Korean J. Crop Sci. 51(6): 527~533(2006)

Response of Chickpea to Dual Inoculation with *Rhizobium* and Arbuscular Mycorrhiza, Nitrogen and Phosphorus

A.R.M. Solaiman†, M.N. Molla, and M.D. Hossain

Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University Gazipur 1706, Bangladesh

ABSTRACT : The response of chickpea (*Cicer arietinum* L.) to dual inoculation with *Rhizobium (R)* and arbuscular mycorrhiza (AM), nitrogen (N) and phosphorus (P) was studied on spore abundance and colonization of AM, nodulation, growth, yield attributes and yield. In all the parameters of the crop the performance of *Rhizobium* inoculant alone was superior to control. Dual inoculation with *Rhizobium* and AM in presence of P performed the best in recording number of spore 100 g^{-1} rhizosphere soil and root colonization, number and dry weight of nodule, dry weights of shoot and root, number of pod $plant^{-1}$, number of seed pod^{-1}, seed and stover yields of chickpea. The maximum seed yield of 3.33 g $plant^{-1}$ was obtained by inoculating chickpea plants with *Rhizobium* and AM in association with P. From the view point of nodulation, growth, yield attributes and yield of chickpea, dual inoculation with *Rhizobium* and AM along with P was considered to be the balanced combination of nutrients for achieving the highest output from cultivation of chickpea in Shallow Red Brown Terrace Soil of Bangladesh.

Keywords: chickpea, *Rhizobium*, arbuscular mycorrhiza, nitrogen, phosphorus

Chickpea (*Cicer arietinum* L.) is one of the protein rich legume crops. This crop has occupied sixth position both in production (10,380 mt.) and acreage (34,370 ac.) in Bangladesh (Anonymous, 2004). Although predominantly consumed as a pulse, dry chickpea is also used in preparing a variety of snack foods, sweets and condiments (Saxena, 1987). Nutritionally, chickpea is relatively free from various antinutritional factors, has a high protein digestibility and is richer in P and calcium than other pulses (Ramalho – Ribero and Portugal Melo, 1988). Because of its higher fat content and better fiber digestibility chickpea holds great promise as a protein and calorie source for animal food for both ruminants and nonruminants (Cordesse, 1988). Two types of symbiotic association can form with microorganisms and the leguminous crops in which *Rhizobium* is involved in atmospheric N fixation and AM fungi is concerned with the uptake of P (Abdel – Fattah, 1997). Arbuscular mycorrhizal fungi increase uptake of immobile nutrients, especially phosphorus and micronutrients (Douds and Miller, 1999). Many tropical legume crops are in fact highly mycorrhizal dependent (Adholeya *et al.*, 1995). Normally legume crops have less extensive root systems and are dependent on colonization by native AM fungi for their nutritional needs (Benthlenfalvay and Newton, 1991). The root system of chickpea can be colonized by AM fungi and nodulated by N fixing bacteria. These two microbes are beneficial to the legume crop and the possibility of a direct interaction between the fungus and bacterium was considered to supply a good amount of N and P. A positive response of inoculation of chickpea with AM and *Rhizobium* was observed in respect of AM colonization and spore population, nodulation, growth and yield (Solaiman *et al.*, 2005, Khanam *et al.*, 2005). However, further study is needed to confirm these findings. Keeping these facts in mind, the present investigation was carried out to assess the response of chickpea to dual inoculation with *Rhizobium* inoculant and AM fungi, N and P on spore abundance and colonization of AM, nodulation, growth, yield attributes and yield of chickpea.

†Corresponding author: (Phone) 880-2-9205310-14 Extn. 2040
(E-mail) arm_solaiman@yahoo.com <Received June 1, 2006>

