Characterization and evaluation of *Rhizobium* isolated from root nodules of pea (*Pisum sativum* L.) and its effect on growth and yield of pea

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF

Master of Science (Agriculture)

in

Soil Science and Agricultural Chemistry

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Submitted by
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CERTIFICATE

To

The Joint Registrar (Academic),
Office of the Registrar (Academic),
Banaras Hindu University,
Varanasi-221 005 (India)

Through: The Head, Department of Soil Science and Agricutural Chemistry,
Institute of Agricultural Sciences,
BHU, Varanasi, 221 005.

Sir,

I have great pleasure in forwarding the thesis entitled "**Characterization and evaluation of *rhizobium* isolated from root nodules of pea (*Pisum sativum* L.) and its effect on growth and yield of pea"** submitted by **Mr. Shubham Sambhav Kamlapuri**, **Examination Roll No. 18412SAC017; Enrolment No. 365559** in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in **Soil Science and Agricultural Chemistry**, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) and placing on record that he has completed the requisite residential requirements as contained in the statutes of the university.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance and supervision. The data presented in thesis, to the best of my knowledge and belief, are genuine and original.

Thanking you,

Yours faithfully,

Forwarded by

(Prof. Janardan Yadav)
Supervisor

Head

BHU capital of knowledge

स्वच्छ भारत
एक कदम स्वच्छता की ओर

Characterization and evaluation of *Rhizobium* isolated from root nodules of pea (*Pisum sativum* L.)

by

Shubham Sambhav Kamlapuri

Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science (Agriculture)
in
Soil Science

DEPARTMENT OF SOIL SCIENCE AND AGRICUTURAL CHEMISTRY
INSTITUTE OF AGRICULTURAL SCIENCES
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Contents

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
°C	:	Degree centigrade
/	:	Per
@	:	At the rate of
B	:	Boron
C.D.	:	Critical difference
cm	:	Centimetre
CRD	:	Completely Randomized Design
Cu	:	Copper
d. f.	:	Degree of freedom
DAP	:	Di ammonium phosphate
DAT	:	Days after transplanting
dSm^{-1}	:	Deci Siemen per meter
e.g.	:	For example,
EC	:	Electrical conductivity
et al.	:	And others
Fig.	:	Figure
FYM	:	Farm Yard Manure
g	:	Gram
ha	:	Hectare
HI	:	Harvesting index
hrs	:	Hours
i.e.	:	Which is to say, in other words
K	:	Potassium
kg	:	Kilogram(s)
m^{-2}	:	Per square metre
Max	:	Maximum
mg	:	Milligram
Min	:	Minimum
mL	:	Milliliter
Mn	:	Manganese
N	:	Nitrogen
No.	:	Number
No. m^{-2}	:	Number per square metre
NS	:	Non-significant
pH	:	Puissance he hydrogen
ppm	:	Parts per million
SEm±	:	Standard error of mean

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Chapter I

INTRODUCTION

Pulses are among the ancient food crops with evidence of their cultivation for over last 8,000 years. Besides being a rich and the cheapest source of dietary protein and a valuable animal feed, they also play a key role in improving and sustaining soil productivity on account of biological nitrogen fixation. Being the largest producer in the world, India accounts (25 % of global production), consumer (27% of world consumption) and importer (14%) of pulses. It produces 14.76 million tons from an area of 23.63 million hectares. However, about 2-3 million tons of pulses are imported annually to meet the domestic consumption requirement. Though pulses are grown in both kharif and rabi seasons. Rabi pulses contribute more than 60% of the total production. Thus, there is a need to increase production and productivity of pulses in the country by more intensive interventions.

Garden pea *(Pisum sativum* L.) is one of the most popular and remunerative vegetable of rabi season. It is the second most important legume crop of the world (Pawar *et al.*, 2017). It belongs to the family Leguminoceae. In Asia and Europe, it is known to be one of the oldest pulse crops cultivated. Being a legume, it helps in maintaining soil fertility, and also the capacity of fixing atmospheric nitrogen through root nodule bacteria incited by *Rhizobium* Pea provides a variety of vegetarian dishes and hence it is liked throughout the world. Janich *et al.* (1969) described association of genus *Pisum* with man at least from Stone Age. It may be classified into two classes' viz. (1) garden or table pea *{Pisum sativum* var. *hortense.).* Instead of Green seeds, this type is also used for vegetable purpose and for canning and (2) field pea *(Pisum sativum* var. a*rvense).* Mature seeds of this type are used as 'dal', and also used for green manuring. Garden peas are harvested in an immature condition to be cooked as green peas to provide a delicious dish, or to be canned or frozen for subsequent during off season. It is mixed with dry fodder namely 'karabi' or 'bhusa' to make a palatable feed for the animals. It is very much nutritive and contains 7 to 9 % protein, 56.6 to 62.1% carbohydrates, 1.5 to 1.8 % fat and appreciable proportion of calcium, iron, phosphorus and vitamins B1, B2 and niacin (Choudhury, 1967).

India is the 5th largest producer of peas in the world. Apart from India, other major producers of peas are USA, China, France, UK etc. The major peas growing states in India are Uttar Pradesh, Bihar, Haryana, Punjab, Himachal Pradesh, Orissa and Karnataka. In Uttar Pradesh, it is cultivated throughout the state in area of 23.3 thousand hectare having production of 2511.38 thousand tonnes, which shares 46.37 % of the total production of the country (National Horticulture Board, 2017-2018). Aligarh, Kashganj, Varanasi, Bundelkhund etc. are the some of the major area in the state.

The various agronomical practices like sowing time, *Rhizobium* inoculation, spacing, seed rate, fertilizer application, insect pest and disease control, weed management etc. plays an important role in maximization of pea production per unit area. The continuous use of NPK fertilizers under intensive cropping system has caused adverse effects on soil physical, chemical and biological properties 'thereby' affecting the sustainability of crop production, besides causing environmental pollution (Virmani, 1994).

For sustainable production system, microbial inoculants are vital resources, which can boost the overall nutrient turnover for the crop production. Microorganisms induce many biochemical transformations in the soil. These include mineralization of organically bound form of nutrients, exchange reactions, fixation of atmospheric nitrogen and various other changes leading to better availability of nutrients already present in the soil. Nutrient enrichment of soil by nitrogen fixing symbiotic bacteria present in legumes roots has been known for centuries. Scientific demonstration of this symbiosis was started in 19thcentury and it established the fact that bacteria present in nodules on legume roots are responsible for fixing atmospheric nitrogen.

Bioinoculants play an important role as these are eco-friendly, low cost and non-bulky agricultural inputs (Sen and Patel, 2004). Use of microbial fertilizers as a source of N and P can also minimize dependence on chemical fertilizers. Moreover, consumption of chemical fertilizer nitrogen not only depletes nonrenewable energy resources but also leads to environmental pollution (Peoples *et al*., 1994). So, to enhance biological nitrogen fixation and crop productivity, rhizobial inoculants have been considered to be of prime importance. The symbiosis is not only beneficial for the associates but ultimately when the legume gets benefited, the human economic concern is benefited. Rhizobial inoculants have contributed to increase yield and N2 fixation in legume crops that represents 70-80 % of total nitrogen in plants (Catroux*et al*., 2001).

Being a legume crop pea has an excellent advantage of fixing atmospheric nitrogen symbiotically with *Rhizobium*, a bacterium, which converts atmospheric nitrogen into the available form. The *Rhizobium* inoculation is a cheap, easy and safe method of supplying nitrogen to legumes and the yield of peas can be considerably increase by using an effective inoculum properly. Like other bacteria, Rhizobia multiply in laboratory culture and now it is possible to produce them on a commercial scale for inoculation purposes (Singh and Choubey, 1971).

Rhizobium culture is undoubtedly the best known and popular biofertilizer used in leguminous crop like pea. It fixes atmospheric nitrogen symbiotically with leguminous vegetable crops or with other legume crops. They usually enter into root hairs, multiply inside and form nodules. The beneficial effect of *Rhizobium* inoculation under the field conditions is noticed by increase in the yield of the subsequent vegetable crops.

The *Rhizobium* (family Rhizobiaceae) in an aerobic rod shaped (0.5~0.9 x 1.2-3 microgram), gram negative, motile with one polar or sub-polar flagella or 2-6 *peritrichous* flagella. Thecells of *Rhizobium* are host specific and invade the root nodules symbiotically. The bacteroide zone becomes pink due to accumulation of leg-hemoglobin in the nodular tissues. These bacteroides are remained in *in situ* condition.

According to Hardy and Holsten (1972) nodule bacteria in association with leguminous plant can fix 90 x 10^6 metric tonnes of nitrogen annually. Interaction among soil micro-organisms, competition for their survival, nutrition and soil environment are some of the important factors which affect survival of *Rhizobium* in the soil and rhizosphere of leguminous plants. These factors are known to play an important role in the formation of nitrogen fixing nodules depending on compatibility of host and rhizobial strain used.

In eastern part of Uttar Pradesh, pea is one of the important pulses grown in rabi season for green vegetable as well as for grains. The amount of nitrogen as required by this crop is mostly dependent upon symbiotic N_2- fixation. The amount of nitrogen being fixed by pea is governed by the efficiency of indigenous rhizobial strain present in soil and other physic-chemical properties of soil and micro-climatic condition of the area. Beside that inoculation of pea seed with *Rhizobium* have shown more effectiveness for N_2 management. Since, effective rhizobial strains of *Rhizobium* well tested for Varanasi region is not available while, strains brought from other area may not be highly effective, therefore, keeping these facts in view the present research work entitled "**Characterization and evaluation of *Rhizobium* isolated from root nodules of pea (*Pisum sativum* L.) and its effect on growth and yield of pea"** has been undertaken with following objectives:

1. Isolation of *Rhizobium* from the root nodule of the healthy pea plants grown in different part of district Varanasi.
2. Initial screening of effective *Rhizobium* isolates using Leonard jar technique.
3. Selection of the most effective *Rhizobium* isolates based on response of nodulation, plant growth and yield of pea under pot experiment.
4. Studies on effect of rhizobia isolates on Avaiable N content and its effect on physio-chemical properties of soil.

Chapter II

REVIEW OF LITERATURE

Legumes play an important role in agriculture. Their importance can be broadly divided into economical, biological and nutritional. The biological role of legumes was firmly established by Beijerink (1888) who isolated the bacteria from the root nodules and named the organism as *Rhizobium.* It has been estimated that every hectare of land has 6400 kg of elemental nitrogen in the atmosphere. Most of the plants belonging to leguminosae can fix atmospheric nitrogen through the legume-rhizobial symbiosis.

Biological nitrogen fixation (BNF) has been estimated to contribute more than 175 million tonnes of N out of which legume N_2 fixation accounts for almost 40% (Burns and Hardy, 1975). Although, the role of legumes in improving soil fertility was known since long, it was only much later that the organism responsible was *Rhizobium.* Presently, only 8.9% of the 14000 or so known species of leguminous plants have been examined for nodulation and less than 0.5% have been studied relative to their symbiotic relationship with nodule forming bacteria. During last five decades, a large number of workers namely Brockwell *et al. (1982);* Howieson and Ewing (1986) and Keyser *et al.* (1992) have listed desirable characteristics in rhizobial strains for inoculant production and utilization. These include not only the ability to fix nitrogen (N) but also the genetic stability, compatibility with agro-chemicals tolerance to stress etc. Another aspect of its importance is the inoculum production technology. Perusal of literature reveals that more emphasis is being given to biological nitrogen fixation (BNF) technology for improving leguminous crop.

2.1 *Rhizobium* as plant growth promoting rhizobacteria

Most of the inoculation studies have focused on free-living diazotrophs, although a few reports indicate that rhizobia can act as plant growth promoting rhizobacteria (PGPR). PGPR influence crop growth and development and yield directly by producing plant growth regulators and by changing the physiological status and morphological characteristics of inoculated roots (Edson *et al*., 2008 and Geneva *et al*., 2006) that favored improved nutrient uptake.

Ham *et al.* (1971) reported that inoculation of effective *Bradyrhizobium japonicum* strains on to soybean was important for maximizing the crop in the soils which lack or are low in indigenous *B. japonicum*. Likewise earlier Erdwan and Wilkins (1928) reported that inoculation of a leguminous crop with effective and appropriate strains of *Rhizobium* is necessary when it is grown in soil for the first time.

Hafeez *et al. (*2004) studied growth response to Co-inoculation by *Bradyrhizobium* strains and *Azotobacter* which increased the rate of seedling emergence by 3-9%, shoot dry weight by 48%, biomass by 75% and N-uptake by 57% due to inoculation with both the *Rhizobium leguminosarum* and *Azotobacter*. The results showed that *Bradyrhizobium* strains promoted cotton growth through efficient nutrient uptake, which was mainly related to increased root growth due to effect of indole 3-acetic acid (IAA) produced by these strains.

Albayrak *et al*. (2006) demonstrated the effect of inoculation with *Rhizobium leguminosarum* on seed yield components of common vetch. It was found that inoculated common vetch cultivars gave higher biological yield (8.5%), seed yield (7.6%), straw yield (10.4%), pod length (25.5%), number of seed per pod (16.2%), number of pods (28.4%), main stem length (3.5%) and thousand seed weight (5.5%) when compared with non- inoculated leguminous cultivars.

Sogut and Tahsin (2006) studied the effect of *Rhizobium* inoculation and N-fertilizer on N accumulation and yield in soybean (*Glycine max*. L.). Their result indicated that inoculation increased N content and dry matter in seed and vegetative parts (stems and leaves), N harvest index and seed yield. Also the interaction between inoculation and soybean cultivars (maturity group) showed that inoculation was more effective on the late maturity cultivars for seed yield.

Ali *et al.* (2008) carried out a pot experiment in a net house to see the response of the inoculation with different plant genotypes. Three varieties of garden pea viz BARI-1, BARI-2, BARI-3 and rhizobial inoculums were used for the experiments. Each variety was tested with and without inoculation. Their results showed increase in nodule number, root weight, shoot weight compared to uninoculated plants.

Babajide *et al.* (2008) conducted greenhouse experiments to determine the effect of rhizobial and mycorrhizal species on growth, nodulation and biomass yield of soyabean. Factorial experiments with two levels of soil (sterile and non-sterile) inoculation with *Glomus clarum* (with and without) and inoculation with rhizobia strains and control was maintained. The effect of combined nodulation was particularly more effective with combined (mycorrhiza + *Rhizobium*) inoculation which had high plant height (62.8 cm), stem circumference (2.94 cm), number of leaves (39.0), shoot dry weight (4.6 g) while compared to control had 33.2 cm, 0.60 cm, 15.0 and 4.4 g respectively with increase in nodulation.

Garcia *et al.* (2008) showed the potencial of *Azospirillum* to enhance the nodulation and plant growth of legumes when co-inoculated with *Rhizobium*. In this study, on farm field experiment in different regions of Cuba were conducted to evaluate agronomic relevance of the differences in response to *Azospirillum-Rhizobium* co-inoculation between the two genotypes. Their results showed that *Azospirillum-Rhizobium* co-inoculation as compared to the single *Rhizobium* inoculation increased the amount of fixed N and yield across the sites.

Edson *et al.* (2008) evaluated a method by spraying inoculant of *Bradyrhizobium* over soyabean plants. It was observed that inoculation by spraying on covering increases nodulation and plant dry matter in 45 and 60 days after plant emergence and also significantly increased the grain yield and N content as compared to uninoculated control.

