EFFECTS OF *BRADYRHIZOBIUM*, NITROGEN AND MICRONUTRIENTS (MOLYBDENUM AND BORON) ON BLACKGRAM (*Vigna mungo* L.)

A THESIS

BY

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DEPARTMENT OF SOIL SCIENCE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

SHER-E-BANGLA NAGAR, DHAKA-1207

JUNE 2009

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This is to certify that thesis entitled "EFFECTS OF *BRADYRHIZOBIUM*, NITROGEN AND MICRONUTRIENTS (MOLYBDENUM AND BORON) ON BLACKGRAM (*Vigna mungo* L.)" submitted to the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka-1207 in partial fulfillment of the requirements for the degree of Master of Science (M.S) in Soil Science embodies the result of a piece of *bonafide* research work carried out by **Poritosh Chandra Paul**, Registration No. 01032 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged by him.

Dated: 01 June 2009
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Dedicated to

My

Beloved Parents

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June 2009

The Author

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Abstract

A pot experiment was conducted at the Net House and Laboratory of Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during September to December 2007 to study the effect of nitrogen, molybdenum, boron and *Bradyrhizobium* inoculant on growth, nodulation, yield, yield contributing characters, nitrogen uptake of blackgram. The variety, BARI Mash-3 and *Bradyrhizobium* inoculant (BARI RVm-301) were used in the present experiment. There were twelve treatment combinations viz. T_1: Control (non-inoculated and non-fertilized control), T_2: PKMo, T_3: PKB, T_4: *Bradyrhizobium* inoculant, T_5: N, T_6: PKMo + *Bradyrhizobium* Inoculant, T_7: PKB + *Bradyrhizobium* Inoculant, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + *Bradyrhizobium* Inoculant and T_{12}: NPKMoB laid out in a Complete Randomized Block Design with 3 (three) replication. Nitrogen @ 50 kg N ha^{-1}, triple super phosphate @ 22 kg P ha^{-1}, muriate of potash @ 42 kg K ha^{-1}, boron @ 1 kg B ha^{-1} and molybdenum @ 1 kg Mo ha^{-1} were applied as per treatments of the experiment as recommended levels. Gypsum @ 20 kg S ha^{-1} and zinc sulphate @ 5 kg ha^{-1} were applied as basal in all pots except in control pot. After 30, 45 and 60 days of sowing, 2 plants were uprooted from each pot to study nodulation, dry matter production and plant growth. At maturity, yield and yield contributing characters were recorded. *Bradyrhizobium* inoculation increased significantly the number of nodules, nodule weight, root and shoot length, seed and stover yield, yield attributes, nitrogen and protein content of blackgram compared to non-inoculated control. *Bradyrhizobium* inoculation in presence of Mo and B recorded the highest nodule number and nodule weight, and also seed and stover yield. *Bradyrhizobium* inoculation alone or in presence of Mo or boron also recorded higher nodulation over other treatment combinations. *Bradyrhizobium* inoculation was better than nitrogen in almost all the parameters studied. Molybdenum and boron performed better results. This result indicated that the use of *Bradyrhizobium* inoculants with molybdenum and boron appeared to be an effective method for successful blackgram production.

CHAPTER I

INTRODUCTION

Pulse, a protein rich agricultural crop, plays an important role in human nutrition. It is the cheapest source of protein for the poor and called the poor men's meat. Pulses, being leguminous crops, are capable of fixing atmospheric nitrogen in the soil and enrich soil fertility. Thus, they are considered as soil building crops (Kumar *et al.*, 1963). Many of the pulse seeds are consumed when they are at green stage. It is taken mostly in the form of soup and "dal". Sometimes, it is grown as green manuring crops and cover crops (Shaikh, 1977). The green plants, the dried stems and leaves after separation of grain and the husks of seeds are the valuable food to the livestock. It is an excellent source of easily digestible protein.

At present, pulses are beyond the reach of the poor peoples because of its high price. In Bangladesh per capita daily consumption of pulses is only 12 g while the World Health Organization (WHO) suggests 45 day^{-1} $capita^{-1}$ (BARI, 1998) for a balanced diet. So, the people of Bangladesh take pulses in their daily diet far below than the recommendation. To maintain the supply of this level, the Government of Bangladesh has to expend a huge amount of foreign currency every year. The pulses crop is not only food crop but also a soil building one as it belongs to the Leguminosae family sub-family Papilionaceae. The stem and leaves of the pulses are used in preparing a concentrate feed called *Bhushi* which is rich in protein. The husks of the seeds are also used as feed for milch cows. Some pulses such as cowpea, mungbean, blackgram are used as green manuring crops. As a whole, pulse crop could be considered as an inevitable component of sustainable agriculture.

Blackgram (*Vigna mungo* L.) is one of the important pulse crop grown in Bangladesh. It is one of the pulse crops in Bangladesh for human consumption, animal fodder as well as soil building purpose. It is widely grown in the Indian subcontinent as a source of protein.

This legume can obtain nitrogen (N) from the atmosphere by fixation in their root nodules in symbiosis with soil rhizobia, and thus have the potential to yield well in N-deficient soils (Hafeez *et al*., 1988). This characteristic is particularly important in developing countries due to the relatively high cost and/or restricted availability of chemical N fertilizers. Furthermore, incorporation of residues into the soil from a N-fixing legume crop may provide organic N for the subsequent benefit of a cereal crop (Roasales *et al*., 1998). High cost and environmentally risky chemical fertilizers cause serious and continuous problem for increasing blackgram production in developing countries including Bangladesh. These problems are likely to become serious in future. Biological nitrogen fixation (BNF) resulting from symbiosis between legume crops and root nodule bacterium *Rhizobium/Bradyrhizobium* can ameliorate the situation by reducing the N-fertilizer inputs required to ensure productivity.

Blackgram is one of the most important pulse crops although not in terms of area (23,482 ha) and production (17, 000 t) but its consumption is quite high as a common pulse in Bangladesh (BBS, 2006). It is an important source of protein and several essential micronutrients. It is used as 'dhal' and as ingredient in preparation of many foods in terms in Bangladesh.

Now-a-days a number of organisms like *Rhizobium* have been identified to use as biological agent for fixing atmospheric nitrogen by process with legume crops and make available to the plants. Bangladesh Agricultural Research Institute (BARI) has isolated some *Rhizobium* strains for some pulse crops. It has already selected some *Bradyrhizobium* strains especially for blackgram. To reduce the production cost and to fulfill the demand, more pulse production could be achieved through seed inoculation with *Bradyrhizobium* strains which is known to influence biological fixation, growth and yield of pulses. In Bangladesh, inoculation with *Bradyrhizobium* increased 28-58% higher nodulation, 9-17% higher dry matter production, 20-32% higher grain yield and 9-14% higher stover yield over non-inoculated control (Bhuiyan *et al.*, 2009). Maximum yields were obtained when fertilizers are applied along with *Rhizobium* inoculation (Bali *et al.*, 1991). By growing blackgram in Bangladesh, there is a large scope of utilizing the biological nitrogen fixing technology for obtaining protein rich food legume and also to improve nitrogen fertility of the soil of this country.

In Bangladesh, most of the pulses are grown in *rabi* season. But in fact, due to cultivation of wheat and other high yielding rice crops, farmers have lost their interest to produce pulses because of low output per unit of resource invested. Increasing irrigation facilities for boro rice cultivation further reduced the area under pulses. Therefore, attention should be given to increase per unit yield through adoption of improved cultural technologies including application of biofertilizer in blackgram, sowing time, land preparation, sowing method, water management and improved cultivar etc. Blackgram can be easily grown in Kharif-II and Kharif-I season which can solve partial protein requirement.

Micronutrients play an important role in increasing yield of pulses and oilseed legume through their effects on the plant itself and on the nitrogen fixing symbiotic process (Bhuiyan *et al.*, 1996a; 1996b). But deficiencies of these nutrients have been very much pronounced under multiple cropping systems due to excess removal thereby necessity their exogenous supply.

Molybdenum is essential for N_2-fixation and the amounts required are so small that the seed of grain legumes can contain sufficient molybdenum for the growth of one generation of plant (Harris *et al.*, 1965). The nitrogen fixing enzyme nitrogenase is composed of molybdenum and iron. Without adequate quantities of these elements, nitrogen fixation cannot occur. Molybdenum is a constituent of one of the two proteins, which together form Mo-nitrogenase, and therefore a sufficient supply of Mo is essential for nitrogen fixation in legumes. Effects of molybdenum application on different grain legumes nodulation have been documented (Bhuiyan *et al.*, 1996a, 1996b, 1997a, 1997b, 1998a, 1998b, 1999a, 1999b, 2005a).

Boron is essential micronutrient for cell division in the process of nodule formation (Mulder, 1948). Boron is essential for plants, has been considered a nonessential nutrient for rhizobia. The deficiency of B affects some grain legumes (Rerkasem *et al.*, 1987). The deficiency of boron and response of different grain legumes have been reported by many researchers (Rerkasem *et al.*, 1987; Bhuiyan *et al.*, 1999a, 1999b, 2005a etc.).

The soils of different parts of Bangladesh are more or less deficient in boron and molybdenum as well as nitrogen fixing bacteria (*Rhizobium* spp.), which causes poor yield of pulses (Bhuiyan *et al.*, 1997a, 1999a; Khanam *et al.*, 1994). Research results on pulses are available in relation to single application of molybdenum, boron or rhizobial inoculum and their interactions (Bhuiyan *et al.*, 1999a, 1999b, 2005). Informations are not available regarding the effect of inoculation with different micronutrients (Mo and B) and their interactions on the nodulation and yield of blackgram. The present investigation was, therefore, undertaken to evaluate the response of blackgram to *Bradyrhizobium* inoculant, nitrogen, molybdenum and boron with the following objectives:

i) To investigate the effect of *Bradyrhizobium* inoculation, nitrogen, molybdenum and boron on the growth, nodulation, yield and nitrogen uptake, and other yield contributing characters of blackgram.

ii) To find out the suitable combination of *Bradyrhizobium*, nitrogen, molybdenum and boron for blackgram production.

CHAPTER II

REVIEW OF LITERATURE

Biofertilizers are microorganisms which benefit the plants by providing nitrogen or phosphorus or rapid mineralization of organic materials. Of the biofertilizers, the use of *Bradyrhizobium* was studied in Bangladesh at large extent. Work on other biofertilizers is very little. Only limited number of research works has so far been carried out on the combined use of *Bradyrhizobium* inoculation on blackgram (*Vigna mungo* L.) and other pulse crops. However, available information on the contribution of *Bradyrhizobium* inoculation, nitrogen, molybdenum and boron on blackgram has been reviewed in this chapter.

2.1 Effect of nitrogen

Effect of nitrogen on blackgram and other legumes have been presented below:

Sharma *et al.* (1995) carried out a field trial in the monsoon season of 1991 at Gwalior, Madhya Pradesh with blackgram (*Vigna mungo*) cv. JU-2 treated with 0, 15 or 30 kg N, 0, 30 or 60 kg P_2O_5 and 0 or 60 kg S ha^{-1}. Application of N and P, either alone or with S, increased Mn, Zn, Cu and Fe contents in seeds and straw and the available Mn and Zn content in soil. Application of 30 kg N + 60 kg P_2O_5 + 60 kg S ha^{-1} gave the highest trace element content in blackgram. Soil available Cu content decreased with increasing N and P applications but increased with S application. Soil available Fe increased with increasing N and P applications and decreased with increasing S applications.

Bhalu *et al.* (1995) conducted a field experiment during the rainy season of 1990 at Junagadh, Gujarat with blackgram (*Vigna mungo*) and seed was inoculated with *Rhizobium* or not inoculated and given 10, 20 or 30 kg N and 20, 40 or 60 kg P_2O_5 ha^{-1}. Seed inoculation increased seed yield (471 vs. 434 kg ha^{-1}). Seed yield increased with up to 20 kg N (464 kg) and 40 kg P_2O_5 (475 kg). N and P uptakes and seed protein content increased with increasing N and P rates. Net return was the highest with seed inoculation.

Ali *et al.* (1995) carried out field trials at Mianchannu in 1992 and Layyah in 1993 on sandy loam soils low in OM, N and P and *V. mungo* was given no fertilizers or 50 kg N, 50 kg N + 50, 75, 100 or 125 kg P_2O_5 or 50 kg N + 125 kg P_2O_5 + 50 kg K_2O ha^{-1}. NPK gave the highest number of pods $plant^{-1}$ (23.03-23.75) and seed yield (1080-1082 kg ha^{-1}) but was not significantly better than 50 kg N + 75 kg P_2O_5, which gave the highest 1000-seed weight (49.30 and 42.75 g in the 2 trials, respectively). Straw yields did not differ significantly among the treatments. Seed protein content was the highest with NPK.

Trivedi (1996) carried out field trials in the rainy seasons of 1990-91 at Gwalior, Madhya Pradesh, India with *P. mungo* (*Vigna mungo*) cv. Jawahar Urd-2 and was given 0-30 kg N, 0-60 kg P_2O_5 and 0 or 60 kg S ha^{-1}. Seed yield, net returns and N, P and S contents in seed increased with rate of N, P and S applications.

Mathan *et al.* (1996) conducted a field experiment during the monsoon seasons of 1991-92 at Coimbatore, Tamil Nadu where a total of 7 treatments compared the effect of applying in various combinations N, P, K, FYM, NAA and seed inoculation with Rhizobium were used on blackgram (*Vigna mungo*) cv. CO 5 yield. In 1992, seed treatment with fungicides was also included. The application of 25 kg N as urea + 50 kg P_2O_5 ha^{-1} as single superphosphate + 750 kg enriched FYM + 6.25 t FYM + foliar application of 25 kg diammonium phosphate at flower initiation and 15 days later + seed inoculation with *Rhizobium* produced the highest seed yields of 0.72 t ha^{-1} in 1991 and 0.62 t in 1992, compared with the control yields of 0.42 and 0.40 t, respectively. The seed crude protein content was increased by 14.5 and 15.4% in the highest yielding treatment compared with the controls.

Trivedi *et al.* (1997a) conducted a field experiment to study the effect of nitrogen, phosphorus and sulfur on yield and nutrient uptake of blackgram (*Vigna mungo*) at Gwalior, Madhya Pradesh during the 1990-91 kharif (monsoon) seasons. Application of 30 kg N, 60 kg P_2O_5 and 60 kg S ha^{-1} increased yield, net profit and nutrient uptake.

Trivedi *et al.* (1997b) carried out a field experiment on blackgram (*Vigna mungo*) where 0, 15 or 30 kg N ha^{-1}; 0, 30 or 60 kg P_2O_5 ha^{-1} and 0 or 60 kg S ha^{-1} were given on sandy loam soil at Gwalior, Madhya Pradesh, during the rainy seasons (kharif) of 1990 and 1991. Increasing levels of N, P_2O_5 and S significantly increased the seed and stover yields. Increases in mean seed yield over controls were 217, 273 and 109 kg ha^{-1} with 30 kg N, 60 kg P_2O_5 and 60 kg S ha^{-1}, respectively. The application of N with S significantly enhanced the yield. Increasing levels of N, P_2O_5 and S significantly increased the total uptake of N, P and S. The maximum net return (Rs. 3893 ha^{-1}) and BCR (2.01) was obtained with $N_{30}P_{60}S_{60}$.

Gunjkar *et al.* (1999) carried out an experiment on blackgram (*Vigna mungo*) at Parbhani during kharif (monsoon) 1994 and reported that blackgram gave seed yields of 658, 870 and 921 kg ha^{-1} with 0, 25 and 50 kg N ha^{-1}, and 768, 800, 836 and 863 kg ha^{-1} with 0, 25, 50 and 75 kg P_2O_5 ha^{-1}, respectively.

Maldal and Ray (1999) conducted a field experiment where mungbean cv. B105, B1 and Hoogly local were untreated, seed inoculated with *Bradyrhizobium* and 20, 30, or 40 kg N ha^{-1} as urea were given. The results revealed that nodulation was greatest with inoculation in B105 and Hoogly local while it was decreased by inoculation and N treatment in B105.

Sharma *et al.* (1999) conducted a field experiment in 1997/98 in Himachal Pradesh and *V. mungo* cv. Pant U-19 was seed inoculated with one of eleven *Rhizobium* strains or not inoculated and given 0 or 20 kg N ha^{-1}. Seed yield was the highest with inoculation with a local strain (1.30 t ha^{-1}). The application of 20 kg N gave higher seed yield than no N (1.24 vs. 1.14 t). Application of nitrogen and inoculation increased nodulation and nodule dry weight $plant^{-1}$, crop growth rate and relative growth rate. No significant effects were observed in protein content of seed and straw but a slight improvement was observed over control.

Sharma *et al.* (2000) carried out a field experiment during kharif 1997 at Palampur, Himachal Pradesh, India where *V. mungo* was seed inoculated with 1 of 9 *Rhizobium* strains and given 0 or 20 kg N ha^{-1}. Growth, yield and dry matter accumulation increased with N application and *Rhizobium* inoculation, with the local strain giving the best results.

An investigation was carried out by Singh *et al.* (2002) to study the effect of N, P and K application on seed yield and nutrient uptake by blackgram at Central Agricultural University, Imphal, Manipur, India during 1998 and 1999. In the grain yield, response of blackgram to the various treatments combinations of N (0 and 15 kg ha^{-1}), P (0, 30 and 60 kg ha^{-1}) and K (0 and 20 kg ha^{-1}), the highest yield was obtained from the application of 15:60:20 kg $N:P_2O_5:K_2O$ ha^{-1} which was at par with control and this might be due to higher values of organic carbon, N, P_2O_5 and K_2O in the soil. The total uptake of nutrients by blackgram was associated with higher biomass production.

Srinivas and Shaik (2002) studied the effects of N (0, 20, 40 and 60 kg ha^{-1}) and P (0, 25, 50 and 75 kg ha^{-1}) along with seed inoculation with *Rhizobium* culture on the growth and yield components of greengram were determined in a field experiment conducted during the kharif seasons. Plant height generally increased with increasing rates of P and with increasing rates of N up to 40 kg ha^{-1} followed by decrease with further increase in N. Number of seeds per pods, 1000-seed weight, seed and haulm yields generally increased. Seed inoculation with *Rhizobium* resulted in higher values for the parameters measured relative to the control. The interactions effects between N and P were not significant for the number pods plant^{-1}, pod length and seed haulm yield.

Asraf *et al.* (2003) conducted a field experiment to study the effects of seed inoculation of a biofertilizer and NPK application on the performance mungbean cv. NM-98 at Faisalabad in Pakistan. The treatments consisted of the seed inoculation of *Rhizobium phaseoli* singly or in combination with 20:50:0, 40:50:0 or 50:50:50 NPK kg ha^{-1} (urea), P (single super phosphate) and K (potassium sulphate) were applied during sowing. The tallest plants (69.93 cm) were obtained with seed inoculation + 50:50:0 kg NPK ha^{-1}. Seed inoculation + 50:50:0 or 50:50:50 kg ha^{-1} resulted in the highest number of pods plant^{-1} (28.97, 56.00, 63.90 and 32.56, respectively) and seed yield (1053, 1066, 1075 and 1072 kg ha^{-1}). Harvest index was highest with seed inoculation in combination with NPK and 40:50:0 (25.23), 50:50:0 (24.70) or 50:50:50 (27.5). Seed inoculation along with NPK at 30:50:0 kg/ha was optimum for the production of high seed yield by mungbean cv. NM-98.

A pot study was conducted by Sheikh *et al. (2003)* to investigate the effects of seed inoculation with *Rhizobium* sp. and N:P:K (at 0:0:0, 5:15:10, 10:30:20, 15:45:30 and 20:60:40 kg ha^{-1}) on growth and physiomorphological traits of blackgram cv. PU 19 irrigated with thermal power plant waste water (TPPW), sewage waste water (SW) and ground water (GW). Sampling was conducted at the vegetative (25 days after sowing, DAS), flowering (40 DAS), and fruiting (55 DAS) stages. Both SW and TPPW were efficient for improving the yield, vegetative growth, physiological characteristics and yield. Waste water application increased the leaf nitrate reductase activity, carbonic anhydrase [carbonate anhydrase] activity and total chlorophyll content of *Rhizobium*-inoculated plants. The optimum fertilizer treatment was N: P: K at 10:30:20 kg ha^{-1} and NPK-treated plants showed better performance under inoculated conditions than uninoculated.

A field experiment was conducted by Reddy *et al.* (2005) during *rabi* 2002-03 on a clay loam soil at the Agricultural Research Station, Warangal, Andhra Pradesh, India to study the influence of foliar fertilizer application of 2% urea on the yield of urdbean with nine treatments. The results revealed that significantly higher plant height, yield attributes, N uptake, seed yield and net returns were observed with 2% urea spray at 30, 40 and 60 days after sowing and was superior to all other treatments.

2.2 Effect of *Bradyrhizobium* inoculation

Effect of *Bradyrhizobium* inoculation on blackgram and other legumes have been presented below:

Bhalu *et al.* (1995) coducted a field experiment during the rainy season of 1990 at Junagadh, Gujarat with blackgram (*Vigna mungo*) and seed was inoculated with *Rhizobium* or not inoculated and given 10, 20 or 30 kg N and 20, 40 or 60 kg P_2O_5 ha^{-1}. Seed inoculation increased seed yield (471 vs. 434 kg ha^{-1}). Seed yield increased with up to 20 kg N (464 kg) and 40 kg P_2O_5 (475 kg). N and P uptakes and seed protein content increased with increasing N and P rates. Net return was highest with seed inoculation.

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Sharma *et al.* (2000) carried out a field experiment during kharif 1997 at Palampur, Himachal Pradesh, India where *V. mungo* seed was inoculated with 1 of 9 *Rhizobium* strains and given 0 or 20 kg N ha^{-1}. Growth, yield and dry matter accumulation increased with N application and *Rhizobium* inoculation, with the local strain giving the best results. They also found that dry matter production was increased by about 50% due to *Bradyrhizobium* inoculation.

Singha and Sarma (2001) conducted an experiment in India on blackgram cv. T-9 to study the effect of different levels of P fertilization and *Rhizobium* inoculation of seeds on yield and nutrient uptake. Application of P significantly increased the grain and straw yield and N, P and K uptake. P at 45 kg ha^{-1} produced the highest grain and straw yield and was at par with the application of 25 and 35 kg P ha^{-1}. N uptake increased from 20 to 30 kg ha^{-1} with application of 25 to 45 kg P ha^{-1}, respectively. *Rhizobium* inoculation significantly increased the number (2.2%) and mass (9.5%) of root nodules $plant^{-1}$ compared to the control indicating increased efficiency of the crop to fix the atmospheric N.

