

Soil Science

Effectiveness of micronutrient application and *Rhizobium* inoculation on growth and yield of Chickpea

Shrila Das*, Navneet Pareek, K.P. Raverkar, R. Chandra and Aditya Kaustav¹

Department of Soil Science, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

¹Division of Sample Survey, IASRI, Pusa Campus, New Delhi, India

*E.mail: shrila34040@gmail.com

Paper no: 66 Received: 17th January 2012 Received in revised form: 13th June 2012 Accepted: 19th September 2012

Abstract

The field investigation was carried out to improve the inoculated *Rhizobium* efficiency by applying different micronutrients on nodulation, growth and uptake of N & P and yield of chickpea during *Rabi* seasons of 2006-07 at the Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar. Fourteen treatments consisting combinations of micronutrients *viz.* Zinc, Boron and Molybdenum, with and without *Rhizobium* sp. inoculation, were laid out in randomized block design (RBD) in triplicates. *Rhizobium* sp. inoculation gave significant increases of 33.72, 26.11 and 4.56 per cent in nodule dry weight and 15.59, 14.25 and 1.90 per cent in plant dry weight at 45, 75 and 120 DAS, respectively. Among the two levels of ZnSO₄, 25 kg ZnSO₄/ha was found superior to 10 kg ZnSO₄/ha for different studied parameters. Application of 10 kg Borax/ha was found better than 5 kg Borax/ha for chickpea. Seed treatment of Mo with 0.5 kg Na₂MoO₄/ha was found sufficient to meet the crop need. *Rhizobium* inoculation in combination with different micronutrients recorded higher nodulation, plant dry weight, grain and straw yield and uptake of N and P than the treatments of only micronutrients or *Rhizobium* alone. The highest nodule dry weight of 235, 616 and 1476 mg/plant was recorded with treatment of 5 kg Borax/ha + *Rhizobium* at 45, 75 and 120 DAS, respectively. The treatment with 0.5 kg Na₂MoO₄/ha + *Rhizobium* gave the highest plant dry weight of 4.22, 9.12 and 11.35 g/plant at 45, 75 and 120 DAS, respectively. The highest grain yield of 2977 kg/ha and straw yield (7111 kg/ha) was recorded due to inoculation with *Rhizobium* + 10 kg Borax/ha and *Rhizobium* + 5 kg Borax/ha, respectively. Significant variations in total N and P uptake due to *Rhizobium* inoculation and application of micronutrients were also observed and it varied from 122.83 kg/ha and 10.67 kg/ha in uninoculated control to 203.90 kg/ha and 22.02 kg/ha in 5 kg Borax/ha + *Rhizobium*, respectively.

@2012 New Delhi Publishers. All rights reserved

Keywords: Boron, Chickpea, Grain, Molybdenum, Nodulation, *Rhizobium*, Straw, Yield, Zinc

Chickpea (*Cicer arietinum* L.), a grain legume, play essential role in ensuring nutritional security and environmental safety, as they have inbuilt mechanism to fix atmospheric di-nitrogen. Besides legume—*Rhizobium* symbiosis is an important facet of symbiotic nitrogen fixation which is exploited to benefit

agriculture and its sustainability (Brahmaprakash and Sahu, 2012). Therefore, its cultivation is gaining importance not only in India, but also all over the world because of its nutritional benefits. Chickpea grain provides about 18-22% protein, 4-10% fat and 52-70% carbohydrate (Ali and Kumar, 2003).

Rhizobium nodulating chickpea is highly specific in infectivity and do not show affinity with any member of known cross inoculation groups. Survey of root nodulation status in chickpea crop at 2482 farmer's field carried out under AICRP on pulses, indicated good nodulation only at 12.5 per cent locations, while 54.8 per cent and 31.4 per cent locations showed poor and moderate nodulation, respectively (Pareek and Chandra, 2003). Response to inoculation and efficiency of inoculation varies with the strains of *Rhizobium*, plant genotype and environmental conditions (Vincent, 1974). These situations suggest the need of inoculation for establishment of effective strains of *Rhizobium* in soil to cause adequate infection in chickpea for good nodulation, N₂-fixation and growth of the crop (Choudhary *et al.*, 2005).

Mineral nutrient deficiencies limit nitrogen fixation by the legume-*Rhizobium* symbiosis, resulting in low legume yields. Adequate supply of nutrients including micronutrients is also essential for proliferation and survival of *Rhizobium* in soil and establishment of effective associations. Several reports indicated the positive effects of micronutrient application on nodulation and nitrogen fixation (O'Hara *et al.*, 1988). Nutrient limitations to legume production result from deficiencies of not only major nutrients but also micronutrients such as molybdenum (Mo), zinc (Zn), and boron (B) (Bhuiyan *et al.*, 1999). However, the effect depends on the micronutrient status and effectiveness of *Rhizobium* strain in the soil. Zinc (Zn), Boron (B) and Molybdenum (Mo) are the most important micronutrients, as they perform several physiological functions in the plant to cause adequate infection for good nodulation, N₂-fixation and growth of the crop. Several study indicated that there is a necessity of application of micronutrients wherever deficient under intensive cultivation of legumes as it is directly involved in biological nitrogen fixation through nitrogenase enzyme activity (Gupta and Sahu, 2012). The present paper communicates the impact of different micronutrients application on efficacy of introduced chickpea rhizobia.