Al-Mujahidy *et al.* (2013) discovered five *Rhizobium* strains that are proficient in fixing a higher measure of nitrogen alongside various development factors.

Rhizobium strains may be connected in proficient nodulation of legumes for nitrogen fixation.

Osei *et al.* (2018) reported that among N-fixing living organisms, *Rhizobium* is one which fixes atmospheric nitrogen. For small farmers, the identification of native rhizobia for effective inoculation of legumes is key for enhancing modest nitrogen fixation technologies in Africa's semiarid area. From root nodule of groundnut plants grown on farmer's fields, 150 *Rhizobium* were discovered and 30 *Rhizobium* separates which were in the end found to adjust groundnut plants.

2.2 Significance of Biological Nitrogen Fixation (BNF)

Fujita *et al*. (1992) reported that legume/cereal intercropping increases grain yield more than their monoculture. At the point when manure N is limited, biological nitrogen fixation (BNF) is the main source of nitrogen in cereal-legume mixed cropping systems. Nitrogen moves from legume to cereal to improve the cropping system's yield and proficiency of N use. The utilization of nitrate-tolerant leguminous crop, whose BNF is believed to be influenced by the use of combined N, may improve the amount of N available for the cereal component.

Peoples *et al*. (1992) reported that the levels of nitrogen fixation accomplished by legume and non-legume associations and evaluates their role as a source of nitrogen in tropical and sub-tropical farming. They talk about elements affecting nitrogen fixation and recognize potential methodologies for improving the measure of nitrogen fixed.

Wani *et al.* (1995) reported that biological nitrogen fixation is dependent upon ecological, physical, organic and nutritional factors, insignificant incorporation of any nitrogen-fixing plant system does not guarantee expanded contributions to the soil nitrogen pool. In semi-arid tropics, Stover is additionally expelled to sustain atmospheric nitrogen, most legumes may be relied upon to drain soil nitrogen. Legumes impact the yields in succeeding grain crops have been reported. Such an advantage is because of nitrogen contribution from legumes through biological nitrogen fixation and soil nitrogen saving effect.

2.3 Isolation and characterization of native root nodulating bacteria

Vincent (1970) isolated pure cultures of *Rhizobium* by washing and surfacesterilizing the nodules and then streaking milky fluid from a crushed nodule on a suitable agar medium.

Pandher and Kahlon (1978) observed that none of the rhizobial isolates grew at pH 3.0 but most of them showed maximum growth at pH 6.5 to 8.0. Though the pH beyond 8.5was not lethal, it however did not support the growth. When all the isolates were subjectedto keto lactose test, some of the isolates were found to be growth on the medium. Incontrast, based on C-utilization pattern (15 carbohydrates) a remarkable metabolicdiversity was observed among the rhizobial isolates.

Siddaramaiah & Bagyaraj, (1981) isolated rhizobia from root nodules of horsegram grown in different parts of Karnataka. Morphological, physiological and nodulation tests were made to confirm their identity. Twenty isolates of rhizobia thus obtained were screened for their symbiotic response with horsegram grown at Bangalore in microplots. Five isolates proved promising for horsegram. These five isolates were studied for their performance in different soils and agro-climatic conditions of Karnataka by taking up a multi-locational field trial at 4 different horsegram growing areas. Strain UASB 87 was found to be uniformly effective in all the places.

Bromfied and Kumar (1983) studied on slow and fast growing rhizobial isolates of *Cajanus cajan* and *Cicer arietinum* were compared in terms of colony characteristics andutilization of carbon sources. Results showed that fast growing isolates from *Cajanus cajan* and *Cicer arietinum* formed 3-6 mm diameter colonies on YEMA after four days and slow growers which produced colonies of 1 mm diameter after 7-10 days at 28^0 C. The fast growing *Rhizobium* sp. from *Cajanus cajan* utilized a wider range of carbon sources than slow growing isolates. In culture media, slow growing isolates from *Cajanus cajan* produced a near-neutral to alkaline reaction (pH 6.6-7.5) whereas the fast growers from Cicer arietinum produced an acidic reaction (pH 4.4 -5.6).

Fuhrmann (1990) isolated 360 isolates of *Bradirhizobium japonicum* from soybean *Glycine max* (L.) nodules taken from18 locations in Delaware. Further isolates were subjected to morphological characterization.

Amarger *et al.* (1994) isolated 287 isolates of *Rhizobium* nodulating *Phaseolus vulgaris* in France from four geographically distant field conditions.

Navarro *et al*. (2000) isolated the *Rhizobium spp.* strains from beans *(Phaseolus vulgaris*) nodules of Andalusian soils with no record of recent bean cultivation and found that no association between the presence of Rhizobia nodulating bean and the chemical or textural properties of the soils. Most of the isolates were more effective than the reference strains *Rhizobium leguminosarum bv. Phaseoli* TAL1121, *R. etli* type strain CFN42 and *R. tropici* type strain CIAT899. Additionally, strong interaction between the bean cultivar and the native Rhizobia populations were observed.

Shamseldin and Warner (2004) also isolated two *Rhizobium* strains, EBRI-2 and EBRI-26, from Egypt and were further tested for nodulation and competitiveness on bean using *Rhizobium tropici*, CIAT 899 G as the competing strain.

Workalemahu (2009) isolated total 44 rhizobial strains and their symbiotic properties such as nodule number, fresh weight, colour and plant growth were recorded. Further, total of 12 strains with good symbiotic properties were characterized for their morphological and physiological traits on Yeast Extract Mannitol Agar (YMA) medium. The results showed that presence of diversity in morphological, physiological and symbiotic properties among the rhizobial strains.

Al-judy and Majeed (2013) isolated rhizobia from root nodules of chickpea. These isolates were identified and characterized on the basis of colony morphology and biochemical tests including gram staining, catalase and oxidase tests.

Bhattacharya *et al.* (2013) stated that *Rhizobium* plays an important role in agriculture by including Nitrogen-fixing nodules on the roots of legume plants. Described the physiological and biochemical characterization of *Rhizobium* species isolated from root nodules of *Pisum sativum*. The *Rhizobium sp*. were rod shaped, gram negative, acid and mucous producing. They were found to be temperature and pH sensitive. It utilizes glucose and starch as sole carbon source. *Rhizobium sp.* was unable to hydrolysis the gelatin and showed sensitivity against different antibiotics such as tetracycline, kanamycin and streptomycin. The isolates were also showed demonstrable antibacterial activity against *Streptococcus sp.*, *E.coli* and *Pseudomonas sp.*

Deshwal and Chaubey (2014) experimented by isolating 81 *Rhizobium* strains from the root nodules of *Pisum sativum* and characterized by standard biochemical tests. Average generation time was between 3.0 to 3.6 hours which indicated that isolated rhizobia were fast grower. All strains were gram-negative and failed to absorb red colour when cultured in YEMA containing congo red. Strains showed positive for urea hydrolysis and gelatinase activity. All the strains utilized D-glucose, mannitol, D-fructose, L-arabinose as fermentation sugar. Results confirmed that isolated strains were *Rhizobium leguminosarum*.

Mukesh *et al*. (2015) reported fenugreek (*Trigonella foenum-graecum* L.) is known to fo rm a symbiosis with nitrogen-fixing bacteria (rhizobia). Characterized the *Rhizobium* bacteria nodulating fenugreek in the east and north shewa of Ethiopia, where the plant is commonly grown, and found that All isolated strains were fast growing on Yeast Extract Mannitol Agar (YEMA) plates. After three days of incubation at 30° C, the rounded colonies had mucous transparent to creamy appearance. Most isolates were able to produce acid after 72 hours of incubation and only three isolates slightly absorbed Congo red. Heterotrophic competence among isolates was also observed from their utilization capabilities of various carbon and nitrogen sources. Interestingly, the isolates showed strong tolerance to different eco-physiological parameters (pH, temperature, salt concentration, and intrinsic antibiotic resistance). Plants inoculated with different isolates shows differences with respect to nodule number and dry weight and shoot dry weight. Two isolates from North Shewa, representing 12.5 % of the isolates, were highly effective (> 80 %) in forming symbiosis with fenugreek whereas, 67 % were found to be effective (50-80 %). More shoot dry weight was observed for which isolates from North Shewa were inoculated, indicating that some soil-related factors such as pH might have affected the symbiotic effectiveness.

Niste *et al.* (2015) isolated twenty rhizobacteria strains from red clover (*Rhizobium trifolii*) and alfalfa (*Sinorhizobium meliloti*). The isolates were investigated for their morphological, physiological and symbiotic properties among the rhizobial strains. Phenotypically all isolated strains had the same colony morphology. The colour and texture was watery to translucent. The isolates were fast-growing and failed to absorb Congo red. Isolates utilized wider range of car absorb Congo red. Isolates utilized wider range of carbohydrates as sole source of carbon such as glucose, arabinose, mannose, mannitol, and maltose.

Rasool *et al*. (2015) isolated the *Rhizobium* sp. from the root nodules of *Vicia sativa* and characterized them by means of various biochemical tests. These Strains showed positive for catalase, urease and oxidase activity. The strain also utilized D-glucose and sucrose as a carbon source but not lactose.

Upadhayay *et al.* (2015) isolated fifteen rhizobial strains from the root nodule of *P. sativum* and *L. culinary* from Tarai agro-ecosystem. All strains were gram negative and did not absorb red colour when cultured on YEMA containing Congo red. In the keto-lactose test yellowish zone of Cu_2O was not found. The isolates showed no growth on the glucose peptone medium after one day which is indicating character of rhizobia. Thirteen isolates were fast growers and only two were slow growers which are confirmed by bromothymol blue test. Results confirmed that isolated strains were *Rhizobium.*

Bantu *et al*. (2016) collected six *Rhizobial* isolates from root nodules of *Phaseolus vulgaris*, majority of isolates were rapid growers within medium where, as few found to 8 grow slow. On Yeast Extract Mannitol Agar (YEMA) medium colonies found round mucoid and white. Microscopic examination resulted that bacteria were non motile, gram negative, and rod shaped. Isolate was characterized based on growth behaviour on medium.

2.4 Screening of efficient root nodulating bacteria

A number of growth parameters of the host and associated *Rhizobium* are considered to select an efficient strain of *Rhizobium.* There are different approaches available for the assessment of nitrogen fixation of which one approach is based on growth on N-free medium. Effectiveness is measured either directly by determining amount of nitrogen fixed or indirectly by measuring the plant dry weight, these two parameters are highly correlated (Erdman and Means, 1952). Anatomical analysis showed that the effectively nodulated plants contained substantially the same amount of persistent nitrogen fixing bacteroid tissue irrespective of number of nodules formed. With fully effective strain of bacteria the amount of nitrogen fixed per unit of active tissue was independent of nodule number indicating that characteristics like number and weight of nodules had no relation to the nitrogen fixing efficiency of rhizobia (Nutman, 1967).

Nambiar *et al*. (1982) reported that the main criterion for effectiveness was the amount of nitrogen fixed, analysed directly or indirectly by determining the plant dry weight, nodule formation not necessarily resulting in effective symbiosis with particular host but the amount of nitrogen fixed by a strain was important.

Kalhapure *et al.* (2003) reported that inoculation of *Bradyrhizobium japonicum* has increased the seed germination, nodulation and other growth parameters like number of branches per plant, dry weight per plant in soybean as compared to uninoculated control.

Appunu and Dhar (2006) isolated eight acid tolerant strains of *Bradyrhizobium* from soybean plants grown on acid soils in Madhya Pradesh, India. Further, symbiotic effectiveness of these strains were determined under the polyhouse conditions in sterilized soil (pH 4.5). Highest and lowest symbiotic characters, dry matter production and nitrogen improvement per plant were observed. In comparison to control, an increase of 4.60- 38.62 % in total plant dry weight was observed with strain inoculated treatments. Maximum enhancement in total plant dry weight (38.62 %) was noticed with the strain PSR011, followed by PSR007 (30.34 %), TSR011 (28.58 %), ASR003 (21.37 %) and ASR002 (15.17 %). Highest nitrogen content (2.20%) per plant was estimated in strain PSR011 inoculated plants, while lowest (1.70 %) was with NSR005 and NSR008 inoculated plants, which was 37.50 and 6.25 % higher than that of control plants, respectively.

Kalita *et al.* (2006) reported that inoculation of *Rhizobium* was found most effective in production of more number of nodules, nodules dry weight, shoot and root dry weight and nitrogen content in both blackgram and greengaram.

Karaca and Uyanoz (2012) evaluate the symbiotic effectiveness of native *Rhizobium* isolated strains on number of nodule, weight, and morphological properties of dry bean (*Phaseolus vulgaris* L.). The pot experiment results revealed that native isolated strains were more effective than control, there was no significant difference among the native isolated strains. However, all the treatments differed significantly ($P<0.05$) in terms of root weight (4 to 5 folds) in dry bean plants (Yunus 90) compared to control. Further more, inoculations with *R. tropici* (CIAT899), native *Rhizobium* and their mixture were more effective on shoot weight (2.48 to 3.36 folds) of dry bean compared to the control. The highest nodule number was obtained from reference strain (*R. tropici* CIAT899), mixture (co-inoculants) and native strain 1, respectively. The highest nodule weight was observed in isolated strains of 1, 3, 5 and 69 than those of the other isolated strains.

Rajpoot *et al.* (2013) reported that rhizobia isolated from root nodules of *Vigna radiata and* characterized morphologically and biochemically to a certain its physiology under normal conditions. Three bacterial strains (Rp1, Rp2 and Rp3) were tested for their effect on root, shoot and number of nodules *Vigna radiate* plants under green house condition. Comparatively out of three strains Rp1 strain was found be most effective in positively enhancing the growth of the plant.

2.5 Method of inoculation

The practice of inoculating the seed with cultures of rhizobia was suggested by Voelcker (1956). The number of method of inoculation has been developed since then. Iswaran et al. (1972) suggested lime pelleting to be most effective method of the seed inoculation with Rhizoium. Due to the buffering action of pelleting agents a neutral zone formed around seed and established. The practice of inoculating the seed with cultures of rhizobia was suggested by Voelcker a congenial environment for successful entry of the Rhizobium into the roots at many points and hence resulted in profused nodulation.

Hamdi *et a*l., (1978) observed that pelleting the seeds with calcium carbonate after inoculation improved the survival of rhizobia on seed, even when the seeds were treated with the fungicides. Nambiar *et al*. (l981) observed that application of liquid inoculants just below the seeds improved the germination of groundnut compared to seed inoculation or granular inoculation. Chamber (1983) obtained highest nodulation of soybean inoculated with a solid mixture or a concentrated liquid broth in furrow.

2.6 Effect of rhizobium inoculation on legume

Since the beginning of the present century, legumes inoculation had been practiced for larger growth and yield, Voelcker (1896). Modhok (1941) observed higher yields in legumes from inoculated seeds. Saric (1953) carried out the field experiment with a number of varieties of soyabean using five strains of *Rhizobium spp*. for inoculation. The inoculation treatment increased vegetative matter by 9.66 to 40.82 per cent, root material by 18.86 to 46.09 per cent and gram yield by 15.85 to 62.75 per cent depending on the variety. Ogneve (1953) obtained larger yields of leguminous crops by inoculation with cultures of root nodule bacteria.

