"INTERACTIVE EFFECT OF RHIZOSPHERE BACTERIAL CONSORTIA ON PERFORMANCE OF CHICKPEA"

M.Sc. (Ag) Thesis

by

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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
COLLEGE OF AGRICULTURE
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INDIRA GANDHI KRISHI VISHWAVIDYALAYA
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"INTERACTIVE EFFECT OF RHIZOSPHERE BACTERIAL CONSORTIA ON PERFORMANCE OF CHICKPEA"

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by

Dharmaraj Porte

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CERTIFICATE – I

This is to certify that the thesis entitled **"Interactive effect of rhizosphere bacterial consortia on performance of chickpea"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Agricultural Microbiology)** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Dharmaraj Porte** under my/our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/ published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him

Chairman

Date: 3-7-17

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

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Member (Dr. K.P. Varma)

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CERTIFICATE – II

This is to certify that the thesis entitled "**Interactive effect of rhizosphere bacterial consortia on performance of chickpea**" submitted by **Dharmaraj Porte** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** in the **Department of Agricultural Microbiology** has been approved by the external examiner and student's advisory committee after an oral examination.

Date: 25/07/17

25/07/17

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"Education plays fundamental role in personal and social development and teacher plays a fundamental role in imparting education. Teachers have crucial role in preparing young people not only to face the future with confident but also build up it with purpose and responsibility. There is no substitute for teacher pupil relationship". I start in the name of God-who has bestowed upon me all the physical and mental attributes that I possess and skills to cut through and heal a fellow human

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College of Agriculture, Raipur (C.G.)

Dharmaraj Porte

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Abbreviations	Detail
%	Percent
^{0}C	Degree Celsius
CD	Critical difference
cm	Centimeter
Cm2	Square centimeter
@	At the rate
g	Gram
Ha-1	Per hectare
Hrs	Hours
Kg	Kilogram
L	Liter
M	Meter
Ml	Milliliter
Mg	Milligram
Kg ha-1	Kilogram per hectare
N	Nitrogen
Ppm	Per part million
SEm±	Standard error of mean
µgml-1	Microgram per milliliter
DAS	Day after sowing
DW	Dry weight
Fig.	Figure
FW	Fresh weight
NPK	Nitrogen, Phosphorus and Potassium

THESIS ABSTRACT

a) Title of the Thesis:	Interactive effect of rhizosphere bacterial Consortia on performance of chickpea.
b) Full Name of the Student:	**Dharmaraj Porte**
c) Major Subject:	Agricultural Microbiology
d) Name and Address of the: Major Advisor	Dr. Ravindra Soni (Assist. Professor-Agril. Microbiology) Department of Agricultural Microbiology, IGKV, Raipur, (C.G.) 492012
e) Degree to be Awarded:	"Master of Science in Agriculture" (Agricultural Microbiology)

Signature of the Student

Signature of Major Advisor

Date: 3/7/17

Signature of Head of the Department

ABSTRACT

In the present study a pot experiment was conducted with 9 treatments to evaluate performance of chickpea under influence of two microbial consortia along with individual members of the consortia. These two consortia C1 (GmR8+AZO137+ASL3+ASL4) and C2 (GmR8+AZO137+ASL47) were selected from previous study. Among all the treatment, C1 (GmR8, Azo137, ASL3&ASL4) increased significantly increase the shoot length (36.10 cm), shoot weight (9.59g) as compared to the control (27.10 cm, 7.06g, respectively) after 45 DAS. Further C1 also has maximum root length (27.00 cm) and root biomass (7.30g) as compared to other treatments. Highest nodulation was observed with treatment T5 i.e. Chickpea+GmR8+75%NPK i.e. 39.33 nodule /plant, however nodulation in treatment T3 associated with C1 were also significant (38.00) as compared to control (12.33). Highest N accumulation % was observed in plant Treatment T5 (9.48/plant) were also significant (9.14) as compared to control

(1.87) it has been found that in treatment T3 50% flowering occurred within (32day). Over all study reveals the better performance of the consortia over individual bacteria. Further it was also interesting that nodulation by rhizobium culture (GmR8) was not much affected when inoculated along with other PGPR.

शोध ग्रंथ सारांश

(अ) शोध का शीर्षकः	चने के प्रर्दशन पर राइजोस्फियर मिश्रित जीवाणुओं का परस्पर प्रभाव
ब) छात्र का पूरा नामः	धर्मराज पोर्ते
(स) प्रमुख विषयः	कृषि सूक्ष्मजीव विज्ञान
(द) प्रमुख सलाहकार का नाम व पताः	डॉ. रविन्द्र सोनी, (सहा. प्राध्यापक) कृषि सूक्ष्मजीव विज्ञान विभाग,कृषि महाविद्यालय रायपुर, (छत्तीसगढ़)
(इ) डिग्री से सम्मानित किया जाना हैः	कृषि स्नातकोत्तर (कृषि सूक्ष्मजीव विज्ञान)

मुख्य सलाहकार के हस्ताक्षर

दिनांक 3-7-17

छात्र के हस्ताक्षर

विभाग प्रमुख के हस्ताक्षर 6.7.17

सारांश

इस शोध अध्ययन में, चने की वृद्धि में दो सूक्ष्मजीव कंर्सोसिया के प्रभाव को जानने के लिए 9 ट्रीटमेंट्स के साथ एक पॉट एक्सपेरिमेंट को ग्लास हाऊस में किया गया। ये दो कंर्सोसिया सी 1 एवं सी 2 पूर्व में किये गये अध्ययन के आधार पर लिये गये है। सभी परीक्षणों (ट्रीटमेंट्स) का तुलनात्मक अध्ययन करने पर यह पाया गया कि कंर्सोसिया सी 1 वाले ट्रीटमेंट के पौधों की तने की लंबाई (36.10सेमी.), तने का भार (9.59 ग्रा.) एवं कंट्रोल (27.10सेमी. और 7.06 ग्रा.) व अन्य की तुलना में अधिकतम था। पुनः सी 1 वाले ट्रीटमेंट में जड. की लंबाई (27.00 सेमी.) तथा जड. का जैव भार (7.30 ग्रा.) भी अन्य ट्रीटमेंट की तुलना में अधिकतम था। सभी परीक्षणों में से परीक्षण संख्या टी 5 (चना+जीएमआर+75 : एनपीके) में अधिकतम नोड्यूल पाये गये हांलाकि परीक्षण संख्या टी 3 में भी (38.00) कंट्रोल (12.33) की तुलना में बहुत अच्छें नोड्यूल संख्या पायी गयी। इसके अतिरिक्त ट्रीटमेंट टी 3 जो कि कंर्सोसिया सी 1 से संबंधित में : नाइट्रोजन स्थिरीकरण पौधें में (9.14) तथा परीक्षण टी 5 में (9.48) तथा कंट्रोल (1.87) की तुलना में अधिकतम था। इसी तरह नोड्यूल में भी परिक्षण टी 5 में जो कि राइजोबियम आइसोलेट्स से संबंधित है जिसमें नाइट्रोजन स्थिरीकरण (5.17) अधिकतम पाया गया, इसके अतिरिक्त परीक्षण टी 3 में 50: फूल की अवधि भी सबसे कम दिन (32 दिन) पायी गयी। पूरी शोध के उपरांत यह निष्कर्ष निकाला जा सकता हैं कि सभी परीक्षणों में टी 3 उत्तम हैं।

CHAPTER - I
INTRODUCTION

In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know how to use Plant growth promoting rhizobacteria (PGPR) that can biologically fix nitrogen, solubilize phosphorus and induce some substances like plant hormones that could contribute to the improvement of plant growth. PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. A large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and Serratia have reported as PGPR to enhance plant growth (Kloepper *et al*., 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995). The direct growth promotion of plants by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. On the other hand, the identification, selection and application of suitable beneficial microorganisms can increase the options to deal with growing problems (Kilian *et al*., 2000), and can be also environmentally sound (Woitke *et al*., 2004).

The use of PGPR for sustainable agriculture has increased tremendously in various parts of the world. The complex interactions between the root and associated microorganisms also enhance the plant growth and yield (Sylvia *et al*., 1998). Recently more paper and publication available on the field of plant growth-promoting bacteria (Ahmed *et al*., 2016) which enhance plant growth either by direct or indirect mechanisms. Plant growth promoting rhizobacteria that have been successfully involved in promoting the growth of crops such as canola, soybean, lentil, pea, wheat, radish and chickpea have been isolated. Synergetic effect have been studied in desi and kabuli chickpea under field condition using *Mesorhizobium* sp. (LGR -33) (*Meso*) and native potential PGPR *Pseudomonas* sp. along with

reference strain *Pseudomonas diminuta* (LK884) to enhance bio-enhancing activity, symbiotic parameters and grain yield.

Chickpea (*Cicer arietinum*) is the most important staple food in several developing countries including India. Legumes especially chickpea occupies special position regarding nutrition as well as soil fertility and improvement.

It has the ability to grow well in poor soils as well as to improve them because of its efficient N fixation system (Neumann *et al*., 2011). About 65 per cent of global area with 68 per cent of global chickpea production is contributed by India (Reddy and Mishra, 2010). The production is still not adequate to meet the domestic demand due to its low productivity (8.50kg/ha). The major causes for low productivity of chickpea in india are low yield potential and susceptibility of improved present day cultivars to various biotic and abiotic stresses (Gowda *et al*., 2011). Chhattishgarh is agricultural state; it contributes only 3% of total pulse production in India. Further, agroclimatic condition of this state is suitable for chickpea production. It is also a top ranked pulse crop in Chhattisgarh. Hence, the present work is proposed to reduce the ill effects of chemical fertilizers by formulating some eco-friendly bacterial consortia, enable to enhance chickpea growth and yield considering chickpea among the major crops of Chhattisgarh.

Objectives:

1. Comparative effect of individual bacterial isolate on Chickpea performance.
2. Comparative effect of selected bacterial consortia on chickpea performance.
3. Effect of microbial consortia on nodulation behavior of chickpea under controlled conditions.

CHAPTER - II
REVIEW OF LITERATURE

The technology of plant production always faced fast-growing food and energy demands, but driven by a new approach, the answer for those demands must be socially and environmentally conscious. Typical feature of modern intensive agriculture worldwide is to increase agricultural productivity by the application of external chemical inputs including fertilizers, pesticides, fungicides, and herbicides. Due the necessity to reduce chemical products (chemical fertilizers, pesticides, and supplements), aiming sustainable agriculture and protecting the environment, the use and research of microorganisms have been focused in the whole world (Vale et al., 2010). Kloepper and Schroth (1978) first defined the term plant growth-promoting rhizobacteria (PGPR) to describe soil bacteria that colonize the roots of plants and enhance plant growth following inoculation onto seeds. For the past several decades, research dedicated to improve crop yield and plant growth with microbial inoculants mainly focused on the symbiotic rhizobia which have been successfully used worldwide for the establishment of the nitrogen-fixing symbiosis with legumes (Reddy 2013; van Veen *et al*., 1997).

Microorganisms are ubiquitous in nature. However, their diversity, distribution and community structure depends on various biotic and abiotic factors. As a consequence, they exhibit spatial and environmental variations and formed distinct communities under different ecosystems. Unfortunately, majority of microorganisms are unable to grow under *in vitro* conditions, and therefore, the accurate enumeration of their diversity is still lacking (Soni *et al*., 2010; Suyal *et al*., 2015a). However, an advance in the molecular biology led to the emergence of high throughput next-generation sequencing (NGS) techniques which facilitate precise identification and characterizations and of microbial communities in specific environment. Microorganisms play an important role in the growth and development of plants. However, they are often exposed to biotic stresses *viz*. pathogens, foreign metabolites as well as environmental stresses *viz*. temperature, pH, moisture content, nutrient availability etc. (Mendes *et al*., 2013). Therefore, they exhibit a wide range

of adaptive features to sense and response the stresses. Moreover, they can modify their signal transduction networks to cope with stress conditions. Plant-growth-promoting rhizobacteria (PGPR) can be classified as bio-fertilizers and is one of the most cost-effective and sustainable way to increase nutrient uptake, plant productivity and immunity.