The nodulation characteristics of 8 varieties of blackgram (*Vigna mungo*) were studied by Reddy and Mallaiah, 2001. *Rhizobium* sp. was isolated from the T-9 cultivar of the crop. The effect of three different methods of *Rhizobium* inoculation on the nodulation of blackgram was studied. The initiation of nodulation was early and the numbers of nodules formed were more in the seed inoculation method than in soil inoculation or seedling inoculation methods. Three isolates of *Rhizobium*, viz. VM isolate, AH isolate and SG isolates, isolated respectively from blackgram, *Arachis hypogaea* and *Sesbania grandiflora*, were used to study their effect on nodulation and nitrogen content of blackgram cultivar T-9. In plants inoculated with the VM isolate, nodules appeared 12 days after sowing, and a maximum of 84 nodules $plant^{-1}$ were found during the reproductive stage of the crop. The nitrogen content of the nodules at the reproductive stage was 4.5%. The nitrogen content of the shoot was 1.9% at the vegetative stage (25-day-old plants), 3.0% at the reproductive stage (45-day-old plants) and 1.0% at the harvesting stage. The nitrogen content of the fresh seeds was 5.78% in the inoculated plants, while that in uninoculated controls was only 2.72%. The plants inoculated with the AH isolate showed better nodulation and nitrogen content compared to the plants inoculated with the VM isolate. However, the SG isolate completely failed to produce nodules on blackgram.

Tomar *et al.* (2001) conducted a field experiment at the G.B. Pant University Research Station, Ujhani, Uttar Pradesh, India, during kharif 1994-95 to study the effect of *Rhizobium*, vesicular arbuscular mycorrhiza (VAM, *Glomus caledonium*) and phosphate solubilizing bacteria (PSB, *Pseudomonas striata* strain P-27) inoculation, with and without P, on blackgram (*Vigna mungo*) seed yield. Phosphorus application in soil with medium P content (5.4 mg kg^{-1}) increased nodulation, grain yield, N and P in plant and grain over no phosphorus control. Forty kilograms of P_2O_5 each hectare recorded an increase of 20.6% in nodule dry weight, significant increases of 0.35 g kg^{-1} in N concentration and 1.28 g kg^{-1} in P concentration of plant over 20 kg P_2O_5 ha^{-1}. Similar significant increases of 0.59 g kg^{-1} in grain yield and 0.54 and 0.23 g kg^{-1} in N and P concentrations of the grain, respectively, over 20 kg P_2O_5 ha^{-1} were also obtained with higher dose. Inoculation of *Rhizobium* + VAM + PSB at all the stages of plant growth recorded maximum increases in all the parameters studied. *Rhizobium* gave the highest and 21.0% more nodule number, 34.7% more nodule dry mass, 0.73 g kg^{-1} more N in grain and 4.2% higher grain yield over PSB.

A field experiment was conducted in Vamban, Tamil Nadu, India by Nagarajan and Balachandar (2002) during the kharif season of 1998 to study the effects of organic amendments on the nodulation and yield of blackgram cv. Vamban 1. The treatments consisted of *Rhizobium* (strains CRU 7 for blackgram and CRM 11 for greengram) seed inoculation, 15 t farmyard manure (FYM ha^{-1}, FYM + *Rhizobium*, 5 t compost ha^{-1} (prepared from leaves and twigs of *Sesbania sesban*, *S. grandiflora*, *Cassia fistula*, *Cassia auriculiformis*, and *Claricidia* (*Gliricidia*) along with cowdung and rock phosphate), compost + *Rhizobium*, 5 t biodigested slurry ha^{-1}, and biodigested slurry + *Rhizobium*. In general, seed inoculation of *Rhizobium* and application of organic amendments enhanced biomass, root nodulation, and grain yield. Biodigested slurry at 5 t ha^{-1} + *Rhizobium* gave the greatest plant height (42.7 and 53.7 cm for blackgram and greengram, respectively), nodule number (23.3 and 24.0), nodule weight (45.3 and 42.3 mg), and grain yield (758.3 and 732.0 kg ha^{-1}).

Sarkar *et al.* (2002) inoculated the seed of blackgram with strains of *Bradyrhizobium* viz. M-10, 129-USA, 480-M, and MK-5 before sowing in a field experiment conducted to determine the cultivars and *Bradyrhizobium* strain for suitable use in the locality. Cultivars M-16 recorded longer roots and higher root volume $plant^{-1}$, number of nodules $plant^{-1}$ and test weight compared to A-43. The interaction effects between cultivar A-43 and *Bradyrhizobium* strain MK-5 resulted in the highest root volume $plant^{-1}$ (1.30), number of nodules $plant^{-1}$ (7.03) and test weight (4.23 g), whereas the interaction effects between cultivar A-43 and *Bradyrhizobium* strain 480-M resulted in the longest roots (14.72 cm). Correlation coefficient studies showed high correlation between seed yield and dry weight, and root weight. Root length and root volume were inversely correlated with test weight.

Sattar and Ahmed (1995) carried out a field experiment on mungbean (*Vigna raidata* L.) to study the response of inoculation with *Bradyrhizobium* inoculants incorporating BINA 403, BINA 407, RCR 3824 and RCR 3825 strains as single and mixed culture. They observed that *Bradyrhizobium* inoculation increased the number of nodules, nodule weight seed, hay and total protein yield significantly compared to uninoculated treatments.

Behari *et al.* (1995) carried out an experiment with *Vigna radiata* cv. K851 and B165. Seeds were treated with 10 different *Bradyrhizobium* strains (applied alone or in pairs) and found that there was a significant interaction between different mixtures of strain and cultivars in terms of nodulation. Seed yields were generally improved by using multi-strain inoculants.

Thakur and Panwar (1995) conducted a field trial where *Vigna radiata* cv. Pusa-105 and PS-16 were given seed inoculation of both. They found that inoculation either singly or combined increased plant height compared with no inoculation.

Shukla and Dixit (1996a) laid out a field trial to study the response of summer mungbean to *Rhizobium* inoculation. *Rhizobium* inoculation delayed 50% flowering, whereas it increased number of branches plant^{-1}, plant height and the dry matter accumulation.

Shukla and Dixit (1996b) stated a field experiment where greengram cv. Pusa Baisakhi was seed inoculated with *Rhizobium* or not inoculated, sown in rows, 20, 30 and 40 cm apart and given 0-60 kg P_2O_5 ha^{-1}. They found that seed inoculation increased seed yield.

Patra and Bhattacharyya (1997) carried out a field trial with *Vigna radiata* cv. B-1, *Bradyrhizobium* and urea (25 kg ha^{-1}). They found that all treatments increased nodulation compared control. They also reported that the highest nodules were given by *Bradyrhizobium* + urea.

Das *et al.* (1997) conducted field trials where *Vigna radiata* cv. Noyagrah local seed were inoculated with *Rhizobium* and/or VAM culture, which was applied at the rate of 15 kg ha^{-1}. They observed that number of nodules was increased with dual inoculation compared with uninoculated control.

Goel *et al.* (1997) carried out an experiment with *Vigna radiata* cv. K851, where seed were inoculated with one or both of 2 bacteriocin-producing *Rhizobium* strains, VRF10 (poor nodulating capacity) and VRF57 (superior nodulating capacity), VRF57 fewer nodules, but nodule biomass was much greater than for VRF10. When the two strains were inoculated together, VRF57 formed a higher proportion of nodules than VRF10, even when inoculated at a much lower ratio. This indicated that a factor other than bacteriocin affected its competitiveness.

Sharma and Khurana (1997) studied the effectiveness of single and multistrain inoculants in field experiments with summer mungbean variety SML 32. They found that number of nodules; nodule dry biomass and grain yield were better in multistrain inoculants. On an average, single strain and multistrain *Rhizobium* inoculants increased the seed yield by 10.4% and 19.3%, respectively compared to the uninoculated control.

Ghosh and Poi (1998) carried out a pot experiment where soybeans, groundnuts, mashkalai (*Vigna mungo*), mung (*Vigna radiata*) and lentil (*Lens esculentus*) were inoculated with *Bradyrhizobium*, *Bacillus polymixa* and *Glomus fasciculatum* in different combinations. They found that nodulation and population of microorganisms in the rhizosphere were highest from combined inoculation with all three microorganisms.

Gupta *et al.* (1998) reported that nodule occupancy of *Bradyrhizobium* strain S 24 was increased by 60% over the uninoculated control.

Provorov *et al.* (1998) observed that seed inoculation of mungbean (*Vigna radiata*) with strain CIAM 1901 augmented the herbage mass by 46.6%, seed mass by 39.2%, 1000-seed weight by 16%, seed N content by 58.3%, seed starch content by 30.0% and number of root nodules by 254%.

Patra and Bhattacharyya (1998) conducted a pot experiment to assess the effects of *Rhizobium* seed inoculation on *Vigna radiata* cv. B1. They observed that seed inoculated plants exhibited significantly high root and shoot weights compared to the uninoculated control plants. From a field trial, they also reported that plant seed inoculated with *Rhizobium* plus urea fertilizer produced significantly high yield compared to the control.

Maldal and Ray (1999) conducted a field experiment where mungbean cv. B105, B1 and Hoogly local were untreated, seed inoculated with *Bradyrhizobium* and 20, 30, or 40 kg N ha^{-1} as urea were given. The results revealed that nodulation was greatest with inoculation in B105 and Hoogly local while it was decreased by inoculation and N treatment in B105.

Paul (1999) conducted a pot experiment where mungbean cv. PS-16 was seed inoculated singly with 5 *Rhizobium* strains and exposed to 3 water regimes. She found that seed yield was not increased by inoculation under excess water or normal irrigation conditions seed yield was increased by inoculation.

Thakur (1999) conducted field experiments at Tendani, Chhindwara (Madhya Pradesh) during the rainy seasons of 1991 and 1992 to evaluate the effects of P, S and *Rhizobium* on growth and yield of blackgram. Inoculation of *Rhizobium* culture on the surface of dry seeds before sowing helped to improve the seed and straw yields. Significant increases in seed and straw yields were observed up to 40 kg P_2O_5 and 20 kg S ha^{-1}, mainly due to improvement in plant height, branches $plant^{-1}$ and pods $plant^{-1}$.

Upadhyay *et al.* (1999) conducted a field experiment where green gram was seed inoculated with *Rhizobium* or not inoculated and 0-60 kg P_2O_5 ha^{-1} was applied. They observed that seed yield was higher with inoculation (2.02 vs. 1.87 t ha^{-1}) and increased with up to 40 kg P_2O_5 (2.01 t ha^{-1}).

Kavathiya and Pandey (2000) conducted a pot experiment with *Rhizobium* on mungbean (*Vigna radiata* cv. K 851) and found that nodule plant^{-1} increased significantly over uninoculated control. They also reported that maximum seed germination (96.6%), plant height (24.6 cm), fresh shoot weight (5.33 g), fresh root weight (4.42 g) and nodulation (69 healthy nodules plant^{-1}) was recorded in the *Rhizobium* treatment.

Chowdhury *et al.* (2000) carried out a pot experiment during kharif in 1995 with mungbean in IPSA, Salna, Bangladesh where mungbean line NM-92 was inoculated with *Rhizobium* strain TAL 303. They found that dry matter production was increased by about 50% due to *Bradyrhizobium* inoculation.

Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India, during the pre-kharif season to study the effect of *Bradyrhizobium* inoculation, P (at 0, 20 kg ha^{-1}) and Mo (at 0, 0.5 and 1 kg ha^{-1}) on the number of nodules plant^{-1} of summer greengram cv. T-44. Inoculation and application of P and Mo significantly influenced the number of nodules plant^{-1}, dry matter accumulation in the shoot, crop growth rate and plant height comparing with control.

Tomar *et al.* (2001) conducted a field experiment at the G.B. Pant University Research Station, Ujhani, Uttar Pradesh, India, during kharif 1994-95 to study the effect of *Rhizobium*, vesicular arbuscular mycorrhiza (VAM, *Glomus caledonium*) and phosphate solubilizing bacteria (PSB, *Pseudomonas striata* strain P-27) inoculation, with and without P, on blackgram (*Vigna mungo*) seed yield. Phosphorus application in soil with medium P content (5.4 mg kg^{-1}) increased nodulation, grain yield, N and P in plant and grain over no phosphorus control. Forty kilograms of P_2O_5 each hectare recorded an increase of 20.6% in nodule dry weight, significant increases of 0.35 g kg^{-1} in N concentration and 1.28 g kg^{-1} in P concentration of plant over 20 kg P_2O_5 ha^{-1}. Similar significant increases of 0.59 g kg^{-1} in grain yield and 0.54 and 0.23 g kg^{-1} in N and P concentrations of the grain, respectively, over 20 kg P_2O_5 ha^{-1} were also obtained with higher dose. Inoculation of *Rhizobium* + VAM + PSB at all the stages of plant growth recorded maximum increases in all the parameters studied. *Rhizobium* gave the highest and 21.0% more nodule number, 34.7% more nodule dry mass, 0.73 g kg^{-1} more N in grain and 4.2% higher grain yield over PSB.

Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate root nodulation and plant growth characters of cowpea, blackgram and greengram and observed that root nodulation and plant growth characters of cowpea, blackgram and mungbean were uniformly better at category A locations.

El-Kramany (2001) conducted a pot trial to investigate the combined impact of biological (*Bradyrhizobium vigna* and *Azotobacter vinelandii*) and NPK fertilizers (25, 50 and 100%) on mungbean cultivars (Kawmy-1, VC-4, VC-9 and King) and found that the combined application of *B. vigna* and *A. vinelandii* increased seed yield, 100-seed weight and biological yield of mungbean significantly over uninoculated control.

Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and phosphate soluble bacteria (PSB) on nodulation and grain yield of mungbean cv. B-1 in field trial conducted in West Bengal. Seeds of mungbean were inoculated with strains of *Rhizobium*, i.e JCa-1 and M-10 strains, at a population of 28.20 x 10^6 and 32.66 x 10^6 cells ml^{-1}, respectively, phosphate solubilizing bacteria containing *Bacillus polymyxa* and *Pseudomonus striata* at a population of 7 x 10^8 cells ml^{-1} at the time of the sowing. The plants inoculated with *Bradyrhizobium* strains and PSB showed increased rate of nodulation and N content. The percentage increased in seed yield over control was observed to be highly significant in plants inoculated with *Bradyrhizobium* strains and PSB.

Malik *et al.* (2002) studied the effects of seed inoculation with *Rhizobium* and P application (at 0, 30, 50, 90 and 110 kg ha^{-1}) on the growth, seed yield and quality of mungbean cv. NM-98 in a field experiment conducted at Faisalabad in Pakistan during the autumn of 2000. Seed inoculation with *Rhizobium* and application of 70 kg ha^{-1}) resulted in the highest number of pods plant^{-1} (22.47), number of seed pod^{-1} (12.06), 1000-seed weight (42.27 g) and seed yield (1158 kg ha^{-1}). Plant height at harvest was highest when inoculated with *Bradyrhizobium* (68.13 cm).

Srinivas and Shaik (2002) studied the effects of N (0, 20, 40 and 60 kg ha^{-1}) and P (0, 25, 50 and 75 kg ha^{-1}) along with seed inoculation with *Rhizobium* culture on the growth and yields components of greengram were determined in a field experiment conducted during the kharif seasons. Number of seeds pods^{-1}, 1000-seed weight, seed and haulm yields generally increased. Seed inoculation with *Rhizobium* resulted in higher values for the parameters measured relative to the control. The interactions effects between N and P were not significant for the number pods plant^{-1}, pod length and seed haulm yield. Plant height generally increased with increasing rates of P and with increasing rates of N up to 40 kg ha^{-1} followed by decrease with further increase in N.

Perveen *et al.* (2002) conducted a field experiment to observe rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiata*). The treatments were single, dual and combined inoculants of *Bradyrhizobium*, *Azotobacter chroococcum* and *Aspergillus*. The maximum root dry weight (0.37 g plant^{-1}) and seed yield (6.6 g plant^{-1}) were observed with single *Bradyrhizobium* sp.

Osunde *et al.* (2003) tested the response of two mungbean cultivars (TGX1456-2E) and TGX1660-19F) to *Bradyrhizobium* inoculation in a two year trials in the farmers' fields of Nigeria. Cultivar effect of plant height and nodulation number was significant only in the first cropping season of the trial. Inoculation increased 40% seed yield in the first cropping season, while no such yield differences occurred in the second season. The proportion of nitrogen derived from nitrogen fixation ranged from 27% to 50% in the both cropping seasons and this was dependent on crop management on the farmer's field, rather than any cultivar or inoculation effect.

A study was conducted by Kumari and Nair (2003) to isolate efficient native strains of *Rhizobium* or *Bradyrhizobium* spp. and to develop suitable package of practices recommendations for their efficient use. The initial isolation of *Bradyrhizobium* spp. was done from seven different locations in Kerala, India, where the soil was generally acidic in nature. A total of 26 isolates (13 each from blackgram (*Vigna mungo*) and greengram (*V. radiata*) were obtained and were screened for nodulation efficiency. The experiment was conducted in complete randomized block design with three replications for each isolate using unsterilized soil of pH 4.89 without any amendments such as applications of FYM or chemical fertilizers. It was also repeated under amended soil conditions. The selected isolates were further evaluated under field (Vellayani and Kayamkulam) conditions along with a package of practices recommendation (POP) developed by the Kerala Agricultural University. The extent of root nodulation, plant growth and yield were more in blackgram and greengram where *Bradyrhizobium* inoculation was done along with the POP recommendation. At Vellayani, the nodule number, plant dry weight and yield in blackgram were significantly high in the treatment combination of POP KA-F-B-6. At Kayamkulam, significant increases were obtained only in nodule number, nodule dry weight and yield. The results indicated that for acidic soils, the mere development of efficient native strains of *Rhizobium* or *Bradyrhizobium* alone was not sufficient but it should be along with a package of practices recommendation consisting of application of organic manure and liming to neutralize the soil pH.

Ashraf *et al.* (2003) conducted a field experiment to observe the effects of seed inoculation of a biofertilizer and NPK application on the performance mungbean cv. NM-98 studied in Faisalabad, Pakistan. The treatments consisted of the seed inoculation of *Bradyrhizobium phaseoli* singly or in combination with 20:50:0, 40:50:0, 50:50:0, or 50:50:50 kg NPK ha^{-1} (urea), P (single superphosphate), and K (potassium sulphate) were applied during sowing. Seed inoculation + 50:50:0 or 50:50:50 kg ha^{-1} resulted in the highest number of pods plant^{-1} (28.97, 56.00, 63.90 and 32.56, respectively) and seed yield (1053, 1066, 1075 and 1072 kg ha^{-1}). Harvest index was the highest with seed inoculation in combination with NPK and 40:50:0 (25.23), 50:50:0 (24.70) or 50:50:50 (27.5). Seed inoculation along with NPK at 30:50:0 kg ha^{-1}) was optimum for the production of high seed yield by mungbean cv. NM-98. The tallest plants (69.93 cm) were obtained with seed inoculation + 50:50:0 kg NPK ha^{-1}.

Sriramachandrasekharan and Vaiyapuri (2003) coducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on the nodulation, growth, and yield of blackgram cv. ADT 3. Plant height (40.3 cm), number of nodules plant^{-1} (36.4), effective number of nodules plant^{-1} (24.5), nodule dry weight (20.2 g), root length (23.5 cm), shoot weight (0.82 g), root weight (0.32 g), number of pods pot^{-1} (18.2), pod yield pot^{-1} (55.6 g), root weight (0.32 g), and stover yield pot^{-1} (90.1 g) was the highest with 2.50 ppm carbofuran and decreased thereafter with further increase in carbofuran concentration. Irrespective of the levels of carbofuran, *Rhizobium*-inoculated blackgram showed better growth and higher pod yield (50.3 g) and stover yield pot^{-1} (81.1 g) than the uninoculated crop.

A study was undertaken by Tanwar *et al.* (2003a) conducted in Rajasthan, India during kharif 1996 to investigate the effect of P (0, 20, 40 and 60 kg ha^{-1}) and biofertilizers

(*Rhizobium* sp. and *Bacillus megaterium* var. *phosphaticum*) on the nutrient content and uptake of blackgram. The biofertilizers were applied singly or in combination. The crop yield, N and P contents, and N and P uptake increased with increasing P rate up to 80 kg ha^{-1}. Inoculation with the combination of the biofertilizers resulted in higher yield, N and P content, N and P uptake of the grain and straw compared to no inoculation and individual inoculation.

An experiment was carried out by Bhuiyan *et al.* (2005b) with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during kharif-I 2001 and kharif-I 2002 seasons to find out the time of nodule initiation, nodulation pattern and their size distribution by where five mungbean varieties viz. BARI Mung-2, BARI Mung-4, BARI Mung-5, BINA Mung-2 and Barisal local, and rhizobial inoculum (*Bradyrhizobium* strain BAUR-604) was used. The nodules were first visible at 9 DAS, when only a few small nodules were observed. The number of nodules increased progressively with the increasing growth period and reached the peak at 42 DAS (i.e. at 50% flowering stage). Inoculated plants recorded higher number of nodules than uninoculated plants at all the sampling dates. The results suggested that nodule initiation in the roots of mungbean varieties started at 9 days of sowing seeds (DAS), reached the peak at 42 DAS and thereafter started reducing in numbers until 70 DAS due to spontaneous degeneration.

Bhuiyan *et al.* (2006) carried out a field study with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during kharif-I 2001 and kharif-I 2002 seasons to observe nodulation pattern and nodule dry matter production at different growth periods of mungbean. They found that inoculated plant produced significantly higher nodule number (17.51 $plant^{-1}$ in 2001 and 17.61 $plant^{-1}$ in 2002) at 42 DAS compared to uninoculated plant (11.35 $plant^{-1}$ in 2001 and 11.52 $plant^{-1}$ in 2002). The lowest number of nodules (3.50 $plant^{-1}$ in 2001 and 3.47 $plant^{-1}$ in 2002) was produced at 14 DAS with uninoculated plants.

Bhuiyan *et al.* (2007a) carried out an experiment with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during kharif-I 2001 and kharif-I 2002 seasons to find out the time of nodule initiation, nodulation pattern and their size distribution. The number of nodules increased progressively with the increasing growth period and reached the peak at 42 DAS (i.e. at 50% flowering stage). The number of nodules of 2–4 mm size started to decline after 42 DAS sharply and in case of <2 mm size nodules, the declining was noticeable after 56 DAS, while the bigger nodules were increased up to 63 DAS. The results suggested that nodule initiation in the roots of mungbean varieties started at 9 days of sowing seeds (DAS), reached the peak at 42 DAS and thereafter started reducing in numbers until 70 DAS due to spontaneous degeneration. Higher number of nodule in different sizes (<2.0 mm, 2.1-4.0 mm and >4 mm) was observed in BARI mung-2 at different DAS in both the years. *Bradyrhizobium* inoculation produced 8.8 (<2.0 mm), 8.5-8.6 (2.1-4.0 mm) and 0.2-0.4 (>4 mm) nodules $plant^{-1}$, while uninoculated plant produced 5.7 (<2.0 mm), 5.6 (2.1-4.0 mm) and 0.1-0.2 (>4 mm) nodules $plant^{-1}$.