MATERIALS AND METHODS

The experiment was carried out at Bangabandhu Sheikh Mujbur Rahman Agricultural University, Gazipur, Bangladesh using chickpea variety BARI Chola-5 as the test crop. The soil was silty clay loam having sand 17.7%, silt 46.9%, clay 35.4%, organic carbon 0.93%, pH 6.80, CEC 15.30 meq 100 g^{-1} soil, total nitrogen 0.067%, available P 11.30 ppm, available K 0.31 meq 100 g^{-1} soil, exchangeable Ca 6.60 meq 100 g^{-1} soil, exchangeable K 7.32 meq 100 g^{-1} soil, exchangeable Mg 3.10 meq 100 g^{-1} soil, exchangeable Na 0.73 meq 100 g^{-1} soil, number of *Rhizobium* 4.1 x 10^6 g^{-1} soil. The collected soil samples were autoclaved at 121°C for 20 minutes before use. Five-kilogram autoclaved soil were put in each earthen pot. Nitrogen 0.27 g pot^{-1} in the form of urea, phosphorus 0.40 g P_2O_5 pot^{-1} in the form of triple super phosphate, inoculant containing arbuscular mycorrhizae 60 g pot^{-1} were applied to pot according to the treatments assigned. Potassium 0.48 K_2O pot^{-1}, sulphur 0.28 g S pot^{-1} and molybdenum 0.01g Mo pot^{-1} were applied as basal dose in the form of muriate of potash, zypsum and sodium molybdenum, respectively. Roots of sorghum (*Sorghum vulgare* L.) with rhizosphere soil propagules were used as AM inoculant which was collected from Soil Microbiology Section, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. Strain RCa-201 was used as *Rhizobium* inoculant. Counts of viable rhizobia in the *Rhizobium* inoculant were taken following the Drop Plate Method of Miles and Misra (1938). An amount of 1.12 g *Rhizobium* inoculant containing 1.5×10^9 cells g^{-1} was mixed with 56g seeds with the help of gum arabic. The experiment was laid out in a complete randomized design (CRD) with three replications. There were ten treatment combinations, viz. T_1: control, T_2: Arbuscular mycorrhiza (AM), T_3: *Rhizobium* (R), T_4: AM + R, T_5: AM + R + N, T_6: AM + R + P, T_7: AM + N + P, T_8: AM + N, T_9: AM + P and T_{10}: AM + R + N + P. Six seeds of chickpea treated with mercuric chloride for surface sterilization were sown in each pot. A layer of AM inoculant was first placed in each pot filled with sterilized soil and was covered with a thin soil layer of 2 cm in which seeds were sown. Pots were watered up to saturation to allow the soil and inoculant to settle down in the pots. Two healthy seedlings were allowed to grow pot^{-1} after germination of seeds. To maintain soil moisture pots were watered whenever necessary. Weeding and mulching were done as and when necessary ensuring the normal growth of the chickpea plant. The pots were carefully observed regularly to record any change of plant growth. During the growth period no pesticide was used as the plants were free from pests.

With minimum disturbance of roots the plants were carefully uprooted at 50 per cent flowering stage so that no nodules were left in the soil. The roots were washed with tap water and finally rinsed with distilled water. The nodules from the roots were separated and then nodule number and weight were recorded. The root, nodules and shoot were first air-dried and then oven-dried at 65°C for 72 hours. According to Koske and Gemma (1989) one hundred (100) root segments of 1 cm long were stained for assessing AM colonization. The spore abundance was counted following the Wet Sieving and Decanting Method (Gerdemann and Nicolson, 1963) and the percentage of AM root colonization was estimated by Root Slide Technique (Read *et al.*, 1976). All data were analyzed in the computer using IRRISTAT program.

RESULTS AND DISCUSSION

Root colonization

The maximum root colonization (47%) was found with the treatment T_6 (AM + R + P) and T_{10} (AM + R + N + P) which was significantly higher over other treatments (Table 1). The second highest root colonization was found in T_4 (AM + R). Control treatment gave the lowest colonization. Per cent root colonization was increased significantly by dual inoculation than single inoculation. This result has the resemblance with the result of Khanam *et al.* (2005) who observed that root colonization percentage in chickpea was higher due to dual inoculation of the crop with *Rhizobium* and AM in presence of P compared to single inoculation with *Rhizobium* or AM. The results are also in good agreement with Andrade *et al.* (1998) who reported increased colonization in pea due to dual inoculation with *Rhizobium* and AM as compared to single inoculation with *Rhizobium* or AM. Balachandar and Nagarjan (1999) found higher root colonization in greengram by dual inoculation with *Rhizo-*

Table 1. Root colonization and abundance of spore in rhizosphere soil of chickpea.

