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EFFECTS OF INOCULATION WITH ARBUSCULAR- MYCORRHIZAL FUNGI AND PHOSPHORUS ON GROWTH, YIELD AND NUTRIENT UPTAKE OF MUNGBEAN GROWN IN STERILE AND NON-STERILE SOIL

M. K. RAHMAN, S. M. KABIR, G. M. MOHSIN[1], M.D. ALAM and R.MANDAL
Department of Soil, Water and Environment, University of Dhaka, Dhaka 1000, Bangladesh.
[1]East West Seed (Bangladesh) Ltd., Joydebpur, Gazipur, Bangladesh.

Effects of inoculation with arbuscular-mycorrhizal (AM) fungi and phosphorus fertilization on dry weights of shoot, root and seed, and uptake of N and P by the shoots of mungbean (*Vigna radiata* **L.**) were studied in pots in sterile and non- sterile soil under water- stressed (60%WHC) and unstressed conditions in a net house. The inoculation with *Glomus mosseae* increased dry weight of shoot and root, seed and N and P content of mungbean. Results revealed that *G. mosseae* inoculum (50g per 3 kg soil) can substitute 40 kg P ha^{-1} in yield of mungbean.

Keywords: Growth, *Glomus mosseae*, Mungbean, N and P uptake

Introduction

Mycorrhizal associations are known to improve growth and yield of crops under nutrient deficient condition of soil. The fungi have an important role particularly in economy and efficient use of phosphatic fertilizers[1,2]. The main effect of agricultural practices on the formation of mycorrhizas is generally related to the changes in plant species and at the rate of phosphatic fertilizer application[3]. Low phosphorus availability in most tropical soils had led to obligate mycotrophy of many crops *viz.* mungbean. Bangladesh is a tropical country and is deficient in available phosphorus. However, farming is intensive, diverse and dynamic. Mungbean is very popular as a good source of soup for human consumption and dry straw as feed for cattle. Moreover, it helps to increase the organic matter content of soils too. Mungbean is cultivated in winter when Bangladesh faces extreme water shortage. Evidences are available that plant- water relations could be enhanced by mycorrhizal colonization. The effects of the soil moisture status on arbuscular mycorrhizal (AM) colonization of crop plants have been investigated because it was suggested that colonization by AM fungi improved the drought resistance of the plants[4,5].

Total agricultural land of Bangladesh is 9 million hectares of which the farm holdings are about 11 millions. Therefore, Bangladesh is in compelling circumstances to utilize its land resource to its maximum potential capacity and at the same time sustaining the high production level[6]. Because of the low availability of P in Bangladesh soils, there is a need to exploit the potential of *G mosseae* (Nicol. and Gerd.) Gerd and Trappe under different soil conditions. Literature review shows that scanty information is available on the role of AM fungi on growth, yield and nutrient uptake of mungbean in soils of Bangladesh which are generally deficient in available P or have an insoluble form of P during winter when the moisture availability is scarce.

In view of this, the present experiment was carried out to evaluate the effect of inoculation with *G. mosseae* and phosphorus fertilization on growth, yield and N and P uptake by mungbean (var. Kantimung) grown in sterile and non- sterile soil under water- stressed and unstressed conditions.

Materials and Methods

Soil: Surface soil (0 to 20 cm depth) was collected from the charlands of the river Padma near the village Kartikpur under Dohar Upazila in Dhaka district. The soil sample was air- dried, sieved (<3mm) and stored in polyethylene bags. The physicochemical properties of the soil were determined following standard methods (Table 1)[7-11]. A portion of soil was autoclaved at 121°C in a pyrex beaker for 3h. The sequence of sterilization was 1h autoclaving and 24h cooling.

Pot experiment: Three kg of soil was taken into each sterilized earthen pot (22.5 cm diameter × 18.0 cm height). The pots and saucers were sterilized with 20% sodium hypochlorite solution.

Basal dressings of nitrogen (30 kgha^{-1}) and potassium (50 kgha^{-1}) were added as urea and muriate of

Table 1. Some physicochemical characteristics of the soil used in the pot experiment.

