COMPARATIVE STUDIES OF DIFFERENT RHIZOBIUM ISOLATES INFECTING CHICKPEA (Cicer arietinum L.) variety BDN-9-3.

BY

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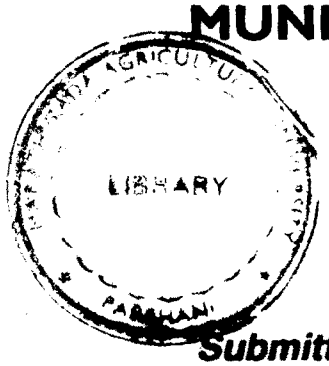
B. Sc. (Agri.)



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Affectionately Dedicated
to my
FATHER SHRI. VITTHALRAO RAMRAO MUNDHE
MOTHER SOW. PADMINBAI VITTHALRAO MUNDHE

****

CANDIDATE'S DECLARATION

I, hereby declare that the dissertation or part thereof has not been previously submitted by me to any other University or Institution for a degree or diploma.

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CERTIFICATE I

This is to certify that shri. **Mundhe Shrikant Vitthalrao** has satisfactorily prosecuted his course of research for pariod of not less than four semisters and that the dissertation entitled " **COMPARATIVE STUDIES OF DIFFERENT *RHIZOBIUM* ISOLATES INFECTING CHICKPEA (*Cicer arietinum* L.) VAR. BDN-9-3."** submitted by him is the result of original research work and is of sufficiently high standard to warrent it's presentation of the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

Place - Parbhani

Date - 30/05/1997

(Dr. K.S. Kulthe)
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CERTIFICATE II

This is to certify that the dissertation entitled **" COMPARATIVE STUDIES OF DIFFERENT *RHIZOBIUM* ISOLATES INFECTING CHICKPEA (*Cicer arietinum* L.) VAR. BDN-9-3."** submitted by shri. **Mundhe Shrikant Vitthalrao** to the Marathwada Agricultural University, Parbhani in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **Plant Pathology** has been approved by the student's advisary committee after viva-voce examination in collaboration with the external examiner.

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Parbhani

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INTRODUCTION

The chickpea (*Cicer arietinum* L.) belongs to family leguminoceae originated from south-west Asia is an important pulse crop grown in India.It is cultivated in countries belonging to tropical and sub-tropical climatic regions.In india it is cultivated on large scale in Punjab, Hariyana, Utter Pradesh, Bihar, Madhya Pradesh, Rajesthan, Andhra Pradesh, West Bengal and Karnatak.(Vaidya *et.al.* 1978).

It is mostly cultivated in rabi sesaon, as it is favoured by cool climate and morning dew . In Maharashtra it is grown as rabi pulse crop in black soils covering **433.6** thousand hactares area and total production **205.9** thousand tonnes with an average of **475** Kg. (Annonymous, 1995).

Chickpea is good source of protein containing about nineteen per cent protein in grains on dry weight basis. It provides quite a large number of amino acids, mallic acid and oxalic acid which are highly useful for intestinal disordes . It is known to supply **358** calories energy from 100 gram of kernals which is more than any legume crop except Lupin and Groundnut (Lokhande, 1982).

Legumes have played a prominant role in increas-ing soil nitrogen level,there by boosting agricultural pro-

duction. This is obviously due to their ability to fix atmosphiric gaseous nitrogen in a symbiotic association with rhizobia. This is particularly more important since the cost of fertilizer nitrogen has increased several fold so also most of fertilizer nitrogen drain off or leach from soil after application there by increases the cost of production. Now a days there is trend to avoid chemicals those may be fertillizer or pesticides so as to avoid pollution. Chemical fertilizers also affects adversely soil natural condition and microflora in it.

The another important limiting factor in chemical nitorgen fertilizers is that they are imported which require foreign exchange. Biological nitrogen fixation there fore,has greater relevance. Thus, use of more and more biotertillizers can reduce the stress on import and there by save the foreign exchange.

India is the world's largest producer of chickpea with 65 per cent of world's production . However,in India and also in Maharashtra per hactare production is quite low . The low productivity is due to various reasons, among which less efficient population of native rhizobia is

one of the important factors. The rhizobia nodulating chickpea are highly specific in infectivity and do not show affinity with any of members of known cross inoculation groups (Gaur and Sen, 1979) . This situation suggests the need of inoculation and establishment of efficient strains of *Rhizobium* infecting chickpea. Such strains must be able to produce effective nodules and able to compete local rhizobia.

Keeping in view these needs various *Rhizobium* isolates are used in presesnt investigation with following objectives on chickpea variety BDN-9-3

1) To isolate local *Rhizobium* isolate from chickpea.
2) Nodulation on host
3) Fresh and dry weight of nodule.
4) Fresh and dry weight of plant.
5) Root and shoot length of plant.
6) Nitrogen content of plant.

REVIEW OF LITERATURE

Chapter : II

REVIEW OF LITERATURE

2.1 General.

The symbiotic relationship of *Rhizobium* in chickpea was reported in past by many workers. Raju (1936) showed that *Rhizobium* infecting chickpea is highly specific in their nodulating ability and nodulate only chickpea crop . However, Gaur and Sen (1979) reported that some strains of chickpea rhizobia could nodulate *Sesbania bipinosa* and *Sesbania sesban*.

Boonkard and Weaver (1982) reported reduction in native population of cowpea rhizobia after flooding. Rupela, *et. al.* (1987) also reported that the rhizobial population reduces to the extent of 100 folds, when fields were flooded with water. This suggested a necessity of artificial inoculation of rhizobia in chickpea are grown after flooding. Rupela and Saxena (1987) have shown that population below 10^2 per gram of soil do not cause nodulation in chickpea. Response to the artificial inoculations were evident in the fields with such low level of native rhizobial population.

The inoculation of *Rhizobium* with seed have given equal yield with half of the recommanded dose of

fertillizer with that of full fertillizer dose without *Rhizobium* inoculation (Shinde *et. al.* 1985).

2.2 Isolation of native *Rhizobium*.

Rhizobium are usualy isolated by using simple isolation technique. The surface sterlized nodules are washed with sterile water and crushed in water blank. The bacterial suspension so obtained is diluted serially and used for plating. Bacterial suspension is streaked on solidified medium in petriplate. Yeast extract manitol agar medium was found to be most satisfactory and has been used by many workers (Vincent, 1970; Vyanjane, 1973; Islam and Alfandi, 1980; Patil and Shinde, 1980; Wyanne *et. al.*, 1975; Jadhav, 1985; Rupela and Saxena, 1987; Pawer, 1987;). *Rhizobium* grows well on yeast extract manitol agar medium producing characteristic well isolated watery transluscent colonies. The *Rhizobium* do not absorb congored dye and there by rhizobia can be well differentiated from other contaminants (Vincent, 1970).

Vincent, (1970) also suggested few criteria for the purpose of identification such as colony character, reaction to congored, gram reaction, cell morphology , growth on alkaline medium, growth on lactose medium, nodulation on host etc.

2.3 Comparison of *Rhizobium* isolates.

The chickpea *Rhizobium* strains interaction shown that chickpea response to efficient strains. This has been reflected in increase in various nitrogen fixing traits like nodule number,nodule mass, acetylene reduction activity, plant dry matter content, protein content , leg-haemoglobin content and seed yield. (Rai and Singh, 1977; Patil and Shinde, 1980; Sharma *et. al.* 1981; Gupta *et. al.* 1982 ; Vaishya and Gajendragadkar, 1982; Balsubramanian *et. al.*, 1983; Giller *et. al.*, 1985; Pawar, 1987).

Medhane and Patil (1974) reported an increase in seed yield by 24 to 26 per cent with the inoculation of eight strains of *Rhizobium*. The various strains have shown different effect in seed yield of chickpea (Meharchandani and Rana,1977)

Islam (1979) reported an incrcase in number of nodules from one to eighteen and in seed yield from 1.25 to 1.51 tonnes per hactare in chickpea with the inoculation of eight strains of *Rhizobium*. Rai and Singh (1979) reported that seed yield of chickpea increased by 13.9 to 29.8 per cent when inoculated with eight strains of chickpea *Rhizobium* Gf-9 gave the highest yield increase followed by strain

Gf-2, Gf-6,Gf-7 and Gf-8 Where 26 to 28 per cent increase was observed. However, there were no significant differences in nodulationand nodule mass. Ibrahim and Shaikh (1980) shown that strain 161-A was superiar over strain Cb-1189 in incrcasing the seed yield.

Khatting and Ghonsikar (1981) studied five chick-pea *Rhizobium* strains. They found that nodulation was highest with strain GA-7 while seed yield and nitrogen uptake with PBN strain. Batra and Rao (1985) While studying four strains of *Rhizobium* observed that increase in seed yield was 48.2 per cent with a strain HAU, 24.5 per cent by strain IARI and 23.6 per cent with strain LSAU -80.

Brine r *et. al.*,(1985) studied the interaction between five strains of *Rhizobium* and four chickpea culti-vars. They found that increase in all growth parameteres and nitrogen content was noticed with the inoculation of strains 300,395 and 398.

2.4 Parametres of *Rhizobium* strain evaluation :

Several parametres such as nodule number, nodule freshweight,nodule dry weight, volume of nodules, root

length, shoot length, plant dry weight, plant fresh weight, nitrogen content of plant, protein content of seed, oil content of seed, yield of pods, straw yield , leg-haemoglobin content of nodules, nitrate reductase activity of intact nodules, ureides content of leaves, tetrazolium reductase activity etc. have been used by different workers for evaluating nitrogen fixing ability of *Rhizobium* strains (Law and Strajidom,1974; Annonymous, 1996; Kulkarni *et. al.*, 1975 ; Dalela and Lodha,1976; Hamdi *et. al.* , 1978; Gaur *et. al.*, 1980 ; Mundada *et. al.*, 1980; Nambiar and Dart, 1980; Elkan *et. al.*, 1981; Teixeira *et. al.*,1981; Kremer and Paterson, 1980; Kremer and Paterson,1982; Kadam and Desi, 1983; Sharma *et. al.*, 1989; Vaishya and Dube, 1991; Rawat *et. al.*, 1991; Chandra, 1995; Palwe and Kawle, 1996)

Various workers observed that artificial inoculation of *Rhizobium* inoculation increase nodulation and seed yield. (Patil and Shinde, 1980; Iswaram *et. al.*, 1971; Islam, 1979; Sahu and Behara, 1972; Shriram and Samual, 1976; Khatting and Ghonsikar,1981).