Treatment	Per cent root colonization	Number of spore 100 g^{-1} soil
T_1 : Control	0.00 g†	0.00 h
T_2 : Arbuscular mycorrhiza (AM)	20.33 f	30.00 g
T_3 : *Rhizobium* (R)	0.00 g	0.00 h
T_4 : AM + R	36.67 b	46.33 c
T_5 : AM + R + N	33.33 c	44.33 d
T_6 : AM + R + P	46.67 a	65.00 a
T_7 : AM + N + P	30.00 d	41.33 ef
T_8 : AM + N	26.67 e	43.00 de
T_9 : AM + P	30.00 d	40.67 f
T_{10} : AM + R + N + P	46.67 a	50.67 b
CV (%)	2.60	2.60

†Means followed by common letter(s) in a column are not significantly different at 5 % level by DMRT

bium + AM. Pandey *et al.* (1998) also reported increased per cent root colonization by dual inoculation of *Rhizobium* + AM in cowpea.

Number of spore 100 g^{-1} soil

The effects of different treatment combinations significantly increased spore population over control (Table 1). Treatment T_6 (AM + R + P) recorded the highest number of population (65.0 100 g^{-1} soil). The effect of this treatment was significantly different to other treatments. Abruscular mycorrhizal colonization, either individually or in combination with *Rhizobium* or dual inoculation along with N and P fertilizers significantly increased spore population over control. Khanam *et al.* (2005) supported these observations. They reported that dual inoculation of chickpea with *Rhizobium* and AM along with P fertilizer significantly increased spore population over control. Similar results were also found by Tarafdar and Rao (1997) who found that the numbers of AM spores in the rhizosphere soil of clusterbean, mungbean and mothbean were increased significantly upon AM inoculation. Treatment receiving *Rhizobium* alone and control did not show any spore in this study.

Nodulation

Treatment T_6 receiving *Rhizobium* along with AM and P gave the highest nodule number (12.5 $plant^{-1}$) but the effect of this treatment was statistically similar to T_{10} (Table 2). The effects of *Rhizobium* inoculant alone was significantly higher over all the treatments except T_4, T_5, T_6 and T_{10}. The maximum nodule number was obtained with AM + R + P that might be attributed to greater availability of P, which was crucial for nodulation (Hayman, 1986). This is likely as dual inoculation might helped plant in increased P uptake which was essential for nodulation. Dual inoculation produced significantly higher nodule number and nodule weight as compared to single inoculation. Zheng and Song (2000) documented similar result. Burity *et al.* (2000) recorded significantly increased nodulation due to dual inoculation over single inoculation with *Rhizobium.* In case of dry weight of nodule, the highest weight (0.054 g) was also recorded with the treatment T_6 which was statistically similar to T_9 and T_{10}. EL-Didamony and Abdel-Fattah (1998) observed that dual inoculation of *Vicia faba* with AM fungus and rhizobia significantly increased the number of nodules, the biomass production and the total nitrogen.

Plant height

The effects of different treatments on plant height of chickpea were found significant (Table 2). The highest plant height (30.33cm) was recorded with the treatment T_6 receiving AM + R + P. This result differed significantly with all other treatments. Balachandar and Nagarajan (1999) found the highest shoot length in greengram by dual inoculation of *Rhizobium* and AM along with 50% recommended N and P fertilizers. In this study single inoculation of chickpea either with *Rhizobium* or AM could not perform well in recording plant height.

Root length

There were significant variations in root length of chick-

Table 2. Effect of different combinations of arbuscular mycorrhiza, *Rhizobium* inoculant, nitrogen and phosphorus on growth of chickpea.

Treatment	Number of nodule $plant^{-1}$	Dry weight of nodule (g) $plant^{-1}$	Plant height (cm)	Root length (cm)	Number of branch $plant^{-1}$	Dry weight of shoot (g) $plant^{-1}$	Dry weight of root (g) $plant^{-1}$
T_1 : Control	1.33 f[†]	0.013 e	19.17 f	20.60 g	3.33 e	1.13 i	1.00 e
T_2 : Arbuscular mycorrhiza (AM)	4.50 e	0.021 de	22.83 de	37.95 e	3.60 d	1.50 g	1.27 d
T_3 : *Rhizobium* (R)	8.00 c	0.023 cd	21.00 e	46.83 b	3.67 cd	1.36 h	1.30 d
T_4 : AM + R	10.33 b	0.031 bc	21.00 e	41.00 d	4.23 b	1.70 f	1.57 c
T_5 : AM + R + N	11.00 b	0.033 b	24.33 cd	34.67 f	4.00 bc	2.07 e	1.63 c
T_6 : AM + R + P	12.50 a	0.054 a	30.33 a	52.67 a	4.83 a	3.23 a	2.17 a
T_7 : AM + N + P	4.00 e	0.019 de	25.33 bc	40.00 d	3.67 cd	2.40 d	1.80 b
T_8 : AM + N	6.00 d	0.023 cd	25.67 bc	44.33 c	4.87 a	2.83 c	1.87 b
T_9 : AM + P	5.00 de	0.037 ab	26.00 bc	37.36 e	3.63 d	2.80 c	1.53 c
T_{10} : AM + R + N + P	11.40 ab	0.044 a	26.67 b	46.46 b	4.67 a	2.90 b	2.06 a
CV (%)	10.40	17.10	4.50	2.40	4.60	1.70	5.80

