STUDY ON SYNERGISTIC EFFECT OF ENDOPHYTIC BACTERIA AND *MESORHIZOBIUM* IN CHICKPEA (*Cicer arietinum*)

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UNIVERSITY OF AGRICULTURAL SCIENCES

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In partial fulfillment of the requirements for the award of the Degree of

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In

AGRICULTURAL MICROBIOLOGY

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Affectionately dedicated

TO

My beloved parents

K. Mallegowda Lakshmamma

And

G. Parashivamurthy sharadamma

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES,
GKVK CAMPUS, BENGALURU -560065

CERTIFICATE

This is to certify that the thesis entitled **"Study on synergistic effect of endophytic bacteria and *Mesorhizobium* in Chickpea (*Cicer arietinum)*"** Submitted by **Ms. SUSHMA, P. ID No. PALB 3149**, for the degree of **Master of Science (Agriculture)** in **Agricultural Microbiology** to the University of Agricultural Sciences, Bengaluru, is a record of research work done by her during the period of her study in this University under my guidance and supervision. This thesis has not previously formed the basis for award of any degree, diploma, associate ship, fellowship or other similar titles.

Bengaluru
July, 2015

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(L. Krishna naik)

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(Mudalagiriyappa)

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"Gratitude is the memory of the heart"

In every one's life, the day arises when one has to share the feelings in words. Sometimes, the words become unable to express the feelings of the mind, because, the feelings of heart are beyond the reach of the words. When, I come to complete this manuscript, so many memories have rushed through my mind which is full of gratitude's to those who encouraged and helped me at various stages of this research. It gives me immense pleasure to record my feelings at this place.

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BENGALURU

July, 2015 ***(Sushma, P)***

STUDY ON SYNERGISTIC EFFECT OF ENDOPHYTIC BACTERIA AND *MESORHIZOBIUM* IN CHICKPEA

(*Cicer arietinum)*

SUSHMA, P.

ABSTRACT

An investigation was carried out to study the synergistic effect of endophytic bacteria with *Mesorhizobium* on chickpea. Large number of endophytic bacteria have been isolated from different parts of chickpea. Fifteen endophytic bacteria were from nodules, roots and seeds of chickpea by using nutrient oxide agar media. Different morphological and biochemical tests were carried out for endophytic bacterial isolates their compatibility with *Mesorhizobium* UASB 835 were tested. Among fifteen three endophytic bacterial isolates were selected based on morphological and biochemical tests and their compatibility with *Mesorhizobium* UASB 835 of chickpea. Molecular characterization was done by using 16S rRNA sequencing for the three compatible endophytic bacterial isolates. Isolates CPN 4 was identified as *Enterobacter sp.* CPR 2 as *Enterobacter hormeachie* and CPS 1 were identified as uncultured gamma *Proteobacteria.* Effect of co-inoculation of endophytic bacteria with *Mesorhizobium* in different treatment combination were studied under green house condition using sterilized soil for their synergistic effect on chickpea. Inoculation of endophytic bacteria and *Mesorhozobium* were done by seed treatment and soil drenching method. The significant difference seen among the treatments. The plant height, nodulation, nodule number, nodule dry weight, plant biomass, rhizosphere bacterial population and plant nitrogen content was recorded highest in treatment T13 receiving the inoculation of CPN+ CPR+ CPS+ *Mesorhizobium*. The results revealed that, co-inoculation of nodule , root and seed endophytic bacteria with *Mesorhizobium* on chickpea encourages more robust crop with better plant health, disease resistance, biomass and ability to cope with environmental stresses.

July 2015

Dept.of Agri. Microbiology
UAS, GKVK, Bengalure

K. Nagaraju
Chair person

ಕಡಲೆ (ಸೀಸರ್ ಆರಿಟಿನುಂ) ಯ ಮೇಲೆ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿ ಮತ್ತು ಮೀಸೊರೈಜೊಬಿಯಂ ಸಹಕ್ರಿಯೆಯ ಪರಿಣಾಮದ ಬಗ್ಗೆ ಅಧ್ಯಯನ

ಸುಷ್ಮ, ಪಿ.

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿ ಮತ್ತು *ಮೀಸೊರೈಜೊಬಿಯಂ* ಸಹಕ್ರಿಯೆಯ ಪರಿಣಾಮವನ್ನು ಕಡಲೆ ಸಸ್ಯದ ಮೇಲೆ ಅಧ್ಯಯನ ನಡೆಸಲಾಯಿತು. ಕಡಲೆಯ ವಿವಿಧ ಭಾಗಗಳಿಂದ ಗರಿಷ್ಟ ಸಂಖ್ಯೆಯ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳು ವರದಿಯಾಗಿದೆ. ೧೫ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳನ್ನು ಕಡಲೆ ಸಸ್ಯದ ಗಂಟುಗಳು, ಬೇರು ಮತ್ತು ಬೀಜದಿಂದ ನ್ಯೂಟ್ರಿಯೆಂಟ್ ಅಗರ್ ಮಾಧ್ಯಮ ಬಳಸಿ ಪ್ರತ್ಯೇಕಿಸಲಾಯಿತು. ಪ್ರತ್ಯೇಕಿತ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳಿಗೆ ವಿವಿಧ ಆಕೃತಿ ಮತ್ತು ರಾಸಾಯನಿಕ ಪರೀಕ್ಷೆಗಳನ್ನು ನಡೆಸಲಾಯಿತು ಹಾಗೂ ಅವುಗಳ ಹೊಂದಾಣಿಕೆಯನ್ನು *ಮೀಸೊರೈಜೊಬಿಯಂ*–ಯುಎಎಸ್‌ಬಿ ೮೩೫ ಜೊತೆ ಪರೀಕ್ಷಿಸಲಾಯಿತು. ೧೫ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳಲ್ಲಿ ೩ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳನ್ನು ವಿವಿಧ ಆಕೃತಿ, ರಾಸಾಯನಿಕ ಪರೀಕ್ಷೆಗಳು ಮತ್ತು ಹೊಂದಣಿಕೆ ಪರೀಕ್ಷೆಯ ಆಧಾರದ ಮೇಲೆ ಆಯ್ಕೆ ಮಾಡಿಕೊಳ್ಳಲಾಯಿತು. ಆಯ್ದ ೩ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳನ್ನು ೧೬ ಎಸ್ ಆರ್.ಆರ್.ಎನ್.ಎ ಆಣ್ವಿಕ ಪರೀಕ್ಷೆಯ ಮೂಲಕ ಸಿ. ಪಿ. ಎನ್ ೪ ನ್ನು *ಎಂಟಿರೋಬಾಕ್ಟೇರ್ ಹೊರ್ಮೊಯಿಚಿ*, ಸಿ. ಪಿ. ಆರ್ ೨ ನ್ನು *ಎಂಟಿರೋಬಾಕ್ಟೇರ್* ಸ್ಪೀಸಿಸ್ ಮತ್ತು ಸಿ. ಪಿ. ಎಸ್ ೧ ನ್ನು *ಗಾಮ ಪ್ರೋಟಿಯೋಬಾಕ್ಟೇರ್* ಎಂದು ಗುರುತಿಸಲಾಯಿತು. ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿ ಮತ್ತು *ಮೀಸೊರೈಜೊಬಿಯಂ* ಸಹಾರೋಗಣುಗಳನ್ನು ವಿವಿಧ ಚಿಕಿತ್ಸೆ ಸಂಯೋಜನೆಯಲ್ಲಿ ಹಸಿರುಮನೆ ಅಧ್ಯಯನವನ್ನು ಕಡಲೆ ಸಸ್ಯದ ಮೇಲೆ ಕೈಗೊಳ್ಳಲಾಯಿತು. ಎಂಡೊಫೈಡಿಕ್ ಅಣುಜೀವಿ ಮತ್ತು *ಮೀಸೊರೈಜೊಬಿಯಂ*ನ್ನು ಕಡಲೆಗೆ ಬೀಜಲೇಪನ ಮತ್ತು ಮಣ್ಣು ನೆನೆಸುವಿಕೆಯಿಂದ ಸೇರಿಸಲಾಯಿತು. ಸಸ್ಯದ ಎತ್ತರ ಗಂಟು ಗಂಟಾಗುವಿಕೆ, ಗಂಟುಗಳ ಸಂಖ್ಯೆ, ಗಂಟುಗಳ ಒಣ ತೂಕ, ಸಸ್ಯ ಜೀವರಾಶಿ, ರೈಜೊಸ್ಪಿಯರ್ ಅಣುಜೀವಿಗಳ ಸಂಖ್ಯೆ ಮತ್ತು ಸಸ್ಯ ಸಾರಜನಕದ ಪ್ರಮಾಣವು ಚಿಕಿತ್ಸೆ ಟಿ–೧೩ (ಸಿ. ಪಿ. ಎನ್ ೪ + ಸಿ. ಪಿ. ಆರ್ ೨+ ಸಿ. ಪಿ. ಎಸ್ ೧ + ಮೀಸೊರೈಜೊಬಿಯಂ) ನಲ್ಲಿ ಹೆಚ್ಚಾಗಿ ಕಂಡು ಬಂದಿದೆ. ಪಲಿತಾಂಶಗಳ ಪ್ರಕಾರ ಗಂಟುಗಳು, ಬೇರೆ ಮತ್ತು ಬೀಜದ ಎಂಡೊಫೈಟಿಕ್ ಅಣುಜೀವಿಗಳು ಮತ್ತು *ಮೀಸೊರೈಜೊಬಿಯಂ* ಸಹಇನಾಕ್ಯುಲೇಶನ್ ಸಸ್ಯದ ಧೃಡತೆ, ರೋಗ ನಿರೋದಕ ಶಕ್ತಿ, ಸಸ್ಯ ಜೀವರಾಶಿ ಮತ್ತು ಪರಿಸರದ ಒತ್ತಡಗಳಿಗೆ ಸರಿಹೊಂದಲು ಸಹಕಾರಿಯಾಗಿದೆ.

ಜುಲೈ ೨೦೧೫

ಸೂಕ್ಷ್ಮಜೀವಿ ಶಾಸ್ತ್ರ ವಿಭಾಗ — ಕೆ. ನಾಗರಾಜು

ಕೃ. ವಿ. ವಿ. ಬೆಂಗಳೂರು – ೬೫ — (ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು)

Isolation and Characterization of Endophytic Bacteria and *Mesorhizobium* from Chickpea (*Cicer arietinum*)

Sushma. P and K. Nagaraju

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Introduction

Chickpea (*Cicer arietinum)*is an important pulse crop belonging to the family Leguminaceae and is rich in protein and some of the essential amino acids. It enriches the soil through symbiotic nitrogen fixation from atmosphere. It contains 21% protein, 61.5% carbohydrates, 4.5% fat and is also rich in calcium, iron and niacin.

In India Chickpea covers about 8.25 million ha which is 40% of total pulse area with annual production of 7.05m tons contributing 50% of pulses production in the country.

Endophytic bacteria live inside the plant tissues and do not cause visible damage or morphological changes to their hosts. They can benefit the host plants in a variety of ways, such as producing IAA (Indole Acetic Acid), fixing nitrogen, dissolving phosphates, producing siderophores, suppressing phytopathogens by competition in the invasion sites and by secreting antibiotic compounds and by helping the symbiotic *Rhizobium* to form nodules with hosts.

Objectives

Isolation and Characterization of endophytic bacteria and *Mesorhizobium* from Chickpea.

Material and Methods

Isolation of endophytic bacteria from roots, seed and nodules and *Mesorhizobium* from roots and nodules has been done. Nodules, seeds and roots were surface sterilized and placed in Nutrient oxidized agar media with cut surface touching the agar. Plates were incubated for two to four days at 27^0 C.

Enumeration of endophytic bacterial population was done by standard plate count method. The bacterial isolates were selected based on distinct colony morphology.

Isolation of *Mesorhizobium* was done by using YEMA (Yeast Extract Mannitol Agar) with congo red media.

Morphological and biochemical characterization of the endophytic bacteria were done. Biochemical tests like Amylase production, Urease test, Phosphate solubilization, Gelatin utilization, Casein hydrolysis, Hydrogen sulfide production, Catalase test, Citrate utilization tests were carried out by using appropriate methods.

Conformation tests for *Rhizobium* has been done by growing the *Rhizobium* in Glucose Peptone Agar media, Lactose broth and Hoffers Alkaline media.

Results

Table 1

Microscopic characteristics of endophytic bacteria

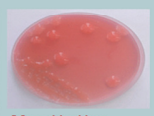
Plant part	Isolates	gram stain	Cell shape	Motility
Nodules	CPN1	+	rod	+
	CPN2	+	rod	+
	CPN3	-	Short rod	-
	CPN4	-	rod	+
	CPN5	+	rod	+
	CPN6	+	rod	+
	CPN7	-	rod	+
	CPN8	+	rod	+
Root	CPR1	-	coccid	-
	CPR2	-	rod	+
	CPR3	+	Short rod	-
	CPR4	+	Short rod	-
	CPR5	-	bacilli	-
	CPR6	-	rod	-
seed	CPS1	-	rod	+

Morphological characteristics:

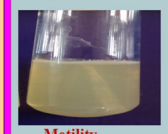
Most of the colonies were dirty white smooth glister colonies, some were white powdery dotted colonies, few were pigmented and non pigmented colonies.

Plates

Endophytic bacteria

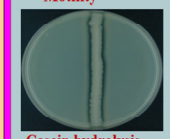


Mesorhizobium

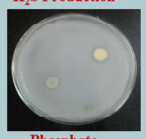


Motility

H_2S Production



Casein hydrolysis



Phosphate solubilizing zone

Table: 2

Biochemical characteristics of endophytic bacteria

Plant part	Isolates	Amylase production	Urease test	Phosphate solubilization	Gelatin utilization	Casein production	Hydrogen sulfide production	Catalase test	Citrate utilization
Nodules	CPN1	-	+	+	-	+	+	+	+
	CPN2	-	+	+	-	+	+	+	+
	CPN3	+	+	+	-	+	+	+	+
	CPN4	+	-	+	-	+	+	+	+
	CPN5	+	-	+	+	+	+	+	+
	CPN6	-	-	+	+	+	+	+	+
	CPN7	-	+	+	+	+	-	+	+
	CPN8	-	-	+	-	+	-	+	+
Root	CPR1	-	-	+	-	+	+	+	+
	CPR2	-	+	+	-	+	+	+	+
	CPR3	-	+	+	-	+	+	+	+
	CPR4	+	+	+	+	+	+	+	+
	CPR5	-	+	+	-	+	+	+	+
	CPR6	-	-	+	-	+	+	+	+
seed	CPS1	+	+	+	+	+	+	+	+

Mesorhizobium has showed gram negative reaction and negative reaction for the confirmatory tests, glucose peptone test, lactose broth and hoffers alkaline test.

Discussion

The number of endophytic bacteria recorded were more in nodule followed by root and seed.

Eight bacterial isolates were Gram positive and seven isolates were Gram negative. However, Zienniel *et al*.,(2002) reported an equal presence of Gram positive and Gram negative bacteria.

Ten isolates were motile and five isolates were non motile although endophytes follow water fluxes for passive movement and they also able to move inside the plant (Taghavi *et al*., 2009).

Bacterial isolates showed different reactions for different biochemical tests. All the endophytes showed positive results for Catalase test , Casein hydrolysis, Citrate utilization test and phosphate solubilization test and both positive and negative results in remaining tests.

Isolated bacterial strain was confirmed by confirmatory test as *Rhizobium*.

Summary

In present study different endophytic bacterial isolates isolated from nodules, roots and seeds vary in microscopic, morphological and biochemical characteristics.

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I INTRODUCTION

Chickpea [*Cicer arietinum*] is an important pulse crop belonging to the family Leguminaceae. India is the largest Chickpea producing country accounting for 70 per cent of the global Chickpea production. Chickpea is rich in protein and some of the essential amino acids and enriches the soil through symbiotic nitrogen fixation from atmosphere. It contains 21 per cent protein, 61.5 per cent carbohydrates, 4.5 per cent fat and is also rich in calcium, iron and niacin. In India this covers about 8.25 million hectares which is 40 per cent of total pulse area with annual production of 7.05 MT contributing 50 per cent of pulses production in the country (Mirza *et al.,* 2013).

Plants are generally associated with diverse microorganisms. Endophytic organisms are those that colonize internal tissue showing no external sign of infection or negative effect on their host (Schulz and Boyle, 2006). Plants constitute vast and diverse niches for endophytic organisms. Of the nearly 300000 plant species that exist on the earth, each individual plant is host to one or more endophytes (Strobel *et al*., 1993). But most likely, there is not a single plant species devoid of endophytes. Only a few of these plants have ever been completely studied relative to their endophytic biology.

India is having a large and diverse agricultural sector, accounting, on an average, of 16 percent GDP (Gross Domestic Product) and 10 per cent of export earnings. India's arable land area of 159.7 million ha is the second largest in the world however progress in organic agriculture is very slow. Only 41,000 ha of area has been converted to organic which is mere 0.03 per cent of cultivated area. To reach the global standards a qualitative and quantitative sustainable agriculture which adopts organic and dynamic cropping system is essential, it can be achieve through management of new biotechnologies and crop production strategies.

In combination of endophytic bacteria with *Mesorhizobium* have multitude applications that enhance agricultural production: they enhance Chickpea growth, disease resistance, pest management, fix atmospheric nitrogen, and improve biomass of the plants (Verma *et al.,* 2013).

Endophytic bacteria have been isolated from a large diversity of plants. Organisms like *Bacillus, Enterobacter, Klebsiella, Pseudomonas, Burkholderia, Pantoea, Agrobacterium, Methylobacterium spp*. Are the commonly isolated endophytic bacteria's from plants such as rice, wheat, maize, cotton, clover, potato, sugarcane, tomato, cucumber, and wild grass (Bacon and Hinton, 2006). The precise role of endophytes in plants is not yet known. However, their capability to thrive within the host tissues away from microbial competition and environmental degradation has made endophytes potential candidates for use in agriculture.

