

A Seminar Paper  
on  
Performance of Biofertilizers on Growth and  
Yield of Chickpea

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# Performance of Biofertilizers on Growth and Yield of Chickpea

## Abstract

Biofertilization is a technology for sustainable crop production. It has many beneficial effects on chickpea production. Biofertilizers are not chemical fertilizers rather these are carrier based preparations containing beneficial microorganisms and when incorporated in soil, enhance specific microbial growth in rhizosphere, play vital role in nutrient mineralization, increase nutrient accumulation ultimately increase crop yield without any deterioration of nature. Biofertilizers reduce chemical fertilizer use thus improve soil fertility and minimize cost of production. The inoculation of seeds with *Rhizobium ieguminosorum* is known to efficiently increase nodulation, nitrogen uptake, growth and yield parameters of chickpea. The coinoculation of *Rhizobium* and phosphate solubilizing bacteria (PSB) also enhance nodulation, crop growth, seed protein and yield in chickpea. They play vital role in N<sub>2</sub>-fixation and phosphate solubilization simultaneously. Combined application of biofertilizers such as *Bacillus lentus* + *Pseudomonas putida* + *Trichoderma* significantly enhance N, P, K and Mg accumulation in chickpea seed, increase nutritious value of chickpea. Besides Biofertilization simultaneous application of nitrogen and microelement spraying have positive effects on growth indices and yield attributes of chickpea. Moreover biofertilizers effectively control many soil borne pathogens and provide us healthy crop. Many *Rhizobium* strains have the capacity to produce volatile compounds to inhibit growth of soil borne pathogens like *R. solani*.

**Key words:** Biofertilizer, chickpea, *Rhizobium*, coinoculation , nodulation, yield, and *R. solani*.

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# CHAPTER I

## Introduction

### 1.1 Background

Pulses are the second most important group of crops after cereals. India, China, Brazil, Canada, Myanmar and Australia are the major pulse producing countries. About 75% of the global chickpea area falls in India (FAOSTAT, 2010). Chickpea (*Cicer arietinum* L.) belongs to the family leguminosae. It is an important protein rich grain legume cultivated in the world. It is a rabi crop and the third major pulse crop in Bangladesh. Chickpea seeds contain essential amino acids like isoleucine, leucine, lysine, valine, and phenylalanine. The proteins in chickpea are highly digestible (70 - 90%) (Jain *et al.*, 2003). It is also a helpful source of zinc and folate. It is rich in dietary fibre (6%) and thus, a healthy source of carbohydrates. It provides dietary calcium (49-53 mg/100 g) and mineral contents such as phosphorous (340 mg/100 g), magnesium (140 mg/100 g), iron (7mg/100 g) are also high. Moreover, chickpeas are low in fat content and most of it is polyunsaturated fatty acid (source: United States Department of Agriculture (USDA) National Nutrient Database, Standard Reference No: 18 & 21).

Biofertilizers are carrier based preparations containing beneficial microorganisms in a viable state intended for seed or soil application to improve soil fertility and plant growth by increasing the number and biological activity of beneficial microorganisms in the rhizosphere. They improve soil fertility level by fixing atmospheric nitrogen, solubilizing insoluble soil phosphates and releasing plant growth substances in the soil (Venkateshwarlu, 2008). Biofertilizers are cost effective, ecofriendly, and renewable sources of plant nutrition (Khan *et al.*, 2007). These are also known as microbial inoculants. There are different types of microbial inoculants. Some important inoculants are *Rhizobium* inoculants, *Azotobacter* inoculants, Arbuscular mycorrhiza (AM), blue green algae inoculants, azolla, phosphate solubilizing bacterial (PSB) inoculants etc. *Rhizobium* inoculants are widely used as biofertilizer to enhance Chickpea growth & yield as they fix atmospheric nitrogen symbiotically. *Rhizobium* inoculation increased nodulation and seed yields upto 35% (Bhuiyan *et al.*, 1998). Gupta and Namdeo (1996b) found that seed inoculation with *Rhizobium* increased chickpea seed yields by 9.6-27.9%.

## 1.2 Rationale of the study

Nitrogen (N) deficiency is a major limiting factor for high yielding crops all over the world (Salvagiotti *et al.*, 2008; Namvar *et al.*, 2011). Deficiency of nitrogen results reduced growth rate, chlorosis, and reduced yield (Caliskan *et al.*, 2008; Erman *et al.*, 2011). Extending the role of biofertilizers such as *Rhizobium* can reduce the use of chemical fertilizers and decrease adverse environmental effects. Biofertilization has great importance in eliminating environmental pollution (Chemining wa, Vessey, 2006; Erman *et al.*, 2011). Chickpea can meet a significant portion (4–85%) of its N requirement through symbiotic N<sub>2</sub> fixation process when grown in association with effective and compatible *Rhizobium* strains (Togay *et al.*, 2008). The inoculation of seeds with *Rhizobium* is known to increase nodulation, N uptake, growth and yield parameters of legume crops (Erman *et al.*, 2011; Namvar *et al.*, 2011). The average yield of chickpea is about 746 kg/ha which is low due to lack of high yielding varieties and suitable rhizobial strains capable of fixing atmospheric nitrogen through biological nitrogen fixation process. A good number of varieties of chickpea have been developed by Bangladesh Agricultural Research Institute (BARI). There is a vast opportunity to increase its production by exploiting better colonization of the roots and rhizospheres through application of effective nitrogen fixing bacterial strains to the seed or rhizosphere. This can reduce chemical nitrogenous fertilizer use, which is very costly in developing countries like ours.

The coinoculation of *Rhizobium* and phosphate solubilizing bacteria (PSB) also enhance nodulation, plant height, seed protein and yield in chickpea. *Rhizobium* and PSB play vital role in N<sub>2</sub>-fixation and P-solubilization. The inoculation of efficient strains of P-solubilizing bacterial species such as *Bacillus megaterium* bv. *phosphaticum*, *Bacillus polymyxa*, *Penicillium digitatum*, *Pseudomonas* and *Aspergillus* in the rhizosphere has been recorded increase phosphorus availability in the soil (Gaur, 1990).

Soil-borne fungal pathogens cause many diseases of chickpea and reduce yields greatly. It has become a serious problem. Different bacterial strains such as *Bacillus*, *Pseudomonas* and recently the *Rhizobium* spp. have been found to control various soil-borne plant pathogenic fungi (Siddiqui, 2006). Among the *Rhizobium* group, *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, and *Sinorhizobium meliloti* reported to remarkably inhibit the growth of pathogenic

fungi such as *Macrophomina phaseolina*, *R. solani*, and *Fusarium* sp., in both legume and non-legume plants. *R. solani* is a soil-borne fungal pathogen, which causes serious losses in many different agricultural crops worldwide (Domsch *et al.*, 2007). The control of soil-borne pathogens is difficult because of their ecological behavior, their broad host range and high survival rate as dormant spores such as chlamydospores and sclerotia under different environmental conditions. Besides all of these, rhizobia are reported to produce plant growth regulators such as auxins, cytokinins and gibberellins like substances that stimulate plant growth (Sheng, 1993).

