

Original research Article

EFFECT OF COWDUNG, NPK AND *RHIZOBIUM* INOCULA ON NODULES NUMBER, YIELD CONTRIBUTING ATTRIBUTES AND YIELD OF SUMMER MUNGBEAN (*Vigna radiata*) IN ACID SOIL

ABSTRACT

A field experiment was conducted to see the effect of cowdung, NPK and *Rhizobium* inocula on nodules number, yield contributing attributes and yield of summer mungbean (var. Binamoog-8), in acid soil at the farm of Agricultural Training Institute, Khadim Nagar, Sylhet from April to July, 2014. Seven treatments were viz. Control (without *Rhizobium* inocula and fertilizers), Cowdung (CD), CD+RI, NPK, NPK+RI, $\frac{1}{2}$ (NPK)+RI and *Rhizobium* inocula (RI). The rate of Urea-N, TSP-P and MoP-K were approximately 14, 14 and 17.5 kg ha⁻¹, respectively. CD was around 8 t ha⁻¹ and RI used approximately 45 g kg⁻¹ seed. The randomly selected 5 plants were uprooted at four times in 30, 45, 60 and 90 days after sowing (DAS) for nodules. The individual ripened pod was picked at 15 days interval from 45 to 90 DAS. Treatments had significant effects on all studied characters except percent sterile pods plant⁻¹ at 75 DAS, number of seeds pod⁻¹ and 1000 seeds weight (g). Sole application of cowdung around 8 t ha⁻¹ performed better in growth character, yield contributing characters which influenced to produce higher seed and stover yield (705 and 2031 kg ha⁻¹), respectively. The highest nodules number were counted when crop treated with CD+RI and $\frac{1}{2}$ (NPK)+RI which did not reflect on crop productivity for suppression of N₂ fixing capacity in acid soil. Application of *Rhizobium* in alone or with other fertilizers had no significant influenced in yield due to soil acidity with coarse texture. The heavy rainfall also limits the higher productivity.

Keywords: Mungbean, Cowdung, *Rhizobium* inocula, yield, acid soil, limitation

1. INTRODUCTION

Mungbean (*Vigna radiata*) belongs to the pea family Fabaceae which is an important pulse crop among the pulses for its high nutritional value. In Bangladesh average pulse intake by the Bangladeshi people is 14.68 g/person/day [1] but a minimum intake of pulse by a human is recommended of 80.0 g per day [2]. Mungbean can be cultivated in two seasons of Bangladesh i.e. Rabi and Kharif-1. Rabi season beginning in October-November and Kharif-1 season is in February-March. In prior, it was cultivated only in winter season but recently a few developed summer varieties are introduced by the researchers of Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA). Mungbean is highly susceptible to waterlogging condition and cause full damaged of matured pods for high rainfall. The plant gives higher yield in winter season due to favor of environment. The nitrogenase enzyme and nif gene activity of *Rhizobium* was reduced in acid, coarse textured soil with high rainfall because the bacteria is aerobic and prefer neutral pH. The soil poor nutrient sources with above limitations played antagonistic behavior of soil microbes by receiving fertilizers and soil nutrients from soil instead of no more fixation of nitrogen from atmosphere. Soil acidity constrains symbiotic N₂ fixation in both tropical and temperate soils, limiting *Rhizobium* survival and persistence in soil and reducing nodulation [3] and [4]. The leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N₂ fixation [4]. The high rainfall with rainy days is observed in experimental site of Bangladesh from April to Sept. during the cropping period.

For above environmental constraints, the lower yield was recorded in study area. In mungbean, cultivation liming may be adopted for raising soil pH is increased fertilizer and *Rhizobium* effectivity. In Bangladesh, the total production of winter mungbean was 0.116 million M tons with an average of 960 kg ha⁻¹ under 0.121 million ha land. Whereas, the total production of summer mungbean was 0.065 million M tons with an average of 1250 kg ha⁻¹ under 0.052 million ha land [5]. The crop has not been commercially cultivated in acid soil of Bangladesh and there are no published studies on mungbean plant. The research was undertaken to evaluate the effect of cowdung, NPK and *Rhizobium* inocula on nodules number, seed yield contributing attributes and yield of summer mungbean in acid soil.