Iswaram *et. al.* (1971) observed significant increase in nodule numbner due to *Rhizobium* inoculation in Mung-bean crop. Patil and Shinde (1980) observed incrcased nodulation with *Rhizobium* inoculation in chickpea.Similar

trend was noticed by Singh *et. al.* (1979) in Pigeon pea cultivars.

Shriram and Samual (1976) reported that *Rhizobium* strains increase nodulation which was reflected in higher dry matter production, higher nitrogen uptake and it's translocation to the seed.

Khatting and Ghonsikar (1981) studied five strains of chickpea and found that strain G-7 increased nodulaton while PBN strain incracased seed yield and nitrogen uptake.

Vaishya and Dube (1991) conducted the experiment with twenty three Cultivars of *Rhizobium* and GJ-315 Variety of chickpea. They found that these twenty three strains significantly increased nodule number , nodules dry weight and grain yield over the control. These isolates also significantly differ among themselves also.

Rawat *et. al.* (1991) reported that *Rhizobium* strain H-45 produced significantly more number of nodules over control and other treatments. Chandra (1995) reported significantly more number of nodules and nodule dry weight in case of *Rhizobium* inoculation over the control.

Palve and Kawle (1996) while working with soybean *Bradyrhizobium* Symbiosis found that *Bradyrhizobium* significantly increased nodule number, dry weight of nodule and plant dry weight over the control.

Raju and Varma (1984) reported that there was significant increase in nodule number and nodule dry weight per plant due to *Rhizobium* treatment alone or *Rhizobium* in combination with lower level of nitrogen i.e. upto 15 Kg N_2 per hactare over the rest of the treatment of gram.

Gajendragadkar and Vaishya (1983) shown with three years experimentation significant increase in nodule number.

Vaishya *et. al.*(1983) found that seed inocultion with *Rhizobium* significantly increased the number and weight of nodules. Singh *et. al.* (1975) reported that application of nitrogen and phosphorus increased number of nodules and dry weight of effective nodules per plant. Balsubramaniam *et. al.*(1980) showed that multistrain inoculation gave more nodules and higher nodule dry weight . similar trend was observed by Sawase and Patil (1984).

Nair, *et. al.*(1976) reported incrcase in mean dry weight of nodules due to inoculation over uninoculated control. Saxena *et. al.*(1975) reported increase in fresh weight of nodules in inoculated plant over uninoculated plant of pigeonpea. More *et. al.* (1981) reported higher nodule weight due to higher *Rhizobium* in groundnut rhizosphere.

Patil and Medhane (1974) reported increase in dry matter content in chickpea plant due to seed inoculation of different *Rhizobium* strains over uninoculated control. Reddy *et. al.*(1978) concluded that dry weight of black gram was increased when seeds were treated with *Rhizobium* inoculam.

Maurya and Sanoria (1982) observed that the *Rhizobium* inoculation signiticantly increase dry weight per plant in case of Bengal gram.

Sharma *et. al.* (1989) reported that *Rhizobium* inoculation have signiticantly increased plant height, branches, pods, root nodules per plant over the uninoculated control .

Raut and Kohire (1991) conducted experiment with various phosphorus fertillization level and alone *Rhizobium* treatment during 1980-81, 1981-82,and 1982-83. The nodule number, nodule wieght, dry matter per plant, nitrogen accumulation and yield per hectare were found to be significantly increased in *Rhizobium* inoculation treatment over the uninoculated control during all these three years experimentation individually and also in pulled analysis.

2.5 Nitrogen content of plant.

The *Rhizobium* inoculation to the pulse crop in general increases the total nitrogen content of plant (Kudbe, 1976; Shriram and Samuel, 1976; Azcon *et. al.*,1979; More *et. al.*, 1981; Raju and Varma, 1984; Sahu and Behara, 1972; Singh and Prasad, 1976).

Sahu and Behara (1972) observed that the nitrogen content of root, shoot and grain protein of groundnut, cowpea and green gram were considirably increased due to *Rhizobium* inoculation.

Kubde (1976) reported that *Rhizobium* inoculation alone or in combination with phosphorus and sulpher sig-

nificantly increased nitrogen and phosphorus content of gram.

Shriram and Samuel (1976) concluded that all rhizobial inoculant except IARI culture increased nitrogen up take by plant and it's transloction to the seed in chickpea.

More *et. al.* (1981) reported that *Rhizobium* inoculation supplimented with 15 Kg nitrogen per hectare have Shown maximum nitrogen uptake in peanut. Similar results in green gram were reported by Raju and Varma (1984).

Maurya and Sanoria (1982) Shown that *Rhizobium* inoculation significantly increased nitrogen and phosphorus content of seed in Bengal gram.

Prasad and Sanoria (1984) reported that *Rhizobium* inoculation significantly increased grain protein over uninoculated control in Bengal gram. They observed that the crude protin was 24.18 per cent in case of *Rhizobium* treatment where as it was 23.08 per cent in uninoculated control.

Raut and Kohire (1991) conducted experiment with various phosphorus level and alone *Rhizobium* inoculation treatment during 1980-81,1981-82 and 1982-83. The nodule number, nodule weight, dry matter per plant, nitrogen accumulation and yield per hactare was found to be significantly increased in *Rhizobium* inoculation treatment over the uninoculated control during all these three years experimentation individually and also in pulled analysis.

MATERIALS AND METHODS

Chapter :III

MATERIAL AND METHODS.

3.1 Isolation of native *Rhizobium* isolate and sub-culturing of *Rhizobium* isolates.

The plumpy pinkish nodules were selected for isolation from the chickpea plants grown in field of Agronomy Dept. Marathwada Agricultural University Parbhani, during Rabi season 1995-96. For isalation of *Rhizobium* single nodule was used. The nodule was separated carefully from root leaving small portion of root attached to the nodule. Nodule was further washed in running tap water to remove adhering soil particles. The nodule was surface sterillized by immursing in 95 per cent ethanol for five minutes and 0.1 per cent mercuric chloride for three minutes. Further, the nodule was washed in six successive changes of sterile water with the help of sterillized glass rod and allowed to stand for about five minutes in starile water for soaking. The suspension obtained by crushing nodule was streaked aseptically on surface of acidified congored yeast extract manitol agar (YEMA) medium in petridish. Composition of YEMA is given in Table -1. As the congored stain was not absorbed by *Rhizobium* but it is absorbed by other bacteria Viz.*Agrobacterium* thereby, they were differentiated from *Rhizobium*.

The plates were incubated at 28 ± 2^{o}C temprature in BOD incubator, The plates were examined after three days of

Table - 1

Composition of congored yeast extract manitol agar medium.

Ingrediants	Quantity
Distilled water	1 liter
Manitol	10.0 g
Agar-agar	15.0 g
Pot. hydrogen phosphate	0.5 g
Sodium sulphate	0.1 g
Magnesium sulphate	0.2 g
Yeast extract	0.5 g
pH	7

incubation daily. The bacterial colonies appeared within five to seven days of incubation. The Well isolated, raised, translusent colonies were selected and transferred aseptically to YEMA Slants in test tubes with the help of sterile inoculating needle. Sub-culturing was done till uniform colonies typical of rhizobia were obtained. The isolates were also mentained on YEMA slant throughout the period of investigation.

The isolates received from the Micro-biologist (pulses), Agriculhural Research station, Badnapur, Dist. Jalna were also multiplied on YEMA broth. The yeast extracts manitol broth prepared and filled in conical flasks having capacity 250ml. Nearly 150ml broth was added in each conical flask. The identical growth was taken from sub-culture and added in broth in aseptic condition . The whole procedure was done in isolaton chamber. The conical flasks were then kept on shaker and contineous shaking was given to provide aereation to *Rhizobium*. The shaking was given for seven days.

The isolate obtained from root nodule of chick-pea plant collected from field of Department of Agronomy,

Marathwada Agricultural University, Parbhani was used and called as local culture i.e. Parbhani local.

Congored 1.0 gm of Congored dissoved in 400ml of distilled water and sterillized 10ml of this congored solution added in 1 liter of sterillized medium before plating.

3.2 Pot culture experiment.

The two pot culture experiment were conducted using twenty three isolates plus one alone nitrogen dose treatment along with one no nitrogen no *Rhizobium* inoculation i.e.con- trol. Thus randamised block design experiment with twenty- five treatments having four replictions was conducted.

For nodule studies sand culture experiment was undertaken. For growth characters the soil, sand and farm yard manure based medium was used to conduct the experiment. In both the experiments the treatments and replictions numbers were same. Details of treatments were given as follows.

*G-7	G-13	G-14	G-16	G-19	G-22	G-29
G-38	G-41	G-43	G-47	G-49	G-51	G-61
G-64	G-69	G-72	G-75	G-83	G-87	G-103
G-110	PBN local		Nitrogen		Control	

*Chickpea *Rhizobium* isolate.

3.2.1. Sand culture expderiment.

The ability of isolates based on the production of nodules on chickpea was ascertained in pot culture trial conducted in screen house, Department of Plant Pathology, MAU Parbhani . The earthen pots of 9" size were used for trial. The sand was sterillized in auto-clave at 1.5 Kg/cm^2 vapour pressure with $121_{\emptyset}C$ temperature for 30 minutes and used for experiment. The pots were filled with sterillized sand. The surface sterilized seeds of chickpea variety BDN-9-3 were sown in these pots. Ten seeds were sown in each pot and finally five plants per pot were maintained. Pots were watered with sterillized water. To provied different nutrients for growth of plant, a nutrient solution (Table-2) was added at the rate of 100 ml per pot at the sowing time and 5ml per pot every day there after.

The cultures under test were incoulated in the form of *Rhizobium* isolates sub-cultured and multiplied on yeast extract manitol broth for one week.

Twenty two cultures plus one local i.e. in all twenty three cultures were tested in this experiment. In control pots with sterile water at the rate of 5ml per pot was added.

Table - 2

Statement showing composition of long Ashton nutrient solution.