Bhuiyan and Mian (2007) conducted experiments with or without *Bradyrhizobium* in five mungbean varieties at Bangladesh Agricultural University Farm during kharif-I 2001 and kharif-I 2002 seasons to observe nodulation, biomass production and yield of mungbean. Five mungbean varieties viz. BARI Mung-2, BARI Mung-4, BARI Mung-5, BINA Mung-2 and Barisal local, and rhizobial inoculum (*Bradyrhizobium* strain BAUR-604) was used for the study. Application of *Bradyrhizobium* inoculant produced significant effect on nodulation, shoot dry weight, seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. Inoculated BARI Mung-2 produced the highest nodulation, dry matter production, seed and stover yields.

Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/ without *Bradyrhizobium* inoculation at the Bangladesh Agricultural University Farm during Kharif-I 2001 and Kharif-I 2002 seasons to observe shoot dry matter production and nitrogen uptake by mungbean at different growth stages. Significant influences of the mungbean varieties were observed on dry matter production and nitrogen uptake. *Bradyrhizobium* inoculant significantly increased dry matter production. The highest dry matter production plant^{-1} at 77 DAS was recorded in *Bradyrhizobium* inoculated plots. Inoculated BARI Mung-2 produced the highest shoot weights.

Shil *et al.* (2007) reported that seed yield, plant height, pod length, pods plant^{-1}, seeds pod^{-1}, 1000-seed weight were the highest in full doses of fertilizers while control plants recorded the lowest yield and also other parameters.

Bhuiyan *et al.* (2008a) carried out field studies with and without *Bradyrhizobium* with five mungbean varieties to observe the yield and yield attributes of mungbean. They observed that application of *Bradyrhizobium* inoculant produced significant effect on seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. *Bradyrhizobium* inoculation also significantly increased pods plant^{-1}, seeds pod^{-1} and 1000-seed weight. Inoculated BARI Mung-2 produced the highest seed and stover yields as well as yield attributes such as pods plant^{-1} and seed pod^{-1}.

Bhuiyan *et al.* (2008b) conducted a field experiment at Regional Agricultural Research Station, Jamalpur on blackgram and reported that inoculated plants gave significantltly higher nodule number, nodule weight, shoot weight and seed yield compared to non-inoculated plants.

2.3 Effect of boron

Boron is an essential micronutrient for cell division in the process of nodule formation (Mulder, 1948). It is one of the most important micronutrients which is required for growth and development of plant. It helps in fruit setting and grain formation. The development of anthers and pollen is affected by boron deficiency. In boron deficient crop, the pollen does not accumulate starch and the nuclei when present are abnormal. It has been suggested that boron deficiency affects pollen development during the pollen mother cell stage (Canhong and Rerkasem, 1992). Thus boron deficiency can cause yield reduction by reducing grain set through impaired development of anther and pollen grain (Sansance and Benchawan, 1989). Rerkasem *et al.* (1991) observed that the poor grain set in wheat reduced grain yield by 40-50% on soils with low boron content (0.08-0.12 mg B kg^{-1}). Grain yield of wheat is depressed by poor number of grains ear^{-1} which may result from boron deficiency (Abedin *et al.*, 1994). The problem can be corrected by boron application to the soil. It has been reported that the crop responds better to soil application than to foliar application (Chatterjee *et al.*, 1980). The added boron causes higher formation of grains resulting in higher grain yield of wheat (Jahiruddin, 1991; Hossain *et al.*, 1994; Khan *et al.*, 1996). Improvement in grain set and 1000-grain weight in wheat by boron application was also observed by Singh and Singh (1976).

Boron enhances uptake and translocation of sugar and is implicated in carbohydrate metabolism. This might be another reason for high grain weight and higher grain yield of wheat due to boron application to wheat. Plant height, number of spikes m^{-2}, number of filled grains $spike^{-1}$, 1000-grain weight, grain yield and straw yield were found to increase by boron application up to 3 kg B ha^{-1} (Rahman, 1989). Application of boron at the rate of 2.9 kg ha^{-1} also increased the number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$ (Saleh *et al.*, 1982).

Bhuiyan *et al.* (1996a) conducted field experiment on Grey Terrace Soil of Gazipur, Bangladesh to evaluate the effect of rhizobial inoculum, molybdenum and boron on the growth, yield and economic performance of soybean. They observed that the highest nodulation, dry matter yield and grain yield were recorded with the treatment of $P_{22}K_{42}B_1$ + inoculum. Inoculation with molybdenum, boron and molybdenum, and only inoculum also gave better performance in producing nodule numbers, nodule weight, shoot and root dry weight, stover and grain yield. Inoculation either with boron or with molybdenum increased nodulation and yield than the combined application of boron or and molybdenum.

An experiment was conducted by Bhuiyan *et al.* (1997a) on Grey Terrace Soil of Gazipur, Bangladesh to observe the effect of rhizobial inoculum, Mo and B on the nodulation, yield and economic performance of groundnut. They reported that *Rhizobium* inoculation in presence of P, K, Mo and B fertilizers significantly increased nodulation, dry matter and nut yield of the crop.

Field experiment were conducted by Bhuiyan *et al.* (1998b) at Agricultural Research Station, Thakurgaon, Bangladesh to find out the effect of Mo, B and rhizobial inoculant on nodule number, nodule and shoot weights, and stover and grain yields of lentil. Significant differences were observed in all the characters studied. Rhizobial inoculant, Mo and B together produced significantly higher nodule number, nodule and shoot weights, and stover and grain yields over control. Crops response to B was better than the Mo. Grains yield of lentil were 115 and 94% higher over uninoculated and unfertilzed control during to *rabi* seasons.

Seven different treatment combinations of rhizobia, Mo and B fertilizers along with a control were evaluated by Bhuiyan *et al.* (1998a) at the Agricultural Research Station, Rajshahi to investigate their effect on nodule number, nodule and shoot weight, and straw and seed yield, in order the select a suitable combiantion of treatment for better chickpea growth and yield. Significant differences were observed in all characters studied. *Rhizobium* inoculation with Mo and B produced significantly higher nodule number, nodule and shoot weight and straw and seed yield compared with control. Seed yield was 107 and 148% higher than control in two consecutive growth seasons.

An experiment was conducted by Bhuiyan *et al.* (1999a) on Himalayan Piedmont Soils of Dinajpur and Thakurgaon, Bangladesh to observe the effect of MoB and rhizobial inoculum on nodulation, yield and economic performance of chickpea. Inculation with *Rhizobium* strain (RCa-220) in presence of P, K, Mo and B fertilizers significantly increased nodulation, top dry matter and seed yield of the crop. The magnitude of increases in seed yield over control was 143% and 86% at Dinajpur and 132% and 174% at Thakurgaon for two consecutive *rabi* season.

Mishpra *et al.* (2001) conducted an experiment on the effect of nutrient management and plant growth regulators on the yield and economics of chickpea in Madhya Pradesh, India during the *rabi* season of 1998-99. Seeds and stover yields were higher in B and cephalexin treatments compared to the other growth regulator treatments. Boron and P with S treatments gave the highest net returns.

A field experiments were conducted by Singh *et al.* (2002) in sandy loam calcareous soil in Uttar Pradesh, India during 1995-96 to study the effect of boron application on yield of pea (cv. Rachna) and blackgram (cv. PV-19). Boron was applied as borax at the rates of 1, 2, 3 and 4 kg borax ha^{-1}, with a control. Application of borax up to 4 kg borax ha^{-1} significantly increased the grain yield of black gram. The maximum yield was 15.42 q ha^{-1} and minimum grain yield of 1.65 q ha^{-1} was found in the control. The additional grain yield over the control was 280, 431, 899 and 1377 kg ha^{-1} at 1.0, 2.0, 3.0 and 4.0 kg borax ha^{-1}, respectively. Application of B progressively increased the grain yield of pea from 510 to 1843 kg ha^{-1} up to 4 kg borax ha^{-1}.

Bharti *et al.* (2002) carried out a field experiment in Bihar, India during the winter of 1997-98 to observe the effects of B (0, 1.5 and 2.5 kg ha^{-1}) application on the yield and nutrition of chickpea (cv. BG256). They reported that the mean seed yield, and seed stover N and B content increased, whereas stover yield decreased with increasing B rates.

Ali *et al.* (2002) reported that yield losses of varying magnitude in chickpea, e.g., 22-50% due to iron (Fe) up to 100% due to boron (B), and 16-30% due to sulphur (S). Genotypic differences in response to application of Fe, B and zinc (Zn) have also been found among chickpea genotypes.

Bharti *et al.* (2003) conducted a field trail in Muzaffarpur, Bihar, India during the 1997-98 *rabi* season on chickpea cultivar (BG256) to study the effect of boron. The treatments comprised 0, 1.5 and 2.5 kg B ha^{-1}. The number, dry weight, nitrogenase activity, leghaemoglobin content and active iron content of the nodules increased with B application. Boron at 1.5 kg ha^{-1} was optimum for most of the characteristic.

Janakiraman *et al.* (2004) conducted a field experiments to determine that groundnut growth and yield were significantly higher when iron, zinc and boron were applied with recommended doses of NPK fertilizers. Combined application of 50 kg N ha^{-1}, 25 kg P_2O_5 ha^{-1}, 25 kg K_2O ha^{-1} along with $FeSO_4$ 10.0 kg ha^{-1}, $ZnSO_4$ @ 5.0 kg ha^{-1} and borax @ 1.0 kg ha^{-1} maximized the groundnut yield and net return.

Chitdeshwari and Poongothai (2004) prevailed the response of groundnut to the soil application of Zn, B, S and Mo, and also the seed treatment with Zn, B and S. A substantial yield increase was obtained with the soil application of Zn at 5 kg ha^{-1} in combination with B at 1.0 kg ha^{-1} and S at 40 kg ha^{-1}. The yield increase over the control was 24.2% for TMV 7 and 14.8% for JL 24. Zinc had the most pronounced effect on yield, followed by S and B.

Bhuiyan *et al.* (2005b) conducted field experiments on Calcareous Brown Floodplain Soils of Jessore to study the effect of *Rhizobium* inoculation and micronutrients (Mo and B) on the growth, yield and economic performance of soybean. Inoculation with P, K and B fertilization produced the highest nodule number, nodule and shoot dry weight, stover and seed yield of the crop. Inoculation either with B or Mo increased nodule number, nodule weight and seed yield than combined application of B and Mo.

Niranjana (2005) conducted a field experiment to investigate the effect of B (1 g kg^{-1} seed), Zn (2 and 4 g kg^{-1} seed) and Mo (2 and 4 g kg^{-1} seed) as seed treatments on the growth and yield of groundnut cv. KRG-1 on Alfisol, which was deficient in Zn (0.46 mg kg^{-1}) and Mo (0.032 mg kg^{-1}). He observed that the micronutrients showed significant effect on yield, oil content and growth parameters. The Zn at 4 g + Mo at 2 g kg^{-1} seed treatment recorded the highest pod yield of 24.99 q ha^{-1} and growth parameters, total number of nodules (57.4) and their dry weight (100.2 mg $plant^{-1}$), number of effective nodules (27.80) and their dry weight

(70 mg plant^{-1}) as well as root length (13.66 cm) and its dry weight (887 mg), over the control. The extent of increase was 24.11% over the control.

Johansen *et al.* (2005) found that chickpea grown on residual soil moisture after rice harvest is a promising crop for the High Barind Tract (HBT), an uplifted, slightly undulating area in northwestern Bangladesh where the soils have an acid surface horizon (pH 4.5-5.5 at 0-10 cm). To determine which elements could be limiting to chickpea. A subtractive design was used in which the absence of sulphur (S), boron (B), zinc (Zn) or molybdenum (Mo) was compared to a complete nutrient control. Only Mo was found to be limiting, giving a grain yield response of 73%.

Nassar (2005) conducted an experiment to evaluate the effect of foliar application of boron, zinc, manganese or iron on the seed and pod yields of groundnut as well as on the nutrient, oil and protein content of seeds. Boron was applied at rates of 75, 150 and 300 mg litre^{-1} as boric acid, whereas zinc, manganese and iron were applied at rates of 150, 300 and 600 mg litre^{-1} in EDTA form. Foliar spraying with 600 mg Fe, 600 mg Zn, 300 mg Mn and 150 mg B litre^{-1} gave the highest seed and pod yields and recorded the highest seed nutrient, oil and protein contents.

Johnson *et al.* (2005) found that the primary micronutrient problem in grain legumes is B deficiency, while in rice (*Oryza sativa*), Zn deficiency is more important, and wheat (*Triticum aestivum*) suffers from both deficiency. A series of field experiments was carried out over two seasons to compare soil fertilization and micronutrient seed priming as methods of improving Zn and B nutrition of each crop. Micronutrient treatments were evaluated for their effect on grain yield micronutrient content. Soil B fertilization increased B content of the grain on lentil (*Lens culinaris*), chickpea (*Cicer arietinum*).

Srivastava *et al.* (2005) observed that in absence of applied B, there was no yield as any pods formed, in comparison to a yield of 300 kg ha^{-1} in the full nutrient treatments. There was yellowing of younger leaves and typical 'little leaf' symptoms when B was omitted. A critical concentration range of 15-20 ppm B was found for the shoot tips of chickpeas.

Singaravel (2006) conducted a pot trial experiments to determine the effects of the different fertilizers on the growth and yield of groundnuts cv. VRI 2. He found that the recommended NPK in combination with 25 kg zinc sulfate ha^{-1}, 10 kg borax ha^{-1} and 10 t composted coir pith ha^{-1} gave the highest values for plant height, dry matter production during the flowering, post-flowering and harvesting stage of the crop. Number of pods $plant^{-1}$, crop yield, and N, P, K, Zn and B uptake of the groundnuts also increased.

Rao *et al.* (2006) carried out a field study to evaluate the response of mustard to zinc (0.5% zinc sulfate), boron (1.0 ppm borax) and molybdenum (0.1% ammonium molybdate) application in addition to the recommended NPK and FYM alone. Combined application of Zn, B and Mo gave the highest values for most yield attributes, closely followed by B and Mo. However, integrated use of B and Mo recorded the highest 1000-seed weight and seed yield, accounting for 24% increase over the recommended NPK and 56% increase over FYM alone.

Shil *et al.* (2007) found that boron played major role in augmenting yield. The highest mean yield (1.23 t ha^{-1}) was obtained with 2 kg ha^{-1} B and 1 kg ha^{-1} Mo, which was 52% higher over control. The optimum economic dose of boron was found to be 1.76 kg ha^{-1}.

2.4. Effect of molybdenum

Molybdenum plays a vital role in legume production. It is an essential micronutrient for all plants, being necessar for the formaiton of nitrate reductase. However, in legumes, it plays an additional role in symbiotic nitrogen fixation. Effect of Mo and B on different grain legumes have been reported by various researchers (Pradhan and Sarkar, 1985; Pal, 1986; Verma *et al.*, 1988; Tiwari *et al.*, 1989; Miah *et al.*, 1992; Bhuiyan *et al.*, 1996a, 1996b, etc.). But in the legumes, it plays an additional role in the symbiotic nitrogen fixation. But research studies on the influence of molybdenum on blackgram are limited. Respnse of molybdenum and boron on different grain legumes have been reported by some researchers (Muralidharan and George, 1977; Kulkarni *et al.*, 1989; Brodrick and Giller, 1991; Giller and Wilson, 1991; Robson, 1993; Kanzaria and Patel, 1985; Rekrasem *et al.*, 1987; Miah *et al.*, 1992; Bhuiyan *et al.*, 1996b; Bhuiyan *et al.*, 1998a; Bhuiyan *et al.*, 1999b; Bhuiyan *et al.*, 2005b). However, some of the information on the effect of Mo regarding blackgram and other legumes yield and uptake by blackgram and other legume crops are cited here:

Sharma *et al.* (1995) observed that application of 30 kg N + 60 kg P_2O_5 + 60 kg S ha^{-1} gave the highest trace element content in black gram. Soil available Cu content decreased with increasing N and P applications but increased with S application. Soil available Fe increased with increasing N and P applications and decreased with increasing S applications.

Chaudhary and Das (1996) conducted an experiment in Uttar Pradesh, India and found that P, S and Mo application significantly increased the canopy, nodule count, yield of rainfed blackgram (*Vigna mungo*), yield of succeeding safflower and reduced splash loss and conserved more soil water. Water stable aggregates, infiltration rate, organic carbon, total N, available P, K, S and Mo in soil increased considerably after the harvest of blackgram but decreased after the harvest of succeeding safflower. Plant canopy showed significant positive relationship with nodule count, soil water conservation, water stable aggregates and infiltration rate but showed significant negative relationship with splash loss.

Solaiman and Habibullah (1990) stated that at 50% flowering stage of groundnut, *Rhizobium* inoculant treatment in presence of P, K and Mo gave significantly higher amount of total dry matter yield plant^{-1} compared to the treatment of 100 kg N ha^{-1} at farm fertility.

Solaiman *et al.* (1991) carried out an experiment with two varieties of lentil, Utfala and Mymensingh local. They reported that 2 kg Mo ha^{-1} when applied with *Rhizobium* inoculant was found stimulating in respect of nodulation and dry matter production of the crop.

Olufajo and Adu (1991) conducted a field experiment on soybean (cv. Samsoy) inoculated with *Bradyrhizobium japonicum* and Mo application @ 270 g Mo ha^{-1} and reported increased nodulation.

Singh *et al.* (1992) noted that application of Mn and Mo, either singly or in combination with *Rhizobium* culture significantly increased grain yield of cowpea. Protein content of grain and available nitrogen in soil also increased with increasing dose of Mn and Mo with *Rhizobium* inoculation.

Singh *et al.* (1992) carried out field experiments to assess the effects of Mn and Mo with *Rhizobium* culture on yield and protein content of cowpea (*Vigna unguiculata*). They found that application of Mn and Mo, either singly or in combination with *Rhizobium* increased yield. Grain protein and soil available nitrogen also increased with *Rhizobium* inoculation and increasing doses of Mn and Mo.

Yanni (1992) reported that seed yields of chickpea, lentil and *Lupinus albus* were generally the highest due to Mo application.

Ali *et al.* (1993) observed that soybean cv. Clark was supplied with the equivalent of 0, 30 or 60 kg P feddan^{-1} plus 0, 5 or 10 ppm Mo. The highest DM yield plot^{-1}, number of nodules and N and Mo uptake pot^{-1} came from the rates of 10 ppm Mo + 60 kg P $feddan^{-1}$ [1 feddan =0.42 ha].

Maurya *et al.* (1993) reported from a pot experiment that *Rhizobium* inoculation with Mo in urdbean (*Vigna mungo*) increased nodule number and weight, 1000-seed weight and N uptake.

Sattar *et al.* (1993) conducted six field experiments on two chickpea varieties (Nabin and Hyprosola) with *Rhizobium* inoculation as affected by TSP (60 kg P_2O_5 ha^{-1}), MP @ 30 kg K_2O ha^{-1}) and ammonium molybdate (@ 1 kg Mo ha^{-1}) at 6 locations. They observed that fertilizer application stimulated the activity of *Rhizobium* specially in producing root nodules and yield of the crops.

Johal and Chahal (1994) noted that nodule numbers and dry weight of mungbean were the greatest with 5 ppm Mo. Nodule leghaemoglobin content increased with up to 5 ppm Mo as did nodule nitrogenase activity.

Sinha *et al.* (1994) observed that lentil cv. B77 was given 0.6 or 1.2 kg B ha^{-1} as borax, 4.4 kg Zn ha^{-1} as zinc sulphate or 0.5 kg Mo ha^{-1} as sodium molybdate singly or in various combinations. Compared with the control yield, application of the trace elements increased seed yield by between 14 and 55%. Application of Mo + Zn gave the highest seed yield of 2.29 t ha^{-1}, the highest net return

Seven different treatments of rhizobia, molybdenum and boron fertilizers along with a control were evaluated by Bhuiyan *et al.* (1996b) at Regional Agricultural Research Station, Ishurdi, Pabna to find their effects on nodule number, shoot weight and stover yield and nut yield and also to select the suitable combination of rhizobial inoculum, Mo and B micronutrients for better growth and yield of groundnut. They opined that rhizobial inoculation with Mo and B produced significantly higher number, nodule weight, shoot weight, stover yield and nut yield.

Experiment were conducted at Central Farm of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur by Bhuiyan *et al.* (1997b) to determine the effect of rhizobial inoculum, B and Mo on the growth, yield and economic performance of chickpea. They noted that ***Rhizobium*** inoculum along with P, K, B and Mo gave significantly higher nodule number, nodule weight, shoot weight, stover yield and seed yield. *Rhizobium* inoculum in presence or absence of P, K, Mo or B also gave better performance in producing nodule number and nodule weight.

Mandal *et al.* (1998) observed that dry matter yield of lentil was increased by the application of lime, P and Mo. Plant dry matter pot^{-1} was the highest with 100% lime + 50 mg P + 1 mg Mo. Yield response to Mo application was the highest, followed by lime and P.

Rosolem and Caires (1998) reported that a high N-uptake had been observed in limed plots probably due to an increase in molybdenum availability.

Seven different treatment combinations of rhizobia, Mo and B fertilizers along with a control were evaluated by Bhuiyan *et al.* (1998a) at the Agricultural Research Station, Rajshahi to investigate their effect on nodule number, nodule and shoot weight, and straw and seed yield, in order the select a suitable combiantion of treatment for better chickpea growth and yield. Significant differences were observed in all characters studied. *Rhizobium* inoculation with Mo and B produced significantly higher nodule number, nodule and shoot weight and straw and seed yield compared with control. Seed yield was 107 and 148% higher than control in two consecutive growth seasons.

An experiment was conducted by Bhuiyan *et al.* (1999b) in the light soil of Tobacco Research Station, Rangpur, Bangladesh to study the effect of rhizobial inoculum and micronutrients (Mo and B) on the growth, yield and economic performance of chickpea. A significant increased over control in nodule number, nodule mass, shoot mass, stover yield and seed yield due to rhizobial inoculation in the presence of P, K, Mo and B. Molybdenum or B application with P, K and *Rhizobium* also resulted in higher nodule number, nodule mass, stover yield, and seed yield then the other treatments except PKMoB + Inoculum. *Rhizobium* without any chemical fertilizer also gave significantly higher nodule number and mass then the uninoculated control.