The role of endophytic microorganisms in plants can be divided into two categories based on types of activity, growth promotion and disease control. Endophytic bacteria are believed to elicit plant promotion in one of two ways:

1. Directly by producing phytohormones such as auxin or cytokinin or by producing the enzyme 1-aminocyclopropane- 1- carboxylate (ACC) deaminase, which lowers plant ethylene levels (Anu, 2012).

2. Indirectly by preventing pathogen infections via antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by establishing the plants systemic resistance (Anu, 2012).

Other beneficial effects of endophytes to plants include helping plants acquire nutrients, via nitrogen fixation, phosphate solubilisation or iron chelation, increased drought resistance, thermal protection, survival under osmotic stress, suppressing phytopathogens by competition in the invasion sites and by secreting antibiotic compounds (Ryan *et al.*, 2008) and by helping the symbiotic rhizobia to form nodules with unspecific hosts (Liu *et al.*, 2010). *Rhizobium* is an efficient N-fixing bacteria which fixes N in root nodules of legumes.

Co-inoculation of endophytes with PGPR and *Mesorhizobium* species have shown to increase root and shoot weight, plant vigor, nitrogen fixation and seed yield in various legumes (Valverde *et al.*, 2006; Yadegari *et al*., 2008; Verma *et al*., 2012). The effect of multiple inoculations with symbiotic N_2-fixing *rhizobium*, endophytic bacteria has rarely been tested. Also, there is little information available on possible synergistic effects on Chickpea. *Rhizobium* has been reported to be a key element for plant establishment under xeric and nutrient unbalanced condition (Chaudhary *et al*., 2011).

The study of plant associated microorganisms and combined inoculation is of great importance, for example biological control of pathogens, plant growth promotion or isolation of active compounds. Understanding the diversity of plant bacterial associations in combination and their role in plant development is necessary if this association are to be manipulated to increase crop production, conserve biodiversity and sustain agro-ecosystems.`

A renewed interest in the internal colonization of healthy plants by rhizobial and non-rhizobial (endophytes) has arise their potential for exploitation in agriculture becomes apparent. Exploitation of co-inoculation of *Mesorhizobium* with endophyte plants interactions can result in promotion of plant health and can play role in low input sustainable agriculture for crops. Considering the enormous potential of the endophytesa research programme has been framed to study the synergistic effect of endophytic bacteria and *Mesorhizobium* in plant growth promotion of Chickpea with the following objectives.

1. Isolation and characterization of endophytic bacteria and *Mesorhizobium* from Chickpea.

2. Study on synergistic effect of endophytic bacteria and *Mesorhizobium* in Chickpea.

II REVIEW OF LITERATURE

Plant – microbe interactions that promote the plant development and plant health have been the subject of considerable interest. Plants constitute vast and diverse niches for endophytic organisms. Among the microorganisms endophytic bacteria occupy the internal tissues of plants without causing damage to their hosts. An understanding of the mechanisms enabling these microorganisms to interact with plants.

The term endophyte (Gr. Endon, within; *phyton*, plant) was first coined by De Bary, in 1866. An endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and / or intracellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Wilson, 1995). The presence of endophytes was reported by Vogl in 1898 who revealed a mycelium residing in the grass seed *Loliumt emulentum.*

2.1 Isolation and characterization of endophytic bacteria and *Mesorhizobium*

The genotypic diversity of indigenous bacterial endophytes within stem of tropical maize (*Zea mays* L.*)* was determined in field and greenhouse experiments by Rai *et al*. (2007). Endophytes were found in most of the growing seasons at population ranging from $1.36\text{-}6.12\times10^5$ colony- forming units per gram fresh weight of stem.

In-plant and ex-plant a populations of endophytic species could be differentiated by biochemical characteristics (Van Peer *et al*., 1990; McInroy and Kloepper 1991) showed that the endophytic bacterial populations in corn stems ranged from 1×10^3 initially to 1×10^{10} CFU/ml after 10 weeks of sowing.

Endophytic bacteria have been isolated from a large diversity of plants by Sturz*et al*. (2000). Mundtz and Hinkle (1976) reported as many as 46 different endophytic bacterial species from 27 plant species.

Thirty five endophytic bacteria were isolated from surface sterilized stems, roots, and nodules of wild and cultivated soyabean varieties by Hung *et al*. (2007) and reported genetic variation was more among endophytes isolated from *Glycine max* tissues than from *G. soja.*

Seventy-seven endohytic bacterial isolates were isolated from roots, stems and leaves of black nightshade plants (*Solonum nigrum*) grown in two different native habitats in Jena, Germany by Long *et al*. (2008).

Thirty two isolates of endophytic bacteria were obtained by Magnani *et al*. (2010) out of which most of the bacteria isolated belonged to *Enterobacteriaceae* and *Pseudomonaceae,* demonstrating niche specificity. The *Enterobacter* genus was most frequently found in the stems of sugarcane.

The diversity of bacterial endophytes associated with ginseng plants of varying age in Korea was investigated by Thamizhvendan *et al.* (2010). Although a mixed composition of endophyte communities were recorded from ginseng based on the results of 16S rDNA analysis, bacteria of the genus *Bacillus* and *Staphylococcus* dominated in one year old and 4 year old plants, respectively. Phylogenetic analysis revealed four clusters, *Actinobacteria, á- Proteobacteria*, and *ɣ- Proteobacteria*, with *Firmicutes* being predominant.

Endophytic bacteria are found in roots, stems, leaves, seeds, fruits, tubers, ovules, and also inside legume nodules (Hallman *et al*., 1997; Sturz *et al*., 1997). In most of the plants, roots have the higher number of endophytic bacteria compared to above ground tissues (Rosenblueth and Martinez, 2004). Endophytic bacteria are able to penetrate and become systemically disseminated in the host plant, actively colonizing the apoplast, conducting vessels (Hallman *et al*., 1997) and occasionally the present intracellular spaces.

Bacterial endophytes were found in variety of plants, such as sugar beat (Dent *et al*., 2004), prairie plants, agronomic crops (Zinniel *et al*., 2002), potato varieties (Sessitch *et al*., 2002), total 81 different bacterial species were reported to form endophytic associations with plants.

Araujo *et al*., 2002; Zinniel *et al*., 2002 isolated *Enterobacter* and has been identified as endophytes of several plants like citrus, soyabean and other crop plants.

Bacteria belonging to the genera *Bacillus* and *Pseudomonas* were easy to culture and cultivation dependent studies have been identified them as frequently occurring endophytes (Seghers *et al*., 2004)

Patel *et al*. (2012) isolated and characterized bacterial endophytes from root and stem of *Lycopersicon esculentum* plant which was collected from different regions of Gujarat. Total 18 isolates of endophytic bacteria were selected in which, only HR7 endophyte of tomato was identified as *Pseudomonas aeruginosa* by 16S rDNA.

Bacillus have been reported as maize kernel endopphytes (Rijavec, 2007) and have been isolated not only from maize (Chelius, 2001; McInory and Kloepper, 1995) but also from (Garbeva *et al*., 2001; Izumi *et al*., 2008; Lian *et al*., 2008; Lo'pez *et al*., 2010).

Rice endophytic bacteria belong to the following groups*; Pseudomononas sp*. (You and Zhou, 1989), *Azoarcus sp*. (Hurek *et al*., 1994), *Burkholderia sp*. (Engelhard *et al*., 2000, Olivares *et al*., 2011), *Rhizobium leguminosarum* (Yanni *et al*., 1997), *Serratia sp.* (Sandhiya *et al*., 2005), *Klebsiella sp*. (Rosenbluet*h et al*., 2004) and *Azorhizobium caulinodan, Enterobacter* (Engelhard *et al*., 2000).

The growth stimulation by the microorganism can be a consequence of nitrogen fixation (Hurek *et al*., 2002; Iniguez *et al*., 2004; Sevilla *et al*., 2001).

Endophytic bacteria are found in legume nodules as well. In red clover nodules, some species of rhizobia were found including *Rhizobium, Agrobacterium rhizogenes*, in addition to *R. leguminosarum bv. Trifolii*, which is the normal clover symbiont (Struz *et al*., 1997). Some γ *Proteobacteria* are co-occupants with the specific rhizobia in Hedysarum plants nodules (Benhizia *et al*., 2004). In most cases, the endophytic bacteria are able to form nodules.

Zecchin *et al*. (2014) reported six endophytic bacteria of corn roots and were identified as *Bacillus sp.* and *Enterobacter sp*, by sequencing of the 16S rRNA gene. *Bacillus* and *Enterobacter* increased the root volume by 44 per cent and 39 per cent, respectively.

Montanez *et al.* (2012) reported that Maize (*Zea mays L.*) plants can establish beneficial associations with various nitrogen fixing and plant growth promoting bacteria (PGPB). Twenty-two putative endophytic bacteria isolated from maize plants were identified and characterized as genera *Rhanella, Pantoea, Rhizobium, Pseudomona, Herbaspirillum, Enterobacter, Brevundimonas and Burkholderia*.

Struz *et al*. (1997) isolated 15 bacterial species from red clover nodules and estimated endophyte population densities to be in the range of 10^8 viable bacteria per gram of gram of fresh nodule.

Cho *et al*. (2007) reported 13 bacterial genera among 63 isolates from the interior of ginseng roots cultivated in three different areas and demonstrated marked regional differences in microbial community.

Aravind *et al*. (2012) isolated 80 isolates of endophytic bacteria from different varieties of black pepper *(Piper nigrum L.*) grown at different locations in India. Theses isolates were tentatively grouped into *Bacillus sp*. (932 strians), *Pseudomonads* (26 strains), *Arthrobacter sp*. (20 strains), *Micrococcus sp.* (10 strains), *Curtobacterium sp*. (one strain), *Serratia* (one strain), *Enterobacter* and twenty unidentified strains based on morphology and biochemical tests.

Surette *et al*. (2003) were reported up to 360 endophytic isolated strain from *Daucuscarota*, and they were classified into 28 genera, with *Pseudomonas, Staphylococus*, and *Agrobacterium* being predominant.

Peng *et al*. (2008) reported twelve facultatively anaerobic, endophytic diazotrophs isolated from surface-sterilized roots of the wild rice species *Oryza latifolia* and characterized by phenotypic and molecular methods.

Suman *et al*. (2001) isolated 853 endophytic strains from aerial tissue of four agronomic crop species and 27 plant species. A majority of the microorganisms were isolated (689 strains) from corn and sorghum; 45 strains were screened from soyabean and wheat, and 119 strains were obtained from 27 different host species of grasses, forbs,

legumes and wild flowers. As a whole, fewer isolates were recovered from perennial plants from the agronomic crops.

Saine *et al*. (2013) isolated endophytic bacteria from roots (12isolates) and nodules (76isolates) of Chickpea. Among the endophytic bacteria, 50% from roots and 93.4% from nodules were Gram positive spore formers. Large number of endophytes from roots and nodules solubilize the phosphate.

Three of the 14 endophytes improved soyabean nodulation and plant weight when co-inoculated with *B. japanicum* (Bai *et al*., 2002)

The 16s rRNA gene sequence of strain NRCPB10T showed highest similarity (98.95%) to that of *Rhizobium radiobacter* NCPPB 2437T, followed by *Rhizobium larrymoorieii* AF3-10T (97.7%) and *Rhizobiumrubi* IFO 13261(97.4%). Phylogenetic analysis of strain NRCPB10T based on the house keeping gene recA and atp D confirmed its position as distinct from recognized *Rhizobium spp*. Levels of DNA-DNA relatedness b/w strain NRCPB10T and *Radiobacter* ICMP5785T.*Radiobacterlarrymomooreii* LMG 21410T and *Radiobacterrabi* ICMP6428T were 51.03, 32.6 and 27.3 per cent respectively (Pandey *et al*., 2012).

Palaniappan *et al*. (2010) isolated 39 endophytic bacterial strains from the nodule of *Lespedeza* sp. The strains were identified by using 16S rRNA gene sequence analysis as belonging to *Alphaproteobacteria, Betaproteobacteria, Actinobacteria, and Firmicutes* phylum with nine different genera *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Dyella*, *Methylobacterium*, *Enterobacterium, Microbacterium*, *Rhizobium*, and *Staphylococcus*. Gene *nod*A amplification showed positive results only for strains from *Bradyrhizobium* and *Rhizobium* genera.

Characterization of 34 endophytic bacterial isolates associated to root nodules collected from legumes in the arid zone of Tunsia by 16S rRNA polymerase chain reaction. In that *nif H* sequences are most similar to those of *Sinorhizobium meliloti*, were detected within strains related to the genera *Microbacterium , Agnomyces* , *Starkeya* and *Phyllobacterium*. But other strains did not induce any nodules when inoculated on wide spectrum of host legume species *M. atropurpureum* and no *nodA* gene could be amplified by PCR (Zakhia *et al*., 2006).

Seo *et al*. (2010) isolated 264 colonies of endophytic bacteria from the interior of young radish leaves and roots Endophytic bacteria from the phylum *Proteobacteria* were predominant in the leaf (61.3%) and root (52.1%) samples.

Mirza *et al*., 2006 obtained four *Rhizobium* strains were obtained from root and four from the nodules of Chickpea. Which were characterized morphologically and biochemically.

Root nodule symbiosis is characterized by two major evolutionary inventions; the intercellular uptake of bacteria and the formation of specialized organs, the nodules. The

later provides a suitable micro environment for *nitrogenase* activity and allow for a protected, controlled development of high bacterial population densities. Nodules are thus resembles micro fermentors within the host plant that are optimized for symbiosis maintenance (Markmann and Parniske, 2009).

Hung and Annapurna (2004) carried out investigation to analyze the phenotypic and genotypic diversity in the bacterial endophytes of two species of soybean *viz.Glycine max* and *G. soja*. Preliminary characterization of the 65 endophytes showed that approximately equal percentage of gram positive (49%) and gram negative (51%) bacteria were present out of which approximately (80%) were motile.

Endophytic bacterial diversity was estimated in Mexican husk tomato plant roots by amplified rDNA restriction analysis and sequence homology comparison of the 16S rDNA genes. Sixteen operational taxonomic units from the 16S rDNA root library were identified based on sequence analysis, including the classes *Gamma proteobacteria, Beta proteobacteria, Actinobacteria* and *Bacilli*. The predominant genera were *Stenotrophomonas* (21.9%), *Microbacterium* (17.1%), *Burkholderia* (14.3%), *Bacillus* (14.3%), and *Pseudomonas* (10.5%). In a 16S rDNA gene library of the same plant species rhizosphere, only common soil bacteria, including *Stenotrophomonas, Burkholderia, Enterobacter, Bacillus*, and *Pseudomonas*, were detected. endophytic bacterial diversity within the roots of Mexican husk tomato plants is a subset of the rhizosphere bacterial population, dominated by a few genera (Santacruz *et al*., 2010).

Bhat *et al*. (2013) reported nine different isolates of Rhizobium were isolated from Mung bean root nodules, cultivated in different Mung bean using YEMA media. All the isolated isolates of Rhizobium failed to show a growth on glucose peptone agar medium as well as Hoffer`s alkaline broth. All test isolates gave a negative test on ketolactose medium.

2.2 Synergistic effect of endophytic bacteria and *Mesorhizobium*

Mirza *et al.* (2006) reported two strains of plant growth-promoting rhizobacteria belonging to genus *Enterobacter* were also isolated from Chickpea roots and *Rhizobium* and *Enterobacter* B strains produced the plant growth hormones. Co-inoculation of *Rhizobium* strain with *Enterobacter* resulted in maximum increase in plant biomass and nodulation, as compared with the control. Whereas the combination of *Rhizobium* with *Enterobacter* A was more efficient in growth promotion of Chickpea.

The *Rhizobium* isolated from *Trigonella foenumgraecum* (fenugreek) is known for its dietary protein source, medicinal properties and symbiotic nitrogen fixation by *Rhizobium* present in its root nodules. The present study reported the characterization of a *Rhizobium* strain isolated from root nodules of fenugreek. The *Rhizobium* isolates were rod shaped, gram negative, acid and mucous producing (Singh *et al*., 2008)

Bressan and Borges, (2004) stated that application of endophytes by root tissue and prooned root dip + soil drench + seed treatment is best method of application of endophytes to crop plants.

Endophytic bacteria are believed to elicit plant growth promotion, nitrogen fixation, phosphate solubilization, siderophore and phytohormone production. It increases nodulation and growth in a wide variety of legumes. Recently, endophytic bacteria have drawn great attention as potential bioinoculants. Endophytic isolates on growth and nodulation of blackgram and greengram by pot culture studies, so as to explore the possibility of development of biofertilizers for enhancing crop productivity of legumes for its use in sustainable agriculture (Uma *et al*., 2014)

Pre sowing inoculation of mung bean seed with different endophytic bacteria increase the nodulation and seed yield. Nodulation was highest when seeds are inoculated with *Rhizobium*+ PGPR than *Rhizobium* alone (Bansal, 2009).

Hung *et al*. (2007) studied the effect of endophytes on soyabean growth and development. These isolates enhanced the total plant biomass by more than 80% over that of uninoculated control.

In the greenhouse experiment by Olivera *et al*. (2011) reported two *Bacillus* and two *Pseudomonas* strains were examined for their ability to promote common bean growth (*Phaseolus vulgaris L.)* when co-inoculated with *Rhizobium phaseoli*. Co-inoculation with *Rhizobium* and *Pseudomonas sp* or *Bacillus sp.* improved shoot dry weight, nitrogen and phosphorus contents in bean plants, compared to inoculation with *Rhizobium* alone.