Ingrediants	Quantity
$MgSo_4.7H_2o$	0.233 g
K_2HPo_4	0.175 g
Nafe Edta	0.041 g (5 ppm $Fecl_3$)
K_2So_4	0.277 g
$CaSo_4$	0.630 g
Distilled water	1 liter
pH	6.8
Trace element solution	
$Mncl_4.4H_2o$	1.81 g
$CuSo_4.5H_2o$	1.08 g
$ZnSo_4.7H_2o$	0.22 g
H_3Bo_3	2.86 g
$Na_2Moo_4.2H_2o$	0.286 g
Distilled water	1 liter

1ml of trace element solution per liter of nutrient solution.

On the fortyfifth day of plant germination, plant were depotted and nodulation studies were undertaken. Nodulation studies consist of nodule number, nodule fresh weight and nodule dry weight.

3.2.2. Soil culture experiment.

A soil pot culture experiment was conducted in screen house,Department of Plant Pathology, MAU Parbhani . The earthen pots used were 9" size. The soil, sand and farm yard manure were taken in praportion of 50:25:25.

This mixture was then sterillized at 121°C with 1.5 Kg/cm_2 vapour pressure in autoclave for 30 minutes. Then pots were filled with this mixture.

Ten surface sterillized seeds of chickpea cv BDN-9-3 were sown in each pot *Rhizobium* strains multiplied on yeast extract manitol broth were applied just below the seed at the rate of 2 ml per hill. In control treatment sterile water was applied. The pots were watered with sterile water. After germintion thinning was done and five plants per pot were maintained .

On the forty-fifth day of the plant germination the plants were depotted and used to measure different

growth contribu ting characters Viz plant height, root length, fresh weight of plant, dry weight of plant and nitrogen content of plant.

3.3 Details of observation.

The observations were recorded by using different units and measurements, treatment and replication wise. The average of five plants was taken.The data obtained from various experiments was subjected to statistical analysis as per methods described by Panse and Sukhatme (1967).

3.3.1. Comparative studies of Rhizobium isolates for nodulation

For various observations on nodulation viz. nodule number,nodule fresh weight and nodule dry weight the nodules were collected per plant treatment and replication wise after depotting of plants from sand culture experiment.

a) Nodule number.

The plants grown in sand culture experiment were depotted carefully . The root system was washed taking care that no nodules are lost in the process and physical count of number of nodules was done.

b)Fresh weight of nodules.

After uprooting the plants from sand culture experiment were washed carefully. The nodules of each plant were separated and weights were taken on electronic weighing balance

c)Dry weight of nodules.

The nodules were separated carefully from roots and dried in oven at 60°C for 5 hrs. The last constant weight was recorded as dry weight.

3.3.2. Comparative studies of *Rhizobium* isolates for growth parameters.

The growth parametres viz plant height, root length, plant fresh weight and dry weight etc. were measured from plants depotted from soil experiment. The data obtained was based on average of five plants per treat-ment per replication.

a) Plant height.

Before depotting the plant, height was measured from soil base to the top of plant, by using a metre scale.

b) Root length.

After depotting the root length was measured by metre scale from stem root joint to the tip of tap root.

c) Fresh weight of plant.

After depotting, roots were washed and the whole plant weight was measured by using electronic weighing balance.

d) Dry weight of plant:-

The plants were oven dried at 60 $^{o}C \pm 2^{o}C$ for 5 hrs. The last constant weight was taken as dry weight of plant .

3.3.3 Nitrogen content of plant.

The oven dried plant (Root + shoot + nodules) was ground to powder form and nitrogen content was estimated by Kjeldhal method (A.O.A.C. 1955)

bKjeldhal method of Nitrogen estimation.

Principle :- $(NH_4)_2So_4$ present in H_2So_4 H_2O_2 extract is decmposed by adding conc NaoH. The NH_3 evolved is absorbed in boric acid solution. The ammonium borate formed is titrated with std. acid, From Volume of std. acid % N is Calculated.

Reagents for digestion

Conc. HNo_3 Conc. H_2So_4 Conc. $HClo_4$

Reagents for Distillation :-

1)o.o2N H_2So_4 - Take 0.556ml of H_2So_4 and pour it in small amount of water make up the volumes to 1000 ml

2)40 per cent sodium hydroxide (NaoH)

3) Boric acid: Indicator solution - Place 80gm of pure boric acid (H_3Bo_3) in a five liter flask marked to indicated a volume of 4 litre, add about 3800 ml of water and heat and swirit the flask until the H_3Bo_3 is dissolved, cool the solution, add 80 ml of mixed indicator solution prepared by dissolving 0.0990 gm of bromocresol green and 0.066gm of methyl red in 100 ml of ethonol.Then add 0.1N NaoH contineously until the solution assumes reddish purple tinge(pH5.0) and make the solution to 4 litres by adding water mix the solution before use.

Procedure:

(A) Digestion

1) Weigh 0.5 gm of finely ground plant sample and transfer it into a 50ml long necked reflux flask.

2)Add 4.4ml of digestion soultion

3)Heat very genthy until the material reaction subsidis then most strongly

4)Continue heating until the H_2So_4 is drive off and the H_2So_4 is refluxing down the neck of flask.

5)Continue the digestion until all traces of yellow colour due to residual organic matter has gone and then heat for a further 20 minutes (This will genrally need 1.3 hrs).

6)Allow to cool and carefully dilute with water.

7)Cool again and dilute to 50 ml in voulmetric flosk with water.

8) Digest is ready for nitrogen estimation.

B)Distillation:

1)Pipette out 10 ml of digested material into distillation flask(500 ml).

2)Take 10 ml Boric acid indicator solution in beaker and placce it under condenser. Ensure that tip of condenser dip into solution.

3) Add 10 ml of 40 per cent sodium hydroxide in distillation flask.

4)Start heating after making required connection.

5)Finnally insure that all nitrogen is received which is tested by a piece of red litmus paper.

6)Stop distillation.

7)Titrate it against 0.02 N H_2So_4 till green colour changes

to faint redish purple. Note down the burrete reading and calculate percent nitrogen in plant samples.

Reactions:

1) $H_3Bo_3 + 3NH_4OH \rightarrow (NH_4)_3Bo_3 + 3H_2O$

2) $2(NH_4)_3Bo_3 + 3H_2So_4 \rightarrow 3(NH_4)_2So_4 + H_3Bo_3$

3) $3H_2So_4 = (NH_4)_3Bo_3$

$$\text{PER CENT N} = \frac{(\text{VOL. OF } H_2So_4 * N) * 0.014 * \text{VOL OF MACE} * 100}{\text{VOLUME TAKEN} * \text{WEIGHT OF SAMPLE TAKEN}}$$

RESULTS

Chapter -IV

RESULTS

The results of the different experiments conducted in present investigation are presented in this chapter.

4.1 Sub-culturing of *Rhizobium* isolates and their multiplication :

The twenty two isolates of *Rhizobium* were obtained from microbiologist (pulses) Agricultural research station, Badnapur. One culture was isolated from chickpea crop grown in field of Department of Agronomy, Marathawada Agricultural University, Parbhani. These isolates were flourishly multiplied on yeast extract manitol agar medium having nutral pH. with incubation temperature of 30+2^{O}C within seven days. All the isolates produced circular transluscent watery raised colonies with entire margin. These isolates were then multiplied on yeast extract manitol broth. The isolates grown well in seven days.

4.2 Pot culture experiment.

The two pot culture expriments Viz. sand culture and soil (soil+sand+FYM;50:25:25 ratio) culture experiments were conducted having twenty five treatments with four replications. Twenty five treatments consists of twenty two isolates from Agricultural Research Station Badnapur, one local from Parbhani i.e. isolated in laboratory of

Department of Plant Pathology, M.A.U., Parbhani, then one treatment of nitrogen application and control without nitrogen without *Rhizobium* treatment. Out of ten seeds sown per pot nearly five to seven seeds were germinated per pot. Five plants of chickpea per pot were mentained for experimental purpose .

4.3 Effect of *Rhizobium* inoculation and nitrogen application on nodule parametres:

3.1 Nodule number:

The data pertaining nodule number in chickpea was presented in table-3 and depicted graphically (graph I).

It is evident from the data that twenty three *Rhizobium* isolates have shown significant increase in number of nodules per chickpea plant. The *Rhizobium* isolate G-72 induced the highest numbar of nodules per plant (30.50) followed by isolate G-51 (27.50) and isolate G-69 (22.50). These three meritorious isolates also significantaly differ from each other amo n g themselves.