Parameters	Values	Parameters	Values
pH (1:2.5 w/v H_2O)	8.0	Cation exchange	
Organic carbon (%)[a]	0.20	capacity(meq/100g)[f]	8.9
Available N (mg100g^{-1} soil)[b]	4.04	Field capacity (%)	31.1
Total N (%)[c]	0.07	Particle size(%)[g]	
C/N ratio	2.86	Sand	4.8
Available P ($\mu g g^{-1}$)[d]	4.0	Silt	76.61
Total P (%)	0.04	Clay	18.95
Exchangeable K ($\mu g g^{-1}$)[e]	12.0	Texture	Silt loam
Total K (%)	0.65		

[a]Wet- oxidation method [7], [b]Extractable in 2*M* KCl [8], [c]By Kjeldahl extraction [3], [d]By ascorbic acid blue colour method [9], [e]Extractable in 1*M* ammonium acetate (pH 7.0), [f]Leaching tube technique [10], [g]Hydrometer method [11].

Table 2. Effect of *G mosseae* inoculation and phosphorus fertilization on shoot and root dry matter yield (g pot^{-1}) of mungbean grown in sterile and non- sterile soil under water- stressed and unstressed conditions.

Treatments	Water- stressed condition				Unstressed condition			
	Sterile soil		Non- sterile soil		Sterile soil		Non- sterile soil	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
M_1.Control (without AM and P)	0.83[a]	0.36[a]	1.64[a]	0.55[a]	1.27[a]	0.29[a]	2.74[a]	0.45[a]
M_2. *Glomus mosseae*	2.01[b]	0.61[b]	3.57[b]	0.89[b]	3.77[b]	0.61[b]	3.46[b]	0.61[b]
M_3. 40 kg P ha^{-1}	1.31[c]	0.38[ac]	1.98[c]	0.56[a]	2.77[c]	0.44[c]	3.87[c]	0.69[b]
M_4. 80 kg P ha^{-1}	1.43[c]	0.54[d]	2.79[d]	0.69[c]	2.82[c]	0.50[c]	4.00[c]	0.63[b]
LSD at 5%	0.24	0.05	0.18	0.06	0.44	0.06	0.20	0.11

abcd Data bearing different superscripts within the same column differ significantly.

Table 3. Effect of *G mosseae* inoculation and phosphorus fertilization on seed yield (g pot^{-1}) of mungbean grown in sterile and non- sterile soil under water- stressed and unstressed conditions.

Treatments	Water- stressed condition		Unstressed condition	
	Sterile soil	Non- sterile soil	Sterile soil	Non- sterile soil
M_1.Control (without AM and P)	0.88[a]	0.70[a]	0.82[a]	0.83[a]
M_2. *Glomus mosseae*	1.12[b]	1.02[b]	1.03[b]	0.99[b]
M_3. 40 kg P ha^{-1}	0.98[ac]	0.73[ac]	1.15[c]	0.98[b]
M_4. 80 kg P ha^{-1}	1.03[bc]	0.90[d]	1.19[c]	1.07[c]
LSD at 5%	0.10	0.08	0.10	0.06

abcd Data bearing different superscripts within the same column differ significantly.

Table 4. Effect of *G. mosseae* inoculation and phosphorus fertilization on nitrogen uptake (mg pot^{-1}) by the shoots of mungbean grown in sterile and non- sterile soil under water- stressed and unstressed conditions.

Treatments	Water- stressed condition		Unstressed condition	
	Sterile soil	Non- sterile soil	Sterile soil	Non- sterile soil
M_1.Control (without AM and P)	13.61[a]	31.32[a]	21.72[a]	51.90[a]
M_2. *Glomus mosseae*	28.74[b]	61.04[b]	61.83[b]	54.25[a]
M_3. 40 kg P ha^{-1}	19.65[c]	38.21[c]	36.01[c]	68.50[b]
M_4. 80 kg P ha^{-1}	22.45[c]	43.80d	36.66[c]	73.60[b]
LSD at 5%	3.70	2.28	2.55	8.25

abcd Data bearing different superscripts within the same column differ significantly.

Table 5. Effect of *G. mosseae* inoculation and phosphorus fertilization on phosphorus uptake (mg pot^{-1}) by the shoots of mungbean grown in sterile and non- sterile soil under water- stressed and unstressed conditions.