Mehrotra and Lehn (1970) found that inoculation of moong, urd and soybean in rainy season crops, have given extremely encouraging results. Inoculation of these legumes with efficient strains, have shown very promising effect, even m tropical soils. In case of mung, yield increased from 637-750 kg per hectare due to inoculation, while in urd the increase was from 900-1081 kg per hectare. Similar increase in yield and nitrogen content of mung has been reported by Nair et al, (1970). Nagre (1982) reported significant increase in the yield of mung bean and black gram with seed inoculation by Rhizobium inoculant. This increase was statistically similar to one obtained by application of 20 kg per hectare in the form of nitrogen fertilizers.

Hungria *et al.* (2000) reported that the co-inoculation of *Rhizobium tropici* in relationship with nitrogen on the conduct of various beans, developed in an ensured environment. *Phaseolus vulgaris* seeds were co-inoculated with *Rhizobium tropici*. Common bean improved shoot dry matter production 76.12% when co-inoculated with rhizobacteria. In the common bean nitrogen use efficiency is higher when co-inoculated with *Rhizobium tropici*, affirming that co-inoculation alone is adequate to give the nitrogen important to plant advancement.

Hafeez *et al.* (2001) studied the nodule occupancy of most efficient strain of *Rhizobium* M-55 which had good competitive ability and has occupied almost 50 *per cent* of nodules in comparison with less effective strains and improved the biomass and nitrogen yield significantly. They recommended that strains M-55 and K-92 as good *Rhizobium* inoculants of commercial quality because of their effective competitiveness with high efficient N2 fixation.

Handerson and Atkins (2003) in their study on *Rhizobium* reported that antibiosis, mobility and nodulation efficiency are to be considered as important characters that make a strain more competitive than the others. Selection of superior *Rhizobium* strains, having increased nitrogen fixation capacity with competitiveness, ability to withstand environmental stress is required to increase crop growth and yield.

Tippannavar *et al.* (2004) in their field trial found that *Rhizobium* strains HF 274 and HD 177 has increased the nodule dry weight, chlorophyll, seed and total biomass yield of chickpea. The isolate GR-4 was found more efficient strain of *Rhizobium* for chickpea in northern dry zone of Karnataka.

Pawar *et al.* (2014) reported the effect of root nodulating bacteria which were isolated from root nodules of Soybean (*Glycine max L.*) on yeast extract mannitol agar (YEMA) medium. The isolates were identified by cultural, morphological and biochemical characterization. *Rhizobium* isolates were found to be rod shaped, gram negative, acid and mucous producing. Optimum temperature and pH of the *Rhizobium* was found to be 36°C and 7.0 respectively. While evaluating antibacterial and antifungal activity of *Rhizobium* against rhizospheric bacteria and pathogenic fungi (*Aspergillus niger and Fusarium oxysporum*), it was found that *Rhizobium* did not inhibit the rhizospheric bacteria but inhibited the growth of pathogenic fungi which indicates that *Rhizobium* may secrete antifungal compounds. In pot assays of *Rhizobium*, seed inoculation was more effective than soil inoculation. In case of seed germination on the growth of Bengal gram (*Cicer arietinum*), Moth beans (*Vigna aconitifolia*), Green gram (*Phaseolus aureus Roxb*.) and Peas (*Pisum sativum*) crop plants, it was noted that enhanced growth rate was obtained; Hence *Rhizobium* can be used as bio inoculant.

2.7 Effect of inoculation on seed germination

Neelamegam *et al.* (2006) reported that application of *Rhizobium* has improved the seed germination, seedling growth and productivity of blackgram. Murat and Ibrahim (2009) conducted a field experiment during 2005-06 and 2006-07 in Turkey to determine the effects of *Rhizobium* inoculates at different levels of phosphorus on the yield and nutrient uptake of field pea (*Pisum sativum* sp. *Arvense* L.). Phosphorus application and *Rhizobium* inoculation showed significant effect on germination.

2.8 Effect of inoculation of Rhizobium on growth and yield attributes

Chomchalow (1970) reported that *Rhizobium* inoculation significantly increased the plant height, number of nodules, dry matter and nitrogen uptake after three, six, nine and twelve weeks of growth in peanut. Field experiment conducted by Habish and Hassan (1974), in two localities in two growing season on the effect of inoculating haricot bean showed that a local strain of *Rhizobium* significantly improved nodulation and increased nitrogen content of the plant. Ranadive (1981) observed that the results pertaining to total number of nodules, dry weight of nodules, number of leaves and height of plants are statistically significant at 36 days, 51 days and 66 days of crop growth and at flowering stage of crop but these characters are non significant at 21 days of crop growth.

Bhagyaraj and Hegde (1978) conducted experiment to see response of cowpea to inoculation with some selected strains of *Rhizobium* and observed significant increase in nodulation and grain yield over control.

Pahwa and Patil (1983) conducted pot trail experiment to see the response of some pasture legumes to cowpea Rhizobium inoculation and observed that cowpea *Rhizobium* significantly increased plant height, number of nodules, fresh weight and dry weight of plant.

Barthakur and Sarmah (1984) observed profuse nodulation in soyabean inoculated with *Rhiobium japonicum* strain in acid soils of Johrat. Prabakar and Ramasamy (1990) showed that under acid lateritic soils at Vamban, soyabean seeds treated with *Rhizobium japonicum* significantly increased nodulation, plant growth and yield attributes of soyabean cultivars.

Dravid (1990) reported that *Rhizobium* inoculation increased nodulation and nitrogen fixation in pea. Yang *et al*. (1994) reported that Rhizobia induce the formation of root nodules on the root of leguminous plants. Ray Chaudhuri *et al*. (1997) found an increase of 94 per cent in nodule weight of *Rhizobium* inoculated plant receiving 26 kg per hectare N in soyabean.

Kumar *et al*. (1998) observed that seed inoculation of chick pea with *Rhizobium* strain significantly increased the nodulation and root colonization. Ozdemir *et al*. (1999) studied that *Rhizobium* inoculation significantly increased the nodule number and dry weight in pea.

Yadegari *et al.* (2008) reported that to the impact of co-inoculation with *Rhizobium* and plant development advancing rhizobacteria and, on yield segments and yield of basic bean (*Phaseolus vulgaris* L.) cultivars demonstrated that soil beneficial microorganisms can decidedly influence the symbiotic performance of rhizobia. Co-inoculation with PGPR and *Rhizobium* exhibited a huge increment in the yield.

Tahir *et al.* (2008) reported that inoculation with isolated isolates from white clover at higher altitudes fundamentally increases crop growth, nodule number and total nitrogen content. Variation in nodule number because of inoculation demonstrates that the result of strain for inoculants production should be based on rhizobia characterization to distinguish the best strains.

Chapter III

MATERIAL AND METHODS

The present investigation was carried out in the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi on "**Characterization and evaluation of *Rhizobium* isolated from root nodules of pea (*Pisum sativum* L.) and its effect on growth and yield of pea"**. The root nodulating bacteria were isolated from root nodules of pea grown in different regions of Varanasi. These isolates were characterized and further screened under net house of the department of Soil Science and Agricultural Chemistry, Institute of Agricultural Science. The material and methods which were followed are detailed in this chapter.

3.1 Collection of root nodules sample

Collection of plant

Root nodules were collected from a young and healthy seedling of Pea plant (*Pisum sativum* L.) from farmer's field at different locations of Varanasi district, Uttar Pradesh, India. A total of fourteen root nodule samples were collected from different pea growing regions of Varanasi *viz.,* Baghi, Gangpur, Ramna, Pacheura, Narayanpur, Navgraha, Govindpura, Dharmerpur, Vishunpura.

3.2 Isolation of root nodulating bacteria from root nodule

Procedure described by Vincent (1970) was followed large sized; healthy, pink nodules were selected for isolation. The plant roots along with nodules were washed under running water to clean them off the surface dirt and soil. The nodule was detached carefully along with bits of root intact. Surface sterilization of nodules was made by immersing 95 % alcohol for 5 to 10 seconds to break their surface tension, washed with sterile water then transferred to a solution containing 3 % sodium hypochlorite for 5 minutes after which they were rinsed in five changes of sterile distilled water using sterile forceps. Surface sterilized nodules were transferred into test tubes containing few drops of sterilized water and crushed aseptically with sterile glass rod to get a nodule suspension. A loopful of nodule suspension was streaked on Yeast Extract Mannitol Agar (YEMA) medium with Congo red. The whitish gummy colonies were picked up from the plates **(Plate 1)** and re-streaked for purification purpose. Single *Rhizobium* colony was picked up from the plates and maintained on YEMA slants. The slants were stored at 4^0C in a refrigerator for further studies.

3.3 Morphological and cultural characterization of the root nodulating bacterial isolate

3.3.1 Growth on YEMA medium

The procedure as described by Vincent (1970) was followed. YEMA medium with congo red (2.5 ml of 1 % solution) was prepared and 18 to 20 ml of the medium was dispensed in sterilized petri-plates. After solidification of the medium the pure culture were streaked separately on different plates and incubated the plates for 3 to 7 days at 28^oC.

Observations were recorded after three to five days for colony development and morphology.

3.4 Authentication of isolated cultures

3.4.1 Leonard jar assembly method

Leonard's jar assembly method was conducted to find out the efficiency of the newly isolated *Rhizobium sp.* Authentication is the main test for which Leonard jar Assembly method of Vincent (1970) is used. Compatible strains isolated will form effective nodules that fix nitrogen.

The authentication of all the isolated rhizobial isolates was tested by using Leonard's jar assembly method. The experiment had 11 treatments with 3 replications laid out in Complete Randomized Design (Somsegaran and Hobben, 1985).

3.4.2 Culturing the rhizobia for experiment

The isolated rhizobial isolates from pea were mass multiplied for 5-7 days in YEMA broth and after attaining desired population of *Rhizobium* in the broth it was used in experiment for testing the nodulation efficiency. The selected rhizobial colony was grown in YEMA slant for 3-9 days. Depending on the growth, the culture was checked for purity and transferred to conical flask containing sterile liquid medium and maintained for 4-9 days. This was called as the "Starter Culture". Later the starter culture was transferred to production medium (Vincent, 1970) with pH 6.5- 7.0 and incubated at 30°-32°C for 4-9 day.

3.4.3 Surface sterilization and pre-germination of seeds

Glassware used was thoroughly washed in detergent water, running tap water followed by rinsing in distilled water. The flasks used were tightly plugged with cotton gauge and were covered with aluminium foil. Glassware was then kept in hot air oven at 180^{o}C temperature for one hour. All the media, distilled water solutions etc. were sterilized at 15 lbs per square inch pressure for 20minutes, unless mentioned otherwise. Laminar airflow chamber was sterilized by ultra violet (UV) irradiation for 15 min.

Before sowing, seeds were surface sterilized by immersing in 95 *per cent* alcohol for3 minutes. Then the seeds were treated with 3 *per cent* calcium hypochlorite solution for5 minutes and then washed with sterile water 5-6 times serially. After that seeds were submerged in sufficient water and kept in the refrigerator for 2-3 hour for the seeds to imbibe. Seeds were placed in 0.75 % (w/v) water agar in petri dishes and incubate at 250°C–300°C.

3.4.4 Transfer of pre germinated seeds from seedling agar to Leonard jar

Surface sterilized and pre germinated seeds of pea were placed in perforated germination Leonard jar assembly. Seeds were placed in such way that radicle can be properly pierced through perforations made at the bottom of the jar without damage. After that 1 ml of each rhizobial isolates broth culture was placed on the respective seeds and rack was placed in green house and replenished with nutrient solution whenever required. After 20 days, the initiation of nodules was observed. The best nodulated strain obtained from Leonard jar experiment was selected for further screening in pot experiment under Net house condition.

3.5 Pot culture study

The ten isolates isolated from pea which was authenticated as *Rhizobium* through various morphological, physiological, biochemical and nodulation efficiency studies, were screened for their efficiency with respect to nitrogen fixation, and biomass production in pot culture trials. The pot culture experiments were conducted in the net house of Dept. of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi.

3.5.1 Preparation of pot

Earthen pot of 5 kg capacity were filled with soil which were collected from the agricultural farm, Institute of Agricultural Sciences, Banaras Hindu University and sieved in the net house of the department. There must be 1 or 2 holes at the bottom of the pot to drain out excess water.

3.5.2 Preparation of seeds for sowing

The seeds of pea were surface sterilized with 95 % of alcohol for 3 minutes, the seeds were washed with sterile water and again surface sterilized with 0.2% $HgCl_2$ for 5 minutes and finally washed with distilled sterile water for several times.

3.5.3 Preparation of inoculums

The different isolates of fresh culture (24 hours) of pea *Rhizobium* were grown on 100 ml YEM broth in 250 ml conical flasks for 7 days over rotary shaker at 28^0C. After attaining desired growth, the culture was prepared by the standard method in which 200g ‘gur’ is boiled in 1 ltr of water for 15 min.

In the present trial, the required amount was calculated as per seed quantity. The solution was allowed to cool down and calculated amount of *Rhizobium* culture was added in the solution. The culture was poured on the required seed. These seeds were used for sowing. The pots were kept on cement platform in randomized design under net house condition.

3.6 Treatments

The pot experiment was laid out in Completely Randomized Design having 11 treatments with 3 replications. In each of the pots, 3 seedlings were maintained. The treatment detail of the pot experiment is as below (table 3.1):

S.No.	Treatment	Details	Location
1.	T0	Control	-
2.	T1	Rhzobium isolate-1	Baghi
3.	T2	Rhzobium isolate-2	Gangpur
4.	T3	Rhzobium isolate-3	Gangpur
5.	T4	Rhzobium isolate-4	Pacheura
6.	T5	Rhzobium isolate-5	Navgraha
7.	T6	Rhzobium isolate-6	Dhermmerpur
8.	T7	Rhzobium isolate-7	Dhermmerpur
9.	T8	Rhzobium isolate-8	Navgraha
10.	T9	Rhzobium isolate-9	Govindpura
11.	T10	Rhzobium isolate-10	Vishunpura

3.7 Care after sowing

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

3.8 Harvesting

The seedlings were harvested after 60 days. The plants were removed carefully from the soil with gentle watering, to keep the root nodules intact. The plant tops were immediately cut off and retained for further observations.

3.9 Observation Records

3.9.1 Germination percentage

The germination percentage of pea seeds in the pot was recorded 7 days after sowing in each treatment.

3.9.2 Plant height (cm)

The plant height at 30, 45 and 60 days was recorded and expressed in cm.

3.9.3 Number of leaves per plant

In each pot the number of leaves per plant at 30, 45 and 60 days were recorded.

3.9.4 Nodule studies

A. Number of nodules

In each pot number of nodules was recorded immediately after harvesting. Three plants were taken from each pot at 45 days after sowing. Roots were gently washed in water to remove the adhering soil. Then nodules were counted carefully and the average was calculated.

B. Fresh weight of nodules per plant (mg.)

After counting the nodules of three sample plants from each treatment at 45 days after sowing, nodules were carefully removed with the help of forceps, and then the total nodules of three plants were weighed in electric balance and averaged for getting fresh weight of nodules per plant.

C. Dry weight of nodules per plant (mg.)

Fresh weighed nodules were put up in oven for drying at 65^0C for 48 hours. After 48 hours of drying, the nodules were weighed on electric balance and averaged for getting their actual dry weight.

3.9.5 Days taken to flowering

The days taken to more than 50% flowering were recorded in each treatment and the time taken from sowing to 50% flowering was calculated.

3.9.6 Days taken to marketable maturity

The date of first picking was recorded in each treatment and the time taken from sowing upto marketable maturity was calculated.