Bhattacharyya and Pal (2001a) conducted a field experiment in West Bengal, India, during the pre-kharif season to study the effect *Bradyrhizobium* inoculation, P (at 0, 20 kg ha^{-1}) and Mo (at 0, 0.5 and 1 kg ha^{-1}) on the number of nodules $plant^{-1}$ of summer greengram cv. T-44. Inoculation and application of P and Mo significantly influenced the number of nodules per plant and plant height.

Bhuiyan *et al.* (2005a) conducted field experiments on Calcareous Brown Floodplain Soils of Jessore to study the effect of *Rhizobium* inoculation and micronutrients (Mo and B) on the growth, yield and economic performance of soybean. Inoculation with P, K and B fertilization produced the highest nodule number, nodule and shoot dry weight, stover and seed yield of the crop. Inoculation either with B or Mo increased nodule number, nodule weight and seed yield than combined application of B and Mo.

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Johansen *et al.* (2005) found that chickpea grown on residual soil moisture after rice harvest is a promising crop for the High Barind Tract (HBT), an uplifted, slightly undulating area in northwestern Bangladesh where the soils have an acid surface horizon (pH 4.5-5.5 at 0-10 cm). To determine which elements could be limiting to chickpea. A subtractive design was used in which the absence of sulphur (S), boron (B), zinc (Zn) or molybdenum (Mo) was compared to a complete nutrient control. Only Mo was found to be limiting, giving a grain yield response of 73%.

Rao *et al.* (2006) carried out a field study to evaluate the response of mustard to zinc (0.5% zinc sulfate), boron (1.0 ppm borax) and molybdenum (0.1% ammonium molybdate) application in addition to the recommended NPK and FYM alone. Combined application of Zn, B and Mo gave the highest values for most yield attributes, closely followed by B and Mo. However, integrated use of B and Mo recorded the highest 1000-seed weight and seed yield, accounting for 24% increase over the recommended NPK and 56% increase over FYM alone.

Shil *et al.* (2007) found that boron played major role in augmenting yield. The highest mean yield (1.23 t ha^{-1}) was obtained with 2 kg ha^{-1} B and 1 kg ha^{-1} Mo, which was 52% higher over control. The optimum economic dose of boron was found to be 1.76 kg ha^{-1}.

CHAPTER III

MATERIALS AND METHODS

This chapter deals with the experimental aspect at the work. Details of the experimental materials and methods followed in the study are presented in this chapter. The experiment was carried out during September to December, 2007. This chapter offers a brief description of soil, treatments, design, fertilizer, biofertilizer, intercultural operations, chemical and statistical analysis.

3.1 Objective

The experiment was conducted to study the response of *Bradyrhizobium*, nitrogen, molybdenum and boron on the growth, nodulation, yield, nitrogen uptake and other yield contributing characters of blackgram.

3.2 Experimental site

The experiment was carried out at the Net House of Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3 Collection and preparation of soil sample

The soil used in this experiment was collected from a selected area of Central Farm of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur. The initial surface soils of 0-15 cm depth were collected. The soils were put into polyethylene bag and were taken to the laboratory. Then, it was spread on the floor and sun dried for one month when dried, the clods were broken with hammer to bake it friable. The soil was sieved to remove weeds, stubbles and hard clods. A composite soil sample was kept in polyethylene bag for chemical analysis.

3.4 Soil

The morphological, physical and chemical characteristics of the experimental soil are presented in Tables 3.1.

Table 3.1. Physical and chemical characteristics of the soils

Characteristics	Values
Mechanical fractions:	
% Sand (0.2-0.02 mm)	27.5
% Silt (0.02-0.002 mm)	33.5
%Clay (< 0.002 mm)	39.0
Textural class	Clay loam
Colour	Grey
Consistency	*Sticky and mud when wet*
pH (1:2.5 Soil-Water)	6.3
CEC (cmol kg^{-1})	17.5
Exchangeable K (cmol kg^{-1})	0.22
Exchangeable Ca (cmol kg^{-1})	9.41
Exchangeable Mg (cmol kg^{-1})	7.15
Exchangeable Na (cmol kg^{-1})	0.15
Organic C (%)	0.95
Total N (%)	0.072
Available P (mg kg^{-1})	13.0
Available S (mg kg^{-1})	15.0
Available Zn (mg kg^{-1})	1.59
Available Cu (mg kg^{-1})	0.59
Available Fe (mg kg^{-1})	17.9
Available Mn (mg kg^{-1})	3.5

3.5 Climate

The climate of the experimental site is sub-tropical, wet and humid. Heavy rainfall occurs in the monsoon (Mid April to Mid August) and scanty during rest of the year. The weather data regarding rainfall, temperature and relative humidity prevailed during the study period (November 2006 to April 2007) is presented in App. 4.3.1.

3.6 Crop: Blackgram (*Vigna mungo* L.)

3.6.1 Blackgram variety: BARI Mash-3

BARI Mash-3 was developed by Bangladesh Agricultural Research Institute (BARI) and it was released in 1996 by the National Seed Board for stable and high yield with combined resistant to YMV caused by the yellow mosaic virus and *Cercospora* leaf spot (CLS). Plant height of this variety ranges from 35-37 cm; maximum field duration from 70-75 days and average yield from 1600-1800 kg ha^{-1}. This variety possesses the special characteristic of photo-insensivity and synchrony in maturity. Leaves are trifoliate, alternate, and green. Leaf pubescence is present. Petioles are short and purple-green. The corolla is yellowish-green. The raceme position is under the canopy. Mature pods are black with dense pubescence. Seeds are drum-shaped and blackish. It has a 100-seed weight of about 4.8 g. Because of its wide adaptability, the cultivar was recommended for three different blackgram growing seasons [*kharif-II* (August-October), *kharif-I* (February-May) and late rabi (January-April)] for cultivation in all blackgram growing areas in Bangladesh. Seeds of BARI Mash-3 have 86.6% kernel content, but produce 78.5% head dhal (intact kernel after splitting) using the traditional method of dehulling. It takes about 22 minutes to cook and shows solid dispersion of 23.8%. BARI Mash-3 contains 23.9% protein and 46.8% carbohydrate (Bakr *et al.*, 2004).

3.7 Year: 2007 (Kharif-II)

3.8 Treatments: 12 (Twelve)

T_1: Control (Uninoculated and unfertilized)

T_2: PKMo ($P_{22}K_{42}Mo_1$ kg ha^{-1} i.e. $P_{110}K_{210}Mo_5$ mg 10 kg^{-1} soil)

T_3: PKB ($P_{22}K_{42}B$ kg ha^{-1} i.e. $P_{110}K_{210}B_5$ mg 10 kg^{-1} soil)

T_4: Inoculum (1.5 kg Inoculum ha^{-1} i.e. 7.5 mg Inoculum 10 kg^{-1} soil)

T_5: N (N_{50} kg ha^{-1} i.e. N_{250} mg 10 kg^{-1} soil))

T_6: PKMo + Inoculum ($P_{22}K_{42}Mo_1$ kg ha^{-1} i.e. $P_{110}K_{210}Mo_5$ mg 10 kg^{-1} soil + 7.5 mg Inoc.)

T_7: PKB + Inoculum ($P_{22}K_{42}B_1$ kg ha^{-1} i.e. $P_{110}K_{210}B_5$ mg 10 kg^{-1} soil + 7.5 mg Inoc.)

T_8: NPKMo ($N_{50}P_{22}K_{42}Mo_1$ kg ha^{-1} i.e. $N_{250}P_{110}K_{210}Mo_5$ mg 10 kg^{-1} soil)

T_9: NPKB ($N_{50}P_{22}K_{42}B_1$ kg ha^{-1} i.e. $N_{250}P_{110}K_{210}B_5$ mg 10 kg^{-1} soil)

T_{10}: PKMoB ($P_{22}K_{42}Mo_1B_1$ kg ha^{-1} i.e. $P_{110}K_{210}Mo_5B_5$ mg 10 kg^{-1} soil)

T_{11}: PKMoB+Inoc. ($P_{22}K_{42}Mo_1B_1$kg ha^{-1} i.e. $P_{110}K_{210}Mo_5B_5$ mg 10kg^{-1} soil+7.5 mg Inoc.)

T_{12}: NPKMoB ($N_{50}P_{22}K_{42}Mo_1B_1$ kg ha^{-1} i.e. $P_{110}K_{210}Mo_5B_5$ mg 10 kg^{-1} soil)

3.9 Treatment combination and experimental design

There were 12 treatment combinations for each test pot. The experiment was laid out in a complete randomized design (CRD) with three replications and total treatment combinations were 36.

3.10 Pot preparation

To conduct the experiment (35 x 25 cm^2) earthen pots were collected and each pot was poured with 10 kg finely ground of sieved soil. Triple super phosphate, muriate of potash, gypsum and zinc sulphate (Monohydrate) was applied as recommended at the time of pot filling.

3.11 Fertilizer application

Gypsum and zinc sulphate were applied according to the fertilizer recommendation guide (BARC, 2005). Gypsum @ 20 kg S ha^{-1} and zinc sulphate @ 5 kg ha^{-1} were applied as basal in all pots except in control pots. Half amount of urea and full doses of other fertilizers were applied one day before of seeds sowing. The rest half amount of urea was applied at 20 days after sowing (DAS). Nitrogen @ 50 kg N ha^{-1}, triple super phosphate @ 22 kg P ha^{-1}, muriate of potash @ 42 kg K ha^{-1}, boron @ 1 kg B ha^{-1} and molybdenum @ 1 kg Mo ha^{-1} were applied as per treatments of the experiment as recommended levels.

3.12 Preparation and amendment of peat material

The peat soil was collected from Gopalgonj and the pH was measured by glass electrode method. The pH of the peat soil was 4.5 and it was adjusted to 6.8 by adding $CaCO_3$. Fifty grams of amended peat having 8 percent moisture was taken in each polyethylene bag and the bags were sealed up. Then they were sterilized by autoclaving for three consecutive days for one hour each day. The sealed peat was ready for inoculation.

3.13 Inoculum preparation

The rhizobial inoculant was prepared in the Soil Microbiology Laboratory of the Bangladesh Agricultural Research Institute (BARI) using the broth culture. The *Bradyrhizobium* strain (BARI RVm-301) was collected from the stock culture of the laboratory. Yeast extract mannitol broth was prepared in a 500 mL Erlenmeyer flask. The liquid medium was sterilized for 30 minutes at 121^0 C at 15 PSI. The medium was kept for cooling. After cooling, a small portion of *Rhizobium* culture was aseptically transferred from agar slant to the liquid medium in the flask with the help of a sterile inoculation needle. The flask was then placed in the shaker at 28^0 C under 120 rpm to enhance rhizobial growth. After 4-5 days, the medium in the flask showed dense growth and then the broth culture was taken out from the shaker. From this ready broth, 30 mL were taken out by sterile syringe and injected into the polyethylene packet having the sterile peat. Finally, the moisture percent of the packet was adjusted to 50 percent. The inoculated packets were then incubated at 28^0C for two weeks to make them ready for seed inoculation.

3.14 Viability count of *Bradyrhizobium*

Viability count of bradyrhizobia in the inoculant was made one day before injecting the peat following plate count method (Vincent, 1970). The average number of bradyrhizobia was approximately above 10^8 cells g^{-1} in the inoculant. The initial soil bradyrhizobial population was below 10^3 cell g^{-1} of soil.

3.15 Procedure for inoculation

Inoculation was done just before sowing. Healthy blackgram seeds @ 20 seeds for each pot were taken into polyethylene bags separately and a little amount of the sticker solution (4% gum acacia solution) was added to each bag with sterilized pipettes. It was followed by addition of desired peat based *Bradyrhizobium* inoculant to each polyethylene bag and mixed thoroughly for uniform distribution and good adherence of inoculant on the surface of each seed.

3.16 Seed sowing

Healthy blackgram seeds (BARI Mash-3) with inoculated and non-inoculated was sown on 5th September 2007 taking 20 seeds in each pot following dibbling method. After sowing the seed, the soil was saturated with water.

3.17 Intercultural operation

The seedlings of the crop emerged out within 3-4 DAS. Thinning and first weeding were done 5 days after sowing of seeds. In thinning one of the seedlings was removed from each in which both the planted seeds germinated. The germinated seedlings were removed for training. Uprooting was not done since this injures the adjacent seedlings that were left behind. Finally three plants were kept in each pot for final harvesting of the crop. Second weeding was done 20 days after the first weeding. After second weeding 12 plants in each pot was allowed for growth. Necessary water was added to the pots at a regular interval of 7 days until crop maturity to maintain proper moisture content. Pest did not infest the blackgram crop. No disease was observed in the pot experiment.

3.18 Collection of samples

The plant samples were collected at 30, 45 and 60 DAS to observe the response of *Bradyrhizobium*, nitrogen, molybdenum and boron on the growth, nodulation, dry matter production and leaf number of blackgram. The following observations were made regarding the growth, yield and nutrient content from the sample plants during the course of experiment.

3.18.1 Plant

Plant samples were collected at 30, 45 and 60 DAS to record data on nodule and shoot parameters. Three plants from each pot were selected randomly and uprooted carefully by digging soil with the help of "khurpi". All possible precautions were taken to minimize the loss of nodules.

3.18.2 Study on nodulation

The plants uprooted for sampling were washed in running water cautiously to make them free from adhering soil particles and dipped in fresh water contained in a tray to avoid shrinkage of nodules. The nodules were counted, kept separately pot-wise and their dry weights were recorded.

3.18.3 Nodule number and mass

The data on nodule number and nodule mass were recorded by taking 3 randomly selected plants from each pot at different DAS. The data on nodule mass were expressed in mg plant^{-1} on oven dry basis.

3.18.4 Shoot weight and root weight

After separation of the roots, the dry shoot and root weights of three selected plants were recorded.

3.18.5 Shoot length and root length

Shoot length and root length of the plant samples of three selected plants were recorded

3.18.6 Leaf number

Leaf number of the three selected plant were recorded.

3.18.7 Harvesting and data recording on yield and yield contributing characters

Yield data were collected from each pot. The seeds and stover were dried and weighed adjusting at 14% moisture content and yields were converted to g plant^{-1}. The following parameters were recorded:

i) Pod yield (g plant^{-1})

ii) Seed yield (g plant^{-1})

iii) Stover yield (g plant^{-1})

iv) Plant height (cm)

v) Pod length (cm)

vi) Pods plant^{-1}

vii) Seeds pod^{-1}

viii) 100-seed weight (g)

3.18.8 Estimation of N

The N concentrations in seed and stover were determined by micro-Kjeldahl method.

3.19 Plant analysis

3.19.1 Collection and preparation of plant samples for chemical analysis

Plant sample (seed and stover) was collected from bulk harvest. The seed and stover was washed under running tap water followed by rinsing with distilled water to remove surface contamination. The seed and stover was immediately air-dried and was chopped off into smaller pieces. Plant sample were dry at 70-80°C for 72 hours. To obtain homogenous powder, the samples were finely ground and passed through a 60-mesh sieve. The samples were stored in polyethylene bags for N determination.

3.19.2 Chemical analysis of plant samples

Seed and stover of blackgram was analyzed for determination of N concentrations following the methods described below:

Nitrogen

The plant sample (0.1 g grain seed and stover) was digested with conc. H_2SO_4, hydrogen peroxide and K_2SO_4-catalyst mixture (K_2SO_4: $CuSO_4$. $5H_2O$: Se = 10: 1: 0.1) at 200°C for one and a half-hour.

3.20 Nutrient uptake

Nitrogen uptake by seed and stover of blackgram was computed from the respective chemical concentration and dry matter yields of seed and stover.

3.21 Soil analysis

Methods of soil analysis are presented in Table 3.2.

Table 3.2. Methods used for soil analysis

Soil Properties	Methods
Soil texture	Hydrometer method (Black, 1965). The texture class was determined using Marshall's Triangular Coordinates of USDA system
pH	Glass-electrode pH meter with 1:2.5 soil-water ratio (Jackson, 1973).
Organic carbon	Wet digestion method (Nelson and Sommers, 1982). The organic matter was oxidized by 1N potassium dichromate and the amount of organic carbon in the aliquot was determined by titration against 0.5N ferrous sulphate heptahydrate solution in presence of 0.025 M O-phenanthroline ferrous complex.
Total N	Microkjeldhal method (Bremner and Mulvaney, 1982). Soil sample was digested with conc. H_2SO_4 in presence of K_2SO_5 catalyst mixture (K_2SO_4: $CuSO_4$: Se = 10:1:1). Nitrogen in the digest was estimated by distilling the digest with 10N NaOH followed by titration of the distillate trapped in H_3BO_3 indicator solution with 0.01N H_2SO_4.
NH_4^+-N	Extracted by 2M KCl solution (1:10 soil-extractant ratio). The aliquot was steam distilled with MgO and Devardas alloy (Keeney and Nelson. 1982).
CEC	Sodium acetate saturation method (Rhoades. 1982). The soil was leached with an excess of 1 M sodium acetate solution to remove the exchangeable cations and saturate the exchange material with sodium. The replaced sodium was determined by flame photometer.
Available P	Extracted by 0.5M $NaHCO_3$ (pH 8.5) and determined calorimetrically using molybdate blue ascorbic acid method (Olsen and Sommers, 1982).
Available K	Extracted by repeated shaking and centrifugation of the soil with neutral 1M NH_4OAc followed by decantation. The K concentration in the extract was determined by flame photometer as outlined by Knudsen *et al.* (1982).
Available S	Extracted by 500 ppm P solution form $Ca(H_2PO_4)_2$. H_2O and estimated by turbidity method using $BaCl_2$ (Fox *et al.*, 1964.
Available Zn	Extracted by 0.05N HCl solution and determined directly by AAS (Page *et al.*, 1989).
Available Cu Mn and Fe	Extracted by 0.005M DTPA solution and directly measured by AAS (Lindsay and Norvell, 1978).
Bulk density	Core sampling procedure (Black, 1965).
Water holding capacity	Determined gravimetrically using brass box following the method of Klute as described by Black (1965).

3.22 Calculation of protein concentration and protein yield

Protein concentration of blackgram seed and stover was determined by multiplying the concentration of nitrogen (%) in blackgram seed and stover with 6.25.

Protein yield by blackgram seed/stover was competed from protein concentration of seed/stover and seed/stover yields.

3.23 Statistical analysis

The collected data were analyzed statistically and Duncan's Multiple Range Test (DMRT) using a computer IRRISTAT and M-stat package programmes (Freed, 1992) adjudged the means. The correlation co-efficient and regression analysis were done for different variables wherever needed using Microsoft EXCEL programme 1997.

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was carried out during September to December 2007 in the net house of Soil Science Division of the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur with a view to observe the response of *Bradyrhizobium*, nitrogen, molybdenum and boron on the growth, nodulation, yield, nitrogen uptake and other yield contributing characters of blackgram. The results are presented and discussed in this chapter.

4.1 Effect of nodulation

4.1.1 Nodule on main root plant^{-1}

Nodulation is an important factor for biological nitrogen fixation by *Bradyrhizobium* inoculants in blackgram. Nodule is formed on roots of blackgram plants through infection by *Bradyrhizobium* bacterium and it grows inside the nodules, and can fix atmospheric nitrogen. The results on the production of nodule on main root plant^{-1} under different treatments were presented in Figure 4.1 and App. 1. The highest nodules on main root plant^{-1} (5.00, 12.51 and 14.29) were recorded in PKMoB + Inoculum at 30, 45 and 60 DAS, respectively (Fig. 4.1 and App. 1). All the treatments showed significant result in forming nodules on main root than control treatment at 30 DAS. At 45 DAS, the nodules form on main root by control treatment was identical with N, NPKB and NPKMoB, and at 60 DAS, identical with PKMo, PKB, N, NPKMo and NPKB. The highest nodules on main root (14.29 plant^{-1}) were produced at 60 days after sowing (DAS) in PKMoB + Inoculums treatment which indicates that nodule formation on main root increased with the passage of time. At 30 and 45 DAS, the numbers of main root nodules were also observed in PKMoB + Inoculum treatment and the lowest nodules were noted in control. All the fertilizer treatments receiving inoculum, inoculum plus molybdenum, inoculum plus boron and inoculum plus molybdenum plus boron showed statistically significant response in nodule formation on main root over control.

Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate root nodulation of cowpea, blackgram and mungbean, and observed that root nodulation of cowpea, blackgram and mungbean were uniformly better when *Bradyrhizobium* inoculant was applied. Kumari and Nair (2003) found that the extent of improvement in root nodulation was more in blackgram and greengram inoculated with *Bradyrhizobium*. Tanwar *et al.* (2002) reported that the interaction between P rate and biofertilizers was significant in regard to the number of nodules. The inoculation of both biofertilizers along with the application of 60 kg P_2O_5 gave the highest number of nodules $plant^{-1}$ (40.5). Tomar *et al.* (2003) observed that P application and inoculation treatments increased the nodulation in blackgram.