2. MATERIAL AND METHODS

The experiment was conducted at the farm of Agricultural Training Institute (ATI), Khadim Nagar, Sylhet in Bangladesh during kharif-1 on 26 April to 31 July in 2014. The soil belongs to the Brown Hill soils under agro-ecological zone of Northern and Eastern Piedmont Plains (AEZ-22). The soil textural class was loamy sand with pH 5.2. The nutrient content of control soil was SOM (1.26 %), total N (0.13 %), exchangeable K (0.12 meq⁻¹ 100 g), available P (11 µg g⁻¹), available S (9.45 µg g⁻¹), available B (0.11 µg g⁻¹) and soil pH was 5.21. The variety of *Vigna radiata* was Binamoog-8 collected from Bangladesh Institute of Nuclear Agriculture (BINA). The treatments were Control (without *Rhizobium* inocula and fertilizers), Cowdung (CD), CD+RI, NPK, NPK+RI, ½(NPK)+RI and *Rhizobium* inocula (RI). CD was applied approximately 8 t ha⁻¹; the nutrients N, P and K were applied approximately at 14, 14 and 17.5 kg ha⁻¹ from the sources of urea, TSP and MoP, respectively. The nutrient content of cowdung was N (1.01 %), P (0.32 %), K (0.26 %), S (0.21 %) and organic carbon was 11.1 %. *Rhizobium* inocula was applied as seed inoculant around 45 g kg⁻¹. The experiment was laid out at a randomized complete block design (RCBD) with three replications. The unit plot size was 3 m × 2 m with 10 cm surrounding area and 1 m proper drainage system. The seed inoculation was done by coated seed with mixing the molasses (20 g kg⁻¹) and *Rhizobium* inocula (45 g kg⁻¹ seed). The seed rate was 30 kg ha⁻¹ with 25 cm × 10 cm Spacing. The un-inoculated seed was sown after the inoculated seed at the same day. The different intercultural operations and plant protection measures were taken when needed, especially drainage, roofing by polythene sheet in early seedling stage and weeding were done. Five plants were selected randomly, uprooted from each plot carefully at 30, 45, 60 and 90 DAS for nodules data. The effective and non-effective nodules were distinguished by observation of colour with naked eye and using compound microscope. The yellow or black ripen pods were harvested at 15 days intervals from 45 to 90 DAS in each plot separately. The four times harvested seed was sun dried on 12 % moisture level and converted seed yield separately into (kg ha⁻¹). At final harvest period (90 DAS), the remaining 225 plants plot⁻¹ were harvested separately from 3 m × 2 m plot area as stover yield and converted into (kg ha⁻¹). The mean was calculated and analysis of variance was performed by F-variance test and mean differences were adjudged by Duncan's Multiple Range Test [6].

3. RESULTS AND DISCUSSION

3.1 Number of nodules plant⁻¹

The total number of nodules plant⁻¹ varied significantly for different treatments (Table 1). The highest number of nodules was found at 45 DAS and 60 DAS. After 60 DAS the nodules number were gradually decreased due to nodule deformation for bacterial inactivity. The result was agreed with the observations of [7]. A significant variation was observed in case of higher nodule number during harvest. The highest number of nodules plant⁻¹ were recorded from cowdung with RI and ½ (NPK) with RI which agreed with the observation of [8]. It is defined that the nodules number depends on soil microbes with their activity [9].

types of fertilizer used, crop duration [7] and mineralization of organic fertilizers. Cowdung produced higher number of nodules due to slow nitrogen release at earlier stage which agreed with the observations of [10]. The inhibitory effect of N fertilizer on nodulation also addressed by Pons *et al.*, 2007 [11].