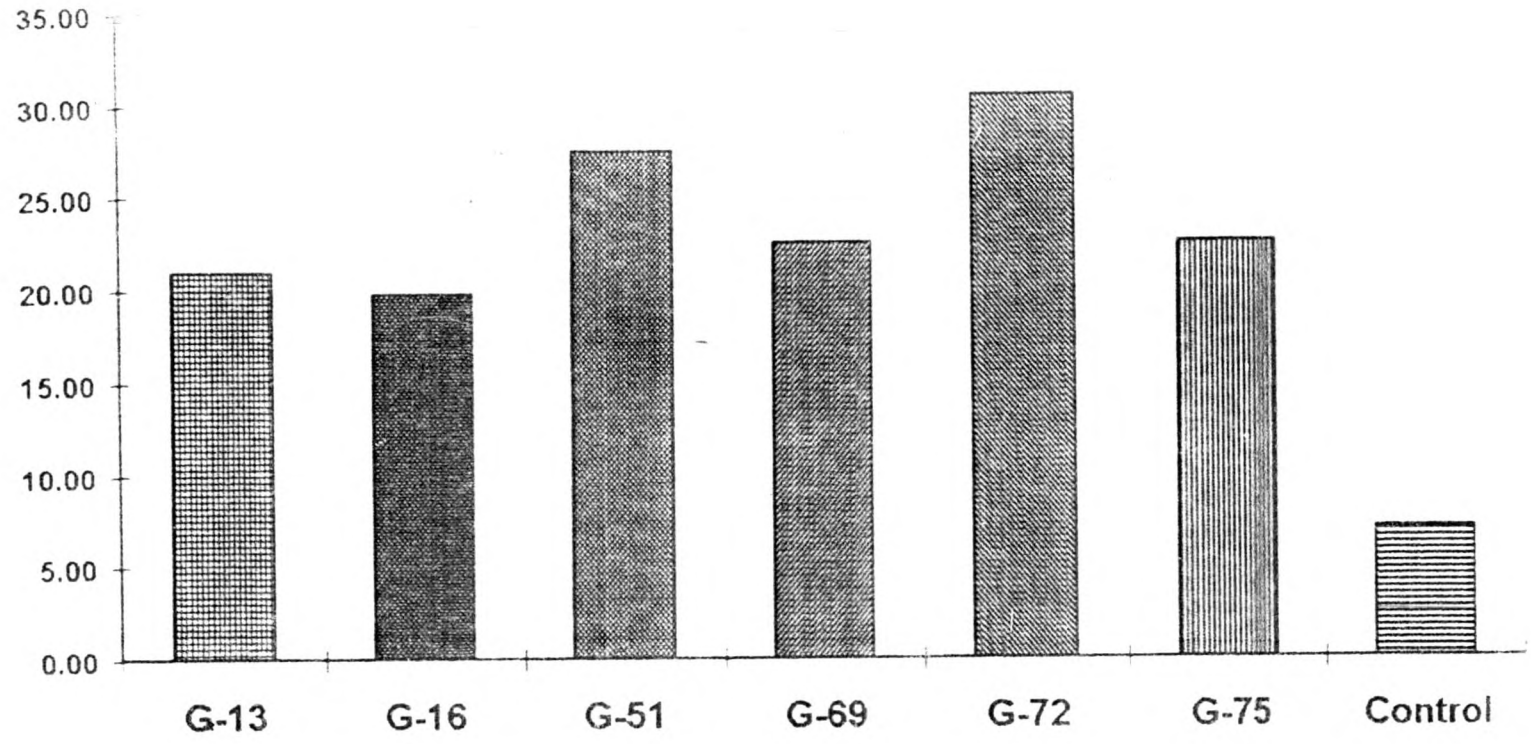
The data shows that the nodule numbers was increased more or less significantly over the control indicated that application of *Rhizobium* increased nodule number. However alone nitrogen application reduce the nodules per plant.

Table - 3

Nodule number per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean nodule number	Increase (%) over control	
1.	G-7	13.00	84.39	
2.	G-13	21.00	197.67	
3.	G-14	11.75	66.67	
4.	G-16	19.75	180.14	
5.	G-19	15.75	123.40	
6.	G-22	10.00	41.84	
7.	G-29	14.50	105.67	
8.	G-38	19.25	173.05	
9.	G-41	13.00	84.39	
10.	G-43	12.50	77.30	
11.	G-47	14.25	102.13	
12.	G-49	14.00	98.78	
13.	G-51	27.50	290.07	II
14.	G-61	16.25	130.49	
15.	G-64	19.25	173.05	
16.	G-69	22.50	219.15	III
17.	G-72	30.50	332.62	I
18.	G-75	22.50	219.15	
19.	G-83	18.00	155.32	
20.	G-87	15.75	123.40	
21.	G-103	17.00	141.13	
22.	G-110	19.50	176.59	
23.	PBN(local)	16.00	126.95	
24.	Nitrogen	6.05	-ve	
25.	Control	7.05	----	
S.E.	±	0.454		
C.D.	at 5%	1.57		

Graph I



Effect of different chickpea *Rhizobium* isolates on number of nodules per plant (Topmost six treatments along with control).

4.3.2 Fresh weight of nodule.

The data pertaining to fresh weight of nodules per plant as influenced by inoculation of different *Rhizobium* isolates in chickpea are presented in table-4 and depicted graphically (graph II).

The data clearly indicated that significant difference exists among the treatments. The significant increase in fresh weight was noticed in all the *Rhizobium* inoculation treatments over the control. The increase in nodule fresh weight was influenced by increase in number of nodules and also increase in nodule size.

The highest nodule fresh weight was obtained in isolate G-72 (374 mg) followed by isolate G-64 (366mg) and isolate G-51 (362mg).

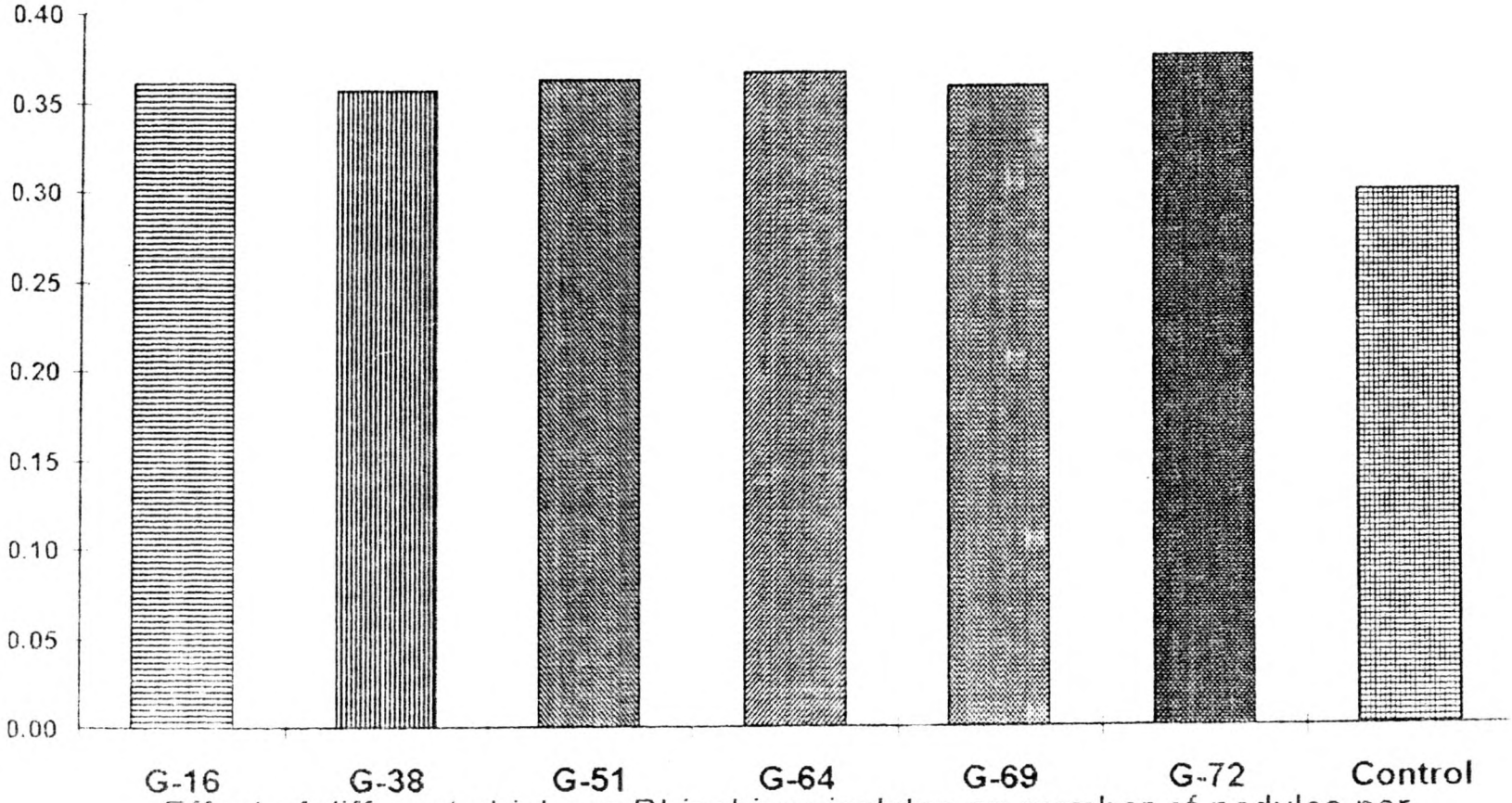
Reduction in nodule fresh weight was noticed in treatment where only nitrogen was applied. This indicate that nitrogen application adversely affect the *Rhizobium* activity.

Table - 4.

Nodule fresh weight per plant of chickpea (var- BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean nodule fresh weight (gm)	Increase (%) over control	
1.	G-7	0.339	17.30	
2.	G-13	0.348	20.41	
3.	G-14	0.316	9.34	
4.	G-16	0.360	24.57	
5.	G-19	0.349	20.76	
6.	G-22	0.340	17.64	
7.	G-29	0.353	22.15	
8.	G-38	0.356	23.18	
9.	G-41	0.356	23.18	
10.	G-43	0.355	15.92	
11.	G-47	0.343	18.69	
12.	G-49	0.335	15.92	
13.	G-51	0.362	25.26	III
14.	G-61	0.336	16.26	
15.	G-64	0.366	26.64	II
16.	G-69	0.358	23.88	
17.	G-72	0.374	29.41	I
18.	G-75	0.355	22.83	
19.	G-83	0.346	19.72	
20.	G-87	0.366	16.26	
21.	G-103	0.342	18.33	
22.	G-110	0.346	19.72	
23.	PBN(local)	0.340	17.64	
24.	Nitrogen	0.297	-ve	
25.	Control	0.298	----	
S.E.	±	0.00092		
C.D. at 5%		0.00319		

Graph II



Effect of different chickpea Rhizobium isolates on number of nodules per plant (Topmost six treatments along with control).

4.3.3 Nodule dry weight.

The data pertaining with nodule dry weight of plants are presented in table-5 and depicted graphically (graph III).

The data clearly indicated that substantial increase in nodule dry weight was observed in twenty three treatments, where *Rhizobium* isolates were applied over the control (uninoculated) and alone nitrogen application treatment.

The significant differences among isolates are also been observed. The inoculation of isolate G-72 produced highest nodule dry weight (22.06mg). The isolate G-64 and isolate G-16 both stand second in merit with having nodule dry weight (18.99mg) per plant. The third position was of isolate G-75 (18.15mg). Nitrogen alone application adversely affect the nodule dry weight (12.06mg).