[†]Means followed by common letter(s) in a column are not significantly different at 5 % level by DMRT

pea recorded with different treatments (Table 2). The highest root length (52.67 cm) was recorded with AM + R + P. This might be due to the adequate supply of N from biological fixation by *Rhizobium* and proper supply of P fertilizer, which increased vegetative growth and greater translocation of photosynthate and thereby root growth, which is in agreement with the findings of Balachandar and Nagarajan (1999), Naqvi and Mukerji (1998). All the treatments recorded significantly higher root length over control.

Number of branch plant^{-1}

With respect to number of branch plant^{-1}, the highest number (4.87) was recorded with the treatment T_8 receiving AM + N which was statistically similar to T_6 receiving AM + R + P and T_{10} receiving AM + R + N + P (Table 2). The effects of this treatments were superior to rest of the treatments of this study. Prasad (2000) reported enhanced plant growth of chickpea through increased branching due to double symbiosis with AM and *Rhizobium*. Barea *et al.* (1996) recorded higher degree of branching due to double symbiosis with AM and *Rhizobium*.

Dry weight of shoot

There was a significant variation in dry weight of shoot with different treatments (Table 2). The highest dry weight of shoot (3.23 g) was obtained with the treatment T_6 receiving AM + R + P and the effect of this treatment was statistically superior to other treatments. This results has the resemblance with the results of Solaiman *et al.* (2005) who reported that dual inoculation of chickpea with *Rhizobium* and AM in presence of N and P produced the highest dry weight of shoot. In this study the lowest dry weight of shoot was obtained in control.

Dry weight of root

Different treatments had significant influence on dry weight of root of chickpea (Table 2). The highest dry weight of root (2.17 g) was recorded by the treatment T_6 receiving AM + R + P and the effect of this treatment was statistically similar to T_{10} but significantly higher over other treatments. Control gave the lowest dry weight of root. This has an agreement with Nurlaeny *et al.* (1996) who recorded greater root weights of inoculated plants compared to uninoculated one's.

Number of pod plant^{-1}

Data in Table 3 revealed that significantly higher number of pod plant^{-1} was obtained with *Rhizobium* + AM + P. Double symbiosis (AM+R) increased pod number significantly compared to single inoculation either with AM or *Rhizobium*. Control treatment gave the lowest number of pod plant^{-1}. Das *et al.* (1997) recorded increased number of pod plant^{-1} in greengram by dual inoculation compared to control. Similar results were documented by Carling *et al.* (1996) who reported that AM fungi and P fertilization increased pod of groundnut.

Number of seed pod^{-1}

Plant receiving *Rhizobium* and AM in combination with P

Table 3. Effect of different combinations of arbuscular mycorrhiza, *Rhizobium* inoculant, nitrogen and phosphorus on yield attributes and yield of chickpea.