Materials and methods

A field experiment during *Rabi* seasons of 2006-07 was carried out to find out the effect of different micronutrients *viz.* Zinc, Boron and Molybdenum, with and without *Rhizobium* sp. inoculation on nodulation, growth, uptake of N & P and yield of chickpea at the Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar. The experimental field soil was sandy loam having pH 6.92, available N and P of 163.07 and 12.33 kg/ha and 0.84, 1.33 and 0.30 mg/kg available Zn, B and Mo, respectively.

Fourteen treatments consisting combinations of different

micronutrients (Zinc, Boron, Molybdenum), with and without *Rhizobium* sp. inoculation, were laid out in randomized block design (RBD) in triplicates to evaluate the response of chickpea to micronutrients and *Rhizobium* sp. (strain LN-7007) inoculation.

The sowing of seed was carried out using 100 kg/ha seed rate of chickpea (Cultivar 'Pant G-186') with net plot size 1.8 m × 3.0 m. Seeds were treated with the respective *Rhizobium* sp. (LN-7007) inoculants by following the standard procedure. Zinc sulphate and Borax, as a source of zinc (Zn) and boron (B), respectively were applied in the soil according to the selected concentration in their respective plots whereas, for molybdenum (Mo) application, first seed was treated with sodium molybdate salt and then inoculated with *Rhizobium* sp. using carboxyl methyl cellulose (CMC) as adhesive. The treated seeds were sown immediately in pre-opened furrows at 3-4 cm depth. A uniform basal dose of nitrogen (20 kg/ha) and phosphorus (40 kg P₂O₅/ha) were applied as per treatment through urea and SSP, respectively, in furrow prior to seed sowing. No potash was added. The recommended agronomic practices were followed throughout experiment for raising the crop.

For different observations samples of soil and plant were taken before and after sowing and after harvest of crop, like collection of soil samples (0-15 cm depth), processing of collected soil samples, analysis of processed samples for their physico-chemical properties, collection of plant samples at different intervals *viz.* 45, 70 and 120 DAS (for counting of nodules, nodule and plant dry weight). Grain and straw yield were also observed at maturity and both grain and straw samples of chickpea collected from each plot at harvest for yield and N, P uptake.

The obtained experimental data were statistically analyzed by applying analysis of variance (ANOVA) technique (Panse and Sukhatme, 1978). The differences among treatments were compared by applying 'F' test of significance at 5 per cent level of probability.

Results and discussion

Effect of Rhizobium inoculation

Inoculation of *Rhizobium* sp. improved the nodule number and their dry weights at different intervals (Figure 1). The numbers of nodules were higher by 14.28, 9.14 and 4.85 per cent and nodule dry weight by 33.72, 26.11 and 4.56 per cent due to inoculation with *Rhizobium* at 45, 75 and 120 DAS, respectively over the uninoculated control. *Rhizobium* sp. inoculation also gave numerical increases of 1.90 to 15.59 per cent in plant dry weight at different intervals (Fig. 1). The non-

Table 1: Effect of *Rhizobium* sp. inoculation and application of micronutrient(s) on total N and P uptake (kg/ha) and grain and straw yield (kg/ha) of Chickpea

Treatment	Total N uptake	Total P uptake	Grain Yield	Straw Yield
Uninoculated Control	122.83	10.67	2033	5497
<i>Rhizobium</i>	161.09	15.18	2606	5602
10 kg ZnSO ₄ /ha	149.26	14.47	2379	6064
25 kg ZnSO ₄ /ha	154.01	14.61	2444	6074
10 kg ZnSO ₄ /ha + <i>Rhizobium</i>	181.34	17.74	2555	6271
25 kg ZnSO ₄ /ha + <i>Rhizobium</i>	190.12	17.74	2583	6101
5 kg Borax/ha	140.87	14.21	2277	5666
10 kg Borax/ha	146.87	15.34	2453	5805
5 kg Borax/ha + <i>Rhizobium</i>	203.90	22.02	2826	7111
10 kg Borax/ha + <i>Rhizobium</i>	202.18	21.49	2977	6244
0.5 kg Na ₂ MoO ₄ /ha	140.04	13.39	2231	6348
1.0 kg Na ₂ MoO ₄ /ha	137.23	15.33	2299	5663
0.5 kg Na ₂ MoO ₄ /ha + <i>Rhizobium</i>	187.33	18.41	2647	6166
1.0 kg Na ₂ MoO ₄ /ha + <i>Rhizobium</i>	176.80	16.46	2609	5759
C.D. at 5 %	31.95	2.85	4.60	NS

