Legume Res., **37** (2) : 180 - 187, 2014
DOI: 10.5958/j.0976-0571.37.2.027

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

NUTRIENT MANAGEMENT IN MUNG BEAN (*VIGNA RADIATA* L.) THROUGH SULPHUR AND BIOFERTILIZERS

Lal Bahadur* and D.D. Tiwari

Department of Soil Science and Agricultural Chemistry,
C. S. Azad University of Agriculture and Technology, Kanpur-208 002, India

Received: 12-12-2012 Accepted: 27-04-2013

ABSTRACT

To study the effect of nutrient management in mung bean through sulphur and biofertilizers, a field experiment was carried out during 2006 and 2007. The treatments contained four levels of sulphur i.e. 0, 15, 30, 45 kg ha^{-1} and four levels of biofertilizers *viz.* Uninoculated, *Rhizobium*, PSB and *Rhizobium* + PSB with 4 replications in factorial RBD. Results indicated that application of sulphur @ 30 kg ha^{-1} significantly increased the plant height (44.6 cm) , primary branches (5.18), effective pods plant^{-1} (27.8), grains pods^{-1} (11.0), 1000 grain wt. (35.7 g), grain and stover yield (12.0 and 28.1 q ha^{-1}), nitrogen uptake (86.05 kg ha^{-1}), phosphorus uptake (10.06 kg ha^{-1}), sulphur uptake (7.71 kg ha^{-1}) and quality parameters like protein (24.56 %) and methionine (5.96 g/ 100 g N) in comparison to sulphur @ 15 kg ha^{-1}. Application of sulphur @ 15 kg S ha^{-1} significantly and linearly decreased the PSB population. Actinomycetes population was not significantly affected by the treatments. However, sulfur application showed a diminishing trend in the population of actinomycetes. *Rhizobium* and PSB inoculation also showed significant response to all the parameters and showed better response when co-inoculated (*Rhizobium* + PSB). However, total bacterial population (41.7 x 10^{6} g^{-1} soil), *Rhizobium* like organism (RLO) population (13.9 x 10^{3} g^{-1} soil) and *Azotobacter* population (12 x 10^{3} g^{-1} soil) significantly increased up to the level of 15 kg S ha^{-1}. *Rhizobium* and PSB inoculation showed significant response to all the parameters and showed better response when co-inoculated with *Rhizobium* + PSB.

Key words: Actinomycetes, *Azotobacter*, Biofertilizers, Mung bean, PSB, *Rhizobium*, Sulphur.

INTRODUCTION

Sulphur a key element of higher pulse production, is required in the formation of proteins, vitamins and enzymes. Sulphur is a constituent of the amino acids- cystine, cysteine and methionine. Besides, it is also involved in various metabolic and enzymatic process including photosynthesis, respiration and the process of biological nitrogen fixation. Deficiency of sulphur in Indian soil is widespread due to extensive use of sulphur free fertilizer coupled with extensive cultivation of high sulphur demanding crops. Sulphur deficiency has been found in 41 per cent soils of the country (Singh, 2001). In general, sulphur requirement of the crop is about the same as that of their P requirement and is between 1/10 - 1/7 of their N needs (Saraf, 1988). Root nodule bacterium-*Rhizobium* has the ability to fix atmospheric nitrogen in symbiotic association with legumes. They normally enter the root hairs, multiply there and form nodules. The amount of nitrogen fixed varies with the strain of *Rhizobium*, the plant species and environmental conditions. Because of nitrogen fixation legumes are self-dependent for their N-requirement and play a significant role in maintaining the nitrogen balance in the soil. They also improve both physical properties such as soil aggregate stability, bulk density and biological properties of soil. They also have a favorable influence on the availability of P, K and micronutrients. The efficiency of phosphatic fertilizer is very low (15- 20%) due to its fixation in soil. Native soil phosphorus is also mostly unavailable to crops because of its low solubility. The introduction of efficient P solubilizer in the rhizosphere has been

*Corresponding author's e-mail: lbyadav_2007@rediffmail.com, lb.yadav@nbri.res.in and Address: National Botanical Research Institute, Lucknow- 226 001,

found to increase the availability of phosphorus from both applied and native soil P. The phosphorus, which gets fixed in the soil, is made available to crops by the actions of microorganisms, which solubilize insoluble/ fixed forms of phosphate in the form which are readily taken up by the plant.

