Ferti-fortification and Quality Enhancement in Chickpea through Integrated Application of Inorganic and Organic Sources of Nutrients

THESIS

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BY

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Transforming Education Transforming India

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Certification

This is to certify that the thesis entitled "**Ferti-fortification and Quality Enhancement in Chickpea through Integrated Application of Inorganic and Organic Sources of Nutrients**" submitted in partial fulfilment of the requirements for the degree of Master of Science with major in Agronomy of the Department of Agronomy, School of Agriculture and Food Technology, Lovely Professional University, Phagwara, is a record of bona-fide research carried out by Devendra kumar, Registration No. 11212166 under my supervision and on part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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DECLARATION

I hereby declare that this thesis is a presentation of my own work and has been generated by me as the result of my own research work and efforts. This thesis is submitted by me in partial fulfillment of the requirement for the award of degree M.Sc. in Agronomy from Lovely Professional University, Phagwara, Punjab comprises only my original work and due acknowledgement has been made in the text to all other material used.

This thesis work was done under the guidance of my advisor.

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CONTENTS

List of table

List of plates

List of figure

Abbreviation

N	Nitrogen
P	Phosphorus
K	Potassium
BC	Benefit cost of cultivation
BNF	biological nitrogen fixation
Rhz	Rhizobium
RDF	Recommended dose of fertilizer
CD	Critical difference
DAS	Days after sowing
DMA	Dry Matter Accumulation
RLWC	Relative leaf water content

Abstracts

The present investigation was carried out at lovely professional University, Jalandhar, India during 2013-14 to enhance nutritional States, quality and productivity in chick pea through Rhizobium. The field experiment was done in a randomized block design with three replications, comparing seven treatments involving different doses of Rhizobium culture, inorganic NPK + FYM, recommended fertilizer doze and absolute control. To study the various growth parameters, yield, plant water status, quality parameters, agronomic efficiency of nitrogen, various root parameters and economic analysis of the experiment. The study revealed that treatments involving Rhizobium inoculation along with inorganic NPK lead to significantly heights plant growth and productivity as compare with recommended dose fertilizer. Similarly maximum increase in quality parameter was recorded under Rhizobium containing treatments. Rhizobium inoculation enhancing overall plant root and shoot parameter. Benefit cost (B:C) ratio, maximum profitability was registered under “Rhz + 75%N + 100% PK” followed by “Rhz + 100% NPK”. Moreover Rhizobium inoculated treatments indicated an increase in yield of about 50% .Thus the use of Rhizobium in production can play an important role in enhancing crop quality and productivity.

Keywords: Nitrogen, Rhizobium, chickpea, cost benefit ratio.

Chapter Number

1

Introduction

The facts reveal that on one hand, the world population is increasing continuously whereas, on the other hand, food grain production is not increasing proportionately due to various factors such as decline in soil fertility and repercussions arising from climate change phenomenon as manifested by unpredictable patterns of rainfall and temperature. The major reason for poor soil health in India seems to be the unbalanced nutrient application. Amongst, various strategies to cope with above situation, soil test based integrated nutrient management holds the key to reverse above trend leading to restoration of soil fertility and in turn, boosting crop production and productivity.

Nitrogen is one of the most essential nutrients required by plant globally. It is an integral component of many compounds such as chlorophyll, nucleotides, alkaloids, enzymes, hormones and vitamins, etc. which are essential for plant growth processes. (Brady 2012). Although N is abundant in atmosphere, yet it is the most limiting nutrient for most crops and soils. Besides being limited, this nutrient has low use efficiency as a large proportion of N applied to the soil through fertilizers get lost by way of leaching, denitrification and volatilization. So, there is a dire need to develop technology that would improve N use efficiency on one hand, and improve soil health on the other hand.

Fertilizer nitrogen has contributed tremendously towards increasing food production, yet even with best agronomic practices, the recovery of fertilizer nitrogen hardly exceeds 30-60 per cent, because most of the applied nitrogen gets leached and becomes unavailable for plant use. A number of approaches aimed at increasing N use efficiency have been developed in India and abroad, but none of the strategies is equally effective under different situations. Therefore, there is an urgent need to attempt some alternative approach to tackle the problem of low N use efficiency.

No doubt, mineral fertilizers have played a vital role in enhancing agricultural productivity but, excessive applications of chemical fertilizers are producing detrimental effect on environment vis-a-vis soil health. Besides above factors, very few farmers use biofertilizers. Moreover, increasing cost of chemical fertilizers further hinders resource-poor farmers to apply recommended nutrient doses, causing multiple nutrient deficiencies. Based on these facts, it was decided to develop an integrated nutrient management (INM) technology for chickpea involving biofertilizer i.e. *Rhizobium*. The above technology would increase N use efficiency and thus economize fertilizer doses. The occurrence and activity of soil microbes have their bearing on soil fertility as they help in nutrient mineralization in soil and in turn maintaining soil health.

In above context, the use of *Rhizobium* can go a long way in addressing the above issues because of its unique characteristics such as being low cost, environment-friendly and easy to use sources of nutrients especially N in the soil plant system. Besides, improving crop quality and sustaining soil health this biofertilizer also produce a number of useful substances such as vitamins, antibiotics, growth promoting hormones, etc. which benefit plants in several ways.

The *Rhizobium* has unique ability to convert atmospheric nitrogen into ammonia GT

Through the biological nitrogen fixation (BNF) process (Michiels and Vanderleyden 1994). The Rhizobia are mostly associated with legume roots (Geurts and Franssen 1996, Freiberg *et al.* 1997), but occasionally, found within the endorhizosphere of non-leguminous hosts also (Yanni *et al.* 1997). The low cost of *Rhizobium* inoculants and high returns from the BNF process are some of the reasons for the world wide use of *Rhizobium* inoculants for various legume crops (Shantharam and Mattoo 1997).

Chickpea (*Cicer arietinum* L.) is an important grain legume crop grown throughout the world. It is a highly nutritious pulse and places third in the importance list of the food legumes that are cultivated throughout the world. It contains 25% proteins, which is the maximum provided by any pulse and 60% carbohydrates so can help people improve the nutritional quality of their diets. Chickpea is also a good source of vitamins (especially B vitamins) and minerals like potassium and phosphorus. Through symbiotic nitrogen fixation, crop meets up to 80% of the soil’s nitrogen needs, so farmers have to apply less nitrogen fertilizer than they do for other non-legume crops.

Amongst various pulses chickpea occupies 2nd rank in area and third in production among throughout the world. It is cultivated on about 11.9 million hectares area involving production potential of 8.5 million metric tons annually. India is the largest producer of this pulse contributing around 70% of the world's total production. In India, area under this crop is 8.56 million hectare with production level of 6.8 million metric tons involving 858 kg ha^{-1} productivity. Chickpea is grown in the drier areas of the country as they are best suited for its production.

Chickpea is also grown in certain belts of Punjab due to well suited agro-climatic conditions, fetching high premium to farmers. But, above crop suffers due to nutritional poorness especially P and K. The erratic and ill distributed rainfall pattern is another constraint for chickpea production. Currently, information on the role of *Rhizobium* in enhancing N use efficiency, quality and drought resistance are lacking in the region and need to be generated urgently so that necessary recommendation can be made to the farmers of the regions. Therefore, the present investigation entitled **"Ferti-fortification and Quality Enhancement in Chickpea through Integrated Application of Inorganic and Organic Sources of Nutrients"** has been carried out at Experimental Farm, Department of Agriculture, Lovely Professional University Phagwara.