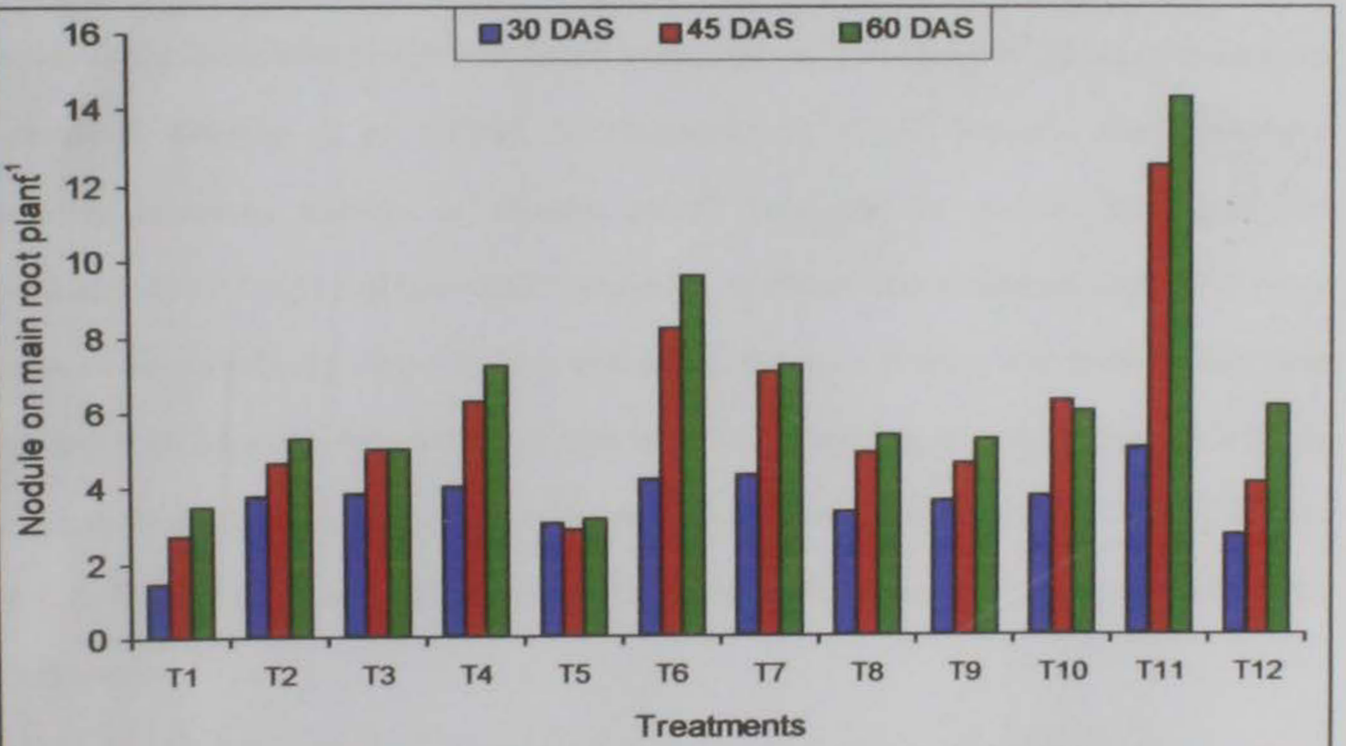

Fig. 4.1. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on main root nodule in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

4.1.2 Nodule on branch root plant^{-1}

Higher number of nodules (3.11 plant^{-1} at 30 DAS and 8.03 plant^{-1} at 45 DAS) on branch root was recorded in PKMoB + inoculum which were significantly higher over all other treatment combinations (Fig. 4.2 and App. 1). At 60 DAS, the highest nodule on branch root (9.03 plant^{-1}) was noted in PKMo + inoculum which were identical with PKMoB + Inoculum but statistically different from all other treatment combinations. Lower number of nodule on branch root plant^{-1} was recorded in each case than the main root in all the DAS. Among the treatments, PKMo + *Bradyrhizobium* and PKB + *Bradyrhizobium* showed similar performances in nodule formation on the branch root at 30 and 45 DAS. Nitrogen has no significant impact on nodule formation on branch root. Tomar *et al.* (2001) reported that phosphorus application increased nodulation in blackgram. The above result was similar with the result at Bhuiyan *et al.* (2006). Jayakumar *et al.* (1997) reported that *Rhizobium* inoculation increased number of nodules plant^{-1} compared to control. Nagarajan and Balachandar (2001) reported that seed inoculation of *Rhizobium* enhanced root nodulation. Tanwar *et al.* (2002) reported that the interaction between P rates and biofertilizers was significant with regard to the number of nodules. The inoculation of both biofertilizers along with the application of 60 kg P_2O_5 gave the highest number of nodules plant^{-1} (40.5). Tomar *et al.* (2003) observed that P application and inoculation treatments increased the nodulation in blackgram.

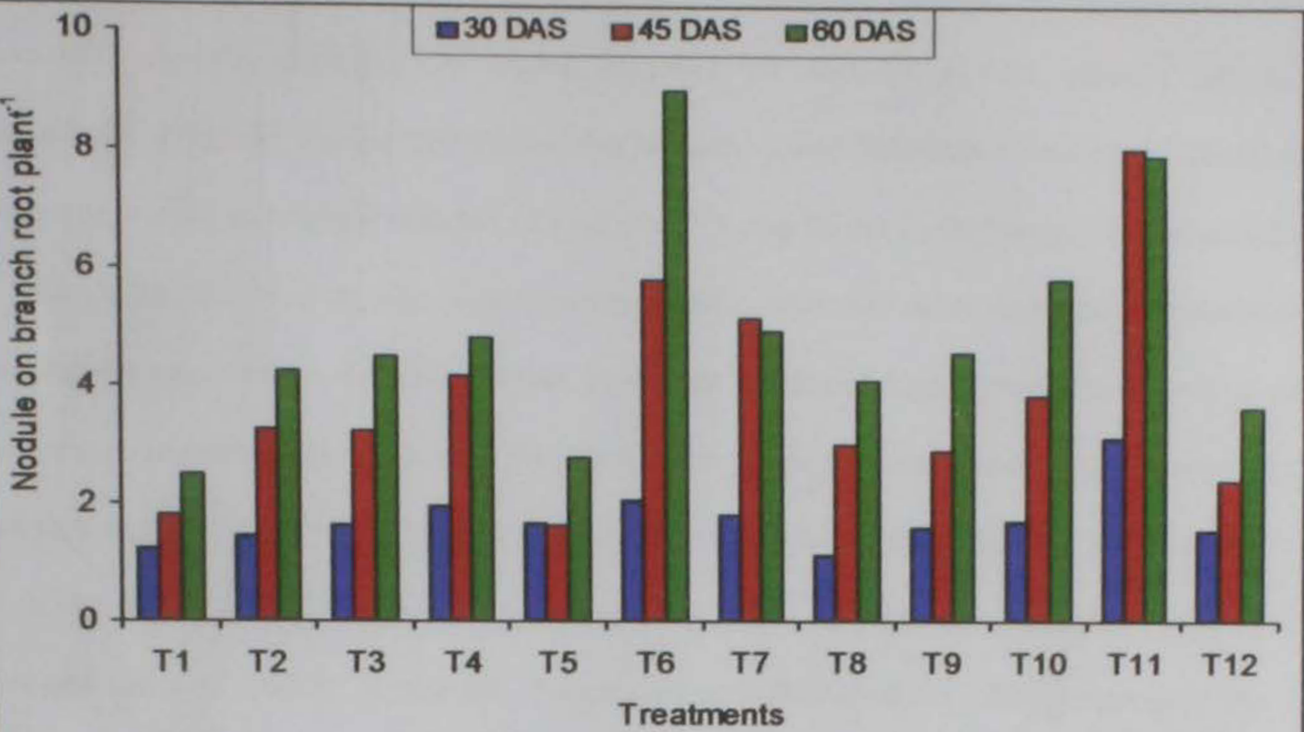

Fig. 4.2. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on branch root nodule in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

4.1.3 Total nodule number plant^{-1}

The highest number of nodules (8.11 at 30 DAS, 20.50 at 45 DAS and 22.22 at 60 DAS) was recorded in the treatment, PKMoB + Inoculum (Fig. 4.3 and App. 1). Among the treatments, *Bradyrhizobium* inoculated plants produced significantly higher number of nodules plant^{-1} than the non-inoculated control plants or N treated plants. It was observed that the highest number of total nodules of 22.22 plant^{-1} was recorded at 60 DAS, which indicates production of nodules plant^{-1} increased with the increasing of time upto 60 DAS. At 30 DAS, the highest number of total nodules 8.11 was noted in PKMoB + Inoculum treatment was which was significantly higher over all other treatment combinations. The second highest number of total nodules was observed in PKMo + Inoculum treatment and the lowest number of total nodules was noted in control. At 45 DAS, the highest number of total nodules (20.50 nodules plant^{-1}) was found in PKMoB + Inoculum treatment that was significantly higher over all other treatment combination. This might be due to synergistic effect of micronutrients with *Bradyrhizobium* inoculant for nodule formation. The treatment, PKMo + Inoculum showed significantly higher number of nodules (14.03 plant^{-1}) which was statistically different from other treatments but inferior to PKMoB + Inoculum. At 60 DAS, the highest total number of nodules (22.22 plant^{-1}) was found in the same treatment (PKMoB + Inoculum) which was also significantly higher over all other treatment combinations. Among the treatments, *Bradyrhizobium* inoculant produced higher number of nodule plant^{-1} than the micronutrients (Mo and B). *Bradyrhizobium* in combination with micronutrient molybdenum or boron should better performance in nodule formation than NPKMo or NPKB at all the three DAS. Nitrogen showed very low effect on nodule formation. It indicates that nitrogen reduced nodule formation. Nagarajan and Balachandar (2001) reported that seed inoculation with *Rhizobium* enhanced root nodulation. Bhattacharyya and Pal (2001) found that inoculation with *Rhizobium* significantly influenced the number of nodules plant^{-1}. Tomar *et al.* (2001) noted that inoculation of *Rhizobium* induced the highest and 21.0% more nodule number in blackgram. Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate root nodulation of cowpea, blackgram and mungbean, and observed that root nodulation of cowpea, blackgram and mungbean were uniformly better at Category. Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and found that inoculation with *Bradyrhizobium* strains increased rate of nodulation and N content. Kumari and Nair (2003) found that the extents of improvement in root nodulation were more in blackgram and greengram inoculated with *Bradyrhizobium*. Bhuiyan and Mian (2007) also found that application of *Bradyrhizobium* inoculant induced significant effect on nodulation. Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India, during the pre-kharif season to study the effect *Bradyrhizobium* inoculation, P (at 0, 20 kg ha^{-1}) and Mo (at 0, 0.5 and 1 kg ha^{-1}) on the number of nodules plant^{-1} of summer greengram cv. T-44 and observed that *Bradyrhizobium* inoculation and application of P and Mo significantly influenced the number of nodules plant^{-1}.

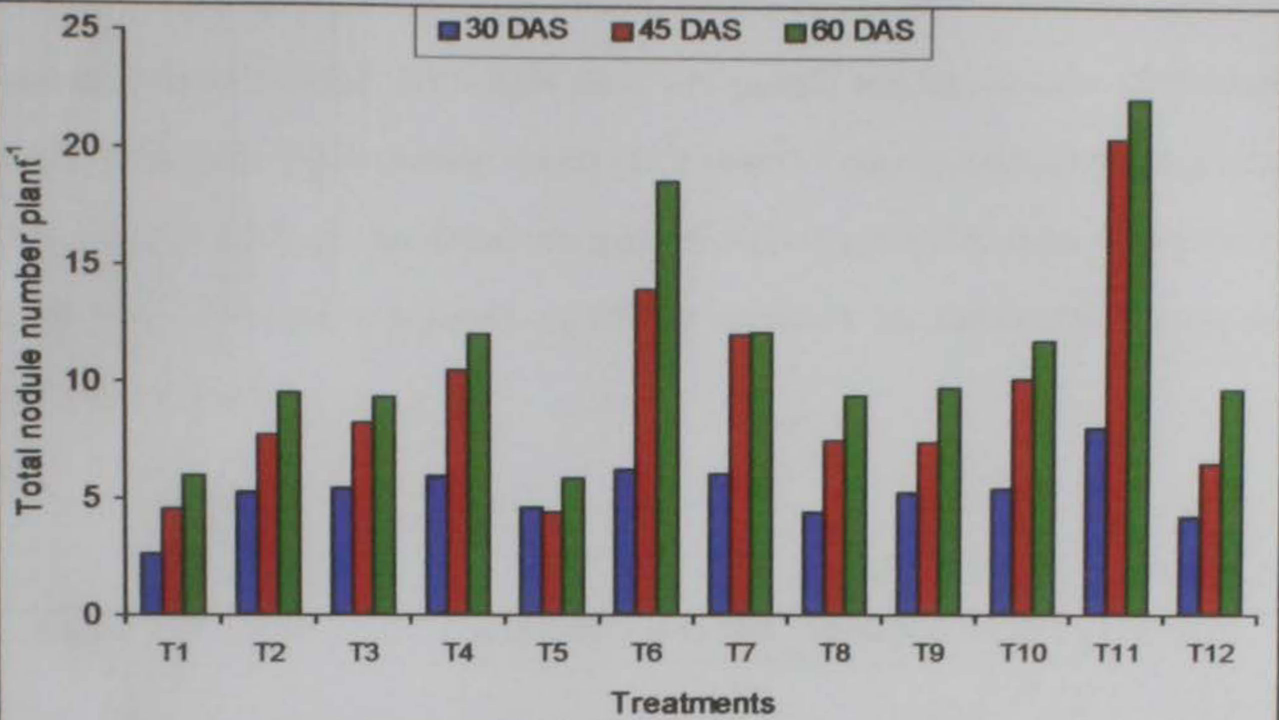

Fig. 4.3. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on total nodule number in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5: N, T_6: PKMo + Inoculum., T_7: PKB + Inoculum, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + Inoculum, T_{12}: NPKMoB

4.1.4 Nodule weight

The highest nodule dry weight (4.00 mg plant^{-1} at 30 DAS, 46.15 mg plant^{-1} at 45 DAS and 65.66 mg plant^{-1} at 60 DAS) was recorded in T_{11} (PKMoB + *Bradyrhizobium*) which was significantly different from all other treatments (Fig. 4.4 and App. 1). Nodule weight increases with the passage of time. Among the treatments, only *Bradyrhizobium* produced higher nodule weight than nitrogen at all the dates of nodule collection. The second highest nodule weight (30.07 mg plant^{-1} at 45 DAS and 48.86 mg plant^{-1} at 60 DAS) was noted in PKMo + Inoculum. The plant which did not take any chemical fertilizers or inoculant recorded the lowest nodule weight at all the three DAS. Nitrogen showed very low effect on nodule weight. When molybdenum and *Bradyrhizobium* inoculated simultaneously showed better result on nodule dry weight. Nagarajan and Balachandar (2002) reported that biodigested slurry at 5 t ha^{-1} + *Rhizobium* produced the highest nodule dry weight (42.3 mg). Tomar *et al.* (2001) who reported that application of phosphorus increased nodule weight in blackgram. Singha and Sarma (2001) reported that *Rhizobium* inoculant had 9.5% higher nodule dry weight than non-inoculated control. Sharma *et al.* (1999) found that application of inoculants increased nodule dry weight plant^{-1}. Nagarajan and Balachandar (2001) reported that *Rhizobium* gave higher nodule weight (45.3 and 42.3 mg) in blackgram and greengram. Tomar *et al.* (2001) found that *Rhizobium* gave the highest and 34.7% more nodule dry mass. Kumari and Nair (2003) observed significant increases in nodule dry weight due to *Rhizobium* inoculation.

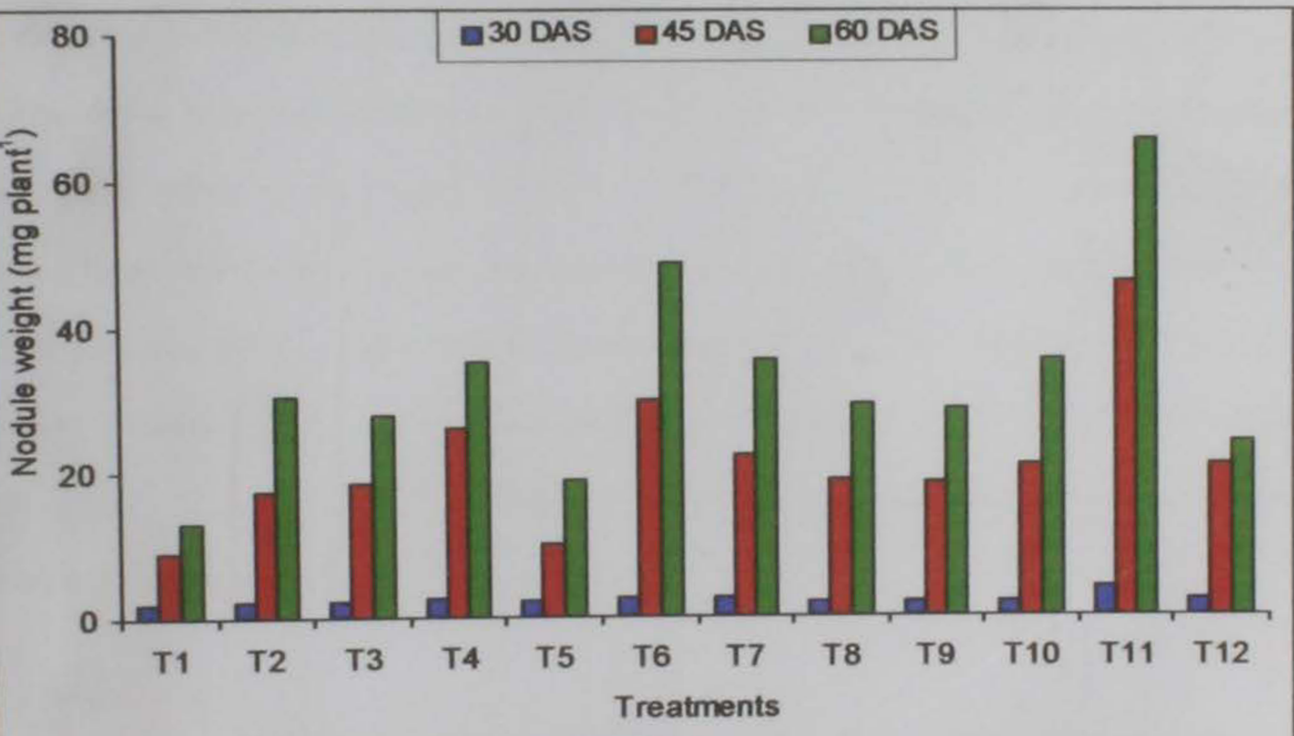

Fig. 4.4. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on nodule weight in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5: N, T_6: PKMo + Inoculum., T_7: PKB + Inoculum, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + Inoculum, T_{12}: NPKMoB

4.2 Root and shoot dry weight

4.2.1 Root weight

Chemical or biofertilizer have no significant effect on root weight at any of the growth stage (Fig. 4.5 and App. 2). The highest root weight (0.17 g plant^{-1}) was obtained in T_{11}, and T_{12} and T_7 at 60 DAS while the non-inoculated and non-fertilized control (T_1) gave the lowest root weight. Among the treatments there was no significant difference but all the treatments produced higher root weight than over control at 60 DAS. Root weight increases with increasing of time. The results are in agreement with the findings of Singh and Singh (2004). Kavathiya and Pandey (2000) conducted a pot experiment during the summer season of 1992-1993 in Gujrat, India and reported that fresh root weight (4.42 g) were recorded in the *Rhizobium* inoculation treatment. Perveen *et al.* (2002) conducted a field experiment to observe the effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiata*) and reported that the maximum root dry weight (0.37 g plant^{-1}) was observed in inoculation with single *Bradyrhizobium* sp. only. Sharma *et al.* (2000) reported that seed inoculated with 1 of 9 *Rhizobium* strains increased dry matter accumulation. Tomar *et al.* (2003) observed that P application and inoculation treatments increased the plant biomass in blackgram.

4.2.2 Shoot weight

The highest shoot weight (2.37 g plant^{-1}) was obtained in T_{11} (PKMoB + *Bradyrhizobium*) and the lowest (1.63 g plant^{-1}) was obtained in T_1 (Control) (Fig. 4.6 and App. 2). *There was no significant difference among the different treatments in shoot weight* at 30 and 60 DAS but shoot weight g plant^{-1} at 45 DAS showed significant difference among the treatments. At 45 DAS, the treatment PKMoB + Inoculum (T_{11}) and NPKMoB produced the highest shoot weight (1.02 g plant^{-1}) while the lowest shoot weight was found in T_1 (Control treatment). Kavathiya and Pandey (2000) reported that maximum fresh shoot weight (5.33 g) was recorded in the *Rhizobium* treated plot. Bhattacharyya and Pal (2001) conducted

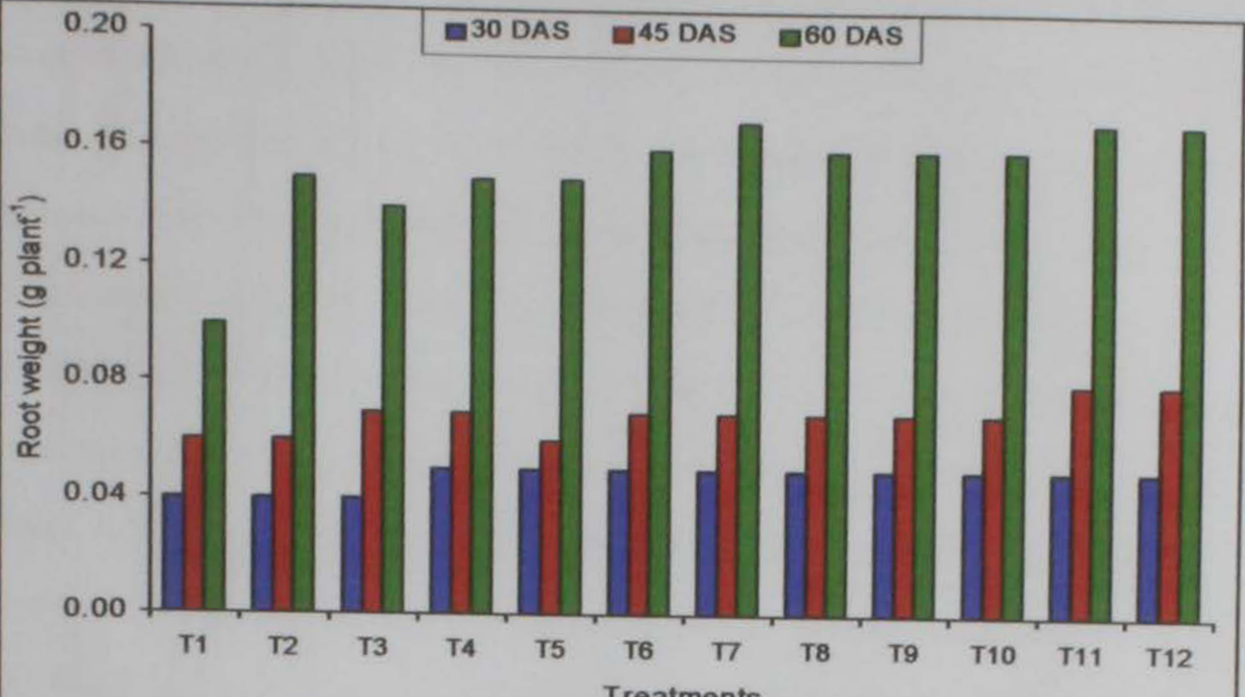

Fig. 4.5. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on root weight in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