2.1 Nitrogen Fixation

Nitrogen is a major limiting nutrient for the growth of the plant. Nearly 78 % of atmosphere gas constituents are represented by nitrogen in molecular form (N_2). Some prokaryotic organisms are able to assimilate the N_2 from atmosphere and convert in absorbed form as NH_3, and this process is called biological nitrogen fixation (BNF) (Reis *et al*., 2006). In atmosphere the amount of free nitrogen present accounts to $4x10^{21}$ gN out of which around 2.5×10^{11} kg NH_3 is fixed annually by biological means (Schlesinger 1991). Furthermore, microbes play an important role in biological nitrogen fixation. The first evidence for nitrogen fixation by *Pseudomonas* like microorganisms has been reported by Anderson in 1955. However, a large number of bacteria from genera *Acinetobacter, Azospirillum, Arthrobacter, Bacillus, Burkholderia, Erwinia , Enterobacte r, Flavobacterium , Pseudomonas, Rhizobium,* and *Serratia* are associated with the rhizosphere and are able to exert a beneficial effect on plant growth including nitrogen fixation (Egamberdiyeva 2005 ; Tilak *et al*., 2005 ; Shahi *et al*., 2011).

Rhizobium is the main contributor to the symbiotic nitrogen fixation in legume crops. Moore and Moore (1992) have divided it into four groups. They are fast-growing *Rhizobium,* slow-growing *Bradyrhizobium* and *Azorhizobium*), and *Sinorhizobium*. Further, *Azospirillum, Herbaspirillum,* and *Acetobacter diazotrophicus* are associated with the roots of Gramineae family. However, free-living nitrogen fixers exist in the rhizosphere zone of plants. Free -living Microorganisms involved in nitrogen fixation are *Azotobacter* ,*Clostridium, Rhodospirillum , Anabaena , Klebsiella* , and *Nostoc*.

2.2 Phosphate solubilisation

Phosphorus is a plant macronutrient that has a vital role in plant metabolism, ultimately affects on crop yields. It is also important for the functioning of key enzymes that control the metabolic pathways. It is expected that about 98 % of Indian soils contain insufficient amounts of available phosphorus, which is essential to support plant growth (Vassilev and Vassileva 2003). Phosphorus in $H_2 PO_4^-$ and HPO_4^{2-} can be absorbed by plants, but unfortunately they are present in bound form with organic or inorganic molecules which are unavailable to plants (Smyth 2011). P fertilizers are required for crop production, but only a small part of P is utilized by plants, rest is converted into insoluble fixed forms (Rodriguez and Fraga 1999). Solubilization of insoluble P by microorganisms was firstly reported by Pikovskaya (1948).

The second essential element in plants' necessity is the phosphorus (P), being only nitrogen's behind (Kucey 1988), and making up for about 0.2 % of a plant's dry weight. Several PGPR strains such as *Pseudomonas, Bacillus, Burkholderia , Rhizobium* and *Flavobacterium* have been reported to have the ability to solubilize such insoluble inorganic phosphate compounds.

2.3 Phytohormone production

The ability to synthesize phytohormones is widely distributed among plant-associated bacteria, and 80 % of the bacteria isolated from plant rhizosphere are able to produce plant growth promoting substances. Several PGPR were involved in the synthesis of phytohormones, viz. indoleacetic acid (IAA) and gibberellins, which enhance root and shoot development in plant, thereby increasing the plant biomass for better alleviation of abiotic stress conditions (Patten and Glick 2002). Among PGPR species, *Azospirillum* is one of the best-studied IAA producers, and other bacteria belonging to genera *Aeromonas, Burkholderia*, *Azotobacter* (Ahmad *et al*., 2008; Chennappa *et al*., 2013), *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhizobium* (Ghosh *et al*., 2010) species have been isolated from different rhizosphere soils.

Patten and Glick (2002) reported the role of IAA-producing *P. putida* in the development of the host plant root system. *P. fluorescens* can produce cytokinins as reported by Garcia *et al*., (2001). Many endophytes like *F. fujikuroi, Sphaceloma manihoticola, Phaeosphaeria sp., Neurospora crassa, Cladosporium sp.,Penicillium sp., Gliomastix murorum, Arthrinium phaeospermum*, and Aspergillus fumigatus have been reported as growth promoters.

2.4 Siderophore production

Iron is a vital nutrient and occurs as Fe^{3+} in the aerobic environment and forms insoluble hydroxides and oxyhydroxides. These insoluble forms are not accessible to both plants and microbes. Generally, endophytes synthesize low molecular weight compounds termed as siderophores that sequester Fe 3+ since they have high Fe 3+ affinity constants and mobilize the irons present (Zhang *et al*., 2008 ; Vendan *et al*., 2010).

Siderophore-producing microbial inoculants have been shown to have a direct plant growth-promoting effect in various crops in the past. Biocontrol strains of PGPR produce siderophores that have high affinity for iron so that fungal pathogens are unable to survive in the rhizosphere of the host plant due to lack of iron.

Siderophore production by certain Pseudomonas spp. also has a secondary effect by triggering systemic acquired resistance (SAR). The direct benefits of bacterial siderophores on the growth of plants have been demonstrated by using radio-labeled ferric siderophores as a sole source of iron and showed that plants are able to take up the labeled iron by a large number of plant growth-promoting bacteria including *Aeromonas, Azadirachta, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Rhizobium, Serratia,* and *Streptomyces sp.* (Vendan *et al*., 2010; Loaces *et al*., 2011; Verma *et al*., 2014, 2015b ; Pedraza 2015).

2.5 Stress tolerance

Microbes confer several benefits to the plants and help them to alleviate the stress. Therefore, exploration of stress tolerant bacteria can help in sustainable agricultural plans in environmentally unfavorable conditions. The ACC deaminase activity of *Achromobacter piechaudi* was shown to confer tolerance to water deficit in tomato and pepper (Mayak *et al*., 2004). Similarly, (Arshad *et al*., 2008) obtained that a strain of *Pseudomonas* spp. with ACC deaminase activity partially eliminated the effect of drought stress on the growth of peas (*Pisum sativum L.*). Furthermore**,** inoculation of *Azospirillum* with wheat under drought stress conditions resulted in a significant increase in water content (Creus *et al*., 2004; Pereyra *et al*., 2012; Saghafi *et al*., 2013; Kasim *et al*., 2013). Several plant growth promoting metal resistant microorganisms viz. Pseudomonas (Kahn *et al*., 2014), Commamonas (Rani *et al*., 2013), Proteus and Bacillus (Saluja *et al*., 2011 and 2012), *Staphylococcus, Planococcus,* and Vibrio (Zampieri *et al*., 2016), Mycorrhiza (Dhawia *et al*., 2016), Actinomycetes (Taj *et al*., 2016) are reported. Cold adapted microorganisms constitute a major fraction of Earth's biomass and perform crucial roles in biogeochemical cycles (Siddiqui *et al*., 2006). These bacteria synthesize cold-shock proteins (Csps) and cold acclimation proteins (Caps) in response to low temperature. In addition to this, they are able to synthesize housekeeping proteins even under cold conditions. Several earlier reports reveal successful implementation of the cold tolerant plant growth promoting rhizobacteria (Rani *et al*., 2013; Suyal *et al*., 2014a). Effective existence of bacteria in saline environment due to excessive accumulation of secondary metabolites may result in better root colonization and plant growth (Hirsch, 2010; Karlidag *et al*., 2011). Bacteria-induced salt tolerance in plants has been observed for several PGPR strains (Mayak *et al*., 2004; Barriuso *et al*., 2008; Zhang *et al*., 2008).

The increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves cause significantly yield losses with great risks for future global food security (Christensen and Christensen, 2007). Nehra *et al*., 2007) reported that heat resistant/tolerant mutants of *Rhizobium* sp. (*Cajanus*) can tolerate thermal stress and can fix atmospheric N2 more efficiently

than the parent strain under natural high temperature conditions. Further, inoculation with thermotolerant PGP *Pseudomonas putida* strain AKMP7 alleviated heat stress and consequently improve the growth of wheat plant in the presence of heat stress (Ali *et al*., 2011).

2.6 Microbial Consortia

In most cases, a single PGPR exhibits multiple growth-promoting attributes including biocontrol ability (Vessey 2003). PGPRs are commonly used to improve crop yields and help in sustainable agriculture. Further, they possess potential in solving environmental problems including phytoremediation to decontaminate soils and waters (Tilak and Manoharachary,2015). Fluorescent pseudomonads and bacilli form a major group among PGPRs along with other bacteria like *Acetobacter, Actinoplanes, Agrobacterium, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Cellulomonas, Clostridium, Enterobacter, Erwinia, Flavobacterium, Pasteuria, Serratia, Xanthomonas,* etc. Bioinoculants represent those living microbes which when amended to the agricultural soil result plant growth promotion through providing plant nutrition and plant protection, stimulating plant hormone production, raising minerals uptake, weathering of soil minerals, etc (Bashan Holguin and 1997 ; Sullivan 2001). It generally comprises either individual microbial strain or a group of different beneficial microorganisms as consortia having positive impact on plant growth. Van Veen *et al*., 1997) critically reviewed the reasons for poor performance of agricultural bioinocula in natural environments and in the rhizosphere of host plants and suggested that instead of using a single strain, for a single trait, use of multiple microbial consortia for multiple benefits, can also thrive together in unique ecological niches in ideal proportions. On the other hand it has been found that these bacteria would also interact synergistically by providing nutrients, removing some inhibitory products, or stimulating each other through physical or biochemical mechanisms. Microbial consortium is a group of different species of microorganisms that act together as a community.

For developing a consortium one can choose microorganisms that are resistant to environmental shock, fast acting, synergistically active, producing natural

enzymatic activity, easy to handle, having long self life, good sustainability, non-pathogenic, noncorrosive of consistent quality and economical. Combinations of bio-control strains are expected to result in a higher level of potential to suppress multiple plant diseases.

Development of consortia with consistency under field conditions and long shelf life will help to commercialize the technology successfully and could pave the way for successful commercialization of the technology. These formulations are customized according to the requirement and depend on soil type, cropping systems, and microorganism function for better outcome (Roesti *et al*., 2006; Ahmad *et al*., 2013). Seed bacterization of tomato and chili with a talc-based consortia comprising of P. fl uorescens and P. chlororaphis performed better in reducing the incidence of damping-off (Kavitha *et al*., 2003). Inoculation with a consortium of several bacterial strains could be an alternative to inoculation with individual strains, likely reflecting the different mechanisms used by each strain in the consortium. The co-inoculation of soybean and common bean (*P. vulgaris L*.) with *rhizobia* and *A. brasilense* inoculants showed good results for improving sustainability (Hungria *et al*., 2013). In field trials, the co-inoculation of soybean with *B. japonicum* and *A. brasilense* species resulted in outstanding increases in grain yield and improved nodulation compared with the non-inoculated control.

Efficient germination is the basic criteria for growth and yield of the crop. Seed inoculation with bacterial consortia significantly enhanced the germination of seed (Akhtar *et al*., 2016). Previous work has been carried out on the effect of N2 fixers, P solubilizers as single and/or consortia on several crops (Leo Daniel *et al*., 2012).

2.7 Chickpea & PGPR

In context to feed nutritious food to burgeoning human population, pulses play a significant role, as these are rich in vitamin, mineral and protein (protein tablets). However, their production and productivity is still very low in India. In India, chickpea accounts for about 45% of total pulses produced in the country. Similar to the case of other pulses, India is the major producing country for

chickpea, contributing for over 75% of total production in the world. Rajasthan, Maharashtra, Uttar Pradesh and Andhra Pradesh contributed about 14%, 10%, 9% and 7%, respectively.