A study was performed by Stajkovic *et al*. (2009) to asses the effects of non rhizobial endophytes from the surface sterilized root nodules of alfalfa (*Medicago sativa L.*) on its growth. Co-inoculation of all non-rhizobial strains with *S. meliloti* positively influenced nodule number, but was without any significant effect on growth parameters with respect to inoculation with *S. meliloti* alone. However, single inoculation with non-rhizobial strains caused significant increase in shoot and root parameters compared to uninoculated plants, indicating that non-rhizobial strains posses some plant growth promoting potential.

Jha and Kumar (2007) isolated and characterized endophytic diazotrophic bacteria from a semi aquatic grass (*Typha australis*). This significant increase in root/ shoot length and chlorophyll a content.

Tilak *et al*. (2005) reported that PGPR, in combination with *Rhizobium* affects the growth and nitrogen fixation in Pigeonpea by inducing occupancy of introduced *Rhizobium* in the nodules of legume. The seeds treated with combination of *Rhizobium* and PGPR increases the growth, nodulation and enzyme activity than *Rhizobium* alone.

Omarjee *et al.* (2004) isolated endophytic bacteria from stalks of sugarcane, *Saccharum officinarum*in papua New Guinea. Endophytic bacterial communities extracted juice from averaged 6× 10^4 CFU/ ml, which is 25 times greater than the number of endophytic bacteria found in commercial varieties in South Africa. In this case the isolated *Burkholderia spp*. are opportunistic endophytes that are capable of utilizing organic compounds within sugarcane for their growth and survivalence.

Remans *et al*. (2008) reported *Azospirillum spp*. have shown potential to enhance nodulation and plant growth of legumes when co-inoculated with *Rhizobium*. Two genotypes of common bean (*Phaseolus vulgaris L.*) BAT477 and DOR364, contrasting in nodulation response to *Azospirillum* when co-inoculated with *Rhizobium*. It was observed that *Azospirillum–Rhizobium* Co inoculation as compared to single *Rhizobium* inoculation increased the amount of fixed nitrogen and the yield of DOR364 across all sites.

Pankaj *et al*. (2010) investigated lentil seeds inoculation with two cold tolerant *Pseudomonas sp*. strains (NARs1, PGERs17) and *R. leguminosarum*-PR1, individually or in combination, Inoculation with *Pseudomonas sp*. strain PGERs17 exhibits more vigorous vegetative growth, synergistic relationship existed between *Pseudomonas sp*. strain NARs1 and *R. leguminosarum*-PR1.

Zahir *et al*. (2011) reported that the use of three plant growth-promoting rhizobacteria strains containing ACC-deaminase (*Pseudomonas jessenii, Pseudomonas fragi, and Serratiafonticola*) and *Rhizobium leguminosarum*. Synergistic/co inoculation effect of *P. jessenii* with *R. leguminosarum* was more pronounced compared to that with *P. fragi* and *S. fonticola*. It increased the number of pods per plant, number of nodules per plant, dry nodule weight, seed yield.

Verma *et al*. (2013) reported that dual inoculation *Mesorhizobium* sp. and PGPR were found significantly better for nodulation, plant growth and yield of chickpea over control. Significant nodulation (62% and 86%), dry weight of root (44 and 57 %), yield and nitrogen 65% were co-inoculation of *Mesorhizobium sp* and *Pseudomonas aeruginosa* as PGPR.

Zecchin *et al*. (2014) reported six isolated endophytic bacteria of corn roots were identified as *Bacillus sp*. and as *Enterobacter sp*, by sequencing of the 16S rRNA gene. Four of the strains, CNPSo 2476, CNPSo 2477, CNPSo 2478 and CNPSo 2480 were positive for the nitrogen fixation ability evaluated through the acetylene reduction assay and amplification of nif H gene. Two has showed outstanding skills for the production of IAA, siderophores and lytic enzymes.

A field experiment was carried out during the rabi season by Tagor *et al*. (2013) to find out the effect of *Rhizobium* and phosphate solubilizing bacterial (PSB) inoculants on symbiotic traits, nodule leghemoglobin, and yield of five elite genotypes of chickpea. Among the chickpea genotypes, IG-593 performed better in respect of symbiotic parameters including nodule number, nodule fresh weight, nodule dry weight, shoot dry weight, yield attributes and yield.

III MATERIAL AND METHODS

3.1 Location

Laboratory experiments were conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru which is located at an altitude of 930 meters above MSL and between 12^{o}.51 N latitude and 77^{o}.35 E longitude.

3.2 Glass wares

Borosil made glass wares were cleaned with soap solution and soaked in chromic acid solution for 4-6 h then rinsed with distil water for two to three times, dried and sterilized in hot air oven at 160 ^{o}C for 3 h before use.

3.3 Chemicals

The analytical grade (AR) chemicals of Hi- media, Qualigens, BDH, E-Merck and sigma were used for media preparation and biochemical studies.

3.4 Composition of media, reagents, buffers and solutions

The composition of reagents, buffers and different media used are given in appendix.

3.5 SAMPLE COLLECTION

3.5.1 Sample site and Chickpea crops used

The samples were collected from MRS, Hebbal and Zonal Agricultural Research Station at University of Agricultural Sciences, GKVK, Bengaluru. The root and nodule samples were collected at the stage of 80 per cent flowering and seeds were collected after the harvest.

3.5.2 Enumeration of endophytic bacteria and *Mesorhizobium*

Standard plate count method

The endophytic bacteria and *Mesorhizobium* were enumerated by modifying the isolation procedure described by Sturz *et al.* (1999) and Gyaneshwar *et al.* (2001). One gram of root, nodule and seed samples was crushed under aseptic conditions with sterile pestle and mortar in 9 ml of sterile water. From this, 1ml of aliquot is transferred to test tube and it is serially diluted up to 10^{5} and 1ml of each dilution was transferred to nutrient agar and YEMA with congored plates with 3 replications and incubated for three to four days at 27 ^{o}C. Number of colonies grown on the media are enumerated.

3.6 Isolation of endophytic bacteria and *Mesorhizobium*.

3.6.1 Preparation of media

The nutrient oxide agar media was used for the isolation of endophytic bacteria. The media was prepared by dissolving all the chemicals and pH of the media adjusted to 7.0 and sterilized by autoclaving at 121 ^{o}C for 15 minutes and cooled to 50 ^{o}C. Solidified media was prepared by the addition of two per cent Oxoid purified agar before autoclaving. Glycerol stock (90 %) was used for storing the cultures for a longer time at refrigerated condition (-4 ^{o}C).

3.6.2 Isolation of endophytes from nodule, root and seed and *Mesorhizobium* from nodule.

The isolation of endophytes was done according to the procedure given by Bacon *et al*. (2002). The randomly selected plants were uprooted manually and washed in running tap water and surface sterilized root sections of 1 to 2 cm length were excised using flame sterilized scalpel. The nodules and seeds were similarly prepared. All the samples were blotted dry with filter paper and then weighed to have final sample of 0.5 g blotted. The surface sterilization of the nodules, root pieces and seeds was done with the following immersion sequence: 70 per cent ethanol for 1min, 3 per cent sodium hypochlorite for 5 minutes in young plants and 10 min in old plants wash for 1 min, they were then rinsed four times with sterile water and dried in laminar flow. Surface disinfection parameters like selection of disinfectant were optimized prior to experimentation. The cut ends of the surface sterilized segments were removed with flame sterilized scalpel and were placed in nutrient oxide agar media with the cut surface touch the agar and *Mesorhizobium* was isolated from placing the nodule sample in YEMA with congored media. Plates were incubated for three to four days at 27 ^{0}C the bacterial isolates were purified by streak plate method and selected for further studies.

3.7 Selection of endophytic bacterial population and *Mesorhizobium*

The endophytic bacterial isolates were selected based on the distinct colony morphology. All the isolates were purified by streaking on nutrient agar and YEMA media plates. Long term storage of the purified isolates were done at -60 ^{0}C in respective broths and short term storage for further characterization was done on respective media plates and test tubes.

3.8 Characterization of the endophytic isolates

3.8.1 Morphological tests

The following morphological tests *viz.,* cell shape, Gram reaction and motility were carried out to characterize the endophytes.

3.8.2 Cell shape (Aneja, 2006)

The purified cultures, at log phase were observed microscopically for the cell morphological characteristics.

3.8.3 Gram staining (Hucker and conn, 1923)

Gram staining was carried out as per modified Hucker's method. The slides were viewed with the light microscope under oil immersion. Gram positive bacteria appear violet and Gram negative bacteria appear pinkish red.

3.8.4 Motility in liquid media (Aneja, 2006)

The endophytic isolates were grown in semi solid agar stab and motility is observed by its growth in medium.

3.8.5 Biochemical test

The following biochemical tests like Amylase production, Urease test, Phosphate solubilization, Gelatin utilization, Casein hydrolysis, Hydrogen sulfide production, Catalase test, Citrate utilization, oxide test, indole production and MR-VP tests were carried out using appropriate methods.

3.8.5.1 Amylase production test (Aneja, 2006)

The endophytic bacterial isolates were streaked on starch agar media and incubated at 25 ^{o}C in inverted position for 3- 4 days. Surface of the plates was flooded with iodine solution with a dropper for 30 seconds. Plates were observed for clear zone surrounding the microbial colonies.

3.8.5.2 Urease test (Aneja, 2006)

The endophytic bacterial isolates were inoculated to the urea agar slants and incubated for 24- 48 hours. Colour of the media changes to red color.

3.8.5.3 Phosphate solubilization test (Aneja, 2006)

Microorganisms capable of producing a halo or clear zone due to solubilization of phosphate in the surrounding medium. Which were streaked on Sperber's medium and plates were incubated at 30 ^{0}C for 4-6 days. The isolates which showed the clear zone were considered as P solubilizer. The clearing zones were measured in mm and recorded.

3.8.5.4 Gelatin utilization test (Aneja, 2006**)**

Gelatin is a protein hydrolyzed by enzymes and is tested by gelatin liquefaction. The test cultures were stab inoculated into nutrient gelatin deep tubes, incubated at refrigerated condition for 48 h and observed for gelatin liquefaction.

3.8.5.5 Catalase activity (Aneja, 2006)

A loop full of 24 h old culture of endophytic isolates maintained on nutrient agar slants were transferred to a glass tube containing 0.5 ml distilled water and mixed thoroughly with 0.5 ml of 3 percent hydrogen peroxide solution and observed for the presence of the effervescence.

3.8.5.6 Citrate utilization test (Seeley and Vandemark, 1981)

The isolates were inoculated to the test tubes having Simmons Citrate agar medium and incubated for 48 h at 30 ^{o}C. Simmons citrate agar contained citrate as its only carbon and energy source. The presence of growth and change of color from green to blue due to pH change indicates positive reaction.

3.8.5.7 Casein hydrolysis (Aneja, 2006)

24h old cultures were streaked on Skim milk agar medium plates, incubate the plates for 24- 48 h at 37 ^{o}C in inverted condition. Presence of clearing around the line of growth is observed.

3.8.5.8 Hydrogen sulfide production test (Seeley and Vandemark, 1981)

Sulfide indole motility (SIM) agar stabs were inoculated with the isolates of endophytic bacteria and incubated at 30 ^{o}C for 48 h. Black coloration along the line of stab inoculation indicate the H_2S production.

3.8.5.9 Indole production (Seeley and Vandemark, 1981)

The endophytic isolates were inoculated into sterilized glucose tryptone broth taken in test tubes were incubated at 30 ^{o}C after 48 h of incubation, 0.3 ml of Kovacs reagent was added and mixed well. The reddening of the alcohol layer within few minutes indicates indole production.

3.8.5.10 Oxide tests (Aneja, 2006)

The endophytic isolates were streaked on the Trypticase soy agar media and incubated at 30 ^{o}C in an inverted position for 48 h. After incubation period, 2-4 drops of para-aminodimethyl aniline oxalate solution were added on the streaked area and the plates were observed for the color change from pink to maroon and finally to purple within 30 sec which indicates a positive reaction.

3.8.5.11 Methyl Red and VogesProskauer test (Seeley and Vandemark, 1981)

Methyl Red and Voges Proskauer test (MA-VP) broth prepared in two sets was inoculated with the endophytic bacterial isolates and incubated for 48 h at 30 ^{o}C. To the first set of tubes, few drops of an alcoholic solution of methyl red were added. The development of distinct red color was indicative of positive reaction for MR test.

α- naphthol solution (5 per cent solution in 70 per cent ethyl alcohol) was added to the second set of tubes and shaken gently for 15 min. the positive reaction of acetyl methyl carbinol production was indicated by development of red color. This indicates positive result for the VP test.

Mesorhizobium UASB-835 reference strain was taken from AICRP on Chickpea scheme for further studies, compatibility test and treatment for the green house experiment.

3.9 Assessment of compatibility between endophytic bacteria and *Mesorhizobium* .

Endophytic bacterial isolates were streaked in 'T' shape or in perpendicular to one other and with *Mesorhizobium* UASB-835. The compatible isolates show no zone of inhibition between the colonies and non compatible isolates shows clear zone of inhibition between them.

3.10 Molecular characterization of endophytic bacteria

After compatibility test, the compatible three endophytic bacterial isolates were identified up to species level by 16S rRNA sequencing.

3.11 Synergistic effect of endophytic bacteria and *Mesorhizobium*

3.11.1 Surface sterilization of Chickpea seeds

Chickpea seeds of variety JG11 were surface sterilized with 70 per cent ethanol for 1 min, 3 per cent Sodium hypochloride for 3 min followed by 70 per centethanol and wash for 1 min, then seeds were rinsed in sterile distilled water three times and blot dried.

3.11.2 Seed germination test

The surface sterilized seeds were placed on plate containing wet blotting paper and kept for 5-7 days and allowed it to germinate.

3.11.3 Preparation of endophytic bacterial isolates and *Mesorhizobium*

The endophytic bacteria were grown on nutrient broth and *Mesorhizobium* in YEMA broth with constant shaking at 150 rpm for 48 h at room temperature to get approximately 10^6 CFU/ ml. The bacterial cells harvested by centrifugation at 10000 rpm for 15 minute and biofertilizer was made using talc. This was used as bacterial inoculums.

3.11.4.1 Place of experiment.

The pot culture experiments as detailed below were conducted in the green house of ZARS, UAS, GKVK, Bengaluru. The soil having the properties of pH 6.5, EC 0.30 dsm^{-1} available nitrogen 458 Kg/ha , available P_2O_5 40 Kg/ha and K_2O 190 kg/ha was passed through a 4 mm sieve and mixed along with farmyard manure at 2:1 proportion and filled in pots @ 10 kg/pot.

3.11.4.2 Source of seeds

Seeds of variety JG-11 was obtained from ZARS, UAS, GKVK, Bengaluru and same seeds were taken for the experiment.

3.11.5 Sowing

The surface sterilized seeds were treated with single and combination of organisms and dibbled in pot and allowed to grow.

3.11.6 Experimental details

T_1: Control.
T_2: *Mesorhizobium.*
T_3: Nodule endophytic bacteria.
T_4: Seed endophytic bacteria.
T_5: Root endophytic bacteria .
T_6: Root endophytic bacteria +*Mesorhizobium.*
T_7: Nodule endophytic bacteria + *Mesorhizobium.*
T_8: Seed endophytic bacteria + *Mesorhizobium.*
T_9:Root endophytic bacteria + nodule endophytic bacteria .
T_{10}: Root endophytic bacteria + seed endophytic bacteria.
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria.
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria.
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium.*
Replication: 3. **Design**: Completely Randomized Design

3.12 Treatment of endophytic bacteria and *Mesorhizobium*

Endophytic bacterial isolates and *Mesorhizobium* UASB-835were inoculated according to treatments. The grown isolates were added equally (v/v) with water and finally mixed at the time of inoculation. The inoculations were done by seed treatment and soil drenching of organisms using sterile distilled water after 15 days of sowing.

3.13 After care

Watering was done regularly.

3.14 Observations recorded

3.14.1 Plant height

Plant height at 30 days and 60 days and after harvest was recorded and expressed in cm. After harvest, the plants were uprooted carefully and were dipped in water for 30 min to loosen the soil. These were used to record the observation.

3.14.2 Nodule number

After uprooting of plant, number of nodules per plant were counted and recorded at pod formation stage.

3.14.3 Root length

After harvesting of plant, root length was recorded in cm.

3.14.4 Plant Biomass

3.14.4.1 Fresh weight of root and shoot

The fresh weight of roots and shoots were recorded using an electronic balance and the mean was calculated and expressed in gram.

3.14.4.2 Dry weight of shoot and root

The shoot and root samples were oven dried at 60 ^{o}C for 4-6 days and dry weight of samples recorded and expressed in gram.

3.14.6 Estimation of plant nitrogen content.

3.14.6.1 Preparation of sample for estimation of nitrogen

Total nitrogen was estimated by kjeldahl distillation method (Jackson, 1973). One gram plant sample was digested in digestion flask using 15 ml conc. H_2SO_4. The digested materials were distilled using 40 per cent NaOH and liberated ammonia was trapped in 4 per cent Boric acid. This was titrated against 0.2 N H_2SO_4 and per cent plant N content was determined using the following formula.

$$\%N = \frac{(T-B) \times N \times 0.014 \times 100}{\text{Weight of the sample}}$$

T=Titration value, B= Blank, N= Normality of the acid

3.14.7 Microbial analysis

The rhizosphere soil samples were collected at 30, 60 DAS and after harvest were analyzed for bacterial population by standard plate count using nutrient agar media (Aneja, 2006).

3.15. Statistical analysis:

The statistical analysis of the data of the experiment was carried out as described by Panse and Sukhatme (1985) for completely randomized design, the critical differences at five per cent level of significance were worked out wherever the "F" test were significant.

IV EXPERIMENTAL RESULTS

The present study encompasses the isolation and characterization of endohytic bacteria and their synergistic effect with *Mesorhizobium* in Chickpea. Fifteen endophytic bacterial isolates were obtained from different parts of Chickpea and three compatible endophytic bacteria were selected for further studies in green house condition to know the synergistic effect with *Mesorhizobium* were analyzed for their plant growth promoting potential and the result are presented here under.