3.2 Number of effective nodules plant⁻¹

The highest number of effective nodules plant⁻¹ were recorded due to combined application of cowdung with *Rhizobium* (Table 2). Whereas, the lowest number was observed in control except at 90 DAS. So, it is concluded that the effectiveness of nodules depend on *Rhizobium* along with cowdung and 50 % of NPK application and the number was highest at 60 DAS. The nodule effectiveness was reduced because nitrogenase enzyme and nif gene activity was suppressed by soil acidity, coarse soil, N fertilizer, soil nutrient and rainfall which supported with Brockwell *et al.*, 1995 [12]. The total rainfall (1593 mm) and rainy days (84 days) were observed at study plot from May to July in 2014.

Table 1. Effect of cowdung, NPK and *Rhizobium* inocula on the number of nodules plant⁻¹ at 30, 45, 60 and 90 DAS

Treatment	Number of nodules plant ⁻¹			
	30 DAS	45 DAS	60 DAS	90 DAS
Control	1.90d	3.10d	2.44f	1.20de
CD	2.20d	6.47c	7.20e	5.87a
CD+RI	13.90a	17.98a	16.27b	4.07b
NPK	1.90d	6.79c	9.17d	4.14b
NPK+RI	3.60cd	6.17c	7.82e	2.54cd
½(NPK)+RI	6.47b	13.10b	17.64a	2.74bc
RI	5.80bc	6.88c	11.19c	0.47e
Level of significance	0.01	0.01	0.01	0.01
CV (%)	18.80	13.11	5.10	18.94

In a column figure(s) bearing common letter(s) do not differ significantly whereas figure(s) bearing dissimilar letter(s) indicate significantly different by DMRT. Here, CD = Cowdung, RI = *Rhizobium* inocula.

3.3 Number of pods plant⁻¹

The highest and the lowest total number of pods (57.78 and 21.85) plant⁻¹ were produced in CD and control treatments, respectively (Table 3). Similarly, the highest number of pods (5.27, 16.07, 24.50, and 11.94) plant⁻¹ were produced due to cowdung application at 45, 60, 75 and 90 DAS, respectively. Whereas, it was lowest due to control. Cowdung supplied different macro and micronutrients to plant in exchangeable form through microbial activities, reduced nutrient lose, improved soil conditions helped to produce the highest pods in acid

soil than other treatments. The result was corroborated with the findings of [13], [14]. The negative effect of *Rhizobium* inocula in acid soil due to pedo-abiotic effect had reduced mungbean productivity which supported with Otieno *et al.*, 2009 [15] and Mohammadi *et al.*, 2012 [16].

Table 2. Effect of cowdung, NPK and *Rhizobium* inocula on the number (no.) of effective nodules plant⁻¹ at 30, 45, 60 and 90 DAS

Treatment	Effective nodules plant ⁻¹							
	30 DAS		45 DAS		60 DAS		90 DAS	
	No.	% of total	No.	% of total	No.	% of total	No.	% of total
Control	0.44d	23.15	1.07d	34.52	1.03c	42.22	0.17c d	14.17
CD	0.68d	30.91	2.16c	33.39	2.52b	35.0	1.55a	26.41
CD+RI	4.82a	34.68	6.45a	35.88	5.70a	35.04	1.01b	24.82
NPK	0.61d	32.11	2.08c	30.64	2.98b	32.50	1.12a b	27.06
NPK+RI	1.08d	30.0	1.75c d	28.37	2.50b	31.97	0.67b c	26.38
½(NPK)+RI	2.83b	43.74	4.62b	35.27	6.31a	34.76	0.63b cd	23.0
RI	1.10c	18.97	2.25c	32.71	3.19b	28.51	0.11d	23.41
Level of significance	0.01	-	0.01	-	0.01	-	0.01	-
CV (%)	14.43	-	12.30	-	8.79	-	26.96	-

In a column figure(s) bearing common letter(s) do not differ significantly whereas figures bearing dissimilar letter(s) indicate significantly different by DMRT. Here, CD = Cowdung, RI = *Rhizobium* inocula.