### 1.3 Objectives

Considering the above facts, the following objectives were undertaken:

- To review the effects of different biofertilizers on growth and yield of chickpea
- To study the antagonistic activity of different *Rhizobium* strains against *R. solani* , and
- To examine the effects of biofertilizer (*Rhizobium*) on growth and yield of chickpea in relation to inorganic nitrogen fertilization and micro element spraying.



## **CHAPTER II**

### **Materials and Methods**

This seminar paper is completely a review paper. Therefore, it has been prepared from the secondary sources or findings of the research works done throughout the world. Information and findings were collected from various Journals, books, papers, publications etc. at different libraries under different research institutes and universities like BRRI, BARI and BSMRAU. For collecting recent information Internet browsing was also practiced. Good suggestions, valuable information and kind consideration from my honorable major professor, course instructors and other resource personnel were taken to enrich this paper. After collecting necessary information, it has compiled and arranged chronologically for better understanding and clarification.

## CHAPTER III

### Review of Findings

#### 3.1 Effect of *Rhizobium* inoculation on different varieties of chickpea

Two field experiments were carried out during two consecutive *rabi* seasons with the aim of assessing the effect of *Rhizobium* inoculation on four varieties of chickpea viz., BARI Chola-3, BARI Chola-4, BARI Chola-5 and BART Chola-6. Each variety was tested with and without *Rhizobium* inoculation (Bhuiyan *et al.*, 2008).

##### 3.1.1 Effects of different varieties on nodulation and yield of chickpea

The effects of 4 chickpea varieties on nodule number, nodule weight, 1000-seed weight, stover yield and seed yield have been presented in Table 1. Among the varieties, BARI Chola-3 gave significantly higher nodule number, nodule weight and stover yield. BARI Chola-6 produced higher 1000-seed weight in first year. The highest seed yield was recorded in BARI Chola-4, which was significantly higher than other varieties in first year but similar to all other varieties in second year (Bhuiyan *et al.*, 2008). Gupta and Namdeo (1996a) have also reported varied in nodulation and yield of chickpea due to use of different varieties.

**Table 1:** Effects of different varieties on nodulation and yield of chickpea.

Variety	Nodules /plant	Nodule wt. (mg/plant)	1000-seed wt. (g)	Stover yield (t/ha)	Seed yield (t/ha)
<u>1<sup>st</sup> year</u>					
BARI Chola-3	42.6a	288a	138a	2.42a	1.08c
BARI Chola-4	24.4c	144c	122b	2.15b	1.35a
BARI Chola-5	24.6c	172b	113c	2.34a	1.23b
BARI Chola-6	30.3b	186b	144a	2.17b	1.11c
SE±	2.43	10.0	2.82	0.07	0.05
<u>2<sup>nd</sup> year</u>					
BARI Chola-3	46.9a	239a	162a	2.01a	1.27
BARI Chola-4	36.4b	169c	143b	1.84b	1.29
BARI Chola-5	31.4c	175bc	134c	1.57c	1.12
BARI Chola-6	43.0a	192b	160a	1.84b	1.19
SE(±)	1.44	7.2	2.12	0.05	NS

Means followed by different letters are significantly different at 5% level by DMRT

NS: Not significant

(Source: Bhuiyan *et al.*, 2008).

### 3.1.2 Effects of *Rhizobium* inoculants on nodulation and yield of chickpea

Table 2 shows the effects of rhizobial inoculation on nodule number, nodule weight, 1000-seed weight, stover yield and seed yield. Plants inoculated with *Rhizobium* showed significant results and gave higher nodule number, nodule weight, stover yield and seed yield compared to control (uninoculated).

**Table 2:** Effects of *Rhizobium* inoculants on nodulation and yield of chickpea.

Inoculants	Nodules /plant	Nodule wt. (mg/plant)	100-seed wt. (g)	Stover yield (t/ha)	Seed yield (t/ha)
<u>1<sup>st</sup> year</u>					
Uninoculated	25.8 b	167 b	129	2.06 b	1.09 b
Inoculated	35.2 a	229 a	129	2.48 a	1.29 a
SE(±)	1.72	7.1	NS	0.05	0.04
<u>2<sup>nd</sup> year</u>					
Uninoculated	32.1 b	176 b	147 b	1.73 b	1.15 b
Inoculated	46.8 a	212 a	153 a	1.90 a	1.29 a
SE(±)	1.02	5.08	1.50	0.04	0.04

Means followed by different letters are significantly different at 5% level by DMRT

NS: Not significant

(Source: Bhuiyan *et al.*, 2008).

### 3.1.3 Interaction effects of varieties and *Rhizobium* on nodulation and yield of chickpea

Table 3 shows the interaction effects of varieties and rhizobial inoculation on nodule number, nodule weight, 1000-seed weight, stover yield and seed yield. The highest nodule number, nodule weight and stover yield were recorded in case of BARI Chola-3 with inoculation, but the highest seed yield was observed in inoculated BARI Chola-4.

**Table 3:** Interaction effects of varieties and rhizobial inoculant on nodulation and yield of chickpea.

Treatments	Nodules /plant	Nodule wt. (mg/plant)	1000-seed wt. (g)	Stover yield (t/ha)	Seed yield (t/ha)
1 <sup>st</sup> year					
BARI Chola-3xU	35.2	260	138	2.17	0.98
BARICHola-3xI	50.0	316	138	2.66	1.17
BARICHola-4xU	22.8	126	122	1.94	1.25
BARI Chola-4xI	26.0	162	122	2.36	1.45
BARI Chola-5xU	20.6	132	111	2.14	1.11
BARICHola-5xI	28.6	212	116	2.54	1.35
BARI Chola-6xU	24.4	148	148	2.00	1.03
BARI Chola-6xI	36.2	224	140	2.34	1.19
SE(±)	ns	ns	ns	ns	ns
CV (%)	15.9	10.2	4.4	6.3	8.7
2 <sup>nd</sup> year					
BARI Chola-3xU	36.8	217	158	1.97	1.23
BARI Chola-3xI	57.0	261	166	2.05	1.31
BARI Chola-4xU	29.3	150	141	1.67	1.20
BARICHola-4xI	43.5	188	146	2.00	1.39
BARI Chola-5xU	27.3	158	129	1.51	1.03
BARICHola-5xI	35.5	193	139	1.63	1.21
BARI Chola-6xU	35.0	179	159	1.75	1.14
BARI Chola-6xI	51.0	204	160	1.93	1.26
SE(±)	ns	ns	ns	ns	ns
CV (%)	10.4	10.5	4.0	8.2	12.8

U= Uninoculated, I= Inoculated, NS= Not significant

(Source: Bhuiyan *et al.*, 2008).

