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NODULATION, GRAIN YIELD AND GRAIN PROTEIN CONTENTS AS AFFECTED BY RHIZOBIUM INOCULATION AND FERTILIZER PLACEMENT IN CHICKPEA CULTIVAR BITTLE-98

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ABSTRACT

Two fertilizer application methods i.e. Fertilizer broadcast (F_1) and Band placement (F_2) and five rhizobium inoculation strains along with a control; Uninoculation (I_0), Mezo rhizobium (I_1), Rhizobium (I_2), Biozote (I_3), Agro bacterium (I_4) and Biofertilizer (I_5) were studied in split plot arrangements. Main plot consisted of two fertilizer application methods whereas rhizobium inoculation strains were kept in sub-plots. The study was carried out at the Arid Zone Research Institute, Bhakkar, during Rabi (winter) of 2004-05 and 2005-06 with the objectives to determine the appropriate method of fertilizer application with the selection of Rhizobium strain for exploitation of its natural symbiosis with chickpea for effective nodulation, maximum grain yield and grain protein contents in chickpea under the agro-climatic conditions of Bhakkar, Punjab, Pakistan. Yield and yield components were significantly affected by fertilizer band placement and seed inoculation. Maximum number of pods plant^{-1} (36.50) and100 grain weight (29.19 g) was recorded in fertilizer band placement. Fertilizer band placement increased grain yield by 10 % (3279 to 3772 kg ha^{-1}) during 2004-05 and 5.96 % (2979 to 3560 kg ha^{-1}) during 2005-06 over fertilizer broadcast. Nodules plant^{-1} was also increased 27 % by seed inoculation. Inoculation with Biofertilizer increased 25.0 and 27.77% yield of chickpea over check (uninoculated) during 2004-05 and 2005-06 study seasons, respectively. It was concluded that, fertilizer band placement along with Biofertilizer inoculation was the best for higher yield and grain protein contents in chickpea.

Key Words: **Fertilizer application method, inoculation, Biofertilizer, *rhizobium, Biozote.***

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INTRODUCTION

Chickpea not only plays an important role in human diet but also improves soil fertility by fixing the atmospheric nitrogen (Siddiqi and Mahmood, 2001, Kantar *et al*. 2007). There are many factors involved in achieving high crop yield. Nutritional imbalance and poor nodulation appears to be the distinct ones, which can be effective in a single crop season. Farmers have a wrong notion that chickpea being a legume crop, does not need any nutrition and usually grow it on marginal land, without applying any fertilizer. This seems to be an important reason for its low grain yield in Pakistan. Growers generally do not apply fertilizer to chickpea; however, some progressive farmers apply a little amount of nitrogenous and phosphatic fertilizer through broadcast as a starter dose. Application of phosphorus to legume crops improves grain yield considerably (Hussain, 1983). Grain yield of chickpea was increased significantly with Rhizobium and phosphorus application (Raut and Kohire, 1991).

The phosphorus requirement is greater for healthy crop growth with efficient root system and profuse nodulation. Phosphorus also plays a key role in pod filling and ultimately enhances the grain yield. Gupta *et al.* (1998) and Reddy *et al*. (1993) reported that phosphorus application significantly increased the yield in red chickpea. Most of the soils in Pakistan are alkaline and calcareous in nature. Such soils bind phosphorus tightly. Therefore, Phosphatic fertilizer is required nearly everywhere to support efficient plant growth and more losses of fertilizer were recorded from broadcasting (Timmons *et al.* 1973). To avoid economic loss and soil depletion, it is necessary to determine the proper method of fertilizer application in chickpea. Shahzad *et al.* (2003) reported that fertilizer placement below the seed yielded significantly higher results, followed by side drilling on both sides of the seed rows, while minimum seed yield was recorded with the broadcast method.