Treatments	Water- stressed condition		Unstressed condition	
	Sterile soil	Non- sterile soil	Sterile soil	Non- sterile soil
M_1.Control (without AM and P)	1.00[a]	2.13[a]	1.90[a]	3.84[a]
M_2 *Glomus mosseae*	2.61[b]	4.64[b]	6.41[b]	5.54[b]
M_3. 40 kg P ha^{-1}	1.70[c]	2.70[ac]	4.15[c]	5.42[b]
M_4. 80 kg P ha^{-1}	1.86[c]	3.91d	4.51[c]	6.40[b]
LSD at 5%	0.68	0.73	0.94	1.03

abc Data bearing different superscripts within the same column differ significantly.

potash, respectively. Fifty gram crude inoculum of *G. mosseae* (fragments of heavily infected maize roots, soil, hyphae *etc.*) were applied to the surface of 2.9 kg soils in the pots as a thin layer and then 100 g soil was spread over the surface of the inoculum. *G. mosseae* inoculum was obtained from the curtsey of the Department of Plant and Soil Science, University of Aberdeen, Scotland, UK. Equivalent amount of soil was added in pots where no AM was inoculated. Phosphorus (triple super phosphate) @ 40 and 80 kg P ha^{-1} was applied separately in sterile and non- sterile soil. Treatments used with three replications were as follows: M_1.Control (without AM and P), M_2.*G. mosseae*, M_3.40 kg Pha^{-1} and M_4.80 kg Pha^{-1}.

Two sets of pots were arranged separately under water-stressed and unstressed conditions following a randomized block design in the net house of the Department of Soil, Water and Environment, University of Dhaka. Seven water soaked seeds of mungbean were sown in each pot and after four days of emergence only 5 seedlings were allowed to grow. Water- stressed condition (60% field capacity) was maintained gravimetrically and pots under unstressed condition received water daily in the morning.

Harvesting and analysis: Twelve-week- old plants were harvested and separated into shoots, roots and seeds. Roots were washed and fine roots were kept in small vials in 50% ethanol solution for assessing the fungal infection. Shoots and roots were air- dried, oven- dried (65°C) for 72h, weighed, ground (<1mm) in a mechanical grinder and stored in air- tight polyethylene bags (16cm × 10cm). Seeds were sun-dried, weighed and kept in polythene bags. Ground shoot (0.1g) was digested with 5ml concentrated sulphuric acid and 2 ml 4% (v/v) solution of perchloric acid (62%) in concentrated sulfuric acid for N and P analyses. The digest was cooled and diluted to 100 ml with distilled water[12]. Finally N content of shoot was determined by using Kjeldahl semi micro steam distillation apparatus. The content of P in the digest was determined by the ammonium (acid molybdate- ascorbic acid- potassium antimony tartrate) molybdate blue colour method in a Cecil spectrophotometer[9].

For mycorrhizal colonization assessment, root pieces (1.5 cm long) were cleared in 0.5*M* KOH solution for 30 min at 90° C in water bath, then rinsed in water and soaked in 0.06 *M* HCl for 24h. After soaking, the roots were stained in an acidic glycerol solution containing

0.05% trypan blue for 30 min at 90°C in a water bath. The roots were destined and stored in acidic glycerol[13]. The stained root pieces were mounted on sterile membrane filters on a microscopic slide and a cover slip was placed on the top. The mounted root pieces were observed under a light microscope. The presence or absence of infection in the root pieces was recorded and then percentage infection was calculated[14]. The results were analyzed statistically.

Results and Discussion

Effects on plant growth and yield: Application of *G.mosseae* and P fertilizer on growth and yield of mungbean showed an appreciable change. The difference in yield values for shoot and root (Table 2) and seed (Table 3) varied significantly ($P<0.05$). Dry weights of shoot were found to be 142.1, 57.8 and 72.3 ; 117.7, 20.7 and 70.1; 196.8, 118.1 and 122.0 ; and 26.3, 41.2 and 46.0% higher in M_2, M_3 and M_4 treatments than the control (M_1) in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively. The highest weight of shoot was 2.01, 3.57, 3.77 and 4.0g pot^{-1} obtained in M_2, M_2, M_2 and M_4 treatments, respectively. Dry weights of root (Table 2) were 69.4, 5.5 and 50.0; 61.8, 1.8 and 25.4; 110.3, 51.7 and 72.4; and 35.5, 53.3 and 40.0% higher in M_2, M_3 and M_4 treatments over the control (M_1) in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively. The highest quantity of roots were 0.61, 0.89, 0.61 and 0.69 g pot^{-1} recorded in M_2, M_2, M_2 and M_3 treatments in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively. Seed yields (Table 3) were 27.3, 11.3 and 17.0 ; 45.7, 4.3 and 28.6 ; 25.6, 40.2 and 45.1 ; and 19.3, 18.1 and 28.9% higher in M_2, M_3 and M_4 treatments over control (M_1) in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively, (Table 2). The highest quantity of seeds were 1.12, 1.02, 1.19 and 1.07 g pot^{-1} obtained in M_2, M_2, M_4 and M_4 treatments in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively. On the whole mycorrhizal plants produced better dry matter and seed yield than non- mycorrhizal one. Ganry *et al.*[15] concluded that *G.mosseae* increased dry weight of soyabean plants.