3.9.7 Plant biomass

A. Determination of fresh weight and dry weight of shoot and root

The fresh weight of shoot and root was recorded immediately after harvesting. The shoot and root samples were oven dried at 600^0C for 72 hours and dry weight of the plants were recorded.

3.10 Pod character and Yield

3.10.1 Number of pods per plant

The total number of pods per plant from the selected plants were calculated after each picking, summed up and averaged.

3.10.2 Length of Pod (cm)

Three pods were selected randomly from each treatment and their lengths were measured with the help of scale to find out the mean pod length.

3.10.3 Number of grains per pod

After each picking, three pods were randomly selected from each treatment and number of seeds was counted per pod and average was calculated to find out mean grains per pod.

3.10.4 Yield

3.11 Soil test at start and at end of the experiment

Soil sampling was done from different pots as per the soil sampling techniques. The composite soil sample was prepared by mixing these samples. This composite soil sample was subjected to analysis. The same procedure was repeated at the end of the experiment, but samples were taken from each treatment and were analyzed separately.

3.12 Chemical parameters

3.12.1 Available nitrogen (kg ha^{-1})

Available nitrogen in the soil was determined by alkaline permanganate method as described by Subbaiah and Asija (1956) and expressed as kg ha^{-1}.

Procedure: Take 20 g of soil sample was weighed and transferred in a 800 ml Kjeldahl flask. The sample was moistened with 10 ml distilled water, washing down the soil adhering to the neck of flask. 100 ml 0.32% $KMnO_4$ was added to it. Few glass beads were put in to Kjeldahl flask to avoid bumping, followed by addition of 2-3 ml of Paraffin liquid to avoid frothing during heating. In a 250 ml conical flask, 25 ml of 2 % boric acid mixed indicator was taken and placed under the receiver tube. Tap water was run continuously in the condenser. 100 ml of 2.5 % NaOH was added to the flask and immediately the rubber stopper was attached in the flask. Heater was switched on and distillation was continued until about 100 ml of blue colour distillate was collected. The flask was removed containing the distillate and the heater was switched off. The distillate was then titrated against 0.02 N H_2SO_4 until pink colour developed.

Calculation:

$$\text{Mineralizable N (kg ha}^{-1}\text{)} = \frac{(S - V) \times 0.02 \times 14 \times 10^6 \times 2.24}{1000 \times 20}$$

Where,

S - Sample titration reading

V - Blank titration reading

3.13 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 and at five *per cent* level of significance were worked out.

Chapter IV

EXPERIMENTAL RESULTS

In this study attempts were made to isolate the native root nodulating bacteria from root nodules of pea (*Pisum sativum* L.) The selected isolates were characterized and identified based on morphological and biochemical characteristics. The efficient root nodulating bacterial isolates were screened in pot culture experiment under net house condition of the Department of Soil Science and Agricultural Chemistry. The results obtained in these studies are presented in this chapter. The results of studies carried out on growth, nodulation and yield of pea are described in this chapter with the help of tables and graphs.

4.1 Isolation and enumeration of *Rhizobium* from root nodules of pea

The rhizobia were isolated from root nodules of pea plants obtained from different pea growing locations such as Baghi, Gangpur, Ramna, Pacheura, Narayanpur, Navgraha, Govindpura, Dharmerpur, Vishunpura. Fourteen *Rhizobium* isolates were obtained from the root nodules of pea (*Pisum sativum* L.) by plating of the suspension on YEMA with Congored medium and the isolates were checked for purity and maintained on YEMA slants for further studies. The isolates were characterized and identified as *Rhizobium* by following various biochemical and nodulation tests. Nomenclature of the isolates was carried out representing their parent plant (first letter), region of origin (second letter) and isolate number (numeric figures) as presented in (Table 4.1).

Table 4.1

S.No.	Place	No. of isolate	Name of isolate	Treatment
1.	Baghi	1	PBI-1	*Rhizoium* isolate-1
2.	Gangpur	2	PGI-1	*Rhizoium* isolate-2
			PGI-2	*Rhizoium* isolate-3
3.	Pacheura	1	PPI-1	*Rhizoium* isolate-4
4.	Navgraha	2	PNI-1	*Rhizoium* isolate-5
			PNI-2	*Rhizoium* isolate-8
5.	Dhermmerpur	2	PDI-1	*Rhizoium* isolate-6
			PDI-2	*Rhizoium* isolate-7
6.	Govindpura	1	PGI-1	*Rhizoium* isolate-9
7.	Vishunpura	1	PVI-1	*Rhizobium* isolate-10

PBI- Pea Baghi Isolate, PGI-1 Pea Gangapur Isolate-1, PGI-2 Pea Gangpur isolate-2, PPI- Pea Pacheura Isolare, PNI-1 Pea Navgraha Isolate-1, PNI-2 Pea Navgraha Isolate-2, PDI-1 Pea Dharmmerpur Isolate-1, PDI-2 Pea Dharmmerpur Isolate-2, PGI-Pea Govindpur Isolate, PVI- Pea Vishunpura Isolate.

4.2 Characteristics of native root nodulating bacterial isolates

The native root nodulating bacterial isolates were further characterized for their identification. The details regarding the characteristics of native root nodulating bacterial isolates are given below.

4.2.1 Morphological characterization of native root nodulating bacterial isolates

Root nodulating bacterial isolates from pea were tested for growth characteristics on Yeast Extract Mannitol Agar medium with congo red **(Plate 2)**. All root nodulating bacterial isolates were observed as circular to round, muccoid and watery to white translucent colonies in appearance. Isolates took 2-3 days to show colonies on YEMA plates depicting that these isolates were fast growing**.**

4.3 Nodulation efficiency of native root nodulating bacterial isolates

Plant infection test was carried out in leonard jar assembly for the purpose of authentication of root nodulating bacterial isolates, which confirmed that the isolates as *Rhizobium.* Since ten isolates out of fourteen isolates formed effective nodules on the pea within 45 days Table (4.2). PDI-1 (11plant^{-1}) produced highest number of effective nodules on pea plant followed by PDI-2 (10plant^{-1}). No nodulation was observed in case of uninoculated (control) treatment.

Table 4.2 Effect of different *Rhizobium* isolates on nodulation of pea plants in leonard's jar assembly

Treatments	No. of nodules at 45 days
Control	0.000
Rhizoium isolate-1	6.000
Rhizoium isolate-2	5.000
Rhizoium isolate-3	5.667
Rhizoium isolate-4	7.667
Rhizoium isolate-5	8.333
Rhizoium isolate-6	11.000
Rhizoium isolate-7	10.000
Rhizoium isolate-8	4.667
Rhizoium isolate-9	6.667
Rhizobium isolate-10	5.333
SEm±	0.426
CD (0.05)	1.251

4.4 Effect of inoculation of rhizobial isolates on pea in pot experiment under net house condition

The pot culture experiment was conducted with eleven treatments as indicated in material and methods. Among fourteen isolates only ten isolates *viz.,* PBI, PGI-1, PGI-2, PPI, PNI-1, PNI-2, PDI-1, PDI-2, PGI, PVI were selected for screening under net house condition and the observation were recorded at 30, 45 and 60 days after sowing are discussed below.

4.4.1 Effect of inoculation of rhizobia on germination percent of pea.

The data in the table 4.3 showed the effect of inoculation of *Rhizobium* isolates on germination percentage of pea in pot experiment under net house condition. From the table 4.3 we can clearly see that the germination percentage was maximum in *Rhizobium* isolate -6 (83.33%) which was at par with *Rhizobium* isolate -4 (73.33%) and *Rhizobium* isolate -7 (76.667%). These strains significantly varied with remaining strains followed by *Rhizobium* isolate -5 (70.00%) and Rhizobium strain-9 being at par with *Rhizobium* isolate -1(66.67%), *Rhizobium* isolate -3 (63.333%) and *Rhizobium* isolate -10 (60%), Lowest germination percentage was obtained in control (56.667%).

Table 4.3 Effect of inoculation of *Rhizobium* isolates on germination percentage of pea in pot experiment under net house condition.

Treatments	Germination % in pot experiment
Control	56.667
Rhizoium isolate-1	66.667
Rhizoium isolate-2	56.667
Rhizoium isolate-3	63.333
Rhizoium isolate-4	73.333
Rhizoium isolate-5	70.000
Rhizoium isolate-6	83.333
Rhizoium isolate-7	76.667
Rhizoium isolate-8	56.667
Rhizoium isolate-9	70.000
Rhizobium isolate-10	60.000
SEm±	4.144
CD (0.05)	12.154

4.4.2 Effect of inoculation of rhizobial isolates on plant height (cm) of pea at different intervals.

On plant height of pea in pot experiment, effect of rhizobial isolates inoculation on at 30, 45 and 60 DAS is presented in Table (4.4). Significant difference was observed at all three stages among the treatments with respect to plant height. At 30 days after sowing the maximum plant height was observed in *Rhizobium* isolate -6 (17 cm) followed by *Rhizobium* isolate -7 (15.75 cm). The next best treatments were *Rhizobium* isolate *-4* (15.25 cm) and *Rhizobium* isolate -5 (15.167 cm). The lowest plant height was observed in case of contol (11.50 cm).

At 45 days after sowing the maximum plant height was observed in *Rhizobium* isolate -6 (32.833 cm) followed by *Rhizobium* isolate -7 (30.5 cm). The next best treatments were *Rhizobium* isolate -4 (29.0 cm) and *Rhizobium* isolate - (28.75 cm). The lowest plant height was observed in the control (20.333 cm).

The same trend was followed at 60 days after sowing and there was a significant difference among the treatments. The maximum plant height was observed in *Rhizobium* isolate -6 (40.5 cm) followed by *Rhizobium* isolate -7 (38.333 cm). The lowest plant height was observed in control (30.0 cm).

Table 4.4 Effect of inoculation of *Rhizobium* isolates on plant height (cm) of pea in pot experiment under net house condition at different intervals

Treatments	**30 DAS**	**45DAS**	**60DAS**
Control	11.500	20.333	30.000
Rhizoium isolate-1	14.000	28.000	36.750
Rhizoium isolate-2	13.083	26.083	33.000
Rhizoium isolate-3	13.833	27.833	35.517
Rhizoium isolate-4	15.250	29.000	38.250
Rhizoium isolate-5	15.167	28.750	37.417
Rhizoium isolate-6	17.000	32.833	40.500
Rhizoium isolate-7	15.750	30.500	38.333
Rhizoium isolate-8	11.833	23.667	32.500
Rhizoium isolate-9	14.417	28.833	36.833
Rhizobium isolate-10	13.417	27.500	33.500
SEm±	0.326	0.66	0.471
CD (0.05)	0.955	1.936	1.381

Note: Each value is average of three replications. DAS: Days after sowing

4.4.3 Effect of inoculation of rhizobial isolates on the number of leaves per plant of pea at different intervals.

The observation recorded on number of leaves per plant at 30, 45, 60 DAS is presented in Table (4.5). The number of leaves per plant at 30 DAS differed significantly due to treatments. However, the number of leaves per plant ranged from 15.333 in control to 23.667 in *Rhizobium* isolate-6 followed by *Rhizobium* isolate -7 (22.667 cm). The number of leaves per plant at 45 DAS differed significantly among the treatments. Maximum number of leaves per plant (23.667) was recorded in *Rhizobium* isolate -6. The lower number of leaves (18.667 cm) was registered by control. The number of leaves per plant at 60 DAS also differed significantly among treatments. Highest number of leaves per plant (33) was recorded in *Rhizobium* isolate -6 whereas; lower numbers of leaves (28.00) were registered by control.

Table 4.5 Effect of inoculation of R*hizobium* isolates on the number of leaves per plant of pea in pot experiment under net house condition at different intervals

Treatments	30 DAS	45DAS	60DAS
Control	15.333	18.667	28.000
Rhizoium isolate-1	16.667	20.667	30.667
Rhizoium isolate-2	15.667	19.667	29.333
Rhizoium isolate-3	16.333	20.333	30.000
Rhizoium isolate-4	18.000	21.667	31.667
Rhizoium isolate-5	17.667	21.333	31.000
Rhizoium isolate-6	19.000	23.667	33.000
Rhizoium isolate-7	18.333	22.667	32.000
Rhizoium isolate-8	15.667	19.333	29.000
Rhizoium isolate-9	17.000	21.000	31.000
Rhizobium isolate-10	16.000	20.000	29.333
SEm±	0.560	0.628	0.503
CD (0.05)	1.641	1.841	1.474

Note: Each value is average of three replications.

4.4.4 Effect of inoculation of rhizobial isolates on the nodules characteristics.

A. Number of nodules per plant

The data pertaining to nodule characters have been presented in Table 4.6. The highest number of nodule (20.00) was recorded in treatment *Rhizobium isolate*-6. The next superior treatment was *Rhizobium isoalte*-7 with value 17.667 nodules per plant. The treatments *Rhizobium isolate*-4 and *Rhizobium isolate*-5 with values 16.00 and 14.667 nodules per plant were superior to the control (3.333 nodules per plant).

B. Fresh weight of nodules

Significantly highest fresh weight of nodules was recorded in *Rhizobium* isolate -6 (301.5 mg). The treatment *Rhizobium* isolate -7 (285.067 mg) was also superior to rest of the other treatment with value 285.07 mg. Treatments *Rhizobium* isolate -4, *Rhizobium* isolate -5, and *Rhizobium* isolate -9 were *at par* among themselves with values 280.933, 268.733 and 260.733 mg, respectively. The treatments *Rhizobium* isolate -1, *Rhizobium* isolate -3 were *at par* with values 238.20, and 172.10 mg, respectively, but superior to the control (56.167 mg), *Rhizobium* isolate -2 (149.133 mg) and *Rhizobium* isolate -8 (120.10 mg).

C. Dry weight of nodules per plant

Highest dry weight of nodules per plant was observed in treatment *Rhizobium* isolate -6 (123.8 mg) which was closely followed by treatments *Rhizobium* isolate -7, *Rhizobium* isolate and *Rhizobium* isolate -5 with values 114.833, 107.533 and 105.567 mg, respectively and varies significantly at par with each other. The minimum dry weight was recorded in control has value 20.50 mg.

Table 4.6 Effect of inoculation of *Rhizobium* isolates on number of nodules per plant, fresh and dry weight of nodules of pea in pot experiment under net house condition.

Treatments	No. of nodule per plant	Fresh wt. (mg)	Dry wt.(mg)
Control	3.333	56.167	20.500
Rhizoium isolate-1	13.000	238.200	98.467
Rhizoium isolate-2	10.000	149.133	83.300
Rhizoium isolate-3	11.667	172.100	92.500
Rhizoium isolate-4	16.000	280.933	107.533
Rhizoium isolate-5	14.667	268.733	105.567
Rhizoium isolate-6	20.000	301.500	123.900
Rhizoium isolate-7	17.667	285.067	114.833
Rhizoium isolate-8	7.333	120.100	47.067
Rhizoium isolate-9	14.000	260.733	104.967
Rhizobium isolate-10	11.333	167.133	91.200
SEm±	0.550	2.616	1.337
CD (0.05)	1.615	7.673	3.921

4.5 Effect of inoculation of rhizobial isolates on Biomass per plant at 60 days after sowing.

4.5.1 Effect of inoculation of rhizobial isolates on the shoot fresh weight

The shoot fresh weight of pea plants upon inoculation with rhizobial isolates at 60 days after sowing showed significant difference among the treatments are presented in Table (4.7)**.** The maximum shoot fresh weight was observed in *Rhizobium isolate*-6 (6.267 g plant^{-1}) followed by *Rhizobium isolate*-7 (6.067g plant^{-1}). The next best treatment was *Rhizobium isolate*-4 (5.667 g plant^{-1}) followed by *Rhizobium isolate*-5 (5.60g plant^{-1}). The least shoot fresh weight was observed in control (3.2 g plant-1).