Chickpea requires about 13 to 41 kg Nitrogen ha^{-1} for growth and development. Nitrogen (N2) fixing *Rhizobium* bacteria could increase yield at a low cost (Kucuk and Kıvanc, 2008). Nitrogen (N) applications are generally not necessary because it fixes (60-80 percent of its requirements) atmospheric N through symbiosis with nitrogen fixing bacteria (*Rhizobium Cicer*). The bacteria, however, should be present in the soil for N fixation (Rhinhart *et al*. 2003). If full benefit from grain legume crop is to be achieved in terms of maximum yield and soil improvement, the seed should be inoculated with its own specific and suitable *Rhizobium* strain before planting. El-Hadi and Sheikh (1999) reported that *Rhizobium* inoculation application significantly increased total nodule numbers $plant^{-1}$, 100 grain weight, yield (70-72%) and protein contents of chickpea grains. Rhizobium plus phosphate-solubilizing bacteria significantly improved crop yield components (Meena, *et al*. 2001). Kyei -Boahen *et al*. (2002) reported that grain yield and quality was improved up to 50% in chickpea with seed inoculation. Karadavut and Ozdemir (2001) showed that Rhizobium significantly increased seed yield (20% higher than control), total above ground dry matter and number of pods $plant^{-1}$ in chickpea. Fatima, *et al*. (2008) narrated that *Rhizobium* inoculum generally increased plant growth, yield and yield components and nitrogen fixation in chickpea.

Inoculation Strains

Chickpea inoculation strains of different origin were received from following National Institutes.

Name of inoculants	Donor Institute
Meso Rhizobium	Nuclear Institute for Biology and Genetic Engineering (NIBGE), Faisalabad
Rhizobium	Nuclear Institute for Biology and Genetic Engineering (NIBGE), Faisalabad
Biozote	National Agricultural Research Centre (NARC), Islamabad
Agro Bacterium	Nuclear Institute for Biology and Genetic Engineering (NIBGE), Faisalabad
Biofertilizer	Ayub Agricultural Research Institute (AARI), Faisalabad

So it is essential that chickpea cultivars together with rhizobium strains be evaluated for their ability to produce nodules and nitrogen fixation potential of produced nodules. The *Rhizobium* strain should have an ability to colonize in soil, tolerate environmental stresses, form effective nodules and should have no deleterious effects on ecosystem. Therefore, the following experiments were conducted to sort out suitable fertilizer application method with minimum losses and selection of effective *Rhizobium* strain for higher yield and improved grain protein content.

MATERIALS AND METHODS

A field experiment was conducted at the Arid Zone Research Institute, Bhakkar, Punjab, Pakistan during Rabi (winter) seasons of 2004-05 and 2005-06 on sandy loam soil with field capacity and permanent wilting point values of 14.67 and 5.40 % on volume basis, respectively. The objectives of the research were to study the nodulation, yield productivity and grain protein contents of chickpea as affected by fertilizer application methods and Rhizobium inoculation strains. The sandy loam soil (Table I) was well prepared before sowing of crop because the soil was virgin and currently brought under cultivation. Second years' experiment was laid out on same land. The chickpea variety Bittle-98 was sown in a randomized complete block design with split plot arrangements. Two fertilizer application methods i.e. broad cast and band placement were kept in main plots while five rhizobium strains alongwith the control were randomized in sub plots. The sub plot size was 5 m x 1.8 m. Seeds of cv. Bittle-98 of chickpea were treated with different rhizobium strains. The graded seed at the rate of 75 kg ha^{-1} was sown with a single row drill. The recommended dose of fertilizer (22 Kg N and 57 Kg P_2O_5 ha^{-1}) was applied according to the above- mentioned methods in the form of urea and triple super phosphate at the time of seed bed preparation. The crop was inter-cultured twice at 35 and 70 days after sowing. Data was recorded for the number of nodules $plant^{-1}$ on days 100, 120 and 140 after sowing; the number of pods $plant^{-1}$ were recorded on 10 randomly selected plants from each plot and the average number of grains pod^{-1} were recorded from 20 randomly selected pods taken from ten randomly selected plants. Average weight of three samples were recorded for 100 grain randomly taken from grain yield of each treatment where as grain yield (kg ha^{-1}) was recorded on plot basis leaving the side rows as non-experimental. The seed N content was determined by Kjeldahl's method and the estimated N content multiplied by a factor of 6.25 to compute the total protein content (Jackson, 1962). Data were subjected to Analysis of variance (Steel *et al*. 1997) to determine the significance of differences between treatments. The Least significant difference (LSD) test was applied for comparison of means of individual treatments.