4.4 Effect of *Rhizobium* inoculation and nitrogen application on growth parametres of chickpea plant.

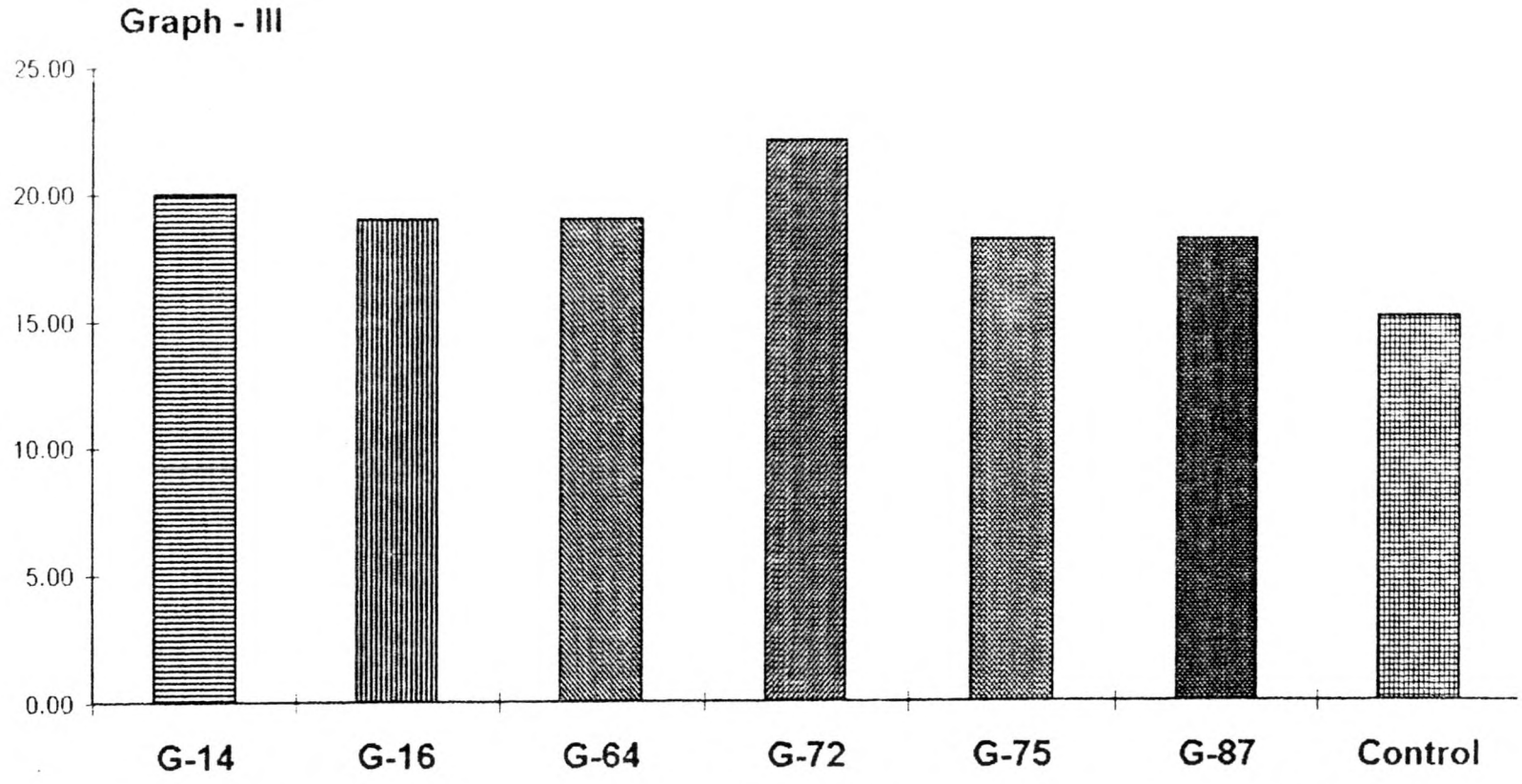
4.4.1 Plant height:

The relevant data on plant height as observed on forty fifth day is presented in table -6 and depicted graphically (graph IV).

Table - 5

Nodule dry weight per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean nodule dry weight (mg)	Increase (%) over control	
1.	G-7	17.99	18.98	
2.	G-13	18.04	19.31	
3.	G-14	19.99	18.98	
4.	G-16	18.99	25.59	
5.	G-19	18.03	19.25	
6.	G-22	17.05	12.76	
7.	G-29	18.04	19.31	
8.	G-38	17.06	12.83	
9.	G-41	16.06	6.21	
10.	G-43	17.06	12.83	
11.	G-47	18.03	19.24	
12.	G-49	18.02	19.18	
13.	G-51	18.03	19.24	
14.	G-61	18.02	19.18	
15.	G-64	18.99	25.59	II
16.	G-69	18.05	19.38	
17.	G-72	22.06	45.89	I
18.	G-75	18.15	20.03	III
19.	G-83	18.09	19.64	
20.	G-87	18.11	19.78	
21.	G-103	18.08	19.58	
22.	G-110	18.12	19.84	
23.	PBN(local)	18.08	19.58	
24.	Nitrogen	12.06	-ve	
25.	Control	15.12	----	
S.E.	±	0.017		
C.D.	at 5%	0.057		



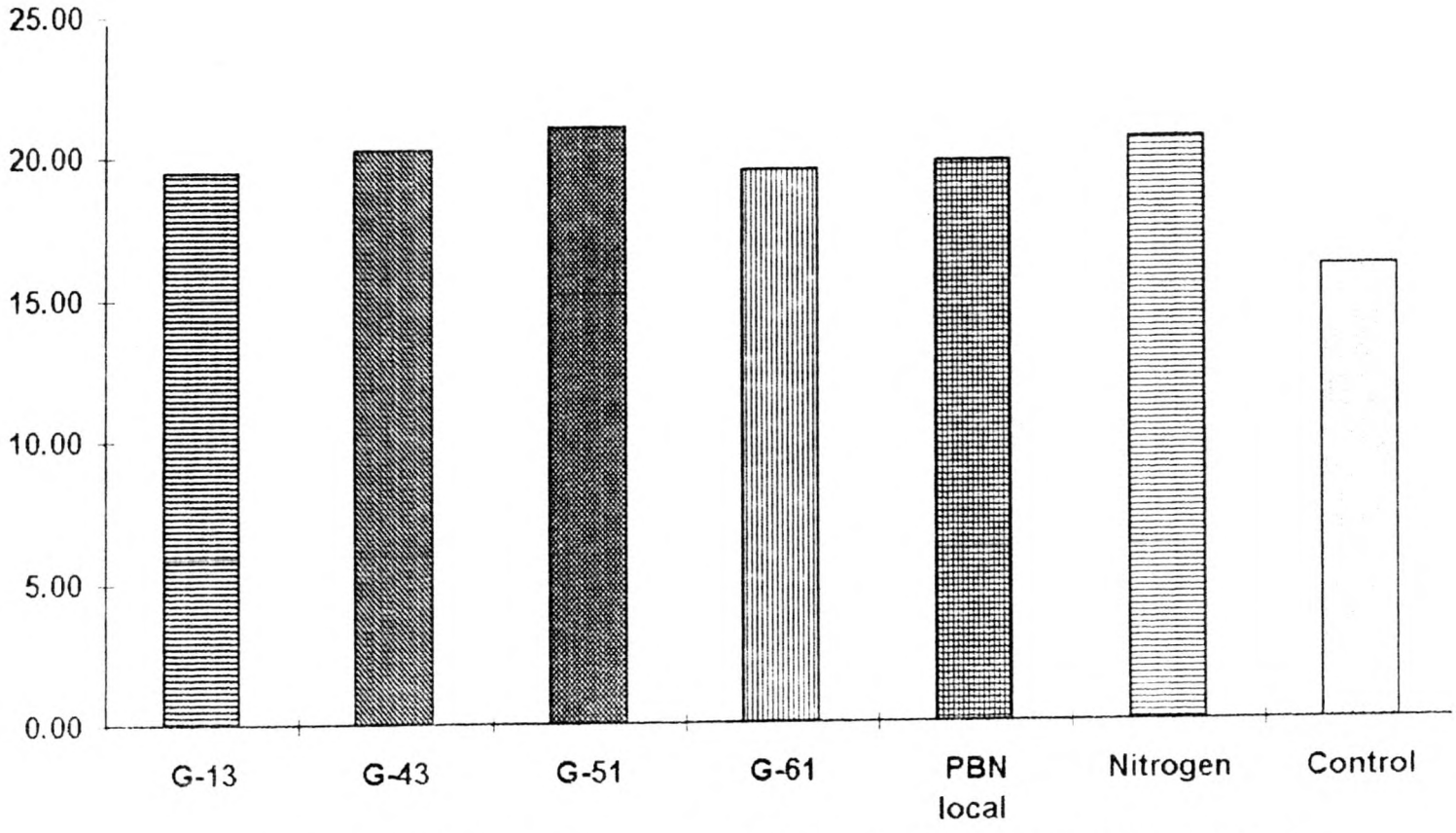
Effect of different chickpea *Rhizobium* isolates on noduledry weight per plant (Topmost six treatments along with control).

Table - 6

Plant height per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean plant height (cms)	Increase (%) over control	
1.	G-7	18.75	17.18	
2.	G-13	19.50	21.87	
3.	G-14	19.00	18.75	
4.	G-16	18.25	14.06	
5.	G-19	19.00	18.75	
6.	G-22	18.50	15.63	
7.	G-29	18.50	15.63	
8.	G-38	18.25	14.06	
9.	G-41	19.25	20.31	
10.	G-43	20.25	25.56	III
11.	G-47	19.50	21.87	
12.	G-49	20.25	25.56	
13.	G-51	21.00	31.25	I
14.	G-61	19.50	21.87	
15.	G-64	17.00	6.25	
16.	G-69	19.00	18.75	
17.	G-72	19.50	21.87	
18.	G-75	19.50	21.87	
19.	G-83	19.50	21.87	
20.	G-87	16.75	4.68	
21.	G-103	18.50	15.62	
22.	G-110	16.75	4.68	
23.	PBN(local)	19.75	23.44	
24.	Nitrogen	20.50	28.13	II
25.	Control	16.00	----	
S.E.	±	0.804		
C.D. at	5%	N.S.		

Graph - IV



Effect of different chickpea *Rhizobium* isolates on plant height per plant (Topmost six treatments along with control).

The data presented in table shown that there is significant increase in height of plant. The chickpea plant in control treatment had an average height of sixteen cm. The highest height was obtained in treatment number thirteen (G-51 isolate) followed by treatment number twenty four i.e. alone nitrogen application and G-47 isolate.