MATERIALS AND METHODS

The field experiment was carried out at Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during 2006 and 2007. The soils of experimental field may thus be characterized as sandy loam (Sand- 58%, Silt- 29% and Clay- 13%), pH- 7.6, EC- 0.32, organic carbon- 5.3 g kg^{-1}, available nitrogen (186 kg ha^{-1}), phosphorus (23.5 kg ha^{-1}) and potassium (272 kg ha^{-1}) and sulphur (11.8 kg ha^{-1}). The treatment of the experiment was four levels of sulphur i.e. 0, 15, 30, 45 kg ha^{-1} (S^0) and four levels of biofertilizers viz. Uninoculated, *Rhizobium*, PSB and *Rhizobium* + PSB. The design of the experiment was Factorial RBD with 4 replication and crop variety - PDM-11. DAP was applied @ 100 kg ha^{-1}, which provided 18 kg N and 46 kg P_2O_5. Elemental Sulphur and DAP were applied as basal at the time of sowing of seed. After preparation of field, sowing of the seed of mung bean @ 20 kg ha^{-1} was done keeping 30 cm distance from row to row and maintained 10 cm from plant to plant. Thinning of plants ensured proper spacing. After harvesting of mung bean, grain and stover yield was recorded. In grain and stover, nitrogen was determined by modified Kjeldahl's method as described by Jackson (1967), for phosphorus, the finally grind samples were digested with tri- acid mixture of conc. nitric acid, sulphuric acid and perchloric acid in the ratio of 9:4:1 and determined by the method described by Chapman and Pratt (1961) colorimetrically and for sulphur, plant samples were digested in di- acid (HNO_3+ $HClO_4$) in the ratio of 4:1 and sulphur was estimated by turbidimetric method as described by Chaudhary and Cornfield (1966). For quality characteristics, methionine was estimated by the colorimetric method as given by Horn *et al.* (1946) and protein content was obtained by multiplying the nitrogen content with the factor 6.25. The rhizospheric population of bacteria, Actinomycetes, *Azotobacter* and phosphate solubilizing bacteria were analyzed by dilution and plate count methods using specific media at 45 days of sowing. The interaction effect of sulphur and biofertilizer was negative in all the cases, so it was not given in the results and discussion.

RESULTS AND DISCUSSION

Yield and yield attributing characters: Results indicated that application of sulphur @ 30 kg ha^{-1} significantly increased the plant height (44.6 cm), primary branches (5.18), effective pods $plant^{-1}$ (27.8), grains $pods^{-1}$ (11.0), 1000 grain wt. (35.7 g) in comparison to sulphur @ 15 kg ha^{-1} showing the value of 42.5 cm, 4.86, 25.9, 10.4 and 34.2 g. Further increase in suphur @ 45 kg ha^{-1} did not increase significantly the yield attributing characters of mung bean. In case of biofertilization, *Rhizobium* or PSB inoculation significantly increased the plant height, primary branches, effective pods $plant^{-1}$, grains $pods^{-1}$ and 1000 grain weight in comparison to control. However, maximum response was recorded with dual inoculation of *Rhizobium* + PSB showing the value of plant height (46.7 cm), primary branches (6.11), effective pods $plant^{-1}$ (29.8), grains $pods^{-1}$ (11.5) and 1000 grain weight (36.0).Dual inoculation of *Rhizobium* and PSB showed that these biofertilizers are compatible with each other and have a synergistic effect.

The results of the present study showed that sulphur was responsible for general improvement in the growth and development of the plant, which had an overall favourable effect on grain yield. The response of sulphur was up to 30 kg ha^{-1} in the case of grain yield of mung bean. Further increase in the dose of sulphur numerically increased the grain yield but it could not reach up to the level of significance. Maximum and optimum grain yield 12.0 q ha^{-1} was recorded with 30 kg S ha^{-1} as compared to 11.1 q ha^{-1} with 15 kg S ha^{-1} and 9.7 q ha^{-1} in control (Table 1). Significant increase in grain yield due to sulphur application in the present investigation corroborates the findings of Aulakh and Chhibba (1992), Singh and Tarafdar (2002).