RESULTS AND DISCUSSION

Nodules Plant^{-1}

Data pertaining to the number of nodules plant^{-1} are presented in Table II. It is evident that differences between the means of treatments were significant. The number of nodules plant^{-1} were counted as 22.33 and 26.04 in band fertilizer application method as compared to 20.89 and 24.50 in broadcast fertilizer application method during 2004-05 and 2005-06, respectively. Thus fertilizer band placement increased nodules plant^{-1} by 6.57 % over fertilizer broadcast. The inoculums also increased the number of nodules plant^{-1} from 19.41 (control) to 23.35 (2004-05) and 21.82 to 28.96 (2005-06). Thus nodules plant^{-1} were increased by 10, 11, 15, 19 and 27 % over the control by inoculation of *Agro bacterium*, *Meso rhizobium*, *Rhizobium*, Biozote and Biofertilizer, respectively. Inoculation with Biofertilizer (commercial inoculum) gave maximum increase of 27 % in number of nodules plant^{-1} and similarly the seed yield was increased up to 25 %. It was also observed that the number of nodules plant^{-1} was higher during the 2005-06 season and this could be attributed to the cultivation of previous pulse crop which might have increased the microbial activities. The interaction between fertilizer application methods and Rhizobium inoculations were also significant and maximum number of nodules plant^{-1} (24.40 and 29.90) were recorded in fertilizer banding together Biofertilizer inoculums and the minimum number of nodules plant^{-1}(18.18 and 21.46) were observed in fertilizer broadcast along with no grains inoculation during 2004-05 and 2005-06, respectively. An increase of 34 % was recorded in the number of nodules plant^{-1} with the interactive effect of fertilizer banding with inoculation (Table II). Similarly, nodules plant^{-1} increased from 17 to 39 % by inoculating the seed with different strains during 2005-06. This may be due to the reason that inoculation increased the rhizobial activities and in turn nodules number which increased the N uptake by the crop plants, thus the yield. Tippannavar and Desai (1992), Shah *et al*. (1994) and Biswas *et al*. (2003) reported that seed inoculation increased the number of nodules plant^{-1}. These results are also in conformity with the findings of El-Hadi and Sheikh (1999) as they reported that Rhizobium inoculation significantly increased total nodules number plant^{-1}. Yadav *et al.* (1994) observed an increase in the number of nodules and grains yield due to grains inoculation. Roy *et al*. (1995) also reported that grains inoculation increased the nodules number per plant and gave the highest harvest index and 100 grains weight.

Table I ***Physico-chemical characteristics of soil at experimental site at Arid Zone Research Institute, Bhakkar, Punjab, Pakistan***

Physico-chemical characteristics		Values				
Textural Class	Unit	Sandy Loam (Before sowing)	Sandy Loam (After harvesting)		Sandy Loam (After harvesting)	
			2004- 05		2005- 06	
			FBC	FP	FBC	FP
pH	-	8.2	8.0	8.0	8.0	8.0
Ec	dSm^{-1}	0.44	-	-	-	-
Available P	kg	3.34	4.75	3.50	2.90	2.75
Available K	kg	66	65	55	60	60
Organic matter	%	0.27	0.33	0.32	0.34	0.35
N	%	0.014	0.017	0.016	0.017	0.018
Field capacity	% by volume	14.60	14.64	14.65	14.67	14.67
Bulk density	$g\ m^{-3}$	1.26	1.26	1.26	1.26	1.26
Permanent wilting point	% by volume	5.4	5.4	5.4	5.4	5.4

FBC: Fertilizer broadcast **FP**: Fertilizer placement

Source: Soil and Water Testing Laboratory, Ayub Agricultural Research Institute, Faisalabad, Punjab, Pakistan