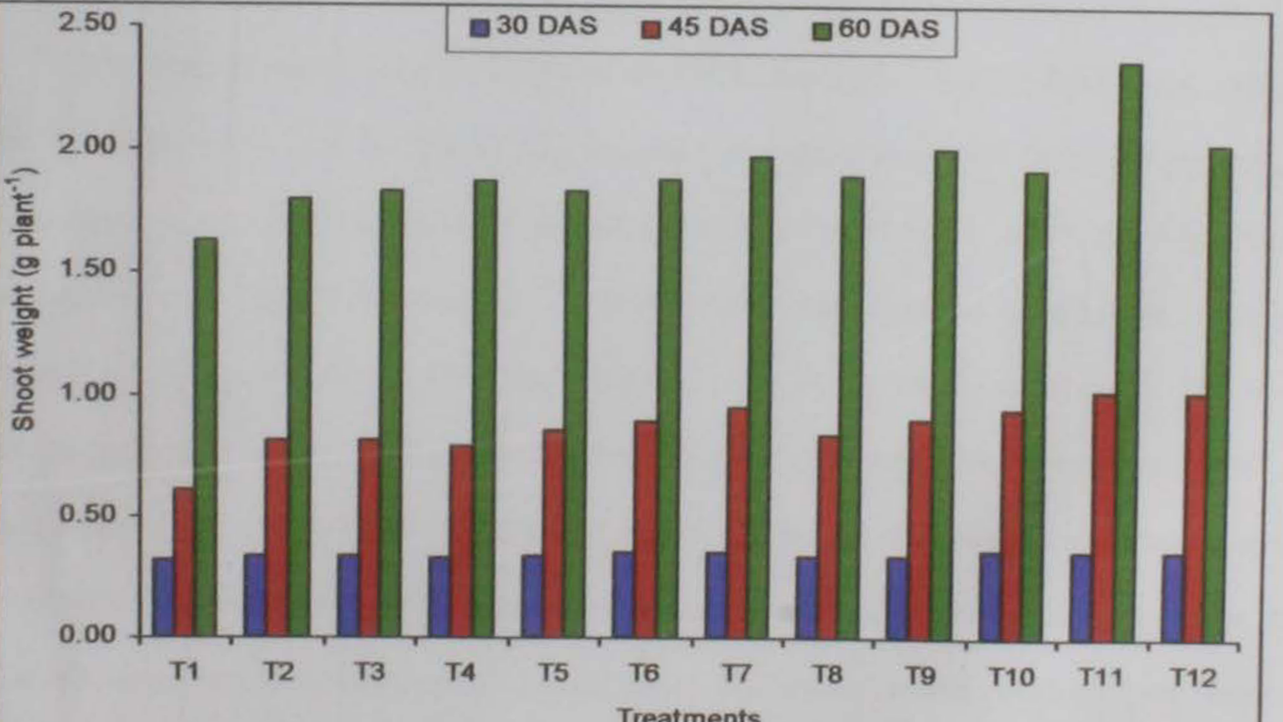

Fig. 4.6. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on shoot weight in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

a field experiment in West Bengal, India, during the pre-*kharif* season of 1998 to study the effect of *Rhizobium* inoculation and reported that inoculation significantly influenced dry matter accumulation in the shoot. Singh and Singh (2004) found that dry matter yield increased with the application of phosphorus. Jayakumar *et al.* (1997) reported that *Rhizobium* inoculation increased the dry weight of plants compared to controls. Srivastav and Poi (2000) found that inoculation with M-10 strain in greengram resulted in the highest dry matter production. Sharma *et al.* (2000) reported that seed inoculated with 1 of 9 *Rhizobium* strains increased dry matter accumulation. Manivannan *et al.* (2003) reported that *Rhizobium* seed treatment produced markedly higher dry matter. Singha and Sarma (2001) reported that P at 45 kg ha^{-1} with *Rhizobium* inoculation produced the highest straw yield and was at par with the application of 25 and 35 kg P ha^{-1}. Tomar *et al.* (2003) observed that P application and inoculation increased the plant biomass in blackgram.

4.3 Root and shoot length and leaves

4.3.1 Root length

The highest root length (8.25 cm at 45 DAS and 9.55 cm at 60 DAS) were obtained from T_{12} (NPKMoB) and the lowest root lengths were noted in control (Fig. 4.7 and App. 3). The second highest root length at 45 and 60 DAS were found in T_{11} (PKMoB + Inoculum). The highest root length observed in T_{12} (NPKMoB) was identical to all other treatments except T_1, T_2, T_3 and T_4 at 45 DAS and except T_1, T_2, T_3, T_4 and T_5 at 60 DAS. The lowest root lengths were obtained in T_1 at all the DAS. At 60 DAS, the highest root length (6.67 cm) was recorded in T_{11} (PKMoB + *Bradyrhizobium*) which was identical to all other treatment combinations. It indicates that combination application of B or Mo and *Bradyrhizobium* has a good influence on root growth of blackgram plant. The above results confirmed the results of Sharma *et al.* (2000) reported that plant growth was increased with *Rhizobium* inoculation, with the local strain giving the best results. Sarker *et al.* (2002) reported that bradyrhizobial strain 480-M resulted the longest roots (14.72 cm). Jayakumar *et al.* (1997) carried out a pot culture experiment where blackgram (*Vigna mungo*) plants were raised from: uninoculated seeds; uninoculated seeds with 250 g pot^{-1} coir-pith compost applied at 15 and 45 days after sowing and found that inoculated seeds increased root length compared to controls (43.3 cm) was found in T_{12} (NPKMoB) which was indicated to all other treatments except T_1, T_2, T_3 and T_4.

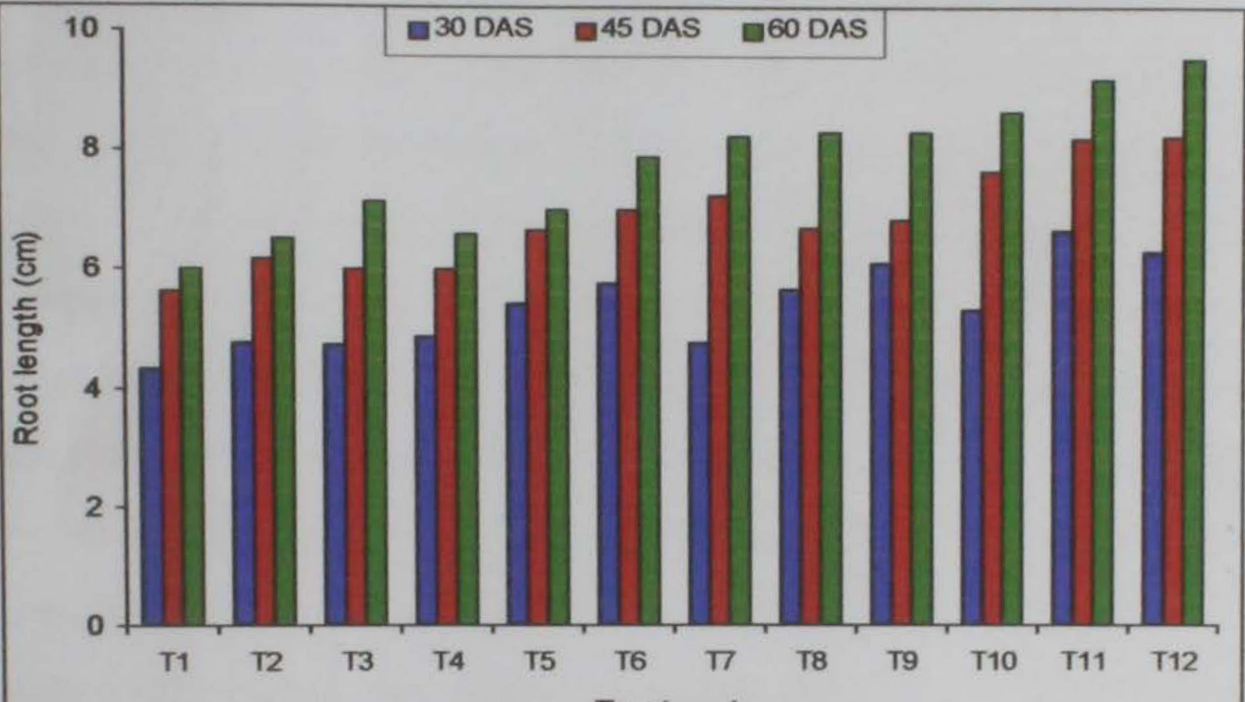

Fig. 4.7. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on root length in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

4.3.2 Shoot length

Bradyrhizobium inoculant alone or in combination with PKMoB had no effect on shoot length at 30 and 60 DAS. Through the highest shoot length (50.4 cm) was obtained from T_{12} (NPKMoB) which was statistically similar with all the treatments (Fig. 4.8 and App. 3). The lowest shoot length was obtained from T_1 (control) at all the DAS. At 45 DAS, the highest shoot length (43.3 cm) was found in T_{12} (NPKMoB) which was indicated to all other treatments except T_1, T_2, T_3 and T_4. Mahmud *et al.* (1997) reported that plant height was significantly increased with increasing phosphorus application. Similar results were observed by Maqsood *et al.* (2001) and Kumar *et al.* (2000). Thakur and Panwar (1995) conducted a field trial where seeds of *Vigna radiata* cv. Pusa-105 and PS-16 were inoculated with inoculant. They found that inoculation either singly or combined increased plant height compared with non-inoculated control treatment. Bhattacharyya and Pal (2001) reported that application of rhizobial inoculum influenced plant height in comparison to control. Nagarajan and Balachandar (2001) conducted a field experiment and observed that seeds treated with bio-digested slurry at 5 t ha^{-1} + *Rhizobium* produced the highest plant height (53.7 cm). Sarkar *et al.* (2002) inoculated the seed of blackgram with strains of *Bradyrhizobium* viz. M-10, 129-USA, 480-M, and MK-5 before sowing in a field experiment conducted to determine the suitable cultivars and *Bradyrhizobium* strain for use in the locality. Cultivars M-16 recorded longer roots and higher root volume $plant^{-1}$, number of nodules $plant^{-1}$ and test weight compared to A-43. 480-M resulted in the longest roots (14.72 cm). Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate plant growth characters of cowpea, blackgram and mungbean and observed that and plant growth characters of cowpea, blackgram and mungbean were uniformly better with inoculation. Kumari and Nair (2003) found that the extent of improvement in plant growth was more in blackgram and mungbean inoculated with *Bradyrhizobium*. Sriramachandrasekharan and Vaiyapuri (2003) conducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on growth of blackgram cv. ADT 3. *Rhizobium*-inoculated blackgram showed better growth than the non-inoculated crop. Mahmud *et al.* (1997) reported that plant height was significantly increased with increasing phosphorus application. Similar results were observed by Maqsood *et al.* (2001) and Kumar *et al.* (2000). Ashraf *et al.* (2003) found that the tallest plants (69.93 cm) were obtained with seed inoculation. Kumari and Nair (2003) observed that the extent of plant growth were more in blackgram and greengram where *Bradyrhizobium* inoculation was done. Sriramachandrasekharan and Vaiyapuri (2003) reported that *Rhizobium*-inoculated blackgram showed better growth than the non-inoculated crop.

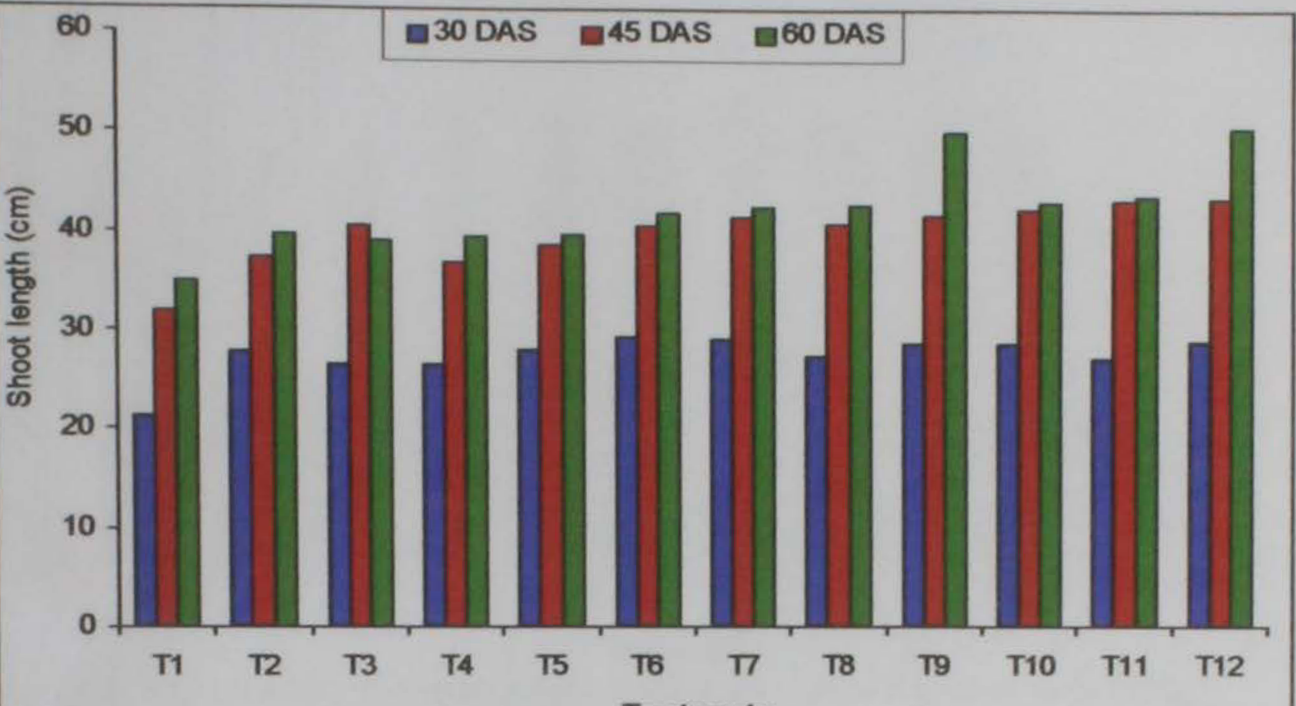

Fig. 4.8. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on shoot length in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5: N, T_6: PKMo + Inoculum., T_7: PKB + Inoculum, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + Inoculum, T_{12}: NPKMoB

4.3.3 Leaves plant^{-1}

Fertilizers did not show any remarkable influence on the number of leaves plant^{-1} at 30, 45 and 60 DAS, respectively through the highest leaves plant^{-1} was obtained from T_{11} (PKMoB + *Bradyrhizobium*) and the lowest leaves plant^{-1} was obtained from T_1 (control) at 30 DAS (Fig. 4.9 and App. 3). At 45 and 60 DAS, the highest number of leaves were found in T_{11} (PKMoB + Inoculum) and the lowest leaves were found in control. Sriramachandrasekharan and Vaiyapuri (2003) conducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on the nodulation, growth, and yield of blackgram cv. ADT 3. *Rhizobium*-inoculated blackgram showed higher leave plant^{-1} than the non-inoculated crop.

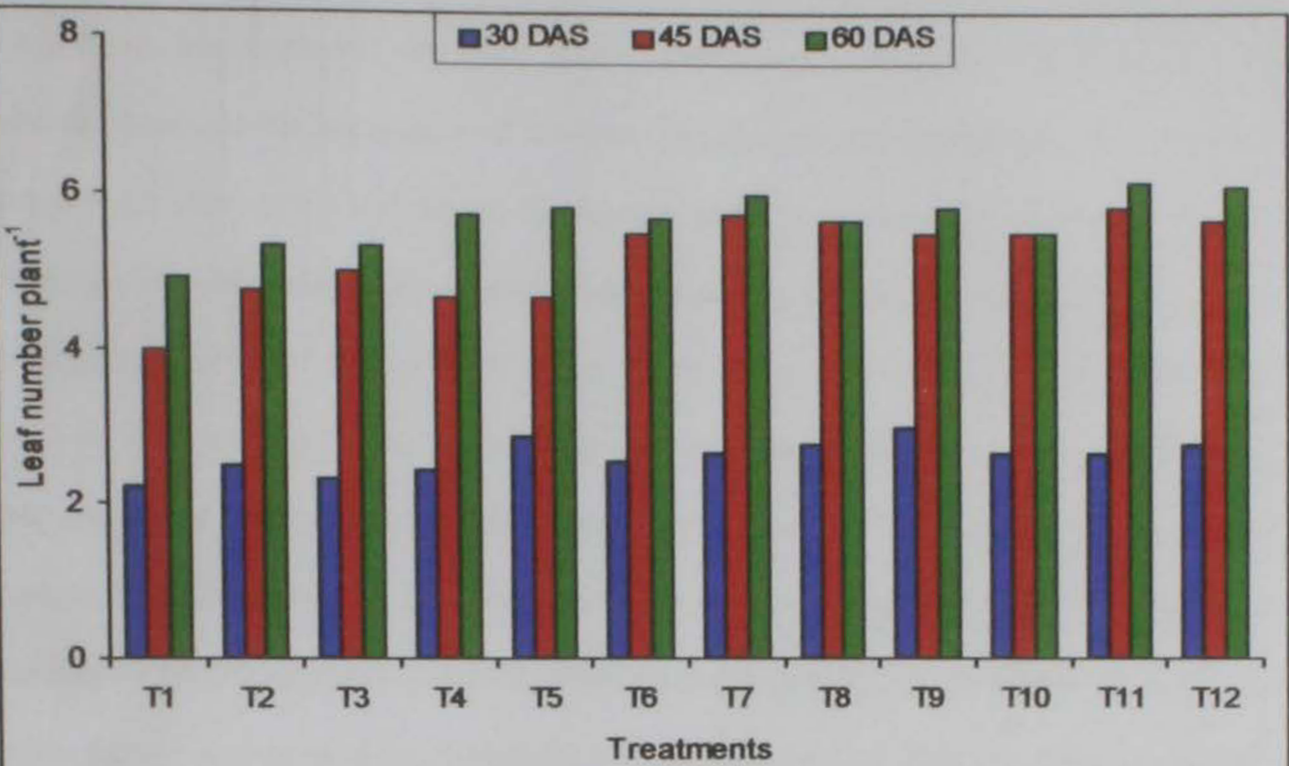

Fig. 4.9. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on leaf number in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5: N, T_6: PKMo + Inoculum., T_7: PKB + Inoculum, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + Inoculum, T_{12}: NPKMoB

4.4 Growth and yield attributes

4.4.1 Plant height

The highest plant height (44.3 cm) was obtained from T_{12} (NPKMoB) which was identical to all other treatments but significantly higher than T_1 (control) and Inoculum treatment (Table 4.1). Among the nutrient elements and *Rhizobium*, B micronutrient gave 38.9 cm plant height, which was higher than the other nutrient elements N (37.2 cm), Mo (38.1) and *Bradyrhizobium* (35.0 cm). But, combined application of both the micro nutrient element with *Bradyrhizobium* gave better plant height (40.7 cm) than individual application of Mo, B or *Bradyrhizobium*. Mahmud *et al.* (1997) reported that phosphorus application increased plant height in blackgram. The result corroborated with the findings of Maqsood *et al.* (2001) and Kumar *et al.* (2000). Thakur and Panwar (1995) found that inoculation either singly or combined increased plant height compared with no inoculation. Bhattacharyya and

Pal (2001) reported that application of rhizobial inoculum influenced plant height comparing with control. Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate plant growth characters of cowpea, blackgram and mungbean, and observed that plant growth characters of cowpea, blackgram and mungbean were uniformly better when *Rhizobium* inoculant was applied. Sriramachandrasekharan and Vaiyapuri (2003) conducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on growth of blackgram cv. ADT 3 and observed that *Rhizobium*-inoculated blackgram showed better growth than the non-inoculated crop. Sharma *et al.* (2000) reported that the growth was increased with *Rhizobium* inoculation and the local strain gave the best results. Nagarajan and Balachandar (2001) reported that *Rhizobium* gave the greatest plant height (42.7 and 53.7 cm for blackgram and greengram, respectively). Bhattacharyya and Pal (2001) reported that *Rhizobium* inoculation significantly influenced plant height. Malik *et al.* (2002) studied that plant height at harvest was the highest when inoculated with *Bradyrhizobium* (68.13 cm). Kumari and Nair (2003) observed that the extent of plant growth were more in blackgram and greengram where *Bradyrhizobium* inoculation was done.

4.4.2 Pod length

All the fertilizer treatments showed insignificant response in terms of pod length. The highest pod length (4.23 cm) was recorded in treatment T_{12} (NPKMoB) that was identical to all other treatment combinations but different from T_1 and the lowest pod length of 3.28 cm was recorded in control plot (Table 4.1). Shil *et al.* (2007) reported that the highest pod length was the highest in full doses of fertilizers while control plants recorded the lowest pod length. Srinivas and Shaik (2002) studied that the interactions effects between N and P were not significant for pod length.

Table 4.1. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on yield attributes of blackgram

Treatment	Plant height (cm)	Pod length (cm)	Pods plant^{-1}	Seeds pod^{-1}	100-seed weight (g)
T_1: Control	29.3c	3.28b	4.89c	2.25	5.08
T_2: PKMo	38.1ab	3.67ab	6.77abc	3.17	5.13
T_3: PKB	38.9ab	3.80ab	7.05ab	2.97	5.33
T_4: Inoculum	35.0bc	3.86a	6.94ab	3.78	5.54
T_5: N	37.2ab	3.79ab	6.55bc	3.70	5.63
T_6: PKMo+Inoculum	40.7ab	3.80ab	7.88ab	3.70	5.59
T_7: PKB+Inoculum	40.7ab	4.20a	7.33ab	3.67	5.40
T_8: NPKMo	38.0ab	3.80ab	7.55ab	3.86	5.64
T_9: NPKB	38.1ab	3.87a	7.66ab	3.51	5.62
T_{10}: PKMoB	38.9ab	4.13a	7.55ab	3.49	5.57
T_{11}: PKMoB+Inoculum	40.3ab	4.16a	8.55ab	4.01	5.89
T_{12}: NPKMoB	44.3a	4.23a	8.78a	3.93	5.81
LSD (0.05)	6.4	0.50	1.83	NS	NS
CV (%)	9.9	7.6	14.8	19.4	12.2

In a column, means followed by a common letter are not significantly differed at 5% level by DMRT

NS = Not significant

4.4.3 Pods plant^{-1}

Number of pods plant^{-1} in different treatments showed significant variation. The highest pod number (8.78) was obtained from T_{12} (NPKMoB), which was significantly higher than control (T_1) (4.89) but identical to all other treatments (Table 4.1). Between two micronutrient, B showed better performance (7.05 pods plant^{-1}) than Mo but combined application of both the micronutrient Mo and B with inoculum (*Bradyrhizobium*) gave better pods number (7.88 and 7.33, respectively) rather than individual application of both the micronutrient. Malik *et al.* (2002) reported that seed inoculation with *Rhizobium* resulted in the highest number of pods plant^{-1} (22.47). Ashraf *et al.* (2003) observed that seed inoculation + 50 : 50 : 0 or 50 : 50 : 50 kg N: P: K ha^{-1} resulted in the highest number of pods plant^{-1} (28.97, 56.00, 63.90 and 32.56, respectively). Bhuiyan *et al.* (2008a) reported that *Bradyrhizobium* inoculation in mungbean plots also significantly increased pods plant^{-1}.

