* spp. *Bacillus* spp. *Penicillium* spp. and 3 strains of *Aspergillus* spp.) from the rhizospheric soils of chilli and tomato plants. He found that *Penicillium* spp. solubilized tricalcium phosphate 1045 µg ml⁻¹ and *Pseudomonas* spp. solubilized 985 µg ml⁻¹ after 14 days of incubation in Pikovskaya's broth medium and in all cultures phosphate solubilization ability was decreased due to organic acid production.

Joshi and Bohra (2011) isolated 72 rhizobacterial isolates from mungbean rhizospheric soil on King's B medium, from various locations near Jodhpur. All the isolates were screened for their ability to solubilize insoluble phosphate on Pikovskaya's medium. All the isolated strains were screened for P-solubilization on Pikovskaya's medium. The medium contained TCP as sole source of phosphate. Four isolates were able to solubilize phosphate ranging from 42.69 $\mu\text{g TCP ml}^{-1}$ to 90.10 $\mu\text{g TCP ml}^{-1}$. MrbIII 7 has shown the largest zone of solubilization and MrbI 12 has shown the smallest zone of solubilization. However, isolate MrbIII 7 and MrbI 4 spread considerably along the zone of solubilization, thus creating a larger zone of clearance.

Fengling *et al.* (2011) studied on isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* spp. YCWA18 from daqiao saltern on the coast of yellow sea of china, which can also perform phosphate-solubilization ability. The fastest growth was observed when the culturing temperature was 28°C and the concentration of NaCl was 6% (w/v). It was found that the bacterium can survive at a concentration of NaCl up to 20%. At the optimum condition, the bacterium solubilized 283.16 $\mu\text{g ml}^{-1}$ phosphorus in 11 days. The growth of the bacterium was concomitant with a significant decrease of acidity in the medium.

Kumar *et al.* (2010) isolated 6 PSB from paddy fields of eastern Uttar Pradesh, India and identified as members of *Enterobacter* and *Exiguobacterium* genera. Of the 6 isolates, *Enterobacter* sp. exhibited high level of phosphate solubilization in liquid medium. *Exiguobacterium* sp. showed increased phosphate solubilization efficiency under alkaline pH.

Keneni *et al.* (2010) isolated the PSB from the rhizosphere of fababean of Ethiopia and their abilities on solubilizing insoluble phosphates. Five efficient PSBs were selected for further study based on their ability in forming a higher clear zone diameter than the other isolates. These isolates were identified based on phenotypic characters. And the results revealed that all the PSB isolates significantly ($P \leq 0.01$) solubilized a higher amount of Tri Calcium Phosphate (TCP), Egyptian Rock Phosphate (ERP), and Old Bone meal (OB) over the uninoculated control. The highest amount of solubilization was achieved for TCP with Meh-305 (39 mg per 50 ml) followed by ERP with Meh101 (31 mg per 50 ml).

Vyas and Gulati (2009) isolated nineteen phosphate-solubilizing fluorescent *Pseudomonas* strains. *P. fluorescens*, *P. poae*, *P. trivialis* and *Pseudomonas* spp. produced gluconic acid, oxalic acid, 2-ketogluconic acid, lactic acid, succinic acid, formic acid, citric acid and malic acid in the culture filtrates during the solubilization of

Tricalcium phosphate, Mussoorie rock phosphate, Udaipur rock phosphate and North Carolina rock phosphate. The strains differed quantitatively and qualitatively in the production of organic acids during solubilization of phosphate substrates.

Oliveira *et al.* (2009) reported several phosphate solubilizing microorganisms from maize rhizospheric soil. The strains of bacteria, fungi and most of the actinobacteria were confirmed as PSM with high 'P' solubilization activity. Among the bacterial isolates, B17 and B5, identified as *Bacillus* sp. (B17) and *Burkholderia* sp. (B5) respectively, were the most efficient 'P' solubilizing strains from P-Ca source culture solution solubilizing 67% and 58.5% of the total 'P' respectively. The B5 isolate also had the highest reduction in pH in the growth solution to 4.46.

Trivedi and Pandey (2008) characterized *Bacillus megaterium* strain B388, isolated from rhizosphere soil of pine belonging to a temperate Himalayan location. The plant growth promotion by the bacterium has been evaluated through petridish and broth based assays. The isolate solubilized TCP under *in vitro* conditions; maximum activity ($166 \mu\text{g ml}^{-1}$) was recorded at 28°C after 15 days of incubation.

2.3.2 Ammonia Production

Prasad *et al.* (2013) isolated and identified twenty six *Pseudomonas* sp. and were *in vitro* screened for PGPR properties. The results revealed that all *Pseudomonas* isolates were positive for IAA production, 76.9% for phosphate solubilization, 92.3% for ammonia, 88.46% for siderophores, 80.76% for HCN productions. Eight isolates showed inhibition potential against *Rhizoctonia solani* and *Sclerotium rolfsii*

Kaur and Sharma (2013) isolated 35 rhizobacterial isolates. Among ten isolates were identified and characterized as *Pseudomonas* sp. These were screened for growth promotion activities, production of IAA, ammonia (NH_3), HCN, siderophore, phosphate solubilization, catalase and seed germination on water agar medium along with reference strain PGPR LK884 (*Pseudomonas diminuta*). Maximum amount of IAA was produced by PGPR-3 ($70.05 \mu\text{g ml}^{-1}$) followed by PGPR-2 ($66.79 \mu\text{g ml}^{-1}$) as compared to PGPR LK 884 ($61.58 \mu\text{g ml}^{-1}$) in the presence of L-Tryptophan as precursor of IAA.

2.3.3 IAA Production

Hossain *et al.* (2016) isolated ten isolates of bacteria designated as SS01, SS02, SS03, SS04, SS05, SS06, SS07, SS08, SS09 and SS10 from rhizosphere of chickpea. All the isolates produced IAA in various ranges. On the contrary, SS01 was found to be a weak IAA producer in comparison to the minimum IAA producer isolates viz. SS02, SS03, SS04, SS05 and SS09. SS06 and SS07 produced the medium quantity IAA producer. On the other hand, only SS10 produced the highest IAA among the isolates.

Dweipayan *et al.* (2015) developed a simple, quick and reliable method for the detection and quantitation of indole-3 acetate (IAA) and indole-3-butyrate (IBA), an auxin phytohormone produced by rhizobacteria from L-tryptophan (Trp) metabolism using High Performance Thin-Layer Chromatography (HPTLC). Results revealed that the ability of *Pseudomonas putida* strain A (AB667903) to produce Indole-3 Lactic Acid (ILA), IAA and Indole-3 Acetamide (IAM) from Trp, qualitative analysis showed that ILA was the most abundant compound produced ($2.6\text{--}34.0\text{ }\mu\text{g ml}^{-1}$), followed by IAA ($0.7\text{--}10.3\text{ }\mu\text{g ml}^{-1}$) with IAM present in very small amounts ($0\text{--}0.4\text{ }\mu\text{g ml}^{-1}$).

Kamble and Galerao (2015) reported that *Pseudomonas* species were selectively obtained followed by screening for IAA production. Most efficient species were selected for further studies like optimization of growth conditions for *Pseudomonas*, comparative studies for IAA production and effect of IAA on seed germination. The study shows *Pseudomonas* is potential bacterium for IAA production. Further it was observed that within short period of time considerable enhancement in the germination was observed in case of seeds treated with IAA.

Sivasakthi *et al.* (2013) isolated *Pseudomonas fluorescens* and *Bacillus subtilis* and designated as PF-1 to PF-10 and BS-1 to BS-10. The efficiency of *P. fluorescens* and *B. subtilis* for IAA production, Gibberellic acid production, Siderophore production and Phosphate solubilization was estimated. The maximum IAA and Gibberellic acid (GA) production by *P. fluorescens* was recorded by the isolate PF - 8 and the minimum production was observed in PF - 4 isolate. The IAA production, Gibberellic acid production and siderophore production by *B. subtilis* was low when compared to *P. fluorescens*. The maximum phosphate solubilization was recorded by the isolate BS - 8.

Sagervanshi *et al.* (2012) studied on 5 strains of PSB and reported that 2 isolates were gram negative, round shape and 2 isolates were positive reaction to IAA production.

Madhuri (2011) isolated and screened the bacterial isolates from Leguminous plants groundnut i.e. *Arachis hypogaea* L., lucerne i.e. *Cicer arietinum* L., fenugreek i.e. *Trigonella foenumgraecum* L., lucerne i.e. *Medicago sativa* L. Results revealed that out of 60 colonies selected from rhizosphere of groundnut, 40 colonies from rhizosphere of groundnut and 22 colonies from rhizosphere of lucerne showed red colour with Salkowaski reagent on nylon 6'6' membrane indicating production of IAA by the organisms.

Sanjani *et al.* (2011) reported that PSB (*Bacillus* spp., *Pseudomonas* spp.) were positive reaction to IAA production.

Ashraf *et al.* (2011) isolated 12 bacterial strains from root and rhizosphere samples collected from different sugarcane growing areas. 10 strains were identified as *Pseudomonas* and 2 as *Azotobacter* on the basis of colony and cell-morphology. All isolates showed IAA production in growth medium containing tryptophan as a precursor. Though IAA production (4.49 mg l^{-1}) was detected in isolate A17 where as IAA production in strains A4 and all was also significant. Most of the strains showed beneficial effects on root length, root area and plant dry weight.

Upadhyay and Srivastava (2010) isolated *Pseudomonas fluorescens* strain and it was reported that this strain solubilized complexed phosphates and also synthesized phytohormone (IAA).

Jayasudha *et al.* (2010) evaluated forty fluorescent Pseudomonads quantitatively for IAA producing ability in the presence or absence of tryptophan and growth promotion in groundnut in response to seed treatment with high IAA producers was analyzed. IAA producers of the fluorescent *Pseudomonas* group showed significant plant growth when compared with control.