Treatment	Number of pod plant^{-1}	Number of seed pod^{-1}	1000-seed weight (g)	Seed yield (g) plant^{-1}	Stover yield (g) plant^{-1}
T_1 : Control†	2.00 f	0.67 g	48.60 g	0.28 f	1.67 h
T_2 : Arbuscular mycorrhiza (AM)	2.16 f	1.00 f	68.50 e	0.73 e	1.79 g
T_3 : *Rhizobium* (R)	2.83 d	1.67 d	79.76 c	0.80 de	1.90 f
T_4 : AM + R	3.60 c	1.33 e	66.36 f	0.87 d	2.38 c
T_5 : AM + R + N	4.16 b	1.67 d	83.40 b	1.67 b	1.80 g
T_6 : AM + R + P	6.33 a	2.67 a	112.30 a	3.33 a	3.20 a
T_7 : AM + N + P	3.00 d	2.00 c	78.45 c	1.57 b	2.40 c
T_8 : AM + N	2.66 de	2.00 c	65.42 f	1.40 c	2.18 e
T_9 : AM + P	2.33 ef	1.33 e	76.33 d	1.40 c	2.27 d
T_{10} : AM + R + N + P	4.00 b	2.31 b	84.20 b	1.63 b	2.51 b
CV (%)	6.80	4.90	1.50	5.10	1.00

†Means followed by common letter(s) in a column are not significantly different at 5 % level by DMRT

produced the highest number of seeds pod^{-1}. The effect of this treatment was statistically superior to other treatments. *Rhizboium* inoculant alone recorded comparatively higher number of seeds than AM. Control treatment recorded the lowest seed pod^{-1}. Singh (1997) obtained significantly higher seed yield in pigeonpea with AM + R compared with no inoculation.

1000-seed weight

There was a significant effect of different treatments in recording 1000- seed weight of chickpea (Table 3). The highest weight of 1000-seed (112.3 g) was recorded with the treatment T_6 (AM + R + P). The combined effect of *Rhizobium* inoculant, AM and P might have led to better assimilation of N for the plants that resulted the larger seeds. Solaiman *et al.* (2003) reported that *Rhizobium* inoculant significantly increased 1000-seed weight of lentil over control. Solaiman *et al.* (2005) also reported significantly higher 1000-seed weight in chickpea due to inoculation with *Rhizobium* and AM in presence N and P.

Seed yield

The effects of different combinations of *Rhizobium*, AM, N and P on seed yield of chickpea were varied significantly (Table 3). The maximum seed yield of 3.3 g $plant^{-1}$ was obtained with the treatment T_6 receiving AM + R + P. The effect of this treatment was statistically different from other treatments. All the treatments produced significantly higher seed yield compared to control. Increases in seed yield by AM inoculation are in good agreement with the observation of Gaur and Adholeya (2000). In this study dual inoculation (AM + R) gave significantly higher seed yield over single inoculation. Similar finding was reported by Carling *et al.* (1996) who found that AM and P fertilization increased seed weight of groundnut. Solaiman *et al.* (2005) obtained significantly higher seed yield in chickpea through inoculation with *Rhizobium* and AM along with N and P fertilizers.

Stover yield

Data presented in Table 3 show that dual inoculation (AM + R) along with P significantly increased stover yield. All the treatments significantly increased stover yield over control. The highest stover yield (3.2 g) at harvesting stage was recorded with the treatment T_6 receiving AM + R + P which was statistically different with other treatments. The trend of increase in stover yield was similar as that of seed yield $plant^{-1}$. This result has the resemblance with that of Solaiman *et al.* (2005).

REFERENCES

Abdel-Fattah, G. M. 1997. Functional activity of VA-mycorrhiza (*Glomus mosseae*) in the growth and productivity of soybean plants grown in sterilized soil. Folia Microbiologica, 42(5): 495-502.

Adholeya, A., B. N. Johri, and R. K. S. Chauhan. 1995. Effect of VAM-*Rhizobium* interaction on productivity, N-P uptake, dry weight and infection per cent in India mungbean (*Vigna radiata* L. Wilczek). In: Mycorrhizaé, Biofertilizer for the Future. A Adholeya, S. Singh (eds.) Proc. 3rd Natinal Conference on Mycorrhizae, 13-15 March, p. 195-197.

Andrade, G., F. A. A. M. De. Leij, and J. M. Lynch. 1998. Plant method interactions between *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and arbuscular mycorrhizae on pea. Letters in Applied Microbiology 26(4): 311-316.

Anonymous, 2004. Yearbook of Agricultural Statistics of Bangladesh, 2004. Bangladesh Bureau of Statistics, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, pp. 63-67.

Balachandar, D. and P. Nagarajan. 1999. Dual inoculation of vesicular arbuscular mycorrhiza and *Rhizobium* in green gram. Legume Res. 22: 177-180.