4.4.4 Seeds pod^{-1}

No significant response was observed due to application of different fertilizers in seeds pod^{-1}. The highest seeds pod^{-1} (4.01) was obtained from T_{11} (PKMoB + Inoculum) and the lowest seeds pod^{-1} (2.25) was obtained from T_1 (control) (Table 4.1). Bhuiyan *et al.* (2008a) observed that *Bradyrhizobium* inoculation also significantly increased seeds pod^{-1}. Shil *et al.* (2007) reported that seeds pod^{-1} was the highest in full doses of fertilizers while control plants recorded the lowest seeds pod^{-1}. Srinivas and Shaik (2002) conducted trial on seed inoculation in *Rhizobium* culture and reported that *Rhizobium* inoculation in mungbean increased the number of seeds pod^{-1}.

4.4.5 100-seed weight (g)

There was no significant relationship among the different treatments in 100-seed weight. The highest 100-seed weight of 5.89 was obtained from T_{11} (NPKMoB + Inoculum) and the lowest 100-seed weight of 5.08 g was obtained from control treatment (T_1). Srinivas and Shaik (2002) reported that 1000-seed weight generally increased due to rhizobial inoculation. Shil *et al.* (2007) reported that 1000-seed weight was the highest in full doses of fertilizers while control plants recorded the lowest in 1000-seed weight. Bhuiyan *et al.* (2008a) opined that *Bradyrhizobium* inoculation also significantly increased 1000-seed weight. Malik *et al.* (2002) studied that seed inoculation with *Rhizobium* application resulted in the highest 1000-seed weight (42.27g).

4.5 Yield characteristics

4.5.1 Pod weight

All the treatment showed insignificant result in pod weight. The highest pod weight of 3.87 g plant^{-1} was obtained from T_{11} (PKMoB + Inoculum) and the lowest pod weight (1.97

g) was obtained from T_1 (control) (Table 4.2). Mahmud *et al.* (1997) reported that weights of pod plant^{-1} significantly increased with increasing phosphorus application. The results are in agreement with Kumar *et al.* (2000), Maqsood *et al.* (2001), Tomar *et al.* (2001), Singh *et al.* (2002), Patel and Thakur (2003), Singh and Singh (2004) and Singh (2004). Sriramachandrasekharan and Vaiyapuri (2003) conducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on yield of blackgram cv. ADT 3. *Rhizobium*-inoculated blackgram showed higher pod yield (50.3 g) than the uninoculated crop. Kumari and Nair (2003) found that the extents of improvement in yield were more in blackgram and greengram inoculated with *Bradyrhizobium*. Srivastav and Poi (2000) found that NK-4 inoculation into blackgram resulted in the highest grain yield. Sharma *et al.* (2000) reported that yield increased with *Rhizobium* inoculation. Nagarajan and Balachandar (2001) reported that *Rhizobium* gave higher grain yield (758.3 and 732.0 kg ha^{-1}) in blackgram and greengram respectively. Malik *et al.* (2002) studied that seed inoculation with *Rhizobium* resulted in the highest seed yield (1158 kg ha^{-1}). Singha and Sarma (2001) reported that P at 45 kg ha^{-1} with *Rhizobium* inoculation produced the highest grain yield and was at par with the application of 25 and 35 kg P ha^{-1}. Tanwar *et al.* (2002) reported that the interaction between P rate and biofertilizers was significant with regard to the seed yield. The inoculation of both biofertilizers along with the application of 60 kg P_2O_5 gave the highest seed yield. Srinivas and Shaik (2002) studied that the interactions effects between N and P were not significant for seed yield. Tomar *et al.* (2003) observed that P application and inoculation treatments increased grain yields in blackgram.

Table 4.2. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on yield of blackgram

Treatment	Pod weight (g plant^{-1})	Stover weight (g plant^{-1})	Seed weight (g plant^{-1})	Seed yield increase over control (%)
T_1: Control	1.97	2.22c	1.40c	-
T_2: PKMo	3.31	3.36abc	2.31ab	65.0
T_3: PKB	3.41	3.32abc	2.22ab	58.7
T_4: Inoculum	2.72	2.95bc	2.04abc	45.7
T_5: N	2.71	3.54ab	1.96bc	40.0
T_6: PKMo+Inoculum	3.48	3.81ab	2.66ab	92.1
T_7: PKB+Inoculum	3.53	3.74ab	2.58ab	84.3
T_8: NPKMo	3.40	3.83ab	2.49ab	77.9
T_9: NPKB	3.62	3.88ab	2.58ab	84.3
T_{10}: PKMoB	3.35	3.63ab	2.50ab	78.6
T_{11}: PKMoB+Inoculum	3.87	4.47a	2.69a	90.0
T_{12}: NPKMoB	3.71	4.30^a	2.62ab	87.1
LSD (0.05)	NS	1.07	0.63	-
CV (%)	21.8	17.6	15.8	-

In a column, means followed by a common letter are not significantly differed at 5% level by DMRT
NS = Not significant

4.5.2 Stover weight

Different fertilizers had significant effect on stover weight. The highest stover weight of 4.47 g plant^{-1} was obtained from T_{11} (PKMoB + Inoculum) and the lowest of 2.22 g was obtained from T_1 (control) (Table 4.2). Bhuiyan and Mian (2007) reported that application of *Bradyrhizobium* inoculant produced significant effect on stover weight. Nagarajan and Balachandar (2001) reported that seed inoculation of *Rhizobium* enhanced biomass. Sriramachandrasekharan and Vaiyapuri (2003) reported that *Rhizobium*-inoculated blackgram showed higher stover yield pot^{-1} (81.1 g) than the non-inoculated crop. Srinivas and Shaik (2002) opined that seed inoculation with *Bradyrhizobium* culture enhanced haulm yield in mungbean. Singha and Sarma (2001) reported that P at 45 kg ha^{-1} with *Rhizobium* inoculation produced the highest straw yield and was at par with the application of 25 and 35 kg P ha^{-1}. Tomar *et al.* (2003) observed that P application and inoculation treatments increased the plant biomass and yield in blackgram.

4.5.3 Seed weight

The highest seed weight (2.69 g) was obtained from T_{11} (PKMoB + Inoculum) and the lowest seed weight (1.40 g) was obtained from T_1 (control) (Table 4.2). The second highest seed weight (2.62 g) was found in T_{12} (NPKMoB). Application of PKMo + Inoculum showed better performance than NKPMo for seed weight. On the other hand, PKB + Inoculum all showed higher seed yield than NPKB. Only inoculum gave better seed yield than application of only N. Same trend was observed in T_{11} and T_{12} where *Bradyrhizobium* inoculant in combination with PKMoB perform better than NPKMoB. It indicates that instead of applying urea N, *Bradyrhizobium* inoculant gave better result. Kumari and Nair (2003) found that yields were more in blackgram and greengram inoculated with *Bradyrhizobium*. Sharma *et al.* (1999) performed a field experiment in 1997/98 in Himachal Pradesh and *V. mungo* cv. Pant U-19 was seed inoculated with one of eleven *Rhizobium* strains or not inoculated and given 0 or 20 kg N ha^{-1}. Seed yield was highest with inoculation with a local strain (1.30 t ha^{-1}). The application of 20 kg N gave higher seed yield than no N (1.24 vs. 1.14 t). Sriramachandrasekharan and Vaiyapuri (2003) observed that *Rhizobium*-inoculated blackgram showed higher pod yield (50.3 g) than the non-inoculated crop. Mathan *et al.* (1996) conducted a field experiment during the monsoon seasons of 1991-92 at Coimbatore, Tamil Nadu where a total of 7 treatments compared the effect of applying in various combinations N, P, K, FYM, NAA and seed inoculation with *Rhizobium* were used on blackgram (*Vigna mungo*) cv. CO 5 yield. In 1992, seed treatment with fungicides was also included. The application of 25 kg N as urea + 50 kg P_2O_5 ha^{-1} as single superphosphate + 750 kg enriched FYM + 6.25 t FYM + foliar application of 25 kg diammonium phosphate at flower initiation and 15 days later + seed inoculation with *Rhizobium* produced the highest seed yields of 0.72 t ha^{-1} in

1991 and 0.62 t in 1992, compared with the control yields of 0.42 and 0.40 t, respectively. Singha and Sarma (2001) reported that P at 45 kg ha^{-1} with *Rhizobium* inoculation produced the highest grain yield and was at par with the application of 25 and 35 kg P ha^{-1}. Tanwar *et al.* (2002) reported that the interaction between P rate and biofertilizers was significant with regard to the seed yield. The inoculation of both biofertilizers along with the application of 60 kg P_2O_5 gave the highest seed yield. Srinivas and Shaik (2002) studied that the interaction effects between N and P were not significant for seed yield. Tomar *et al.* (2003) observed that P application and inoculation treatments increased grain yields in blackgram. Tanwar *et al.* (2003a) observed that the application of 60 kg P ha^{-1} along with inoculation of *Rhizobium* gave the highest seed yield (10.93 q ha^{-1}).

4.6. Nitrogen and protein content

4.6.1 Nitrogen content in seed

All the treatments varied significantly in nitrogen content in seed. The highest nitrogen content in seed 3.30% was obtained from T_{11} (PKMoB + Inoculum) and T_{12} (NPKMoB), which was significantly different from control, PKMo, PKB, N and PKMoB (Table 4.3). Among the micronutrients (Mo and B), N and Inoculum (*Bradyrhizobium*) the highest N content (3.27%) in seed obtained from *Bradyrhizobium* inoculated plant. Combined application of micronutrient (Mo and B) with *Bradyrhizobium* produce significantly higher N content in seed than individual application of both the micronutrient. Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and reported that the plants inoculated with *Bradyrhizobium* strains and PSB showed increased rate of N content. The lowest N uptake (34.7 kg ha^{-1}) in seed was recorded in non-inoculated control. Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/without *Bradyrhizobium* inoculation and observed that inoculation significantly increased nitrogen uptake of mungbean. Srivastav and Poi (2000) also stated that inoculation with M-10 strain in greengram resulted in the highest nitrogen fixation. Reddy and Mallaiah (2001) opined that the nitrogen content of the fresh seeds was 5.78% in the inoculated plants, while that in non-inoculated controls was only 2.72%. Chatterjee and Bhattacharjee (2002) noted that plants inoculated with *Bradyrhizobium* strains showed increased N content. Tomar *et al.* (2003) observed that P application and inoculation treatments increased N content in blackgram. Tanwar *et al.* (2003b) found that the N contents increased with increasing P rate up to 80 kg ha^{-1} with inoculation in blackgram.

Table 4.3. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on nitrogen and protein content in seed and stover of blackgram

Treatment	N content in seed (%)	N content in stover (%)	Protein content in seed (%)	Protein content in stover (%)
T_1: Control	3.15e	1.43f	19.7e	8.94f
T_2: PKMo	3.19d	1.48d	19.9d	9.25de
T_3: PKB	3.20d	1.46e	20.0d	9.13e
T_4: Inoculum	3.27abc	1.52ab	20.4abc	9.50ab
T_5: N	3.26bc	1.51abc	20.4bc	9.44abc
T_6: PKMo+Inoculum	3.28ab	1.47de	20.5ab	9.19de
T_7: PKB+Inoculum	3.29ab	1.49bcd	20.6ab	9.34bcd
T_8: NPKMo	3.29ab	1.48de	20.6ab	9.25de
T_9: NPKB	3.28ab	1.49cd	20.5ab	9.31cd
T_{10}: PKMoB	3.24c	1.49cd	20.2c	9.31cd
T_{11}: PKMoB+Inoculum	3.30a	1.53a	20.6a	9.56a
T_{12}: NPKMoB	3.30a	1.52ab	20.6a	9.50ab
LSD (0.05)	0.03	0.03	0.19	0.16
CV (%)	0.60	1.0	0.60	1.0

In a column, means followed by a common letter are not significantly differed at 5% level by DMRT

4.6.2 Nitrogen content in stover

All the treatments showed positive response in N content in stover. The highest N content (1.53%) was obtained from T_{11} (PKMoB + Inoculum) and the lowest N content

(1.43%) in stover was obtained from T_1 (control) (Table 4.3). Srivastav and Poi (2000) conducted field experiments to determine the symbiotic efficiencies of greengram (*V. radiata*) and blackgram (*V. mungo*) after inoculation with a native *Bradyrhizobium* strain and the residual effects of 7 *Bradyrhizobium* strains (NG-13/1, M-10, Kuthi AR-1, Jca-1, Caj-3, NK-4 and Caj6/1) in neutral pH soil, in Mohanpur, West Bengal, India. Symbiotic variations of greengram and blackgram were observed due to the host and inoculant strains. Inoculation with M-10 strain in greengram resulted in the highest dry matter production and nitrogen fixation, while NK-4 inoculation into blackgram resulted in the highest nitrogen uptake and grain yield.

4.6.3 Protein content in seed

Protein content in seed showed positive result by different treatments. The highest protein content (20.6%) in seed were obtained from T_{11} and T_{12} which significantly different from control plot (19.7%) (Table 4.3). The interaction effect between micronutrient and *Bradyrhizobium* were significant. Sharma *et al.* (1999) performed a field experiment in 1997/98 in Himachal Pradesh and *V. mungo* cv. Pant U-19 was seed inoculated with one of eleven *Rhizobium* strains or not inoculated and given 0 or 20 kg N ha^{-1}. They reported that no significant effects were observed in protein content of seed and straw but a slight improvement was observed over control. Mathan *et al.* (1996) conducted a field experiment during the monsoon seasons of 1991-92 at Coimbatore, Tamil Nadu where a total of 7 treatments compared the effect of applying in various combinations N, P, K, FYM, NAA and seed inoculation with *Rhizobium* were used on blackgram (*Vigna mungo*) cv. CO 5 yield and reported that seed crude protein content was increased by 14.5 and 15.4% in the highest yielding treatment compared with the controls.

4.6.4 Protein content in stover

Protein content in stover was significantly increased due to the combined application of PKMoB + Inoculum (Table 4.3). The highest protein content in stover (9.56%) was recorded at T_{11} (PKMoB + Inoculum) which was identical to NPKMoB, Inoculum and N but superior to all other treatments. The lowest protein content in stover (8.94%) was recorded at non-inoculated and non-fertilized control.

4.7 Nitrogen uptake and protein yield

4.7.1 Nitrogen uptake by seed

Nitrogen uptake by seed of blackgram was significantly increased due to combined application of fertilizers (Fig. 4.10 and App. 4). The highest N uptake (88.1 mg $plant^{-1}$) was recorded at T_{11} (PKMoB + Inoculum) which was identical all other treatment combinations except Control and N. The lowest N uptake in seed (44.2 mg $plant^{-1}$) was recorded in control. Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/without *Bradyrhizobium* inoculation and observed that inoculation significantly increased nitrogen uptake of mungbean. Srivastav and Poi (2000) found that inoculation with NK-4 into blackgram resulted in the highest nitrogen uptake. Tomar *et al.* (2003) observed that P application and inoculation treatments in blackgram increased N uptake by wheat significantly. Tanwar *et al.* (2003b) found that the N uptake increased with increasing P rate up to 80 kg ha^{-1} with inoculation in blackgram.

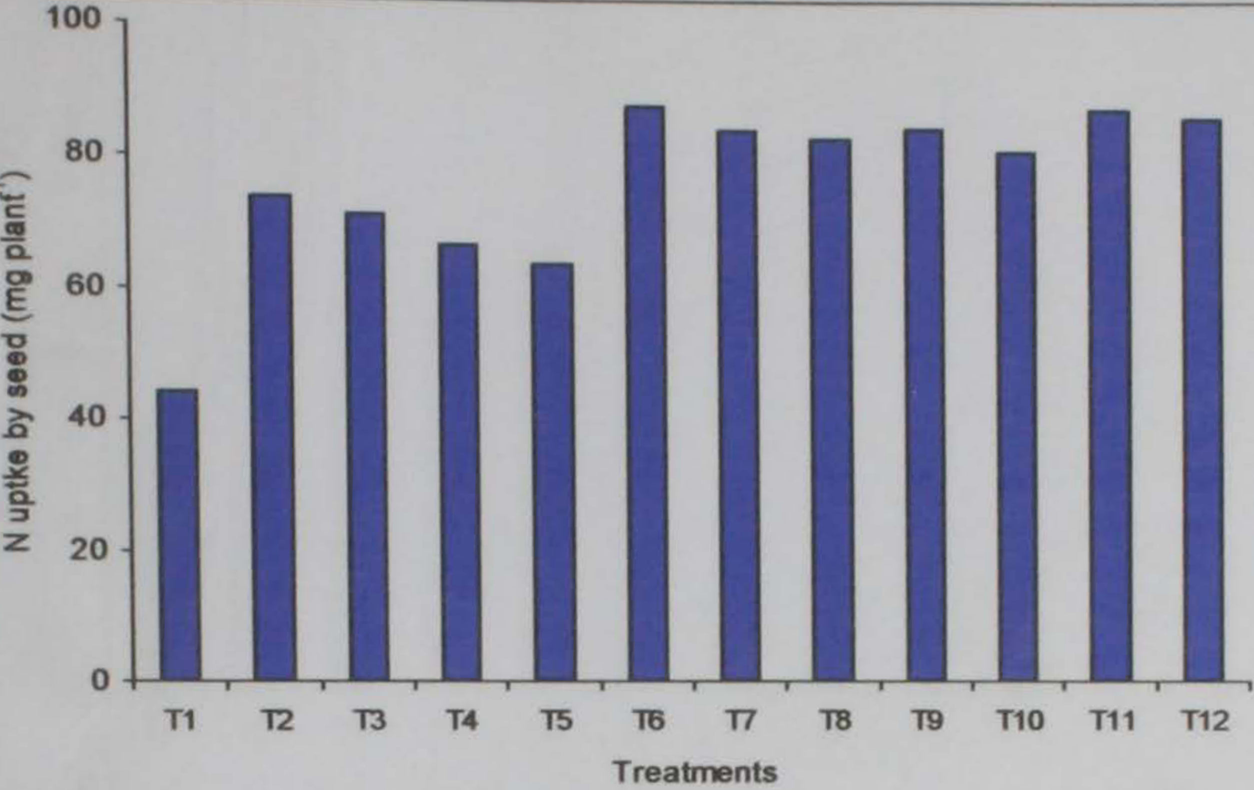

Fig. 4.10. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on N uptake by seed in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5: N, T_6: PKMo + Inoculum., T_7: PKB + Inoculum, T_8: NPKMo, T_9: NPKB, T_{10}: PKMoB, T_{11}: PKMoB + Inoculum, T_{12}: NPKMoB

4.7.2 Nitrogen uptake by stover

Application of fertilizers and *Bradyrhizobium* inoculants showed significant variation in N uptake by stover of blackgram (Fig. 4.11 and App. 4). The highest amount of N uptake in stover (68.3 mg plant) was recorded in T_{11} (PKMoB + Inoculum) which was different from control, PKMo, PKB, Inoculum only. The lowest N uptake in stover (31.7 mg plant^{-1}) was recorded in control. Srivastav and Poi (2000) conducted a field experiment to determine the symbiotic efficiencies of greengram (*Vigna radiata*) and blackgram (*Vigna mungo*) and found symbiotic variations due to the effect of both the host and inoculant strains. Inoculation with M-10 strain in greengram resulted in the highest dry matter production and nitrogen fixation.

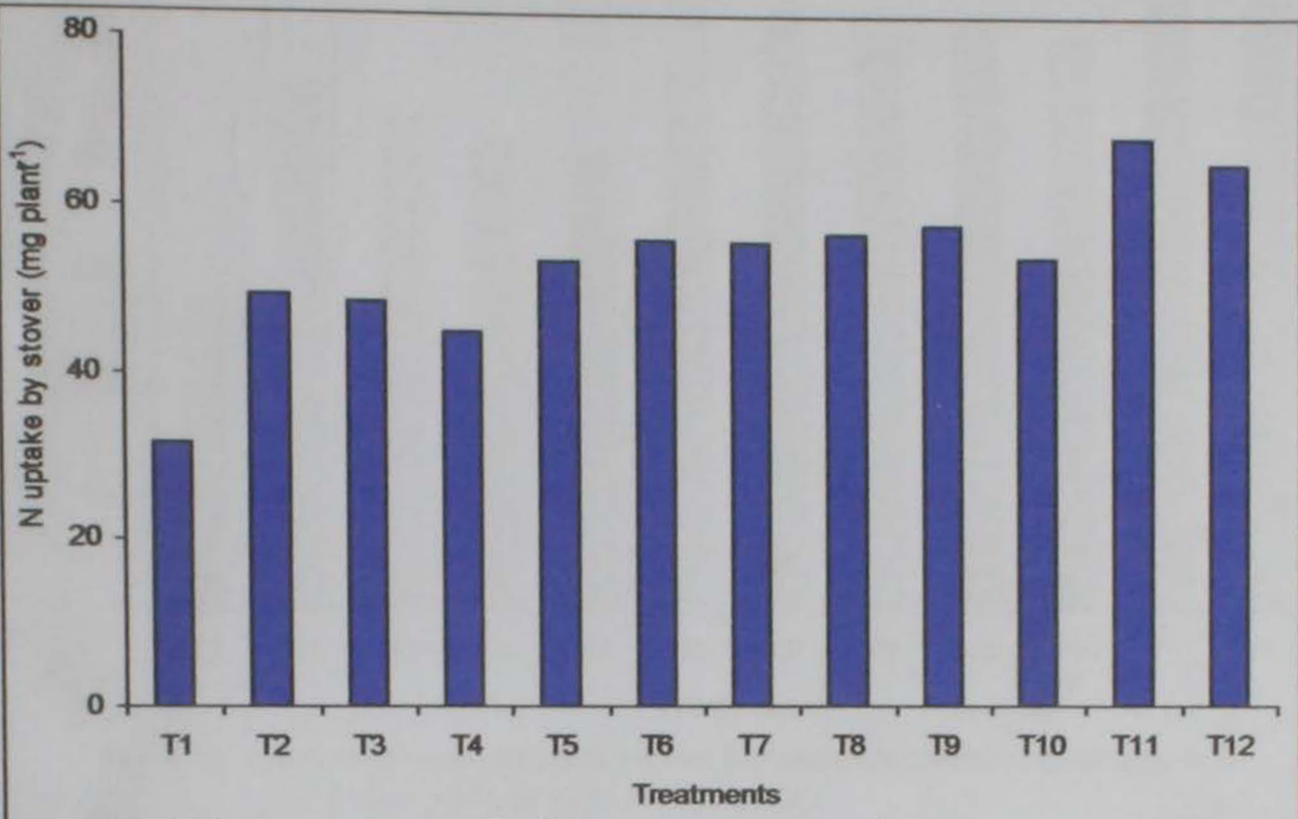

Fig. 4.11. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on N uptake by stover in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

4.7.3 Protein yield by seed

Chemical fertilizers and *Bradyrhizobium* had significant effect on protein yield by seed (Fig. 4.12 and App. 4). The highest protein yield by seed was recorded at T_{11} was (551 mg plant^{-1}) which was identical to all other fertilizer treatments except control and the lowest protein yield by seed (276 mg plant^{-1}) was noted in control. Bhuiyan *et al.* (2007b) found that inoculation significantly increased protein yield by seed.