4.1 Occurrence of endophytic microorganisms in root, nodules, seed and *Mesorhizobium* from nodule of Chickpea

The natural occurrence of endophytic microorganisms in the root, nodules and seeds and *Mesorhizobium* in nodule was studied. The Chickpea plant samples and seeds were collected from ZARS, UAS, GKVK, Bengaluru-65.

4.2 Population of endophytic bacteria in different parts of Chickpea

The observation recorded for enumeration of endophytic bacteria is presented in Table1. The number of population of endophytic bacteria recorded was highest in nodules (5.2×10^5CFU/g) followed by roots (3.96×10^5CFU/g) and least number of endophytic bacteria recorded in seed (2.29×10^5CFU/g) and population count of *Mesorhizobium* was (1.8×10^5CFU/g).

4.3 Naming of isolates

Endophytic bacterial isolates were named according to the plant part from which the endophytes were isolated. Fifteen endophytic bacteria were isolated and named. Eight isolates from nodule were named as CPN1, CPN2, CPN3, CPN4, CPN5, CPN6,CPN7 and CPN8 and from roots six isolates were isolated and named as CPR1, CPR2, CPR3, CPR4, CPR5 and CPR6 and from seeds only one isolate was obtained which was named as CPS1 and recorded in Table 2.

Table1: Population of endophytic bacteria in different parts of Chickpea

Plant part	Population count (No. $\times10^5$CFU/g)
Nodules	5.2
Roots	3.96
Seeds	2.8
Mesorhizobium from nodules	1.8

Table2: Naming of endophytic bacterial isolates

Plant part	Isolates
Nodules	CPN1
	CPN2
	CPN3
	CPN4
	CPN5
	CPN6
	CPN7
	CPN8
	CPN9
Root	CPR1
	CPR2
	CPR3
	CPR4
	CPR5
	CPR6
Seed	CPS1

4.4 The cultural characteristics of the endophytic bacterial isolates

The cultural characters of the isolates on broth and media in the form of surface growth, clouding of broth and colony characters on agar media were studied and presented in table 3.

There was no surface growth in CPN3, CPN4, CPN5, CPN6, CPN7, CPN8, CPR1, CPR2, CPR4, CPS1 and pellicle growth was observed in the isolates CPR3, CPR5, CPR6, CPN1 is none and CPN2 was ring.

The broth cloudiness is heavy in the isolates CPN4, CPN5, CPN8, CPR4, CPR6 and slight growth was seen in the isolates CPN2, CPN3, CPR1, CPR3, CPR5 and CPS1 and no clouding was observed in the isolates CPN1, CPN6, CPN7 and CPR2.

Observation recorded on the colonies characters are CPN1 was showed dirty powdery dotted colonies, CPN2 and CPN5 were showed dirty white slimy glistening colonies, CPN3 white flat wrinkeled colonies and CPN7 yellow raised colonies, CPR5 was pinkish white slimy colonies and CPN4, CPN6, CPN8, CPR2, CPR3, CPR4, CPR6 and CPS1 were white dotted colonies.

4.5 Microscopic characteristics of the endophytic bacterial isolates

The isolates were microscopically examined and observations are presented in the Table 4.

Seven isolates were Gram negative CPN3, CPN4, CPN7, CPR1, CPR2, CPR5, CPR6, CPS1 and eight isolates were Gram positive CPN1, CPN2, CPN5, CPN6, CPN8, CPR3, CPR4 and shape of the isolates were rod shaped CPN1, CPN2, CPN3, CPN4, CPN5, CPN6, CPN7, CPN8, CPR2, CPR3, CPR4, CPR6, CPS1 were Cocci and Bacilli CPR, CPR5 respectively. Seven isolates were endospore formers and eight isolates were non endospore formers and nine isolates were motile and six isolates were non motile.

Table3: Morphological Characteristics of endophytic bacterial isolates

Isolates	Cultures characters on broth		Colony characters in agar media
	Surface growth	Clouding of broth	
CPN1	None	No	White powdery dotted colonies
CPN2	Ring	Slight	Dirty white slimy glister colonies
CPN3	No	Slight	White flat wrinkled colonies
CPN4	No	Heavy	White dotted colonies
CPN5	No	Heavy	Dirty white glistening colonies
CPN6	No	No	White dotted small colonies
CPN7	No	No	Yellow raised glistening colonies
CPN8	No	Heavy	White dotted colonies
CPR1	No	Slight	White dotted colonies
CPR2	No	No	White dotted colonies
CPR3	Pellicle	Slight	White dotted raised colonies
CPR4	No	Heavy	White dotted raised colonies
CPR5	Pellicle	Slight	Pinkish white slimy colonies
CPR6	Pellicle	Heavy	White dotted colonies
CPS1	No	Heavy	White dotted colonies

Table 4: Microscopic characteristics of the endophytic bacterial isolates

Plant part	Isolates	Gram reaction	Cell shape	Motility	Endospore formation
Nodules	CPN1	+	Rod	+	+
	CPN2	+	Rod	+	+
	CPN3	-	Short rod	-	+
	CPN4	-	Rod	+	-
	CPN5	+	Rod	+	-
	CPN6	+	Rod	+	+
	CPN7	-	Rod	+	-
	CPN8	+	Rod	+	-
Root	CPR1	-	Cocci	-	+
	CPR2	-	Rod	+	-
	CPR3	+	Short rod	-	+
	CPR4	+	Short rod	-	-
	CPR5	-	Bacilli	-	-
	CPR6	-	Rod	-	+
Seed	CPS1	-	Rod	+	-

4.6 Biochemical characteristics of the endophytic bacterial isolates

For further characterization of the isolates biochemical tests were carried out and the results are presented in the Table 5 and Table 6.

All the isolates were shown positive for Phosphate solubilization, Casein production, Catalase test, Citrate utilization and MR tests. All isolates showed negative reaction for indole production test and VP test. Nine isolates are positive for oxide test. CPN7 and CPN8 showed negative reaction for Hydrogen sulfide production test, CPN1, CPN2, CPN6. CPN7, CPN8, CPR1, CPR2, CPR3, CPR5, CPR6 showed negative reaction for amylase production test, CPN1, CPN2, CPN3, CPN7,CPR2, CPR3, CPR4, CRP5, CPS1 showed negative reaction for urease test, CPN5, CPN6, CPN7, CPR3, CPS1 are positive for gelatin utilization test.

4.7 Confirmation test for *Mesorhizobium*

Mesorhizobium isolates were white glittery colonies, rod shaped, showed Gram negative reaction, positive for motility, negative for endospore formation. The confirmatory test for *Mesorhizobium* was done which showed negative reaction for the confirmatory tests, Hoffer's alkaline test, Glucose peptone test, Lactose broth.

4.8 Compatibility test of endophytic bacteria and *Mesorhizobium*

The isolates CPN4, CPR2, CPS1 were compatible with each other and with *Mesorhizobium*UASB-835. These isolates were selected for further molecular level identification and studies in green house condition (Plate 1).

4.9 16S rRNA molecular identification of endophytic bacteria.

From 16S rRNA sequencing the endophytic bacteria were identified as *Enterobacter hormaechei*, Gamma *Proteobacter*, *Enterobacter sp* for CPN4, CPR2 and CPS1 respectively are presented in the Table 7.

Table 5: Biochemical characteristics of the endophytic bacterial isolates

Plant part	Isolates	Oxide test	Indole Production	Hydrogen sulfide production	Catalase test	Citrate utilization	MR test	VP Test
Nodules	CPN1	+	-	+	+	+	+	-
	CPN2	+	-	+	+	+	+	-
	CPN3	+	-	+	+	+	+	-
	CPN4	-	-	+	+	+	+	-
	CPN5	+	-	+	+	+	+	-
	CPN6	-	-	+	+	+	+	-
	CPN7	-	-	-	+	+	+	-
	CPN8	-	-	-	+	+	+	-
Root	CPR1	+	-	+	+	+	+	-
	CPR2	-	-	+	+	+	+	-
	CPR3	-	-	+	+	+	+	-
	CPR4	+	-	+	+	+	+	-
	CPR5	-	-	+	+	+	+	-
	CPR6	+	-	+	+	+	+	-
Seed	CPS1	-	-	+	+	+	+	-

Table 6: Biochemical characteristics of the endophytic bacterial isolates

Plant part	Isolates	Amylase production	Urease test	Phosphate solubilization	Gelatin utilization	Casein production
Nodules	CPN1	-	+	+	-	+
	CPN2	-	+	+	-	+
	CPN3	+	+	+	-	+
	CPN4	+	-	+	-	+
	CPN5	+	-	+	+	+
	CPN6	-	-	+	+	+
	CPN7	-	+	+	+	+
	CPN8	-	-	+	-	+
Root	CPR1	-	-	+	-	+
	CPR2	+	+	+	-	+
	CPR3	-	+	+	-	+
	CPR4	+	+	+	+	+
	CPR5	-	+	+	-	+
	CPR6	-	-	+	-	+
Seed	CPS1	+	+	+	+	+

Table 7: Molecular level identification of endophytic bacteria

CPN4	*Enterobacterhormaechei*
CPR2	Gamma *Proteobacteria*
CPS1	*Enterobacter sp.*

4.9.1.1 16s rRNA sequencing of endophytic bacterial isolates (Zheng *et al,*2000)

4.9.1.1.1 Sample CPN-4:

>150518-27_A03_1_785F.ab1 706

GGGGCGTAGAATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCG

GAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA

ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA

ATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTT

TCCAGAGATGGTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGG

CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCG

CAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGAC

TGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGC

CCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAG

CGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATT

GGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGAT

CAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA

CACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGG

GCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGACAGGCAACCC

CAAAAG

4.9.1.1.2Sample CPR-2:

>150518-27_E05_02_785F.ab1 700

GCCTCGATGCCACTGTCTGTTGGGGGCTTTTGGCTTGGGTATCGAAGCTA

ACGCGTTAAGTTCCGCGCCTGGGGAGCGGTGTCGAGATACTGAATCTCAA

GGGATTTCGGGGGGGCCCGCACACGGGGGGGAATGTGGGTTTTATTTCTA

TGCCCCGCAAAAAACTTTCCCTGCCTTTGACGTGTGGAAAACTTTCCAAA

TTTGGTTGGGTGCCTTCGAGAACTCGAACACAGGTGCTGCATGGCTGTCG

TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC

TTGTCCTTAGTTGCCAGCAAAGAATGGGGGGAACTCACTGGAGACCACCG

GTGACAAAACGGTGGAAGGGGGGGATGACCTCATGGCCCTTGGCCCTTAC

GGTTCGGGCTACACACGTACTACAGTGGTAAGGACAGAGGGCTGCCAACG

GGGAACCGAACCCCAATCCCAAAAACCCTATCTGATTCCAGATTGGAATT

CGACTCTCTACTCTATGAATCCCTAATCATTACTAATCGCATATCAGCGT

TGCAGAGGTTAATAGGTTCTGGGGCCTTGTCCCCTCCCCCCGTCACACTG

TGGTAGTTTGTAGCACCATAAGCTAGCCGCTTAGCCTTCGGGAGGCCGCT

TGCCATTCAGTACTGGATTGCTGTGGTAAATGTAACGGAAAAACCCAAAA

4.9.1.1.3 Sample CPS-1:

>150518-27_E03_2_785F.ab1 706

TGGCATTGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGA

GCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAAC

TCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT

TCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAC

CAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCT

GTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCA

ACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTG

CCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCC

TTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCG

ACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGG

AGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCA

GAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACA

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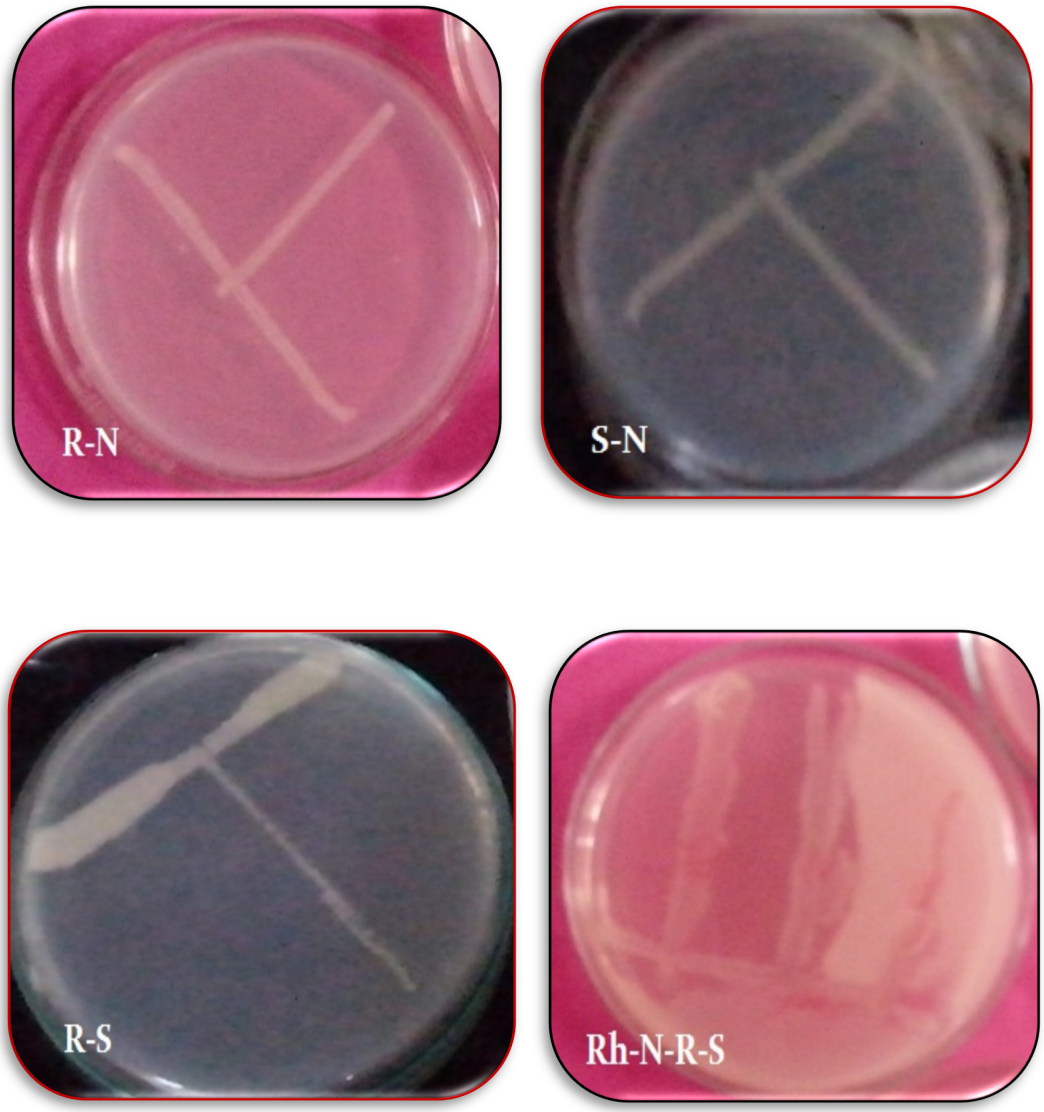
CGTAAA



R- Root endophytic bacteria (CPR2)

S- Seed endophytic bacteria (CPS1)

N-Nodule endophytic bacteria (CPN4)

Rh- *Mesorhizobium* UASB-835

Plate 1: Compatibility test of endophytic bacteria and *Mesorhizobium*

4.10 Synergistic effect of endophytic bacteria and *Mesorhizobium* on Chickpea

The synergistic effect of four isolates CPN6, CPR3, CPS1 and *Mesorhizobium* UASB-835 in combination of different treatments was studied in green house and experiment and results are reported here.

4.10.1 Synergistic effect of endophytic bacteria and *Mesorhizobium* on plant height of Chickpea at different intervals

The observation recorded on 30 DAS, 60 DAS, at harvest on plant height of Chickpea is presented in the Table 8 and Fig. 1.

On the 30 days after sowing, the highest plant height was recorded in T13 (12.66 cm) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by treatment T6 (11.6 cm) Root endophytic bacteria +*Mesorhizobium* and treatment T7 (11 cm) Nodule endophytic bacteria + *Mesorhizobium* and treatment T8 (11 cm) Seed endophytic bacteria + *Mesorhizobium* least plant height was recorded in T1 (6.77 cm) Control.

At 60 days after sowing highest plant height was noted in T13 (22.7 cm) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (21.33 cm) Root endophytic bacteria +*Mesorhizobium* and T8 (19 cm) Seed endophytic bacteria + *Mesorhizobium* and least plant height was recorded in T1 (12 cm) control .

At harvest the maximum plant height was observed in T13 (38 cm) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (36.33 cm) Root endophytic bacteria +*Mesorhizobium* and T8 (36 cm) Seed endophytic bacteria + *Mesorhizobium* and least plant height was recorded in T1 (20 cm) control.

4.10.2 Bacterial population at different interval

The observation recorded at 30 days, 60 days and at harvest on bacterial population is presented in Table 9 and Fig. 2.

At 30 days after sowing the maximum bacterial population was recorded in T13 (55.33×10^5 CFU/g) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (53.3×10^5 CFU/g) Root endophytic bacteria +*Mesorhizobium* and T8 (41.33×10^5 CFU/g) Seed endophytic bacteria + *Mesorhizobium* least bacterial population was recorded in T1 (29.33×10^5 CFU/g) Control.

At 60 days after sowing the maximum bacterial population was recorded in T13 (55×10^5 CFU/g) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (53×10^5 CFU/g) Root endophytic bacteria + *Mesorhizobium* and least bacterial population was recorded in T1 (28×10^5 CFU/g) control.