Table II ***Effect of fertilizer application methods and various inoculation strains on number of nodules plant^{-1} in chickpea***

Rhizobium inoculation strai (I)	2004-05 Fertilizer application methods (FAM)		Means	2005-06 Fertilizer application methods (FAM)		Means
	Fertilizer broadcast (F_1)	Band application (F_2)		Fertilizer Broadcast (F_1)	Band application (F_2)	
Uninoculation (I_0)	18.18d	20.63c	19.41d	21.46j	25.19i	21.82f
Mezo Rhizobium (I_1)	20.80c	22.09b	21.44c	23.73g	25.34d	24.53d
Rhizobium (I_2)	20.83c	22.29b	21.56c	24.93e	26.35c	25.63c
Biozote (I_3)	22.18b	22.52b	22.35b	25.58d	27.88b	26.73b
Agro bacterium (I_4)	21.10c	22.08b	21.59c	23.29h	24.60f	23.94e
Biofertilizer (I_5)	22.31b	24.40a	23.35a	28.03b	29.90a	28.96a
Means	20.89b	22.33a		24.50b	26.04a	

Year	2004-05	2005-06
$LSD_{0.05}$ (Fertilizer application method)	= 1.73	0.80
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 0.44	0.21
$LSD_{0.05}$ (FAM x inoculation strains)	= 0.62	0.30

Pods Plant^{-1}

Number of pods plant^{-1} is an important yield determinant in pulse crops. Data regarding number of pods plant^{-1} are presented in Table III. The analysis of variance showed that the differences among the means of treatments were significant. The maximum number of pods plant^{-1} (29.23 and 36.50) were recorded in fertilizer banding followed by fertilizer broadcast with an average of 27.38 and 34.84 pods plant$^{-1,}$ thus fertilizer banding yielded an increase of 7 and 5% in number of pods plant^{-1} over broadcast during the 2004-05 and 2005-06 study seasons, respectively. This may be due to more availability and less losses of nutrients in band placement as compared to broadcast method. These findings are in agreement with that of Timmons *et al*. (1973) who reported less losses of fertilizer in band placement and better availability of nutrients to crop plants. Rhizobium inoculation significantly increased the number of pods plant^{-1}. Maximum number of pods plant^{-1} (31.73 and 39.99) were recorded in Biofertilizer (chickpea inoculums) followed by Biozote (inoculum) with an average of 28.71 and 36.65 pods plant^{-1} during 2004-05 and 2005-06, respectively. Minimum number of pods plant^{-1}, (26.49 and 32.92) were recorded in uninoculated plots during 2004-05 and 2005-06 study seasons, respectively. On average, the number of pods plant^{-1} were increased by 4, 6, 6, 9 and 20% over control by inoculating the seeds with *Agro bacterium*, *Rhizobium, Meso rhizobium*, Biozote and Biofertilizer, respectively. These results are in confirmation with that of Karadavut and Ozdemir (2001) and Fatima et *al*. 2008 who reported that inoculation significantly increased grain yield (20% higher than control), total above ground dry matter and number of pods plant^{-1} in chickpea. The interaction of fertilizer application methods and inoculation also significantly affected the number of pods plant^{-1} and maximum number of pods plant^{-1} (33.22 and 41.94) were noted in fertilizer banding together with biofertilizer inoculums during both the year of investigation against the minimum of 26.30 and 32.25 pods plant^{-1} in fertilizer broadcast along with the check during 2004-05 and 2005-06, respectively. Average increase in number of pods plant^{-1} due to fertilizer banding x Biofertilizer (F_2 x I_5) was 30 % over control (F_1 x I_0) during 2005-06. Seed inoculation with fertilizer banding might have increased nitrogen supply to crop plants, which ultimately resulted in more number of pods plant^{-1}. These results are in line with those of Ali *et al.* (2003) who reported increased number of pods plant^{-1} by seed inoculation.