Sadaf *et al.* (2009) studied on IAA production and enhanced PGP by indigenous PSB's. IAA and Indole butyric acid were produced by these bacteria in varying concentration with and without the addition of tryptophan. These bacteria showed stimulatory effects on the growth of root and shoot elongation of mungbeans. Three promising isolates CMG854, CMG857 and CMG860 were investigated to establish the effect on plant growth. On a comparative basis isolate CMG860 was most promising in promoting plant growth.

Karnal (2009) reported that 30 fluorescent *Pseudomonas* isolates (15 *P. fluorescens* and 15 *P. aeruginosa*) were characterized on the basis of biochemical tests and PGP activities. *P. fluorescens* AK1 and *P. aeruginosa* AK2 showed the best plant growth-promoting activity. These isolates were tested for their ability to produce IAA in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 $\mu\text{g ml}^{-1}$. For both strains, indole production increased with increases in tryptophan concentration (0.5, 1.2, 4.3 and 9.3 $\mu\text{g ml}^{-1}$; and 0.2, 0.7, 3.8, and 8.3 $\mu\text{g ml}^{-1}$, respectively). *P. aeruginosa* AK2 was less effective in production of IAA than *P. fluorescens* AK1.

2.3.4 Siderophore Production

Marathe *et al.* (2015) isolated two siderophore producing bacterial strains from waste water sample and labeled them as RM1 and RM2. Among these two isolates, RM1 strain was found to produce more siderophore. 16s rRNA analysis and biochemical characterization, identified the RM1 strain as *Pseudomonas aeruginosa* 6A (bc4). Siderophore production was determined by more sensitive and reliable Chrome Azurol-S (CAS) method. Chemical and spectrophotometric assays showed that *P. aeruginosa* 6A strain produced 99% siderophore units.

Veronique *et al.* (2015) studied the detailed process of Pyoverdine I (PVDI) and pyochelin (PCH) siderophores produced by *Pseudomonas aeruginosa* PA01 and the cytoplasmic enzymes involved in each of these two siderophore biosynthesis pathways can form siderophore specific multi-enzymatic complexes called siderosomes associated with the inner leaflet of the cytoplasmic membrane.

Mandal and Kotasthane (2014) isolated fifty nine *Pseudomonas fluorescens* from the rhizosphere and non rhizospheric soil of cave, forest, fallow land and agriculture field in Chhattisgarh region. The amounts of siderophore produce by *P. fluorescens* isolates were screened in iron deficient succinate media and most of them were found positive for the production of much siderophores. One of the isolate from Pakhanjore area “P3” produced highest siderophore.

Sreedevi *et al.* (2014) isolated ten *Pseudomonas* sp. Among isolated strains, three *Pseudomonas* isolates P1, P2 and P3 showed siderophore production on succinic acid medium and CAS agar plate medium. The ability of *Pseudomonas* to grow and to produce siderophores is dependent on the iron content and the type of carbon sources in

the medium. 4 basal media, supplemented with different concentration of iron, were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas* isolates. Maximum siderophore production was 94, 88, 83 units for P1, P2 and P3 isolates respectively. All 3 isolates have showed both type of siderophore production i.e. wine red color formation in supernatant indicated production of hydroxamate type (pyoverdine) while yellow color formation in supernatant showed presence of catecholate or phenolate type (pyochelin) siderophore.

Vanitha and Ramjegathesh (2014) isolated ten different strains of *Pseudomonas fluorescens* from *Coleus* rhizosphere except the pf1 strain and identified by biochemical tests. The mechanism of *Pseudomonas* strains namely the iron-chelating agent (siderophore) and volatiles (HCN) production tests were studied and reacted for siderophore and HCN production.

Kannahi and Kowsalya (2013) evaluated on efficiency of plant growth promoting rhizobacteria for the enhancement of *Vigna mungo* growth. They concluded that the isolates of *pseudomonas* species were strong siderophore production while few isolates of *Bacillus* were able to produce siderophore.

Souza *et al.* (2013) isolated 336 bacterial strains belonging to the genera *Agrobacterium*, *Burkholderia*, *Enterobacter*, and *Pseudomonas* spp. Siderophore and indolic compound producers were widely found among isolates, but one hundred and one isolates were able to solubilize phosphate.

Tailor and Joshi (2012) isolated 7 bacterial isolates from sugarcane rhizosphere. All the isolates were found to produce more than 85% siderophore units. Among them S-11 was found to be the most efficient siderophore producer (96 %). S11 was further characterized and identified as *Pseudomonas fluorescens*. Physicochemical parameters were evaluated for optimum production of siderophores by *Pseudomonas fluorescens* strain.

2.3.5 HCN Production

Karmel *et al.* (2014) isolated, purified indigenous *Pseudomonas* spp. and *Bacillus* spp. and evaluated its ability to produce HCN synthesis and also evaluated the potential of super strains on reduction in the fungal growth of *Macrophomina phaseolina*. Results showed that among these isolates on qualitative analysis of HCN

indicated a strong production of HCN in Pf1 and CPf5. Isolates of *Bacillus subtilis* did not produce HCN. In quantitative estimation, Pf1 and CPf5 recorded the maximum OD value of 0.094 and 0.085 respectively but Bs10 recorded least OD value of 0.015.

Jayaprakashvel *et al.* (2010) isolated twenty four hydrogen cyanide (HCN) producing fluorescent *Pseudomonads* from the rhizosphere of sand dune vegetation from Chennai coastal area and were subjected to spectrophotometric assay to relatively quantify the amount of hydrogen cyanide (HCN) produced by them. Five fluorescent *Pseudomonads* strains designated as AMET1039, AMET1041, AMET1042, AMET1055 and AMET1064 produced more amount of HCN in their volatile fraction.

Mehnaz *et al.* (2009) isolated seven fluorescent *Pseudomonads* from the roots, shoots and rhizosphere soil of sugarcane were shown to produce HCN which had biocontrol ability against *Colletotrichum falcatum* inciting red rot disease in sugarcane in addition to their ability to plant growth.

2.3.6 Antagonistic Activity

Priyanka *et al.* (2017) carried research work to assess antagonistic potential of 24 fluorescent *Pseudomonas* isolates *in vitro* against *R. solani* and *S. rolfsii*. Differences in growth inhibitions of *R. solani* and *S. rolfsii* ranged from 4.44 to 55 and 8.325 to 49.9%, respectively. Confrontation assays revealed isolate P76 exerting antagonism against both *R. solani* and *S. rolfsii*, where as fluorescent *Pseudomonas* isolates (P201, P176, P6, P151 and P248, P216, P85, P167) exerted pathogen specific antagonism against *R. solani* and *S. rolfsii*, respectively. Isolate P205 and P129 showed the lowest inhibitory effect on *R. solani* and *S. rolfsii*, respectively. Variation in quantitative inhibitory data was significant at 0.01 and 0.05% level for *S. rolfsii* and 0.05% for *R. solani*.

Evelise *et al.* (2016) evaluated the biological control and rhizosphere competence of these PGP bacteria. Wheat seeds were inoculated with *Bipolaris cynodontis*, *Drechslera tritici-repentis* and *Fusarium graminearum* and the biocontrol ability of *B. cepacia* 89 was tested. Results revealed that both *B. mycoides* B38 V and *B. cepacia* 89 showed remarkable antifungal activity in addition to other PGP characteristics, representing potential inoculants and biocontrol agents.

Aly *et al.* (2015) revealed that among 116 bacterial isolates, 19 bacterial isolates showed the highest inhibition percentage against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Regarding the antifungal activities, results indicated that isolates B38 and B103 recorded the highest values of chitinase activity, catechol type siderophores and NH₃. Data showed that isolate B103 was the highest producer for HCN followed by the isolates number B13 and B388. Concerning the production of volatile compounds, data indicated that isolates B103 and B38 showed considerable inhibition against pathogenic fungal growth. The most potent isolates were identified as *Pseudomonas fluorescens* (B103) and *Bacillus subtilis* (B38).

Mezeal (2014) isolated four bacterial strains of *Bacillus subtilis* and 5 strains of *Pseudomonas fluorescens* from tomato field soil. The antagonistic microorganisms against the pathogens were observed by dual culture technique. *Pseudomonas fluorescens* isolate was found to show 81.3% and 77.4% of growth inhibition against *Rhizoctonia solani* and *Fusarium oxysporum* respectively while *Bacillus subtilis* was found to show 77.4% and 73.2% of growth inhibition against test pathogens respectively.

Soltani *et al.* (2012) reported three isolates (PA24, PA1, PA18) of Pseudomonads showed *in vitro* antifungal activity against *Rhizoctonia solani*. Results of this study showed that pseudomonads which are native to soils of Iran had the potential to be used for promotion of plant growth and suppression of soil borne plant pathogens.

Lawongsa *et al.* (2012) isolated *Pseudomonas fluorescens* R21 from rhizosphere of rice in Thailand, *Pseudomonas fluorescens* F113 from rhizosphere of sugar beet in Ireland and studied *in vitro* antagonistic activity against phytopathogens.

Kumar *et al.* (2012) isolated seven bacterial isolates from rhizosphere of common bean growing at Uttarakhand, Himalaya. Strain BPR7 was identified as *Bacillus* sp. and strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium solani*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Colletotrichum* sp. *in vitro*. Cell-free culture filtrate of strain BPR7 caused colony growth inhibition of all test pathogens. PGP and antifungal activities of *Bacillus* sp. BPR7 suggest that it may be exploited as potential bioinoculant agent for *P. vulgaris*.