4.5.2 Effect of inoculation of rhizobial isolates on the root fresh weight

At harvest the fresh weight of root of pea plant, showed significant difference among the treatments which are presented in Table (4.7)**.** The maximum root fresh weight was observed in *Rhizobium* isolate -6 (2.767 g plant^{-1}) followed by *Rhizobium* isolate -7 (2.633g plant^{-1}). The least root fresh weight was observed in control (1.120 g plant^{-1}).

4.5.3 Effect of inoculation of rhizobial isolates on the shoot dry weight

The shoot dry weight of pea plants upon inoculation with rhizobial isolates at 60 days after sowing showed significant difference among the treatments are presented in Table (4.7)**.** The maximum shoot dry weight was observed in *Rhizobium* isolate -6 (0.707 g plant^{-1}) followed by *Rhizobium* isolate -7 (0.680 g plant^{-1}). The next best treatment was *Rhizobium* isolate -4 (0.637 g plant^{-1}) followed by *v*-5 (0.633 g plant^{-1}). The least shoot dry weight was observed in control (0.360 g plant^{-1}).

Table 4.7 Effect of inoculation of *Rhizobium* isolates on fresh and dry weight of pea plant, in pot experiment under net house condition.

Treatments	Fresh wt. (g/plant)		Dry wt.(g/plant)	
	Shoot	Root	Shoot	Root
Control	3.200	1.120	0.360	0.127
Rhizoium isolate-1	4.600	1.933	0.577	0.180
Rhizoium isolate-2	3.533	1.533	0.450	0.163
Rhizoium isolate-3	4.200	1.800	0.543	0.177
Rhizoium isolate-4	5.667	2.433	0.637	0.243
Rhizoium isolate-5	5.600	2.367	0.633	0.220
Rhizoium isolate-6	6.267	2.767	0.707	0.273
Rhizoium isolate-7	6.067	2.633	0.680	0.253
Rhizoium isolate-8	3.300	1.200	0.427	0.150
Rhizoium isolate-9	4.867	2.267	0.590	0.207
Rhizobium isolate-10	3.900	1.700	0.483	0.167
SEm±	0.104	0.074	0.011	0.005
CD (0.05)	0.305	0.216	0.032	0.0140

4.5.4 Effect of rhizobial isolates inoculation on the root dry weight

The root dry weight of pea plants upon inoculation with rhizobial isolates at harvest, showed significant difference among the treatments are presented in Table (4.7)**.** The maximum root dry weight was observed in treatment *Rhizobium* isolate -6 (0.273 g plant-^{1}) followed by *Rhizobium* isolate -7 (0.253g plant^{-1}). The next best treatment was *Rhizobium* isolate -4 (0.243g plant^{-1}) followed by *Rhizobium* isolate -5 (0.220 g plant^{-1}). The least shoot fresh weight was observed in control (0.127g plant^{-1}).

4.6 Effect of inoculation of R*hizobium* isolates on flowering of pea in pot experiment under net house condition

4.6.1 Days taken in more than 50% flowering

The data to days taken in more than 50% flowering have been presented in the above Table 4.8. Days to more than 50% flowering was significantly influenced by different treatments. Minimum days taken in more than 50% flowering i.e. 47.333 were recorded in treatment *Rhizobium* isolate -6, which was *at par* with *Rhizobium* isolate -5, *Rhizobium* isolate -7, *Rhizobium* isolate -4 with values 47.667, 48.00 and 48.33 days, respectively. These treatments were superior to rest of the other treatments including the control (53.667 days) which has taken maximum days among treatments.

4.7 Inoculation effect of *Rhizobium* isolates on marketable maturity of pea in pot experiment under net house condition

4.7.1 Days taken in marketable maturity

The data regarding days taken in marketable maturity were significantly influenced by different treatments in comparison to the control (Table 4.8). The minimum days taken to obtained marketable maturity is 75.00 days observed in treatment *Rhizobium* isolate-6, whereas, maximum days 82.667 were taken in control. The treatment *Rhizobium* isolate -7 and *Rhizobium* isolate -4 with values 76.667 days and 77.00 days, respectively, were *at par* with each other. Similarly, *Rhizobium* isolate -5 (77.33 days), *Rhizobium* isolate -9 (77.667 days) and *Rhizobium* isolate-1 (78.333 days) were equally effective.

Table 4.8 Effect of inoculation of R*hizobium* isolates on flowering and marketable maturity of pea in pot experiment under net house condition.

Treatments	Days taken in more than 50% flowering	Days taken in marketable maturity
Control	53.667	82.667
Rhizoium isolate-1	49.667	78.333
Rhizoium isolate-2	52.333	80.000
Rhizoium isolate-3	50.333	78.667
Rhizoium isolate-4	48.333	77.000
Rhizoium isolate-5	47.667	77.333
Rhizoium isolate-6	47.333	75.000
Rhizoium isolate-7	48.000	76.667
Rhizoium isolate-8	52.667	81.667
Rhizoium isolate-9	48.667	77.667
Rhizobium isolate-10	50.667	79.333
SEm±	0.402	0.512
CD (0.05)	1.179	1.503

4.8 Effect of inocuation of R*hizobium* isolates on number of pod characters of pea in pot experiment under net house condition.

4.8.1 No. of pods per plant

Data pertaining to number of pods per plant have been presented in Table 4.9. More number of pods was obtained significantly in all the treatments, in comparison to the control. Maximum number of pod was recorded in *Rhizobium* isolate -6 (7.667) whereas; it was 3.667 pods per plant in control. The treatments *Rhizobium* isolate-7, *Rhizobium* isolate-*4* with values 7.00 and 6.667 pods per plant respectively were *at par* among themselves but superior to rest of the treatments.

4.8.2 Length of pod (cm)

Length of pod was also increased significantly in different treatments in comparison to the control (Table 4.9). Longest pod (7.00 cm) was harvested in treatment *Rhizobium* isolate -6 closely followed by treatment *Rhizobium* isolate 7 (6.667 cm). The shortest pod (4.667 cm) was observed in control. The treatments *Rhizobium* isolate -4 (6.667 cm), *Rhizobium* isolate -5 (6.00 cm), *Rhizobium* isolate -9 (6.00 cm), *Rhizobium* isolate -1 (5.667 cm) and *Rhizobium* isolate -3 (5.667 cm) were *at par* among themselves. Treatment *Rhizobium* isolate -10 (5.33 cm) was superior to treatments *Rhizobium* isolate -2 and *Rhizobium* isolate -8 with values 5.00 cm and 4.667 cm respectively.

4.8.3 Number of grain per pod

The results obtained with regard to number of grains per pod have been presented Table 4.9. The results exhibited maximum number of grains per pod (7.33) in *Rhizobium* isolate -6. However, treatments *Rhizobium* isolate -7, *Rhizobium* isolate -4 and *Rhizobium* isolate -5 were *at par* with values 6.667, 6.333, and 6.00 grains per pod. The minimum number of grains per pod was recorded in control 4.333. However, it is shown from the table that number of grains per pod was significantly influenced by all the treatments.

4.8.4 Pod weight (g) per plant

Highest pod weight was obtained in treatment *Rhizobium* isolate -6 (18.23 gm) followed by treatment *Rhizobium* isolate -7 (17.33 gm) which is significantly different with each other. The next superior treatment was *Rhizobium* isolate -4 (16.6 gm) followed by *Rhizobium* isolate -5 (16.367 gm) and varies significantly to rest other treatments. The treatments *Rhizobium* isolate -9 (16.20 gm), *Rhizobium* isolate-1 (15.93 gm) *Rhizobium* isolate -3 (14.033 gm), and other remaining treatments were also significantly superior to the control (9.10gm).

Table 4.9 Effect of inocuation of *Rhizobium* isolates on number of pod per plant, pod length, number of grains per pod and pod weight per plant of pea in pot experiment under net house condition.

Treatments	No. of pod per plant	Pod length (cm)	No. of grains per pod	pod weight (g) per plant
Control	3.667	4.667	4.333	9.100
Rhizoium isolate-1	6.000	5.667	5.667	15.933
Rhizoium isolate-2	5.000	5.000	4.667	12.833
Rhizoium isolate-3	5.667	5.333	5.333	14.033
Rhizoium isolate-4	6.667	6.333	6.333	16.600
Rhizoium isolate-5	6.333	6.000	6.000	16.367
Rhizoium isolate-6	7.667	7.000	7.333	18.233
Rhizoium isolate-7	7.000	6.667	6.667	17.933
Rhizoium isolate-8	4.667	4.667	4.667	11.733
Rhizoium isolate-9	6.333	6.000	5.667	16.200
Rhizobium isolate-10	5.333	5.333	5.000	13.133
SEm±	0.414	0.471	0.492	0.144
CD (0.05)	1.215	1.380	1.444	0.423

4.9 Effect of isolates on available N in the soil after harvest

The data in the table (4.10) clearly showed the effect of isolates on available N content of the soil after harvest. Upon inoculation with *Rhizobium* isolate, significant difference among treatments shown in the fig. *Rhizobium* isolate -6 recorded maximum Av. N with the value (199.33 kg) followed by *Rhizobium* isolate-7 (193.33 kg).The least available N was recorded in the control (164.667 kg).

Table 4.10 Effect of isolates on available N content in soil after harvesting in the pot experiment under net house condition

Treatments	Before Sowing (Kg)	After Sowing (Kg)
Control	160	164.667
Rhizoium isolate-1	160	183.000
Rhizoium isolate-2	160	176.233
Rhizoium isolate-3	160	181.333
Rhizoium isolate-4	160	190.333
Rhizoium isolate-5	160	187.000
Rhizoium isolate-6	160	199.333
Rhizoium isolate-7	160	193.333
Rhizoium isolate-8	160	172.717
Rhizoium isolate-9	160	186.000
Rhizobium isolate-10	160	177.333
SEm±	-	1.147
CD (0.05)	-	3.363