Table III ***Effect of fertilizer application methods and various inoculation strains on number of pods plant^{-1} in chickpea***

***Rhizobium* inoculation strains (I)**	**2004-05** Fertilizer application methods (FAM): **Fertilizer broadcast (F_1)**	**Band application (F_2)**	**Means**	**2005-06** Fertilizer application methods (FAM): **Fertilizer broadcast (F_1)**	**Band application (F_2)**	**Means**
Uninoculation (I_0)	26.30e	26.68e	26.49d	32.25f	33.58e	32.92f
Mezo Rhizobium (I_1)	26.38e	30.82b	28.59b	33.58e	35.68d	34.63d
Rhizobium (I_2)	26.78e	27.33de	27.05cd	35.56d	36.36cd	35.96c
Biozote (I_3)	27.72dc	28.70cd	28.21b	36.15d	37.15c	36.65b
Agro bacterium (I_4)	26.87e	28.66cd	27.76bc	33.45e	34.30e	33.87e
Biofertilizer (I_5)	30.24bc	33.22a	31.73a	38.06b	41.94a	39.99a
Means	27.38b	29.23a		34.84b	36.50a	

Year	**2004-05**	**2005-06**
$LSD_{0.05}$ (Fertilizer application method)	= 1.52	0.80
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 1.13	0.63
$LSD_{0.05}$ (FAM x inoculation strains)	= 1.59	0.89

Grains Pod^{-1}

Grains pod^{-1} was affected significantly by fertilizer application methods and inoculation strains (Table IV). The maximum number of grains pod^{-1} were recorded as 1.904 in band placement and 1.829 in fertilizer broadcast during the 2005-06 study season. On the average, fertilizer banding increased 3.55% number of grains pod^{-1}. Seed inoculation with Rhizobium strains also significantly affected the number of grains pod^{-1}. The maximum number of grains pod^{-1} (1.944 and 1.981) were recorded in inoculation with Biofertilizer closely followed by *Rhizobium* inoculum (1.938 and 1.944) during Rabi (winter) of 2004-05 and 2005-06, respectively. The minimum number of grains pod^{-1} was recorded in uninoculated treatment (1.713 and 1.700) during 2004-05 and 2005-06, respectively. Inoculation with *Meso rhizobium, Agro bacterium*, Biozote, *Rhizobium* and Biofertilizer increased the number of grains pod^{-1} by 6.62, 9.17, 11.37, 13.79 and 15.04 % over the Control, respectively.

The interaction between fertilizer application methods and inoculation were also significant and the maximum number of grains pod^{-1} (1.963) was recorded in band placement with *Rhizobium* inoculums, while the minimum number of grains pod^{-1} (1.713) was recorded in fertilizer broadcast together with the check during 2004-05. Similarly, the maximum number of grains pod^{-1} (1.988) was recorded in band placement with Biofertilizer and the minimum number of grains pod^{-1} (1.675) recorded in fertilizer broadcast cum uninoculated treatments during 2005-06. The treatment F_2 x I_5 yielded 18.68 % increase over control treatment (F_1 x I_0). Increase in the number of grains pod^{-1} was probably due to more availability of nutrients in fertilizer banding and more nodules N-uptake by plants due to inoculation. The promotive effects of seed inoculation on number of seeds pod^{-1} were also observed by Malhur *et al.* (2003), who reported an increase in the number of seeds pod^{-1} by seed inoculation. These results are also in agreement with that of Zai *et al*. (1999) who concluded that Rhizobium inoculation had a significant effects on growth, N contents and uptake in shoots, yield and yield attributes and grains protein content in chickpea.