Table 8: Synergistic effect of endophytic bacteria and *Mesorhizobium* on plant height of Chickpea at different intervals

Treatments	Plant height (cm)		
	30DAS	60DAS	At harvest 80 DAS
T_1: Control	6.77	12.00	20.00
T_2: *Mesorhizobium*	10.00	21.00	33.00
T_3: Nodule endophytic bacteria	7.22	15.26	22.00
T_4: Seed endophytic bacteria	8.33	15.00	24.33
T_5: Root endophytic bacteria	8.6	15.50	26.00
T_6: Root endophytic bacteria +*Mesorhizobium*	11.6	21.33	36.33
T_7: Nodule endophytic bacteria + *Mesorhizobium*	11.00	18.67	35.00
T_8: Seed endophytic bacteria + *Mesorhizobium*	11.00	19.00	36.00
T_9:Root endophytic bacteria + nodule endophytic bacteria	9.50	16.00	30.00
T_{10}: Root endophytic bacteria + seed endophytic bacteria	10.20	16.00	32.00
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	10.00	15.00	32.30
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	10.50	15.50	33.00
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	12.66	22.17	38.00
SEM	0.413	0.712	0.400
CD at 5 %	1.218	2.095	1.162

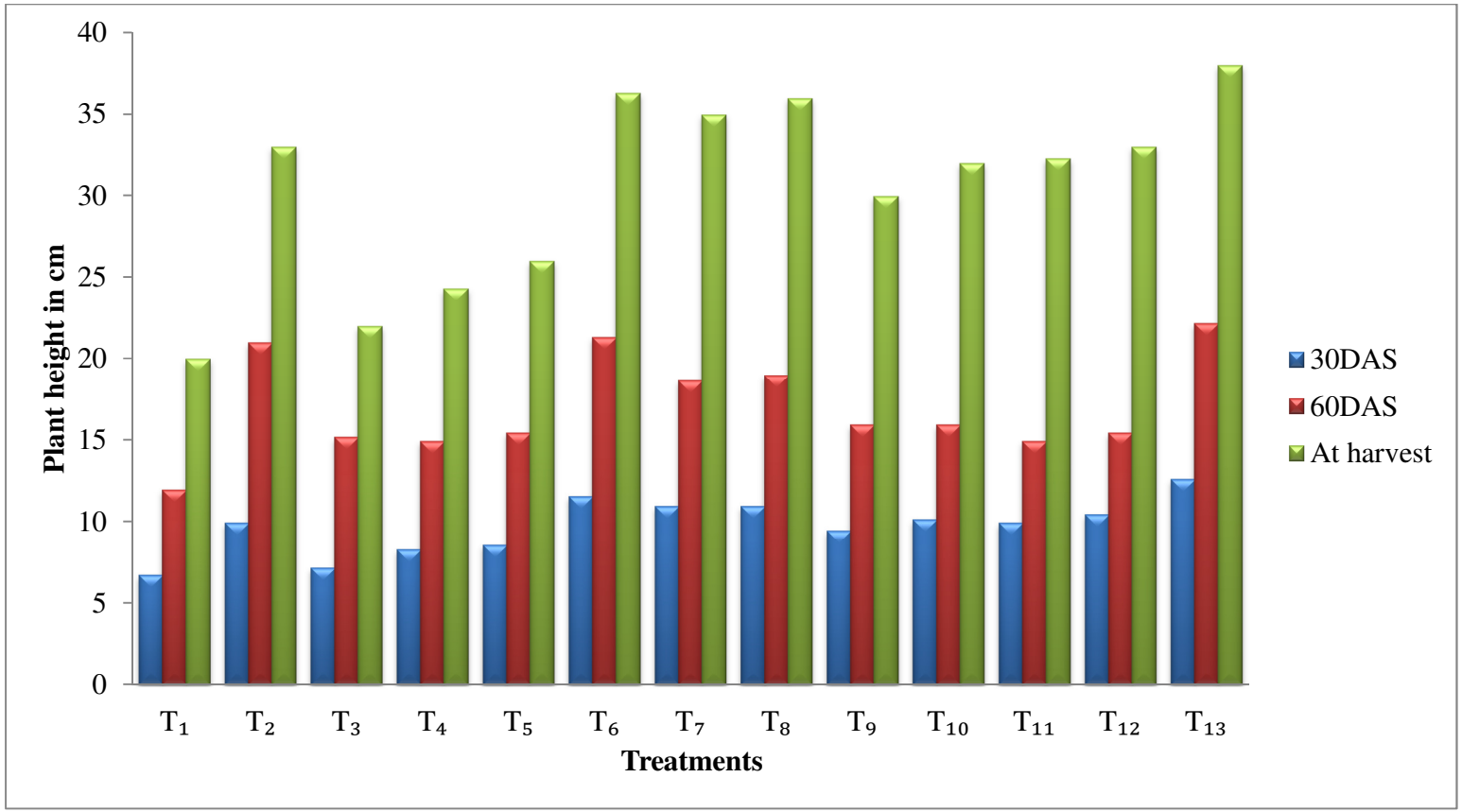


Fig.1: Synergistic effect of endophytic bacteria and *Mesorhizobium* on plant height of Chickpea at different intervals

At the harvest the maximum bacterial population was recorded in T13 (87 $\times 10^5$ CFU/g) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (80 $\times$ 10^5 CFU/g) Root endophytic bacteria +*Mesorhizobium* and least bacterial population was recorded in T1 (16 $\times$ 10^5 CFU/g) control.

4.10.3 Synergistic effect of endophytic bacteria and *Mesorhizobium* on flowering per cent of Chickpea

The results obtained by synergistic effect of endophytic bacteria and *Mesorhizobium* in different treatment combination on flowering percentage was recorded in the Table 10 and Fig. 3.

The average flowering percentage of the treatments varied from 88 per cent to 35.27 per cent. Highest flowering percentage was recorded in the treatment T13 (88%) involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by the treatment T6 (78.67%) Root endophytic bacteria + *Mesorhizobium* and T8 (71.67%) Seed endophytic bacteria + *Mesorhizobium* and lowest flowering percentage was recorded in T1 (35.27%) that is control.

Table 9: Bacterial population in the Chickpea rhizosphere at different interval

Treatments	Population count No. $\times 10^5$CFU/g soil		
	30 DAS	60 DAS	At harvest 80 DAS
T_1: Control	29.33	28	16
T_2: *Mesorhizobium*	35.33	36	53
T_3: Nodule endophytic bacteria	30	30.67	21.67
T_4: Seed endophytic bacteria	41.33	35.33	38.67
T_5: Root endophytic bacteria	30.67	30	40
T_6: Root endophytic bacteria +*Mesorhizobium*	53.33	53	80
T_7: Nodule endophytic bacteria + *Mesorhizobium*	39.33	39.33	62
T_8: Seed endophytic bacteria + *Mesorhizobium*	41.33	41.66	77.33
T_9:Root endophytic bacteria + nodule endophytic bacteria	36.33	36.33	38
T_{10}: Root endophytic bacteria + seed endophytic bacteria	36	31.33	44.67
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	37.67	36	43.33
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	38	37.67	53.33
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	55.33	55	87
SEM	4.280	4.200	6.282
CD at 5 %	12.441	12.532	18.261

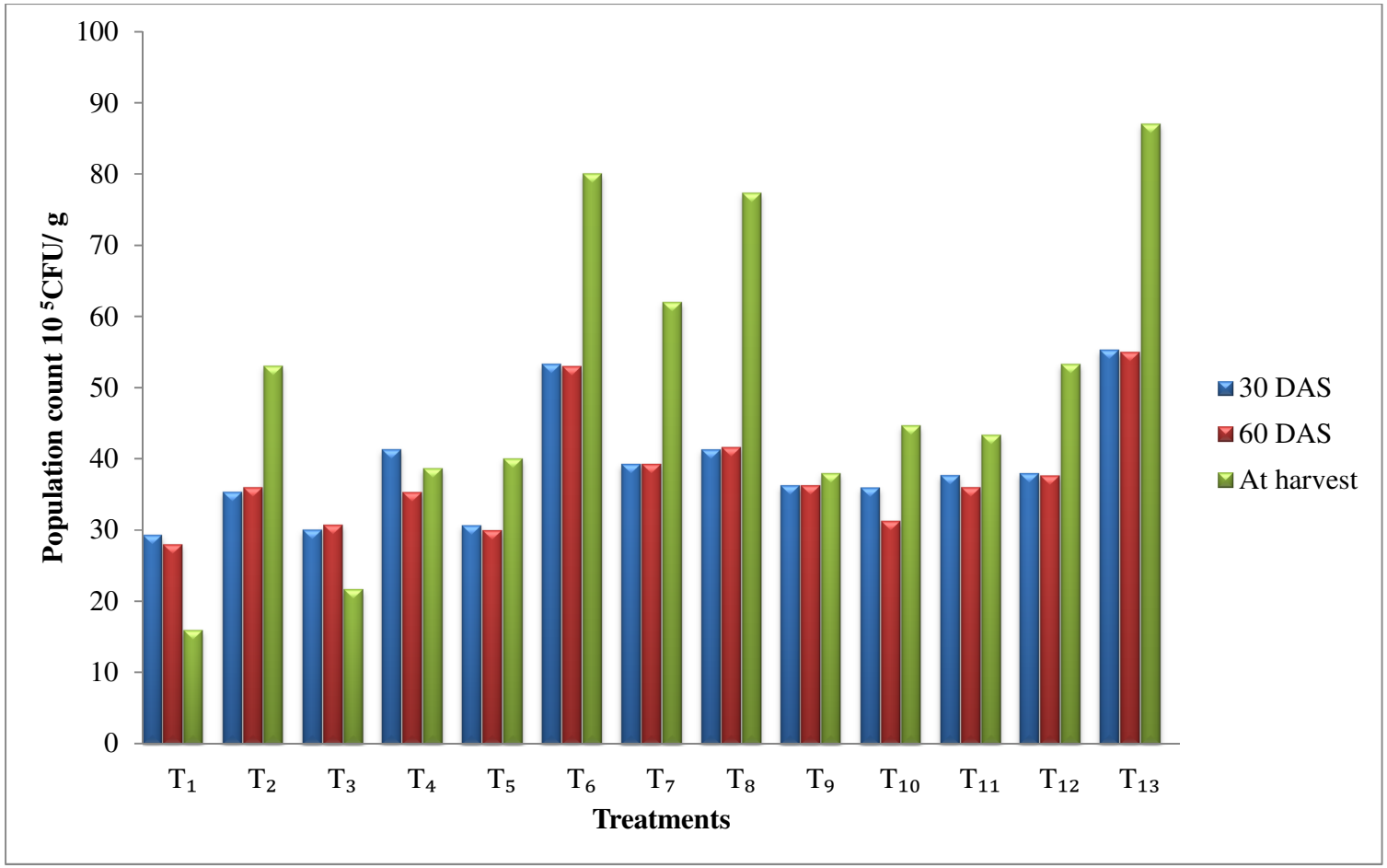


Fig. 2:Microbial population in the Chickpea rhizosphere at different intervals

Table 10: Synergistic effect of endophytic bacteria and *Mesorhizobium* on flowering percentage of Chickpea.

Treatments	Flowering %
T_1: Control	35.27
T_2: *Mesorhizobium*	71.33
T_3: Nodule endophytic bacteria	36.67
T_4: Seed endophytic bacteria	38.33
T_5: Root endophytic bacteria	36.67
T_6: Root endophytic bacteria +*Mesorhizobium*	78.67
T_7: Nodule endophytic bacteria + *Mesorhizobium*	73.33
T_8: Seed endophytic bacteria + *Mesorhizobium*	71.67
T_9:Root endophytic bacteria + nodule endophytic bacteria	68.33
T_{10}: Root endophytic bacteria + seed endophytic bacteria	68.33
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	68
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	66.67
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	88.00
SEM	2.491
CD at 5 %	7.214

4.10.4 Synergistic effect of endophytic bacteria and *Mesorhizobium* on nodule number and nodule dry weight of Chickpea.

The observation noted on the influence of endophytic bacteria and *Mesorhizobium* on nodule number and their dry weight of Chickpea plants are presented in the Table 11, Fig. 4 and Fig. 5.

The highest number of nodules was recorded highest in treatment T13 (38) Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* followed by T6 (36.33) Root endophytic bacteria +*Mesorhizobium* is on par with T2 *Mesorhizobium*, T6 Root endophytic bacteria +*Mesorhizobium*andT7 Nodule endophytic bacteria + *Mesorhizobium,* T8 Seed endophytic bacteria + *Mesorhizobiu*and T9 Root endophytic bacteria + nodule endophytic bacteria and T10 Root endophytic bacteria + seed endophytic bacteria and T11 Root endophytic bacteria + seed endophytic bacteria and T12 Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria and least number of nodules recorded in T1 (20) control.

The highest nodule dry weight was recorded in the treatment T13 treatment having (37.33 mg) Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* followed by T6 (34.87 mg) Root endophytic bacteria +*Mesorhizobium* and T8 (35.27 mg) Seed endophytic bacteria + *Mesorhizobium* lowest nodule weight was recorded in T1 (25.3 mg) control.

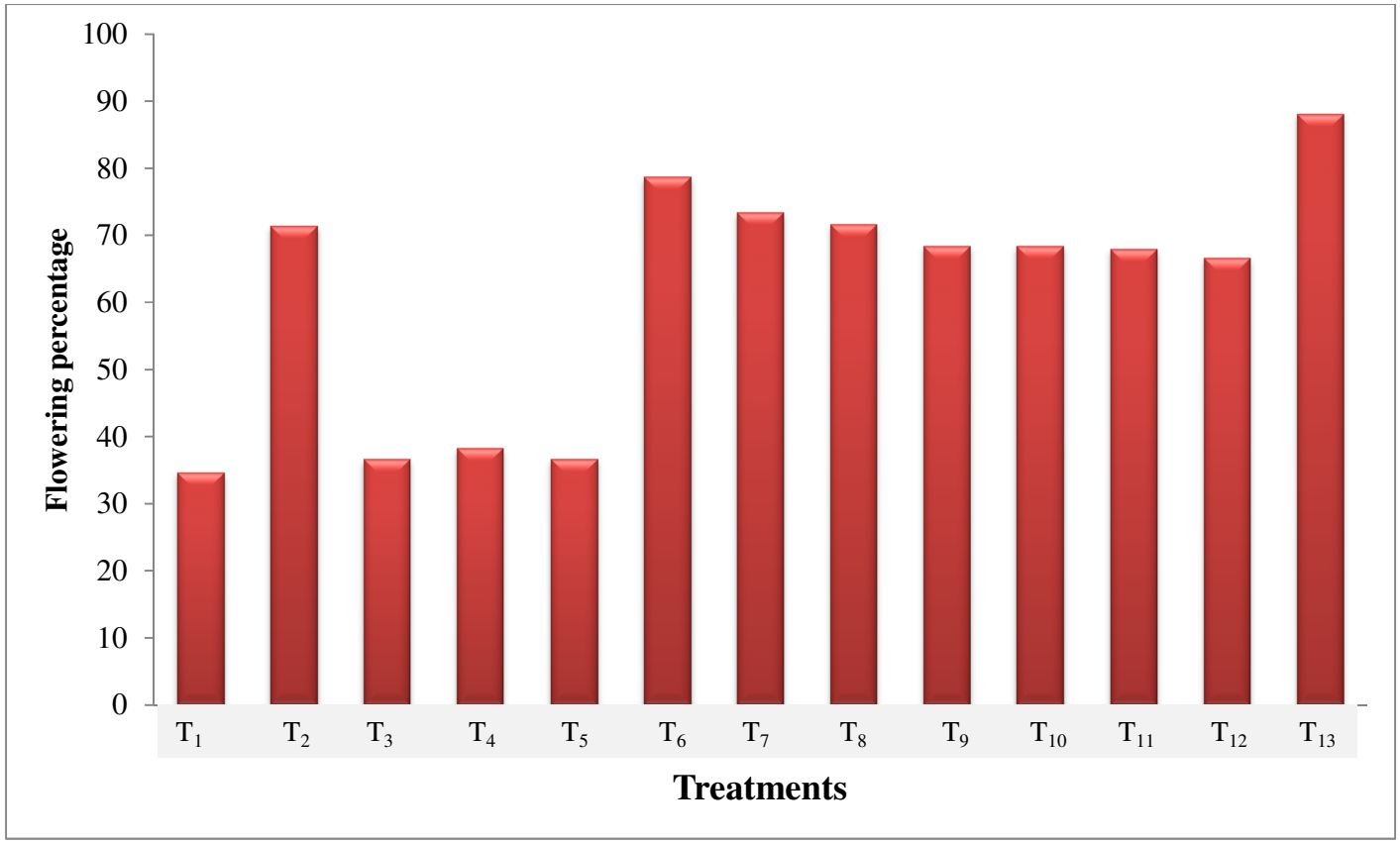


Fig. 3 :Synergistic effect of endophytic bacteria and *Mesorhizobium* on flowering percentage of Chickpea

Table11: Synergistic effect of endophytic bacteria and *Mesorhizobium* on number of nodules and nodule dry weight of Chickpea.

Treatments	No. of nodules/plant	Dry weight of nodules (mg)/plant
T_1: Control	20.00	25.33
T_2: *Mesorhizobium*	33.00	30.33
T_3: Nodule endophytic bacteria	22.00	26.67
T_4: Seed endophytic bacteria	24.00	28.67
T_5: Root endophytic bacteria	26.00	28.67
T_6: Root endophytic bacteria +*Mesorhizobium*	36.00	34.87
T_7: Nodule endophytic bacteria + *Mesorhizobium*	36.00	35.33
T_8: Seed endophytic bacteria + *Mesorhizobium*	35.00	35.27
T_9:Root endophytic bacteria + nodule endophytic bacteria	30.00	30.67
T_{10}: Root endophytic bacteria + seed endophytic bacteria	32.00	30.40
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	32.30	31.21
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	33.00	31.53
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	38.00	37.33
SEM	3.387	0.670
CD at 5 %	9.845	1.947

4.10.5 Synergistic effect of endophytic bacteria and *Mesorhizobium* on fresh and dry weight of Chickpea roots.

The observations recorded on the effect of endophytic bacteria and *Mesorhizobium* on root dry weight and fresh weight is presented in Table 12 and Fig. 6.