significant response of *Rhizobium* sp. inoculation could be because of high population of native rhizobia nodulating chickpea in soil because of its cultivation from time immortal. The high population of native rhizobia probably did not allow the inoculated rhizobia to increase nodule number in soil. Similar findings were also obtained by Pareek and Chandra (2003), Choudhary *et al.*, (2005) and Gupta (2006). Inoculation of *Rhizobium* significantly increased 21.98, 1.87, 23.75 and 29.71 per cent grain yield, straw yield, total N and P uptake by over uninoculated control, respectively (Table 1). It could be attributed to competitiveness and effectiveness of introduced strain of rhizobia, which might have fixed higher amount of atmospheric nitrogen over the native rhizobia. Similar results were reported and supported by Khurana and Dudeja (1997) and Pareek and Chandra (2003), Gupta (1998) and Gupta (2006). Similar response of *Rhizobium* sp. inoculation in chickpea in terms of yield has also been reported by Bharti *et al.*, (2002).

Effect of Micronutrient application

Zinc application alone

Among the two different grades of Zn alone i.e. 10 and 25 kg ZnSO₄/ha applied, treatment 25 kg ZnSO₄/ha gave better results than the treatment 10 kg ZnSO₄/ha. Results were corroborated with the findings of Ahlawat *et al.*, (2007).

Application of zinc produced more nodule number of 5.21, 4.85 and 9.14 per cent with 10 kg ZnSO₄/ha and 2.43, 9.14, and 13.05 per cent with 25 kg ZnSO₄/ha over the uninoculated control at 45, 75 and 120 DAS, respectively (Figure 1). Results corroborated with Yadav and Shukla (1983) who reported that number of nodule in chickpea increased with increasing Zn

application up to 7.5 µg/g soils. Misra *et al.*, (2002) also observed an increase of 55 per cent in root nodulation with application of 20 mg Zn/kg soil. They further reported that application of Zn also favoured the nodule dry weight. Increase in nodule dry weight ranged from 2.75 to 16.87 per cent with 10 kg ZnSO₄/ha and 4.27 to 32.82 per cent 25 kg ZnSO₄/ha was observed over the uninoculated control at different intervals, respectively. Similar results were observed and explained by Ahlawat *et al.*, (2007), that the fertilization of Zn enhanced root growth, nodulation and nodule dry weight. Application of zinc at both levels increased plant dry weight by 23.38, 3.43 and 0.12 per cent with 10 kg ZnSO₄/ha and 27.82, 7.22 and 4.75 per cent with 25 kg ZnSO₄/ha application over the uninoculated control at 45, 75 and 120 DAS, respectively. Yadav and Shukla (1983) also found increased dry matter yield of chickpea with increasing Zn application up to 10 µg/g soil. Similarly, Choudhary *et al.*, (1990) reported that final plant height and dry matter yield were affected significantly following soaking seeds in ZnSO₄ solution resulted higher yield.

Both the level of applied zinc were at par for total N uptake and recorded increase of 17.70 per cent with 10 kg ZnSO₄/ha and 20.24 per cent with 25 kg ZnSO₄/ha application over uninoculated control (Table 1). Similarly, Both the levels of applied zinc were at par in respect of total P uptake, however, its application resulted in increase of 26.26 and 26.96 per cent with 10 and 25 kg ZnSO₄/ha over uninoculated control, respectively. This could be attributed to enhanced supply of Zn in soil leading to better plant growth, nodulation and N₂-fixation. Ahlawat *et al.*, (2007) reported significant response upto 25 kg ZnSO₄/ha application in improving yield of chickpea. However, 10 kg ZnSO₄/ha seems to be the optimum in most of the chickpea growing areas.