Barea, J. M., R. M. Tobar, and C. Azcon-Aguilar. 1996. Effect of a genetically modified *Rhizobium meliloti* inoculant or the development of arbuscular mycorrhizas root nutrient uptake and biomass accumulation in *Medicago sativa*. New-Phytologist. 134(2): 361-369.

Benthlenfalvay, G. J. and W. E. Newton. 1991. Agroecological aspects of mycorrhizae and nitrogen fixing legume symbiosis. *In*: The Rhizophere and plant Growth. D. L. Keister and P. B. Cregan (eds.) Netherlands: Kluwer Academic Publishers, pp. 349 - 354.

Burity, H. A., M. D. C. P. De Lyra, E. S. De Souza, S. A. C. D. Mergulhao, and M. L. R. B. Da Silva. 2000. Effectiveness of inoculation with AMF and *Rhizobium* on *Mimosa caesalpiniifolia* seedlings, under different phosphorus levels. Pesqui. Agropecu. Bras. 35: 801 - 807.

Carling, D. E., R. W. Roncadori, and R. S. Hussey. 1996. Interactions of arbuscular mycorrhizae, *Meloidogyne arenaria*, and phosphorus fertilization on peanut. Mycorrhiza 6: 9 - 13.

Cordess, R. 1988. Value of chickpea as animal feed. In: Proc. Conference Present Status and Future Prospects of Chickpea Crop Production and Improvement in the Mediterranean Countries, 11-13 July 1988, Zaragoza, Spain. J.I. Cubero; M.C. Saxena and J. Wery (eds.). Zaragoza, Spain: Centre Inernational de Hautes Etudes Agronomiques Mediterra neenes.

Das, P. K., P. N. Sahoo, and M. K. Jena. 1997. Effect of arbuscular mycorrhiza and rhizobia inoculation on nutrient uptake, growth attributes and yield of greengram (*Vigna radiata* L. Wilczek). Envoron. Ecol. 15: 830-833.

Douds, D. D. and P. Miller. 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agril. Ecosyst. Enviro. 74: 77-93.

El-Didamony, G. and G. M. Abdel Fattah. 1998. Influence on VA-myucorrhiza on growth and nodulation of *Vicia faba* in soil containing lytic phages. Egyptian J. Microbiol. 33: 153-166.

Gaur, A. and A. Adholeya. 2000. Response of three vegetable crops to AM fungal inoculation in nutrient deficient soils amended with organic matter. Symbiosis 29 : 19-31.

Gerdemann, J. W. and T. H. Nicolson. 1963. Species of mycorrhizal endogone species extracted from soil by wet sieving and decanting method. Trans. Brit. Mycol. Soc. 46: 235 - 246.

Hayman, D. S.1986. Mycorrhizas of nitrogen-fixing legumes. J. Appl. Microbiol. Biotech. 2:121 - 145.

Khanam, D., M. A. U. Mridha, and A. R. M. Solaiman. 2005. Effect of dual inoculation of arbuscular mycorrhiza and *Rhizobium* on root colonizaiton and spore population in chickpea. Bangladesh J. Microbiol. 22(1): 15-18.

Koske, R. E. and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92: 486 - 488.

Miles, A. A. and S. S. Misra. 1938. The estimation of the bactericidal power of blood. J. Hyg. Cambridge 38: 732 - 749.

Naqvi, N. S. and K. G. Mukerji. 1998. Mycorrhization of micro propagated *Leucaena leucocephala* (Lam) de Wit. Symbiosis. 24: 103-113.

Nurlaeny, N., S. Marschner, and E. George. 1996. Effects of liming and mycorrhizal colonization on soil phosphate depletion and phosphate, uptake by maize and soybean grown in two tropical acid soils. Plant and Soil 181 : 275-285.

Pandey, P. N., M. M. Verma, and R. K. Jian. 1998. Response of cowpea (*Vigna unguiculata* Linn. Walp.) to AM fungi *Glomus fasciculatum* and *Rhizobium* inoculation under different sources and levels of phosphorus. Biological Sciences 68 (3/4): 273-278.

Prasad, K. 2000. Growth responses in *Acacia nilotica* L. inoculated with *Rhizobium* and *Glomus fasciculatum* (AM fungi). J. Trop. Forestry 16: 22-27.