Of the twenty four treatments fifteen treatments have shown significant increase over control. However, these fifteen treatments have no significant difference among themselves.

The result indicate that *Rhizobium* inoculation increased chickpea plant height numericaly significant.

4.4.2 Root length:

The data pertaining to root length of plant is presented in table -7 and depicted graphically (graph V).

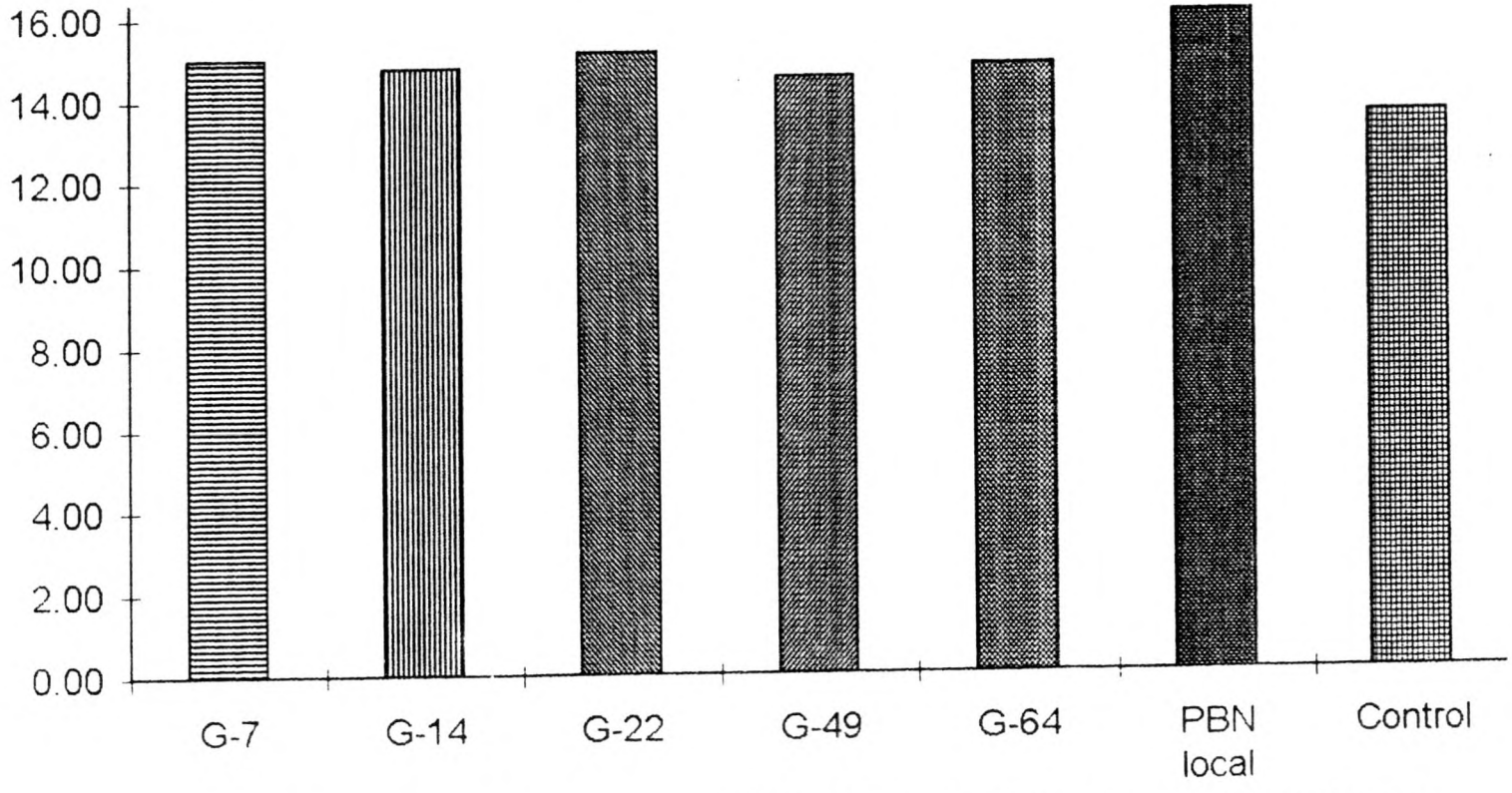
The results of root length are statistically nonsignificant. However, numerical increase in root length was noticed in all twenty three treatments over the control except treatment number eight i.e. isolate G-38.

Table - 7

Root length per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean root length (cms)	Increase (%) over control	
1.	G-7	15.00	11.11	III
2.	G-13	13.75	1.85	
3.	G-14	14.75	9.25	
4.	G-16	14.50	7.40	
5.	G-19	14.25	5.56	
6.	G-22	15.12	12.00	II
7.	G-29	15.00	11.11	
8.	G-38	13.50	------	
9.	G-41	14.00	3.70	
10.	G-43	14.25	5.56	
11.	G-47	13.75	1.85	
12.	G-49	14.50	7.40	
13.	G-51	14.00	3.70	
14.	G-61	14.12	4.59	
15.	G-64	14.75	9.25	
16.	G-69	14.00	3.70	
17.	G-72	14.25	5.56	
18.	G-75	14.50	7.40	
19.	G-83	14.25	5.56	
20.	G-87	14.12	4.59	
21.	G-103	14.00	3.70	
22.	G-110	14.37	6.44	
23.	PBN(local)	16.00	18.51	I
24.	Nitrogen	13.75	1.85	
25.	Control	13.50	----	
S.E.	±	0.50		
C.D. at	5%	N.S.		

Graph -V



Effect of different chickpea *Rhizobium* isolates on root length per plant (topmost six treatments along with control).

The highest root length was observed in chickpea plant inoculated with native *Rhizobium* isolate (16 cms) followed by isolate G-22 (15.12cms) and isolate G-29 (15cms).

4.4.3 Fresh weight of plant:

The relevant data on fresh weight of chickpea plant is presented in table -8 and depicted graphically (graph VI).

Data presented in table-8 shown that there is significant increase in fresh weight of chickpea plant over the control in all twenty four treatments.

The highest plant fresh weight was obtained in isolate G-72 (6.358 g) followed by isolate G-49 (6.223g) and nitrogen treatment (6.218 g).

4.4.4 Dry weight of plant:

The data pertaining to the dry weight of chickpea plants is presented in table-9 and depicted graphically (graph VII).

The inoculation of *Rhizobium* isolates and

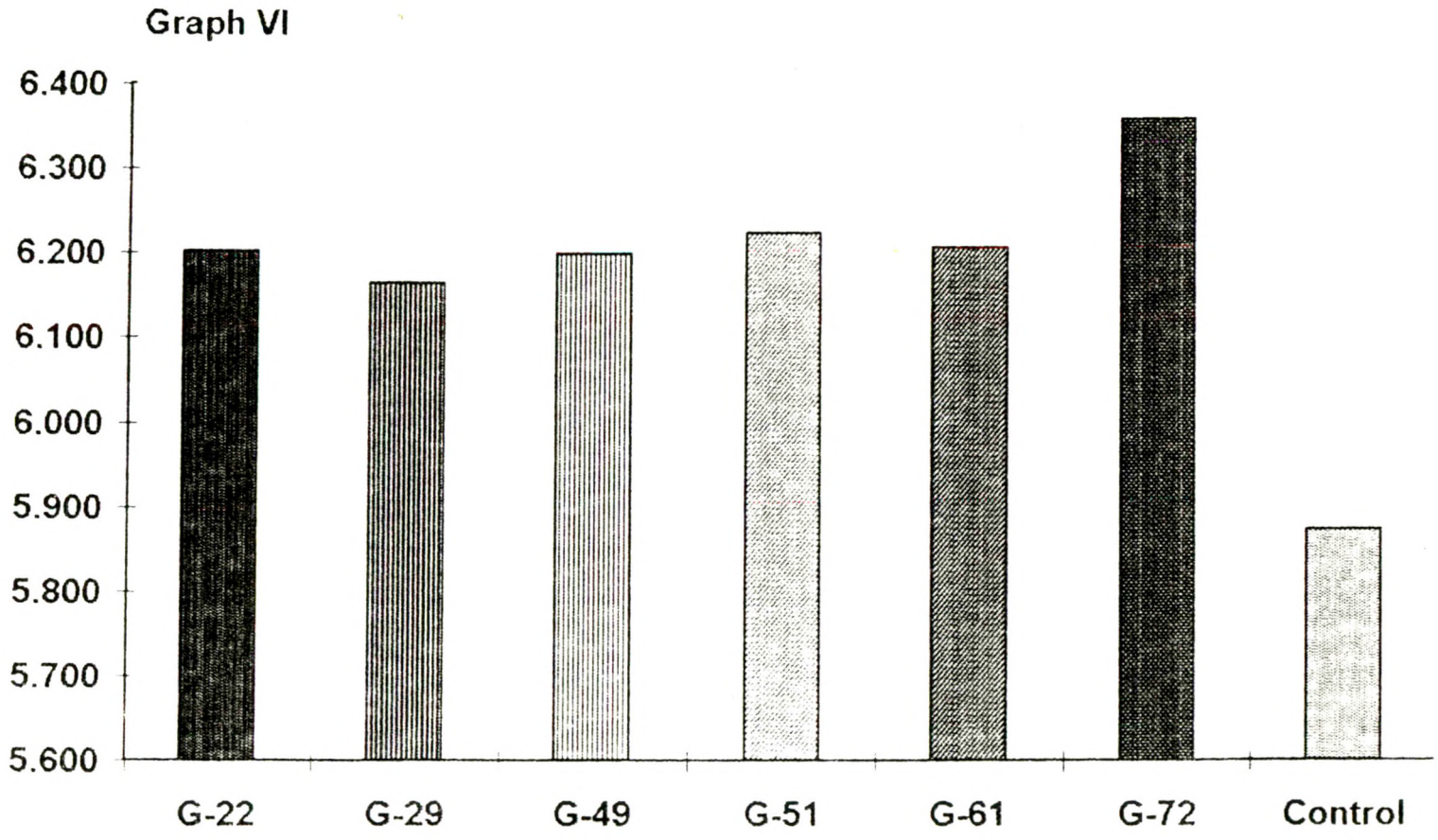
Table - 8

Fresh weight per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean fresh weight of plant (g)	Increase (%) over control	
1.	G-7	6.135	4.46	
2.	G-13	6.135	4.46	
3.	G-14	6.193	5.45	
4.	G-16	6.098	3.83	
5.	G-19	6.185	5.31	
6.	G-22	6.203	5.62	
7.	G-29	6.165	4.97	
8.	G-38	6.140	4.55	
9.	G-41	6.160	4.89	
10.	G-43	6.135	4.46	
11.	G-47	6.173	5.11	
12.	G-49	6.198	5.53	
13.	G-51	6.223	5.96	II
14.	G-61	6.205	5.65	
15.	G-64	6.165	4.97	
16.	G-69	6.160	4.89	
17.	G-72	6.358	8.26	I
18.	G-75	5.955	1.37	
19.	G-83	5.965	1.57	
20.	G-87	6.118	4.17	
21.	G-103	6.093	3.75	
22.	G-110	6.065	3.27	
23.	PBN(local)	6.123	4.27	
24.	Nitrogen	6.128	5.87	III
25.	Control	5.873	---	