On the other hand, the share of Andhra Pradesh and Karnataka has consistently been rising during the past 10 years. Further, states like Jharkhand and Chhattisgarh are expanding their area and production of chickpea crop (AICRP-Chickpea). Due to high nutritive value, chickpea (*Cicer arietinum* L.) is one of the earliest cultivated and third widely grown edible legume in tropical, sub-tropical and temperate regions of the world. Also chickpea play a key role in organic cropping systems. It is one of the most widely grown legumes (Romdhane *et al*., 2007) and forms a highly specific symbiosis with its rhizobial partner (*Mesorhizobium ciceri*) (Khan *et al*., 2006). In such agro ecosystem with limited availability of nitrogen, chickpea potentially constitute both a cash crops and a source of N incorporation into the system via biological nitrogen fixation. (Singh *et al*., 2013) recently showed the synergistic effect of antagonistic fungi *Trichoderma* with combined application of *Pseudomonas* and *rhizobial* strains can protect chickpea from infection by the collar rot pathogen *Sclerotium rolfsii*. Seed treatment with the synergistic microbial consortium had a positive impact on chickpea growth and activation of the phenyl propanoid pathway under the stress of the pathogen S. rolfsii and higher accumulations of antimicrobial polyphenols and flavonoids in a short time is an indication of induced systemic resistance (Singh et al., 2014). The combined inoculation of *Rhizobium* and phosphate solubilizing bacteria has increased nodulation, growth and yield parameters in chickpea (Jain *et al* 1999, Rudresh *et al*., 2005. Kumar *et al.,* 2016) suggested that an improved tolerance of plants to drought, with higher growth and yields, and synergistic effects from the use of consortia were found.

In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know now to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that could contribute to the improvement of chickpea growth.

CHAPTER - III
MATERIALS & METHOD

The brief description of the materials used and the methods adopted during the course of present study entitled "**Interactive Effect of Rhizosphere Bacterial Consortia on Performance of Chickpea**" were given in this chapter. The details of geographical situation, climate during the experiment are briefly described below.

3.1 Experimental site and geographical situation

The experiment was conducted in the glass house of Dept. of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during 2016-2017 with chickpea crop. Raipur is situated in plains of Chhattisgarh at $21^0$16' N latitude and $81^0$36' E longitude with an altitude of 298.56 meter above mean sea level (MSL)., variety. JG 14 (Table no.3.1)

3.2 Climatic conditions

Raipur comes under dry sub humid region, receiving an average rainfall of 1200-1400mm out of which about 85 per cent is received during the rainy season (June to September) and the rest 12 percent during winter season (October-February). The place experiences a short mild winter, January being the coolest. May being the hottest month. Soil surface temperature of this region crosses 60 ºC, air temperature touches to 48 ºC and humidity drops up to 3 to 4 per cent during summer season and mercury level drops to as low as 6 ºC during December and January.

3.3 Procurement of selected plant growth promoting rhizobacteria

Rhizobacterial culture here procured in YEMA media, OKON'S media, JENSEN'S media Slant from Department of Microbiology culture repositing than culture here maintend in YEMA media (for *Rhizobium*), OKON'S media (for *Azospirillium*), and JENSEN'S media (for *Azotobacter*) Slant routinely sub culture in the sample at 15 days interval at 37^0C for 72 hour and Slanted at 40^0C in further

use. During this experiment four rhizobial isolates were compared alone and in combination with the uninoculated control (table no.3.2) the number of treatments was nine replicated three in completely randomized design. Chickpea (JG14) was taken as a test variety. Other details are as follows:

3.3.1 Polythene bag preparation

The medium used for growing chickpea crop was soil (Alfisol) which was well air dried and processed to good physical condition ideal for chickpea growth. This soil was filled in polythene bags @ 6 kg per bags. Soils was collected from a depth of 6 inches (15cm) from soil surface and thoroughly mixed and filled in each polythene bag having 10 kg capacity.

3.3.2 Details of the treatments

Two best consortia from previous study (Nag 2016) were selected along with individual isolates. Chickpea (JG14) in single and dual combinations. Uniform level of Nitrogen, Phosphorus and Potassium @ 20:40:20 kg/ha was applied as basal through urea, single super phosphate and murate of potash respectively as per the treatment decided (Table 3.1 Thus, the experiment comprising the following nine treatments with three replicated.

Tr. No.	Name of Isolate
T1	Chickpea+100%NPK
T2	Chickpea+75%NPK
T3	Chickpea +C1+75%NPK
T4	Chickpea +C2+75%NPK
T5	Chickpea+GmR8+75%NPK
T6	Chickpea+AZO137+75%NPK
T7	Chickpea+ASL3+75%NPK
T8	Chickpea+ASL4+75%NPK
T9	Control

Table 3.1 **Treatments for glass house experiment**

100% NPK = Recommended Dose of Fertilizer (NPK) 20:40:20

C1 = GmR8 – Rhizobium, AZO137 – Azotobacter, ASL3 –Azospirillum, ASL4 – Azospirillum

C2 = GmR8 – Rhizobium, AZO137 – Azotobacter, ASL3 – Azospirillum

3.3.3 Seed treatment

Healthy seeds of chickpea (JG 14) were taken for experimentation. Just before sowing, healthy seeds of chickpea were treated with Thiram @ 3 gm/kg of seed.

3.3.4 Inoculums preparation & seed bacterization

All Plant Growth Promoting rhizobacteria culture on Chickpea growth under controlled condition inoculated separately to 25 ml nutrient broth in 50ml conical flask and incubated at 28±20C for72 hours. Equal volume of the broth culture here then used for the purpose of bacterization of seeds at the time of sowing.

3.3.5 Sowing

About 24 hours before sowing, all the bags were irrigated by unsterilized water. Five holes (2 to 3 cm deep) per bags were made with the help of sterilized glass rod maintaining equal distance from hole to hole. Sowing of 5 seed/ pot was done on 13-12-2016 by placing one seed in each hole with the help of sterilized forcep.

3.3.6 Care after sowing

After germination a population of 3 plants per bags was maintained by thinning out the extra seedlings. Uniform irrigation to all pots was given as and when required.

3.3.7 Application of fertilizer.

The nitrogen (N), phosphorus (P2O5) and potassium (K2O) were applied @20:40:20 kg/ha by using urea, single super phosphate and murate of potash, respectively.

3.4 Observations recorded during experiment

Morphological growth parameters

The following morphological growth parameters were recorded in chickpea subjected to different treatments. The observations were recorded on 3 randomly selected plants in each treatment at 15 days interval.

3.4.1 Heights of plant

Heights of the plants were recorded at different day's interval viz. 15, 30, and 45 days after sowing (DAS) and expressed in centimeters per plant. Similarly Root length was recorded after 45 day.

3.4.2 Duration of 50% Flowering

Duration of 50% flowering here recorded.

3.4.3: Biomass Accumulation

3.4.3.1 Plant biomass

The plants components *viz* shoot & Root were collected from plant at 45 DAS of Chickpea. Fresh biomasses of these were taken and then these were oven dried at 65 ^{0}C for 3-4 days up to the attainment of constant weight. Final dry weights of shoot & Root were recorded.

3.4.3.2 Nodulation study

Roots of uprooted plants were washed carefully so that nodules were not damaged, then no. of nodules and their fresh weight were recorded. After recording the fresh weight, the nodules were kept in small papers bags and were dried in hot air oven at 60^0C till their constant oven dry weight is obtained.

3.4.4 Nitrogen content in plant

The oven dried different plant components viz shoot were ground into powder through Wiley mill and used for N analysis. The nitrogen content in the plant samples was estimated by Micro – Kjeldhal method as described by Jackson (1973) using auto digestion and distillation system. The nitrogen concentrations of each component were multiplied with their respective biomass to obtain nitrogen uptakes.

3.5 Statistical analysis

All observations recorded from this experimental study were tabulated in a systemic manner. The final observations of morphological growth parameters, biomass, and nodulation and Nitrogen uptake by shoot of Chickpea plants were statistically analyzed using ANOVA for Completely Randomized Block Design (CRBD) (Panse and Shukhatme, 1978).

CHAPTER – IV
RESULTS AND DISCUSSION

The investigation with chickpea crop was conducted at Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during the year 2016-17. The topic is **Interactive effect of Rhizosphere bacterial consortia on performance of chickpea**. Pot experiment for evaluation of different (PGPR) especially with reference to plant growth. Nitrogen (N) is a major growth-limiting nutrient, and unlike the case for phosphorus, there is large atmospheric source that can be made biologically available. Root development, nodule formation, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with nutrition. The results obtained from these studies are depicted and discussed in this chapter.

Background of the study

The present investigation is an important part of study which is being carried out in the Department of Microbiology, College of Agriculture, IGKV, Raipur in order to develop effective biofertilizer for different chickpea grown under climatic conditions of Chhattisgarh. Initially the areas of Chhattisgarh having actual need of microbial inoculations have been identified. Then after, a series of experiments were planned for selection of effective isolates for different crops. In this connection, under the present investigation, the studies were conducted to select effective combination of (PGPR) isolates for chickpea growers of Chhattisgarh which is bigger than many states of the country. Similarly, Amarger *et al*.1994; Gupta *et al*. 2000; Gupta *et al.* 2000b; Chowdhury and Gupta, 2003; Shamseldin, and Werner, 2004 and Gupta *et al*. 2005 conducted isolation-screening experiments and selected effective location specific *Rhizobium* isolates on the basis of BNF (Biological nitrogen fixation) parameters.

4.1 Formulation of composite culture

Two consortia selected from previous study (Nag, 2016) for further study based on their plant growth promoting ability. The details are summarized in Table 4.1 & 4.2 and Fig 4.1-4.4.

4.2 Pot Experiment

Pot experiment with natural soil was planned for the influence of combination of (PGPR**)** different growth of chickpea at green house of Department of Microbiology, IGKV, Raipur, Chhattisgarh, during the year 2016-17 in pot containing, 6 kg experimental soil. During this experiment**,** Two rhizobacteria isolates were compared alone and in combination with the uninoculated control. The number of treatments was nine replicated thrice in Complete Randomized Block Design (CRBD). Chickpea (JG- 14) was taken as a test variety. Other details recorded are as follows.

Table 4.1 Summary of selected obtained in the study

Code	Culture	Properties	Resources
GmR8	*Rhizobium*	BNF, siderophore production	selected in this study
AZO137	*Azotobacter*	BNF, IAA, temperature, pH, salt tolerance	selected from previous study (Nag 2016)
ASL3	*Azosprillum*	BNF, IAA	selected in this study selected
ASL4	*Azosprillum*	BNF, siderophore production	in this study

Table.4.2 Details in this study of Bacterial Consortia used in present study

No.	Formulation of Composite culture	Properties
C1	GmR8+AZO137+ASL3+ASL4	BNF, siderophore production, IAA, temperature,pH, salt tolerance.
C2	GmR8+AZO137+ASL4	BNF, siderophore production, IAA,Temperature, pH, salt tolerance.