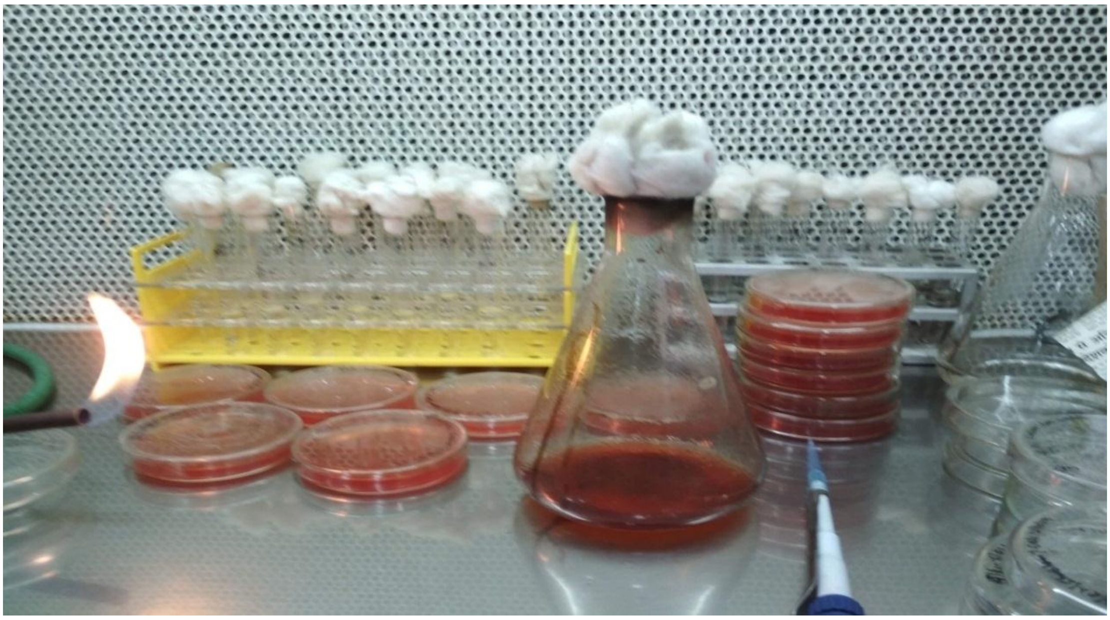

Plate 1 YEMA media with congo red for *Rhizobium* isolation

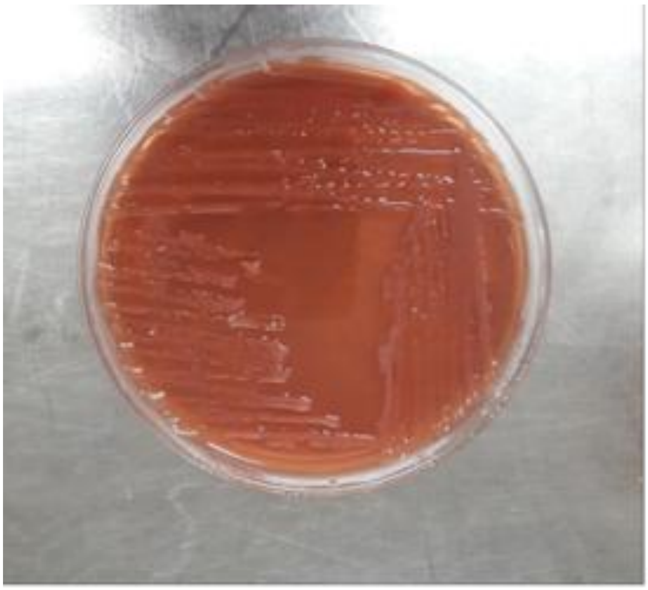

Plate 2 Growth of *Rhizobium* on YEMA medium with congo red

Plate 3 Overall view of pea in pot experiment under net house

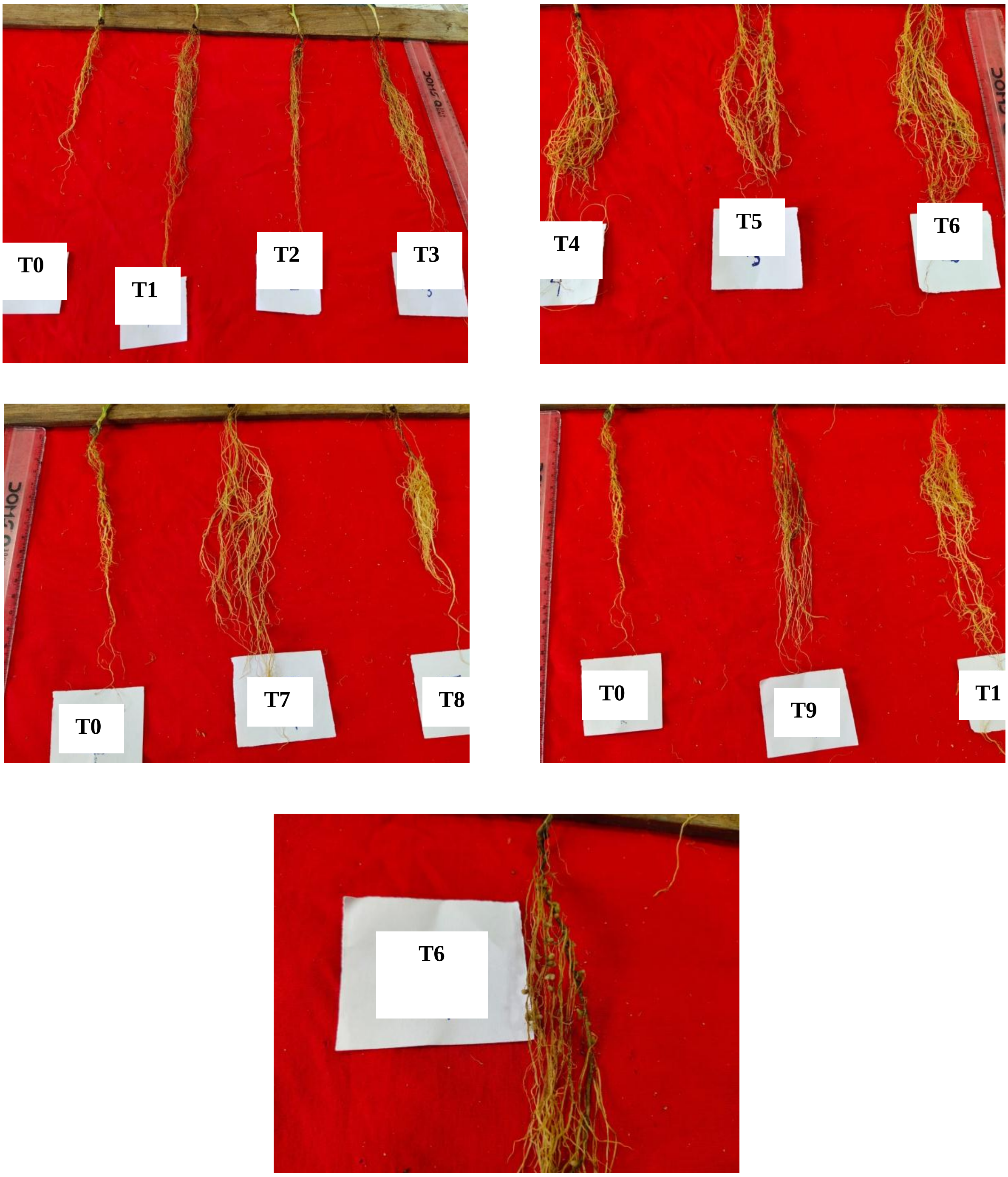

Plate 4 Effect of *Rhizobium* isolates on root biomass of pea

Chapter V

DISCUSSION

Garden pea *(Pisum sativum* L.) is one of the most popular and remunerative vegetable of rabi season and belongs to the family Leguminoceae. Being a legume, it helps in maintaining soil fertility, and also the capacity of fixing atmospheric nitrogen through root nodule bacteria incited by *Rhizobium.* Pea provides a variety of vegetarian dishes and hence it is liked throughout the world. It may be classified into two classes' viz. (1) garden or table pea (*Pisum sativum* var. *hortense.)* and (2) field pea *(Pisum sativum* var. a*rvense).* Mature seeds of this type are used as 'dal', and for green manuring. Garden peas are harvested in an immature condition to be cooked as green peas to provide a delicious dish, or to be canned or frozen for subsequent during off season. It is mixed with dry fodder namely 'karabi' or 'bhusa' to make a palatable feed for the animals. It is very much nutritive and contains 7 to 9 per cent protein, 56.6 to 62.1per cent carbohydrates, 1.5 to 1.8 per cent fat and appreciable proportion of calcium, iron, phosphorus and vitamins B1, B2 and niacin (Choudhury, 1967).

The continuous use of NPK fertilizers under intensive cropping system has caused adverse effects on soil physical, chemical and biological properties 'thereby' affecting the sustainability of crop production, besides causing environmental pollution (Virmani,1994).

For sustainable production system, microbial inoculants are vital resources, which can boost the overall nutrient turnover for the crop production. Microorganisms induce many biochemical transformations in the soil. These include mineralization of organically bound form of nutrients, exchange reactions, fixation of atmospheric nitrogen and various other changes leading to better availability of nutrients already present in the soil. Microbial fertilizers play an important role as these are eco-friendly, low cost and non-bulky agricultural inputs. So, to enhance biological nitrogen fixation and crop productivity, rhizobial inoculants have been considered to be of prime importance.

In ecological restoration of such degraded lands, Legume-*Rhizobium* associations have potential application. Given the potential of legume-*Rhizobium* symbiosis, rhizobial inoculants have been used for legume crops, but the issue is that, the successful establishment of introduced strain on account of competition with the indigenous rhizobial population. It is important to properly characterize and classify rhizobia to achieve full BNF from any legume-*Rhizobium* interaction before they are commercially available for field experiments. (Sahgal and Johri, 2003). The selection of successful rhizobial strain is therefore based on laboratory screening followed by sufficient pot and field trials. Therefore, the current investigation was carried out to isolate and classify pea-infecting rhizobia and to evaluate the promising rhizobial isolates for pea crop growth promotion.

5.1 Isolation and characterization of *Rhizobium* from pea growing areas around Varanasi.

From different pea growing areas *viz;* Baghi, Gangpur, Ramna, Pacheura, Narayanpur, Navgraha, Govindpura, Dharmerpur, Vishunpura. Fourteen root nodulating bacterial isolates were Isolated. The isolates were subjected to various observations. The colony characters on YEMA with congo red medium were watery to white translucent in appearance with varying quality of mucus production. The shape was circular and round with smooth margins. The results are in agreement with the findings of Deshwal and Chaubey *et al*. (2014) that isolated root nodulating bacteria from pea, which were circular round with smooth margined colonies on YEMA medium and colonies in Yeast Extract Mannitol Agar (YEMA) medium, were round mucoid and white. Similar observations were also reported by many researchers (Upadhayay *et al*., (2015); Bantu *et al.,* (2016); Bhattacharya *et al.* (2013).

Cultural characterization of root nodulating bacterial isolates results revealed that all fourteen native root nodulating bacterial isolates did not observe the congo red colour and such nature according to Deshwal and Chaubey, (2014). In the present study the results of all the experiment concur with the observations made by Upadhayay *et al.* (2015) who isolated fifteen rhizobial strains from the root nodule of *P. sativum* and *L. culinaris* from Tarai agro-ecosystem and characterized by standard tests.

5.2 Nodulation efficiency of native root nodulating bacterial isolates

Plant infection test was carried out in leonard jar assembly method, which confirmed that the isolates as *Rhizobium*. This was done for the purpose of authentication of root nodulating bacterial isolates. Out of fourteen isolates, ten isolates formed effective nodules on the pea within 45 days. PDI-1 (11.00 plant-1) produced highest number of effective nodules on pea plant followed by PDI-2 (10.00 plant-1). In case of uninoculated control no nodules were observed (Fig 5.1**).** The results were in accordance with the observations of Vincent, (1990), Jake Halliday, (1984), Somasegaran and Hoben, (1985).

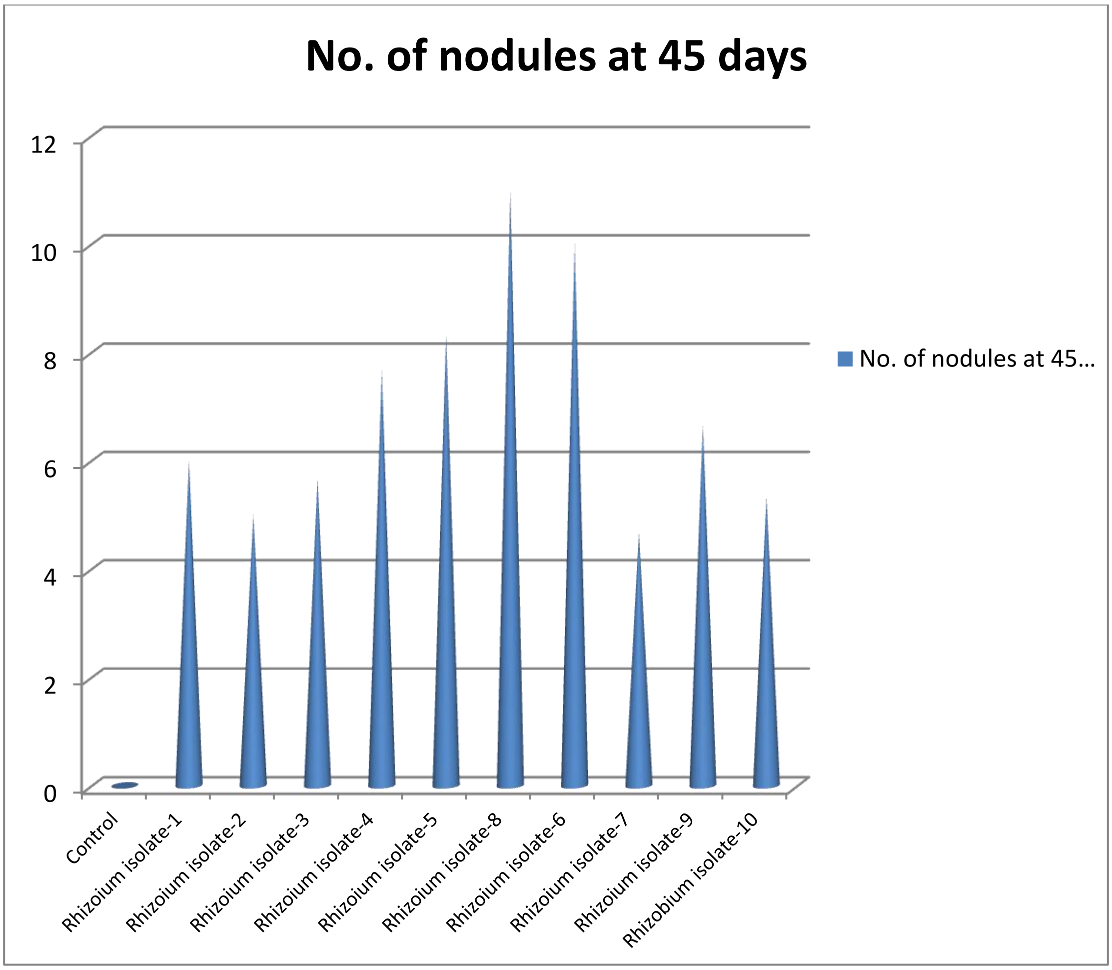

Fig. 5.1 Nodulation efficiency of native root nodulating bacterial isolates

The selected isolates were further screened for their efficiency of nitrogen fixation and biomass production in pot culture experiment under glass house condition. Positive response was clearly brought out in all the inoculated plants when compared with uninoculated plants.

5.3 Effect of inoculation of rhizobial isolates on growth of pea in pot experiment under net house condition

In the pot experiment, the inoculation of rhizobial isolates with varying nitroge n levels had major differences in pea growth parameters such as germination percentage, plant height, number of leaves, fresh and dry weight of shoots and roots, number of nodules.

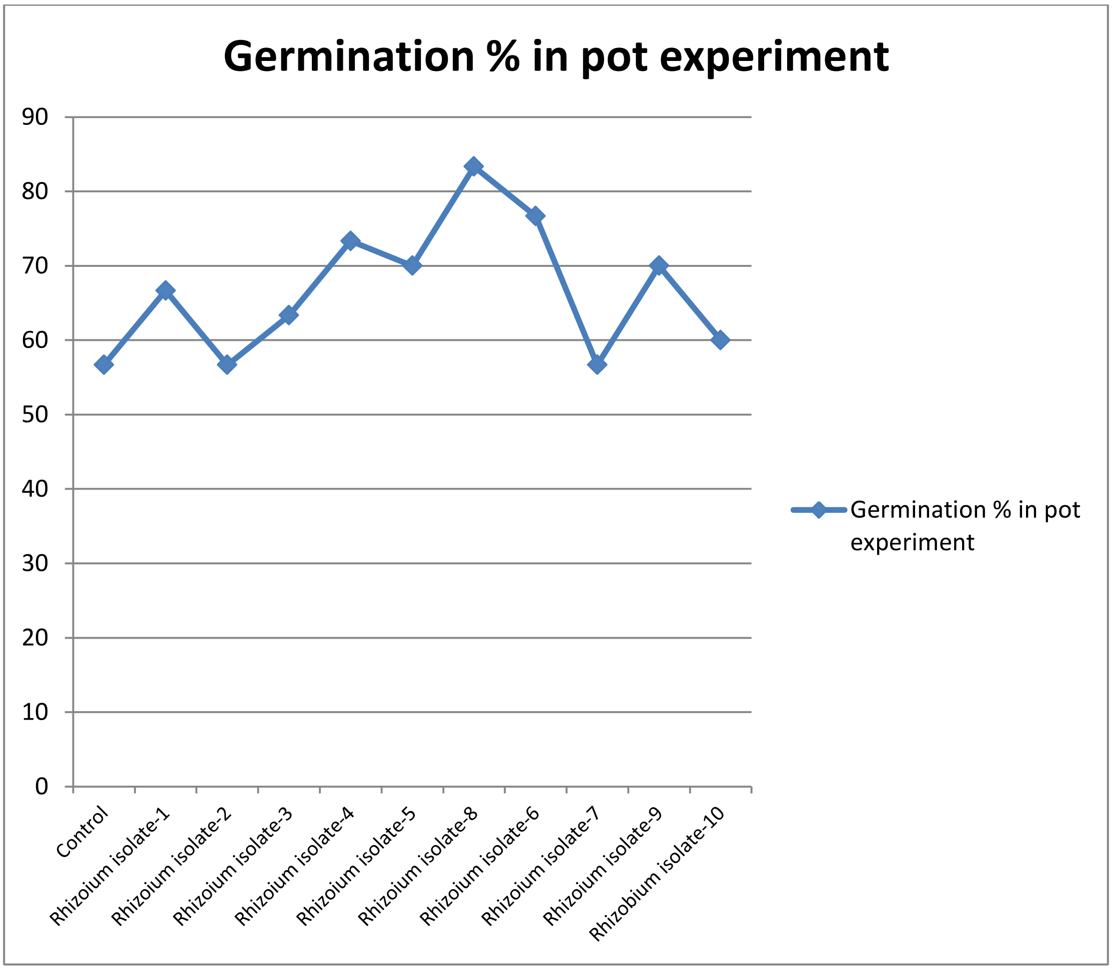

Fig 5.2 Effect of Rhizobium isolates on germination % of pea in pot experiment under net house condition

The effect of inocuation of *Rhizobium* isolates on germination percentage of pea in pot experiment under net house condition in shown in table 4.3 and fig. 5.2. *Rhizobium* isolates -6 being at par with *Rhizobium* isolates -4 and *Rhizobium* isolates -7 recorded maximum germination percentage. *Rhizobium* has enhanced the germination percentage. This finding was in line with Neelamegam *et al.* (2006). Result of inoculation was also reported by Pawar *et al* (2014).