Aim and objectives

Aim: The aim of the experimentation is assessing quality and productivity through inoculation with *Rhizobium* in chickpea with following specific objectives:

Objectives

1. Impact of integrated application of inorganic and organic sources of nutrients on yield attributes crop productivity and crop quality
2. Impact of integrated application of inorganic and organic sources of nutrients on N use efficiency
3. To work out economics of various treatments

Chapter Number

2

Review of Literature

The soil ecosystem is a complex network composed of interactions of thousands of organisms. Among these, soil microbial community is a particularly important component. The soil microbial community is an important biological component of soil function, valued for its role in improving soil health and regulating nutrient availability and thereby, influencing plant production for agriculture and other purposes. Soil microbial communities can also affect the interaction between plants and important aboveground macro fauna.

The association of legumes with rhizobia and its benefits in N nutrition are well known. Above association is a mutually beneficial process. While rhizobia supply N to the plants, plants in turn furnish C for their growth and metabolism through carbohydrates, photosynthethates. Besides above benefits, biofertilizers confer other benefits on the plants such as drought resistance, disease control, etc. The rhizobia benefit the legume crops through the process of biological N_2 fixation. The N_2 fixation by rhizobia in root nodules of legumes is an energy requiring process (Dilworth 1974). The above process is catalysed by the enzymatic complex nitrogen's (Marschner 2002).

The information available on various aspects of the subject is discussed under the following heads*:*

1. Fate of applied N in agricultural soils
2. losses Leaching loss
3. Volatilization and denitrification
4. Importance of Rhizobium in agriculture
5. N economy through Rhizobial inoculation
6. Productivity and quality as influenced by Rhizobial inoculation

2.1 Fate of applied N in agricultural soils

The total N content of soils generally varies from 0.02 to 0.44 per cent. Due to tropical and subtropical climates, Indian soils are generally poor in organic matter and consequently, have low N content. Nitrogen is one of the most essential nutrients required by plant globally. While N is needed by plants in large amounts, it is deficient in most soils all over; therefore, it has to be applied externally. Unfortunately, mineral N applied through fertilizers has low use efficiency. About 50 per cent of N applied to the soil through fertilizers gets lost by way of leaching, denitrification and volatilization. (Jenkinson,1990).

2.2. Leaching loss

The leaching loss of N occur NO_3^- When fertilizer N is applied to soil, it is accumulated as NO_3^- in the soil profile. The accumulated NO_3^- is susceptible to leaching by rain and irrigation water. The nitrate leaching is a serious problem in many irrigated areas especially those having porous soils and involving a high dose of fertilizer N application.

2.3 Volatilization and denitrification losses

The volatilization loss occurs as ammonia (NH_3). The loss can range from 5 to 35 per cent depending on the soil, environment and fertilizer management practices. The above loss is influenced by pH; higher the pH, higher is the volatilizations loss (Mohanty *et al*. 2009). Denitrification loss occurs mostly under anaerobic conditions such as in flooded and low land rice soils. The above loss is influenced by several factors such as NO_3^- as a substrate, organic C supply, aeration status, soil moisture status, soil texture, pH, temperature, etc. (Mohanty *et al.* 2009).

2.4. Importance of Rhizobium in agriculture

The *Rhizobium* belongs to Rhizobiaceae family. The *Rhizobium* has unique ability to convert atmospheric nitrogen into ammonia through the biological nitrogen fixation (BNF) process (Michiels and Vanderleyden 1994). The Rhizobia are mostly associated with legume roots (Geurts and Franssen 1996, Freiberg *et al*. 1997), but occasionally, found within the endorhizosphere of non-leguminous hosts also (Yanni *et* *al*. 1997). The low cost of *Rhizobium* inoculants and high returns from the BNF process are some of the reasons for the world wide use of *Rhizobium* inoculants for various legume crops (Shantharam and Mattoo 1997).

The *Rhizobium* form symbiotic associations with leguminous plants and fixes atmospheric N within the root / stem nodules of their hosts (Brockwell *et al.* 1995, Boivin *et al*. 1997). The process of N fixation is preceded by the nodulation process (Hirsch, 1992).

It is estimated that the rhizobia-mediated BNF process contributes approximately 35 x 10^{12} tones i.e., 47 per cent of the total N fixed annually to the global N budget (Elkan 1992). On an area basis, the *rhizobia-legume* symbiosis contributes 24-584 kg N ha^{-1} per year (Elkan 1992). The low cost of *Rhizobials* inoculants and high return from the BNF process are some of the reasons for their world-wide use for various legume crops (Shantharam and Mattoo 1997).

2.5. N economy through Rhizobial inoculation

In a field experiment conducted by Tippannavar and his associates (2001) on pigeon pea, it was found that seed inoculation with *Rhizobium* reduced N rate by 50 per cent and increased the yield by 50 per cent. They reported the maximum yield (20.10 q ha^{-1}) in case of treatment with 100 per cent N which was found to be at par with *"Rhizobium + 50 per cent N"* (18.67 q ha^{-1}).

Sarma and his colleagues (2003), while working with pea in a sandy loam soil, observed that Rhizobial inoculation of pea seeds along with application of 15 kg N ha^{-1} gave a higher green pod yield than that involving recommended dose of 20 kg N ha^{-1} alone. Further, the above treatment gave a higher B: C ratio over the recommended dose cited above.

A study aimed at determining the residual effect of Rhizobial inoculation of pea with the application of 20 and 40 kg N ha^{-1} in maize crop was carried out by Dubey and Bindra (2008) in an Alfisol. It was observed that the use of three indigenous strains of *R. leguminosarum* saved around 60 kg N ha^{-1} in the maize-based cropping sequence.

2.6. Productivity and quality as influenced by Rhizobial inoculation

Application of SSP in combination with *Rhizobium* inoculation in black gram produced 69.1 per cent increase in nodulation over control in an acidic soil of Tripura (Data and Laskar 1990).

Hadi and Elsheikh (1999) studied the effect of Rhizobium inoculation and nitrogen fertilization on yield and protein content of chickpea (*Cicer arietinum* L.) in marginal soils under irrigation. The results revealed that *Rhizobium* inoculation or N fertilization significantly increased the total nodule number per plant, 100 seed weight, yield and protein content of seeds. Moreover, combined application of *Rhizobium* and 50 kg N ha^{-1} increased crop yield by 70 and 69% in first and second seasons, respectively.

In a field study conducted at Madhya Pradesh with chickpea involving neutral soil conditions, Tomar (2010) found that seed inoculation with *Rhizobium* (20g/kg seed) enhanced productivity by 72% in comparison with Farmers' practice of the area. Further, increase in net returns and benefit cost ratio of chickpea was to the tune of 107 and 16%, respectively.

Ogutcu *et al.* (2010) in a study recorded that chickpea is able to grow and yield well under saline soil conditions following *Rhizobium* inoculation without any reduction in yield.

Singh *et al.* (2011) studied the effect of irrigation application and *Rhizobium* inoculation on plant water use, nodulation and yield of chickpea. Results revealed that *Rhizobium* enhances the production level, improve quality and provide resistance against various stresses like drought and salinity conditions, etc. Besides, application of *Rhizobium* economize nitrogenous fertilizer application rate, as it as ability to fix atmospheric nitrogen and add the same to soil.