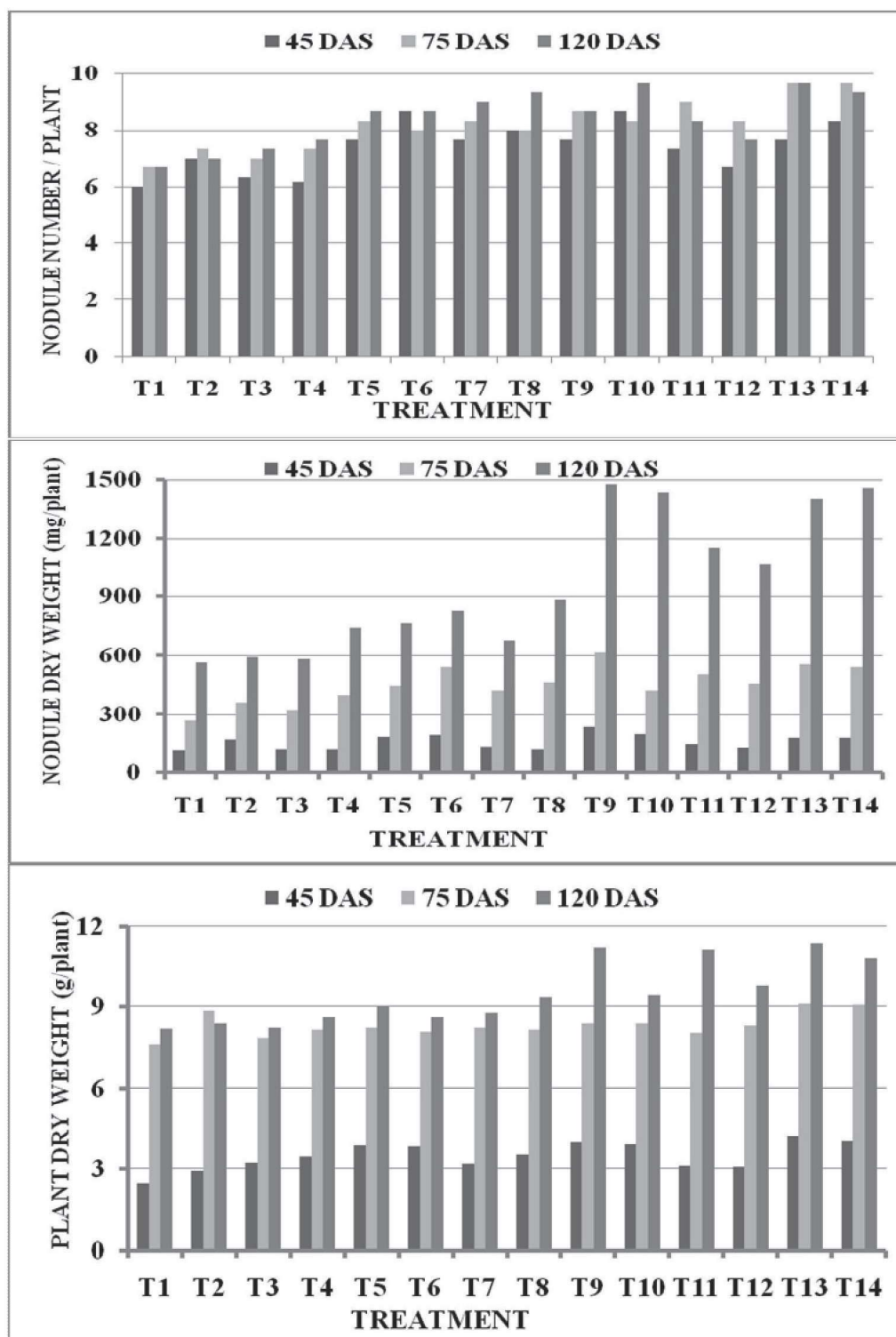


Fig 1: Effect of *Rhizobium* sp. inoculation and micronutrient(s) application on nodule number, nodule dry weight and plant dry weight at 45, 75 and 120 DAS.

Grain yields due to Zn application were numerically higher by 14.54 per cent with 10 kg ZnSO₄/ha and 16.81 per cent with 25 kg ZnSO₄/ha application over uninoculated control (Table 1). Similarly, straw yield due to both level of Zn application were numerically higher by 9.35 and 9.49 per cent with 10 and 25 kg ZnSO₄/ha application over the uninoculated control, respectively. Increase in grain and straw yield due to application of micronutrient could be due to adequate supply of these nutrients. Similar, results were also reported by Sawires (2001) and Choudhary *et al.*, (1990).

Combined application of Zinc with Rhizobium sp

Combined application of *Rhizobium* sp. with Zn found better than *Rhizobium* and Zn alone.

Inoculation of *Rhizobium* in combination with 10 and 25 kg ZnSO₄/ha gave 8.61 to 19.16 per cent and 8.37 to 19.16 per cent more nodule number and 8.15 to 22.71 per cent and 12.88 to 33.33 per cent more nodule dry weight over the *Rhizobium* alone at different intervals, respectively (Fig. 1). The results are in agreement with Singh *et al.*, (2004) who reported increased nodule number and nodule dry weight due to inoculation with *Rhizobium* + Zn, because of their positive effect on nodulation and N₂-fixation. Combined inoculation of *Rhizobium* with 10 and 25 kg ZnSO₄/ha gave 6.88 to 24.16 per cent and 2.67 to 23.17 per more plant dry weight over the *Rhizobium* alone at different intervals, respectively. These results are in the close proximity with the data reported by Singh *et al.*, (2004) who also observed that inoculation of *Rhizobium* and application of micronutrients gave more plant dry weight than *Rhizobium* alone.