S.E. ± 0.074

C.D. at 5% 0.0257



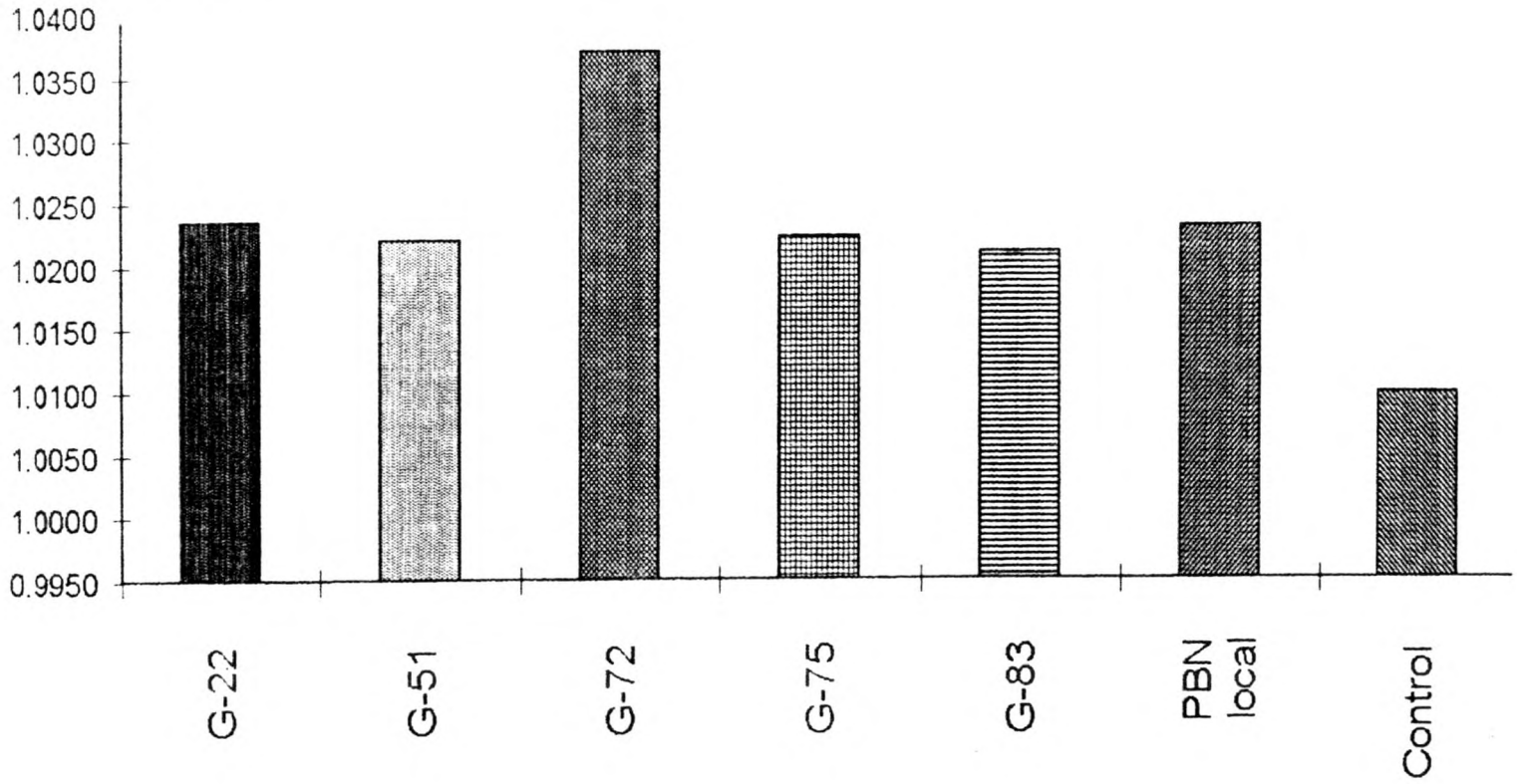
Effect of different chickpea *Rhizobium* isolates on fresh weight per plant (Topmost six treatments along with control)

Table - 9

Dry weight per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean dry weight of plant (g)	Increase (%) over control	
1.	G-7	1.0188	0.90	
2.	G-13	1.0193	0.95	
3.	G-14	1.0197	0.99	
4.	G-16	1.0160	0.62	
5.	G-19	1.0200	1.02	
6.	G-22	1.0235	1.37	II
7.	G-29	1.0153	0.55	
8.	G-38	1.0125	0.28	
9.	G-41	1.0115	0.18	
10.	G-43	1.0123	0.26	
11.	G-47	1.0198	1.00	
12.	G-49	1.0200	1.02	
13.	G-51	1.0220	1.22	
14.	G-61	1.0205	1.07	
15.	G-64	1.0160	0.62	
16.	G-69	1.0135	0.38	
17.	G-72	1.0370	2.70	I
18.	G-75	1.0222	1.24	III
19.	G-83	1.0210	1.12	
20.	G-87	1.0195	0.97	
21.	G-103	1.0200	1.02	
22.	G-110	1.0208	1.09	
23.	PBN(local)	1.0230	1.32	
24.	Nitrogen	1.0205	1.07	
25.	Control	1.0097	---	
S.E.	±	0.00078		
C.D.	at 5%	0.0027		

Graph VII



Effect of different chickpea *Rhizobium* isolates on dry weight per plant (Topmost six treatments along with control).

nitrogen fertilizer alone application to chickpea plants in pot culture experiment significantly increased the dry weight of plants over the control in which no nitrogen applied and no *Rhizobium* inoculation done. This clearly indicated that *Rhizobium* inoculant and alone nitrogen application have significantly increased the plant growth.

The inoculation of isolate G-72 produced highest dry weight of plant (1.037 g) followed by isolate G-22 (1.0235 g) and isolate G-75 (1.0222 g).

4.5 Effect of treatment of different *Rhizobium* isolates on total Nitrogen content of chickpea.

The data pertaining to nitrogen content of chickpea plant was presented in table-10 and depicted graphically (graph VIII).

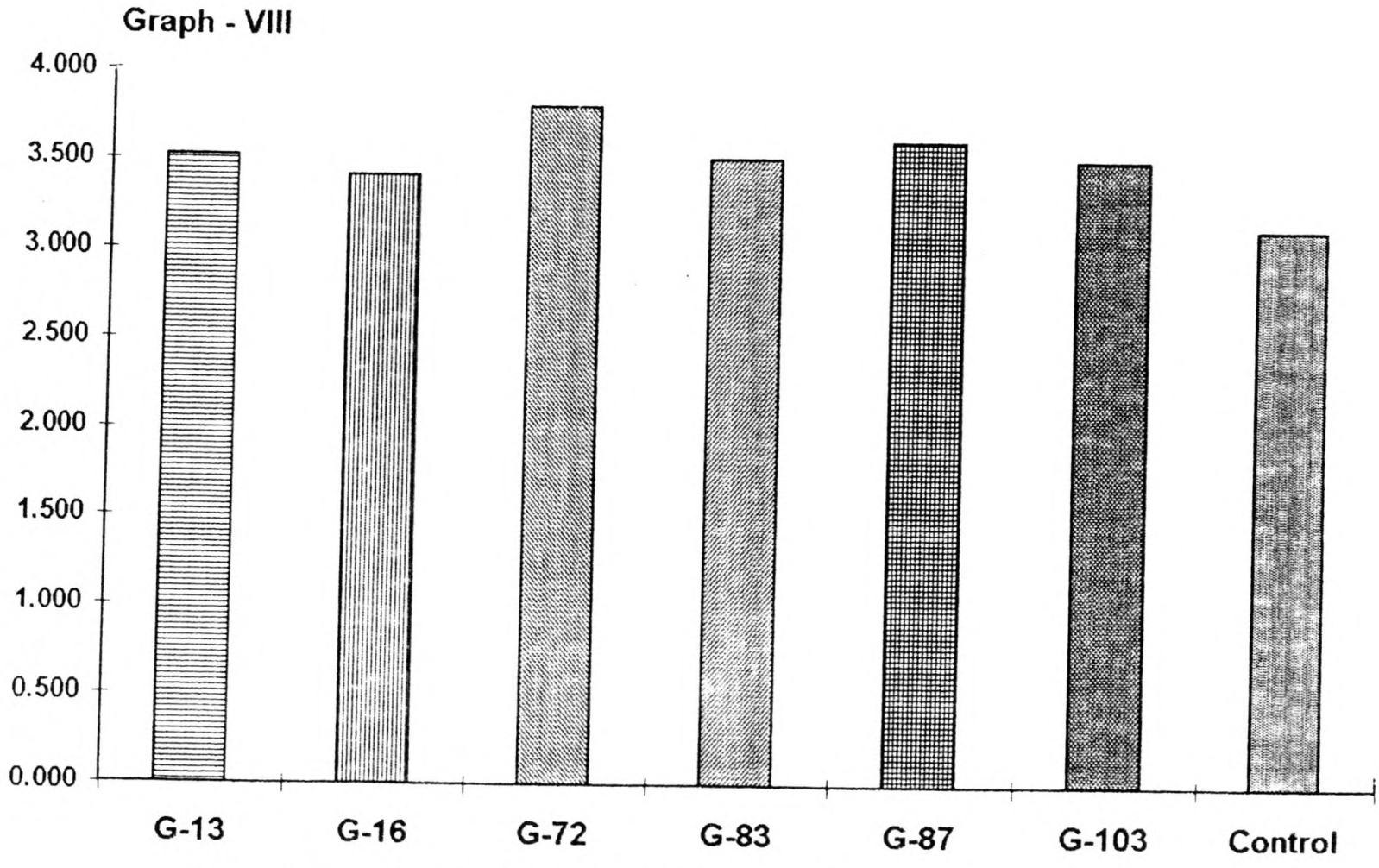
The data clearly indicated that there was substantial increase in total nitrogen content of plant in all twenty four treatment over the control. The significant differences among isolates were also observed.