Above three tables clearly reveal that higher nodule number, nodule weight, stover yield and seed yield were obtained from inoculated plants in comparisons with un-inoculated plants. Among four varieties, the highest nodule number, nodule weight and stover yield were provided by BARI Chola-3 whereas the highest yield was obtained from BARI Chola-4.

### 3.2 Performance of different biofertilizers on nutrient accumulation and yield of chickpea

Four levels of biofertilizers consisted of (B1): PSB (*Bacillus lentus* + *Pseudomonas putida*); (B2): *Trichoderma harzianum*; (B3): PSB+ Fungi (*Bacillus lentus* + *Pseudomonas putida* + *Trichoderma harzianum*); and (B4): control (without biofertilizer) were used to identify most effective biofertilizer (Mohammadi *et al.*, 2010).

Table 4 shows that combined application of biofertilizers (B3) performed best in terms of N, P, K and Mg accumulation in chickpea seed. B1 and B3 showed maximum results in case of Ca content in seed.

**Table 4:** Effect of different biofertilizers on nutrient accumulation in chickpea seed

Treatment	Nitrogen (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)
PSB (B1)	2269 b	271.5 b	1201b	184.3 a	4.32 a
<i>Trichoderma</i> fungi (B2)	2289 b	266c	1176.3 c	183.7 ab	4.27b
PSB + fungi (B3)	2410 a	279.8a	1232.1 a	181.2 b	4.34 a
Control (B4)	2167 c	264.9c	1199.8 b	184.5 a	4.28b

Means followed by different letters are significantly different at 5% level by DMRT

(Source: Mohammadi *et al.*, 2010).

Biofertilizer has significant effect on protein content, nodule number and nodule activity. Results showed that B3 produced the highest grain yield (Table 5). Coinoculation of PSB and fungi performed well than they perform solely.

**Table 5:** Effect of different biofertilizers on grain quality, grain yield and BNF

Treatment	Grain protein (%)	Grain starch (mg.kg <sup>-1</sup> )	Nodule number	Nodule activity (μmol/h)	Grain yield (kg.ha <sup>-1</sup> )
PSB (B1)	14.18b	154.1 a	37 b	8.2 a	1756.1 c
<i>Trichoderma</i> fungi (B2)	14.30b	154.2 a	34 b	8 a	1866.2 b
PSB + fungi (B3)	15.06a	153.6 a	42 a	8.4 a	2560.3 a
Control (B4)	13.54c	152.6 a	26 c	6 b	1310.7 d

Means followed by different letters are significantly different at 5% level by DMRT

(Source: Mohammadi *et al.*, 2010).

### 3.3 Effects of *Rhizobium* and (PSB) inoculants on different chickpea genotypes

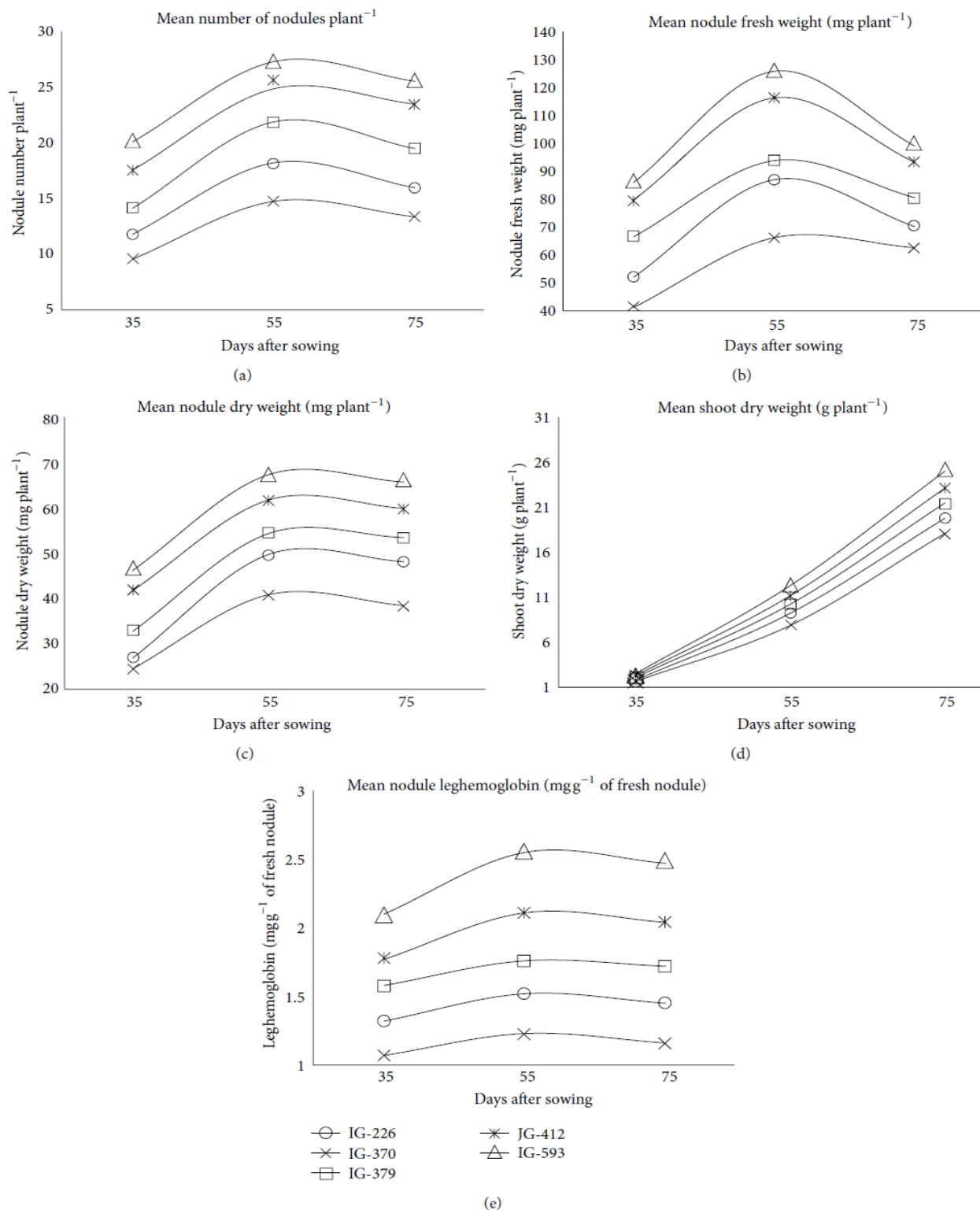
#### 3.3.1 Symbiotic traits

The data on mean nodule number, nodule fresh weight, nodule dry weight, and shoot dry weight at 35, 55 and 75 days after sowing (DAS) have been presented in Figures 1(a), 1(b), 1(c), 1(d). Among the genotypes, IG-593 exhibited the highest nodule number, nodule fresh and dry weight, shoot dry weight and the minimum values were recorded in case of IG-370. The figures indicated that nodule number, nodule fresh weight, nodule dry weight, and shoot dry weight increased significantly at 35 and 55 DAS, but the decline was noted in nodule number, fresh weight, and dry weight of nodules at 75 DAS (Tagore *et al.*, 2013).