3.4 Percent sterile pods plant⁻¹

Application of cowdung decreased pod sterility than other treatments (Table 4). The results are agreed with Mohammadi and Rokhzadi, 2012 [17].

3.5 Number of seeds pod⁻¹

An insignificant result was obtained to the seeds number pod⁻¹ for treatments effect and it ranged from 4.27 to 5.44 (Table 4). The highest was obtained due to cowdung and the lowest was in control and *Rhizobium* inocula.

3.6 1000 seeds weight (g)

The 1000 seeds weight did not significantly affected under different treatments which ranged from 38.03 g to 39.31 g (Table 4). It may be assumed that genetical influence was greater than the treatments effect and cowdung in alone or combined increased soil organic matter

which enhanced seed quality. The observation is corroborated with Mohammadi and Rokhzadi, 2012 [17].

Table 3. Effect of cowdung, NPK and *Rhizobium* inocula on the number of pods plant⁻¹ at different DAS

Treatment	Number of pods plant ⁻¹				Total number of pods plant ⁻¹ during harvest
	45 DAS	60 DAS	75 DAS	90 DAS	
Control	1.81d	7.90c	8.67c	3.47c	21.85
CD	5.27a	16.07a	24.50a	11.94a	57.78
CD+RI	3.87b	15.27a	17.64b	10.43ab	47.21
NPK	3.20bc	12.80b	17.93b	8.34b	42.27
NPK+RI	2.79bcd	12.43b	17.87b	5.18c	38.27
½(NPK)+RI	2.22cd	8.50c	9.70c	4.87c	25.29
RI	1.90d	8.40c	9.10c	3.94c	23.34
Level of significance	0.01	0.01	0.01	0.01	-
CV (%)	15.36	5.55	6.78	17.14	-

In a column figure(s) bearing common letter(s) do not differ significantly whereas figures bearing dissimilar letter(s) indicate significantly different by DMRT. Here, CD = Cowdung, RI = *Rhizobium* inocula.

3.7 Seed yield

A significant variation was observed in seed yield of mungbean due to application of different fertilizers (Table 5). The highest seed yield (705 kg ha⁻¹) was obtained due to cowdung application approximately 8 t ha⁻¹ while, the lowest was (343 kg ha⁻¹) produced by control treatment. A positive effect of cowdung on seed yield also agreed with Umunna and Anselem, 2012 [13] and Mukthar *et al.*, 2012 [14]. *Rhizobium* in alone or with other treatments did not give significant response due to low pH soil [18], coarse textured soil [19], rainfall and poor soil nutrient. An unavailable N₂ fixation by bacteria competed with plant for food sources of soil and the negative effect of *Rhizobium* on yield also documented by Singh *et al.*, 2014 [20] and Otieno *et al.*, 2009 [15].

3.8 Stover yield

A similar pattern was also observed to stover yield of mungbean as seed yield (Table 5). The enhanced plant growth, shoot length and branches number of mungbean was increased stover yield due to cowdung application than other treatments either single or combined. The findings are agreed with the observations of Umunna and Anselem, 2014 [14] and Rahman *et al.*, 2012 [21]. The poor stover yield by *Rhizobium* inocula and control also agreed with Bahadur and Tiwari, 2014 [22] and Otieno *et al.*, 2009 [15].