Rhizobium or PSB inoculation also significantly increased grain yield of mung bean. *Rhizobium* inoculation showed the grain yield of 11.51 q ha^{-1} and PSB inoculation showed 11.19 q ha^{-1} as compared to 10.67 q ha^{-1} in uninoculated (Table 2). Increased grain yield in legumes due to *Rhizobium* inoculation has also been reported by Singh and Tarafdar, 2002. There was further increase

TABLE 1: Effect of sulphur on yield attributing characters and yield of mung bean (mean of two years)

S doses (kg ha^{-1})	Plant height (cm)	Primary branches	Effective pods $plant^{-1}$	Grains $pods^{-1}$	1000 grain wt. (g)	Grain yield (qha^{-1})	Stover Yield ((qha^{-1})
0	40.0	4.36	22.7	9.2	31.2	9.7	25.2
15	42.5	4.86	25.9	10.4	34.2	11.1	26.8
30	44.6	5.18	27.8	11.0	35.7	12.0	28.1
45	45.3	5.25	28.5	11.3	36.5	12.6	28.6
SE (m)+	0.62	0.06	0.55	0.16	0.27	0.22	0.23
CD (P= 0.05)	1.75	0.17	1.57	0.44	0.75	0.60	0.65

TABLE 2: Effect of biofertilizers on yield attributing characters and yield of mung bean (mean of two years)

Biofertilizers	Plant height (cm)	Primary branches	Effective pods $plant^{-1}$	Grains $pods^{-1}$	1000 grain wt. (g)	Grain yield (q ha^{-1})	Stover Yield (q ha^{-1})
Uninoculated	38.6	3.64	21.7	9.3	32.4	10.67	26.21
Rhizobium	44.2	5.52	27.9	11.2	34.8	11.51	27.46
PSB	42.4	4.42	25.4	9.9	34.3	11.19	27.03
Rhizobium + PSB	46.7	6.11	29.8	11.5	36.0	11.99	27.92
SE (m)+	0.61	0.06	0.55	0.16	0.28	0.22	0.23
CD (P= 0.05)	1.74	0.17	1.57	0.44	0.75	0.60	0.65

in grain yield when *Rhizobium* and PSB inoculants were combined together showing the value of 11.99 q ha^{-1} which was significantly higher than single inoculation of *Rhizobium* or PSB or uninoculation. The compatibility between sulphur and biofertilization suggested that direct nutrition of plant by applied sulphur and indirect through utilization of sulphur by nitrogen fixing organism for their growth and activity.

Similar to grain yield, stover yield was also affected by sulphur application. Significant stover yield (28.1 q ha^{-1}) was obtained by sulphur application up to the level of 30 kg S ha^{-1}. This was significantly higher than 15 kg S ha^{-1} (26.8 q ha^{-1}) and without sulphur application (25.2 q ha^{-1}). However, further increase in stover yield due to sulphur application was not effective (Table 1). The increase in crop yield due to sulphur application has also been reported by Singh *et al.* (1992).

Corresponding to grain yield, stover yield was also significantly increased by *Rhizobium* and PSB inoculation. *Rhizobium* inoculation showed the stover yield of 27.46 q ha^{-1} as compared to 26.21 q ha^{-1}, without inoculation. Significant increase in grain and stover yield due to *Rhizobium* inoculation might be due to their higher nitrogen fixing capacity and better nitrogen assimilation than native rhizobia. Inoculation of *Rhizobium* increased the nodulation, which resulted in higher nitrogen fixation and consequently higher grain and stover yield. PSB inoculation also showed significant increase in stover yield of mung bean showing the value of 27.03 q ha^{-1} over control (Table 2). PSB inoculation produces organic acids, which convert tricalcium phosphate to di and monocalcium phosphate with the net result of the enhanced availability of P to the nutrition and improvement of root uptake of N and P. The PSB increases P availability not only to the plants but also increases the efficiency of rhizobia for higher nitrogenase activity. Further increase in stover yield (27.92 q ha^{-1}) was recorded with dual inoculation of *Rhizobium* and PSB.