Coinoculation of *Rhizobium* and PSB has reported to have more significant effect on nodule number and its fresh and dry weight than *Rhizobium* and PSB sole due to synergistic activity of those two types of microbes for biological nitrogen fixation. Similar result has been recorded by Rudresh *et al.*, 2005.

#### 3.3.2 Leghemoglobin Content in Root Nodules

Figure 1(e) shows that the leghemoglobin content in chickpea root nodules increased with the crop age. It was maximum at 55 DAS and then declined at 75 DAS. Among the genotypes, The highest nodule leghemoglobin content of 2.12, 2.6, and 2.45mg g<sup>-1</sup> of fresh nodule were obtained from IG-593 and lowest 1.27, 1.43, and 1.65mg g<sup>-1</sup> of fresh nodule were found in case of IG-370 at 35, 55 and 75 DAS, respectively.



**Fig. 1:** ((a)–(d)) Symbiotic parameters and (e) leghemoglobin content in nodular tissues of chickpea genotypes at different intervals.

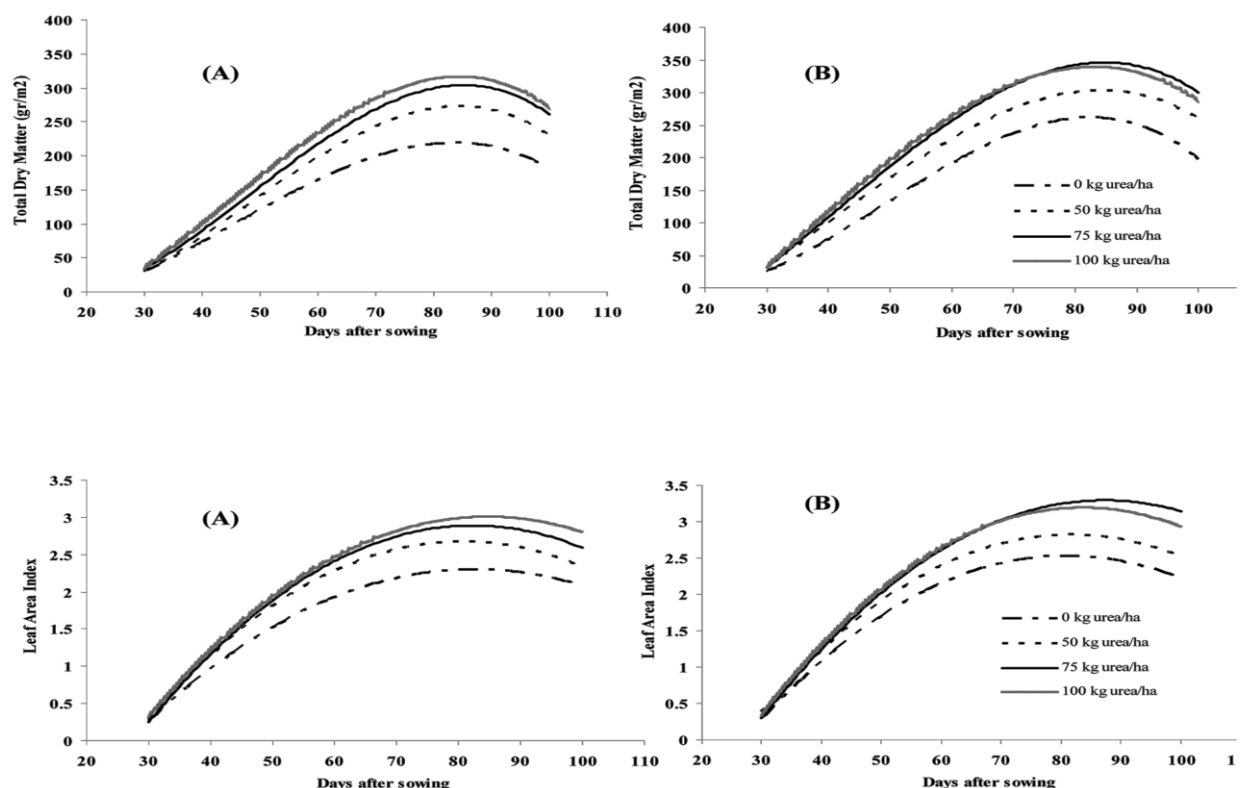
(Source: Tagore *et al.*, 2013).

### 3.4 Effects of biofertilizer (*Rhizobium*) on growth indices of chickpea in relation to inorganic nitrogen fertilization

N application and *Rhizobium* inoculation simultaneously have positive effects on growth indices and yield attributes of chickpea. Plants with N application in lower amount and no inoculation showed less growth indices including total dry matter, leaf area index, crop growth rate, relative growth rate and net assimilation rate whereas the highest values of these indices were observed at the high levels of nitrogen application (100 kg urea ha<sup>-1</sup>) and inoculated plants.

#### 3.4.1 Total dry matter (TDM)

At the early stages of crop growth TDM increased slowly and then increased rapidly with the advancement of plant age (Fig. 2). The rapid increase in TDM at the later stages was due to the development of a considerable amount of leaf area compared to early stages (Yasari and Patwardhan, 2006; Namvar *et al.*, 2011 ).



**Fig. 2.** Influence of different levels of nitrogen application on total dry matter (TDM) and leaf area index (LAI) in non-inoculated (A) and inoculated (B) chickpea.

(Source: Namvar *et al.*, 2011).



The highest values of TDM noted in the application of 75 kg urea ha<sup>-1</sup> in inoculated plants while the non-inoculated plants showed the highest amounts of TDM in the application of 100 kg urea ha<sup>-1</sup> which might be due to the more positive effect of *Rhizobium* presence in usage of 75 kg urea ha<sup>-1</sup> than the application of 100 kg urea ha<sup>-1</sup>. The lowest TDM was observed in non-fertilized plants at both levels of inoculation. Generally, inoculation with *Rhizobium* increased TDM at all levels of N application (Fig. 2). The same kind of results were reported about the effects of N fertilization (Alam and Haider, 2006; Yasari and Patwardhan, 2006; Caliskan *et al.*, 2008) and *Rhizobium* inoculation (Ogutcu *et al.*, 2008; Togay *et al.*, 2008; Namvar *et al.*, 2011) on TDM production of different crops.