Table IV ***Effect of fertilizer application methods and various inoculation strains on number of grains pod^{-1} in chickpea***

***Rhizobium* inoculation strains (I)**	**2004-05 Fertilizer application methods (FAM)**		**Means**	**2005-06 Fertilizer application methods (FAM)**		**Means**
	Fertilizer broadcast (F_1)	**Band application (F_2)**		**Fertilizer broadcast (F_1)**	**Band application (F_2)**	
Uninoculation (I_0)	1.71e	1.71e	1.71c	1.68g	1.73f	1.70e
Mezo Rhizobium (I_1)	1.79de	1.94ab	1.86b	1.68g	1.88d	1.78d
Rhizobium (I_2)	1.91abc	1.96a	1.94a	1.93bc	1.96ab	1.94b
Biozote (I_3)	1.86bcd	1.89abc	1.88b	1.90cd	1.95ab	1.93b
Agro bacterium (I_4)	1.84cd	1.86bcd	1.85b	1.83e	1.93bc	1.88c
Biofertilizer (I_5)	1.94ab	1.95a	1.94a	1.98a	1.99a	1.98a
Means	1.84^{NS}	1.89		1.83b	1.90a	

Year	**2004-05**	**2005-06**
$LSD_{0.05}$ (Fertilizer application method)	= NS	0.03
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 0.06	0.03
$LSD_{0.05}$ (FAM x inoculation strains)	= 0.08	0.05

100-Grain Weight

The data regarding hundred grain weight of chickpea as affected by fertilizer application methods and inoculation are presented in Table V. It is obvious that different treatments significantly affected the 100 grain weight. Maximum 100 grain weight of 28.03 and 29.19 g was recorded in fertilizer banding compared to 27.75 and 28.15 g in fertilizer broadcast during the period of investigation. Hundred grains weight was increased by 2.36 % with fertilizer band placement over fertilizer broadcast. This increase in 100 grains weight may be due to the more availability of nutrients like nitrogen and phosphorus in the rhizosphere and less losses as compared to the broadcast method. These findings are in agreement with those of Timmons *et al*. (1973) who reported less losses of fertilizer in band placement.

Inoculation also increased the 100 grain weight in chickpea. Maximum 100 grains weight of 28.84 and 29.87 g was recorded in Biofertilizer followed by Biozote treatment with 100 grains weight of 28.66 and 29.66 g whereas the minimum 100 grains weight of 26.31 and 27.66 g was recorded in the control treatment during 2004-05 and 2005-06, respectively. On average, 100 grains weight was increased 2.3, 4.6, 7.2, 8.0 and 8.8 % by inoculating seeds with *Agro bacterium, Meso rhizobium, Rhizobium,* Biozote, and Biofertilizer, respectively. The interaction between inoculation and fertilizer application methods showed significant differences and interaction of fertilizer banding with Biofertilizer gave 100 grains weight of 28.80 and 30.22 g closely followed by fertilizer banding plus Biozote as 28.80 and 30.03 g during both the 2004-05 and 2005-06 study period while minimum 100 grain weight of 26.23 and 27.63 g was recorded in check. On average, F_2 x I_5 treatment gave the maximum 100 seed weight of 29.60 g depicting 9.91% increase over control (F_1 x I_0). A maximum gain of 10.48 % in 100 seed weight was achieved with inoculation x fertilizer banding. Better growth and development of crop plants due to seed inoculation and fertilizer banding might have affected the nutrient supply to plant growth resulting in producing more assimilates which might have partitioned more efficiently from source to sink and ultimately gained more seed weight. Similar results were reported by Alam *et al*. (1999), El-Hadi and Sheikh (1999) Meena *et al*. (2001) and Kyei-Boahen *et al*. (2002) who stated that *Rhizobium* inoculation significantly increased 100 seed weight and yield. The results suggest that fertilizer banding and Biofertilizer inoculation contributed much to increase the efficiency of major nutrient and thus led to the higher grains weight and yield.