Jukanti *et al.* (2012) proposed chickpea as a good source of *carbohydrates and protein*. Chickpea has significant amounts of various essential amino acids. Moreover, chickpea is a good source of Ca, Mg, P and K. Thus, provide balanced diet.

Mahdi *et al.* (2012) reported that inoculation of chickpea with *Rhizobium* gave highest height, weight of 1000 seeds and seed yield in comparison with farmers' practice.

Hadi *et al.* (1999) studied the effect of *Rhizobium* inoculation and nitrogen fertilization on yield and protein content of chickpea (*Cicer arietinum* L.) in marginal soils under irrigation. The results revealed that *Rhizobium* inoculation or N fertilization significantly increased the total nodule number per plant, 100 seed weight, yield and protein content of seeds. Moreover combined application of *Rhizobium* and 50 kg N ha^{-1} increased crop yield by 70 and 69% in first and second seasons, respectively

Rajiv *et al*. (2005) reported that garden gave 12.5 and 27.5 per cent higher yield and nodulation following integrated nutrient management.

Kunal *et al*. (2012) reported 9.6 per cent higher chickpea yield *Rhizobium* inoculation in comparison with non-inoculated ones.

In a field experiment with chick pea conducted by Sharma and Khurana (2001) at Ludhiana (India), it was reported that the maximum number and dry weight of nodules and nitrogen's activity occurred at 50 kg N ha^{-1} application in dually inoculated treatments. Further, nodulation and nitrogen's activity were inhibited at 75 kg N ha^{-1} dose.

According to Champawat (1990), dual inoculation in chickpea enhanced considerably the plant growth, N nutrient and root nodulation. A similar result was obtained by Konde and Deshmukh (1996) in chick pea pot experiment conducted at Rahuri (India).

In a field experiment with chickpea, it was found that the seed inoculation with *Rhizobium* increased nodulation, grain yield, dry matter yield and N uptake over uninoculated control (Kumar *et al.* 1998).

The findings of a field experiment revealed that the combined inoculation with *Rhizobium* and AM fungi in lentil (*Lens culinaris cv. Medikus*) increased grain and straw yield, and nutrient (N, P and Zn) uptake than with their individual inoculations. (Reddy and Ahlawat 2001)

It is concluded from above survey of literature that inoculation with *Rhizobium* enhances the production level, improve quality and provide resistance against various stresses like drought and salinity conditions, etc. Besides, application of *Rhizobium* reduce the dose of nitrogenous fertilizer as it as ability to fix atmospheric nitrogen and add it to the soil. So use of Rhizobium in crop production has dual effect on crop productivity, quality and overall soil health.

Chapter Number

3

Rationale of the study

The statistical facts reveal that on one hand, the world population is increasing continuously whereas, on the other hand, food grain production is not increasing proportionately due to various factors such as decline in soil fertility and repercussions arising from climate change phenomenon as manifested by unpredictable patterns of rainfall and temperature. The major reason for poor soil health in India seems to be the unbalanced nutrient application. No doubt, mineral fertilizers have played a vital role in enhancing agricultural productivity but, excessive applications of chemical fertilizers are producing detrimental effect on environment vis-a-vis soil health. Besides above factors, very few farmers use biofertilizers. Moreover, increasing cost of chemical fertilizers further hinders resource-poor farmers to apply recommended nutrient doses, causing multiple nutrient deficiencies. Based on these facts, it was decided to develop an integrated nutrient management (INM) technology for chickpea involving biofertilizer i.e. *Rhizobium*. The above technology would increase N use efficiency and thus economize fertilizer doses. The occurrence and activity of soil microbes have their bearing on soil fertility as they help in nutrient mineralization in soil and in turn maintaining soil health.

Chickpea is also grown in certain belts of Punjab due to well suited agro-climatic conditions, fetching high premium to farmers. But, above crop suffers due to nutritional poorness especially P and K. The erratic and ill distributed rainfall pattern is another constraint for chickpea production. Currently, information on the role of *Rhizobium* in enhancing N use efficiency, quality and drought resistance are lacking in the region and need to be generated urgently so that necessary recommendation can be made to the farmers of the regions. Hence, an initiative is taken to enhance quality of above crops under low input management system to harvest nutritionally rich farm produce for farming communities.

Chapter Number 4

METHODOLOGY

The present study was carried out at the Experimental Farm of the Department of Agriculture, Lovely Professional University, Jalandhar, Punjab (India) during 2013-14 with the broad aim of assessing quality and productivity through inoculation with *Rhizobium* in chickpea (variety- PBG- 1).

The experimental site is characterized as "*Central Plain Zone (PB-3)"* of Punjab. The rainfall in the region varies from 500-800 mm and about 80 per cent of which is received in a short period 3 months *(mid June to mid September)*. Major constraints of the region are declining water table and soil sodicity and salinity. It comprises parts of eight districts of Punjab viz. Amritsar, Tarn taran, Kapurthala, Jalandhar, Ludhiana, Fatehgarh Sahib, Sangrur and Patiala. The soils predominantly belong to Central Alluvial Plain or sandy loam. The major crops grown in the region are mainly wheat, rice, maize, groundnut, cotton, gram, barley, pear and guava.

The experimental site is located at 31º 15' N latitude and 75^0 41' E longitudes at an elevation of 245 m above mean sea level. The climate of the experimental area is characterized as *hot and dry summer and wet and humid monsoons*, distinctly experiences all the four seasons. The soil of experimental field was Sandy loam. The experimental soil was subjected to various estimations before the commencement of experiment, the details of which are given in Table 4.1

Plate 4.1 A general view of the experimental field at 60 DAS

Table 4.1 Initial status of the experimental soil

Sr. No.	Parameter	Status/ Value
1.	Textural class	Sandy Loam
	Mechanical separates (%)	
	Sand	61
	Silt	32
	Clay	7
2.	Chemical properties	
	Soil reaction	Alkaline (pH 8.7)
	Organic carbon(g kg^{-1})	3.6
	Available macronutrients (kg ha^{-1})	
	N	136
	P	14.2
	K	130

4.1 Experimental detail

A total of 7 treatments were evaluated in a randomized block design (RBD) with three replications. The relevant information is given in Table 4.2.

4.2 Lay out and treatment procedures

The field experiment consisted of 7 treatments with 3 replications. The various treatments were laid out in a randomized block design (RBD). There were 21 experimental plots. The size of each plot was 8 m^2. The field preparation was done by applying the primary and secondary tillage, using mould board plough and harrow respectively which were mounted on a tractor. It was followed by planking of the field using planker. Once the field was leveled uniformly, the layout was carried out manually. The treatments were allocated randomly to individual plots, concentrating on a single replication at a time. Macronutrients viz. N, P and K were applied in different plots as urea, Phosphorus (P_2O_5) and Potassium (K) respectively, their amounts varying depending on the treatments.

The *Rhizobium* culture used in the experiment belonged to *Rhizobium leguminosarum L*. and it was obtained from local dealer. *Rhizobium* culture was applied by seed treatment method just before sowing of chickpea seeds. The required amounts of seeds were soaked in water overnight. Just before sowing, the seeds were first treated with *Rhizobium* culture and then left to dry under shade for about 30 minutes followed by sowing.

In order to prepare the field for the experiment, a nominal amount of FYM (*5 t ha^{-1} on fresh weight basis*) was incorporated in all treatments except T_1. The N, P and K fertilizer application in various plots was made on the basis of traditional soil test approach i.e. the grouping of soils into low, medium and high.