#### **3.4.2 Leaf area index (LAI)**

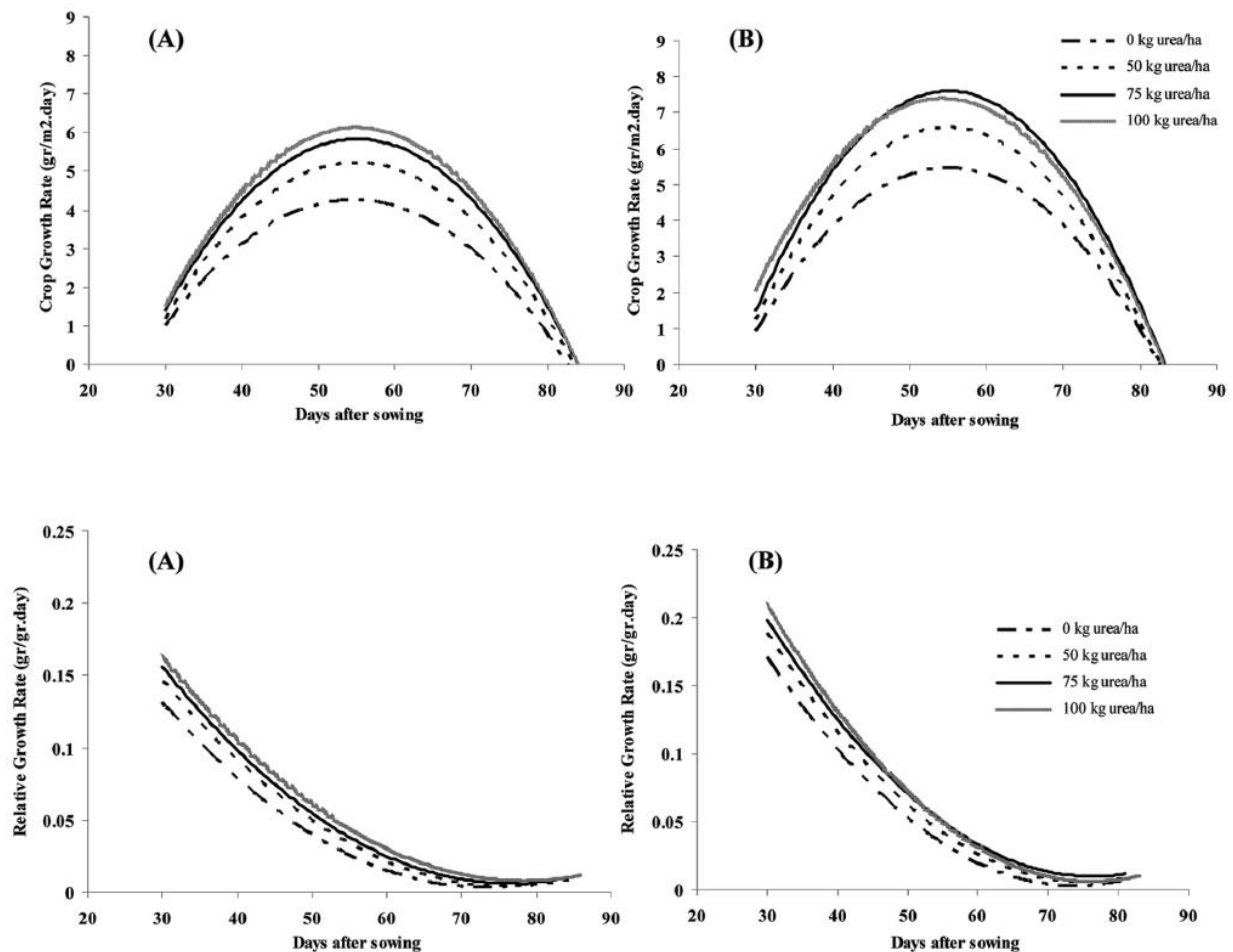
LAI of chickpea showed the same trend as TDM. LAI increased with the increase of N fertilizer rate. The highest values of LAI in inoculated and non-inoculated plants were noticed in application of 75 (22.45% increase over control) and 100 kg urea ha<sup>-1</sup> (19.67% increase over control), respectively. 0 kg urea ha<sup>-1</sup> treated plants showed the lowest LAI at both levels of inoculation (Fig. 2). The decrease of LAI at the later stages of crop growth was probably due to the senescence of older leaves. Similar results were also reported by Alam and Haider (2006), Yasari and Patwardhan (2006) and Namvar *et al.* (2011). Moreover, *Rhizobium* inoculation enhanced the amount of LAI at all levels of N fertilization (Fig. 2). Inoculated plants showed about 6.36% more LAI than the non-inoculated plants.

#### **3.4.3 Crop growth rate (CGR)**

CGR started from lower value, reached a certain peak and then declined at the later stages of growth (Fig. 3). Application of 75 and 100 kg urea ha<sup>-1</sup> showed the highest CGR in inoculated (35.06% increase over control) and non-inoculated (31.33% increase over control) plants, respectively. Non-fertilized and non-inoculated plants were reported to have the lowest CGR (Fig. 3). Inoculation with *Rhizobium* enhanced CGR at all levels of N application. These results are similar to the findings of Alam and Haider (2006).

### 3.4.4 Relative growth rate (RGR)

Fig. 3 also shows that, irrespective of N fertilizer treatments, RGR was high in the early growth stages and showed a declining trend with ageing. RGR enhanced with increasing N fertilizer amount. At both levels of inoculation, the highest RGR were recorded in maximum (100 kg urea  $\text{ha}^{-1}$ ) and the lowest values of RGR were found at minimum (0 kg urea  $\text{ha}^{-1}$ ) rates of N application, respectively. Moreover, inoculation with *Rhizobium* bacteria increased RGR at all levels of N fertilizer use (Fig. 3). These results match with the observations made by Alam and Haider (2006), and Namvar *et al.* (2011).