Supraja *et al.* (2011) isolated fifteen fluorescent *Pseudomonas* isolates from rhizospheric soils of redgram and maize crops in the Rangareddy district. These test isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like phosphate solubilization, production of indole acetic acid (IAA), Hydrogen cyanide (HCN) and siderophore. Due to production of HCN and siderophores, fluorescent *Pseudomonas* isolates inhibited the growth of *Fusarium moniliforme*. All the 15 isolates inhibited the growth of fungal pathogen except MPF-1. Among the 15 isolates, RPF-5 (46.30%) showed highest percent inhibition zone. This study suggested that a few isolates of the fluorescent *Pseudomonas* could be potential biocontrol agents against *Fusarium moniliforme* based on the studies conducted *in vitro*.

Wahyudi *et al.* (2011) isolated 118 *Bacillus* spp. from the rhizosphere of Soybean plant of Cirebon, Indonesia, and further examined for plant growth promoting activities. A total of 90 isolates (76.3%) among them positively produced phytohormone indole acetic acid. The 12 isolates (13.3%) were selected, initially on the basis of germinating seed bioassay in which the root length, shoot length or number of lateral roots of the seedling was enhanced significantly. Among them 12 isolates produced siderophores and 11 isolates (91.7%) were able to solubilize phosphate. Furthermore, 3 isolates (25%) were able to inhibit the growth of *Fusarium oxysporum*, 9 isolates (75%) inhibited the growth of *Rhizoctonia solani*, and 1 isolate (8.3%) of *Bacillus* spp inhibited the growth of *Sclerotium rolfsii*.

Nicolas (2011) isolated *Pseudomonas* sp. from rhizospheric soil of tomato and pepper plants, and was evaluated as potential antagonist of fungal pathogens and tested against the causal agents of stem canker and leaf blight (*Alternaria alternata* sp. *lycopersici*), southern blight (*Sclerotium rolfsii*) and root rot (*Fusarium solani*) strains significantly inhibited *Alternaria alternata* f. spp. *lycopersici*, particularly in 25% TSA medium. Antagonistic effect on *Sclerotium rolfsii* and *Fusarium solani* was greater on Kings B medium.

Rakh *et al.* (2011) isolated eleven *Pseudomonas* sp. from rhizospheric soil and tested for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas cf. monteilii* 9, showed highest antagonistic activity against *Sclerotium rolfsii*. In dual cultures, the *Pseudomonas cf. monteilii* 9 inhibited the *Sclerotium rolfsii* up to 94% in terms of dry weight. *Pseudomonas cf. monteilii* 9 produced diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophores which affected *Sclerotium rolfsii* growth.

Ahmadzadeh and Sharirifi-tehrani (2009) detected the production of HCN by six isolates out of the 41 selected fluorescent *Pseudomonads* having good antagonistic activity against *Rhizoctonia solani* under *in vitro* conditions.

2.4 EFFECT OF PSB ON GROWTH AND YIELD OF CROPS

Harinathan *et al.* (2016) found that, the isolate C2 (*Bacillus* sp.) treated plants recorded maximum total chlorophyll, IAA, starch, fresh and dry weight compared to C7 isolate (*Pseudomonas* sp.) treated plants both in pearl millet and ragi. Maximum fresh weight and dry weight of pearl millet and ragi were recorded when treated with isolate C2 compared to C7 on all schedules of 30 and 45 days. Plant height recorded maximum in pearl millet on 45 days when treated with isolate C7 while ragi recorded maximum growth when treated with C2 isolate.

Lin *et al.* (2016) isolated thirty six PSB strains with the ability to solubilize inorganic P and screened for their PGPR properties. Of all the 36 PSB strains analyzed, three (*Psychrobacter alimentarius* HB15, *Enterobacter ludwigii* HB21, and *Ochrobactrum haematophilum* HB36) were selected for a controlled plant inoculation experiment. Inoculation of tobacco plants with these bacterial strains significantly increased plant dry weight. Additionally, inoculation increased P, K, and N uptake by tobacco seedlings as well as soil P availability.

Thilakarathna *et al.* (2015) found that in finger millet, application of 50% of the recommended inorganic fertilizers, gliricidia green leaf manure and biofertilizers (*Azotobacter* and PSB) produced highest grain yield (3.95 t ha^{-1}) compared to farmers traditional practice (1.76 t ha^{-1}).

Muhammad *et al.* (2015) isolated nineteen isolates of rhizobacteria associated with sunflower (*Helianthus annuus* L.) and screened for phosphate solubilization and indole-3-acetic acid (IAA) production. Two potential PSB isolates with substantial IAA biosynthetic capacity, Ps-5 and Ss-2, were identified. Based on 16S rRNA gene sequence analysis, isolate Ps-5 was identified as *Bacillus* spp and Ss-2 as *Alcaligenes faecalis*. Both strains were found to be metabolically diverse in terms of the number and amount of different carbon substrates they utilized. Ps-5 and Ss-2 were found to be good root colonizers and significantly ($P \leq 0.05$) increased sunflower growth and phosphorus (P) uptake.

Gurdeep and Sudhakara (2015) conducted a two-year field study to test the effects of two PSB, *Pantoea cyripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5), inoculated singly or together with rock phosphate (RP) fertilization on maize and wheat cropping cycle by comparing with chemical P fertilizer (Di Ammonium Phosphate, DAP), mainly in the crop yield, soil fertility and economic returns. Inoculation of PSB together with RP fertilization increased the crop growth in terms of shoot height, shoot and root dry biomass, grain yield and total P uptake in both maize and wheat crops compared to the other treatments.

Ida *et al.* (2015) conducted an experiment to determine the potency of phosphate PSM's and studied the solubilisation of P and P uptake of plant in peat soil. Based on their ribosomal DNA, the best PSM were identified as *Burkholderia gladioli* that yielded the highest growth and maximum uptake of a phosphate by oil palm seedling.

Viruel *et al.* (2014) conducted a pot culture experiment to investigate the effects of seven previously isolated PSB on early development of plants. Seeds were treated with each bacterial strain, and seedlings were harvested 30 days after inoculation. All strains showed a positive effect on plant growth. A significant increment in plant height (45%), shoot dry weight (40%) was determined in plants treated with *Pseudomonas tolaasii* IEXb, while *Pseudomonas koreensis* SP28 has remarkably increased P content compared to the uninoculated control. These results provided baseline information for future studies of *P. tolaasii* IEXb as bioinoculant to promote an eco-friendly and sustainable agriculture.

Reetha *et al.* (2014) collected soil samples from rhizosphere of onion plants from Botanical garden, Department of Botany, Annamalai University. Effect of IAA producing *Pseudomonas fluorescens* and *Bacillus subtilis* bacteria on plant growth was studied by pot culture experiments by using sterilized air dried soil and viable onion seeds. Both bacteria demonstrated increase in root length, shoot length, root and shoot fresh and dry weights, on bacterial inoculated onion seeds over control.

Abdullahi *et al.* (2013) observed that the application of bio-fertilizers and organic manures singly and in combination improved pearl millet yield in low-input agriculture. Results also revealed that bio-fertilizer application reduced the application of organic manure by 50%.

Deshwal and Kumar (2013) reported that selected strains PW-99, PW-136, PW-2, PW-56, PW-18, PW-43, PW-5 and PW-104 effectively showed four major PGPR activities such as IAA, HCN, Siderophore and Phosphorous solubilization. In present study, nine treatments were prepared. Each seed coated with 108 cfu of *Pseudomonas* culture. Results suggested that all *Pseudomonas* strains enhanced plant growth in rice plant. *P. fluorescens* PW-5 produced maximum shoot, root and dry weight by 157.72, 408.06 and 233.84% respectively as compared to control.

Rizvi *et al.* (2013) evaluated the efficacious nature of some oil seed cakes such as neem cake and castor cake, a botanical *Calotropis procera* and PSB, *Pseudomonas fluorescens* singly and in various combinations on the growth and productivity of *Trigonella* plant. Although all the parameters were significantly increased in these treatments, maximum yield were obtained in the combinations of PSB and neem cake.

Prathibha and Siddalingeshwara (2013) observed the effect of PGPR strains, *Pseudomonas* and *Bacillus subtilis* in improving seed quality such as seed germination, vigour index and nutritional quality such as protein content and carbohydrate content of sorghum.

Shruti *et al.* (2013) isolated fluorescent *Pseudomonas* strains from the soil samples collected from Bhojia Institute of Life Sciences, Budh, Baddi. The *Pseudomonas* sp. strain showed positive effect by increasing both roots and shoots length of rice plant as compared with control. Both dry shoots and dry root weight of plants inoculated by the *Pseudomonas* spp. strain exhibited positive result as compared with control. Similarly, both wet shoots and dry shoot weight of plants inoculated by *Pseudomonas* spp. strain also showed the positive results as compared with control.

Phan *et al.* (2013) conducted an experiment on the effect of inoculating *Pseudomonas fluorescens* (1N) bacteria on rice in a greenhouse using 15N labelled urea and variable nitrogen (N) fertilizer levels. At 20 days after sowing (DAS), inoculation halved the fertilizer-N uptake compared to the control, which lacked bacteria. There was a significant increase in dry weight of the rice plants, but no difference in total N uptake. Inoculation with 1N bacteria increased dry matter yield as well as N uptake of rice plants from both fertilizer and non-fertilizer sources at 45 DAS.

Hussain *et al.* (2013) isolated seventy two bacterial isolates from maize rhizosphere. All bacterial isolates were tested for P-solubilization. Out of thirty bacterial isolates, fifteen showing higher phosphatase activity, ACC-deaminase activity and auxin production in liquid culture were selected for further evaluation for their growth promoting activities under axenic conditions. Inoculation with selected bacteria

significantly increased shoot length, root length, shoot fresh and dry weight, and root fresh and dry weights up to 39.7, 58.9, 99, 69.4, 97.7 and 87%, respectively over uninoculated control.