Table 4.2 Detail of treatments evaluated in chickpea during *Rabi* (2013-14)*

Treatment No.	Treatment detail	Treatment code
T_1	No Rhizobium inoculation + No NPK (Absolute control)	No Rhz, $N_0P_0K_0$
T_2	Recommended dose of fertilizer i.e. 20 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1} + 20 kg K_2O ha^{-1}	RDF (100% NPK)
T_3	Rhizobium inoculation + 20 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1} + 20 kg K_2O ha^{-1}	Rhz + 100% NPK
T_4	Rhizobium inoculation + 15 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1} + 20 kg K_2O ha^{-1}	Rhz + 75%N + 100% PK
T_5	Rhizobium inoculation + 10 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1} + 20 kg K_2O ha^{-1}	Rhz + 50%N + 100% PK
T_6	Rhizobium inoculation + 15 kg N ha^{-1} + 30 kg P_2O_5 ha^{-1} + 15 kg K_2O ha^{-1}	Rhz + 75%NPK
T_7	Rhizobium inoculation + 0 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1} + 20 kg K_2O ha^{-1}	Rhz + N_0 + 100% PK

Note:

***Rabi* season:** The season that started from October/ November and ended in March/ April. FYM application @ 5 t ha^{-1} was applied in 7^{th} treatments viz. No application of FYM was made to treatment T_1.Recommended dose of NPK @ 20:40:20 kg ha^{-1}.

4.3 Field operations

The field used for raising chickpea crop was cultivated twice with a tractor and then, it was planked. Now, the field was divided into small plots each of size 8 m^2. Full doses of N, P and K were placed basally in above crop at the time of sowing. The fertilizers and manures were applied as per the treatments scheduled in various plots. The seeds were treated with Bavistin @ 3g per kg of seeds to avoid any fungal disease. The *Rhizobium* treated seeds of chickpea were now sown. The row to row planting distance maintained was 60 cm whereas plant to plant distance maintained within the rows, was 15 cm. In order to manage weeds, pendimethalin was sprayed @ 4 liter ha^{-1} one day after sowing. Further all relevant plant protection measures were followed. The schedule followed for the various farm operations is given in Table.5.3.

Table 4.3 The schedule of various agronomic operations in chickpea

Sr. /No.	Operation	Date
1.	Ploughing and planking of the field	October 10, 2013
2.	Pre sowing irrigation	October 15, 2013
3.	Lay out of the field	October 21, 2013
4.	Seed treatment with Bavistin	October 22, 2013
4.	Seed Inoculation with *Rhizobium*	October 22, 2013
5.	Sowing	October 22, 2013
6.	Fertilizer application	October 22, 2013
7.	Weed cid spray	October 23, 2013
8.	First Nipping	November 28, 2013
8.	Irrigation	December 05, 2013
9.	Gap filling	December 12, 2013
11.	Weeding	December 12, 2013

12.	Second Nipping	December 28, 2013
12.	Carbendazine spray to control collar root	February 10, 2014
13.	Harvesting	April 05, 2014

Plate 4.2 Crop growth at 60 DAS

Plate 4.3 Crop growth at 90 DAS

Plate 4.4 weeding operation

4.4 Soil studies

Table 4.4 Analytical methods employed for soil analysis

Sr./ No.	Parameter	Method employed
1.	Textural class Mechanical separates	International pipette method (Piper1950)
2.	Chemical properties	
	Soil reaction	1: 2.5 soil : water suspension (Jackson 1967)
	Organic carbon	Rapid titration method (Walkley and Black 1934)
3.	Available nutrients	
	N	Alkaline permanganate method (Subbiah and Asija, 1956)
	P	0.5 M $NaHCO_3$, pH=8.5 (Olsen1954)
	K	1 N Neutral ammonium acetate (Black 1965)

4.5 Yield and yield attributing characters

4.5.1 Yield

The dry seed pods harvested from each treatment were weighed out and expressed as seed mean yield per treatment.

4.5.2 Growth observations

In each plot, four chickpea plants were selected randomly, tagged and used for recording growth parameters periodically.

Plant height

Above parameter was recorded three times during crop growth (30 days interval) using a metre scale from ground level to tip of the upper most leaf in extended position.

Dry weight accumulation

The dry matter accumulation was recorded three times during crop growth at 30 day intervals. The randomly selected plants were removed from each plot. Above plant samples were dried in an oven at $60^{o}C$ for 72 hours and their weights were recorded.

4.6 Plant water status (Relative leaf water content)

Six leaves were sampled from each plot. These were brought to the laboratory in tightly closed polythene begs and then their fresh weight were recorded. Now, they were chopped into small pieces and saturated overnight in Petri plates. The saturated leaves were taken out the next day, dried between the folds of the filter paper followed by recording of their turgid weight. The same were now transferred to an oven ($60^{0}C$) and dried for 72 hours after which their weights were taken.

The RLWC was computed from the data involving fresh weight, turgid weight and oven dry weight, using to the method given by Weatherly (1950) as

$$\text{RLWC} \quad \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Fully turgid weight} - \text{Oven dry weight}} \times 100$$

4.7 Root studies

Above studies were carried out at the maximum flowering stage (120 DAS). The root samples were taken by “core break method” (Bohm 1979) to a depth of 0-0.30 m. In the present study, metallic core of size 1532 cm^3 was used. The soil samples with root biomass were kept in water overnight and then, roots were made free from soil by gentle washing under a fine jet of water. The roots were collected on sieves and observations on following parameters were made.

Root volume

The root volume was determined by displacement method given by Mishra and Ahmed (1987). About 500 ml of water was poured into a 1000 ml measuring cylinder; thereafter the roots were transferred into it and the change in water volume reading resulting from the addition of the roots was recorded.

Root nodule count

Freshly collected roots for the given treatment were washed and then the roots bearing nodules were separated out and the total nodule number were recorded for the respective treatment.

4.7.3 Root weight

Root samples collected earlier were dried in an oven at $60^{o}C$ for 72 hours after which their weights were recorded.

4.7.4 Root weight density

Above parameter is the ratio between root dry weight and volume from which the roots were sampled in the field (Mishra and Ahmed 1987). As such, it was worked out using relevant data recorded earlier.

4.8 Laboratory analysis

4.8.1 Plant analysis

Plant samples (leaves and pods) collected at final picking from all the field plots, were air dried and then, dried in an oven at $60^{o}C$ for 72 hours. The dried samples were now ground in a Willey Mill fitted with stainless steel parts, and passed through 1 mm sieve and stored in paper bags for analysis. The analytical procedures employed for the estimation of N, P and K is given in Table 4.8.1.

Table 4.8.1 Analytical methods employed for plant analysis

Sr. No.	Parameter	Method employed	Reference
1.	Nitrogen	Micro-kjeldahl method	Jackson (1973)
2.	Phosphorus	Vanado-molybdo-phosphoric acid yellow colour method	Jackson (1973)
3.	Potassium	Wet Digestion method	Black (1965)

4.9 Quality parameters

4.9.1 Protein content in chickpea seeds

Above parameter was estimated in chickpea seeds through the estimation of total nitrogen (Jackson, 1973), in various samples. The value thus, obtained was multiplied by factor 6.25 to obtain crude protein content.