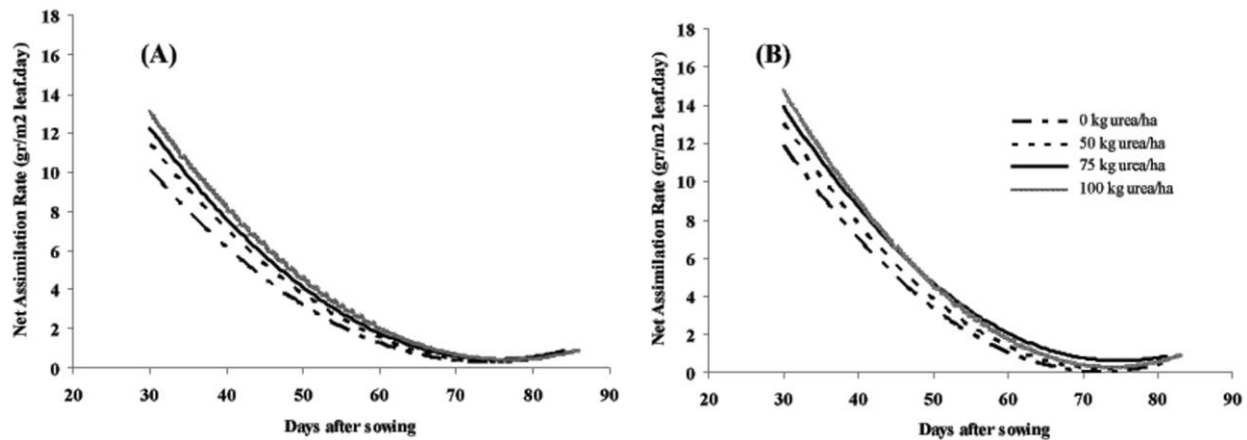


**Fig. 3.** Influence of different levels of nitrogen application on crop growth rate (CGR) and relative growth rate (RGR) in non-inoculated (A) and inoculated (B) chickpea.

(Source: Namvar *et al.*, 2011 ).

### 3.4.5. Net assimilation rate (NAR)

NAR is high at the early stages and significantly declines with increasing age (Fig. 4). NAR remains to be highest when all leaves can obtain full sunlight. Mutual shading of leaves and less photosynthetic efficiency of older leaves reduce NAR with crop ageing (Alam, Haider, 2006; Yasari, Patwardhan, 2006). Usage of 0 showed the lowest and 100 kg urea ha<sup>-1</sup> showed the highest values of NAR, respectively. Moreover, *Rhizobium* inoculation increased NAR at all levels of N application in chickpea plants (Fig. 4).



**Fig. 4.** Influence of different levels of nitrogen application on net assimilation rate (NAR) in non-inoculated (A) and inoculated (B) chickpea.

(Source: Namvar *et al.*, 2011).

### 3.5 Effects of biofertilizer (*Rhizobium*) with inorganic nitrogen fertilization on yield and its components of chickpea

The highest plant height, number of primary and secondary branches, number of pods per plant and number of grains per plant were obtained from the highest level of nitrogen fertilization (100 kg urea ha<sup>-1</sup>) and *Rhizobium* inoculation simultaneously. Application of 75 and 100 kg urea ha<sup>-1</sup> did not show significant difference in these traits. Moreover, the highest grain yield was found in the inoculated plants those were treated with 75 to 100 kg urea ha<sup>-1</sup> (Table 6).

**Table 6.** Effects of nitrogen fertilization and *Rhizobium* inoculation on yield and its components in chickpea

Treatments	PH (cm)	NPB (per plant)	NSB (per plant)	NP (per plant)	NG (per pod)	NG (per plant)	100-GW (gr)	GY (kg ha <sup>-1</sup> )
<b>Nitrogen rates (kg urea ha<sup>-1</sup>)</b>								
0	33.47 c	1.93 c	4.67 c	17.85 c	1.03 b	11.42 c	28.47 a	911.5 c
50	37.43 b	2.31 b	6.61 b	19.42 b	1.11 ab	14.12 b	27.60 ab	1207.3 b
75	44.37 a	2.60 a	8.51 a	20.61 a	1.18 a	16.41 a	26.58 b	1328.2 a
100	45.97 a	2.63 a	9.35 a	21.30 a	1.17 a	16.63 a	26.48 b	1413.6 a
<b><i>Rhizobium</i> inoculation</b>								
Uninoculated	41.25 a	2.26 b	6.80 b	18.97 b	1.08 a	13.38 b	27.21 a	1166.5 b
Inoculated	39.37 a	2.47 a	7.77 a	20.62 a	1.15 a	15.91 a	27.37 a	1263.7 a
Mean	40.31	2.36	7.28	19.79	1.12	14.64	27.28	1215.15
Nitrogen	**	**	**	**	ns	**	**	**
<i>R.</i> Inoculation	ns	*	**	**	ns	**	ns	**
Nitrogen × <i>R.</i> Inoculation	ns	ns	ns	ns	ns	*	ns	*
CV (%)	13.63	10.30	10.96	16.29	11.02	16.10	11.23	12.34

PH: Plant Height, NPB: Number of Primary Branches, NSB: Number of Secondary Branches, NP: Number of Pods, NG: Number of Grains, 100-GW: 100-Grains Weight, GY: Grain Yield. Mean values followed by the same letters in each column and treatment showed no significant difference by DMRT ( $P = 0.05$ ). – \*, \*\* and ns showed significant differences at 0.05, 0.01 probability levels and not significant, respectively.

(Source: Namvar *et al.*, 2011).

The results from the Table 6 clearly revealed that the suitable amounts of nitrogen fertilizer application (i.e. between 75 and 100 kg urea ha<sup>-1</sup>) as basal dose can be advantageous in improving growth, development and total yield of inoculated chickpea.

### 3.6 *Rhizobium* biofertilizer and microelements application on chickpea

#### 3.6.1 *Effects of Rhizobium inoculation and microelements spraying on nodulation*

A field experiment was done to investigate the effects of *Rhizobium* biofertilizer and microelements application on chickpea (Bejandi *et al.*, 2012). Two levels of seed inoculation (with and without inoculation), two levels of microelements application (with and without microelements spraying) were used. Seed inoculation and microelements spraying had significant effects on nodule number, nodule fresh weight, nodule dry weight and active nodule per plant (Table 7) and (Figure 5).