The inoculation of isolate G-72 had significant effect on total nitrogen content ofplant (3.803 per cent) followed by isolate G-87 (3.613 per cent) and isolate G-13 (3.523 per cent).

Table - 10

Nitrogen content per plant of Chickpea (BDN-9-3) as influenced by various isolates of chickpea *Rhizobium* on 45th day after sowing.

Sr. No.	Treatment	Mean nitrogen content of plant	Increase (%) over control	
1.	G-7	3.405	9.13	
2.	G-13	3.523	12.92	III
3.	G-14	3.315	6.25	
4.	G-16	3.413	9.39	
5.	G-19	3.220	3.25	
6.	G-22	3.255	4.33	
7.	G-29	3.315	6.25	
8.	G-38	3.310	6.09	
9.	G-41	3.350	7.37	
10.	G-43	3.208	2.82	
11.	G-47	3.203	2.66	
12.	G-49	3.113	2.98	
13.	G-51	3.215	3.05	
14.	G-61	3.315	6.25	
15.	G-64	3.410	9.29	
16.	G-69	3.503	12.27	
17.	G-72	3.803	21.89	I
18.	G-75	3.408	9.23	
19.	G-83	3.513	12.59	
20.	G-87	3.613	15.80	II
21.	G-103	3.508	12.44	
22.	G-110	3.210	2.88	
23.	PBN(local)	3.410	9.29	
24.	Nitrogen	3.158	1.22	
25.	Control	3.120	----	
S.E.	±	0.006		
C.D.	at 5%	0.019		



Effect of different chickpea *Rhizobium* isolates on nitrogen content per plant (Topmost six treatments along with control).

DISCUSSION

Chapter -V

DISCUSSION

Nitrogenous fertilizers are expensive and need import from other countries and there by require expenses of foreign exchange. This situation is not favorable for economy of developing contries like India. Hence it is necessary to find out maximum possibilities of nitrogen fertilizers substitution by biological nitrogen fixation. The wide gap between the nitrogen requirment and its production, the soil water pollution hazards, where it is being used on large scale, has created reservation in the use of nitrogenous fertilizers. Among the various biological nitrogen fixation system symbiotic nitrogen fixation association of legume *Rhizobium* symbiosis is the most efficient nitrogen fixing system, which account nearly twenty millon metric tones of nitrogen per year (Subbarao, 1982).

Chickpea is the third widely grown grain legume in the world and nodulated by highly specific *Rhizobium* strians. Chickpea *Rhizobia* do not show affinity to any of the members of known cross inoculation groups (Raju, 1936).

The poor nodulation in chickpea accounted for low population of *Rhizobium* in the fields where chickpea's are not included in the cropping pattern or due to exsitance of inefficient *Rhizobial* population. In this view efforts

are needed for searching the efficient strains to see their adapt-ability to our agroclimatic and soil situation of our region.

During the course of investigation different chickpea *Rhizobia* isolates were obtained from microbiologist (pulses) Agricultural Research station, Badnapur and one Parbhani local isolate, isolated from the root nodule on chickpea plant grown in the field of the Department of Agronomy, Marathawada Agriculture University, Parbhani. The isolates were studied for their nodulation characters in sand culture experiment. The isolates studied for their effect on plant growth were studied in soil, sand and farm yard manure (2:1:1) culture experiment.

The *Rhizobium* isolates were found to be significantely superior in almost all parametres over the control. The differences also exist between various isolates also. The nitrogen fixing ability is mostly governed by nodule number, nodule dry weight and presence of leg-haemoglobin. In present investigation all the *Rhizobium* isolates studied increased by nodule number significantly. The highest nodule number was found in isolate G-72 followed

by G-51 and G-69. Similar trend of results are also been reported in India and abroad. (Raju and Varma, 1984; Gajendragadkar and Vaishya, 1983; Vaishya, *et. al.*, 1983; Iswaram, *et. al.*, 1971 ; Choudhary, *et. al.* 1975). Iswaram *et. al.* (1971) observed ten fold increase in nodule number due to *Rhizobium* inoculation in mungbean. Raju and Varma (1984) reported that there was significant improvement in nodule number per plant due to *Rhizobium* treatment.

The application of alone nitrogen reduced the nodule number per plant in present investigation. Similar results are also been reported by Sundaram, *et. al.*(1979); Joseph and Hetherington (1984). Hernandez and Hill (1973) shown that availibility of nitrates (No_3^-) from fertilizers inhibit the production of nodule. This might have been attributed to the tedency of plant to take redily available nitrogen rather than nitrogen through symbiotic process. However, the nodule numbers were found to be increased at lower level of nitrogen (Raju and Varma, 1984).

As the fresh weight and dry weight of nodule is one of the criterians in nodulation character, these studies were undertaken in present investigation. The fresh weight

of nodule was found to be increased significantly due to application of *Rhizobium* inoculants. Similar results are also been reported by Patil and Medhane (1974). In present investigation the *Rhizobium* isolates shown significant increase in dry weight of nodule. The other workers from varios centre also shown similar trend in chickpea and other pulses. (Choudhary *et.al.*, 1975; Patil and Medhane, 1974; Reddy *et.al.* , 1978; Mourya and Sanoria, 1982; Raju and Varma, 1984).

The *Rhizobium* inoculations have increased the plant height and root length. Similar results are also been reported by many workers. (Sharma *et.al.*,1989; Medhane and Patil, 1974; Saxena *et.al.*,1975).

Fresh weight and dry weight of chickpea plants inoculation with *Rhizobium* isolates were found to be increased significantly over uninoculated control in present investigation.

Patil and Medhane (1974) reported that seed inoculation with differnt strains of *Rhizobium* in gram increased the dry weight significantly.

Shansky (1977) reported that the nitrogen application do not stimulate nodulation and dry matter accumulation in soybean crop.

Reddy *et.al.* (1978) concluded that dry weight of black gram increased when seeds were treated with *Rhizobium* inoculation. Raut and Kohire (1991) observed that there was significant increase in dry matter per plant over the control.

Azcon *et.al.* (1979) stated that glomous inoculation plants of *Medicago sativa* , the inoculation of *Rhizobium* was effective and it increased plant dry weight nearly by 50 per cent.

In present investigation it has been observed that inoculation of chickpea seeds with *Rhizobium* isolates increased the nitrogen content of plant. However, isolate G-72 had given highest nitrogen content of plant followed by G-87 and G-13.

Increase in nitrogen content and there by increase in protein content of plant also been reported by

many workers. (Sahu and Behara, 1972; Prasad and Sanoria, More *et.al.*, 1989).

Sahu and Behara (1972) observed that nitrogen content of root, shoot and grain in groundnut, cowpea and greengram were considerly increased due to *Rhizobium* inoculation.

Prasad and Sanoria (1982) reported that *Rhizobium* inoculation significantly increased grain protein content over uninoculated control in Bengal gram.

SUMMARY

Chapter-VI

SUMMERY

Nitrogen is an important plant nutrient playing a key role in growth and crop production. Nitrogen availability of our crops comes through two sources i.e. inorganic chemical fertilizers and biological nitrogen fixation (symbiotic and by free living micro-organism). In present investigation the various *Rhizobium* isolates were studied for their ability of fixing nitrogen symbiotically.

The nitrogen fixation and it's supply to the plant generally expressed in terms of increase in effective nodule number, nodule dry weight, plant dry weight and root-shoot length. Thus, these parameters were considered for study in present investigation. The two pot culture experiments Viz. sand culture and soil, sand, F.Y.M. pot culture experiments were carried. Data pertaining to these growth parametres was subjected to statistical analysis. Results of these data tables were statistically significant except root-shoot length. Over all when all these parametres studied if was found that increase in parametres was noticed in *Rhizobium* isolates inoculations over the uninoculated control. Of the twenty-five treatments studied the effective nodule numbers were highest in gram *Rhizobium* isolate G-72(30.50) followed by G-51 (27.50) and G-69 (22.50). As regards the nodule fresh weight, the highest fresh weight

was recorded in G-72 followed by G-64 and G-51. The isolate G-72 followed by G-64 and G-75 shown the sequencial decrease in nodule dry weight.

The soil, sand and FYM pot culture experiment, the substantial increase in shoot-root length, fresh weight, dry weight and nitrogen content of plant was observed wherever *Rhizobium* isolates were tried over the uninoculated control. As regards the plant height isolate G-51 had shown 21 centimeter shoot length which had shown 31.25 per cent increase over control which was followed by alone nitrogen application and isolate G-43. Observations on root length indicated that highest root length was noticed in Parbhani local isolate followed by G-29 and G-7. When fresh weight was studied it is found that fresh weight of chickpea was more in plant inoculated with G-72(6.35g) followed by G-51 (6.22g) and Parbhani local (6.21g).

The most reliable criteria of growth i.e. plant dry weight was also been undertaken in evaluating chickpea isolates in present investigation. The highest plant dry weight was noticed in G-72 (1.03g) followed by G-22 (1.02g) and G-75(1.02g) . The *Rhizobium* isolate inoculations and alone nitrogen application treatement i.e. in all 24

treatment nitrogen content of plant significantly increased over the uninoculated and no nitrogen given control. This data clearly indicate that *Rhizobium* isolates fixed the nitrogen symbiotically and translocated it into the host plant. Nitrogen content of the plant was increased in isolate G-72 followed by G-87 and G-13.

When all the parametres under study were seen, it is observed that isolate G-72 rank first in merrit.

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