4.7.4 Protein yield by stover

The highest amount of protein yield by stover (427 mg plant^{-1}) was noted in T_{11} (PKMoB + Inoculum) which was statistically different from control, PKMo, PKB and Inoculum only (Fig. 4.13 and App. 4). The lowest protein yield by stover (193 mg plant^{-1}) was recorded in control. Bhuiyan *et al.* (2007b) found that inoculation significantly increased the protein yield in mungbean and blackgram.

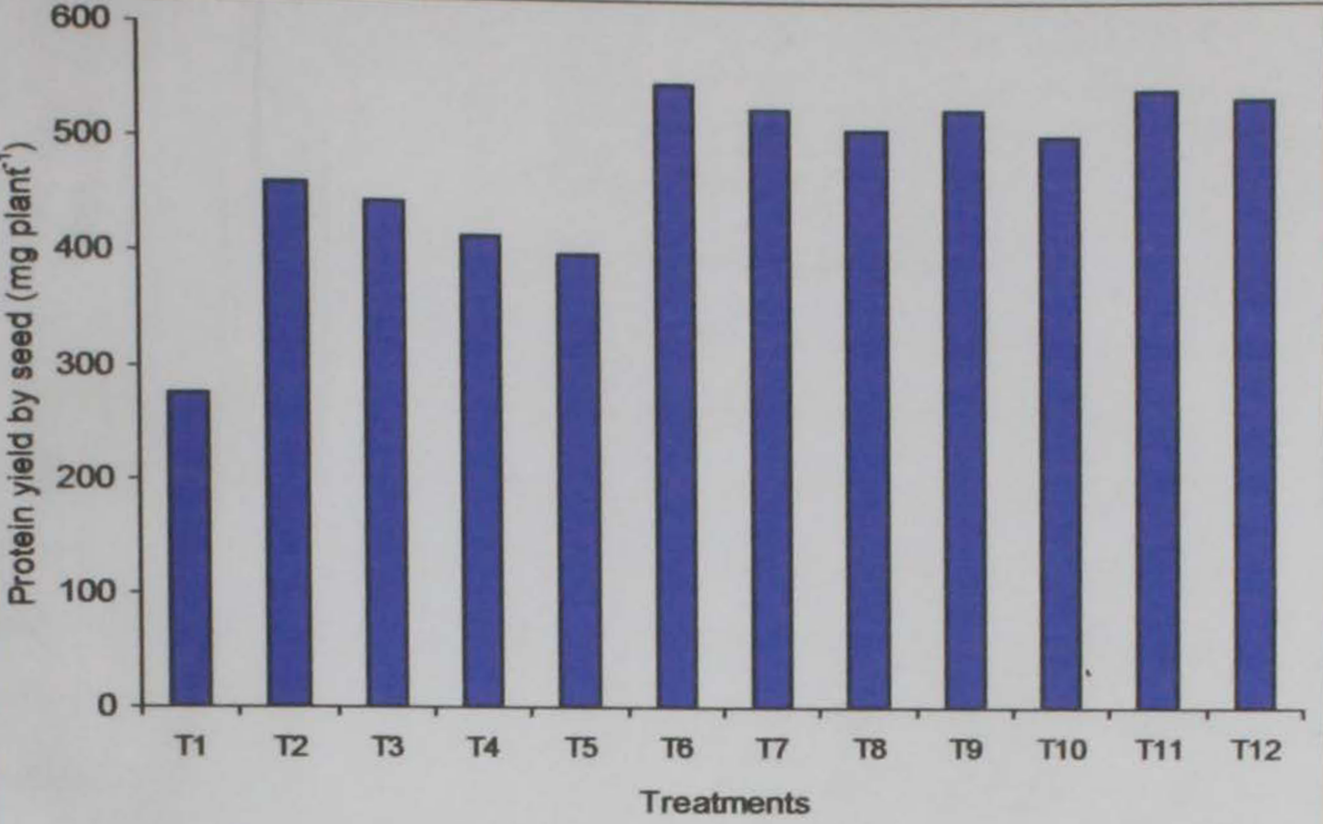

Fig. 4.12. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on protein yield by seed in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

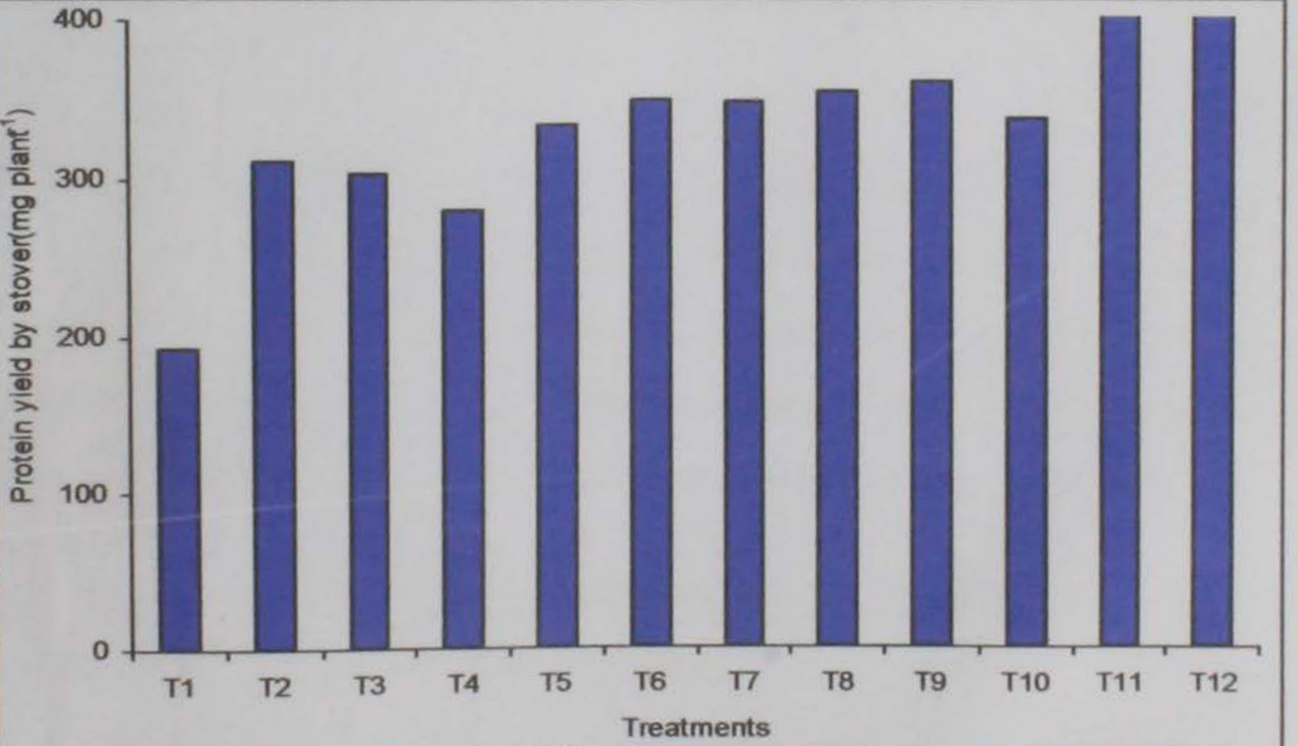

Fig. 4.13. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (Mo & B) on protein yield by stover in blackgram

T_1: Control, T_2: PKMo, T_3: PKB, T_4: Inoculum, T_5 : N, T_6 : PKMo + Inoculum., T_7 : PKB + Inoculum, T_8 : NPKMo, T_9 : NPKB, T_{10} : PKMoB, T_{11}: PKMoB + Inoculum, T_{12} : NPKMoB

CHAPTER V

SUMMARY AND CONCLUSION

A pot experiment was conducted at Net House of Soil Science Division, the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during September to December 2007 to evaluate the effect of *Bradyrhizobium* inoculation, nitrogen, molybdenum and boron on the growth, nodulation, yield and nitrogen uptake, and yield contributing characters of blackgram and to find out the suitable combination of *Bradyrhizobium*, nitrogen, molybdenum and boron for blackgram production followed by CRD technique. Triple super phosphate (22 kg P ha^{-1}), muriate of potash (42 kg K ha^{-1}), gypsum (20 kg S ha^{-1}), zinc sulphate (5 kg Zn ha^{-1}) and boric acid (1 kg B ha^{-1}) were applied as basal dose at the time of pot preparation. All the fertilizers were calculated for 10 kg soil which was used for each pot. Blackgram seeds were inoculated with respective inoculants and were sown on 1^{st} September, 2007. Necessary water was applied to the pot at a regular interval of alternate days upto crop maturity to maintain proper moisture condition.

Data for nodule number, nodule on main root, nodule on branch root, nodule weight, root weight, shoot weight, root length, shoot length and leaf number were recorded at three stage of growth viz. 30. 45 and 60 DAS, and for seed and stover and other yield attributing data characters were taken at the time of plant maturity. Nitrogen content, protein content, nitrogen uptake and protein yield data were taken after chemical analysis of plant and seed samples.

Abridged account of the different plant characters were analyzed statistically and the significant mean differences were adjusted by DMRT.

The highest nodules on main root plant^{-1} (5.00, 12.51 and 14.29) were recorded in PKMoB + Inoculum at 30, 45 and 60 DAS, respectively. All the inoculated treatments showed significant result in informing nodules on main root than control plant. The highest number of nodules (3.11 plant^{-1} at 30 DAS and 8.03 plant^{-1} at 45 DAS) on branch root was recorded in PKMoB + Inoculum which were significantly higher over all other treatment combinations. Nitrogen has no significant impact on nodule formation on branch root. *Bradyrhizobium* inoculated plants produced significantly higher number of nodules plant^{-1} than the non-inoculated control plants or N treated plants. The highest number of total nodules of 22.22 plant^{-1} was recorded at 60 DAS, which indicates production of nodules plant^{-1} increased with the increasing of time upto 60 DAS. It was observed that *Bradyrhizobium* inoculation and application of Mo significantly influenced the number of nodules plant^{-1}. Nodule weight increases with the passage of time. Among the treatments, only *Bradyrhizobium* produced higher nodule weight than nitrogen at all the dates of nodule collection.

The highest root weight (0.17 g plant^{-1}) was obtained in T_{11}, T_{12} and T_7 at 60 DAS while the non-inoculated and non-fertilized control (T_1) gave the lowest root weight. Among the treatments there was no significant difference but all the treatments produced higher root weight than control at 60 DAS. No significant effect on shoot weight with was observed at 30 and 60 DAS due to *Bradyrhizobium* inoculation though the highest shoot weight (2.37 g plant^{-1}) at 60 DAS was obtained in T_{11} (PKMoB + *Bradyrhizobium*) and the lowest (1.63 g plant^{-1}) was obtained in T_1 (Control). The highest root length observed in T_{12} (NPKMoB) was identical to all other treatments except T_1, T_2, T_3 and T_4 at 45 DAS and except T_1, T_2, T_3, T_4 and T_5 at 60 DAS. The lowest root lengths were obtained in T_1 at all the DAS. *Bradyrhizobium* inoculant alone or incombination with PKMoB had no effect on shoot length at 30 and 60 DAS. Leaves plant^{-1} has no significant effect by different fertilizers at 30, 45 and 60 DAS, respectively though the highest leaves plant^{-1} was obtained from T_{11} (PKMoB + *Bradyrhizobium*) and the lowest leaves plant^{-1} was obtained from T_1 (control) at 30 DAS. The highest plant height (44.3 cm) at harvesting stage was obtained from T_{12} (NPKMoB) which was identical to all other treatments but different from T_1 (control) and Inoculum.

All the fertilizer treatments showed insignificant response in terms of pod length. The highest pod length (4.23 cm) was recorded in treatment T_{12} (NPKMoB) that was identical to all other treatment combinations but different from T_1 and the lowest pod length of 3.28 cm was recorded in control plant. All the fertilizer treatments showed significant response in pods plant^{-1} except T_2 and T_5. The highest pod number (8.78) was obtained from T_{12} (NPKMoB) which was significantly different from control (T_1) (4.89) and T_5 but identical to all other treatments. No significant response due to application of different fertilizer was observed for seeds pod^{-1}. There was no significant relationship among the different treatments in 100-seed weight. The highest 100-seed weight of 5.89 was obtained from T_{11} (NPKMoB + Inoculum) and the lowest 100-seed weight of 5.08 g was obtained from control treatment (T_1).

The highest pod weight of 3.87 g plant^{-1} was obtained from T_{11} (PKMoB + Inoculum) and the lowest pod weight (1.97 g) was obtained from T_1 (control). *Bradyrhizobium* inoculaiton had significant effect on blackgram stover weight. The highest stover weight of 4.47 g plant^{-1} was obtained from T_{11} (PKMoB + Inoculum) and the lowest of 2.22 g plant^{-1} was obtained from T_1 (control). The highest seed weight (2.69 g) was obtained from T_{11} (PKMoB + Inoclum) and the lowest seed weight (1.40 g) was obtained from T_1 (control).

All the treatment showed significant response in nitrogen content in seed. The highest nitrogen content in seed (3.30%) was obtained from T_{11} (PKMoB + Inoculum) and T_{12} (NPKMoB), which was significantly different from control, PKMo, PKB, N and PKMoB. The highest N content (1.53%) was obtained from T_{11} (PKMoB + Inoculum) and the lowest N content (1.43%) in stover was obtained from T_1 (control). Protein content in seed showed positive result among the different treatments.

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App. Table 1. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on nodulation in blackgram

Treatment	30 DAS				45 DAS				60 DAS			
	Total nodule number plant^{-1}	Nodule on main root plant^{-1}	Nodule on branch root plant^{-1}	Nodule weight (mg plant^{-1})	Total nodule number plant^{-1}	Nodule on main root plant^{-1}	Nodule on branch root plant^{-1}	Nodule weight (mg plant^{-1})	Total nodule number plant^{-1}	Nodule on main root plant^{-1}	Nodule on branch root plant^{-1}	Nodule weight (mg plant^{-1})
T_1: Control	2.67 e	1.44 f	1.23 cd	2.01 c	4.53 g	2.72 g	1.81 f	9.15 e	6.00 d	3.50 de	2.50 e	13.11 e
T_2: PKMo	5.23 bcd	3.78 bc	1.45 bcd	2.21 bc	7.75 def	4.65 def	3.27 def	17.43 d	9.55 c	5.31 cd	4.24 b-e	30.65 c
T_3: PKB	5.45 bc	3.83 bc	1.62 bcd	2.25 bc	8.25 def	5.03 de	3.22 def	18.56 d	9.33 c	5.00 cde	4.50 b-e	28.00 cd
T_4: Inoculum	6.00 b	4.03 bc	1.97 b	2.55 bc	10.51 cd	6.31 cd	4.20 bcd	26.28 bc	12.07 c	7.23 c	4.84 bcd	35.21 c
T_5: N	4.67 cd	3.00 de	1.67 bcd	2.30 bc	4.50 g	2.88 fg	1.62 f	10.12 e	5.93 d	3.12 e	2.81 de	18.79 de
T_6: PKMo+Inoculum	6.23 b	4.17 b	2.06 b	2.72 b	14.03 b	8.22 b	5.81 b	30.07 b	18.62 b	9.59 b	9.03 a	48.86 b
T_7: PKB+Inoculum	6.11 b	4.28 b	1.83 bc	2.74 b	12.05 bc	7.03 bc	5.17 bc	22.53 cd	12.17 c	7.21 c	4.96 bc	35.51 c
T_8: NPKMo	4.45 cd	3.33 cde	1.12 d	2.07 c	7.52 ef	4.86 de	3.01 def	18.81 d	9.44 c	5.33 cd	4.11 b-e	29.32 cd
T_9: NPKB	5.23 bcd	3.62 bcd	1.61 bcd	2.08 c	7.49 ef	4.57 d-g	2.92 def	18.73 d	9.81 c	5.25 cd	4.58 b-e	28.90 cd
T_{10}: PKMoB	5.45 bc	3.73 bc	1.72 bcd	2.11 c	10.16 cde	6.30 cd	3.86 cde	20.86 cd	11.83 c	6.00 c	5.83 b	35.49 c
T_{11}: PKMoB+Inoculum	8.11 a	5.00 a	3.11 a	4.00 a	20.50 a	12.51 a	8.03 a	46.15 a	22.22 a	14.29 a	7.93 a	65.66 a
T_{12}: NPKMoB	4.23 d	2.67 e	1.56 bcd	2.28 bc	6.51 fg	4.10 efg	2.41 ef	20.87 cd	9.75 c	6.13 c	3.62 cde	24.25 cd
LSD (0.05)	1.02	0.68	0.53	0.49	2.57	1.71	1.53	5.26	2.88	1.95	1.88	10.52
CV (%)	11.3	11.1	18.2	12.0	16.0	17.5	24.0	14.4	15.0	17.8	22.6	18.9

App. Table 2. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on root and shoot dry weight of blackgram

Treatment	30 DAS		45 DAS		60 DAS	
	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})
T_1: Control	0.04	0.32	0.06	0.61c	0.10b	1.63
T_2: PKMo	0.04	0.34	0.06	0.81abc	0.15a	1.80
T_3: PKB	0.04	0.34	0.07	0.81abc	0.14a	1.83
T_4: Inoculum	0.05	0.33	0.07	0.79bc	0.15a	1.87
T_5: N	0.05	0.34	0.06	0.85ab	0.15a	1.83
T_6: PKMo+Inoculum	0.05	0.35	0.07	0.89ab	0.16a	1.88
T_7: PKB+Inoculum	0.05	0.35	0.07	0.95ab	0.17a	1.98
T_8: NPKMo	0.05	0.34	0.07	0.84ab	0.16a	1.90
T_9: NPKB	0.05	0.34	0.07	0.90ab	0.16a	2.01
T_{10}: PKMoB	0.05	0.36	0.07	0.94ab	0.16a	1.92
T_{11}: PKMoB+Inoculum	0.05	0.36	0.08	1.02a	0.17a	2.37
T_{12}: NPKMoB	0.05	0.36	0.08	1.02a	0.17a	2.03
LSD (0.05)	NS	NS	NS	0.19	0.04	NS
CV (%)	21.9	10.2	22.8	12.8	15.1	12.9

App. Table 3. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on root length, shoot length and leaf number of blackgram

Treatment	30 DAS			45 DAS			60 DAS		
	Root length (cm)	Shoot length (cm)	Leaves plant^{-1}	Root length (cm)	Shoot length (cm)	Leaves plant^{-1}	Root length (cm)	Shoot length (cm)	Leaves plant^{-1}
T_1: Control	4.33	21.4	2.22	5.63c	32.0c	4.00	6.00d	35.0	4.93
T_2: PKMo	4.78	27.8	2.50	6.17bc	37.3b	4.75	6.53cd	39.7	5.33
T_3: PKB	4.75	26.6	2.33	6.00bc	40.5ab	5.00	7.16bcd	39.0	5.33
T_4: Inoculum	4.89	26.6	2.44	6.00bc	37.0b	4.67	6.60cd	39.5	5.75
T_5: N	5.44	28.1	2.89	6.66abc	38.70ab	4.66	7.00bcd	39.8	5.83
T_6: PKMo+Inoculum	5.78	29.3	2.56	7.00abc	40.5ab	5.50	7.90abc	41.9	5.70
T_7: PKB+Inoculum	4.78	29.1	2.67	7.25abc	41.5ab	5.75	8.25abc	42.5	6.00
T_8: NPKMo	5.67	27.5	2.78	6.70abc	40.7ab	5.67	8.33abc	42.8	5.66
T_9: NPKB	6.11	28.6	3.00	6.83abc	41.7ab	5.50	8.33abc	50.0	5.83
T_{10}: PKMoB	5.34	28.6	2.67	7.66ab	42.3a	5.50	8.66ab	43.0	5.50
T_{11}: PKMoB+Inoculum	6.67	27.1	2.67	8.20a	43.2a	5.83	9.20a	43.7	6.16
T_{12}: NPKMoB	6.33	29.0	2.78	8.25a	43.3a	5.67	9.55a	50.4	6.10
LSD (0.05)	NS	NS	NS	1.61	4.44	NS	1.63	NS	NS
CV (%)	21.4	9.80	15.3	13.8	6.6	21.3	12.4	15.0	12.8

App. Table 4. Effect of nitrogen, *Bradyrhizobium* inoculant and micronutrients (molybdenum and boron) on nitrogen uptake by seed and stover, and protein yield by seed and stover of blackgram

Treatment	N uptake by seed (mg plant^{-1})	N uptake by stover (mg plant^{-1})	Protein yield by seed (mg plant^{-1})	Protein yield by stover (mg plant^{-1})
T_1: Control	44.2c	31.7d	276c	193d
T_2: PKMo	73.8ab	49.4bc	461ab	311bc
T_3: PKB	71.2ab	48.5bcd	445ab	303bc
T_4: Inoculum	66.7ab	44.9cd	417ab	280cd
T_5: N	64.0bc	53.4abc	400bc	334abc
T_6: PKMo+Inoculum	88.1a	56.1abc	551a	350abc
T_7: PKB+Inoculum	84.5ab	55.8abc	529ab	349abc
T_8: NPKMo	82.9ab	56.8abc	512ab	355abc
T_9: NPKB	84.7ab	57.8abc	530ab	361abc
T_{10}: PKMoB	81.1ab	54.1abc	507ab	338abc
T_{11}: PKMoB+Inoculum	87.7a	68.3a	548a	427a
T_{12}: NPKMoB	86.3ab	65.4ab	540ab	408ab
LSD (0.05)	20.2	15.9	127	100
CV (%)	15.7	17.6	15.7	17.6