Similarly, Micronutrient zinc application at the rate of 10 and 25 kg ZnSO₄/ha in combination with *Rhizobium* resulted in 11.16 and 15.26 per cent more total N uptake and similar of 14.43 per cent more total P uptake over the *Rhizobium* alone, respectively (Table 1). In a pot culture experiment Singh *et al.*, (1984) found that application of *Rhizobium* and Zn in chickpea increased N content and uptake.

Grain yield due to *Rhizobium* inoculation with both levels of Zn application were numerically higher by 20.43 per cent with 10 kg ZnSO₄/ha and 21.29 per cent with 25 kg ZnSO₄/ha over *Rhizobium* alone (Table 1). Similarly, straw yield due to Zn application was also found numerically higher with 10 and 25 kg ZnSO₄/ha over *Rhizobium* alone. Singh *et al.*, (2004) further reported that the content and uptake of Zn, N and P, irrespective of *Rhizobium*, increased significantly with the addition of micronutrients individually or in combination over control.

Boron application alone

In legume B also apparently plays a structural role by maintaining the integrity of cell wall and membranes (Bolanos *et al.*, 1996). Soil application of B at the rate of 5 and 10 kg Borax/ha produced 20.00 to 26.00 and 16.75 to 28.61 per cent more nodule number than the uninoculated control at different intervals (Fig. 1). Bolanos *et al.*, (1996) suggested that B plays an important role in mediating cell-surface interactions that lead to endocytosis of rhizobia by host cells and hence to the correct establishment of the symbiosis between legume and *Rhizobium*. Similarly, B alone applied at the rate of 5 and 10 kg Borax/ha produced 16.91 to 36.05 and 4.27 to 42.54 per cent more nodule dry weight than the uninoculated control at different intervals, respectively. These results corroborates with the findings of Bolanos *et al.*, (1996); Rahman *et al.*, (1999); O'Hara (2001). Similarly, B alone applied at 5 and 10 kg Borax/ha produced 6.48 to 22.00 and 7.22 to 30.05 per cent more plant dry weight than the uninoculated control at different intervals. These results are in agreement with Bolanos *et al.*, (1996); Rahman *et al.*, (1999); O'Hara, (2001).

Non-significant improvements were observed when B alone applied at the rate of 5 and 10 kg Borax/ha resulting in 12.80 and 16.36 per cent more N uptake over the uninoculated control, respectively. Similar result was also found in case of total P uptake registering nonsignificant increase of 24.91 and 30.44 per cent in total P uptake over the uninoculated control, with the treatments of 5 and 10 kg Borax/ha respectively (Table 1). Similar response has been reported by Bolanos *et al.*, (1996).

Grain yield due to both level of B application were numerically higher by 10.71 per cent with 5 kg Borax/ha and 2.98 per cent with 10 kg Borax/ha application over uninoculated control (Table 1). Straw yield due to B application was numerically higher by 17.12 per cent with 5 kg Borax/ha and 5.30 per cent with 10 kg Borax/ha application over uninoculated control. Similarly, Sakal *et al.*, (1998) reported increased grain yield up to 750 kg/ha due to 2 kg B/ha fertilization with an agronomic efficiency of 375 kg grain/kg B. Numerical increase in the yield may be attributed due to proper nutrition of Boron, which play an important role in hormone synthesis and translocation, carbohydrate metabolism and DNA synthesis and probably contributes to additional growth and yield (Kalyani *et al.*, 1993).

Combined application of Boron with Rhizobium

Rhizobium inoculation along with different B levels also favoured the nodule number registering 8.61 to 19.16 and 20.04 to 31.05 per cent more nodule number and 28.08 to 59.72 and 14.28 to 58.60 per cent more nodule dry weight with 5 kg and 10 kg Borax/ha over the *Rhizobium* alone at different intervals, respectively (Fig 1). Application of *Rhizobium* in combination

with micronutrients probably facilitated more root infection and increased the metabolic activity of chickpea. These results are in agreement with Solaiman (1999). Bharti *et al.*, (2002) also reported that the number and dry weight of nodules increased with *Rhizobium* inoculation along with B application in chickpea. *Rhizobium* inoculation with different B levels also favoured the plant dry weight and application of 5 kg and 10 kg Borax/ha registered 25.04 to 26.61 and 11.0 to 25.12 per cent more plant dry weight over the *Rhizobium* alone at different intervals. Solaiman (1999) also reported increased plant dry weight with *Rhizobium* inoculation along with B application.

Rhizobium inoculation in combination with both the B levels of 5 and 10 kg Borax/ha favoured the total N uptake (kg/ha) by 20.99 and 20.32 per cent and total P uptake registering 31.06 and 29.36 per cent over the *Rhizobium* inoculation alone, respectively (Table 1). Similar response has also been reported by Bharti *et al.*, (2002) who reported that inoculation of *Rhizobium* with different levels of B had positive effect on the yield, dry weight and nutrients uptake of chickpea. Results were also in agreement with Singh *et al.*, (2004). Singh and coworkers suggested that combined application of *Rhizobium* and different levels of B improved the dry matter production and yield of chickpea. This may be due to the presence of B could change the affinity with which the bacterial cell surface interacts with peribacteroid membrane glycocalyx relative to its interaction with intercellular plant matrix glycoprotein. Thus B plays an important role in establishment of the symbiosis between host and *Rhizobium*.