Singh and Ghosh (2012) isolated a total of 93 isolates. Out of 93 isolates 30 isolates were selected for their evaluation of phosphate solubilizing potential on Pikovskaya's media. Out of 30 isolates five isolates showed positive result on Pikovskaya's medium producing clear zones. Further, out of five isolates, three isolates individually, and as well as consortia were tested on *Zea mays* in pot under natural environmental conditions. The observations revealed that C (32) D, C (32) E were efficient strains by improving the growth of pot experiments. Further these isolates were used for biofertilizer production.

Qureshi *et al.* (2012) isolated PSB and conducted field trial of PSB with chemical fertilizer and revealed that bacterial inoculum produced significantly higher seed cotton yield 1630 kg ha⁻¹ as compared to 1511 kg ha⁻¹. Also reported that solubilizing efficiency and solubilizing index of bacterial isolates ranged between 233 to 275 % and 3.3 to 3.8 respectively.

Karnwal (2012) reported that inoculation of *Bacillus lentus*, *Bacillus* sp. *Bacillus subtilis*, *Pseudomonas fluorescens*, *Bacillus halodurans*, *Pseudomonas* strains as biofertilizer will be clearly showed greater root and shoot dry weight response in maize and wheat compared to control by different plant growth substances of these rhizospheric bacteria.

Adjanohoun *et al.* (2011) reported that *Pseudomonas fluorescens* and *Pseudomonas putida* are the best plant growth promoting candidates for maize crop improvement on reddish ferrous field. An increased root biomass of 59.57% and 23.40% was recorded with *Pseudomonas fluorescens* and *P.aeruginosa*, respectively.

Ekin (2010) evaluated the effect of application of PSB, *Bacillus* Sp. with and without varying amounts of phosphorus fertilizer on growth and yield of sunflower under field conditions. The application of PSB was able to mobilize phosphate efficiently in the sunflower and improved seed quality and oil yield. It also enhanced the head diameter, seed weight, kernel ratio, oil content and increase in oil yield. However, when PSB was used in conjunction with phosphatic fertilizers, a much greater effect was observed.

Yadav *et al.* (2010) reported five isolates designated as *Pseudomonas aeruginosa* strain BHUPSB02, *Pseudomonas putida* strain BHUPSB04, *Bacillus subtilis* strain BHUPSB13, *Paenibacillus polymyxa* strain BHUPSB17 and *Bacillus boronophilus* strain BHUPSB19 were successfully isolated. An experiment was conducted under plant growth chamber where chickpea plants were grown in plastic cups containing soil and mixed with isolates to investigate the effect on the growth of chickpea plant. Isolates induced production of plant hormones (IAA), phosphate solubilization and ammonia production and enhanced plant growth. Most of isolates resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings.

Susana *et al.* (2009) studied and reported the growth promotion effect on maize and wheat seeds at the sowing time. *Pseudomonas aurantiaca* SR1 colonized the root system of both crops and it persisted at appropriate population densities. It also showed a significant plant growth promoting effect that was reflected in the yield. Another relevant finding was that both crops when inoculated with *P. aurantiaca* SR1, showed higher yields with lower doses of fertilizers than those conventionally applied.

Mohammad *et al.* (2009) studied the effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn *Zea mays* (L) cv. SC604. According to the results, in all fertilizer treatments, application of PSM and PGPR together could reduce P application by 50 % without any significant reduction of grain yield. However, this treatment could not compensate 50 % reduction of N application.

Selvakumar *et al.* (2009) isolated phosphate solubilization and growth promotion by *Pseudomonas fragi*. The identity of the isolate was arrived on the basis of its biochemical features and sequencing of the 16S rRNA gene. Besides solubilizing P it produces IAA and HCN. Seed bacterization with the isolate significantly increased the percent germination, rate of germination, plant biomass and nutrient uptake of wheat seedlings.

Linu *et al.* (2009) screened high PSB *Gluconacetobacter* sp. and *Burkholderia* sp. Seed inoculation of cowpea by these novel phosphate solubilizers helps in improved nodulation, root and shoot biomass, straw and grain yield and phosphorus and nitrogen uptake of the crop. The dehydrogenase, phosphatase and the available P contents of the soil were also stimulated by the inoculation these strains. Among these two bacterial strains best effect on yield was observed with *Burkholderia* sp.

Poonguzhali *et al.* (2008) isolated PSB from the rhizosphere of Chinese cabbage and screened on the basis of their solubilization of inorganic tricalcium phosphate in liquid cultures. Ten strains that had higher solubilization potential were selected, and they also produced indole-3-acetic acid, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and siderophores. Seed bacterization with PSB strains increased the root elongation and biomass of Chinese cabbage in seedling culture, although they had no effect on phosphorus uptake of plants.

Vikram and Hamzehzarghani (2008) isolated 18 bacterial strains. Inoculation of greengram seeds with PSBV-14 phosphate solubilizing bacterial strain recorded the highest nodule number, nodule dry weight, shoot dry matter and total dry matter in greengram plants after 45 days of sowing. In this study majority of PSB isolates were able to improve the growth parameters of greengram significantly compared to rock phosphate control and single super phosphate control.

Turan *et al.* (2007) have studied the effects of PSB (*Bacillus* FS-3) application on phosphorus contents of tomato (*Lycopersicon esculentum*) plant under greenhouse condition with five different phosphorus fertilizer treatments (normal superphosphate, triple superphosphate, di-ammonium phosphate, phosphoric acid, and rock phosphate with and without bacterium, *Bacillus* FS-3) were applied. They found increase in plant shoot and root weight and P uptake were greater with PSB applications than without PSB in all of the fertilizers treatments. They concluded that application of PSB (FS-3) may increase the availability of soluble phosphate by dissolving the inorganic forms of phosphate.

Fernandez *et al.* (2007) studied the effect of PSB, *Burkholderia* sp, *Enterobacter* sp. and four *Bradyrhizobium* sp. was assessed in a soil plate assay by evaluating soil phosphorus (P) availability. The effect of PSB inoculation on growth and nutrient uptake of soybean plants was studied under greenhouse conditions. Plants inoculated with *Burkholderia* sp. had the highest aerial height and showed an appropriate N/P ratio. However, none of the PSB increased P uptake by plants. We concluded that the selection of efficient PSB strains as possible inoculation tools for P-deficient soils should focus on the integral interpretation of soil assays, greenhouse experiments and field trials.

Vikram (2007) reported that inoculation with PSBV-1 recorded, the highest ear head weight, grain yield, P content and P uptake in root and grain of sorghum plants. Inoculation of sorghum plants with PSBV-2 recorded the highest shoot length, shoot

dry matter and P uptake in shoot. Almost all the PSB isolates tested in the present study were able to improve the growth and yield parameter of sorghum significantly compared to absolute control.

Hameeda *et al.* (2006) screened two efficient strains *Serratia marcescens* and *Pseudomonas* sp. Inoculation of these strains with the maize resulted in increase in plant biomass (dry weight) both under glasshouse and field conditions as compared to uninoculated controls. Population of *Serratia marcescens* and *Pseudomonas* sp was traced back from the rhizosphere of maize on buffered rock phosphate (RP) medium and both the strains survived up to 96 days after sowing.

Chandrashekar (2003) observed that the plant growth parameters viz., shoot, root length and number of leaves per plant in green gram plants at 45 days after sowing significantly increased due to inoculation of P-solubilizing bacterial strains along with rock phosphate application as compared to rock phosphate alone (control).

Sundara *et al.* (2002) tested the application of PSB, *Bacillus megaterium* var. *phosphaticum* with varying amounts of P fertilizer on soil available P and sugarcane growth and yield. Population increase of inoculated P solubilizing bacterial strains was observed in the rhizosphere and also the increase in plant available P in the soil was observed. It enhanced tillering, stalk population, stalk weight and cane yield. Use of PSB reduces the use of super phosphate. They found that 50% super phosphate can be replaced by rock phosphate when applied with PSB. They observed that inoculation of PSB improved juice quality and sugar yields.

Biswas *et al.* (2001) conducted field studies under All India Co-ordinated Research Project and reported a significant effect of inoculation of *Bacillus megaterium* and *B. circulans* on rice and wheat crops.

The beneficial aspects of single and combined inoculation of phosphate solubilizing bacteria on crop plants have received adequate attention of researchers. Therefore the combined inoculation with the reduced chemical fertilizers may be an answer to the present problem. The pot culture studies are thus useful for necessitate further investigations.

Chapter V

SUMMARY AND CONCLUSIONS

Phosphate Solubilizing Bacteria (PSB) as inoculants simultaneously increased P uptake by the plant and crop yield. Twelve rhizospheric soil samples were collected from finger millet growing areas of Andhra Pradesh. Preliminary investigations involved isolation of PSB from finger millet rhizospheric soils. Serial dilution technique was followed and spread plate method was used for isolation and enumeration of rhizobacteria.

Sixteen PSB isolates were selected for further experimentation. Among 16 PSB isolates, all the isolates were able to solubilize phosphate quantitatively and qualitatively containing TCP in the range of 41.3 to 189.5 mg l⁻¹ and 5.3 mm to 27.3 mm respectively. BKP2 isolate recorded highest phosphate solubilization both qualitatively and quantitatively (6.61, 189.5 mg l⁻¹). This is due to the availability of higher amount of phosphate solubilizing capacity in the medium and also the ability of the strains.