**Table 7 .** Chickpea studied traits with seed inoculation and microelements application.

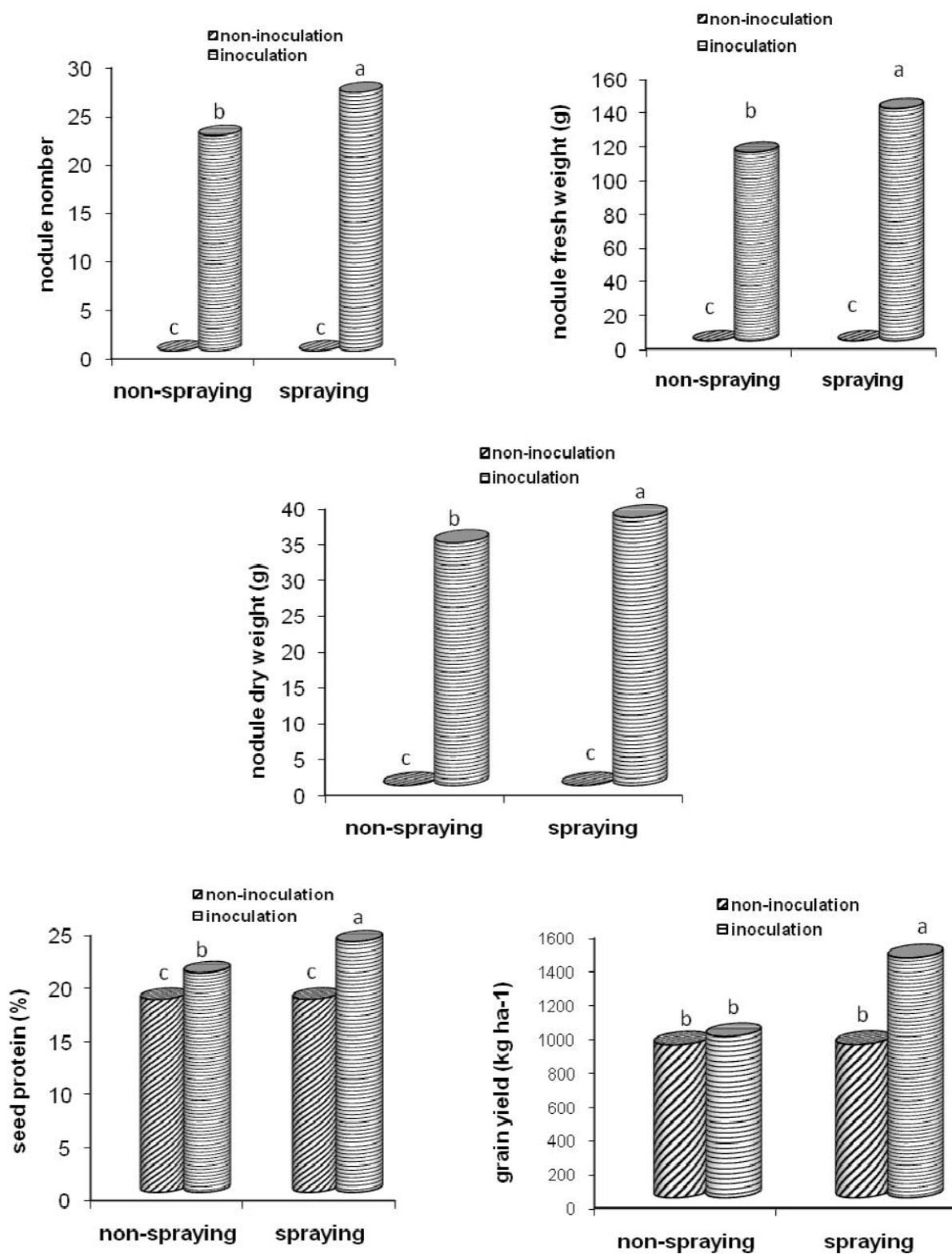
Treatments	Nodule number (No. plant <sup>-1</sup> )	Nodule fresh weight (g plant <sup>-1</sup> )	Nodule dry weight (g plant <sup>-1</sup> )	Active nodule (%)
<i>Rhizobium cic.</i>				
Inoculation	24.44a	131.99a	35.63a	83.5a
Non-inoculation	0b	0b	0b	0b
<b>Microelements</b>				
spraying	13.33a	76.43a	18.69a	89a
Non-spraying	11.11b	55.56b	16.94b	78b
CV (%)	16.29	14.20	11.42	14.5

Means followed by equal do not differ by Duncan's multiple range test, at 5% of probability.

(Source: Bejandi *et al.*, 2012).

#### 3.6.2 *Effects of Rhizobium inoculation and microelements spraying on yield parameters*

Seed inoculation and microelements application showed significant effects on maturity time, chlorophyll content, seed protein, pods per plant, hollow pod and grain yield. The highest values of these traits were observed in the inoculation treatments. The results revealed that time of maturity and hollow pods percentage can be reduced significantly. The highest grain yield and protein content were achieved in seed inoculation with microelements application and the lowest of them were found in non-inoculation and without of spraying microelements (Figure 5) and (Table 8).



**Fig. 5.** Nodulation, seed protein and grain yield of chickpea at two inoculation treatments and two microelements application rates.

(Source: Bejandi *et al.*, 2012).

**Table 8.** Seed inoculation and microelements application effects on studied traits of chickpea

Treatments	Maturity time (day)	Chlorophyll content	Pod per plant	Hollow pod (%)	Seed protein (%)	Grain yield (kg ha <sup>-1</sup> )
<b>Rhizobium cic.</b>						
Inoculation	109b	33.14a	32a	7b	22.15a	1163.06a
Non-inoculation	117a	28.21b	23.32b	14a	18.17b	906.82b
<b>Microelements</b>						
Spraying	119a	34.34a	30.25a	8b	20.89a	1138.05a
Non-spraying	111b	27.01b	25.07b	13a	19.43b	931.83b
CV %	16	9.1	11.25	12	10.61	18.95

(Source: Bejandi *et al.*, 2012).

Means followed by equal do not differ by Duncan's multiple range test, at 5% of probability

### 3.7 The antagonistic activity of different *Rhizobium* strains against *R. solani*

An experiment was conducted to study the antagonistic effect of different *Rhizobium* strains against *R. solani* in dual culture *in vitro* and under greenhouse conditions (Hemissi *et al.*, 2011). 42 *Rhizobium* strains were used to investigate the beneficial effects of rhizobial inoculants on nitrogen fixation, phosphorous uptake and on plant growth promotion. To know the biological control mechanisms, the *Rhizobium* strains ability to produce volatile compounds and to solubilise phosphate were investigated. Among the 42 strains tested, 24 isolates effectively controled *R. solani in vitro* (Table 9). The findings showed that 13 strains produced volatile compounds and 10 strains were able to solubilise phosphorus. Among these The strain S27 successfully control *R. solani in vitro* and in pot experiments (Hemissi *et al.*, 2011). As a result S27 was reported most effective strain as it provided maximum nodule per plant, shoot height and shoot dry weight (Table 10).

**Table 9.** Effects of *Rhizobium* on *R. solani* growth and determination of effective bacterial isolates to solubilise phosphate and to produce volatiles.