Grain yield due to *Rhizobium* inoculation with B application was numerically higher by 7.78 per cent with 5 kg Borax/ha and 12.46 per cent with 10 kg Borax/ha over *Rhizobium* alone (Table 1). Similarly straw yield due to both the levels of B application were numerically higher by 21.22 per cent with 5 kg Borax/ha and 10.28 per cent with 10 kg Borax/ha over *Rhizobium* alone. Similar results were also reported by Bharti *et al.*, (2002).

Molybdenum application alone

Mo application at both the levels (i.e. 0.5 kg Na₂MoO₄/ha and 1.0 kg Na₂MoO₄/ha) increased nodule number ranging from 18.14 to 26.00 per cent with 0.5 kg Na₂MoO₄/ha and 9.90 to 20.00 per cent with 1.0 kg Na₂MoO₄/ha over the uninoculated control at different intervals (Figure 1). Such increase in nodule number due to Mo application was also reported by Islam *et al.*, (1995); could be associated with an increase in nitrate reductase and nitrogenase activities, as Mo is an essential part of these enzymes. Chandra and Kothari (2002) revealed that the application of Mo with increasing doses significantly increased number of nodules/plant. Application of 0.5 kg Na₂MoO₄/ha and 1.0 kg Na₂MoO₄/ha also registered 25.33,

47.11 and 50.99 per cent and 11.11, 41.28 and 46.69 per cent more nodule dry weight over uninoculated control at 45, 75 and 120 DAS, respectively. It suggests that application of Mo allowed synthesis of more nodule tissue due to better supply of Mo from soil to plants and also by maintaining supply of essential metabolites to the nodules (Jongruaysup *et al.*, 1993). Similar results were re-reported by Chahal *et al.*, (1976), who treated pea with molybdenum (30µg/L) and achieved significantly higher nodule number as well as nodule leghaemoglobin content compared to untreated treatments. Application of Mo at both levels increased plant dry weight ranging from 5.95 to 25.94 per cent with 0.5 kg Na₂MoO₄/ha and 8.89 to 15.95 per cent with 1.0 kg Na₂MoO₄/ha application over the uninoculated control at different intervals. Such increase in plant dry weight due to Mo application was also reported by Chandra and Kothari (2002). They revealed that the application of Mo with increasing doses significantly increased plant dry weight.

Applications of 0.5 kg Na₂MoO₄/ha was found slightly better than 1.0 kg Na₂MoO₄/ha registering 12.28 per cent more N uptake than the uninoculated control. However, application of 1.0 kg Na₂MoO₄/ha was found better than 0.5 kg Na₂MoO₄/ha registering 30.39 per cent more P uptake than the uninoculated control. Kumar *et al.*, (2005) also reported that application of 1.0 kg Mo/ha, which was at par with 1.5 kg Mo/ha, significantly enhanced all the yield attributing characters, yield and quality characters of chickpea over the control and 0.5 kg Mo/ha. The N and P content in seeds, haulms and their total uptake and protein content in seeds increased significantly upto application of 1.0 kg Mo/ha over 0.5 kg Mo/ha. Similar response has also been reported by Brodrick *et al.*, (1992). This could be due to better availability of N due to increased N₂-fixation or better root development that might have allowed more absorption of plant nutrients from soil.

Grain yield due to both level of Mo application were numerically higher by 8.87 per cent with 0.5 kg Na₂MoO₄/ha and 11.57 per cent with 1.0 kg Na₂MoO₄/ha over uninoculated control (Table 1). Similarly, straw yield was numerically higher by 13.40 per cent with 0.5 kg Na₂MoO₄/ha and 2.93 per cent with 1.0 kg Na₂MoO₄/ha over uninoculated control. Chandra and Kothari (2002) also reported beneficial effect of the application of Mo with increasing doses on the grain yield, protein content of grain and available N content. Similar results were also re-reported by Chahal *et al.*, (1976), who observed significantly higher yields of green mass and dry matter, compared to untreated treatments by treating pea with molybdenum (30µg/L).

Combined application of Molybdenum with Rhizobium

Seed inoculation with *Rhizobium* and application of 0.5 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ was found better by producing 8.61, 31.05 and 31.05 per cent more nodule number and 5.58, 35.25 and 57.71 per cent more nodule dry weight over the *Rhizobium* alone at 45, 75 and 120 DAS, respectively (Fig. 1). However, Mudholkar and Ahlawat (1979) reported that response of Mo was greater when applied along with P and *Rhizobium* inoculation. *Rhizobium* inoculation with different Mo levels favoured the plant dry weight. Application of Mo 0.5 kg and 1.0 kg per ha registered increase of 3.07 to 30.09 and 2.75 to 38.2 per cent over the *Rhizobium* alone at different intervals. These results are in agreement with Brkić *et al.*, (2004), reported more plant dry weight with *Rhizobium* inoculation + Mo application than *Rhizobium* alone.