Ramalho – Ribero, J. M. C. and I. M. Portugal – Melo. 1988. Composition and nutritive value of chickpea. In: Proc. Conference on Present Status and Future Prospects of Chickpea Crop Production and Improvement in the Meditennaean Countries, 11-13. July 1988. Zanagoza, Spain. J.I. Cubero; M.c. Saxena and J. Wery (eds.). Zaragoza, Spain: Centre International de Hautes Etudes Agronomiques Mediaterraneennes.

Read, D. J., H. K. Koucheki, and J. Hodgaon. 1976. Vesicular arbuscular mycorrhiza in natural vegetation systems. New Phytol. 77: 641 - 653.

Saxena, M. C. 1987. Utilization of food legumes for human consumption. In: Jornadas Portuguesas de Protenaginosas Comunicacoes, 19a 21 de Fevereirode 1986, Deiras, Portugal: Estacao Agronomica Nacional, P. 406-419.

Singh, S. 1997. Interaction of arbuscular mycorrhizal fungi with nodule forming nitrogen fixing organisms. Part-I. Effect of dual inoculation on plant AM and/or nitrogen fixers. Mycorrhiza News 9: 1-13.

Solaiman, A. R. M., M. S. Mahmud, and M. S. Hoque. 2003. Response of lentil to *Rhizobium* inoculant and nitrogen: Yield and yield contributing characters. Bangladesh J. Life Sci. 15(2): 71-77.

Solaiman, A. R. M., M. G. Rabbani, and M. N. Molla. 2005. Effects of inoculation of *Rhizobium* and arbuscular mycorrhiza, poultry litter, nitrogen and phosphorus on growth and yield in chickpea. Korean, J. Crop Sci. 50(4): 256-261.

Tarafdar, J. C. and A.V. Rao. 1997. Response of arid legumes to AM fungal inoculation. Symbiosis 22: 265 - 274.

Zheng, W. W. and Y. N. Song. 2000. Effects of AM and *Rhizobium* on the growth and nitrogen fixation of wingbean. Fujian J. Agril. Sci. 15: 50-55.

Procedure of gum arabic solution preparation

01. 40% solution of gum arabic in 100 ml worm water (nearly boiling) was prepared
02. Seed wetted with adhesive solution (gum arabic) @ 20 ml kg^{-1} seed, mixed thoroughly.
03. Inoculated was added to the seed @ 15 g kg^{-1} seed.
04. Inoculant name : BARI Rca-201, Date : 05-10-2005

Initial status of soil (Kodda, Gazipur Sadar) :

Fertilizer :

Urea : 0.53 g per pot (10 kg^{-1} soil) ÷ 2 = 0.27 g 5 kg^{-1}
TSP : 1.80 g per pot (10 kg^{-1} soil) ÷ 2 = 0.90 g 5 kg^{-1}
MP : 0.95 per pot (10 kg^{-1} soil) ÷ 2 = 0.48 g 5 kg^{-1}

Total

AM x 24 pot (8 treatment x 3 replication)
Rhizobium x 15 pot (5 treatment x 3 replication)
Urea x 12 pot (4 treatment x 3 replication)
TSP x 12 pot (4 treatment x 3 replication)
MP x 30 pot (10 treatment x 3 replication)

Treatment :

T_1 : Control
T_2 : Arbuscular mycorrhiza (AM)
T_3 : *Rhizobium* (R)
T_4 : AM+R
T_5 : AM+R+N
T_6 : AM+R+P
T_7 : AM+N+P
T_8 : AM+N
T_9 : AM+P and
T_{10} : AM+R+N+P

Design :	CRD (10 x 3 sets = 30 pots)
Sowing date :	29-11-2005 (Tuesday)
Seed rate :	Six seeds pot^{-1}, should be taken on 2 seedling in each pot
Amount of soil pot^{-1} :	5 kg
Name of variety :	BARI chola-5
Number of AM :	350 100 g^{-1} soil
Amount of soil with inoculum : (Soils with sorghum root)	60 g pot-1 (210 AM pot^{-1})
Name of inoculum :	BARI AM-1
Name of *Rhizobium* :	BARI Rca-201 (Date of preparation October 5, 2005)

Surface sterilization of chickpea seed :

50 seeds in tuber

↓ Shake with 5 ml of methyl alcohol for 30 seeds

drain

↓ Shake with 10 ml 0.2% $HgCl_2$ for 3-4 minutes

drain

↓ Rinse with sterile water for 6-7 times

drain and place in water agar.