The fresh weight of root was recorded highest in the treatment T13 (2.67 g) having Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 (2.2 g) with Root endophytic bacteria + *Mesorhizobium* and T8 (1.8 g) Seed endophytic bacteria + *Mesorhizobium* and lowest fresh weight of root was recorded in T1 (0.87 g) control.

The dry weight of root was highest in the treatment T13 (1.31 g) involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by treatment T6 (1.12 g) Root endophytic bacteria + *Mesorhizobium* and treatment T8 (0.71 g) having Seed endophytic bacteria + *Mesorhizobium* and the lowest dry weight was recorded in T1 (0.31 g) control .

4.10.6 Synergistic effect of endophytic bacteria and *Mesorhizobium* on shoot fresh and dry weight of Chickpea.

The shoot fresh and dry weights are presented in Table13 and Fig. 7.

The fresh weight of shoot was highest in the treatment T13 involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* (21 g) followed by T6 with Root endophytic bacteria +*Mesorhizobium* (20.67 g) and T8 Seed endophytic bacteria + *Mesorhizobium* (20.33 g) lowest shoot weight was recorded in T1 control (12.57 g).

The dry weight of root was highest in the treatment T13 involving Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (6.71 g) followed by T6 Root endophytic bacteria +*Mesorhizobium* (5.75 g) and T8 Seed endophytic bacteria + *Mesorhizobium* (4.8 g) and the lowest shoot dry weight was recorded in T1 (3.13 g) control.

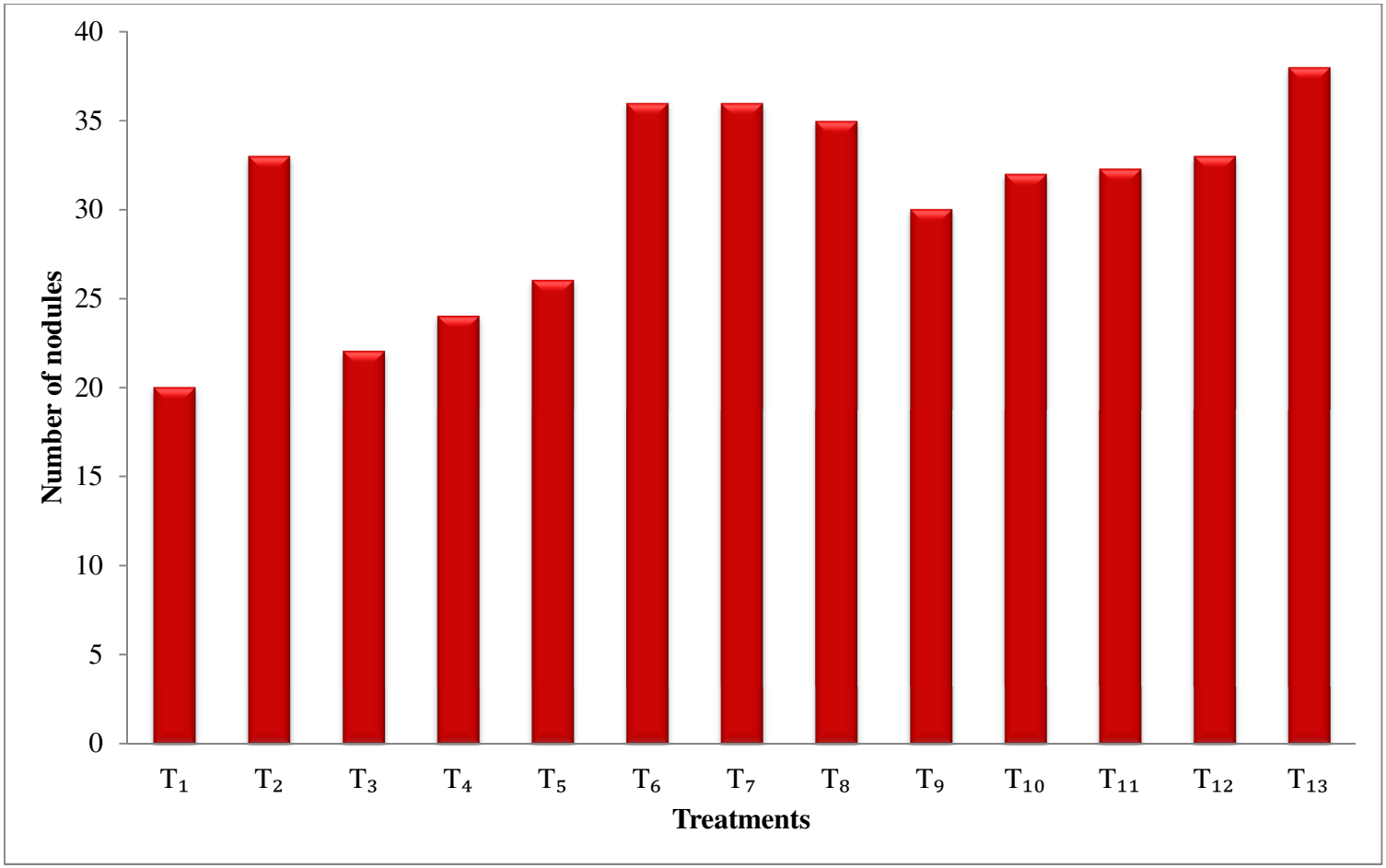


Fig.4 : Synergistic effect of endophytic bacteria and *Mesorhizobium* on nodule number of Chickpea

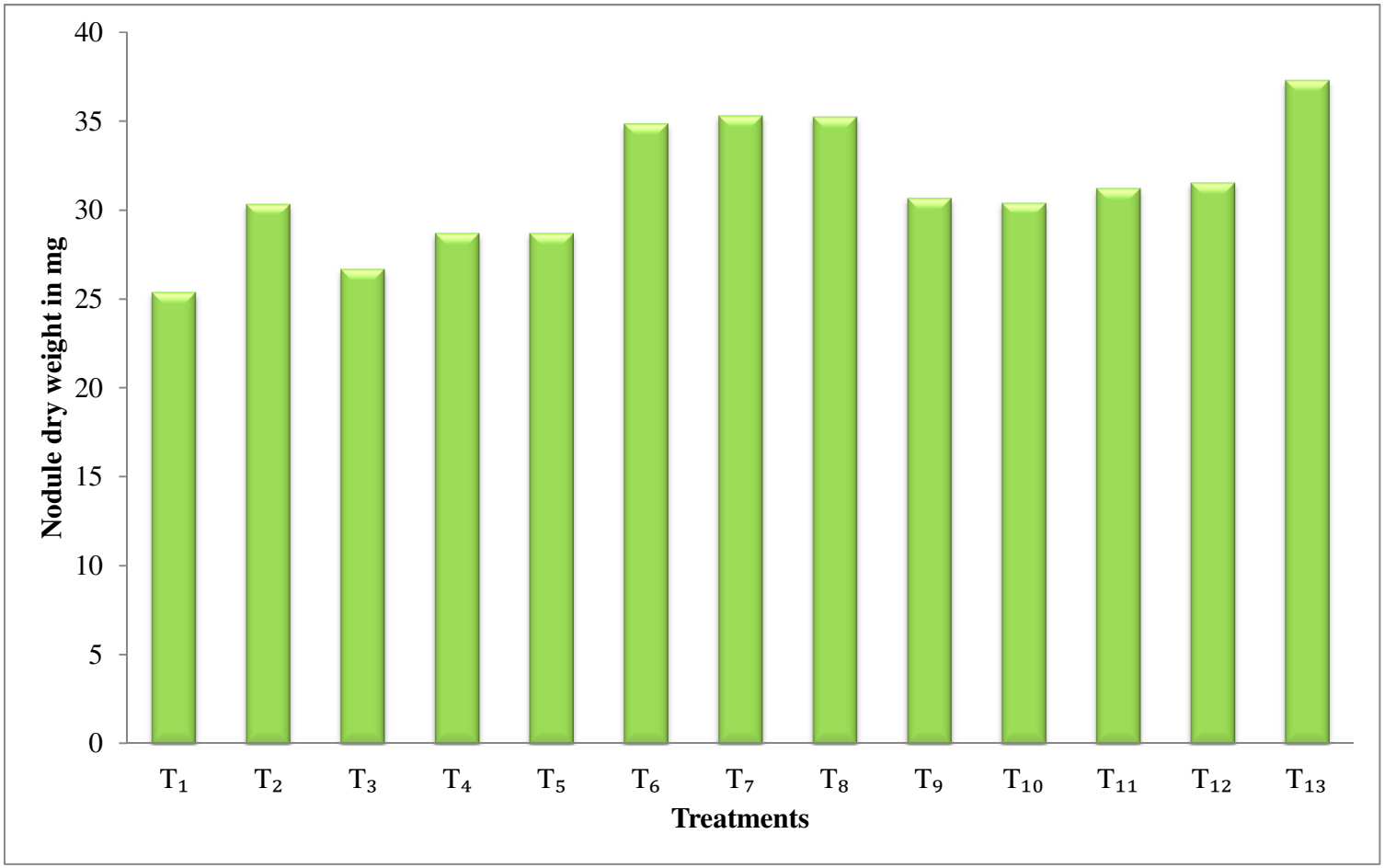


Fig. 5: Synergistic effect of endophytic bacteria and *Mesorhizobium* on nodule dry weight of Chickpea

Table 12: Synergistic effect of endophytic bacteria and *Mesorhizobium* on root fresh and dry weight of Chickpea.

Treatments	**Fresh wt (g)**	**Dry wt (g)**
T_1: Control	0.87	0.31
T_2: *Mesorhizobium*	1.46	0.98
T_3: Nodule endophytic bacteria	0.89	0.43
T_4: Seed endophytic bacteria	1.2	0.43
T_5: Root endophytic bacteria	0.97	0.47
T_6: Root endophytic bacteria +*Mesorhizobium*	2.20	1.12
T_7: Nodule endophytic bacteria + *Mesorhizobium*	1.33	0.67
T_8: Seed endophytic bacteria + *Mesorhizobium*	1.80	0.71
T_9:Root endophytic bacteria + nodule endophytic bacteria	1.28	0.47
T_{10}: Root endophytic bacteria + seed endophytic bacteria	1.14	0.47
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	1.42	0.6
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	1.42	0.63
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	2.67	1.31
SEM	0.190	0.149
CD at 5 %	0.551	0.434

Table13: Synergistic effect of endophytic bacteria and *Mesorhizobium* on shoot fresh weight and dry weight of Chickpea

Treatments	**Fresh wt(g)**	**Dry wt (g)**
T_1: Control	12.57	3.13
T_2: *Mesorhizobium*	13.51	4.0
T_3: Nodule endophytic bacteria	12.77	3.2
T_4: Seed endophytic bacteria	13.08	3.51
T_5: Root endophytic bacteria	15.2	3.34
T_6: Root endophytic bacteria +*Mesorhizobium*	20.67	5.75
T_7: Nodule endophytic bacteria + *Mesorhizobium*	19.97	4.35
T_8: Seed endophytic bacteria + *Mesorhizobium*	20.33	4.8
T_9:Root endophytic bacteria + nodule endophytic bacteria	15.4	4.43
T_{10}: Root endophytic bacteria + seed endophytic bacteria	15.3	3.71
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	15.2	4.21
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	15.77	4.43
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	21.00	6.71
SEM	1.689	0.511
CD at 5 %	4.910	1.486

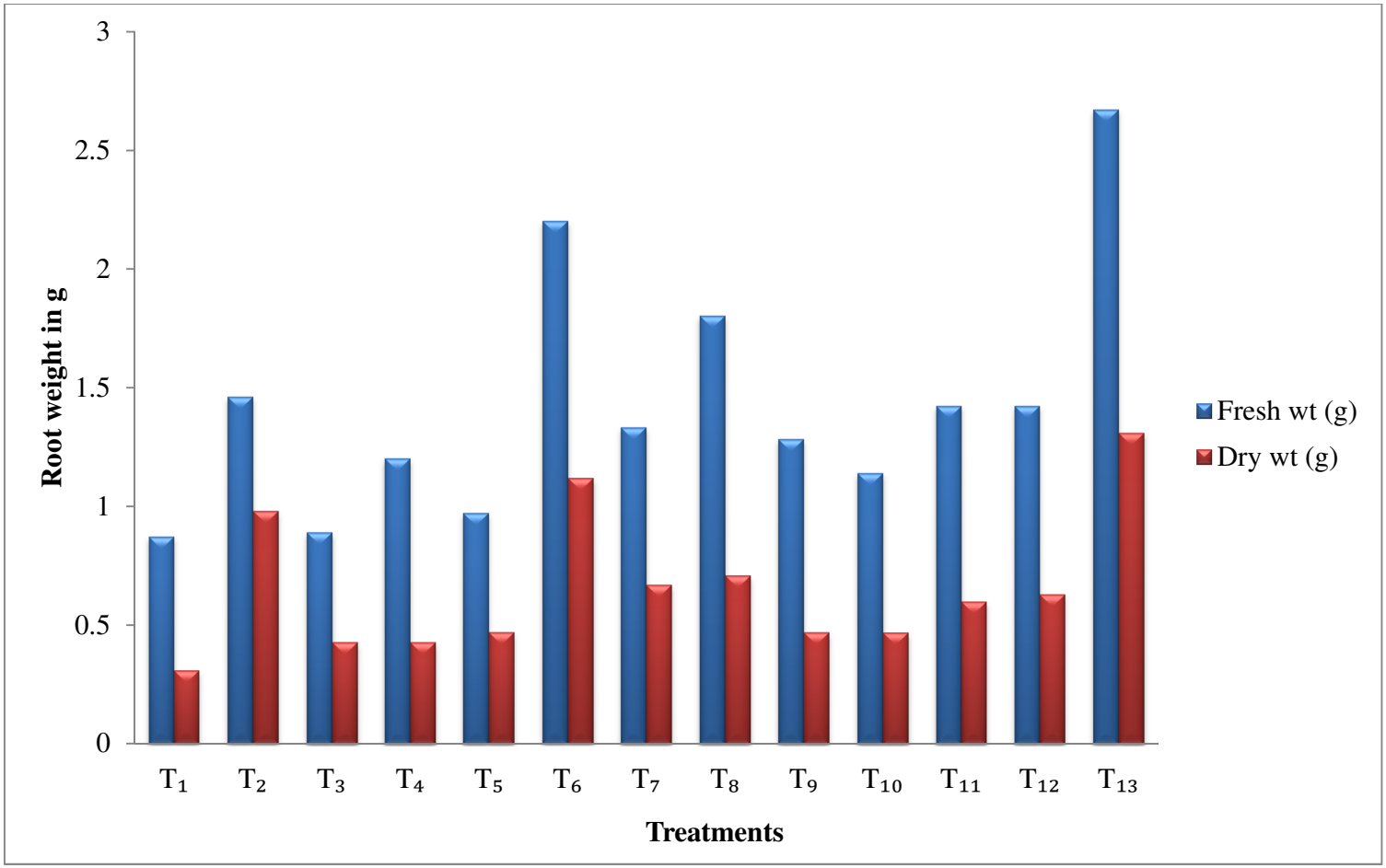


Fig. 6: Synergistic effect of endophytic bacteria and *Mesorhizobium* on root fresh wet and dry weight of Chickpea

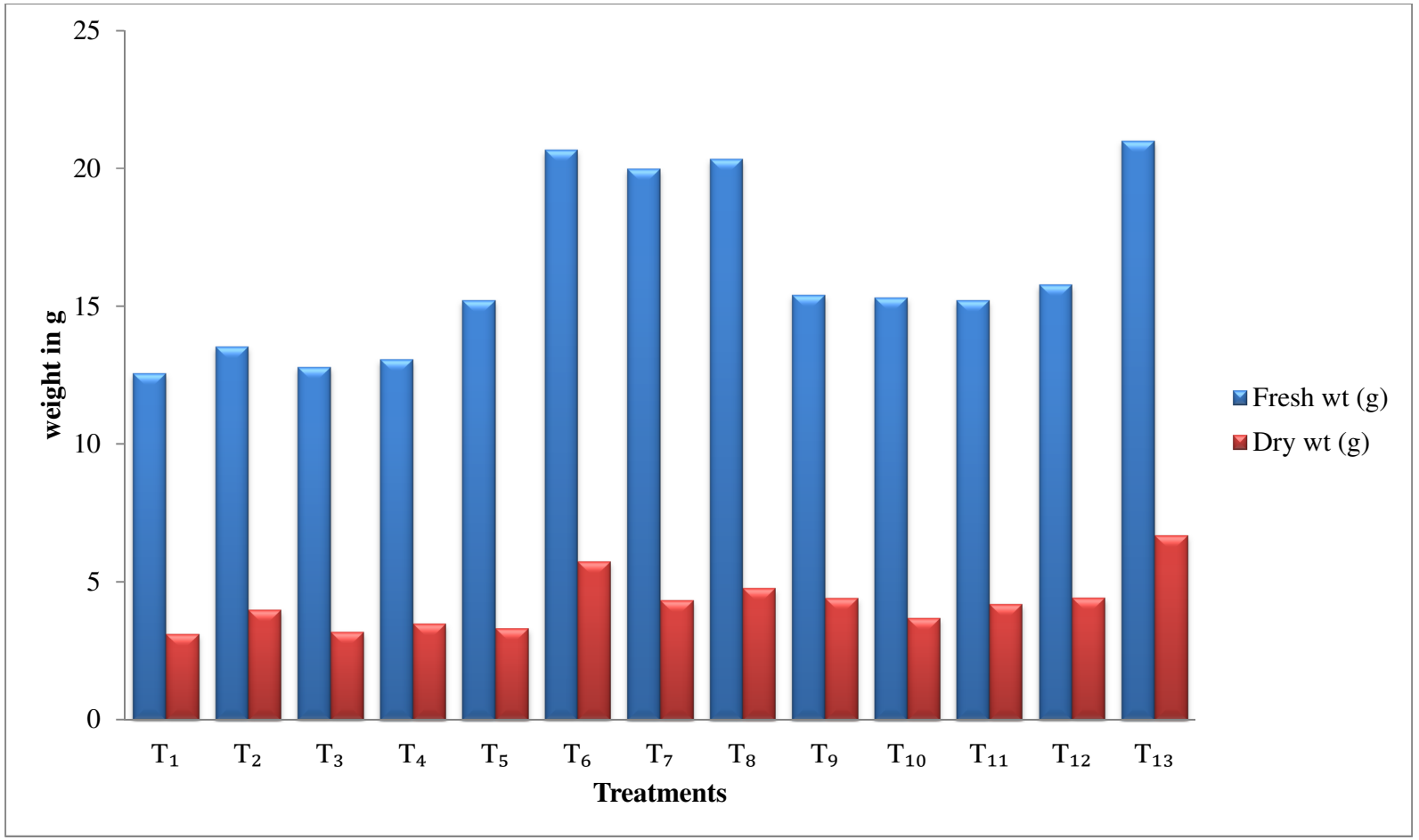


Fig. 7: Synergistic effect of endophytic bacteria and *Mesorhizobium* on shoot fresh weight and dry weight of Chickpea

4.10.7 Synergistic effect of endophytic bacteria and *Mesorhizobium* on root length of Chickpea

The observation taken on the influence of endophytic bacteria and *Mesorhizobium* on root length is presented in Table 14 and Fig. 8.