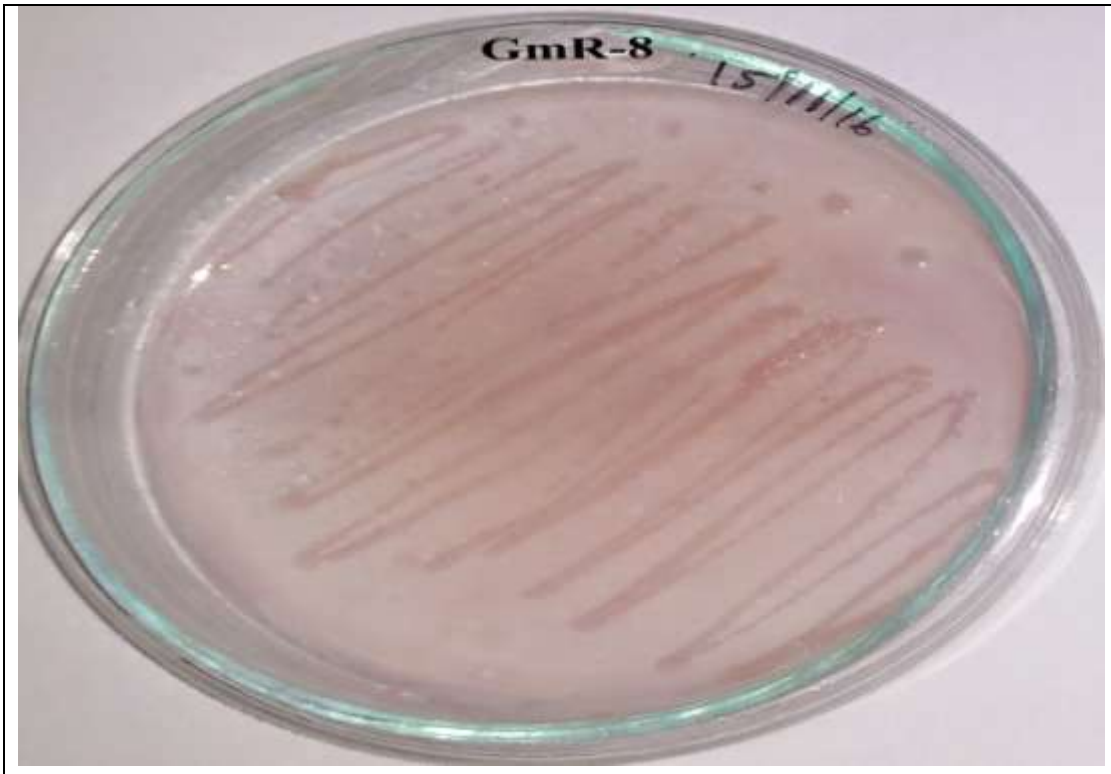


Plate 4.1 Growth of *Rhizobium* Isolates on YEMA Media

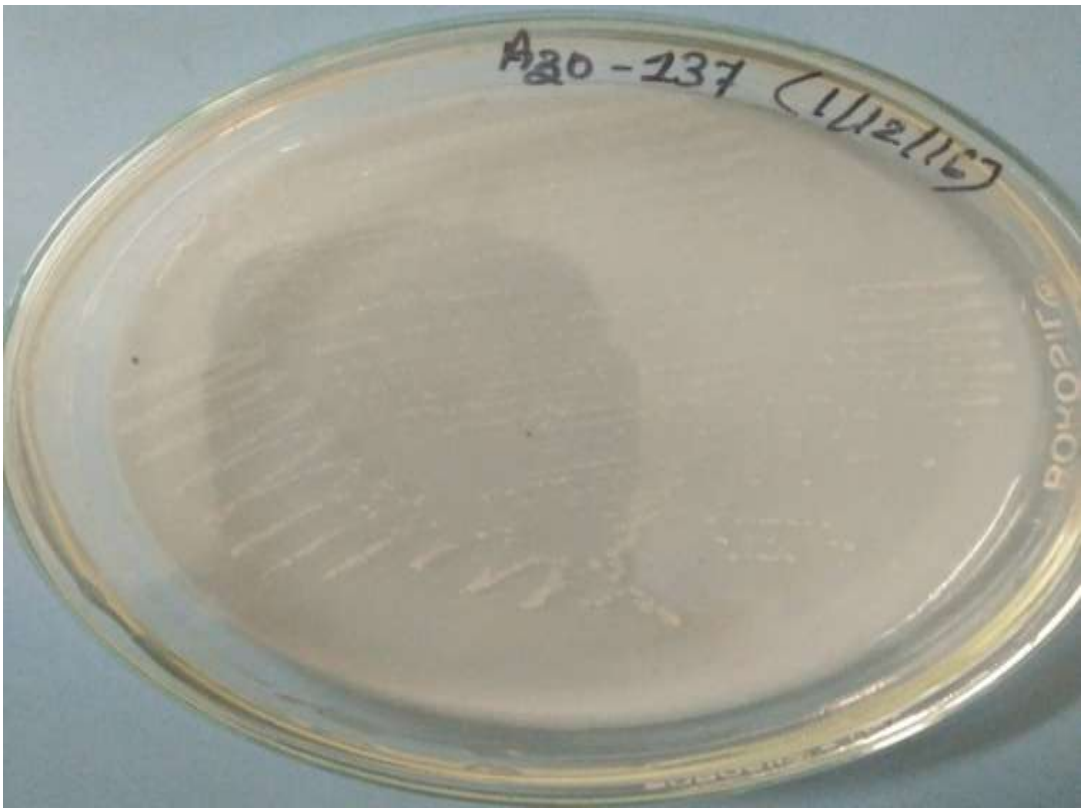


Plate 4.2 Growth of *Azotobacter* Isolates on Jenson's Media

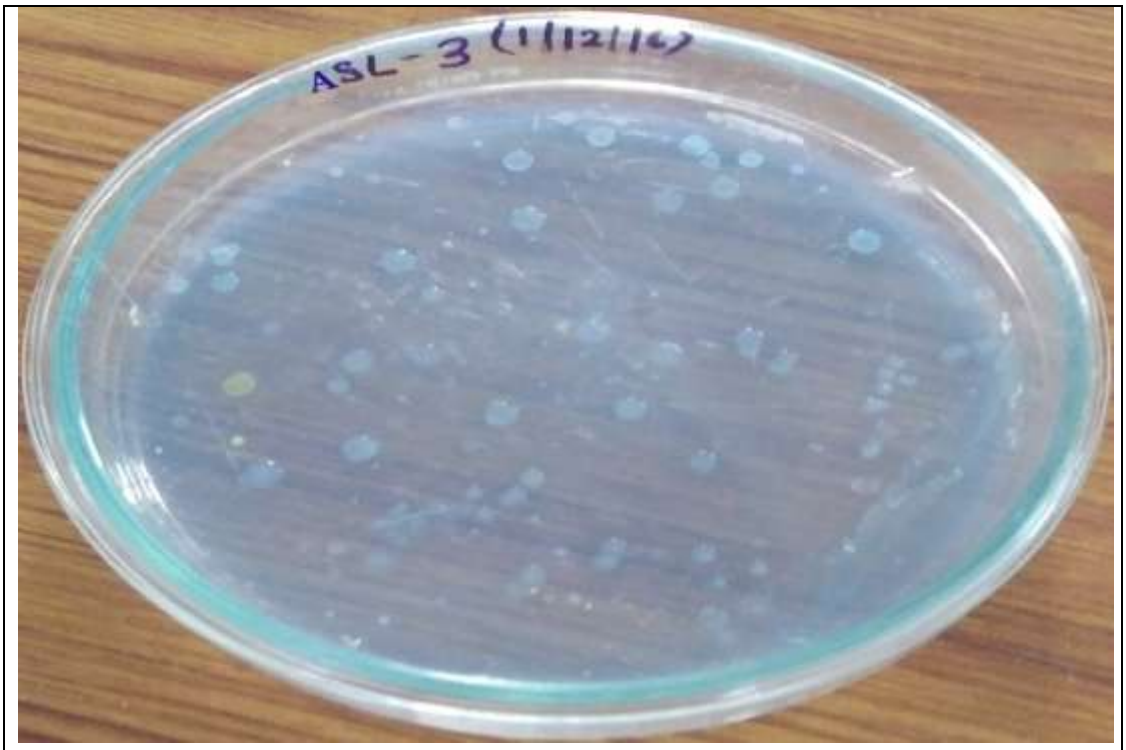


Plate 4.3 Growth of ASL - 3 *Azospirillum* Isolates on Okon's Media

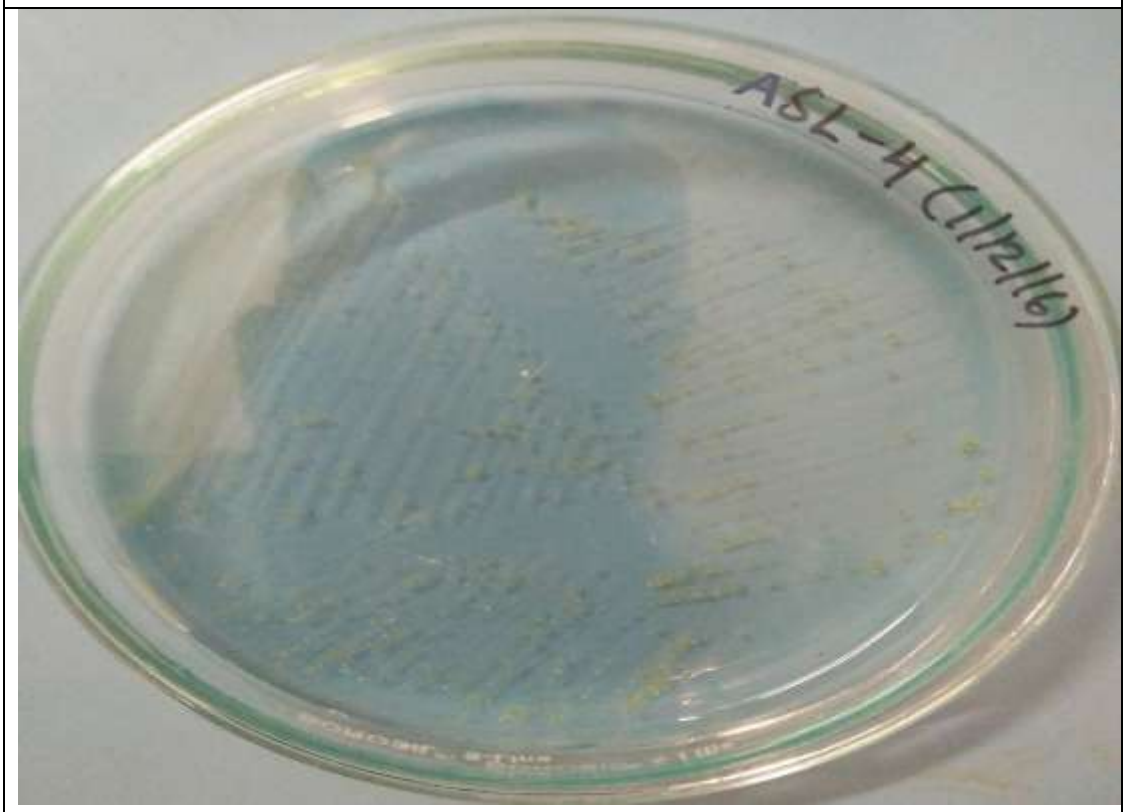


Plate 4.4 Growth of ASL - 4 *Azospirillum* Isolates on Okon's Media

4.2.1 Plant growth parameter

Plant height

Data of plant height recorded at three different stages of (15, 30, and 45 DAS) crop growth presented in Table 4.3 and depicted in Plate 4.1. The critical look in the data that used in different treatments which are early of plant growth. At 15 DAS some of the microbial isolates alone and in combination found significant to enhance the plant growth. However, rest of the microbial isolates alone and in combination found significant affected by applied PGPR at 15 DAS. Performance of treatments T3, T4, T5, T6, T8, T1, and T2 were found good over control respectively. At 30 DAS, plant height increased significantly from T3, T4, T5, T8, T7, T6, T1 and T2 were found better over control respectively. At 45 DAS, plant height increased significantly from T3, T5, T4, T7, T6, T1, T8, and T2 were found better over control respectively. Influence of selected native PGPR isolates on plant height of chickpea plants after different days of sowing is shown in Fig. 4.4.

Observations recorded 15, 30 and 45 DAS on plant height clearly states that increase in plant height in each treatment were positively correlated with each other. Influence of selected native rhizosphere bacterial consortia isolates on plant height of chickpea plants after different days of sowing is shown in table 4.3. Observations recorded 15, 30 and 45 DAS on plant height clearly states that increase in plant height in each treatment were positively correlated with each other. It is clear from the study that plant height increased significantly from 15 to 45 DAS due to inoculation PGPR. This may be because of greater rate of nutrient utilization up to reproductive phase.