Seed inoculation of *Rhizobium* showed better impact with 0.5 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ by producing 14.00 per cent more N uptake by grain and straw over the *Rhizobium* alone and 5.62 per cent more over 1.0 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ + *Rhizobium* application and accumulating 17.54 per cent more total P uptake over the *Rhizobium* alone and 10.60 per cent more over 1.0 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ + *Rhizobium* application (Table 1). Similar response has also been reported by Brkić *et al.*, (2004).

Grain yield due to *Rhizobium* inoculation with Mo application was numerically higher by 1.54 per cent with 0.5 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ and 0.11 per cent with 1.0 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ over *Rhizobium* alone (Table 1). Similarly, straw yield was numerically higher by 9.14 per cent with 0.5 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ and 2.72 per cent with 1.0 kg $\text{Na}_2\text{MoO}_4/\text{ha}$ over *Rhizobium* alone. Pal (1986) has also reported that Seed inoculation in combination with 60 kg P_2O_5 + 1.5 kg Sodium Molybdate/ha gave the highest yields of chickpea. These improvements in plant dry weight, grain and straw yield, N, P and micronutrients content and uptake in chickpea may be because of their synergistic interaction with inoculated and native rhizobia nodulating chickpea by helping in survival in rhizosphere, root colonization, root hair infection and efficiency of N_2 -fixation.

It could be concluded from the study that mineral nutrient deficiencies are limiting legume nitrogen fixation and yield. Besides nutrients application of micronutrients at optimum doses is necessary to harness the maximum benefits of N_2 -fixation for achieving maximum productivity of pulse crops. A dose of 25 kg ZnSO_4/ha and 5 kg Borax/ha and seed treatment with 0.5 kg NaMoO_4/ha and *Rhizobium* was found optimum for chickpea growth, yield, residual soil fertility under Tarai conditions. To ensure full benefit from N_2 -fixation by legume symbioses, successful management strategies should consider the whole legume-*Rhizobium* system and selection for improved inoculants and not only the host plant.

References

- Ahlawat, IPS., Gangaiah, B., and Singh, O. 2007. Effect of fertilizer and stover management on productivity and soil fertility in chickpea (*Cicer arietinum*) – maize (*Zea mays*) cropping system. *Indian Journal of Agricultural Sciences* **75**:400–403.
- Ali., and Kumar. 2003. Chickpea research in India: An overview. In: *Chickpea Research in India* (Ali, M.; Shiv Kumar and Singh, N.B. Eds.). IIPR, pp. 13.
- Bharti, N., Murtuza, M., and Singh, A.P. 2002. Effect of boron-*Rhizobium* relationship on yield, nitrogen and boron nutrition of chickpea. *Journal of Research of Birsa Agricultural University*. **14**:175-179.
- Bhuiyan, M., Khanam, D., and Ali, M. 1999. Chickpea root nodulation and yield as affected by micronutrient application and *Rhizobium* inoculation. *International Chickpea Pegionpea Newsletter*. **6** : 28-29.
- Bolanos, L., Brewin, N.J., and Bonilla, I. 1996. Effect of boron on *Rhizobium*-legume cell-surface interaction and nodule development. *Plant physiology*. **110** :1249-1258.
- Brahmaprakash, G. P., and Sahu, P. K. 2012. Biofertilizers for sustainability. *Journal of the Indian Institute of Science*. **92** (1):37-62
- Brkić, S., Milaković, Z., Kristek, A., and Antunović, M. 2004. Pea yield and its quality depending on inoculation, nitrogen and molybdenum fertilization. *Plant Soil and Environment*. **50**: 39–45.
- Brodrick, S. J. and Giller, K.E. 1992. Root nodules efficient scavengers of molybdenum for N_2 fixation. *Journal of Experimental Botany*. **42** : 679-686.
- Chahal, V.P.S., Gupta, R.P., and Pandher, M.S. 1976. Inoculate and get more yields of gram. *Programming Farming*. **13**: 24-25.
- Chandra, D., and Kothari, M. L. 2002. Effect of modes and nodulation of chickpea grown on loamy sand soil. *Communication of Soil Science and Plant Analysis*. **33**:2905-2915.
- Choudhary, A.U., and Muhammad, N. 1990. Effect of micronutrients on nodulation and seed yield of chickpea (*Cicer arietinum* L.). *Journal of Agricultural Research*. **28**:139-147.
- Choudhary, L. M., Kulthe, K. S., Baig, M. F. N., Dhuppe, M. V., and More, S. S. 2005. Effect of *Rhizobium* isolates on nodulation and growth of chickpea. *Journal of Soils and Crops*. **15**: 323-327.
- Gupta, S.C. 2006. Effect of combined inoculation on nodulation nutrient uptake and yield of chickpea in Vertisol. *Journal of Indian Society of Soil Science*. **54** : 251-254.
- Gupta, S. C., and Sahu, S. 2012. Response of chickpea to micronutrients and biofertilizers in vertisol. *Legume Research - An International Journal*. **35** (3):248-251.
- Islam, M.A., Saha, U.K., Islam, M.S., and Saha, R.R. 1995. Fertilizing chickpea for yield with special emphasis on boron and molybdenum in grey flood plain soils of Bangladesh. *Annals of Bangladesh Agriculture*. **5**:43–49.
- Jongruaysup, S., O'Hara, G. W., Bell, R. W., and Dell, B. 1993. Effect of molybdenum deficiency on nodule initiation, development and N_2 -fixation in black gram (*Vigna mungo* L. Hepper). *Plant and Soil*. **156** : 345-348.
- Khurana, A. L., and Dudeja S. S. 1997. Biological nitrogen fixation