N-Content and uptake in grain and stover: Seed serves as a storage organ for nutrients of the plant. It is well known that the seed is very rich in protein and therefore the nitrogen of seed is expected to be quite high. Results revealed that sulphur application showed an increase in nitrogen content in the order of 3.93 percent in grains and 1.72 percent in stover with 30 kg S ha^{-1}, which was significantly higher than either 15 kg S ha^{-1} or without sulphur application (Table 3). However, sulphur application @ 45 kg S ha^{-1} did not affect the nitrogen content significantly in grains and stover. This treatment indicates that the 30 kg S ha^{-1} was sufficient for protein synthesis in seed. The higher doses of sulphur appeared to be for vegetative growth and plant vigour. It also suggested that sulphur is the first requirement for protein synthesis and seed and stover yield required additional amount of sulphur for growth. Increased nitrogen content in grain and stover of the pulse crop due to sulphur application

TABLE 3: Effect of sulphur on nitrogen and phosphorus content and uptake of mung bean (mean of two years)

S doses (kg ha^{-1})	Nitrogen content (%)		Nitrogen uptake (kg ha^{-1})		Phosphorus content (%)		Phosphorus uptake (kg ha^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
0	3.50	1.46	30.43	33.13	0.384	0.197	3.33	4.46
15	3.73	1.61	37.17	38.90	0.409	0.209	4.07	5.05
30	3.93	1.72	42.58	43.47	0.425	0.216	4.61	5.45
45	3.99	1.75	45.28	45.15	0.431	0.219	4.90	5.64
SE (m)±	0.04	0.017	1.07	0.73	0.004	0.0018	0.11	0.07
CD (P= 0.05)	0.11	0.05	3.06	2.07	0.012	0.005	0.33	0.22

have also been reported by other scientists (Jat and Rathore, 1994, Singh and Aggrawal, 1998).

Significantly increase the nitrogen content in grain and stover was recorded due to *Rhizobium* inoculation showing the value of 3.82 percent over 3.66 percent without inoculation in grain and 1.67 percent with inoculation over 1.49 percent without inoculation in stover of mung bean (Table 4). Our result falls in the line reported by Jat and Rathore (1994). Nodules are phenotypic expression of two genotypes host-legume (macrosymbiont) and *Rhizobium* (microsymbiont), which serve as the seat of nitrogen fixation. The number of nodules of the root system also governs the extent of nitrogen fixation. Similar to *Rhizobium*, PSB inoculation significantly increased nitrogen content in grain and stover, as compared to without inoculation showing the value of 3.78 per cent in grain and 1.62 per cent in stover. The increased nitrogen content in grain and stover of legumes due to PSB inoculation has also been reported by other worker (Romesh and Sabale, 2001). There was further increase of nitrogen content in grain and stover when the both inoculant was combined together. The nitrogen content in grain (3.89 per cent) with *Rhizobium* + PSB was noted as compared to 3.66 percent (without inoculation). The higher nitrogen content in grain and straw due to dual inoculation has also been reported by Dubey (1997). Similar trend was also recorded in the case of nitrogen uptake in grain and stover.

P-Content and uptake in grain and stover: Sulphur application also significantly increased the P-content in grain and stover up to 30 kg S ha^{-1} showing the value of 0.425 percent in grain and 0.214 percent in stover which was significantly higher than that of 15 kg S ha^{-1} or without sulphur application (Table 3). Further increase in rate of sulphur was not beneficial so far as P-content is concerned. The increase in P-content due to sulphur application has also been reported by Jat and Rathore (1994); Singh and Aggrawal (1998). It might be due increasing the sulphur containing amino acid in plant body which ultimately increased the P-uptake. There was significant increase in P-content in grain and stover due to *Rhizobium* inoculation showing the values of 0.408 per cent as compared to 0.394 per cent without inoculation in grain while the value was 0.208 per cent with *Rhizobium* inoculation followed by 0.201 per cent without inoculation in stover (Table 4). The increase in phosphorus content in grain and stover due to *Rhizobium* inoculation has also been reported by Jat and Rathore (1994). PSB inoculation also significantly increased P-content in grain and stover showing the value of 0.420 per cent in grain and 0.214 per cent in stover as compared to without inoculation. These results are also supported by the finding of Romesh and Sabale (2001).There was further increase in P-content in grain and stover when both inoculants were combined together. P-