This observation is in close agreement with Dasgupta, D. *et al.,* (2015) Hilal *et al*., (1990), Alagawadi *et al.,* (1993). Reddy *et al*., (2002), Khalequzzaman and Hossain (2008), they also mentioned plant growth can be increased by inoculation with effective Rhizobium isolates.

4.2.2: Biomass accumulation study

Results of plant biomass are presented in Table 4.4 revealed that at 45 DAS fresh shoot weight increased due to bacterization of plant. The fresh shoot biomass increased significantly from 7.06 g of (control) to 9.59, 9.23, 9.17, 8.96, 8.81, 8.67, 8.60 and 8.17g due to inoculation with (T3, T4, T5, T6, T7, T8, T2, T1 and T9) Among the treatments fresh shoot weight was recorded 8.17 (gm) per plant in case of T2 where as lowest and 9.59g per plant was highest among the inoculated plants which was association with T3.

Table 4.3 Effect of Bacterial Consortia on plant height (cm) of chickpea

Tr. No.	Name of Isolates	(Avg.) Plant height (cm plant-1) at 15 DAS	Plant height (cm plant- 1) at 30 DAS	Plant height (cm plant- 1) at 45 DAS
T1	Chickpea+100%NPK	12.32	23.23	33.20
T2	Chickpea+75%NPK	12.30	22.92	32.70
T3	Chickpea +C1+75%NPK	16.01	27.25	36.10
T4	Chickpea+C2+75%NPK	15.29	26.33	34.90
T5	Chickpea+GmR8+75%NPK	14.40	25.09	35.40
T6	Chickpea+AZO137+75%NPK	13.08	24.31	34.40
T7	Chickpea+ASL3+75%NPK	11.63	24.60	34.50
T8	Chickpea+ASL4+75%NPK	13.02	24.87	33.10
T9	Control	8.35	19.45	27.10
	SE (m)	0.82	1.07	0.69
	CD	2.26	3.17	2.06

The dry weight of plant increased from 1.37g (control) to 2.52, 2.15, 2.08, 2.01, 1.98, 1.92, and 1.37g per plant when chickpea seeds were grown with treatment T3,T4,T5,T6,T7,T8,T2, respectively. Maximum increase in dry weight was observed by isolate T3, followed by T4 (2.15g), followed by T6 (2.08g), value of plant dry weight was 1.37g per plant in case of uninoculated control while highest value of plant dry weight, 2.52g per plant that was observed in seed inoculated with T3.

A

B

Plate 4.5: Performance of Chickpea at 15DAS (A) and 30 DAS (B).

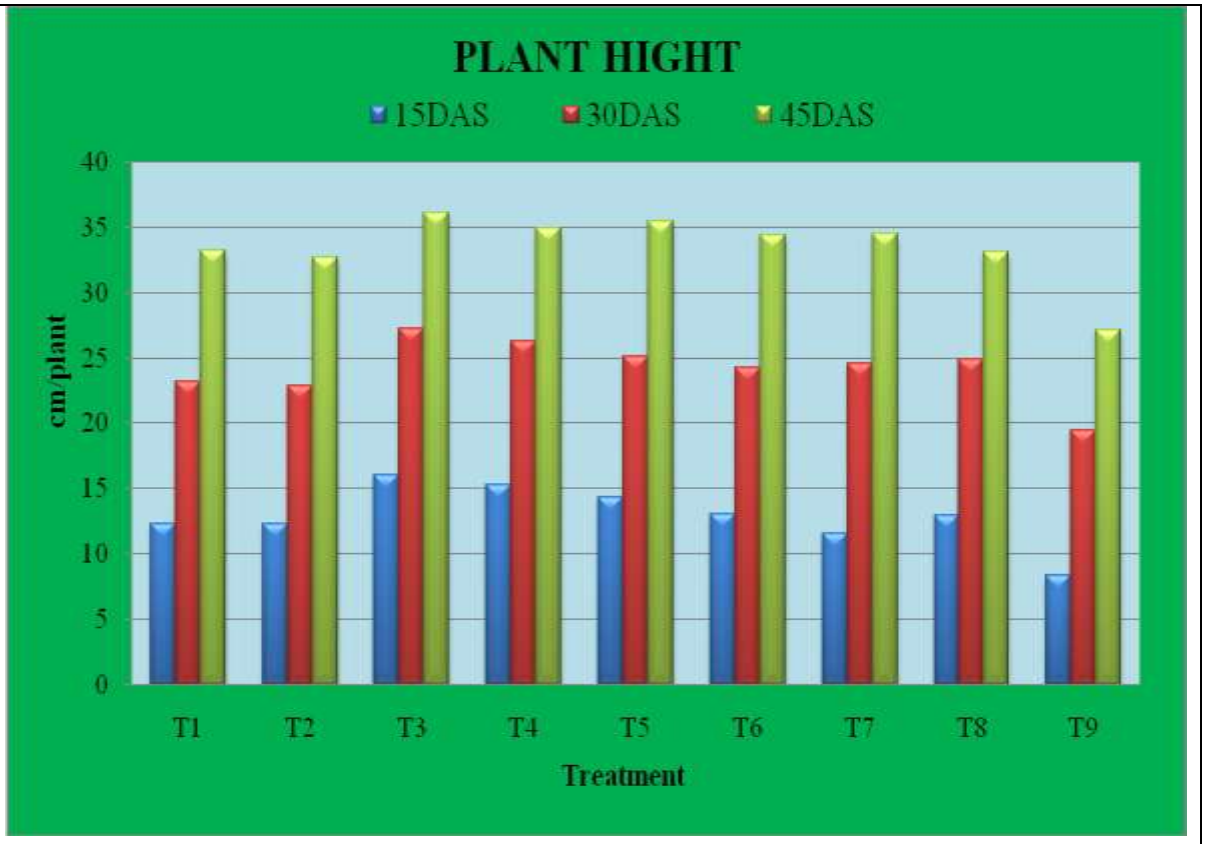


Fig 4.1 Influence of Rhizosphere bacterial consortia on plant height at 15 DAS, 30 DAS and 45 DAS

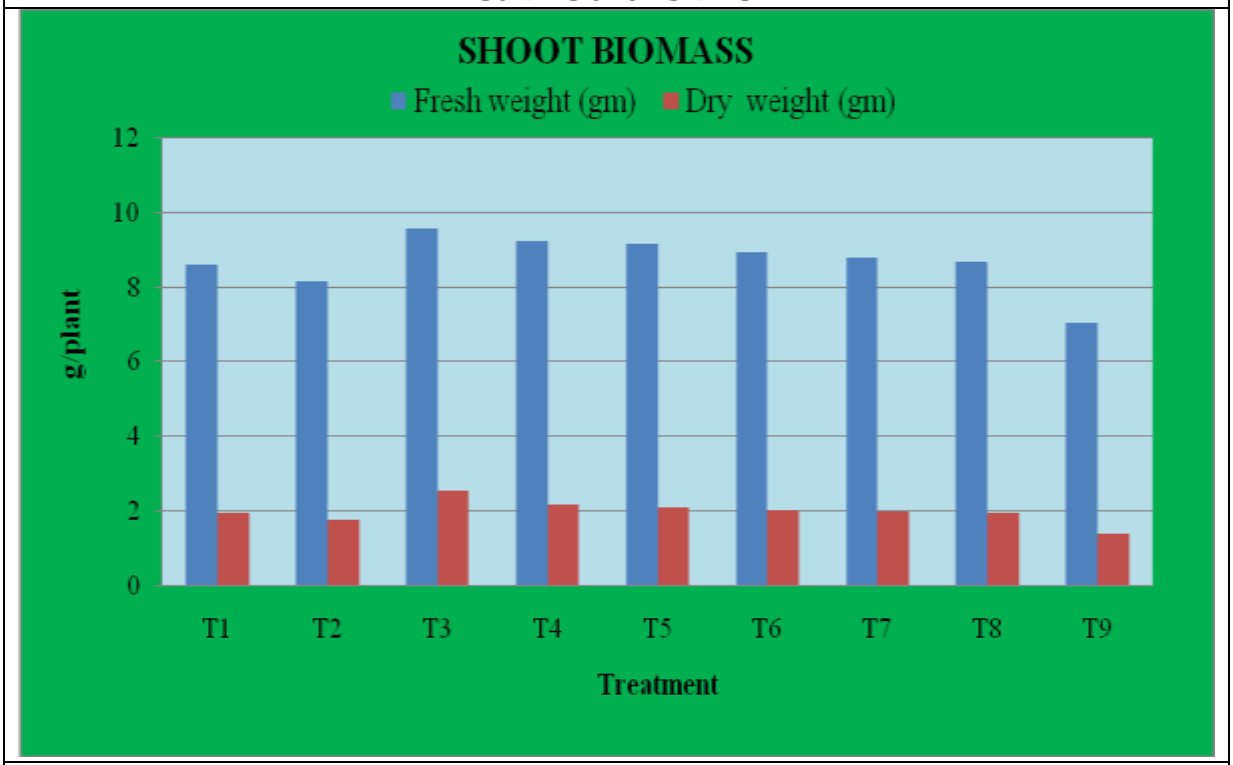


Fig 4.2 Influence of Rhizosphere bacterial consortia on shoot fresh weight and dry weight at 45 DAS

Table.4.4 Effect of composite culture on Fresh weight and dry weight of Chickpea shoot study

Tr. No.	Name of Isolates	Shoot fresh weight (gm plant-1) at 45 DAS (Avg)	Shoot dry weight (gm plant-1) at 45 DAS (Avg
T1	Chickpea+100%NPK	8.60	1.95
T2	Chickpea+75%NPK	8.17	1.77
T3	Chickpea +C1+75%NPK	9.59	2.52
T4	Chickpea+C2+75%NPK	9.23	2.15
T5	Chickpea+GmR8+75%NPK	9.17	2.08
T6	Chickpea+AZO137+75%NPK	8.96	2.01
T7	Chickpea+ASL3+75%NPK	8.81	1.98
T8	Chickpea+ASL4+75%NPK	8.67	1.92
T9	Control	7.06	1.37
	SE (m)	0.36	0.17
	CD	1.09	0.50

There results were in similar trends as in case of fresh weight. Maximum dry weight was associated with T3 whereas T2 was minimum excluding control.

4.2.3 Root length

Data of root length recorded at 45 DAS crop growth presented in Table 4.5 and depicted in Plate 4.3. At 45 DAS some of the microbial isolates alone and in combination found significant to enhance the root growth. However, rest of the microbial isolates alone and in combination found significant affected by applied PGPR at 45 DAS. Performance of treatments T3, T8, T7, T4, T5, T6, T1 and T2 were found well over controls respectively T9 (15.33 cm). Results of root biomass are presented in Table 4.5 revealed that at 45 DAS fresh root weight increased due to bacterization of plant. The fresh root biomass increased significantly from 4.12 (g) of (control) to 7.30, 6.80, 6.48, 6.38, 6.19, 5.94, 5.26, and 4.80 (g) due to inoculation with (T3, T8, T7, T4, T5, T6, T1, and T2). Among the treatments fresh root weight was recorded 4.80 (g) per plant in case of T2 where as lowest and

7.30g per plant was highest among the inoculated plants which was association with T3.

The dry weight of root increased from 0.74 g (control) to 2.71, 2.20, 2.09, 2.07, 2.03, 1.80, 1.39 and 1.37 g per plant when chickpea seeds were grown with treatment (T3, T8, T7, T4, T5, T6, T1, and T2) respectively. Maximum increase in dry weight was observed by isolate T3, followed by T8 (2.20g), followed by T7 (2.09 g), value of plant dry weight was 0.74 (g) per plant in case of uninoculated control while highest value of plant dry weight, 2.71 (g) per plant that was observed in seed inoculated with T3.