The isolates were characterized culturally, morphologically, biochemically and were screened *in vitro* for plant growth promoting attributes. All the 16 PSB isolates were positive for phosphate solubilization. SSP1 and SBP1 isolates recorded strong siderophore production, isolates BVP2 and BKP2 recorded strong ammonia production, moderate HCN and IAA production was observed in five isolates. All the 16 isolates were checked for antagonistic activity against *Rhizoctonia solani* under *in vitro* conditions using dual culture plate technique on PDA medium. Maximum per cent inhibition was observed in the isolate BKP2 (51.49%), followed by isolate SSP1 (44.34%) and the least per cent inhibition was noticed in the isolate BHP2 (25.68%). The isolates inhibited the *R. solani* in dual culture method due to the production of secondary metabolites.

PSB1, PSB2 and PSB3 strains found superior over other isolates based on the phosphorus solubilizing ability qualitatively as well as quantitatively and by determining the biochemical and plant growth promoting rhizobacterial properties of isolates. Therefore, the best 3 PSB strains were identified as *Pseudomonas* sp., (isolate name, BKP2) *Bacillus* sp., (isolate name, SSP1) and *Enterobacter* sp. (isolate name, SBP1).

The bacterial (1.24×10^9 CFU⁻¹ g soil), PSB (1.54×10^8 CFU⁻¹g soil), fungal (2.65×10^3 CFU⁻¹ g soil) and actinomycetes population (3.64×10^5 CFU⁻¹ g soil) was observed to be significantly higher in treatment T₅ having 100 % RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least bacterial (1.01×10^9 CFU⁻¹g soil), PSB (1.06×10^8 CFU⁻¹g soil), fungal (1.33×10^3 CFU⁻¹g soil) and actinomycetes population (1.65×10^5 CFU⁻¹g soil) was observed in T₁₀ (control) at initial stage of crop growth.

The bacterial (2.91×10^9 CFU⁻¹g soil), PSB (3.54×10^8 CFU⁻¹g soil), fungal (5.33×10^3 CFU⁻¹g soil) and actinomycetes population (5.65×10^5 CFU⁻¹g soil) was observed to be significantly higher and increased at flowering stage of crop growth in treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) when compared to initial stage of crop growth and least bacterial population (2.43×10^9 CFU⁻¹g soil), PSB population (2.73×10^8 CFU⁻¹g soil), fungal population (2.63×10^3 CFU⁻¹g soil) and actinomycetes population (4.09×10^5 CFU⁻¹g soil) was observed in T₁₀ (control) which showed a gradual increase in population from initial stage to flowering stage of crop growth.

The bacterial (2.74×10^9 CFU⁻¹g soil), PSB (3.11×10^8 CFU⁻¹g soil), fungal (4.35×10^3 CFU⁻¹g soil) and actinomycetes population (4.66×10^5 CFU⁻¹g soil) was observed to be significantly higher and decreased at harvesting stage of crop growth in treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) when compared to flowering stage of crop growth and least bacterial (2.07×10^9 CFU⁻¹g of soil), PSB (2.52×10^8 CFU⁻¹g of soil), fungal (1.66×10^3 CFU⁻¹g of soil) and actinomycetes population (2.63×10^5 CFU⁻¹g of soil) was observed in T₁₀ (control) which showed a gradual decrease in population from flowering stage to harvesting stage of crop growth. The number of microorganisms found in each soil sample were different because the composition and function of the microbial community was shown to vary according to nutrients, oxygen availability, pollutants and other environmental variables.

The highest root length (23.1cm), no.of fingers per plant (10) and phosphorus content in plant sample (0.183%) was recorded in the treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least in the treatment T₁₀ (control) 17.3 cm, 6.6, 0.131% respectively.

The treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) was found to be significantly high in grain yield (4031.9 kg ha⁻¹) and straw yield (4691.5 kg ha⁻¹) and least was recorded in the treatment T_{10} (control) 2812.4 kg ha⁻¹, 3646.2 kg ha⁻¹ respectively. Leaf dry weight (5.7 g⁻¹ plant), stem dry weight (15.3 g⁻¹ plant), earhead dry weight (18.9 g⁻¹ plant) and total dry matter (39.4 g⁻¹ plant) was observed to be significantly higher in the treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least was recorded in the treatment T_{10} (control) 4.2 g⁻¹ plant, 11.8 g⁻¹ plant, 15.8 g⁻¹ plant, 31.3 g⁻¹ plant respectively. This could be because of higher amount of growth promoting substances such as IAA, ammonia and siderophores released by inoculants.

Soil enzyme activities increased from initial to flowering stage and then after that the enzyme activity got declined at harvesting stage of crop growth. Acid phosphatase (61.6 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (47.4 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (137.0 µg TPF g⁻¹ of soil day⁻¹) and urease (1476.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed to be significantly higher in treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least acid phosphatase (40.4 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (21.6 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (67.3 µg TPF g⁻¹ of soil day⁻¹) and urease (1006.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed in T_{10} (control) at initial stage of crop growth.

Acid phosphatase (86.3 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (68.4 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (194.3 µg TPF g⁻¹ of soil day⁻¹) and urease (1991.3 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed to be significantly higher and increased in treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) at flowering stage when compared to initial stage of crop growth and least acid phosphatase (52.5 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (40.5 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (115.6 µg TPF g⁻¹ of soil day⁻¹) and urease (1724.1 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed in T_{10} (control) which showed a gradual increase in enzyme activities from initial stage to flowering stage of crop growth.

Acid phosphatase (78.5 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (57.5 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (163.3 µg TPF g⁻¹ of soil day⁻¹) and urease (1678.0 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed to be significantly higher at flowering and decreased at harvesting stage of crop growth in treatment T_5

having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) which showed a gradual decrease in enzyme activities from flowering stage to harvesting stage of crop growth. Least acid phosphatase (48.5 µg pNP g⁻¹ of soil h⁻¹), alkaline phosphatase (29.5 µg pNP g⁻¹ of soil h⁻¹), dehydrogenase (97.0 µg TPF g⁻¹ of soil day⁻¹) and urease (1094.6 µg urea hydrolyzed g⁻¹ of soil 4h⁻¹) enzyme activities were observed in T₁₀ (control). Hence there is a positive correlation between phosphate solubilizing capacity and phosphatase activity. This is due to availability of higher amount of phosphorus in the medium and the ability of the strains. The application of balanced amounts of nutrients and manures through inorganic and organic fertilizers improved the organic matter of soil, which corresponded with higher enzyme activity.

Highest pH (7.7) and EC (0.249 dSm⁻¹) activity in soil was recorded in the treatment T₉ having 100% RDN and RDK + (50% RDP+PSB₁+PSB₂+PSB₃) and least pH (7.0) and EC (0.217 dSm⁻¹) in the treatment T₁₀ (control) at initial stage of crop growth. At flowering stage of crop growth pH (8.1) and EC (0.259 dSm⁻¹) activity in soil was recorded highest in the treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) over the treatment T₁₀ (control). pH (8.0) and EC (0.255 dSm⁻¹) activity in soil was recorded highest in the treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least pH (7.1) and EC (0.225 dSm⁻¹) activity in soil was recorded in the treatment T₁₀ (control) at harvesting stage of crop growth. The lowering of pH with addition of organic manures is attributed due to the presence of organic acids and EC of soil is directly related to the ions present in soil. It may be due to the production of acids or acid forming compounds through the decomposition of organic materials.

The available nitrogen (286.3 kg ha⁻¹), phosphorus (38.6 kg ha⁻¹) and potassium (356.0 kg ha⁻¹) in soil was recorded highest in the treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and lowest available nitrogen (146.6 kg ha⁻¹), phosphorus (19.3 kg ha⁻¹) and potassium (217.0 kg ha⁻¹) in soil was recorded in the treatment T₁₀ (control) at initial stage of crop growth. At flowering stage of crop growth, available nitrogen (306.0 kg ha⁻¹), phosphorus (51.4 kg ha⁻¹) and potassium (394.3 kg ha⁻¹) in soil was recorded highest and increased in the treatment T₅ having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) when compared to initial stage of crop growth and lowest available nitrogen (205.0 kg ha⁻¹), phosphorus (26.6 kg ha⁻¹) and potassium (243.6 kg ha⁻¹) in soil was recorded in the treatment T₁₀ (control) which showed a significant increase from initial stage to flowering stage of crop growth. At

harvesting stage of crop growth, available nitrogen (293.6 kg ha^{-1}), phosphorus (45.4 kg ha^{-1}) and potassium (364.0 kg ha^{-1}) in soil was recorded highest and decreased in the treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) when compared to flowering stage of crop growth and lowest available nitrogen (196.0 kg ha^{-1}), phosphorus (23.3 kg ha^{-1}) and potassium (231.6 kg ha^{-1}) in soil was recorded in the treatment T_{10} (control) which showed a significant decrease from flowering stage to harvesting stage of crop growth. Available phosphorus in soil is increased by low-molecular-mass organic acids produced from PSB. The K status in soil is due to the organic manures on decomposition, released the organic acids which have mobilised the native or non-exchangeable forms of K.

Organic carbon (0.843%) in soil was found highest in the treatment T_5 with 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least (0.616%) was recorded in the treatment T_{10} (control) at initial stage of crop growth. At flowering stage of crop growth organic carbon (0.910%) in soil was recorded highest in the treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least (0.652%) was recorded in the treatment T_{10} (control). Organic carbon in soil was recorded highest (0.896%) in the treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) and least (0.623%) was recorded in the treatment T_{10} (control) at harvesting stage of crop growth. The build-up of OC is due to the addition of sloughed roots and the incorporation of leaf litter. Hence, accumulation of organic matter has proved to be very important to soil improvement.

From the study it is concluded that the treatment T_5 having 100% RDN and RDK + (75% RDP+PSB₁+PSB₂+PSB₃) was found to be most effective in all the parameters regarding the enhanced microbial dynamics and soil enzyme activities which in turn increased plant growth parameters, total dry matter content and finger millet yield as well as soil health parameters due to more availability of nutrients and plant growth promoting substances.