Table V ***Effect of fertilizer application methods and various inoculation strains on 100 grain weight (g) in chickpea***

Rhizobium inoculation strains (I)	2004-05 Fertilizer application methods (FAM): Fertilizer broadcast (F_1)	Band application (F_2)	Means	2005-06 Fertilizer application methods (FAM): Fertilizer broadcast (F_1)	Band application (F_2)	Means
Uninoculation (I_0)	26.23e	26.39e	26.31c	27.63i	27.69i	27.66f
Mezo Rhizobium (I_1)	27.59cd	28.79a	28.19b	28.00g	28.54f	28.27e
Rhizobium (I_2)	28.09bc	29.14a	28.61ab	29.02e	29.50c	29.26c
Biozote (I_3)	28.53ab	28.80a	28.66a	29.28d	30.03b	29.66b
Agro bacterium (I_4)	27.17d	26.28e	26.73c	27.82h	29.12e	28.47d
Biofertilizer (I_5)	28.91a	28.98a	28.84a	29.52c	30.22a	29.87a
Means	27.75b	28.03a		28.55b	29.19a	

Year	**2004-05**	**2005-06**
$LSD_{0.05}$ (Fertilizer application method)	= 0.20	0.15
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 0.44	0.072
$LSD_{0.05}$ (FAM x inoculation strains)	= 0.62	0.102

Grain Protein Content

Grain protein content is considered to be an important character in chickpea. Data regarding grain protein content are presented in Table VI. It is apparent that different treatments affected the grain protein content of chickpea significantly. Fertilizer banding increased the protein percentage from 21.05 and 21.39 % in broadcast to 21.72 and 22.24 % during 2004-05 and 2005-06, respectively. It is assumed that availability of more nitrogen and phosphorous in the root zone might have increased grain protein content. Seed inoculation significantly influenced the protein content of chickpea. It increased from 19.04 in uninoculated to 22.62 % in inoculated treatment during 2004-05. Similarly an increase in protein contents from 20.15 to 22.72 % was recorded with inoculation during 2005-06. As nitrogen is a constituent of protein, seed inoculation might have enhanced the nitrogen supply to seed which resulted in higher protein content. Our findings are supported by reports by Ibrahim and Mahmood (1989), who stated that seed inoculation with different strains of bacteria significantly improved pod yield, N content of the plant and average nitrogen recovery. Alam *et al.* (1999) also reported that inoculums significantly increased grains protein content. Kyei-Boahen *et al.* (2002) reported that grain yield and quality was improved up to 50 % in chickpea with inoculation.

Table VI ***Effect of fertilizer application methods and various inoculation strains on grain protein content (%) in chickpea .***

Rhizobium inoculation strains (I)	2004-05 Fertilizer application method (FAM): Fertilizer broadcast (F_1)	Band application (F_2)	Means	2005-06 Fertilizer application Methods (FAM): Fertilizer broadcast (F_1)	Band application (F_2)	Means
Uninoculation (I_0)	18.64g	19.45f	19.04d	19.81g	20.49f	20.15d
Mezo Rhizobium (I_1)	21.24e	22.36b	21.80b	21.54de	22.96ab	22.25b
Rhizobium (I_2)	21.59d	21.76cd	21.68b	22.14cd	22.54bc	22.34ab
Biozote (I_3)	21.20e	21.66d	21.43c	21.32e	20.66f	20.99c
Agro bacterium (I_4)	21.98c	23.26a	22.62a	21.90de	23.54a	22.72a
Biofertilizer (I_5)	21.64d	21.79cd	21.71b	21.66de	23.22a	22.44ab
Means	21.05b	21.72a		21.39b	22.24a	

	2004-05	**2005-06**
$LSD_{0.05}$ (Fertilizer application method)	= 0.25	1.04
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 0.16	0.45
$LSD_{0.05}$ (FAM x inoculation strains)	= 0.22	0.63

Grain Yield (kg ha^{-1})