The root length was recorded highest (15.33 cm) in the treatment in T13 involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* followed by T6 with Root endophytic bacteria +*Mesorhizobium* these two treatments were on par with T8 Seed endophytic bacteria + *Mesorhizobium* and T12 Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria. Lowest root length was recorded in T1 control followed by T3 Nodule endophytic bacteria where these treatments are on par with T4 Seed endophytic bacteria and T5 Root endophytic bacteria + *Mesorhizobium*.

4.10.8 Synergistic effect of endophytic bacteria and *Mesorhizobium* on nitrogen content and biomass Chickpea

The observation recorded on the influence of endophytic bacteria and *Mesorhizobium* on plant nitrogen content and biomass is presented in Table 15, Fig 9 and 10.

The highest plant nitrogen content was recorded in treatment T13 (2.33%) involving Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* followed by T6 (2.1%) Root endophytic bacteria +*Mesorhizobium* is on par with T2 *Mesorhizobium* , T7 Nodule endophytic bacteria + *Mesorhizobium*, T8 Seed endophytic bacteria + *Mesorhizobium*, T12 Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria and T8 (2.04%) lowest was recorded in T1 (0.83%) control.

The highest plant biomass was recorded in the treatment T13 (8.02 g) with Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* followed by T6 (6.87 g) with Root endophytic bacteria +*Mesorhizobium* and T8 (5.51 g) Seed endophytic bacteria + *Mesorhizobium* lowest was recorded in T1 (3.44 g) control.

Table 14: Synergistic effect of endophytic bacteria and *Mesorhizobium* on root length of Chickpea

Treatments	**Root length(cm)**
T_1: Control	7.33
T_2: *Mesorhizobium*	10.67
T_3: Nodule endophytic bacteria	8.00
T_4: Seed endophytic bacteria	8.33
T_5: Root endophytic bacteria	8.33
T_6: Root endophytic bacteria +*Mesorhizobium*	15.27
T_7: Nodule endophytic bacteria + *Mesorhizobium*	12.27
T_8: Seed endophytic bacteria + *Mesorhizobium*	13
T_9:Root endophytic bacteria + nodule endophytic bacteria	11.33
T_{10}: Root endophytic bacteria + seed endophytic bacteria	11.00
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	12.00
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	12.67
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	15.33
SEM	0.974
CD at 5 %	2.83

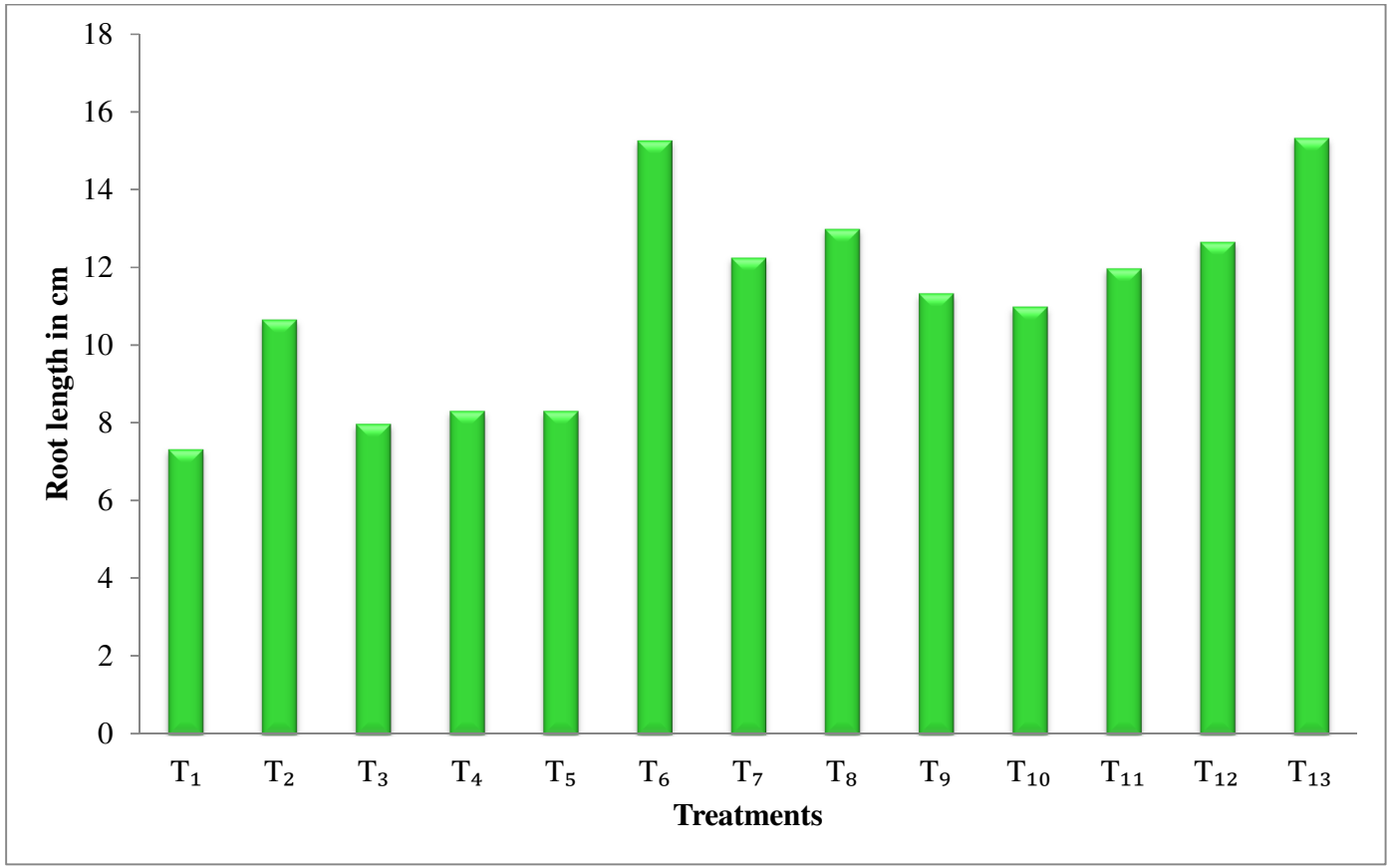


Fig. 8: Synergistic effect of endophytic bacteria and *Mesorhizobium* on root length of Chickpea

Table15: Synergistic effect of endophytic bacteria and *Mesorhizobium* on nitrogen content and plant biomass of Chickpea.

Treatments	% N content	Plant biomass (g)
T_1: Control	0.83	3.44
T_2: *Mesorhizobium*	1.87	4.98
T_3: Nodule endophytic bacteria	1.23	3.63
T_4: Seed endophytic bacteria	1.25	5.24
T_5: Root endophytic bacteria	1.15	3.81
T_6: Root endophytic bacteria +*Mesorhizobium*	2.1	6.87
T_7: Nodule endophytic bacteria + *Mesorhizobium*	1.67	5.02
T_8: Seed endophytic bacteria + *Mesorhizobium*	2.04	5.51
T_9:Root endophytic bacteria + nodule endophytic bacteria	1.41	4.9
T_{10}: Root endophytic bacteria + seed endophytic bacteria	1.41	4.18
T_{11}: Nodule endophytic bacteria + seed endophytic bacteria	1.42	4.81
T_{12}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria	1.87	5.06
T_{13}: Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*	2.33	8.02
SEM	0.261	0.013
CD at 5 %	0.760	0.037

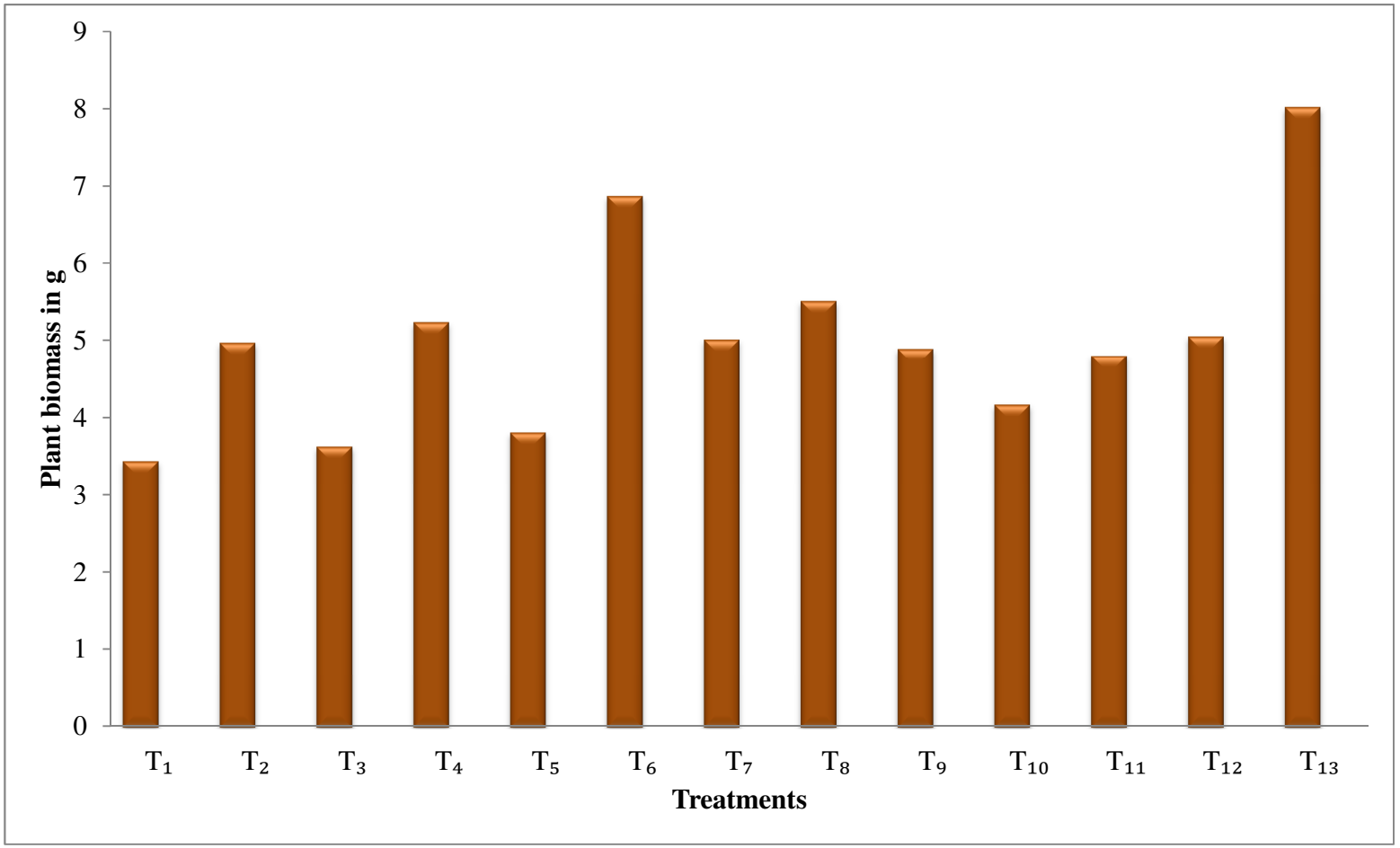


Fig.9: Synergistic effect of endophytic bacteria and *Mesorhizobium* on total plant biomass of Chickpea

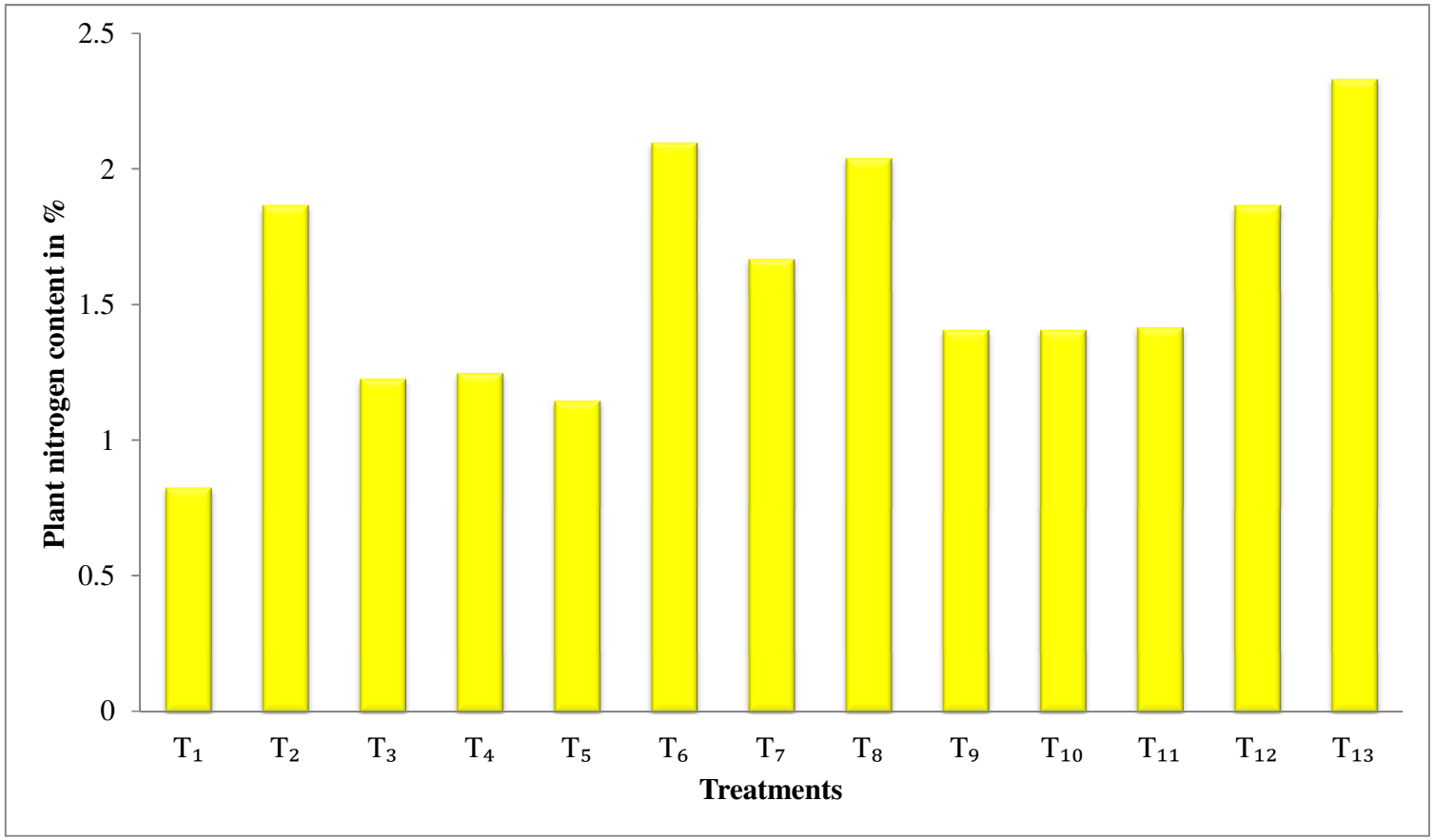


Fig.10: Synergistic effect of endophytic bacteria and *Mesorhizobium* on nitrogen content of Chickpea