The use of chemical fertilizer is increasing to boost up crop production. Simultaneously, cost of chemical fertilizer is increased constantly and only use of inorganic fertilizer is injurious to soil health and soil productivity. Hence the integrated application of PSB biofertilizer along with inorganic fertilizers reduces the cost of fertilizers and increases the productivity of soil compared to inorganic fertilizers alone, thus satisfying all the criteria for crop sustainability.

Table 4.3. Microbial population count from different rhizospheric soils of finger millet in Andhra Pradesh

S. No.	Area	Mandal	Soil type	Bacterial population (CFU×10⁸ g⁻¹ soil)	PSB population (CFU×10⁷ g⁻¹ soil)	Fungal population (CFU×10³ g⁻¹ soil)	Actinobacterial population (CFU×10⁵ g⁻¹ soil)
1	Barlakunta	Bestavaripeta	Red	6.4	4.2	4.3	5.2
2	Singarapalle	Bestavaripeta	Black	7.3	3.7	3.2	3.7
3	Karlagatta	Santhipuram	Red	6.1	4.4	4.7	4.1
4	Venkateswarapuram	Bestavaripeta	Red	8.7	5.1	5.6	3.5
5	Sariki	Saluru	Black	6.9	5.6	4.8	5.6
6	Voppangi	Kurupam	Black	8.2	3.2	3.7	4.4
7	Hanuvayapalle	Bestavaripeta	Red	7.8	4.8	5.7	3.3
8	Neelakantapuram	Kurupam	Black	8.6	3.5	4.9	4.5
9	Gorjapadu	Kurupam	Black	8.1	4.0	3.5	3.9
10	Konapalli	Bestavaripeta	Black	7.7	5.3	5.4	5.4
11	Boyanapalle	Santhipuram	Red	8.9	5.0	4.1	4.8
12	Chinthakampalle	Santhipuram	Black	6.5	4.7	3.6	3.7

Table 4.1. Location details of the isolates from finger millet crop along with latitudes, longitudes and soil type

S.No.	District	Latitudes	Longitudes	Name of the farmer	Village	Mandal	Soil type
1	Prakasam	15°20' 54.4"	79°32' 37.2"	Chitturu Venkata Swamy	Barlakunta	Bestavaripeta	Red
2	Prakasam	15°20' 42.7"	79°32' 31.4"	Dasari Chinna Narayana	Singarapalle	Bestavaripeta	Black
3	Chittoor	13°13' 01.8"	79°06' 01.1"	Korada Kasi Reddy	Karlagatta	Santhipuram	Red
4	Prakasam	15°21' 25.3"	79°35' 29.7"	Kane Chinna Oblesu	Venkateswarapuram	Bestavaripeta	Red
5	Vizianagaram	18°06' 23.9"	83°23' 43.8"	Binna Guruvudu	Sariki	Saluru	Black
6	Vizianagaram	18°09' 27.4"	83°25' 47.7"	Konda Narayana Reddy	Voppangi	Kurupam	Black
7	Prakasam	15°22' 49.3"	79°30' 36.9"	Mallela Subbayya	Hanuvayapalle	Bestavaripeta	Red
8	Vizianagaram	18°07' 36.3"	83°31' 52.9"	Chinta Anjaneyulu	Neelakantapuram	Kurupam	Black
9	Vizianagaram	18°03' 48.2"	83°29' 49.8"	Gudhe Yodhaya	Gorjapadu	Kurupam	Black
10	Prakasam	15°20' 33.4"	79°31' 38.7"	Katta Sambhasiva Rao	Konapalli	Bestavaripeta	Black
11	Chittoor	13°06' 01.2"	79°12' 01.6"	Jangala Venkateswarlu	Boyanapalle	Santhipuram	Red
12	Chittoor	13°09' 01.7"	79°02' 01.4"	Pothuri Chenchu Raju	Chinthakampalle	Santhipuram	Black

Table 4.2. Naming of PSB isolates according to the place of collection

S.No.	Area	Mandal	Soil type	Crop	Abbreviated Names
1	Barlakunta	Bestavaripeta	Red	Finger millet	BBP1, BBP2
2	Singarapalle	Bestavaripeta	Black	Finger millet	BSP1
3	Karlagatta	Santhipuram	Red	Finger millet	SKP1
4	Venkateswarapuram	Bestavaripeta	Red	Finger millet	BVP1, BVP2
5	Sariki	Saluru	Black	Finger millet	SSP1
6	Voppangi	Kurupam	Black	Finger millet	KVP1
7	Hanuvayapalle	Bestavaripeta	Red	Finger millet	BHP1, BHP2
8	Neelakantapuram	Kurupam	Black	Finger millet	KNP1
9	Gorjapadu	Kurupam	Black	Finger millet	KGP1
10	Konapalli	Bestavaripeta	Black	Finger millet	BKP1, BKP2
11	Boyanapalle	Santhipuram	Red	Finger millet	SBP1
12	Chinthakampalle	Santhipuram	Black	Finger millet	SCP1

Table 4.5. Estimation of phosphate solubilization quantitatively for the screening of PSB isolates

Isolate No.	Isolate Name	Soluble P Concentration mg l⁻¹
1	BBP1	41.3
2	BBP2	48
3	BSP1	74.6
4	SKP1	64.2
5	BVP1	44.8
6	BVP2	88
7	SSP1	174.6
8	KVP1	114.7
9	BHP1	138.2
10	BHP2	97.4
11	KNP1	85.6
12	KGP1	112.3
13	BKP1	57.6
14	BKP2	189.5
15	SBP1	146.2
16	SCP1	77.4
CD		0.032
SE(±m)		0.011
CV%		1.212

Table 4.8. Screening of PSB isolates for plant growth promoting activities

Isolate No.	Isolate Name	Phosphate Solubilization	Siderophore Production	Ammonia Production	HCN Production	IAA Production
1	BBP1	++	--	+	+	--
2	BBP2	++	--	+	++	--
3	BSP1	+++	++	--	--	+
4	SKP1	+++	+	+	+	+
5	BVP1	++	--	+	+	++
6	BVP2	+++	+	+++	++	+
7	SSP1	+++	+++	++	++	++
8	KVP1	+++	--	+	+	+
9	BHP1	+++	+	++	--	++
10	BHP2	+++	--	+	+	+
11	KNP1	+++	--	++	+	+
12	KGP1	+++	++	+	--	--
13	BKP1	++	+	--	+	+
14	BKP2	+++	++	+++	++	++
15	SBP1	+++	+++	++	++	++
16	SCP1	+++	--	+	--	+

-No Production

IAA - Indole Acetic Acid

HCN - Hydrogen Cyanide

+ Weak Production

++ Moderate production

+++ Strong Production

Table 4.4. Estimation of phosphate solubilization qualitatively for the screening of PSB isolates

Isolate Name	Phosphate Solubilization		
	Phosphate Solubilization Index (PSI)	Zone Diameter (mm)	
		Solubilization Zone	Culture Diameter
BBP1	4.60	16.6	4.6
BBP2	3.15	5.6	2.6
BSP1	3.18	11.6	5.3
SKP1	4.38	22.3	6.0
BVP1	2.79	11.3	6.3
BVP2	2.76	10.6	6.0
SSP1	5.87	27.3	5.6
KVP1	3.65	5.3	2.0
BHP1	2.62	8.6	5.3
BHP2	3.0	10.0	5.0
KNP1	3.56	13.6	5.3
KGP1	4.17	7.3	2.3
BKP1	4.08	17.3	5.6
BKP2	6.61	10.6	2.3
SBP1	5.60	11.3	3.6
SCP1	4.13	14.6	2.6
CD		0.377	
SE(±m)		0.127	
CV%		1.712	

Table 4.9. Antagonistic activity of PSB isolates against *Rhizoctonia Solani* by dual culture method

Isolate No.	Isolate Name	Per Cent Inhibition (%)	Inhibition Zone (mm)
1	BBP1	37.52	3.00
2	BBP2	30.30	2.00
3	BSP1	38.53	3.00
4	SKP1	34.31	2.00
5	BVP1	33.17	2.00
6	BVP2	39.05	3.00
7	SSP1	44.34	4.00
8	KVP1	38.20	2.00
9	BHP1	33.77	2.00
10	BHP2	25.68	3.00
11	KNP1	34.12	2.00
12	KGP1	32.23	2.00
13	BKP1	35.78	3.00
14	BKP2	51.49	5.00
15	SBP1	42.71	4.00
16	SCP1	35.33	3.00
	Control	0	
CD		0.103	
SE(±m)		0.036	
CV%		0.168	

Table 4.14. Influence of PSB isolates on growth and yield attributes of finger millet crop growth

Treatments	Growth and Yield Attributes			
	Root length (cm)	No. of fingers plant ⁻¹	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)
T₁ - 100% RDF	20.8	7.6	3086.2	3944.8
T₂ - 75% RDP + PSB ₁	21.4	8.6	3484.4	4417.7
T₃ - 75% RDP + PSB ₂	21.2	8.3	3036.4	4255.9
T₄ - 75% RDP + PSB ₃	20.2	7.3	2837.3	4106.6
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	23.1	10.0	4031.9	4691.5
T₆ - 50% RDP + PSB ₁	18.9	8.3	3272.8	4243.5
T₇ - 50% RDP + PSB ₂	18.8	7.6	3011.5	4019.5
T₈ - 50% RDP + PSB ₃	18.0	7.0	2899.5	3957.3
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	21.8	9.6	3720.8	4567.1
T₁₀ - Control	17.3	6.6	2812.4	3646.2
CD	3.347	1.039	337.4	577.8
SE(±m)	1.127	0.350	113.5	194.5
CV%	9.671	7.445	6.111	8.050

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.16. Influence of PSB isolates on total dry matter production of finger millet crop growth