TABLE 4: Effect of biofertilizers on nitrogen and phosphorus content and uptake of mung bean (mean of two years)

Biofertilizers	Nitrogen content (%)		Nitrogen uptake (kg ha^{-1})		Phosphorus content (%)		Phosphorus uptake (kg ha^{-1})	
	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
Uninoculated	3.66	1.49	35.30	35.23	0.394	0.201	3.79	4.73
Rhizobium	3.82	1.67	39.80	41.39	0.408	0.208	4.24	5.16
PSB	3.78	1.62	38.22	39.50	0.420	0.214	4.24	5.21
Rhizobium + PSB	3.89	1.77	42.16	44.53	0.428	0.218	4.64	5.51
SE (m)+	0.04	0.02	1.08	0.73	0.004	0.002	0.11	0.08
CD (P= 0.05)	0.11	0.05	3.06	2.08	0.02	0.005	0.33	0.22

content of 0.428 percent in grain and 0.218 percent in stover were noted which was significantly higher than single inoculation of *Rhizobium* or without inoculation. Higher P-content due to dual inoculation of *Rhizobium* and PSB was also recorded by Dubey (1997). Similar trend was also recorded in the case of phosphorus uptake in grain and stover.

S-Content and uptake in grain and stover: There was a linear increase in the sulphur content in plants with increasing doses of sulphur. Results were consistent during both years. Application of sulphur significantly increased the S-content in grain and stover up to 30 kg S ha^{-1} showing the value of 0.397 per cent in grain and 0.134 per cent in stover, the values being significantly higher than that of 15 kg S ha^{-1} or without sulphur application (Table 5). Further increase in sulphur application did not increase S-content in grain and stover. Increase in sulphur content due to sulphur application in sulphur deficient soil is well understood. The increase in S-content due to sulphur application has also been reported by Singh *et al*. (1992). In case of biofertilization, there were no significant differences recorded in S-content in grain and stover by the single inoculation of *Rhizobium* or PSB during both years (Table 6). Significant differences in S-content in grain was recorded by dual inoculation of *Rhizobium* and PSB showing the value of 0.386 per cent as compared to 0.363 per cent in control.

Owing to increase in both grain and stover yield coupled with sulphur content under the influence of applied sulphur, the increase in sulphur uptake followed the same pattern as that of grain and stover yield. Result was significant up to 30 kg S ha^{-1} showing the value of 4.31 kg ha^{-1}. Further increase the sulphur rate up to 45 kg ha^{-1} did not increase sulphur uptake significantly in grain (Table 5). Similar trends were also noted in case of sulphur uptake in stover and total sulphur uptake. The increase in sulphur uptake due to applied sulphur has been reported by Singh *et al*. (1992), Kachhave *et al*. (1997) and Singh and Aggrawal (1998). In case of biofertilization, *Rhizobium* and PSB inoculation did not increase the sulphur uptake in grain and stover. However, dual inoculation of *Rhizobium* + PSB resulted in significant increase (4.20 kg ha^{-1}) as compared to control (3.51 kg ha^{-1}) in case of grain and stover, the value was 3.54 kg ha^{-1} in comparison to 2.53 kg ha^{-1} in control (Table 6).

Quality parameters: Protein content was significantly increased by the addition of sulphur (Table 5). The significant increase in protein content was recorded with 30 kg S ha^{-1} showing the value of 24.95 %. Further increase in sulphur @ 45 kg ha^{-1} did not increase the protein content significantly. Increase in protein content due to sulphur application has also been reported by other workers like Kamat *et al*. (1981), Aulakh and Chibba (1992) and Singh and Aggarwal (1998). The effect of inoculation on protein content in seeds was found significant. Rhizobization significantly increased the seed protein content to the levels of 23.92 % as compared to

TABLE 5: Effect of sulphur on quality parameters of mung bean (mean of two years)

S doses (kg ha^{-1})	Sulphur content (%)		Sulphur uptake (kg ha^{-1})		Protein content (%) in grain	Methionine (g/ 100g N)
	Grain	Stover	Grain	Stover		
0	0.330	0.112	2.86	2.53	21.90	5.11
15	0.368	0.125	3.66	3.01	23.33	5.59
30	0.397	0.134	4.31	3.40	24.56	5.96
45	0.412	0.138	4.70	3.54	24.95	6.13
SE (m)+	0.007	0.002	0.19	0.092	0.17	0.07
CD (P=0.05)	0.021	0.007	0.54	0.27	0.48	0.21