175
176
177
178
179
180

Table 4. Effect of cowdung, NPK and *Rhizobium* inocula on the number and percent sterile pods plant⁻¹ at two harvest, number of seeds pod⁻¹ and 1000 seeds weight (g)

Treatment	Sterile pods plant ⁻¹				Number of seeds pod ⁻¹	1000 seeds weight (g)
	75 DAS		90 DAS			
	Number	% of total	Number	% of total		
Control	3.35	38.64	2.07ab	59.66	4.27	38.10
CD	4.10	16.74	2.90a	27.81	5.44	39.31
CD+RI	3.09	17.52	2.82a	33.82	5.43	39.30
NPK	3.44	19.19	2.37ab	45.75	5.24	39.11
NPK+RI	3.59	20.09	1.74c	44.17	5.07	39.17
½(NPK)+RI	2.93	30.21	2.62a	53.80	4.37	39.06
RI	3.44	37.81	2.80a	57.50	4.27	38.03
Level of significance	NS		0.05		NS	NS
CV (%)	14.83		18.28		20.20	3.74

181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204

In a column figure(s) bearing common letter(s) do not differ significantly whereas figures bearing dissimilar letter(s) indicate significantly different by DMRT. NS = Not significant. Here, CD = Cowdung, RI = Rhizobium inocula.

Table 5. Effect of cowdung, NPK and *Rhizobium* inocula on seed and stover yield (kg ha⁻¹)

Treatment	Seed yield (kg ha ⁻¹)	Increased over control (%)	Stover yield (kg ha ⁻¹)	Increased over control (%)
Control	343f	-	881e	-
CD	705a	51.35	2031a	56.62
CD+RI	681b	49.64	1906b	53.78
NPK	673c	49.04	1531c	42.46
NPK+RI	647d	46.99	1517c	41.93
½(NPK)+RI	606e	40.65	1397d	36.94
RI	350f	2.00	896e	1.68
Level of significance	0.01	-	0.01	-
CV (%)	0.60	-	1.46	-

In a column figure(s) bearing common letter(s) do not differ significantly whereas figure(s) bearing dissimilar letter(s) indicate significantly different by DMRT. Here, CD = Cowdung, RI = *Rhizobium* inocula.

4. CONCLUSION

In acid soil cowdung fertilizer showed better response to mungbean plant rather than NPK fertilizer and *Rhizobium* biofertilizer. The poor function of *Rhizobium* inocula was observed due to pedo-ecological effect stopped N₂ fixation by microbes ultimately competed with crops for food sources. Further experimentation is needed to confirm the results as one year's is not enough for recommendation.

REFERENCES

1. Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM). A final report in June,13. Desirable dietary pattern for Bangladesh. Dhaka, Bangladesh. 2013:158.
2. FAO (Food and Agriculture Organization). Production Year Book. Rome, Italy. 1999:45-48.
3. Ibekwe AM, Angle JS, Chaney RL and Vonberkum P. Enumeration and nitrogen fixation potential of *Rhizobium leguminosarum* biovar *trifolii* grown in soil with varying pH values and heavy metal concentrations. Agric. Ecosyst. Environ. 1997;61:103-111.