4.10 Nitrogen use efficiency (kg yield kg^{-1} N)

The efficiency of applied N nutrient in different treatments was estimated in the form of N response ratio (agronomic efficiency) by applying the following formula:

$$\text{N Use efficiency (kg yield kg}^{-1}\text{ N)} = \frac{\text{Yield in treated plot (kg ha}^{-1}\text{) - Yield in Absolute control plot (kg ha}^{-1}\text{)}}{\text{N applied (kg ha}^{-1}\text{)}}$$

4.11 Economic analysis

The economic analysis of the experiment was carried out by taking into consideration the prevailing market prices of produce and inputs used.

The unit prices (Rupees per kg) of the inputs are given below:

FYM	N	P_2O_5	K_2O	Rhizobium	Dry Chickpea
0.80	11.54	27.93	8.93	200	38

4.12 Statistical analysis

All the field and laboratory data were analyzed statistically by the methods described by Gomez and Gomez (1984).

Chapter Number 5

Results and Discussions

The experimental results pertaining to the current study entitled *"***Ferti-fortification and Quality Enhancement in Chickpea through Integrated Application of Inorganic and Organic Sources of Nutrients***"* have been presented in this chapter under following headings:

5.1 Effect of different treatments on various growth parameters

5.2 Effect of different treatments on yield attributing characters and yield

5.3 Effect of different treatments on plant water status

5.4 Effect of different treatments on quality parameters

5.5 Effect of different treatments on agronomic efficiency of nitrogen

5.6 Effect of different treatments on various root parameters

5.7 Economic analysis of the experiment

5.1 Effect of different treatments on various growth parameters

The data on various growth parameters i.e. plant height and dry matter accumulation is presented in Fig 5.1.

5.1.1 Plant height

The data presented in Fig 5.1 revealed that at 30 DAS, highest magnitude of increase in plant height was registered under "recommended dose of fertilizer (RDF)" i.e. 100% NPK followed "Rhz + 100% NPK" and "Rhz + 75%N + 100% PK", all of which were observed statistically at par with one another. However, a significant respective increases of 16.5 and 26.5% were recorded under RDF over 'Rhz + 50%N + 100% PK' and 'Rhz + 75%NPK' significant higher the lowest plant height was observed under absolute control (No Rhz $N_0P_0K_0$).

At 60 DAS, highest plant height was recorded under "Rhz + 50%N + 100% PK" followed by "Rhz + 100% NPK" and "Rhz + 75%N + 100% PK". Above treatments gave statistically similar plant height (Fig 5.1).

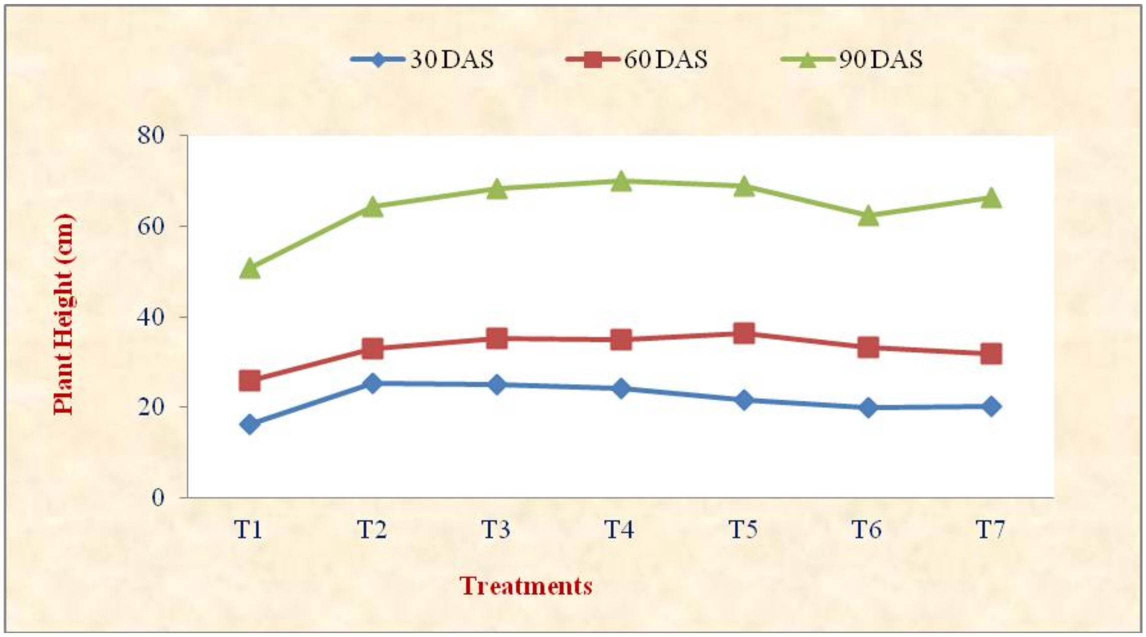

Fig 5.1 Effect of different treatments on plant height at different stages of crop growth

However, treatment "Rhz + 50%N + 100% PK" gave significantly higher (14.5%) plant height in comparison with Rhz + N_0 + 100% PK. The lowest plant height was observed under absolute control (No Rhz $N_0P_0K_0$).

At 90 DAS, highest plant height was noted under "Rhz + 75%N + 100% PK" followed by "Rhz + 50%N + 100% PK", "Rhz + 50%N + 100% PK", Rhz + N_0 + 100% PK" and "Rhz + 100% NPK" (Fig 5.1). Above treatment gave statistically similar plant height. However, treatment "Rhz + 75%N + 100% PK" gave significantly higher 1.44%, 2.48% and 2.45% in respectably RDF the minimum plant height was noted under absolute control.

The present results are in conformity with the findings of Panjebashi *et al*. (2012), who reported significantly higher plant height of chickpea under *Rhizobium* inoculated treatments. *Rhizobium* has a positive effect on biomass production and subsequently enhanced plant height. According to present analysis, *Rhizobium* has increased plant height by enhancing the N content and the rate of photosynthesis (Migahed *et al*. 2004).

5.1.2 Day matter accumulation per pant (g)

It is apparent from Fig. 6.2 that at 30 DAS, highest and similar magnitude of increase in dry matter accumulation was recorded under "recommended dose of fertilizer (RDF)" i.e. RDF (100% NPK) and Rhz + 75%N + 100% PK. Both of above treatments were observed statistically at par with one another. However, a significant increase of 2.56% above parameter was found under Rhz + 100% NPK in comparison with RDF. The lowest dry matter accumulation was found under absolute control (No Rhz $N_0P_0K_0$).