TABLE 6: Effect of biofertilizers on quality parameters of mung bean (mean of two years)

Biofertilizers	Sulphur content (%)		Sulphur uptake (kg ha^{-1})		Protein content (%) in grain	Methionine (g/ 100g N)
	Grain	Stover	Grain	Stover		
Uninoculated	0.363	0.124	3.51	2.53	22.90	5.39
Rhizobium	0.380	0.128	3.98	3.01	23.92	5.76
PSB	0.377	0.127	3.85	3.40	23.62	5.71
Rhizobium + PSB	0.386	0.130	4.20	3.54	24.31	5.88
SE (m)+	0.007	0.002	0.18	0.09	0.17	0.07
CD (P=0.05)	0.020	0.006	0.54	0.27	0.48	0.21

uninoculated (22.90 %). Increased protein content due to *Rhizobium* inoculation also has been reported by Patel and Patel (1991). PSB inoculation also responded significantly over uninoculation showing the value of 23.62 %. Increased protein content due to PSB inoculation was reported by Romesh and Sabale (2001). Dual inoculation of *Rhizobium* and PSB showed maximum protein content in grain of mung bean to the levels of 24.31 %. It showed the synergistic effect of *Rhizobium* and PSB on protein content in grain of mung bean (Table 6).

Linear increase in the methionine content was recorded by the application of sulphur up to 45 kg ha^{-1} but it was significant up to 30 kg S ha^{-1} showing the average value of 5.96 g/ 100 g N (Table 5). Thus, it is clear that the amino acid can be upgraded by the sulphur application (Kamat *et al.*, 1981). In case of biofertilization, *Rhizobium* inoculation significantly increased the methionine content showing the average value of 5.76 g/ 100 g N in comparison with 5.39 g/ 100 g N without inoculation (Table 6). Increase in methionine content due to *Rhizobium* inoculation has also been reported by Tiwari and Misra, 2000). PSB inoculation also responded significantly over control showing the value of 5.71 g/ 100 g N however the maximum methionine content was recorded with dual inoculation of *Rhizobium* + PSB (5.88 g/ 100 g N).

Rhizospheric microflora: Effect of sulphur on rhizospheric population of total bacteria was significant (Table 7) particularly at 15 kg S ha^{-1} which appeared to be the optimum doses for bacterial growth. The total bacterial population at 0 kg S ha^{-1} was recorded as 34.5 x 10^6 g^{-1} soils, which increased to 47.4 x 10^6 g^{-1} soils with 15 kg S ha^{-1}. Sulphur application beyond to this level tended to reduce the population of bacteria. Sulphur functions as a nutritional source for not only the plant but also for microbes. Increase in bacterial population as a whole due to application of sulphur in sulphur deficient soil has been reported by Naidu and Ram (1995), Singh and Tarafdar (2002). In case of inoculation, *Rhizobium* inoculation significantly increased the total bacterial population (41.5 x 10^6 g^{-1} soil) over un-inoculation (33.2 x 10^6 g^{-1} soil) (Table 8). Similar results also has been found by other scientists like Naidu and Ram (1995), Singh and Tarafdar (2002). PSB inoculation alone also increased the total bacterial population over control but it was not significant. However, combined inoculation of *Rhizobium* and PSB significantly increased the total bacterial population (43.7 x 10^6 g^{-1} soil) over control during both years of studied. The increase in the population of total bacteria by *Rhizobium* inoculation is due to higher availability of available nitrogen and carbon, which acts as a food for these microbes. PSB inoculation increased the phosphorus availability by solubilizing the fixed P in the soil, which provides the energy to the microbes for their better proliferation. These findings are in close conformity with the findings of Gupta *et al.* (1992) and Singh and Tarafdar (2002).