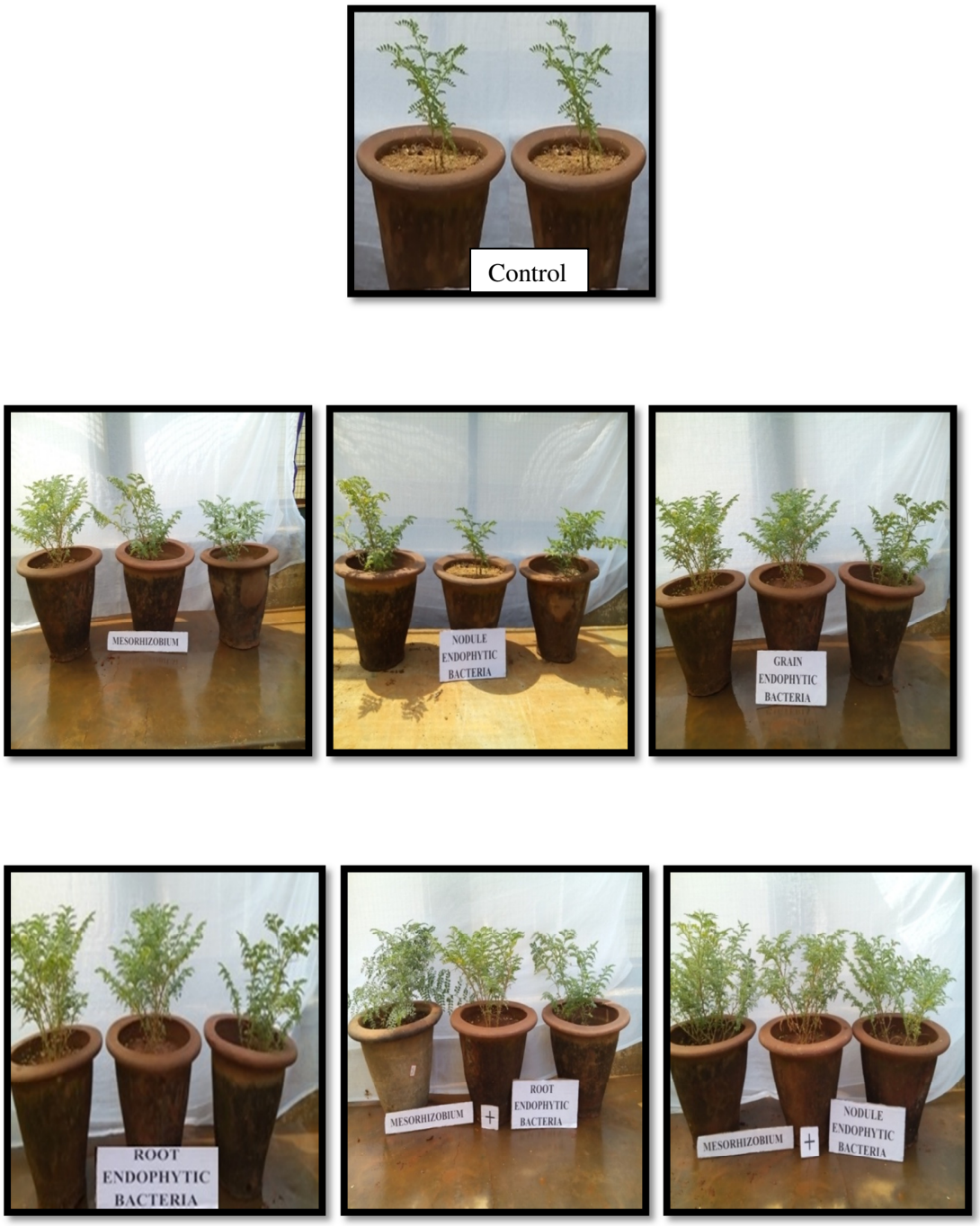


Plate 2: Effect of different treatments on Chickpea plant growth

Plate 2: Effect of different treatments on Chickpea plant growth

V DISCUSSION

All plants are hosts to one or more endophytic microorganism and *Rhizobium*. However, synergistic effect of endophytes with *Rhizobium* on crop plants is one of the least studied biochemical system in nature. Endophytic bacteria primarily reside in the tissues beneath the epidermal cell layers and host tissues are transiently symptomless and inconspicuous (Stone *et al*., 2000) and *Rhizobium* lies in association with the nodules. Conceivably, the microbes live within the intercellular spaces of tissue, and it also seems likely that penetration of living cells may occurs, but it is not easy to observe. There is ample evidence that many endophytic bacteria have beneficial effects on plants (Hallman *et al*., 1997). Growth promotion of plants may be achieved by bacterial production of plant growth regulators such as auxins, cytokinins and gibberellins; nitrogen or other nutrients may be provided by biological nitrogen fixation or mobilized as is the case for phosphorus; moreover, endophytes may confer plant protection against induction of plant defense mechanisms.

The present work was carried out to study the synergistic effect of endophytic bacteria with *Mesorhizobium* on plant growth of Chickpea.

5.1 Occurrence of endophytic microorganisms in the nodules, roots and seeds of Chickpea

In the present study endophytic bacteria were isolated from nodules, roots and seeds. The samples were collected at the stage of pod formation and seeds were collected after harvest. Endophytic bacteria in a single plant host are not restricted to a single species but comprise of several genera and species but *Rhizobium* is restricted. Bacterial endophytes and *Rhizobium* are found in variety of plants. However, different endophytes in Chickpea depend on the plant genotype, plant age, tissue sampled, and also on the season of isolation. Not much work has been done on their synergistic effect in India, Hence the present study was taken.

In the present study were isolated endophytic bacteria from the nodules, roots and seeds and *Rhizobium* from the nodules by plating on agar media. The sterility check was carried out by plating the last washed water confirmed that bacteria obtained on the plates were endophytes (Gyaneswar *et al*., 2001). Samples were discarded if any growth was detected in the sterility check.

Similar studies have also have been reported by Miche and Balandreau (2001) on diversity of endophytes and also have focused on characterization of isolates obtained from internal tissues following disinfection of plant surface with sodium hypochlorite or similar agents

5.2. Enumeration of endophytic bacterial population from different parts of plant

In present study, the population of endophytic bacteria in the nodule was found to be 5.2 CFU× 10^5/g, by root 3.6 CFU× 10^5/g and in seed 2.88 CFU× 10^5/g. Giri and Dudeja (2013) reported that 3.6 CFU × 10^5/g units of endophytic bacteria in roots of Chickpea. in roots of Chickpea. Endophytes are sheltered from environmental stresses and microbial competition in the host plant and they seem to be ubiquitous in plant tissue.

The population of endophytes recorded was more in nodules than in roots and least number of endophytic bacteria were recorded in seeds. The larger endophytic bacterial population in nodule compared to roots and seeds, may be because nodules have high symbiotic association with the microorganisms compare to root. The normal endophytes concentrations can vary between 10^2 to 10^6 CFU/g in plants.

5.3. Characterization of the endophytic bacterial isolates

The diversity of endophytic bacteria isolated from different tissues were assessed using phenotypic characterization methods. Colony morphology gave an indication of the variation among the endophytic bacterial isolates studied which were chosen for their dominance as well as uniqueness or differences with others in colony morphology. Fifteen isolates were selected from three plant part and were named according to the plant part.

Seven of these isolates were Gram positive end eight of these were Gram negative. The Gram positive bacteria were predominant in nodules compared with the root and seeds. However, Zinneil *et al*. (2002) reported an equal presence of Gram positive and Gram negative bacteria in crop plants.

Motility is an important characteristic for endophytic bacteria. Out of fifteen isolates, nine isolates were motile and seven isolates were non motile. Although endophytic bacteria can follow water fluxes for passive movement, they also need to be move inside the plant since endophytes tend to colonize specific plant parts that do always correspond to the port of entry in the plant (Taghavi *et al*., 2009).

5.3.1 Phosphate solubilization ability

The Ability of bacteria to solubilize mineral phosphate has been of interest to agricultural microbiologists, as it can enhance the capability of phosphorous for microbial and plant growth. Bacteria capable producing clear zone due to solubilization of inorganic phosphate in the surrounding media were selected as potential phosphate solubilizer. *Enterobacter* is good solubilizer of phosphate (Anu, 2011).

5.4 Compatibility of endophytic bacterial isolates and *Mesorhizobium*

Among the fifteen endophtic bacterial isolates CPN4, CPR2, CPS1 showed compatibility with each other and with *Mesorhizobium*. Thus proves that with *Mesorhizobium* endophytic bacteria coexists in same plant and there is no antagonistic effect among them hence they showed compatibility.

5.5 Application of bacterial endophyte and *Mesorhizobium*

Greater productivity and competitiveness in agriculture are anticipated to come from increased efficiency through the acquisition and management of new biotechnologies and crop production strategies. A renewed interest in the internal colonization of healthy plants by non rhizobial endophytic bacteria with *Mesohizobium* has arised as their potential for exploitation in agriculture becomes apparent (Kloepper *et al.*, 1992). Several methods of delivery of endophytic bacteria to crop plants are reported which includes seed treatment, seed biopriming, bacterisation and soil drenching.

5.6 Synergistic effect of endophytic bacteria and *Mesorhizobium* on growth of Chickpea.

Significant differences were observed in the growth parameters like plant height, flowering %, population count, fresh and wet weight of root and shoot, root length, number of nodules, nodule dry weight, plant biomass and plant nitrogen content between the treatments.

At 30^{th} days after sowing, the highest plant height was recorded in treatment involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*(12.66 cm) andleast plant height was recorded in Control (6.77 cm) and on the 60^{th} day after sowing highest plant height was recorded in treatment Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (22.7 cm)and least plant height was recorded in control (12 cm) and after harvest highest plant height was recorded treatment Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (38 cm) least plant height was recorded in control (20 cm). Similar work was done by Verma*et al.*, 2013 who revealed that mutual inoculation improves the plant height compare to un inoculated control. It may be because of the growth promoting hormones and nutrient uptake ability. Increase in the vegetative and reproductive growth in all the combination of isolates could have been lead to the better solubilization of phosphorous, development of disease resistance and production of plant growth hormones (Struz *et al.*, 1997).

At the 30 days after sowing the maximum bacterial population was recorded highest in T13 (55.33) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* and least bacterial population was recorded in T1 (29.33) Control and on 60 days after sowing the maximum bacterial population was recorded in T13 (55) Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* least bacterial population was recorded in T1 (28)

control and after harvest the same trend was followed, the highest bacterial population was recorded (87) in treatment involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* and least bacterial population was recorded in control (16). In different intervals the bacterial population count was more in consortium inoculation may be because, in combination they stimulate microbial activity, improved the soil health and their by improvement in rhizosphere microbial activity and increases the population of microorganism.

The average flowering percentage of the treatments varied from 88 per cent to 35.27 per cent. The highest flowering percentage was recorded in the treatment involving Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (88%) and lowest flowering percentage was recorded in control (35.27%). This may be due to the organisms in combination would have enhanced the growth activity and produced some of the metabolites and hormones which improved the early flowering and increased flowering percentage.

The highest number of nodules was recorded was highest in treatment Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* 38 and least number of nodules recorded in control 20. The highest nodule dry weight was recorded in the treatment involving Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (37.33 mg) lowest nodule dry weight was recorded in control (25.3 mg). Rokhzadi and Toashih, (2011) recorded the similar results. It may be due to the action of endophytic bacteria which could have helped in association and forming nodules and fixing the atmospheric nitrogen. Nodules are the site of nitrogen fixation hence in endophytic bacterial association with *Mesorhizobium* recorded the highest number of nodules and nodule dry weight.

Fresh and dry weight of root was recorded highest in treatment and Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* (2.67g and 1.31g) respectively and lowest root weight was recorded in control (0.87g and 0.31g). The fresh weight and dry weight of shoot was recorded highest in the treatment involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* (21 g and 6.71 g) respectively and lowest root fresh weight and dry weight was recorded in control (12.57 g and 3.13 g).may be because microbial activity improved the soil health and better crop growth and improved the shoot and root biomass too, similar results were recorded by Verma *et al*. (2013) in Chickpea in combination with PGPR and *Mesorhizobium* compared with un inoculated control.

The highest plant biomass was recorded in the treatment involving Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium*(8.02g) lowest plant biomass was recorded in control (3.44 g). The root length was recorded highest in the treatment Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* (15.33 cm) and lowest root length was recorded in control (7.33 cm). The increase in root and shoot weight,

biomass and root length because, the consortium inoculation improves the plant height and production of dry matter by producing varies metabolites and growth promoting hormones. Similar results were recorded by Verma *et al*. (2013) in Chickpea crop .

The highest plant nitrogen content was recorded in the treatment involving Root endophytic bacteria + seed endophytic bacteria + Nodule endophytic bacteria + *Mesorhizobium* (2.33%) and lowest was recorded in control (0.83%). This may be due to nitrogen fixation by *Mesorhizobium* which improves plant nitrogen content when it inoculated in combination with endophytic bacteria the nitrogen fixing ability increases. Similar results were recorded by Verma *et al*. (2013) in Chickpea crop .

Under the conditions of experiment the application of inoculation of endophytic bacteria which contained *Enterobacter hormaechei*, Gamma *Proteobacteria* , *Enterobacter sp* and *Mesorhizobium* UASB-835 treatment in different combination studied here, especially the treatments of nodule, root, seed endophytic bacteria and *Mesorhizobium* stimulate growth of Chickpea in comparison with the un inoculated control.

In organic agriculture where we avoid the use of chemicals, the seed bacterization with endophytic bacteria with *Mesorhizobium* is a cheap and viable option to increase the plant growth parameters and total biomass. *Enterobacter hormaechei*, Gamma *Proteobacteria*, *Enterobacter sp* isolated as endophytic bacterias possessed at least one of the different characteristics involved in plant growth promotion. It is better to incorporate the non rhizobial endophytic bacteria with *Mesorhizobium* which improves the growth and development of the plant than uninoculated plants and inoculation of *Mesorhizobium* alone.

VI SUMMARY

Healthy plants carry populations of endophytic bacteria and the plant kingdom represents a vast and relatively unexplored ecological niche for these organisms. Even so, the occurrence of communities of endophytic bacteria, although regularly identified, has received relatively little attention with their synergistic effect with *Rhizobium*. The co inoculation of endophytic bacteria and *Mesorhizobium* in Chickpea are not only desirable for enhancing the growth of crop and also in future course of time they minimize the usage of agricultural inputs, thus saving the costs and reducing pollutants to the environment. Occurrence of endophytic bacteria was studied in Chickpea nodules, roots and seeds. The population was highest in nodule followed by root and least in seeds.

Fifteen isolates isolated from different parts of Chickpea plant were selected for further studies based on their dominance as well as uniqueness and differences with others colony morphology and were named according to the plant part. The phenotypic and biochemical characterization of the isolates were carried out, seven isolates were Gram positive and eight isolates were gram negative. Nine isolates were motile and six isolates were non motile and seven isolates were endospore formers and eight isolates were non endospore formers. Different isolates showed different reactions (positive and negative) for different biochemical tests. Fifteen isolates obtained were tested for compatibility among each other and with *Mesorhizobium* UASB-835. It was found that the three endophytic bacterial isolates were compatible. The compatible organismswere selected for further studies. And these endophytic bacteria were molecularly characterized and identified as *Enterobacter hormaechei*, *Enterobacter sp* , Gamma *Proteobacter*..

A study was taken up under greenhouse condition to evaluate the effect of endophytic bacteria and *Mesorhizobium* on growth and development of Chickpea.

The plant height was significantly highest in the treatment Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* least plant height was recorded in un inoculated control at 30 days, 60 days and after harvest of crop.

The bacterial population was significantly highest in Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* treated plants and least bacterial population was recorded in un inoculated control in 30 days, 60 days and after harvest in Chickpea rhizosphere soil. The highest flowering percentage was observed in Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* treated plants and least flowering percentage observed in un inoculated control. The highest number of nodules and highest nodule dry weight was also recorded highest in Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria +*Mesorhizobium* least nodule dry weight was recorded in un inoculated control.

The highest plant biomass was recorded Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* and least plant biomass was observed in un inoculated control. The highest plant nitrogen content was seen in Root endophytic bacteria + seed endophytic bacteria + nodule endophytic bacteria + *Mesorhizobium* treated plants and least plant nitrogen content was observed in un inoculated control.

The endophytic bacteria and *Mesorhizobium* are essential for Chickpea plant growth promotion. In the present study the synergistic effect of endophytic bacteria with *Mesorhizobium* on Chickpea was determined by assessing the plant growth parameters, plant biomass and nitrogen content. Co inoculation of endophytic bacteria with *Mesorhizobium* encourages more robust crop with better plant health, disease resistance, biomass and ability to cope with environmental stresses.

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APPENDIX

PREPARATION OF MEDIUM

The standard media used in the experimental studies are listed below:

NUTRIENT AGAR MEDIA

Peptone	5.0g
Beef extract	3.0g
NaCl	5.0g
Distilled water	1000.0ml
Agar	15g
pH	7.0

YEAST EXTRACT MANNITOL AGAR MEDIUM

Mannitol	10 g
Yeast extract	0.2 g
Calcium carbonate	3.0 g
Magnesium sulphate 7 H_2O	0.2 g
Di- potassium hydrogen phosphate	0.5 g
Ferric chloride 6 H_2O	0.1 g
Agar	15 g
Congo red 2.5 ml of 1 % solution	
Distilled water	1000 ml
pH	7.0

SPERBER'S MEDIA

Glucose	10g
Yeast extract	0.5g
$MgSO_4.7H_2O$	0.25g
$CaCl_2$	0.1g
Agar	15g
Distilled water	1000ml
pH	7.0

NUTRIENT GELATIN

Beef extract	3.0g
Pepton	5g
Potassium nitrate	1.0g
Distilled water	1000 ml
pH	7.0

SIM MEDIA

Casein digest	20g
Peptic digest	6.1g
Ferrous ammonium Sulphate	0.2g
Sodium thiosulfate	0.2g
Agar	3.5g
Distilled water	1000ml

STRACH AGAR

Beef extract	3.0 g
Soluble starch	10 g
Agar	12 g
Distilled water	1000 ml

COMPOSITION OF STAINS

CRYSTAL VIOLET SOLUTION

Crystal violet	10 g
Ammonium oxalate	4.0 g
Ethanol	100 ml
Distilled water	400 ml

IDOINE SOLUTION

Idione	1 g
Potassium iodide	2 g
Ethanol	25 ml
Distilled water	100 ml

ALCOHOL

Distilled water	5 ml
Ethanol	95 ml

COUNTER STAIN

2.5% Safranin	10ml
Distilled water	100ml