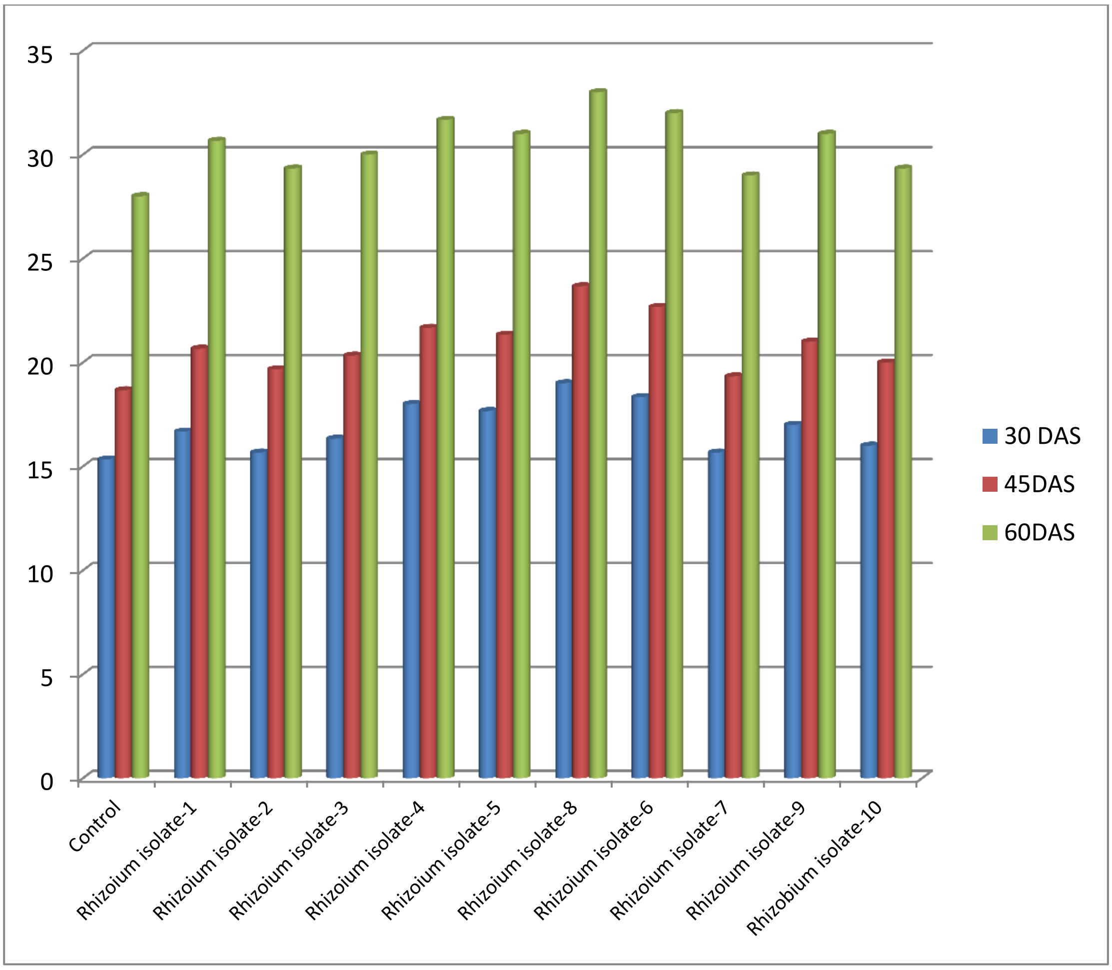

Fig. 5.3 **Effect of Rhizobium isolates on number of leaves of pea plant in pot experiment under net house condition.**

The inoculation of rhizobia had showed significant effect on number of leaves per plant at all the three stages over control (Fig. 5.3). The maximum number of leaves was observed in the treatment *Rhizobium isolate*-6 which was followed by *Rhizobium isolate* -7 and these leaves found broader and greener than the control treatments. The minimum numbers of leaves per plant was found in control.

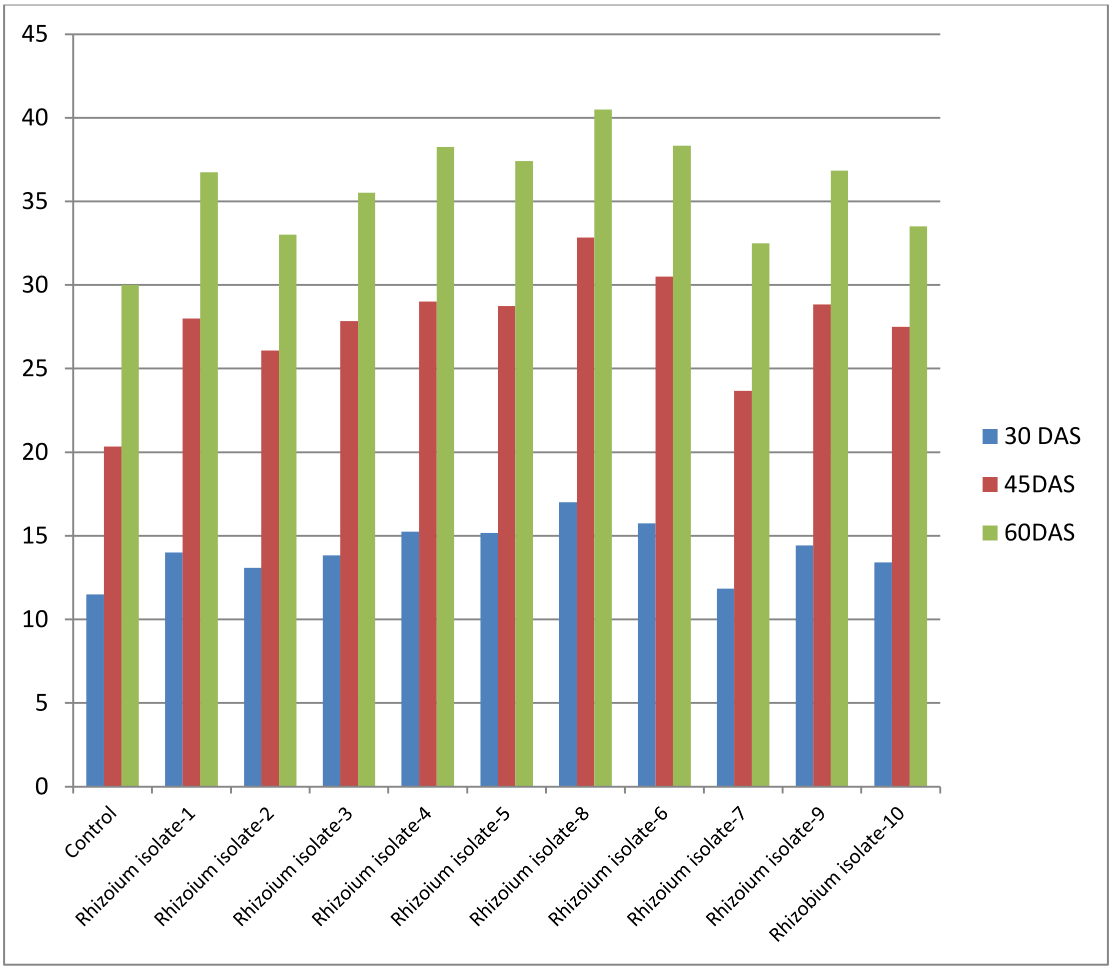

Fig. 5.4 **Effect of Rhizobium isolates on plant height of pea in pot experiment under net house condition**

The effect of inoculation of *Rhizobium* isolates on plant height of pea in pot experiment under net house condition in shown in table 4.4 and fig. 5.4. *Rhizobium* isolates -6 being at par with *Rhizobium* isolates -4 and *Rhizobium* isolates -7 recorded maximum plant height. Inoculation of *Rhizobium* has enhanced the plant height over uninoculated at different interval. This finding was in line with Gupta *et al.* (2005).

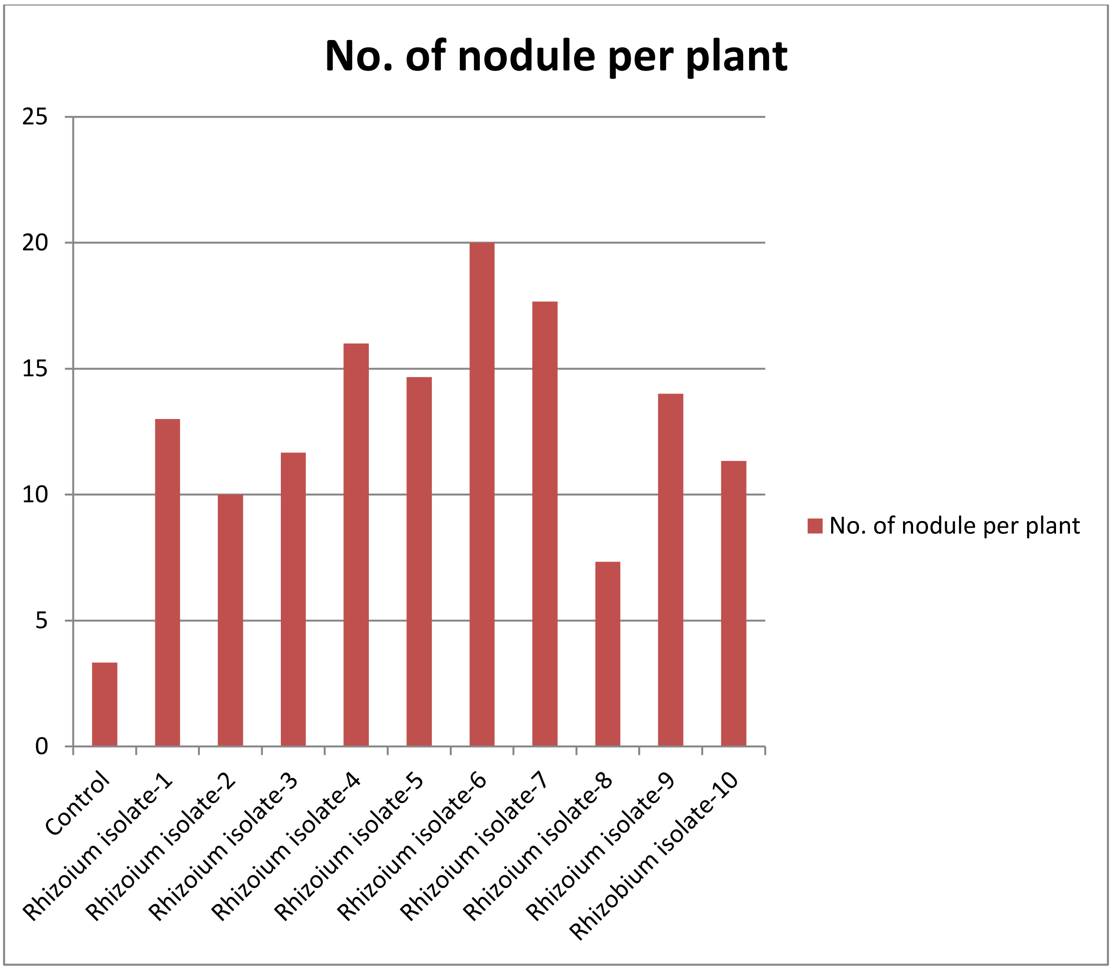

Fig. 5.5 **Effect of inoculation of *Rhizobium* isolates on number of nodules per plant of pea in pot experiment under net house condition.**

The effect of inoculation of *Rhizobium* isolates on number of nodules per plant and fresh weight of nodules of pea in pot experiment under net house condition which is shown in table 4.6 and fig. 5.5 the highest number of nodules (20.00) were recorded in treatment *Rhizobium isolate*-6 and the was recorded in (3.333 nodules per plant). Significantly highest fresh weight of nodules was recorded in *Rhizobium* isolates -6 (301.5 mg whereas least was recorded in control (56.167 mg).

The shoot fresh weight of pea plants upon inoculation with rhizobial isolates at 60 days after sowing showed significant difference among the treatments. Highest fresh weight was recorded in Rhizobium strain-6 (6.267 g per plant). The least shoot fresh weight was observed in control (3.2 g plant-1). Effect of inoculation in case of root fresh weight followed same trend. The maximum root fresh weight was observed in *Rhizobium strain*-6 (2.767 g plant^{-1}) followed by *Rhizobium strain*-7 (2.633g plant^{-1}). The least root fresh weight was observed in control (1.120 g plant^{-1}).

This finding was ine with Appunu and Dhar 2006; Sudaric *et al*., 2008 and Sikora *et al*., 2008. They were reported that the number of nodules can be increased by inoculation with effective rhizobial strains. Better results was observed in inoculated treatment as compared to uninoculated

5.4 Effect of different rhizobial isolates inoculation on Biomass per plant

Inoculation with rhizobial isolates showed significant difference at 60 days after sowing among the treatments which are presented in Table (4.7)**.** The maximum shoot dry weight was observed in *Rhizobium* isolates -6 (0.707 g plant^{-1}) followed by *Rhizobium* isolates -7 (0.680 g plant^{-1}). The least shoot dry weight was observed in control (0.360 g plant^{-1}). In case of root dry weight same trend is observed. The maximum root dry weight was recorded in treatment *Rhizobium* isolates -6 (0.273 g plant-^{1}) and the least was observed in control (0.127g plant^{-1}).

Highest dry weight of nodules per plant was observed in treatment *Rhizobium* isolates -6 (123.8 mg) which was closely followed by treatments *Rhizobium* isolates -7 with value 114.833mg. The minimum dry weight was recorded in control has value 20.50 mg.

Appunu and Dhar, (2006) also reported that inoculation of soybean with *Bradyrhizobium* increases the dry weight of nodule, shoot and root dry matter production. Similar observation were also reorded by Karaka and Uyanoz, (2012) that inoculation of native root nodulating bacteria increases shoot and root dry mass production and nodule dry weight in dry bean.