It was observed that different treatments significantly affected the grain yield in chickpea (Table VII). Maximum grain yield of 3278.84 and 3771.92 kg ha^{-1} was recorded in fertilizer banding as compared to 2978.84 and 3560.84 kg ha^{-1} in fertilizer broadcast during 2004-05 and 2005-06, respectively. On average, fertilizer band placement gave 7.8 % edge in yield of chickpea over fertilizer broadcast. It might be due to the availability of plant nutrients in the vicinity of rhizosphere and less losses of nutrient due to fertilizer banding. These results are in conformity with those of Din *et al.* (1999) who recorded maximum yield in band placement. Seed inoculation also significantly affected the grain yield of chickpea, and on average, increased 10.85, 16.76, 18.18, 20.66 and 25.96 % over control with inoculating the seed with *Agro bacterium, Meso rhizobium, Rhizobium,* Biozote, and Biofertilizer, respectively. This increase in yield may be due to effective nodulation which in turn enhanced the utilization of atmospheric nitrogen and availability of phosphorus towards higher yield. Average chickpea yield from all inoculated plots was increased by 22 % as compared with the uninoculated plots during 2005-06. These findings are in line with those of Sharma *et al.* (2001) who reported 13 % increase in seed yield by inoculation. Our findings are ion conformity with that of Fatima et *al*. (2008), who reported that application of *Rhizobium* inoculum, generally increased growth, yield components and nitrogen fixation.

The interaction of band placement plus Biofertilizer gave the maximum average yield of 3841.09 kg ha^{-1}, a 36.83 % increase over check (broadcast plus uninoculation–2807.14 kg ha^{-1}). These results support the idea that inoculum could be more effective and beneficial in enhancing grain yield in combination with fertilizer and agrees with reports published by Meena *et al.* 2001, who concluded that inoculum singly or in combination with fertilizer increased grain yield in chickpea (Karadavut and Ozdemir, 2001). The greater yield values were observed during the 2005-2006 study period and this might be due to the fact that microbial activities were at minimum level as the field was reclaimed and brought under cultivation for the first time during Rabi (winter) of 2004-05. Consequently, plant growth was retarded during the first year and as the microbial activities were optimum during 2nd year of study on account of previous pulse crop which increased the nodules N-supply to crop plants and hence increased crop growth, number of pods plant^{-1}, grains pod^{-1} and grain yield. It may be concluded from the present study that the use of inoculums along with fertilizer band placement should be encouraged in order to get profitable yield of chickpea under prevailing agro-climatic conditions.

Table VII ***Effect of fertilizer application methods and various inoculation strains on grain yield (kg ha^{-1}) in chickpea***

***Rhizobium* Inoculation strains (I)**	**2004-05**			**2005-06**		
	Fertilizer application method (FAM)		**Means**	**Fertilizer application method (FAM)**		**Means**
	Fertilizer broadcast (F_1)	**Band application (F_2)**		**Fertilizer broadcast (F_1)**	**Band application (F_2)**	
Uninoculation (I_0)	2695.59g	2938.56e	2817.07e	2917.00i	3365..22h	3141.0f
Mezo Rhizobium (I_1)	3005.84de	3373.41b	3189.62c	3627.00f	3766.02d	3696.12d
Rhizobium (I_2)	3038.05d	3377.46b	3207.76c	3676.00e	3830.07c	3753.32c
Biozote (I_3)	3065.89d	3532.24a	3299.06b	3773.01d	3905.11b	3839.08b
Agro bacterium (I_4)	2825.30f	3057.77d	2941.53d	3464.70g	3707.34e	3585.31e
Biofertilizer (I_5)	3216.20c	3616.27a	3416.23a	3905.11b	4059.01a	3982.90a
Means	2974.48b	3315.95a		3560.33b	3771.92a	

	2004-05	**2005-06**
$LSD_{0.05}$ (Fertilizer application method)	= 34.62	29.87
$LSD_{0.05}$ (*Rhizobium* inoculation strains)	= 65.50	29.70
$LSD_{0.05}$ (FAM x inoculation strains)	= 92.64	42.00

CONCLUSION AND RECOMMENDATIONS

From the foregoing accounts, it may be concluded that the application of specific rhizobium inoculum (Biofertilizer) alongwith band placement of chemical fertilizer (NP) improved grain yield and quality of chickpea through a positive effect on agronomic parameters like nodules plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, 100 grains weight and grain protein contents. Therefore, use of rhizobium species (Biofertilizer) alongwith band placement of chemical fertilizer (NP) is recommended for better chickpea crop production. It could be recommended that grains quality being an important character can be improved by inoculation in chickpea.

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