Table 4.5: Effect of Rhizosphere bacterial consortia on performance of chickpea. on Root length, biomass fresh weight and dry weight at 45 DAS

Tr. No.	Name of Isolates	Avg. Root length (cm)	Biomass fresh weight (g plant-^{1}) at 45 DAS	Biomass dry weight (g plant-^{1}) at 45 DAS
T1	Chickpea+100%NPK	19.33	5.26	1.39
T2	Chickpea+75%NPK	17.00	4.80	1.06
T3	Chickpea +C1+75%NPK	27.00	7.30	2.71
T4	Chickpea+C2+75%NPK	24.00	6.38	2.07
T5	Chickpea+GmR8+75%NPK	23.67	6.19	2.03
T6	Chickpea+AZO137+75%NPK	23.40	5.94	1.80
T7	Chickpea+ASL3+75%NPK	24.39	6.48	2.09
T8	Chickpea+ASL4+75%NPK	24.72	6.80	2.20
T9	Control	15.33	4.12	0.74
	SE (m)	0.97	0.51	0.35
	CD	2.89	1.53	1.06

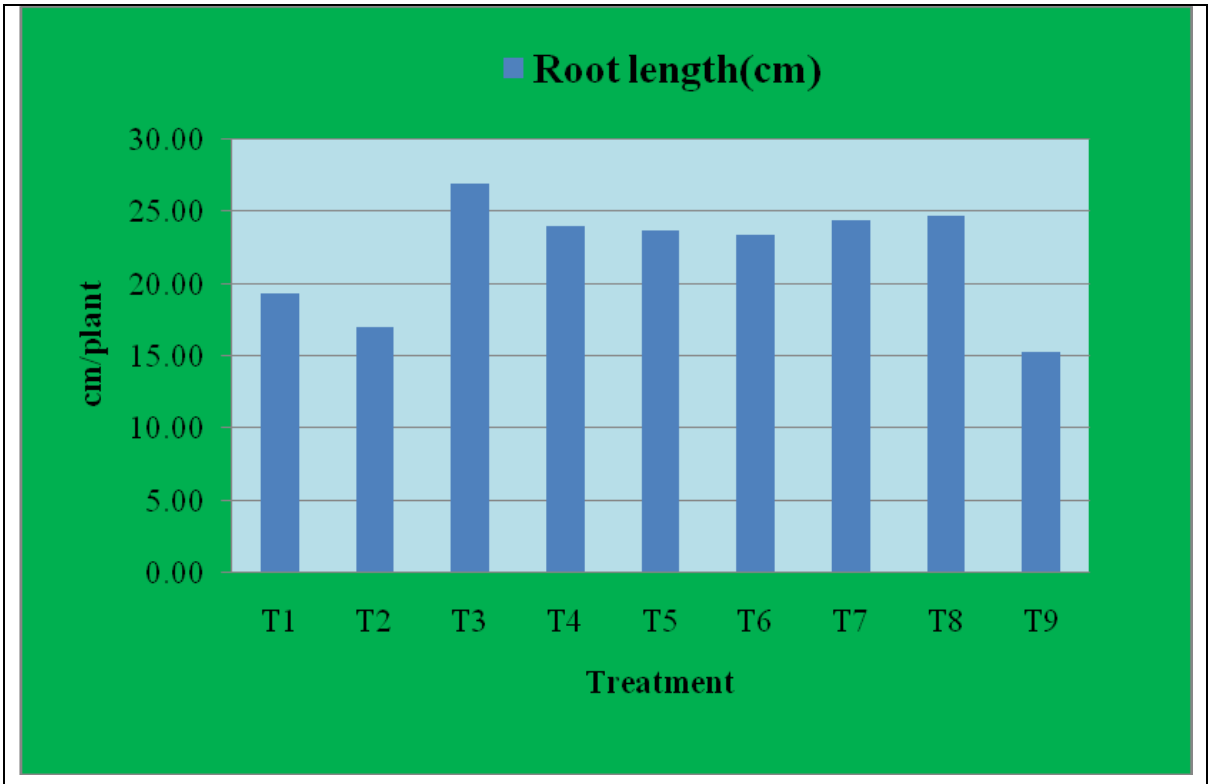


Fig 4.3 Influence of Rhizosphere bacterial consortia on root length at 45 DAS

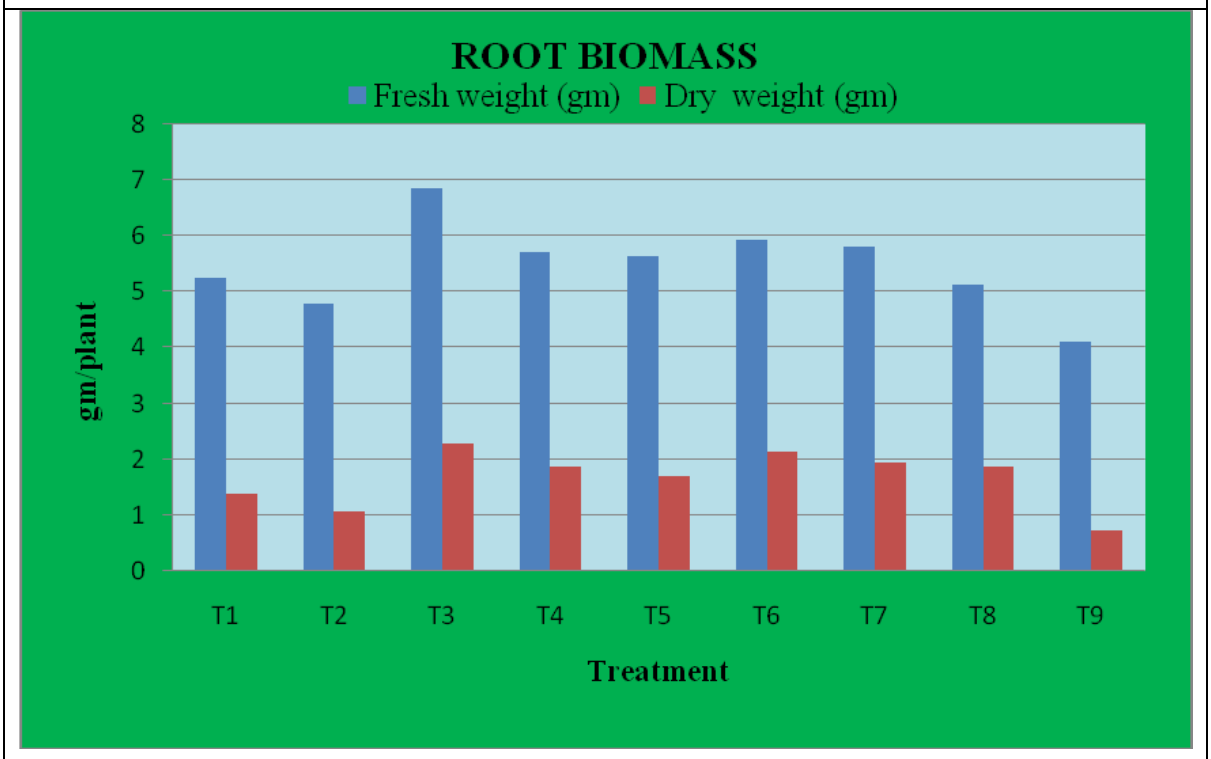


Fig 4.4 Influence of Rhizosphere bacterial consortia on root fresh weight and dry weight at 45 DAS

4.2.4: Nodulation study

Number of nodules

Nodulation of chickpea crop as affected by different soil inoculums as treatments at 45 DAS are presented in Table 4.6. Results indicate that the highest nodulation was found in plants raised from seeds inoculated with the chickpea containing T5. Followed by T3. At 45 DAS number of nodules uninoculates plant was increased from 12.33 to 39.33 due to inoculation of seeds with promising native *Rhizobium* isolates. There was 12.33 nodule in most of the uninoculated control plants. Significantly higher nodulation was observed in T5 (avg nodules per plant are 39.33)

This observation is found PGPR was found significantly better for nodulation, plant growth and yield of chickpea (*Cicer arietinum L.*) over control in a pot and trial. *Rhizobium* strains differ in their ability to nodulate and in their capacity to fix nitrogen. Response differs due to different population levels of native and added strains of *Rhizobium* and due to the competitive ability of native *Rhizobium* with the added strain (Rupela and Dart 1980). Symbiotic nitrogen fixation by *Rhizobium* meets the N2 requirement of the crop and leaves 40-108 kg/ha in the soil (Subba Rao 1976).

Nodule fresh weight

Results of nodules fresh weight are presented in Table 4.6 revealed that at45 DAS fresh nodule weight increased due to bacterization of plant. The fresh nodule weight increased significantly from 18.33 (mg) of (control) to 48.33, 45.00, 42.00, 37.00, 34.00, 32.67, 32.33, and 30.00 (mg) due to inoculation with (T5, T3, T4, T8, T1, T7, T2, and T1) respectively. Fresh nodule weight was recorded 18.33 (mg) per plant as lowest and 48.33 (mg) per plant was highest among the inoculated plants.

Nodule dry weight

Result of nodule dry weight is presented in Table 4.6 The dry weight of plant increased from 6.33 (mg) (control) to 22.67, 19.33, 15.33, 14.00, 12.67,

10.00, and 9.33 (mg) per plant when chickpea seeds were grown with soil inoculums numbered treatment. (T5, T3, T4, T8, T1, T7, T2 respectively.

Maximum increase in dry weight was observed by isolate.T5, followed by T3 (19.33 mg), followed by T4 (15.33 mg), value of nodule dry weight was 6.33 (mg) per plant in case of uninoculated control while highest value of plant dry weight, 22.67 (mg) per plant that was observed in seed inoculated with T3. Hossain et al. (1999) observed that nitrogen up to 20 lb/acre increased the number and weight of nodules.

Table 4.6 Nodulation study of chickpea in Pot Experiment

Tr. No.	Name of Isolates	(Avg.) Number of Nodule (plant-^{1})	Nodule fresh weight (mg plant-^{1}) at 45 DAS	Nodule dry weight (mg plant-^{1}) at 45 DAS
T1	Chickpea+100%NPK	29.67	34.00	10.00
T2	Chickpea+75%NPK	27.00	32.33	9.33
T3	Chickpea +C1+75%NPK	38.00	45.00	19.33
T4	Chickpea+C2+75%NPK	35.43	42.00	15.33
T5	Chickpea+GmR8+75%NPK	39.33	48.33	22.67
T6	Chickpea+AZO137+75%NPK	27.00	30.00	12.67
T7	Chickpea+ASL3+75%NPK	28.67	32.67	9.33
T8	Chickpea+ASL4+75%NPK	30.17	37.00	14.00
T9	Control	12.33	18.33	6.33
	SE (m)	1.22	1.44	0.77
	CD	3.64	4.29	3.72