- 236 4. Brockwell J, Pilka A and Holliday RA. Soil pH is a major determinant of the numbers
237 of naturally occurring *Rhizobium meliloti* in non-cultivated soils of New South
238 Wales. Aust. J. Exp. Agric. 1991;31:211-219.
- 239
- 240 5. All Crop Statistics. Pulse crop 09-10 to 13-14: 2. Website: <http://www.dae.gov.bd in>
241 portal.gov. (Date: 30.6.2014).
- 242
- 243 6. Gomez KA and Gomez AK. Statistical procedures for Agricultural Research. 2nd edn.
244 John Wiley and Sons. New York. 1984:207-215.
- 245
- 246 7. Bhuiyan MAH, Main MH, Islam MS and Islam MR. Effects of inoculation with
247 *Bradyrhizobium* on nodulation pattern, nodule senescence and size
248 distribution in mungbean. Bangladesh J. Agril. Research. 2007;32(2):269-
249 282.
- 250 8. Nagarajan P and Balachandar D. Influence of *Rhizobium* and organic amendments
251 on nodulation and grain yield of blackgram and greengram in acid soil.
252 Madras Agril. J. 2002;88(1):703-705.
- 253
- 254 9. Bhuiyan MAM, Khanam D, Ali ME, Khatun MR, Alam F and Rahman MA. Influence
255 of variety on the response of blackgram to inoculation with elite strains of
256 *Bradyrhizobium*. Annual Res. Reports 2007-08. Soil Sci. Div., Bangladesh
257 Agricultural Research Institute. 2008:333-347.
- 258
- 259
- 260 10. Ganeshamurthy AN and Sammi-Reddy K. Effect of integrated use of farmyard
261 manure and sulphur in a soybean and wheat cropping system on
262 nodulation, dry matter production and chlorophyll content of soybean on
263 swell-shrink soils in Central India. J. Agron. Crop Sci. 2000;185:91-97.
- 264
- 265 11. Pons TL, Perreijn K, Van Kessel C and Werger MJA. Symbiotic nitrogen fixation in a
266 tropical rain forest: 15N natural abundance measurements supported by
267 experimental isotope enrichment. New Phytologist. 2007;173:157-167.
- 268
- 269 12. Brockwell J, Bottomley PJ and Thies JE. Manipulation of rhizobia microflora for
270 improving legume productivity and soil fertility: a critical assessment. Plant
271 Soil. 1995;174:143-180.
- 272
- 273 13. Umunna OE and Anselem A. Application of organic amendments and botanical foliar
274 sprays against bacterial diseases of mungbean (*Vigna radiata*) in Nigeria.
275 Greener J. Agril. Sci. 2014;4(2):52-57.
- 276
- 277 14. Mukhtar AA, Odina EC, Ahmed A, Babaji BA, Aminu-Mukhtar M and Arunash U L.
278 Comparative effects of organic manure sources and rates on performance
279 of groundnut varieties. J. Environ. Sci. and Natural Res. 2014;4(2):155-158.
- 280
- 281 15. Otieno PE, Muthomi JW, Chemining GN and Nderitu JH. Effect of Rhizobia
282 inoculation, farm yard manure and nitrogen fertilizer on nodulation and yield
283 of food grain legumes. J. Biol. Sci. 2009;9(4):326-332. DOI:
284 10.3923/jbs.2009.326.332. Source: DOAJ.
- 285
- 286 16. Mohammadi K, Sohrabi Y, Heidari G, Khalesro S and Majidi M. Effective factors on
287 biological nitrogen fixation. African Journal of Agril. Res. 2012;7(12):1782-
288 1788. DOI: 10.5897/AJARX11.034.<http://www.academicjournals.org/AJAR>.

289
290 17. Mohammadi K and Rokhzadi A. An integrated fertilization system of canola
291 (*Brassica napus* L.) production under different crop rotations. Ind. Crop
292 Prod. 2012;37:264-269.
293
294 18. Shamseldin A. Use of DNA marker to select well adapted *Phaseolus*-symbionts
295 strains under acid conditions and high temperature. Biotechnol. Lett.
296 2007;29:37-44.
297
298 19. Turco RF and Sadowsky MJ. Understanding the microflora of bioremediation. In:
299 Skipper HD and Turco RF (Eds.), Bioremediation: Science and Applications.
300 Soil Science (Special Publication). Madison, WI: Soil Sci. Soc. Am. J.
301 1995;43:87-103.
302 20. Singh AK, Singh PK, Kumar M, Bordoloi LJ and Jha AK. Nutrient management for
303 improving mungbean (*Vigna radiata*) productivity in acidic soil of northeast
304 India. Indian J. Hill Farming. 2014;27(1):62-71.
305
306 21. Rahman M, Imran M and Ashrafuzzaman M. 2012. Effect of inoculant on yield and
307 yield contributing characters of summer mungbean cultivars. J. Environ. Sci.
308 and Natural Res. 5(1): 211-215.
309
310 22. Bahadur L and Tiwari DD. Nutrient management in Mungbean (*Vigna radiata*)
311 through sulphur and biofertilizers. An International J. 2014;37(2):180.
312