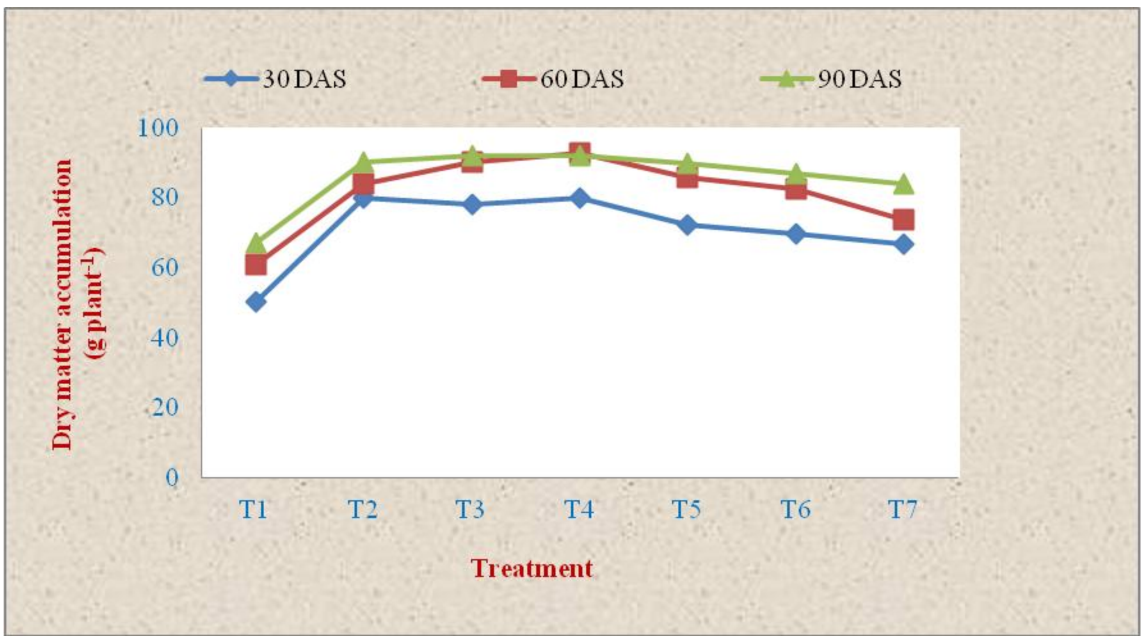

Fig 5.2 Effect of different treatments on dry matter accumulation at different stages of crop growth

At 60 DAS, highest and similar magnitude of increase in dry matter accumulation was recorded under "Rhz + 75%N + 100% PK" and "Rhz + 100% NPK", both of which were observed statistically alike (Fig 5.2). However, above treatments gave significantly higher i.e. 7.8% and 4.76% DMA in comparison with RDF. The lowest DMA were observed under absolute control “No Rhz $N_0P_0K_0$”. The absolute control showed lower dry matter accumulation per plant.

At 90 DAS, highest plant (DMA) was found under “Rhz + 100% NPK”, followed by “Rhz +100% NPK”, both of which were found statically alike (Fig 5.2). However, above treatments gave significant respective increases of 11.06% and 8.10% in comparison with RDF. Likewise, 30 and 60 DAS, at 90 DAS lowest DMA was recorded under control i.e. “No Rhz $N_0P_0K_0$”.

The above trends are attributed to the same reasoning as given under plant height. A higher amount of dry matter accumulation in *Rhizobium* inoculated plants is attributable to more N availability to plants. *Rhizobium*s have a positive effect on biomass production. In a study aimed at investigating the effect of biofertilizer inoculation on field pea in conjunction with different doses of chemical fertilizers, Mishra and his associates (2010) observed that the plant dry weight at 90 DAS increased with each increment in recommended dose of fertilizers (RDF) i.e. 50, 75 and 100 per cent of recommended N, P and K. The dry weights recorded in case of above RDF levels were 21.0, 21.7 and 25.8 respectively. Bai (2014) while working with *Rhizobium* in field grown garden pea under temperate climate involving acid Alfisol, observed that *Rhizobium* inoculated plants gave significantly larger biomass as compared to uninoculated control plants and RDF.

5.2 Effect of different treatments on yield attributing characters and yield

5.2.1 No. of branches plant^{-1}

The data presented in Table 6.1 revealed that none of treatment influenced number of branches per plant significantly.

5.2.2 Hundred seed weight

The data with respect to seed weight (g per 100 seeds) is presented in Table 5.1. None of the treatment influenced 100 seed weight significantly barring absolute control, which gave lowest value in above parameter.

Table 5.1 Effect of treatments on yield attributing characters and yield

Treatment	No. of branches plant^{-1}	100 seed weight	Yield
No Rhz $N_0P_0K_0$	5.9	18.6	7.0
RDF (100% NPK)	6.3	19.0	16.2
Rhz + 100% NPK	6.2	19.2	17.9
Rhz + 75%N + 100% PK	6.1	19.3	18.0
Rhz + 50%N + 100% PK	6.1	19.0	17.7
Rhz + 75%NPK	6.0	18.9	15.3
Rhz + N_0 + 100% PK	5.8	18.7	16.2
CD (5%)	*NS*	*0.07*	*2.65*

5.3.3 Yield

The highest magnitude of increase in chickpea seed yield was registered "Rhz + 75%N + 100% PK" followed by "Rhz + 100% NPK" and "Rhz + 50%N + 100% PK", all of which were observed statistically at par with one another (Table 6.1). Moreover, all above treatments were found statistically at par to RDF, signifies economy in fertilizer N by 25-50%. Likewise, other parameter, lowest seed yield was registered under absolute control.

It is inferred from current experimentation that inoculation with *Rhizobium* can economize soil test based fertilizer N sharply by about 50%. Increased yield of chickpea under *Rhizobium* is owing to improvement of yield components such as plant height, seed weight and dry matter yield. Moreover, *Rhizobium* has ability to fix atmospheric N and make it available to plants, further, adding it to the soil, which in turn enhance soil fertility. The present results are in conformity with the findings of Moradi *et al*. (2010) and Darzi *et al*. (2012).

5.3 Effect of different treatments on plant water status

5.3.1 Relative leaf water content

The effect of various treatments on relative leaf water content was registered non-significant across all the growth stages i.e. 30, 60 and 90 DAS (Fig. 5.3). Above trend is obvious, as crop did not suffer due to moisture stress at important physiological stages viz. flowering and pod formation. The total rainfall was throughout more than adequate and the same was well-distributed. As such, plants indicate higher relative leaf water content or plant water status.

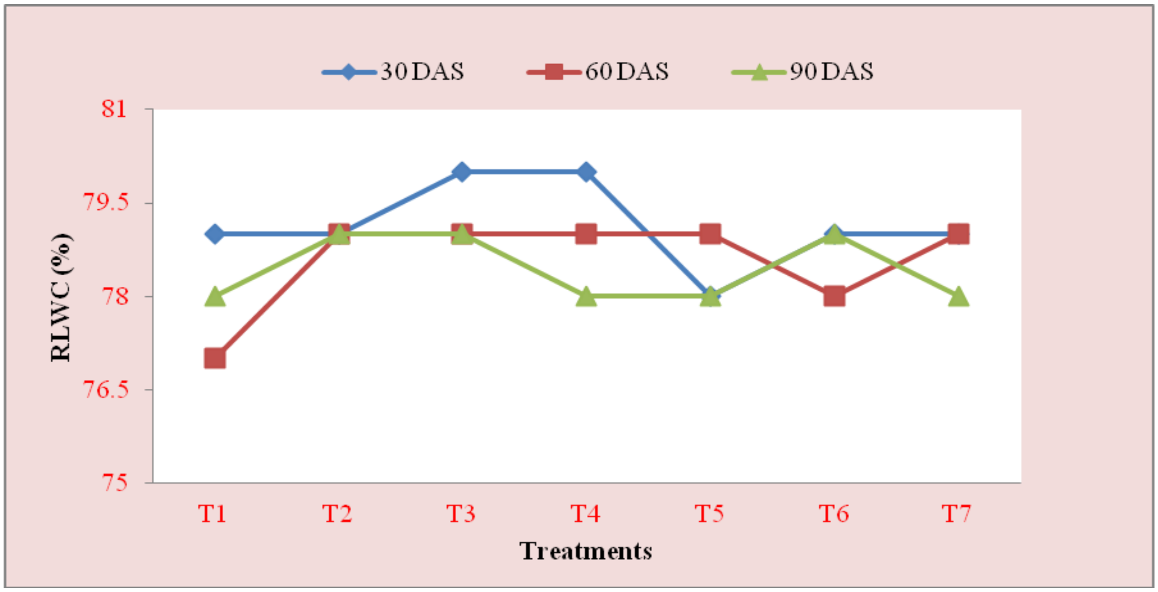

Fig 5.3 Effect of different treatments on relative leaf water content (RLWC) at different stages of crop growth

5.4 Effect of different treatments on quality parameters

The data with respect to quality parameters is presented in Table 5.2.