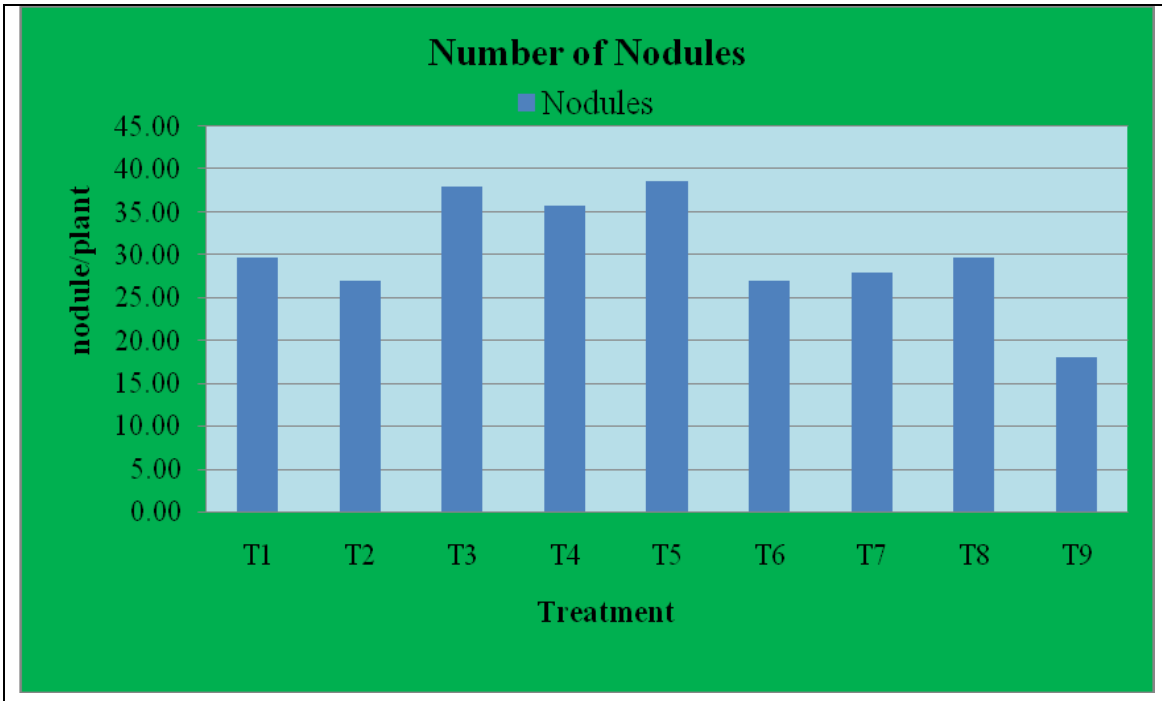


Fig 4.5 Influence of Rhizosphere bacterial consortia on number of nodule at 45 DAS

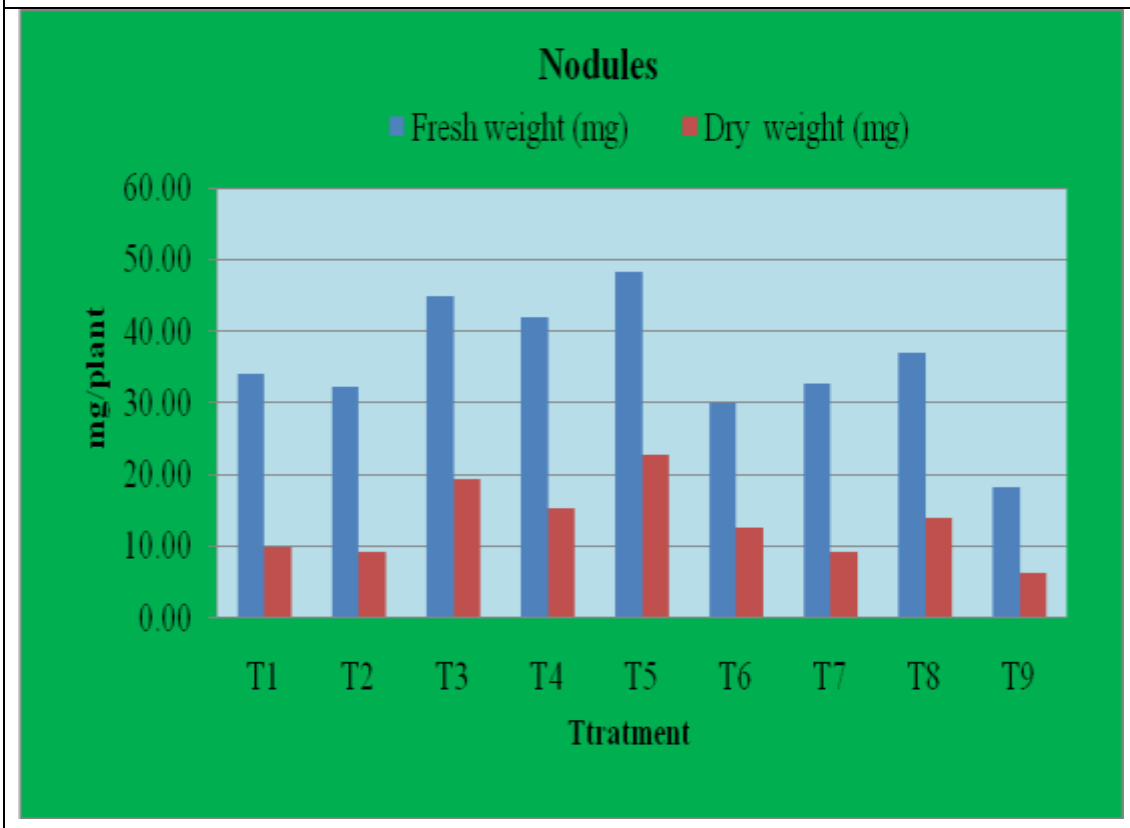


Fig 4.6 Influence of Rhizosphere bacterial consortia on nodule fresh weight and dry weight at 45 DAS

4.2.5: Nitrogen accumulation in shoot of chickpea plants

Data of table 4.7 clearly indicated that N- content (shoot nitrogen content) at 45 DAS was increased from 1.87 at control to 9.48 percent due to seed inoculation. Plant shoot was recorded as highest at T5 (9.48) followed by T4 (7.44) while among inoculated minimum was found at T8 (4.64) respectively. Value of N-content Nodule by 45 DAS of chickpea was increased from 1.14 percent at control to 5.77 percent due to seed inoculation with soil inoculums nodule was recorded as highest at T5 (5.77) followed by T3 (5.56) while among inoculated minimum was found at T2, (2.83) respectively. Lowest value of N – nodule was (2.83) and highest was (5.77) by inoculated plant raised under green house mixed culture conditions (Table 4.7)

Table 4.7 Effect of Rhizosphere bacterial consortia on performance of Chickpea on Nitrogen accumulation at 45 DAS

Tr. No.	Name of Isolates	% of Nitrogen in plant at 45 DAS	% of Nitrogen in nodule at 45 DAS
T1	Chickpea+100%NPK	6.07	3.70
T2	Chickpea+75%NPK	5.59	3.40
T3	Chickpea +C1+75%NPK	9.14	5.56
T4	Chickpea+C2+75%NPK	7.44	4.53
T5	Chickpea+GmR8+75%NPK	9.48	5.77
T6	Chickpea+AZO137+75%NPK	5.71	3.48
T7	Chickpea+ASL3+75%NPK	6.16	3.75
T8	Chickpea+ASL4+75%NPK	4.64	2.83
T9	Control	1.87	1.14
	SE (m)	0.16	0.09
	CD	0.85	0.52

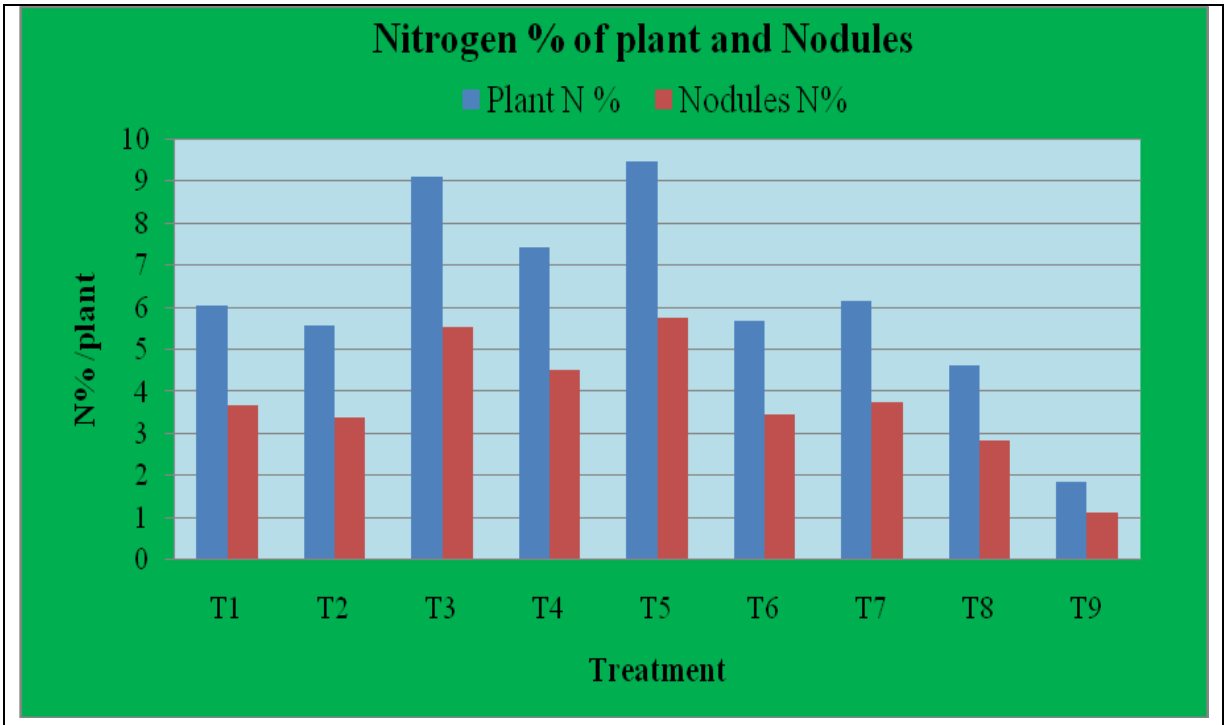
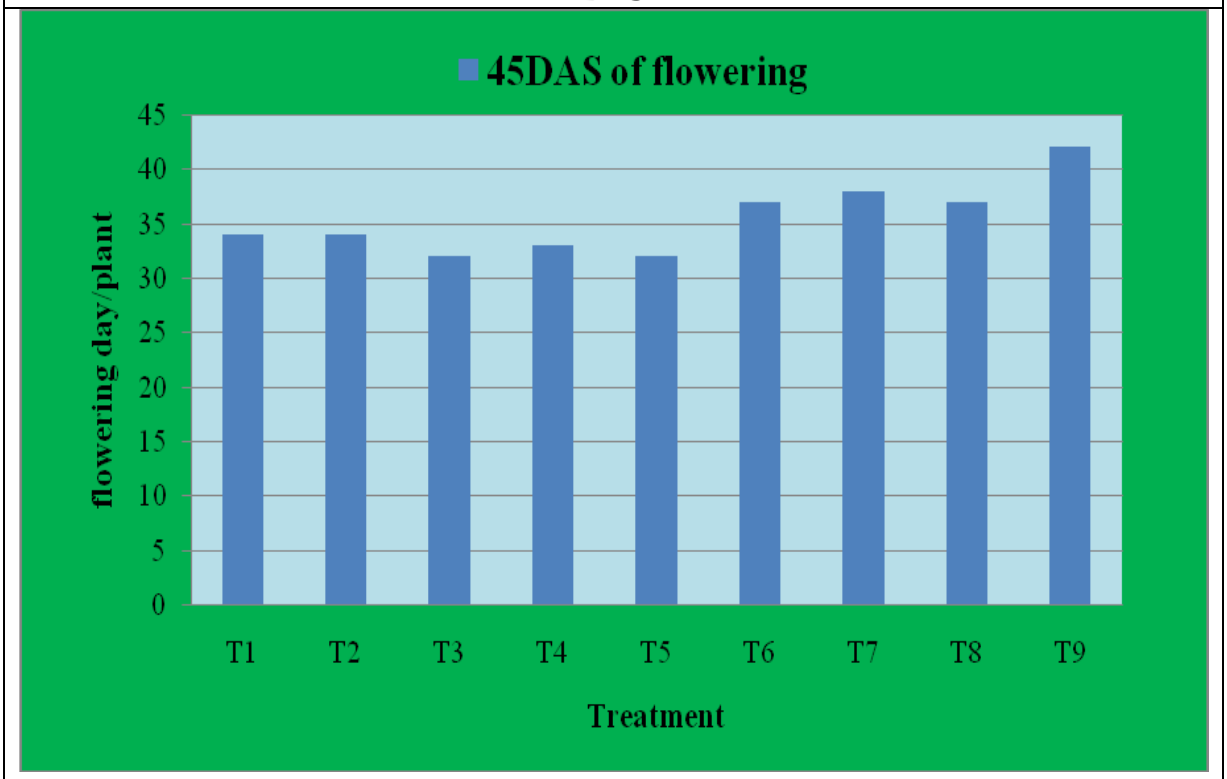


Fig 4.7 Influence of Rhizosphere bacterial consortia on N % of plant and nodule at 45 DAS



4.8 Influence of Rhizosphere bacterial consortia on Duration of 50% flowering at 45 DAS

4.2.6: Duration 50% flowering

Chickpea flowering is duration of by different soil inoculums as treatments at 45 DAS are presented in Table 4.8 Indicates that the first flowering was found in plants raised from seeds inoculated with the chickpea containing T3. Followed by T5, T4, T1, T2, T7, T6, and T9 (42 days) after flowering at 45 DAS. Significantly higher flowering was observed in T3 (32days) in case of uninoculated control while 42 days in flowering starts.