Application of sulphur significantly affected the population of *Rhizobium* like organisms (RLOs) in the rhizosphere of the crop being maximum population 14.2 x 10^3 g^{-1} soil at the level of 15 kg S ha^{-1} (Table 7). Increases in rhizobial population due to sulphur application also have been reported by Singh and Tarafdar (2002). RLOs as counted by cultural plate count method, significantly increased due to *Rhizobium* inoculation showing the value of 14.3 x 10^3 g^{-1} soil over 9.4 x 10^3 g^{-1} soil in uninoculation (Table 8). The increased rhizobial population is well understood in view of its addition and also due to crop response. Similar result has been reported by Rawat and Sanoria (1978), Naidu and Ram (1995) and Singh and Tarafdar (2002). PSB inoculation alone as well as combined with *Rhizobium* significantly increased the rhizobial counts in the root zone of the crop during both years of study, showing the synergistic behavior of these two (Gupta *et al.*, 1992).

Application of sulphur above to 15 kg S ha^{1} significantly and linearly decreased the PSB population in both years of studied (Table 7). *Rhizobium* inoculation did not affect the population of PSB over control. However, single inoculation of the PSB or in combination with *Rhizobium* significantly increased the PSB population in rhizopheric soil. Gupta *et al.* (1992) also reported that higher PSB population due to PSB or dual inoculation of *Rhizobium* and PSB (Table 8).

Application of sulphur significantly increased *Azotobacter* population at 15 kg S ha^{-1} but showed the tendency of decreasing at higher rate of sulphur application (Table 7). The Population of *Azotobacter*

TABLE 7: Effect of sulphur on microbial population in soil after harvest of mung bean (mean of two years)

S doses (kg ha^{-1})	Total bacterial population (x 10^6 g^{-1} soil)	*Rhizobium* like organism (RLO) population (x 10^3 g^{-1} soil)	Phosphate solubilizing bacteria (PSB) population (x 10^3 g^{-1} soil)	*Azotobacter* population (x 10^3 g^{-1} soil)	Actinomycetes population (x 10^4 g^{-1} soil)
0	34.5	11.3	11.0	9.6	15.6
15	47.4	14.2	10.6	12.1	15.8
30	41.2	13.1	8.2	10.0	14.6
45	33.7	12.3	7.3	7.2	13.0
SE (m)±	2.24	0.34	0.74	5.3	1.11
CD (P= 0.05)	6.38	0.94	1.63	1.52	NS

TABLE 8: Effect of biofertilizers on microbial population in soil after harvest of mung bean (mean of two years)

Biofertilizers	Total bacterial population (x 10^6 g^{-1} soil)	*Rhizobium* like organism (RLO) population (x 10^3 g^{-1} soil)	Phosphate solubilizing bacteria (PSB) population (x 10^3 g^{-1} soil)	*Azotobacter* population (x 10^3 g^{-1} soil)	Actinomycetes population (x 10^4 g^{-1} soil)
Uninoculated	33.2	9.4	7.6	9.0	14.2
Rhizobium	41.5	14.3	8.5	10.5	15.0
PSB	38.2	11.2	10.2	9.8	14.5
Rhizobium + PSB	43.7	15.7	11.3	11.0	15.6
SE (m)±	2.24	0.34	0.57	0.53	-
CD (P= 0.05)	6.38	0.95	1.62	1.52	NS

was found as 9.6 x 10^3 g^{-1} soil at without sulphur application which increased to 12.1 x 10^3 g^{-1} soil at 15 kg S ha^{-1}. The result showed that sulphur has worked as a nutritional source of *Azotobacter* proliferation. The result suggested that 15 kg S ha^{-1} was an optimum dose of sulphur for growth of *Azotobacter*. Increase in *Azotobacter* population due to sulphur application has been reported by Singh and Tarafdar (2002). *Rhizobium* inoculation increased the *Azotobacter* cell count but PSB inoculation had no effect on *Azotobacter* population (Table 8). However, dual inoculation showed higher *Azotobacter* population. Increase in *Azotobacter* population due to *Rhizobium* inoculation has also been reported by Rawat and Sanoria (1978) and Singh and Tarafdar (2002).

Actinomycetes population was not significantly affected by the treatments. However, sulphur application showed a diminishing trend in the population of actinomycetes (Table 7). Similar results have also been reported by Naidu and Ram (1995) and Singh and Tarafdar (2002). Single inoculation of *Rhizobium* and PSB did not affect the actinomycetes population (Table 8) but dual inoculation, increased the cells counts numerically. The results corroborate the findings of Naidu and Ram (1995) and Singh and Tarafdar (2002).