Treatments	Total Dry Matter Production (g plant ⁻¹)			
	Leaf	Stem	Earhead	Total Dry Matter
T₁ - 100% RDF	4.6	12.6	16.6	33.0
T₂ - 75% RDP + PSB ₁	5.4	12.9	17.8	35.6
T₃ - 75% RDP + PSB ₂	5.2	12.7	17.3	35.3
T₄ - 75% RDP + PSB ₃	5.0	12.3	17.1	34.3
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	5.7	15.3	18.9	39.4
T₆ - 50% RDP + PSB ₁	5.0	12.6	17.6	35.4
T₇ - 50% RDP + PSB ₂	5.1	12.3	17.2	34.8
T₈ - 50% RDP + PSB ₃	4.9	12.0	16.8	33.2
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	5.5	14.6	18.2	38.4
T₁₀ - Control	4.2	11.8	15.8	31.3
CD	0.094	0.386	0.262	0.846
SE(±m)	0.032	0.130	0.088	0.285
CV%	1.071	1.738	0.880	1.406

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.17. Influence of PSB isolates on acid phosphatase enzyme activity in soil at various stages of finger millet crop growth

Treatments	Acid Phosphatase ($\mu\text{g pNP g}^{-1} \text{ h}^{-1}$)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	42.4	68.4	53.6
T ₂ - 75% RDP + PSB ₁	53.3	77.5	69.5
T ₃ - 75% RDP + PSB ₂	51.7	72.3	64.4
T ₄ - 75% RDP + PSB ₃	48.5	69.5	58.4
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	61.6	86.3	78.5
T ₆ - 50% RDP + PSB ₁	50.3	74.5	65.7
T ₇ - 50% RDP + PSB ₂	47.6	70.5	60.7
T ₈ - 50% RDP + PSB ₃	45.2	66.5	54.5
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	58.6	80.6	70.5
T ₁₀ - Control	40.4	52.5	48.5
CD	0.377	0.489	0.535
SE($\pm m$)	0.127	0.165	0.180
CV%	0.440	0.397	0.500

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.18. Influence of PSB isolates on Alkaline phosphatase enzyme activity in soil at various stages of finger millet crop growth

Treatments	Alkaline Phosphatase ($\mu\text{g pNP g}^{-1} \text{h}^{-1}$)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	24.4	44.5	33.5
T ₂ - 75% RDP + PSB ₁	39.4	58.3	47.2
T ₃ - 75% RDP + PSB ₂	35.4	53.5	43.6
T ₄ - 75% RDP + PSB ₃	30.5	50.5	40.4
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	47.4	68.4	57.5
T ₆ - 50% RDP + PSB ₁	33.4	55.5	44.4
T ₇ - 50% RDP + PSB ₂	30.6	50.6	40.6
T ₈ - 50% RDP + PSB ₃	28.4	47.4	36.4
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	40.7	60.4	50.4
T ₁₀ - Control	21.6	40.5	29.5
CD	0.500	0.588	0.571
SE($\pm m$)	0.168	0.198	0.192
CV%	0.878	0.647	0.785

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.19. Influence of PSB isolates on dehydrogenase enzyme activity in soil at various stages of finger millet crop growth

Treatments	Dehydrogenase ($\mu\text{g TPF g}^{-1} \text{ day}^{-1}$)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	77.3	124.6	102.0
T ₂ - 75% RDP + PSB ₁	120.3	181.0	142.0
T ₃ - 75% RDP + PSB ₂	106.6	165.0	136.0
T ₄ - 75% RDP + PSB ₃	98.6	147.3	125.0
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	137.0	194.3	163.3
T ₆ - 50% RDP + PSB ₁	113.0	172.6	131.0
T ₇ - 50% RDP + PSB ₂	91.0	161.0	116.6
T ₈ - 50% RDP + PSB ₃	82.6	140.6	101.0
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	128.0	187.3	151.3
T ₁₀ - Control	67.3	115.6	97.0
CD	1.771	2.620	1.534
SE($\pm m$)	0.596	0.882	0.516
CV%	1.011	0.961	0.707

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.20. Influence of PSB isolates on urease enzyme activity in soil at various stages of finger millet crop growth

Treatments	Urease (μg urea hydrolyzed $\text{g}^{-1} \text{4h}^{-1}$)		
	At Initial	50% Flowering	Harvesting
T₁ - 100% RDF	1026.3	1758.6	1137.0
T₂ - 75% RDP + PSB ₁	1393.3	1934.0	1582.0
T₃ - 75% RDP + PSB ₂	1284.0	1889.6	1476.0
T₄ - 75% RDP + PSB ₃	1124.3	1850.0	1323.6
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	1476.3	1991.3	1678.0
T₆ - 50% RDP + PSB ₁	1295.3	1907.6	1426.3
T₇ - 50% RDP + PSB ₂	1104.0	1868.0	1293.3
T₈ - 50% RDP + PSB ₃	1097.3	1827.3	1255.6
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	1406.3	1961.3	1605.3
T₁₀ - Control	1006.3	1724.1	1094.6
CD	3.745	9.813	3.718
SE($\pm$m)	1.261	3.303	1.252
CV%	0.179	0.306	0.156

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.10. Influence of PSB isolates on bacterial population at various stages of finger millet crop growth

Treatments	Bacterial Population (CFU×10 ⁹ g ⁻¹ soil)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	1.15	2.66	2.16
T ₂ - 75% RDP + PSB ₁	1.18	2.83	2.65
T ₃ - 75% RDP + PSB ₂	1.10	2.81	2.43
T ₄ - 75% RDP + PSB ₃	1.07	2.72	2.24
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	1.24	2.91	2.74
T ₆ - 50% RDP + PSB ₁	1.07	2.64	2.52
T ₇ - 50% RDP + PSB ₂	1.04	2.72	2.33
T ₈ - 50% RDP + PSB ₃	1.02	2.67	2.17
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	1.17	2.91	2.63
T ₁₀ - Control	1.01	2.43	2.07
CD	0.036	0.032	0.031
SE(±m)	0.012	0.011	0.010
CV%	1.873	0.835	0.754

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.11. Influence of PSB isolates on PSB population at various stages of finger millet crop growth

Treatments	PSB Population (CFU×10 ⁸ g ⁻¹ soil)		
	At Initial	50% Flowering	Harvesting
T₁ - 100% RDF	1.16	3.18	2.56
T₂ - 75% RDP + PSB ₁	1.49	3.24	2.92
T₃ - 75% RDP + PSB ₂	1.43	3.18	2.83
T₄ - 75% RDP + PSB ₃	1.27	3.08	2.76
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	1.54	3.54	3.11
T₆ - 50% RDP + PSB ₁	1.44	3.16	2.83
T₇ - 50% RDP + PSB ₂	1.38	3.05	2.67
T₈ - 50% RDP + PSB ₃	1.18	2.94	2.60
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	1.47	3.44	2.96
T₁₀ - Control	1.06	2.73	2.52
CD	0.050	0.058	0.040
SE(±m)	0.017	0.020	0.013
CV%	2.179	1.077	0.839

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.12. Influence of PSB isolates on fungal population at various stages of finger millet crop growth

Treatments	Fungal population (CFU×10 ³ g ⁻¹ soil)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	1.65	3.35	2.04
T ₂ - 75% RDP + PSB ₁	2.63	5.10	3.65
T ₃ - 75% RDP + PSB ₂	2.35	4.64	2.64
T ₄ - 75% RDP + PSB ₃	1.64	3.68	2.33
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	2.65	5.33	4.35
T ₆ - 50% RDP + PSB ₁	2.35	4.09	2.64
T ₇ - 50% RDP + PSB ₂	2.26	4.36	2.33
T ₈ - 50% RDP + PSB ₃	1.36	3.64	2.10
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	2.32	4.66	3.36
T ₁₀ - Control	1.33	2.63	1.66
CD	0.173	0.084	0.076
SE(±m)	0.058	0.028	0.026
CV%	4.905	1.174	1.638

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.13. Influence of PSB isolates on actinobacterial population at various stages of finger millet crop growth

Treatments	Actinobacterial population (CFU×10 ⁵ g ⁻¹ soil)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	2.35	4.36	3.36
T ₂ - 75% RDP + PSB ₁	3.63	5.10	4.33
T ₃ - 75% RDP + PSB ₂	2.65	5.04	4.64
T ₄ - 75% RDP + PSB ₃	2.34	4.66	3.34
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	3.64	5.65	4.66
T ₆ - 50% RDP + PSB ₁	2.66	4.62	3.33
T ₇ - 50% RDP + PSB ₂	2.33	4.33	3.64
T ₈ - 50% RDP + PSB ₃	2.09	5.63	4.66
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	3.35	5.33	4.33
T ₁₀ - Control	1.65	4.09	2.63
CD	0.061	0.086	0.045
SE(±m)	0.020	0.029	0.015
CV%	1.327	1.030	0.661

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.21. Influence of PSB isolates on pH in soil at various stages of finger millet crop growth

Treatments	pH		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	7.1	7.3	7.2
T ₂ - 75% RDP + PSB ₁	7.5	8.0	7.9
T ₃ - 75% RDP + PSB ₂	7.3	7.8	7.5
T ₄ - 75% RDP + PSB ₃	7.4	7.9	7.7
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	7.6	8.1	8.0
T ₆ - 50% RDP + PSB ₁	7.5	8.0	7.7
T ₇ - 50% RDP + PSB ₂	7.3	7.8	7.5
T ₈ - 50% RDP + PSB ₃	7.4	7.8	7.7
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	7.7	8.0	7.8
T ₁₀ - Control	7.0	7.3	7.1
CD	0.390	0.305	0.287
SE(±m)	0.131	0.103	0.097
CV%	3.065	2.274	2.189