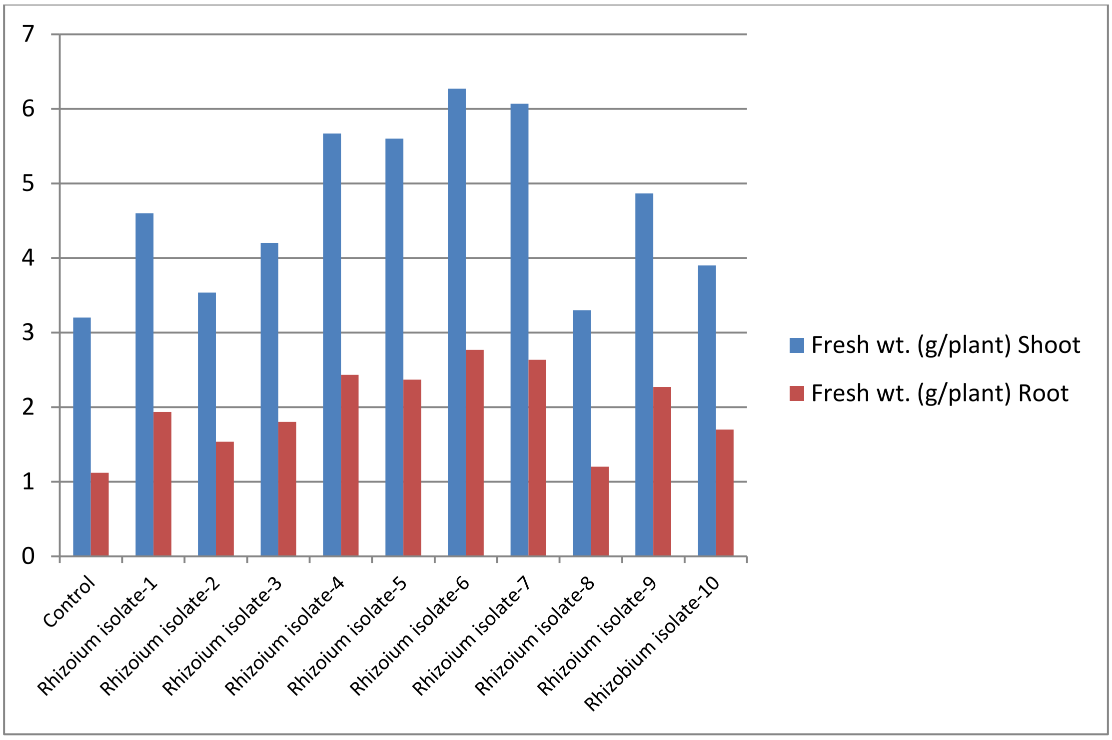

Fig. 5.6 **Effect of inoculation of *Rhizobium* isolates on fresh weight of pea plant, in pot experiment under net house condition.**

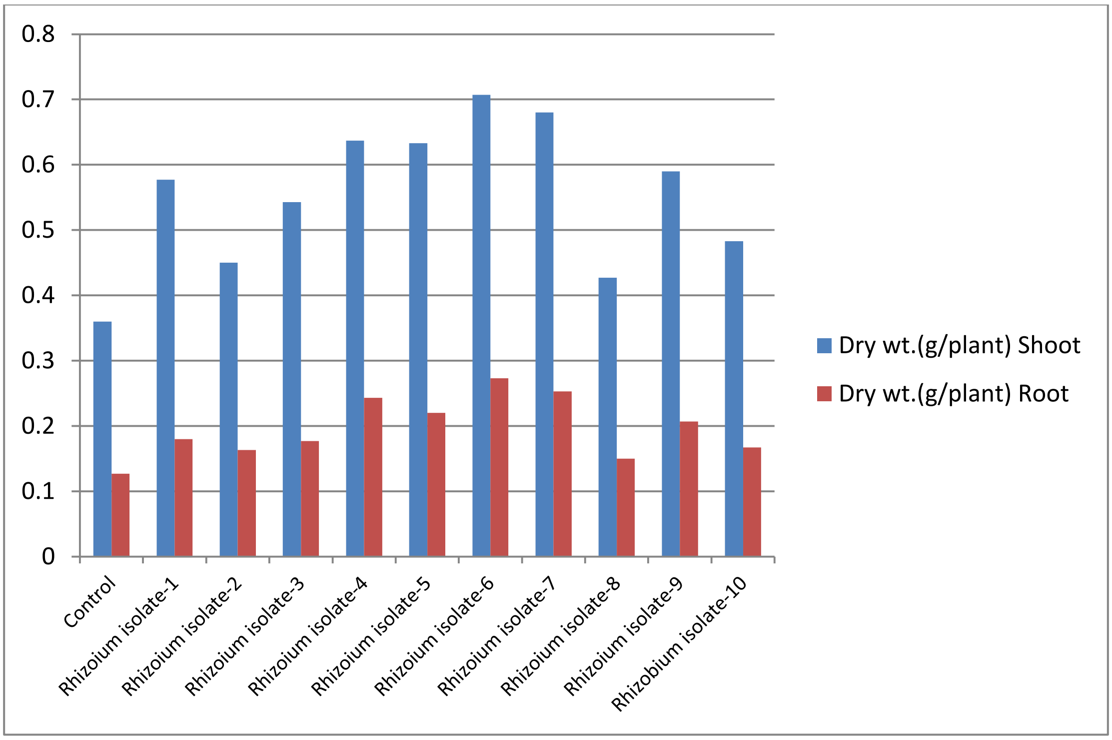

Fig. 5.7 **Effect of inoculation of *Rhizobium* isolates on dry weight of pea plant, in pot experiment under net house condition**.

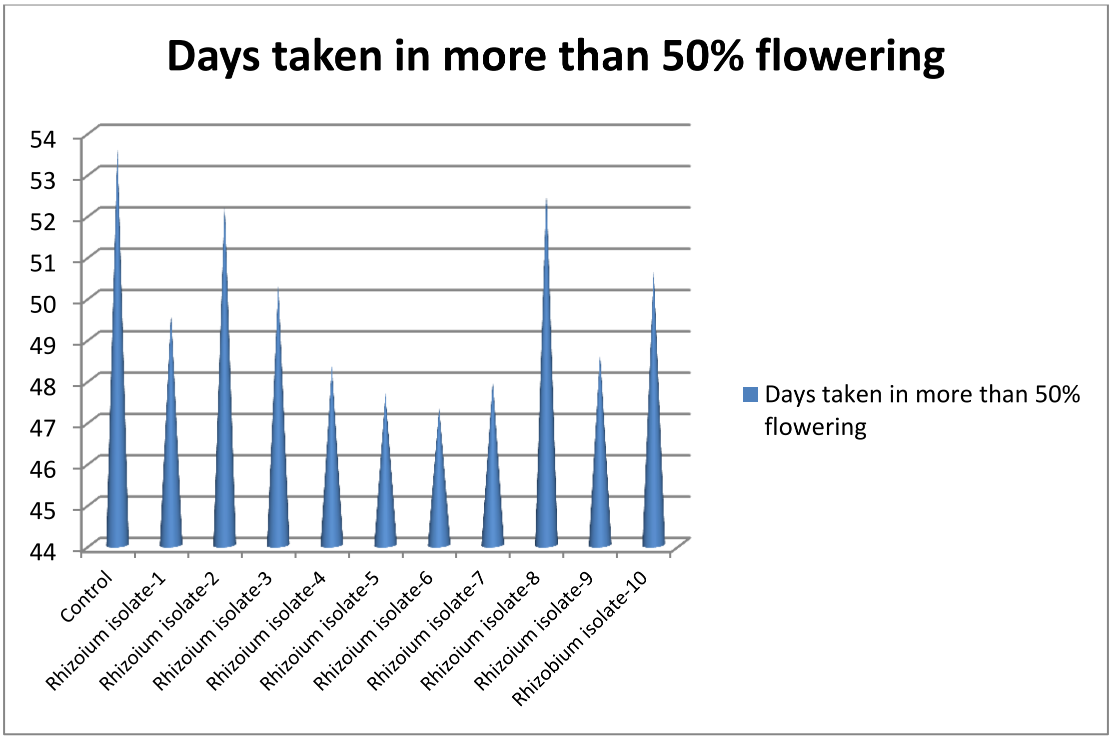

Fig. 5.8 Days taken in more than 50% flowering

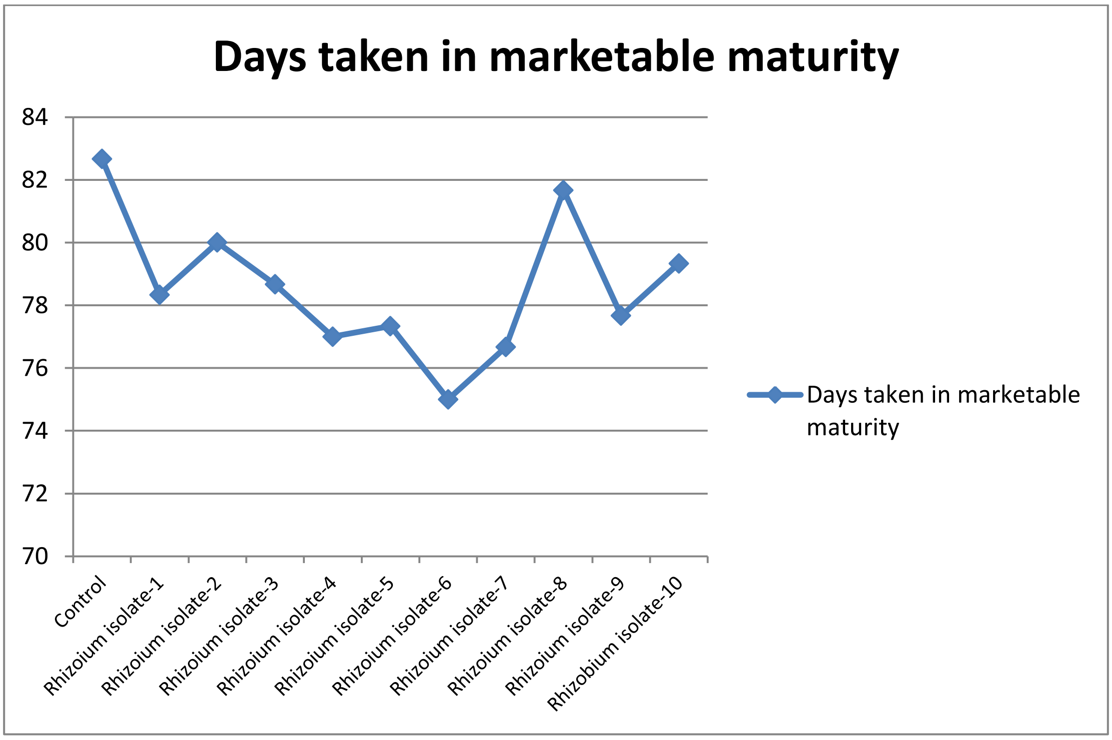

Fig. 5.9 Effect of isolate on marketable maturity

5.5 Effect of rhizobial isolates inoculation on the available nitrogen content of soil after harvest

After harvest, upon inoculation significant increase in available N of the soil was observed shown in the fig. 5.10. The maximum av. N content was recorded in *Rhizobium* isolate-6 and the least was recorded in uninoculated soil.

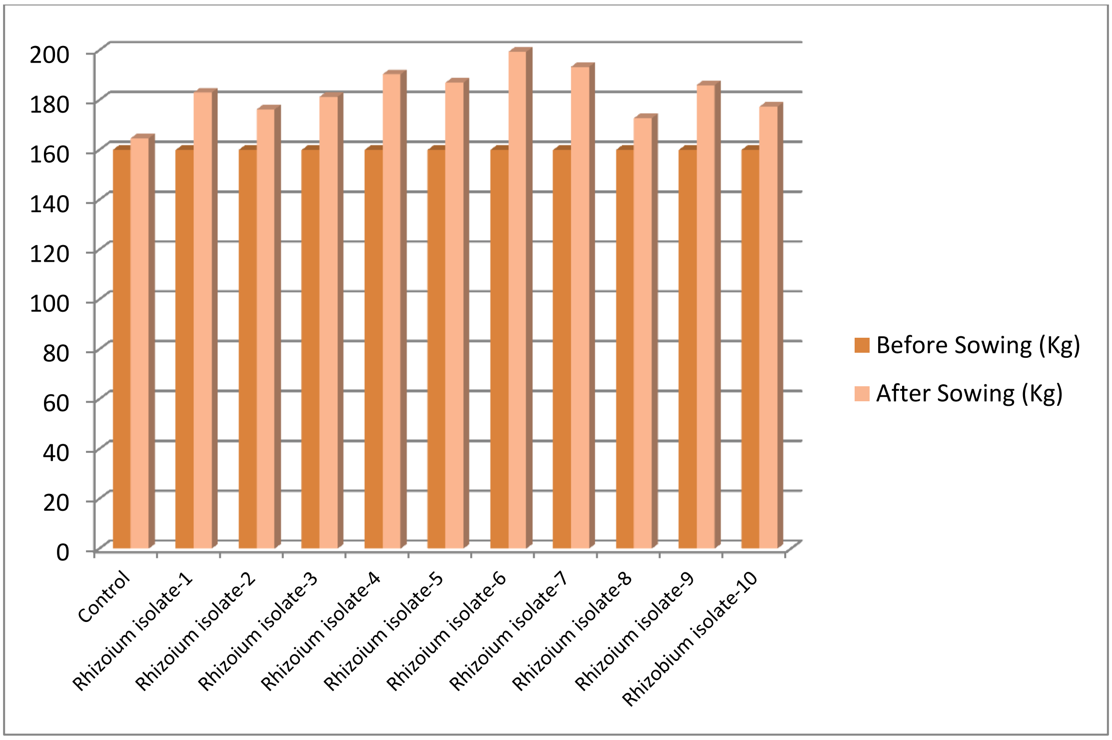

Fig. 5.10 Effect of isolates on available N content of the soil after harvest

SUMMARY AND CONCLUSION

Pea (*Pisum saivum* L.) is an annual plant of the family *Fabaceae*, which is commonly grown in many parts of the world. This herbaceous plant is highly nutritive and contains high percentage of digestible protein (25.6%), essential amino acids, carbohydrates, vitamins and minerals *Rhizobium leguminosarum* are nitrogen-fixing bacteria that lower the plants demand for nitrogen fertilization and thus contribute to environmental protection. Nitrogen-fixing bacteria supply soil with nitrogen and other nutrients. Soil-dwelling microorganisms form a symbiotic relationship with pea plants. In pea, nitrogen fertilization contributes to intensified growth, deferred maturation, leaf health, stem development and luxuriant foliage with the desired dark-green colour. Efforts are being made to improve the yield and qualitative composition of pea to make it amenable to large-scale production. In this study an attempt was made to isolate native *Rhizobium leguminosarum* from different pea growing soils around Varanasi further the best ten nodulated isolates were selected to know their efficiency with varied levels of nitrogen in pot experiment. The laboratory studies and net house studies were conducted in the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Pea rhizobia (*Rhizobium leguminosarum*) were isolated from different locations of traditional pea growing areas around Varanasi. Morphological and biochemical properties of *Rhizobium* isolates were studied. Formation of nodules was studied in leonard jar assembly method. Nodulated pea roots were collected by uprooting the plant carefully from seven pea growing areas such as Baghi, Gangpur, Ramna, Pacheura, Navgraha, Govindpura, Dharmerpur and Vishunpura and fourteen isolates were obtained on YEMA with congored medium namely PBI, PGI-1, PGI-2, PPI, PNI-1, PNI-2, PDI-1, PDI-2, PGI, PVI.

The nodulation studies made in leonrd jar assembly showed that the isolate PDI-1 produced the Highest number of nodules per plant (11.00 plant^{-1}). In pot experiment– *Rhizobium legumiosarum* isolates were inoculated under net house condition. Pot experiment was carried on to know the efficacy of *Rhizobium*.

The experiment results showed that the treatment which received isolate PDI-1 enhanced the growth parameter of pea. Highest germination *per cent* was recorded in the treatment which received isolate PDI-1. The growth parameters of pea such as plant height, number of leaves, at three growth stages *viz.*, 30 DAS, 45 DAS, and 60 DAS were recorded. Highest in the treatment which received isolate PDI-1 and least growth parameters were recorded in treatment with no fertilizer and no inoculum *i.e*, only soil. The number of nodules per plant at 60 DAS was also maximum in the same treatment. Plants treated with isolate PDI-1 showed higher amount of plant fresh weight. Higher fresh weight accumulation in plants can be attributed to better nutrient mobilization by introduced microbial inoculants by synergistic interaction which resulted in luxuriant growth of the crop.

The maximum shoot and root dry weight was found in the plants which were grown in pots treated with isolate PDI-1 and least dry matter was observed in treatment with only soil. Application of microbial inoculant with graded levels of nitrogen has significantly influenced on the increased nutrient uptake by plants treated with isolate PDI-1 and least nutrient uptake by plants was accounted in treatment with only soil.

Thus, considering the results of this experiment, among selected isolates, PDI-1 isolate found best for all the studied parameters. Hence there is need for inoculating pea with effective strain of *Rhizobium* which improves symbiotic nitrogen fixation in order to reduce the use of inorganic fertilizers and to obtain higher yields**.**

Future line of work

The best performing native strain of pea need to be evaluated for its efficiency under field conditions and interaction with other plant growth promoting rhizobacteria (PGPR).

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