Table4.8 Effect of Rhizosphere bacterial consortia on performance of chickpea. on Duration of 50% flowering in 45 DAS.

Tr. No.	Name of isolate	50% Duration DAS
T1	Chickpea+100%NPK	34
T2	Chickpea+75%NPK	34
T3	Chickpea +C1+75%NPK	32
T4	Chickpea+C2+75%NPK	33
T5	Chickpea+GmR8+75%NPK	32
T6	Chickpea+AZO137+75%NPK	37
T7	Chickpea+ASL3+75%NPK	38
T8	Chickpea+ASL4+75%NPK	37
T9	Control	42

	
T1 100% NPK	**T2 75% NPK**
	
T3 C1+75% NPK	**T4 C2+75% NPK**

	
T5 GmR8+75%NPK	**T6 AZO137+75% NPK**
	
T7 ASL3+75% NPK	**T8 ASL4+75% NPK**

Plate 4.6 Root length behavior of chickpea at 45 DAS

T1 100% NPK

T2 75% NPK

T3 C1+75% NPK

T4 C2+75% NPK

T5 GmR8+75%NPK

T6 AZO137+75% NPK

T7 ASL3+75% NPK

T8 ASL4+75% NPK

CONTROL

Plate 4.7 Effect of Rhizosphere bacterial consortia on performance of Chickpea on Plant height inocula at 45 DAS.

Crops grown under rainfed conditions are prone to water stress, due to rapid loss of soil moisture and development of mechanical impedance to root growth (Singh and Rana, 2006). Agriculture could take advantage of symbiotic relationships between plants and microbes to enhance plant productivity and crops by manipulating the composition of soil microbial communities (Woomer *et al*., 2017, Asei *et al.,* 2015). Pérez-Fernández and Alexander (2017) reported that chickpea plants inoculated with indole acetic-producing bacteria and Rhizobium rendered more plant biomass, flowers, and viable seed than those inoculated with only one of the bacterial strains and that the level of IAA produced by the selected strains might have an effect on the total plant performance. The observed promotion in root nodulation of plant in this study could be attributed to the cumulative effects of these rhizobacteria. Similar results were obtained by Wani *et al*., (2007). Further, *Azospirillum* is one of the best characterized genera among associative plant growth promoting rhizobacteria and the bacterial strains of this genus are able to exert beneficial effects on plant growth and yield of many agronomic crops (Okon and Vanderleyden, 1997; Steenhoudt and Vanderleyden, 2000). Similarly, *Azotobacter* spp. are free-living and nitrogen fixing bacteria which under appropriate conditions can enhance plant development and promote the crop yield (Rodelas *et al*., 1999). The positive effect of inoculation with PGPR strains on growth of chickpea was reported by many workers (Valverd *et al*., 2006; Malik and Sindhu 2011; Rokhzadi and Toashih 2011). Present investigation also revealed that use of PGPRs with seed treatment improve seed germination, seedling emergence, seedling vigor and seedling stand over the control. Similar results have been reported in other crops such as potato, radish plants, sorghum and pearl millet (Burr *et al*., 1978; Raju *et al*., 1999; Niranjan *et al*., 2004)

In the present investigation, the number of nodules and weight of nodules were found consistently and significantly higher in the selected consortia-treated pots over un-inoculated control. The mechanisms associated with PGP by bacteria include secretion of PGP hormones such as indole acetic acid (IAA), chelation of iron by producing compounds such as siderophore, solubilization of phosphorous and antagonistic potential against phytopathogens (Panhwar *et al*. 2012; Sreevidya *et al*., 2016).

It can be concluded that the bacterial isolates studied in this investigation were apparently well adapted to the growth of chickpea, in addition to their adaptability in other crops.

CHAPTER – V

SUMMARY AND CONCLUSIONS

The present investigation entitled **"Interactive effect of Rhizosphere bacterial consortia on performance of chickpea)"** was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur, Chhattisgarh during *Rabi* season, 2016-17 comprising 9 treatments (treatments were different bacterial culture and control without inoculate in bacteria.and 3 replications in CRD with the objective to find out the effective Rhizosphere bacterial consortia with chickpea plants variety JG-14.

The results have been furnished and discussed in the preceding chapter. The salient findings of the investigation have been summarized in this chapter in following points:

1. Study under composite culture experiment in 9 treatments and 3 replication of pot culture in glass house conditions at 45 DAS.

2. A 45 days pot experiment was conducted to study to effect of composite culture on chickpea treatment 15 days clearly showed that highest plant height (14.77 cm) was associated with isolate T9 followed by isolate T3 (16.01 cm plant $^{-1}$) and control plant height T9 (8.5 cm plant $^{-1}$). was recorded as lowest. At 30 Days T3 (27.25 cm plant $^{-1}$) was recorded as highest associated with T4 followed by (26.33 cm plant $^{-1}$) and plant height of control was T9 (19.45 cm plant $^{-1}$). At 45 Day was recorded as highest T3 (36.10 cm per plant) followed by T5 (35.40 cm plant $^{-1}$) and plant height of control was 27.10 cm per plant. It is treatment T3 and treatment T5 significant to the other treatment.

3. Shoot Fresh weight of gm is in T3 (9.59 gm) followed by treatment T4 (9.23 gm) Dry weight (gm) is highest in treatment T3 (2.52 gm) followed by treatment T4 (2.15 gm) and control treatment is 1.37 gm is lowest.

4. Root length in treatment T3 (27 cm plant^{-1}) followed by T4 (24 cm plant^{-1}) and average root length in control is T9 (15.33cm plant^{-1}). Fresh root biomass is T3 (7.30 g plant^{-1}) followed by T8 (6.80 g plant^{-1}) and average fresh root biomass in control T9 (4.12 g plant^{-1}). Dry root biomass is T3 (2.71 g plant^{-1}) followed by T8 (2.20 g plant^{-1}) and average control is T9 (0.74 g plant^{-1}).

5. At 45 Day highest nodulation was recorded in T5 (39.33 per plant) followed by T3 (38) and plant nodule of control was 12.33 per plant.

6. Nodule study under pot experiment revealed that the highest fresh nodule weight was T5 (48.33 mg per plant) followed by T3 (45 mg per plant) and fresh weight of control was (18.33 mg) dry weight in T5 (22.67 mg) followed by T3 (19.33 mg) and dry weight of control was 6.33(mg).

7. N percent accumulation highest of plant in treatment T5 (9.48%) followed by T3 (9.14%) and control treatment is 1.87 % is almost lowest from above and nodule N content in T5 (5.77) percent due to seed inoculation with soil inoculums was recorded followed by T3 (5.56) and control was T9 (1.14) percent.

8. At 45 day was recorded as first flowering was duration of T3 32 DAS followed by T4 33 DAS and in control was T9 42 days in flowering starts.

Conclusion;

The result revealed that composite culture C1 i.e. (GmR8+Azo137+ASL3+ASL4) significantly increase the root, shoot length and biomass of seedlings as compared to the control. In nodulation study highest nodulation was observed with treatment T5 i.e. C1+75%NPK however nodulation in treatment associated with C1 composite group were also very good and data also related that there is no much effect on nodulation due to the presence of other member of consortia.

Sugesstion for future;

Investigated carried out that composite consortia is give better performance than any single one biofertilizer and more effective in crop growth. For applied in field condition it is suitable for crops as recommened as compare to chemical fertilizer and single biofertilizer.

1. Combination of plant rhizobia or composite culture are good viable in chickpea crops and may also be effective with other crops to the chhttisgrh region and climate adaptability for the farmer.

2. Composite culture are well known for multiple PGPR activity i.e. N2 production, phosphate solubilization, IAA production as well as siderophore production.

3. It is evident from the study that use of composite culture is better than the individual culture. Farmer field trial should conduct to test the best composition of culture under field condition.

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APPENDIX A

Station: Labhandi Weekly Meteorological Data: Dec.2016 to Feb. 2017

S. No.	Month	Max. Temp. (°C)	Min. Temp. (°C)	Glass house Temp. (°C)	Rain-fall (mm)	Relative Humidity (%)		Vapour Pressure (mm of Hg)		Wind Velocity (Kmph)	Evapo-ration (mm)	Sun Shine (hours)
						I	II	I	II			
1	December 1	28.9	14.3	26.3	0.0	90	39	11.3	11.2	1.5	3.6	7.4
2	2	28.8	11.9	26.2	0.0	83	29	9.5	8.3	2.0	8.1	8.1
3	3	27.5	8.6	25.4	0.0	87	24	7.9	6.1	1.5	5.3	8.5
4	4	28.2	9.9	26.1	0.0	86	26	8.3	7.4	1.1	2.8	7.4
5	January 1	28.6	12.2	27	0.0	90.4	32.4	10.0	8.8	1.5	2.8	6.8
6	2	27.2	11.9	26	5.6	84.6	34.9	9.5	8.5	2.0	3.0	7.0
7	3	28.9	11.8	26.5	0.0	84.6	27.0	9.3	8.0	1.2	3.1	8.0
8	4	29.9	14.3	27	0.0	83.4	29.4	10.9	8.7	1.9	3.6	7.7
9	Feburary 1	31.6	13.1	29	0.0	81.0	26.1	9.7	8.5	1.6	9.2	9.4
10	2	31.0	15.3	28.5	5.6	8.4	32.6	11.2	10.1	2.6	4.2	7.2
11	3	32.9	15.1	30	0.0	76.9	19.9	10.8	6.9	2.1	5.2	9.3
12	4	33.7	15.0	31	0.0	67.1	16.1	9.4	6.0	2.5	6.2	10.2

APPENDIX B

Chemical composition of media

Yeast Extract Mannitol Agar Media (YEMA) for *Rhizobium* (SubbaRao, 1988)

Ingredients	**gm/Lit**
Mannitol	10.0 g
K2HPO4	0.5 g
MgSO4.7H2O	0.2 g
NaCl	0.1 g
Yeast extract	1.0 g
Agar	15.0 g
Distilled water	1000.0ml
Congo red solution	0.5ml
Ph	7.0

Okons Media for ***.Azospirillum***

Ingredients	**gm/Lit**
Malic acid	5.0gm
KOH	4.0gm
K2HPO4	0.5gm
MgSo4	0.1gm
NaCl	0.02gm
CaCl2	0.01gm
FeSo4	0.05gm
Na2MoO4	0.002gm
MnSo4	0.01gm
0.5% BTB	2.0gm

Jensen ‘s Agar Media for *Azotobactor*

Ingredients	**gm/Lit**
Sucrose	20gm
K2HPo4	1gm
MgSo4	0.5gm
NaCl	0.5gm
FeSo4	0.1gm
Na2MoO4	0.001gm
CaCo3	2.0gm
Agar	15.0gm
Distilled Water	1000ml
pH	7.0-7.2

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Awards / Recognition	: NO
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