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.22. Influence of PSB isolates on EC in soil at various stages of finger millet crop growth

Treatments	EC (dSm ⁻¹)		
	At Initial	50% Flowering	Harvesting
T₁ - 100% RDF	0.224	0.234	0.226
T₂ - 75% RDP + PSB ₁	0.237	0.247	0.246
T₃ - 75% RDP + PSB ₂	0.235	0.244	0.239
T₄ - 75% RDP + PSB ₃	0.229	0.236	0.233
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.249	0.259	0.255
T₆ - 50% RDP + PSB ₁	0.234	0.244	0.239
T₇ - 50% RDP + PSB ₂	0.234	0.240	0.236
T₈ - 50% RDP + PSB ₃	0.224	0.232	0.229
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.249	0.254	0.251
T₁₀ - Control	0.217	0.228	0.225
CD	0.010	0.009	0.008
SE(±m)	0.003	0.003	0.003
CV%	2.459	2.057	1.857

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.23. Influence of PSB isolates on available nitrogen in soil at various stages of finger millet crop growth

Treatments	Nitrogen (kg ha ⁻¹)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	164.0	212.0	203.6
T ₂ - 75% RDP + PSB ₁	226.3	284.3	263.0
T ₃ - 75% RDP + PSB ₂	203.6	251.6	232.0
T ₄ - 75% RDP + PSB ₃	194.0	231.6	216.0
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	286.3	306.0	293.6
T ₆ - 50% RDP + PSB ₁	206.6	244.0	232.0
T ₇ - 50% RDP + PSB ₂	194.0	231.0	227.6
T ₈ - 50% RDP + PSB ₃	184.0	217.6	205.6
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	244.3	292.6	273.6
T ₁₀ - Control	146.6	205.0	196.0
CD	3.835	3.973	3.705
SE(±m)	1.291	1.337	1.247
CV%	1.091	0.936	0.922

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.24. Influence of PSB isolates on available phosphorus in soil at various stages of finger millet crop growth

Treatments	Phosphorus (kg ha ⁻¹)		
	At Initial	50% Flowering	Harvesting
T₁ - 100% RDF	21.3	39.3	28.3
T₂ - 75% RDP + PSB ₁	30.6	45.2	39.3
T₃ - 75% RDP + PSB ₂	27.6	42.2	36.5
T₄ - 75% RDP + PSB ₃	25.3	39.4	33.3
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	38.6	51.4	45.4
T₆ - 50% RDP + PSB ₁	26.6	41.6	35.5
T₇ - 50% RDP + PSB ₂	25.6	38.5	32.3
T₈ - 50% RDP + PSB ₃	22.6	34.2	29.5
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	34.6	49.2	41.3
T₁₀ - Control	19.3	26.6	23.3
CD	0.990	0.333	0.406
SE(±m)	0.333	0.112	0.137
CV%	2.117	0.476	0.686

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.25. Influence of PSB isolates on available potassium in soil at various stages of finger millet crop growth

Treatments	Potassium (kg ha ⁻¹)		
	At Initial	50% Flowering	Harvesting
T ₁ - 100% RDF	234.0	296.0	244.0
T ₂ - 75% RDP + PSB ₁	304.6	367.0	352.0
T ₃ - 75% RDP + PSB ₂	292.6	324.6	314.6
T ₄ - 75% RDP + PSB ₃	273.6	315.3	306.0
T ₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	356.0	394.3	364.0
T ₆ - 50% RDP + PSB ₁	294.3	342.6	338.3
T ₇ - 50% RDP + PSB ₂	282.6	314.3	300.6
T ₈ - 50% RDP + PSB ₃	263.3	305.0	282.6
T ₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	332.3	374.6	356.6
T ₁₀ - Control	217.0	243.6	231.6
CD	3.924	4.178	3.758
SE(±m)	1.321	1.406	1.265
CV%	0.802	0.743	0.709

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.26. Influence of PSB isolates on organic carbon in soil at various stages of finger millet crop growth

Treatments	Organic carbon (%)		
	At Initial	50% Flowering	Harvesting
T₁ - 100% RDF	0.626	0.682	0.632
T₂ - 75% RDP + PSB ₁	0.794	0.821	0.814
T₃ - 75% RDP + PSB ₂	0.706	0.755	0.724
T₄ - 75% RDP + PSB ₃	0.694	0.735	0.716
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.843	0.910	0.896
T₆ - 50% RDP + PSB ₁	0.713	0.746	0.727
T₇ - 50% RDP + PSB ₂	0.685	0.725	0.704
T₈ - 50% RDP + PSB ₃	0.677	0.715	0.697
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.823	0.882	0.863
T₁₀ - Control	0.616	0.652	0.623
CD	0.003	0.012	0.004
SE(±m)	0.001	0.004	0.001
CV%	0.274	0.950	0.312

Note: (100% RDN and RDK – T₂ to T₉, RDF- Recommended dose of fertilizer, RDN- Recommended dose of Nitrogen, RDP- Recommended dose of Phosphorous, RDK- Recommended dose of Potassium)

Table 4.7. Biochemical characterization of PSB isolates from rhizospheric soils of finger millet

S. No.	Isolate Name	Indole Test	MR Test	VP Test	Nitrate Reduction	Motility	Citrate Utilization	Catalase	Oxidase	Starch Hydrolysis	Gas and Acid Production	Gelatin Liquefaction	H ₂ S	Carbohydrate Utilization		
														Glucose	Maltose	Lactose
1	BBP1	–	–	–	+	–	+	+	+	+	–	–	–	+	–	+
2	BBP2	–	–	+	–	–	+	+	+	+	+	+	–	+	+	–
3	BSP1	+	+	–	+	+	+	–	–	–	–	+	–	+	+	–
4	SKP1	+	–	+	–	+	–	+	+	–	–	–	+	+	–	+
5	BVP1	+	+	+	+	–	+	+	+	+	–	–	–	+	+	–
6	BVP2	+	+	–	–	+	–	–	–	–	+	–	+	–	–	+
7	SSP1	+	–	+	+	+	+	+	+	+	–	+	–	+	+	+
8	KVP1	+	+	–	–	+	+	+	+	+	+	–	+	+	–	–
9	BHP1	+	–	+	+	–	–	–	–	–	–	+	–	–	+	+
10	BHP2	+	+	–	–	+	+	+	+	+	–	–	+	+	–	–
11	KNP1	+	+	+	+	–	–	–	+	–	–	+	–	–	+	+
12	KGP1	–	–	–	+	–	+	+	+	+	+	–	+	–	–	+
13	BKP1	+	+	+	–	+	–	–	–	–	–	+	–	+	–	+
14	BKP2	+	–	–	+	+	+	+	+	–	+	+	+	–	–	–
15	SBP1	+	–	+	+	+	+	+	–	+	+	–	–	+	+	–
16	SCP1	+	+	+	+	–	–	–	+	–	–	+	–	+	–	+

+ positive

– negative

MR-Methyl Red test

VP-Voges Proskauer's test

H₂S-Hydrogen sulphide test

Table 4.6. Cultural and morphological characteristics of PSB isolates

Isolate No.	Isolate Name	Size	Shape	Color	Elevation	Surface	Margin	Gram Reaction	Shape	Sporulation
1	BBP1	Small	Round	Creamy White	Convex	Smooth shiny	Regular	Negative	Rod	Negative
2	BBP2	Medium	Round	Dull White	Convex	Smooth shiny	Regular	Negative	Cocci	Negative
3	BSP1	Small	Round	Dull White	Convex	Smooth shiny	Irregular	Positive	Rod	Positive
4	SKP1	Very Small	Irregular	White	Convex	Smooth shiny	Regular	Negative	Rod	Negative
5	BVP1	Small	Round	Creamy White	Convex	Smooth shiny	Regular	Negative	Rod	Negative
6	BVP2	Medium	Irregular	Creamy White	Convex	Smooth shiny	Irregular	Negative	Rod	Negative
7	SSP1	Medium	Round	Dull White	Convex	Smooth shiny	Irregular	Positive	Rod	Positive
8	KVP1	Small	Round	White	Convex	Smooth shiny	Regular	Positive	Cocci	Negative
9	BHP1	Small	Irregular	White	Convex	Smooth shiny	Irregular	Negative	Rod	Negative
10	BHP2	Medium	Irregular	Creamy White	Convex	Smooth shiny	Regular	Negative	Rod	Negative
11	KNP1	Very Small	Round	Yellow	Convex	Smooth shiny	Irregular	Positive	Rod	Positive
12	KGP1	Small	Irregular	Dull White	Convex	Smooth shiny	Irregular	Negative	Rod	Negative
13	BKP1	Small	Irregular	Dull White	Convex	Smooth shiny	Irregular	Negative	Rod	Negative
14	BKP2	Medium	Round	Yellow	Convex	Smooth shiny	Regular	Negative	Rod	Negative
15	SBP1	Medium	Irregular	Creamy White	Convex	Smooth	Regular	Negative	Rod	Negative
16	SCP1	Small	Round	White	Convex	Smooth	Irregular	Positive	Cocci	Negative

Table 4.15. Influence of PSB isolates on phosphorus content present in plant sample at various treatments

Treatments	Phosphorus content in plant sample (%)
T₁ - 100% RDF	0.153
T₂ - 75% RDP + PSB ₁	0.173
T₃ - 75% RDP + PSB ₂	0.165
T₄ - 75% RDP + PSB ₃	0.163
T₅ - 75% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.183
T₆ - 50% RDP + PSB ₁	0.163
T₇ - 50% RDP + PSB ₂	0.160
T₈ - 50% RDP + PSB ₃	0.158
T₉ - 50% RDP + PSB ₁ + PSB ₂ + PSB ₃	0.176
T₁₀ - Control	0.131
CD	0.003
SE(±m)	0.001
CV%	1.122