5.4.1 Nitrogen concentration

In general, treatments involving *Rhizobium* inoculation gave significant higher N concentration in chickpea seed comparison with recommended dose of fertilizer i.e. RDF (100% NPK) and absolute control (Table 5.2). A significant respective increases of 19.81% and 16.71% in above parameter were observed under "Rhz + 100% NPK" and "Rhz + 75%N + 100% PK" in comparison with RDF. Similarly treatments Rhz + 50%N + 100% PK, Rhz + 75%NPK and Rhz + N_0 + 100% PK gave significant increases of 10.52%, 8.35% and 10.52%, respectively in N concentration over RDF. The higher N concentration in inoculated treatments might be due to higher nitrogen's enzyme activity and enhanced N_2 fixation (Islam, 1990). Our findings are in agreement with the observation of Tarafdar and Rao (2001) found that the nitrogen's activity in *Rhizobium* inoculation involving treatment was 71 per cent.

Table 5.2 Effect of treatments on quality parameters

Treatment	N (%)	P (%)	K (%)	Crude protein (%)
No Rhz $N_0P_0K_0$	2.13	0.22	1.21	13
RDF (100% NPK)	3.23	0.31	1.76	20
Rhz + 100% NPK	3.87	0.34	1.83	24
Rhz + 75%N + 100% PK	3.77	0.34	1.83	24
Rhz + 50%N + 100% PK	3.57	0.34	1.82	22
Rhz + 75%NPK	3.50	0.33	1.80	22
Rhz + N_0 + 100% PK	3.57	0.34	1.73	22
CD (5%)	*0.22*	*0.022*	*0.09*	*1.41*

5.4.2 Phosphorus concentration

None of the treatment influenced P concentration in chickpea seed except absolute control, which was found significant inferior to all other treatments (Table 5.2). However, treatments involving *Rhizobium* inoculation gave nominally higher (but non-significant) magnitude of p concentration in comparison with RDF, indicating improvement in quality of chickpea in long term or following its continuous use.

5.4.3 Potassium concentration

The different treatments did not influenced K concentration significantly barring absolute control, which gave lowest value of K in chickpea seed due to obvious reason (Table 5.2). However, treatments involving *Rhizobium* inoculation gave nominally higher (but non-significant) magnitude of p concentration in comparison with RDF, indicating improvement in quality of chickpea in long term or following its continuous use.

5.4.4 Crude protein content

In general, treatments involving *Rhizobium* inoculation gave significant higher crude protein content in chickpea seed comparison with recommended dose of fertilizer i.e. RDF (100% NPK) and absolute control (Table 5.2). A significant respective increases of 20% and 20% in above parameter were observed under “Rhz + 100% NPK” and “Rhz + 75%N + 100% PK” in comparison with RDF. Similarly treatments Rhz + 50%N + 100% PK, Rhz + 75%NPK and Rhz + N_0 + 100% PK gave significant increases of 10%, 10% and 10%, respectively in crude protein content over RDF.

The crude protein content depends upon the plant nitrogen concentration. *Rhizobium* inoculation improved nitrogen concentration thereby enhancing the protein content of chickpea pods. Above results are in conformity with the findings of Bagyaraj et al. (1979). Rao et al. (1986) also suggested that seed inoculation with *Rhizobium* enhanced protein content of black gram and green gram.

5.5 Effect of different treatments on agronomic efficiency of N or N response ratio

Above parameter was computed to evaluate biological efficiency of nitrogen applied under various treatments. The relevant information is presented in Table 5.3. It is obvious that, there was an impressive increase in N response ratio due to use *Rhizobium* biofertilizer in concerned treatments. The treatment “RDF (100% NPK)” gave relatively a lower response ratio due to higher N dose. However, in pursuance of the law of diminishing returns, it decreased as the N levels increased, with every additional increment of N. The highest N response ratio was registered under “Rhz + 50%N + 100% PK” followed by “Rhz + 75%N +

100% PK" and "Rhz + 100% NPK" due to increasing N levels from 50 t0 100% of recommended dose.

The general trend of response ratio data can be explained through the law of diminishing returns (Voisin 1962). However, higher response ratio in case of *Rhizobium* involving treatments under varying levels of N is obviously the outcome of higher chickpea productivity.

Table 5.3 Effect of treatments on N response ratio or N response ratio (%)

Treatment	Yield (kg ha^{-1})	N applied (kg ha^{-1})	N response ratio (%)
No Rhz $N_0P_0K_0$	700	0	-
RDF (100% NPK)	1620	20	46
Rhz + 100% NPK	1790	20	54
Rhz + 75%N + 100% PK	1800	15	74
Rhz + 50%N + 100% PK	1770	10	107
Rhz + 75%NPK	1530	15	55
Rhz + N_0 + 100% PK	1620	0	-

5.6 Effect of different treatments on various root parameters

The data on rooting depth, root volume, root dry weight and root weight density at maximum flowering stage in depicted in Table 5.4 and Fig 5.4.

5.6.1 Rooting depth

In general, treatment involving *Rhizobium* inoculation gave higher value of rooting depth in comparison with recommended dose of fertilizer (Table 5.4). The highest magnitude of increase in rooting depth was registered under Rhz + 75%NPK, which gave a significant increase of 38.88% over RDF. Similarly, magnitude of increase in rooting depth following Rhz + 100% NPK treatment was to the tune of 11.11% over RDF. The lowest value of rooting depth was found under absolute control.

The higher rooting depth following *Rhizobium* inoculation is probably due to more N availability in soil through the activity of *Rhizobium.* Moreover, there is more root proliferation and production of high order lateral and in turn more rooting depth. The present results are in conformity with the finding of Bai (2014), who reported altered root morphology with *Rhizobium* inoculation.

5.6.2 Root volume

The treatment wise trend with respect to root volume was found similar as that observed under rooting depth and the treatments involving *Rhizobium* inoculation gave higher value of root volume in comparison with recommended dose of fertilizer (Table 5.4). The highest magnitude of increase in root volume was registered under Rhz + 75%NPK, which gave a significant increase of 26.66% over RDF. Similarly, magnitude of increase in rooting depth following Rhz + 100% NPK treatment was to the tune of 23.33% over RDF. The lowest value of root volume was found under absolute control. *Above trend is ascribed to the same reasoning as given under rooting depth parameter earlier.*

Table 5.4 Effect of treatments on root parameters

Treatment	Rooting depth (cm)	Root volume ($x10^{-6}m^3$)	Root dry weight (g)	Root weight density

No Rhz $N_0P_0K_0$	14	20	3.14	2.05
RDF (100% NPK)	18	30	3.94	2.58
Rhz + 100% NPK	20	37	4.24	2.77
Rhz + 75%N + 100% PK	23	33	4.03	2.63
Rhz + 50%N + 100% PK	24	33	4.05	2.65
Rhz + 75%NPK	25	38	4.26	2.78
Rhz + N_0 + 100% PK	22	33	4.02	2.63
CD (5%)	*2.21*	*4.11*	*0.92*	*0.72*

5.6.3 Root dry weight

It is apparent from Table 5.4 that none of the treatment influenced root dry weight except absolute control, which was found significant inferior to all other treatments. However, treatments involving *Rhizobium* inoculation gave marginally higher (but non-significant) value of above parameter in comparison with RDF, indicating better plant condition.