REFERENCES

Aulakh. M.S. and Chhibba, I.M. (1992). Sulphur in soils and response of crops to its application in Punjab. *Fertil. News* **37:** 33-45.

Chapman, H.D. and Pratt, P.F. (1961). Methods of Analysis for Soils, Plants and Waters. University of California, USA.

Chaudhary, I.A. and Cornfield, A.H. (1966). The determination of total sulphur in soil and plant material. *Analyst* **91**: 528-530.

Dubey, S.K. (1997). Co-inoculation of phosphate solubilizing bacteria with *Bradyrhizobium japonicum* to increase phosphate availability to rainfed soybean on Vertisol. *J. Indian Soc. Soil Sci.* **45:** 506-509.

Gupta, S.B., Vyas, M.K. and Patil, S.K. (1992). Effect of phosphorus solubilizing bacteria and thiram at different levels of phosphorus on soybean and soil microflora. *J. Indian Soc. Soil Sci.* **40:** 854-856.

Horn, M.J., Jones, D.B. and Blum, A.E. (1946). Colorimetric determination of methionine in protein and food. *J. Biol. Chem.* **166:** 313-320.

Jackson, M.L. (1967) Soil Chemical Analysis. Prentice Hall of India, Pvt. Ltd. Delhi.

Jat, R.L. and Rathore, P.S. (1994). Effect of sulphur, molybdenum and *Rhizobium* inoculation on green gram (*Phaseolus radiatus*). *Indian J. Agron.* **39:** 651-654.

Kachhave, K.G., Gawande, S.D., Kohire, O.D. and Mane, S.S. (1997). Influence of various sources and levels of sulphur on nodulation, yield and uptake of nutrients by chickpea. *J. Indian Soc. Soil Sci.* **45:** 590-591.

Kamat, V.N., Kantute, V.G., Puranik, R.B., Kohadkar, W.S. and Joshi, R.P. (1981). Effect of sulphur and molybdenum 0application on yield and protein and S-amino acid contents of green gram. *J. Indian Soc. Soil Sci.* **29:** 225-227.

Naidu M.V.S. and Ram H. (1995) Effect of sulphur and *Rhizobium* inoculation on rhizosphere and non-rhizosphere microflora. *Agropedol.* **5:** 53-58.

Patel, F.M. and Patel, L.R. (1991). Response of green gram varieties to phosphorus and *Rhizobium* inoculation. *Indian J. Agron.* **36:** 295-297.

Rawat, A.K. and Sanoria, C.L. (1978). Soil microbial balance as the possible cause for synergistic effect of *Azotobacter* and *Rhizobium* on *Cicer arietinum*. *Indian J. Microbiol.* **18:** 135.

Romesh, N. and Sabale, R.N. (2001). Effect of Phosphate fertilization, phosphate solubilizer and plant population on yield and quality of summer groundnut (*Arachis hypogea*). *Indian J. Agron.* **46:** 156-161.

Saraf, C.S. (1988). Sulphur fertilization of pulses for yield and quality, TSI-FAI Symposium, Sulphur in Indian Agriculture, March 9-11, New Delhi.

Singh J.P. and Tarafdar, J.C. (2002). Rhizospheric microflora as influenced by sulphur application, herbicide and *Rhizobium* inoculation in summer mungbean (*Vigna radiata* L.). *J. Indian Soc. Soil Sci.* **50:** 127-129.

Singh S, Singh, A.P. and Singh, B. (1992). Direct and residual effect of pyrite on yield, protein content and S uptake by blackgram and lentil in Entisol. *J. Indian Soc. Soil Sci.* **40:** 584-585.

Singh, M.V. (2001). Importance of sulphur in balanced fertilizer in India, *Fertil. News* **46:** 13-18.

Singh, Y.P. and Aggarwal, R.L. (1998). Effect of sulphur sources and levels on yields, nutrient uptake and quality of blackgram (*Phaseolus mungo*). *Indian J. Agron.* **43:** 448-452.

Tiwari, V.N. and Misra, S.K. (2000). Studies on nitrogen and bio-inoculants on biological nitrogen fixation and productivity of lentil. *Indian J. Pulses Res.* **13:** 39-42.