- technology for pulse production in India. Indian Institute of Pulse Research, Kanpur, India. pp18.
- Kumar, B.S.D., Berggren, I., and Martensson, A.M. 2005. Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. *Plant and Soil*. **229** : 25-34.
- Misra, S.K., Upadhyay, R.M., and Tiwari, V.N. 2002. Effect of salt and zinc on nodulation leghaemoglobin and nitrogen content of Rabi legumes. *Indian Journal of Pulses Research*. **15** : 145-148.
- Mudholkar, N. J., and Ahlawat, I.P.S. 1979. Response of Bengal gram to nitrogen, phosphorus and molybdenum. *Indian Journal of Agronomy*. **24** : 61-65.
- O'Hara, G. W. 2001. Nutritional constraints on root nodule bacteria affecting symbiotic N_2 -fixation: a review. *Australian Journal of Experimental Agriculture*. **41**:417-433.
- O'Hara, G. W., Boonkerd, N., and Dilworth, J. 1988. Mineral constraints to nitrogen fixation. *Plant and Soil*. **5** : 93-110.
- Pal, A. K. 1986. Interaction of *Rhizobium* inoculation with phosphate and molybdenum application on chickpea (*Cicer arietinum* L.) at rainfed condition. *Environment and Ecology*. **4**:642-647.
- Panse, V.G., and Sukhatme, P.V. 1978. Statistical methods for Agricultural workers. I.C.A.R., New Delhi.
- Pareek, R.P., and Chandra, R. 2003. Chickpea: Microbiology and nitrogen fixation. In: Chickpea Research in India (Eds. Ali, M.; Shiv Kumar and Singh, N.B.). IIPR, pp. 167-193.
- Rahman, M.H.H., Arima, Y., and Watanabe, K.H. 1999. Adequate range of boron nutrition is more restricted for root nodule development that for plant growth on young soybean plant. *Soil Science and Plant Nutrition*. **45** : 287-296.
- Ratna Kalyani, R., Sree Devi, V., Satyanarayana, N.V., and Madhava Rao, K.V. 1993. Effect of foliar application of boron on crop growth and yield of pigeonpea (*Cajanus cajan* L. Millsp.). *Indian Journal of Plant Physiology*. **36**(4):223-226.
- Sakal, R., Sinha, R.B., Singh, A.P., and Bhogal, N.S. 1998. Responses of some Rabi pulses to B, Zn and S application in farmers fields. *Fertilizer News*. **43** : 39-43.
- Sawires, E. S. 2001. Effect of phosphorus fertilization and micronutrients on yield and yield components of chickpea (*Cicer arietinum* L.). *Annals of Agricultural Science*. **46**(1) : 155-164.
- Singh, B., Laura, R. D., and Gupta, V. K. 1984. Influence of molybdenum, zinc and *Rhizobium* inoculation on dry matter yield and nitrogen content in chickpea. *International Journal of Tropical Agriculture*. **2** : 159-165.
- Singh, M., Chaudhary, S. R., Sharma, S. R., and Rathore, M. S. 2004. Effect of some micronutrients on content and uptake by chickpea. *Agricultural Science Digest*. **24** : 268-270.
- Solaiman, A. R. M. 1999. Influence of *Rhizobium* inoculant, nitrogen and boron on nodulation, dry weight and grain yield of chickpea. *Annals of Bangladesh Agriculture*. **9** : 75-84.
- Vincent, J. M. 1974. Root nodule symbiosis with *Rhizobium*. In: "The biology of Nitrogen Fixation" (Eds. Quispal, A.). North Holland Publishing Company, Amsterdam. pp. 265-341.
- Yadav, O. P. and Shukla, U. C. 1983. Effect of zinc on nodulation and nitrogen fixation in chickpea. *Journal of Agricultural Science*. **101**:559-563.