The increase in root dry weight in inoculated treatments is attributable to the increase in root nodulation. Yadav *et al.* (2007) reported that an increase in number and weight of nodules in *Rhizobium* inoculated plants led to a significant increase in root dry and fresh weights of chick pea in comparison to non-inoculated plants. Similar results were obtained by Moradi *et al.* (2013).

5.6.4 Root weight density

Above parameter is a function of root dry weight and actual root volume i.e. the soil volume from which the roots were collected (1.53 m^3) and measured. It is obvious that the trend observed herein is the same as in case of root dry weight (Table 5.4). The different treatments did not influenced above parameter significantly barring absolute control. The same reasoning as given under root dry weight holds true here also.

5.6.5 Root nodule count

The data presented in Fig. 5.4 revealed that highest numbers of nodules were registered under ‘Rhz + 75%NPK’ followed by ‘Rhz + 50%N + 100% PK’ and ‘Rhz + 75%N + 100% PK”, all of which were found statistically alike to one another. However, above treatments gave significant increases of 24.0, 20.6 and 17.2%, respectively over RDF. The lowest nodules were registered under absolute control (No Rhz $N_0P_0K_0$).

The inoculation with *Rhizobium* ensures the presence of a high density of these organisms in close proximity to the root systems of the seedlings causing the development of high number of nodules especially at the early stage of plant development. The above information is in agreement with the findings of Antoun *et al.* (1998) and Dileep-Kumar *et al.* (2001), who reported that rhizobia are capable of colonizing the roots of legumes and produce plant growth-promoting substances of phytohormonal nature and also exhibit antagonistic effects against many plant pathogenic fungi.

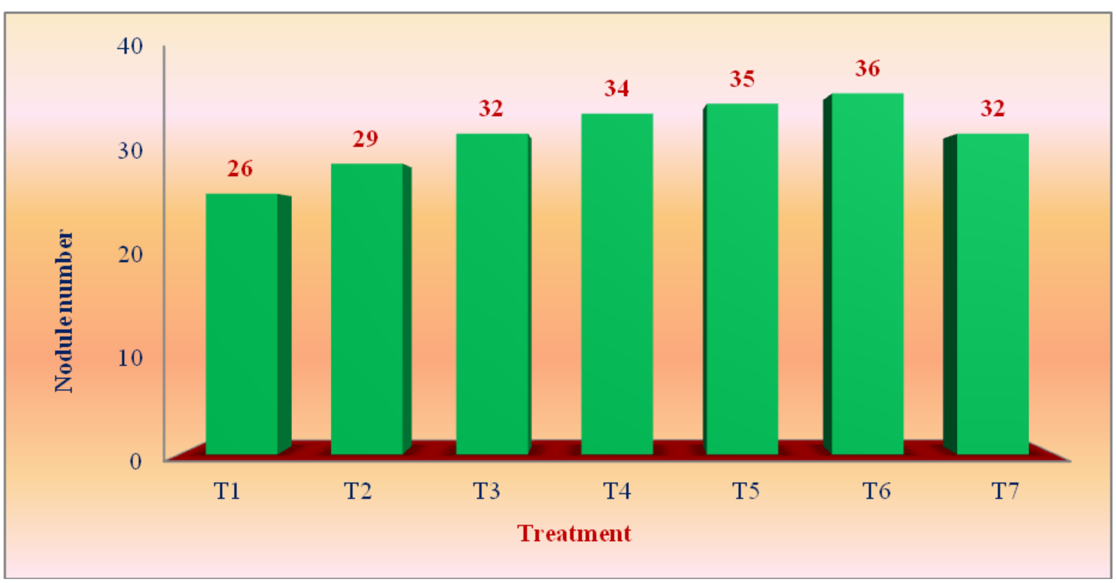

Fig 5.4 Effect of different treatments on root nodule number at maximum flowering stage

5.7 Economic analysis of the experiment

The data presented in the Table 5.5 showed that the maximum net returns were registered under "Rhz + 75% + 100% PK" followed by "Rhz + 100% NPK". Above treatment gave respective increases of 16.00% and 14.89% over RDF. Similarly, magnitude of increase in above parameter following "Rhz + 50%N + 100% PK" treatment was to the tune of 13.35%, over RDF.

As regard benefit cost (B:C) ratio, maximum profitability was registered under "Rhz + 75%N + 100% PK" followed by "Rhz + 100% NPK". Above treatment gave respective increases of 15.34%and 14.00 % over RDF. Similarly, magnitude of increase in above parameter following "Rhz + 50%N + 100% PK" treatment was to the tune of 13.02%, over RDF.

The *Rhizobium* inoculated treatments gave higher productivity and profitability due obvious reasons. Applications of Rhizobium reduce the cost of cultivation and economize the fertilizer N use and in turn enhance profitability to the farmers. The present results are in conformity with the finding of Bai, (2014), who reported higher B: C ratio following *Rhizobium* inoculation.

Table 5.5 Effect of treatments on economics of different treatments

Treatment	Cost of Produce (green pods) (Rs ha^{-1}) (1)	Cost of cultivation (Rs ha^{-1}) (2)	Net returns (Rs ha^{-1}) (3)=(1 – 2)	B:C ratio (3/2)
No Rhz $N_0P_0K_0$	26600	18000	8600	0.48
RDF (100% NPK)	61560	19526	42034	2.15

Rhz + 100% NPK	68020	19726	48294	2.45
Rhz + 75%N + 100% PK	68400	19669	48731	2.48
Rhz + 50%N + 100% PK	67260	19611	47649	2.43
Rhz + 75%NPK	58140	19345	38795	2.01
Rhz + N_0 + 100% PK	61560	19496	42064	2.16

Note:

All kinds of costs including the fixed costs have been considered in working out of cost of cultivation above

Cost of input (Rs. kg^{-1}): *FYM=0.80 N=11.45* P_2O_5 *=27.9* K_2O *=8.45 RHZ = 200*

Cost of produce (Rs. Kg^{-1}): Dry chickpea seed = 38

Chapter Number
6

CONCLUSION

Results of the current study suggest that the practice of Rhizobium inoculation can go a long way in reducing the cost of production directly as well as otherwise. Moreover, its continuous use is going to enhance its nutritional status and crop quality, which is the need of the hour. Above practice led to a reduction in soil test based N requirement in chickpea by about 50 %. Moreover, use of above biofertilizer enhanced N-use-efficiency significantly over recommended dose of fertilizer.

Some of the significant findings are:

Rhizobium inoculation enhanced crop growth and yield productivity significantly.

Rhizobium inoculation led to enhanced nutritional quality i.e. N and crude protein content

Rhizobium inoculation significantly enhance N use efficiency

Rhizobium inoculated plants shows increased rooting depth, root volume and more nodules

Rhizobium inoculation economize fertilizer N dose by about 50%

Rhizobium inoculation enhanced the profitability through reduction in fertilizer N use.

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