Treatments	% Growth Inhibition <sup>(+)</sup>	% Inhibition of fungal growth by volatiles	Phosphate solubilization
S12	41.65 ± 2.10	0.00 ± 0.00	+
S4	46.60 ± 1.75*	0.00 ± 0.00	+
S20	47.17 ± 3.33*	0.00 ± 0.00	+
S28	50.50 ± 3.80*	49.25 ± 1.75*	-
S27	50.52 ± 2.81*	38.25 ± 2.81*	+
S29	51.62 ± 2.80*	32.50 ± 3.80*	-
S30	52.17 ± 2.87*	37.50 ± 2.84*	+
S31	52.17 ± 2.87*	0.00 ± 0.00	-
S32	52.75 ± 1.79*	25.75 ± 1.84*	-
S33	52.75 ± 2.10*	0.00± 0.00	-
S34	53.27 ± 1.81*	38.25 ± 2.87*	+
S35	53.30 ± 1.79*	0.00± 0.00	-
S36	54.40 ± 1.27*	40.75 ± 1.54*	-
S22	54.40 ± 2.84*	0.00± 0.00	+
S37	52.20 ± 1.27*	0.00± 0.00	-
S38	57.72 ± 1.83*	25.00 ± 1.23*	-
S10	57.75 ± 1.79*	25.75 ± 2.87*	+
S16	58.85 ± 1.98*	43.25 ± 3.3*	+
S39	63.85 ± 3.30*	25.00 ± 1.43*	-
S15	67.70 ± 1.27*	37.50 ± 1.54*	+
S40	68.27 ± 1.81*	0.00± 0.00	-
S3	69.37 ± 2.89*	37.50 ± 2.89*	-
S41	69.95 ± 2.89*	0.00± 0.00 -	-
S17	76.15 ± 1.93*	35.75 ± 1.93*	+
S42	77.72 ± 1.83*	40.75 ± 3.3*	-
S1	77.72 ± 2.84*	42.5 ± 2.89*	+
S2	79.95 ± 1.83*	0.00± 0.00	+
Control	0.00	0.000	-

(+) = Percent growth inhibition compared to uninoculated control was determined after 7 days of incubation. Each value is a mean of 3 replicates. Mean values followed by \* were significant (P= 0.05), compared to the control, by Duncan's multiple range test.

(Source: Hemissi *et al.*, 2011).



**Table 10.** Effect of inoculation by *Rhizobium* strains on growth parameters of chickpea infected with *R. solani* under glass house conditions (8 weeks after sowing).

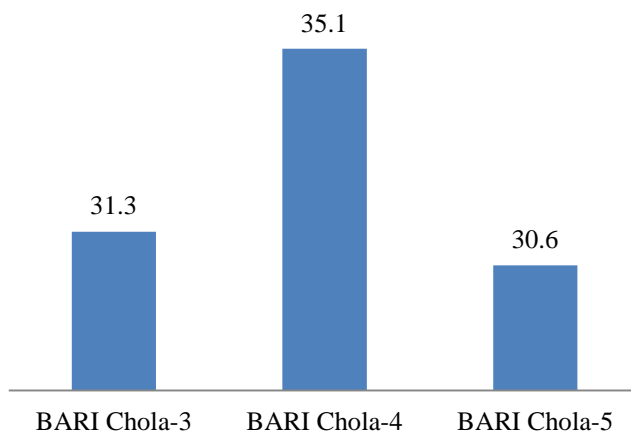
Treatments	Nodules number	Shoot height (cm)	Shoot dry weight (g)
S12	8	32.33 ± 1.15*	7.01 ± 0.40
S4	10	34.33 ± 0.57*	9.26 ± 0.14*
S20	15	35.00 ± 1.15 *	10.63 ± 0.20*
S37	5	21.67 ± 0.57	3.57 ± 0.14
S31	10	28.67 ± 0.57	6.13 ± 0.76
S22	12	31.00 ± 0.57	8.74 ± 0.31*
S28	11	33.00 ± 0.57*	8.22 ± 0.45*
S1	15	33.00 ± 0.57*	5.10 ± 0.21
S30	9	33.33 ± 0.57*	5.41 ± 0.40
S39	15	34.00 ± 0.57*	10.07 ± 0.09*
S35	10	34.67 ± 0.57*	8.76 ± 0.17*
S15	19*	35.33 ± 0.57*	10.04 ± 0.55*
S38	13	35.33 ± 1.15 *	10.81 ± 0.17*
S33	8	35.33 ± 1.54*	8.59 ± 0.09*
S34	7	35.67 ± 0.57*	5.84 ± 0.31
S3	12	36.00 ± 0.57*	9.83 ± 0.76*
S32	7	36.33 ± 1.00*	7.01 ± 0.76
S41	11	36.33 ± 0.57*	8.26 ± 0.21*
S2	12	36.67 ± 0.57*	8.92 ± 0.21*
S16	13	38.67 ± 0.57*	9.86 ± 0.17*
S40	12	39.00 ± 0.57*	8.68 ± 0.31*
S10	20*	39.00 ± 0.57 *	11.02 ± 0.40*
S42	13	40.00 ± 0.57*	9.19 ± 0.55*
Control	0	41.00 ± 0.57*	8.76 ± 0.58*
S29	12	41.33 ± 0.57*	9.16 ± 0.20*
S36	14	42.67 ± 0.57*	11.36 ± 0.21*
S17	22*	43.00 ± 1.00*	10.46 ± 0.55*
S27	25*	45.33 ± 0.15*	11.53 ± 0.45*

Each value is a mean of 3 replicates. Mean values followed by \* were significantly different (P=0.05), compared to the positive control, by Duncan's multiple range test.

(Source: Hemissi *et al.*, 2011).

### 3.8 Mycorrhizal dependency of chickpea

A field experiment was conducted to assess the mycorrhizal dependency of chickpea (Solaimam *et al.*, 2012). According to mycorrhizal dependency 3 varieties of chickpea were highly dependent plants. The range of mycorrhizal dependency is from 30.6 to 35.1 percent (Figure 6). Among the three varieties BARI Chola-4 showed the highest mycorrhizal dependency that was 35.1%, BARI Chola-3 of 31.3% and BARI Chola-5 of 30.6%.



**Fig. 6.** Mycorrhizal dependency of chickpea.

(Source: Solaimam *et al.*, 2012).

## **CHAPTER IV**

### **Conclusion**

- Biofertilizers are eco-friendly, cost effective, more efficient, productive over chemical fertilizers. They reduce chemical fertilizers use in chickpea production through more efficient use of available nitrogen (N) and other nutrient elements in the rhizosphere. Thus protect environment from harmful impact of chemical fertilizers as well as improve soil fertility by maintaining beneficial microorganisms population in the soil. Combined application of biofertilizers provides more effectiveness in terms of growth and yield of chickpea.
- Biofertilizers effectively control many noxious soil borne pathogens and keep chickpea crop free from soil borne diseases caused by those pathogens.
- N fertilizer use with biofertilization has positive effects on growth indices and, consequently, on yield and its attributes of chickpea. Moreover microelement spraying with seed inoculation increase chickpea growth and yield satisfactorily. Further studies regarded to biofertilizers are recommended to discover more advantages of biofertilizers use in chickpea production.

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