Effects on N and P uptake: The uptake of N and P in shoots is presented in Tables 4 and 5, respectively. The variation between in uptake of N and P due to treatments were significant ($P<0.05$). The amount of N taken up by the shoot were 111.1, 44.4 and 64.9; 94.9, 22.0 and 39.8; 184.7, 65.8 and 68.8; and 4.5, 32.0 and 41.8% higher in M_2, M_3 and M_4 treatments over the control (M_1) in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively(Table 4). The highest amounts of N accumulated by shoots were 28.74, 61.04, 61.83 and 73.6 mg N pot^{-1} in M_2, M_2, M_2 and M_4 treatments, respectively, under same conditions (Table 4). Hayman[16] concluded that in legumes nodulation and N-fixation were greatly increased by mycorrhizal inoculation, sometimes beyond that achieved by adding phosphate fertilizer alone. The uptake of P were 161.0, 70.0 and 86.0; 117.8, 26.7 and 83.5; 237.3, 118.4 and 137.3; and 44.2, 41.1 and 66.6% higher in M_2, M_3 and M_4 treatments over the control (M_1) in sterile and non- sterile soils under water- stressed and unstressed conditions, respectively. The highest amount of P uptake by shoots was recorded as 2.61, 4.64, 6.41 and 6.40 mg P pot^{-1} in M_2, M_2, M_2 and M_4 treatments, respectively, under same conditions(Table 5).Nitrogen and phosphorus uptake were higher in *G.mosseae* inoculated treatment except in non- sterile soils under unstressed conditions. Mycorrhizal colonization (29.1 to 48.3%) was observed in *G.mosseae* inoculated plants only. Inoculation with *Glomus* sp. (WUM 16) increased P and Zn contents in the shoots of subterranean clover[17]. Osonobi[18] suggested that AM inoculation enhanced plant growth through the improvement of drought resistance as well as P nutrition in low- P soil under dry condition. However, the soil moisture level is markedly deficient in winter compared to ordinary water- stress in Bangladesh. Further study is required to clarify the influence of the soil moisture status and AM-P interactions in the field.

It may be concluded that *G.mosseae* (50 g inoculum per 3 kg soil) has positive effect on dry matter yield, seed production, and N and P uptake by mungbean and has the ability to replace 40 kg P ha^{-1} fertilization.

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References

1. Rahman MK and Parsons JW 1997, Effects of inoculation with *Glomus mosseae*, *Azorhozobium caulinodans* and rock phosphate on the growth of and nitrogen and phosphorus accumulation in *Sesbania rostrata*. *Biol. Fertil. Soils* **25** 47-52.
2. Rahman MK, Kabir SM, Mohsin GM and Alam MD 2006, Interaction of arbuscular mycorrhizal fungus *Glomus mosseae* and phosphorus on growth and nutrient uptake of maize palnts grown under different

soil conditions. *Bangladesh J. Bot.* **35** 1-7.

3. Abbott LK and Robson AD 1994, The impact of agricultural practices on mycorrhizal fungi. In: CE Pankhurst, BM Doube, VVSR Gupta and PR Grace (Eds.) Soil Biota Management in Sustainable Farming Systems. CSIRO, Australia. pp. 88-95.
4. Ellis JR, Larsen HJ and Boosalis MG 1985, Drought resistance of wheat plants inoculated with vesicular-arbuscular mycorrhizae. *Plant Soil* **86** 369-378.
5. Sylvia DM, Hammond LC, Bennet JM, Haas JH and Linda SB 1993, Field response of maize to a VAM fungus and water management. *Agron. J.* **85** 193-198.
6. Hussain, MS, Elahi SF, Eswaran H, Uddin MJ and Islam S 2003, Bangladesh: In Quest of Resource Management Domains. Publication No. 2. Department of Soil, Water and Environment, University of Dhaka, Bangladesh. pp.83.
7. Jackson ML 1958, Soil Chemical Analysis. Prentice-Hall, Englewood Cliffs, NJ. pp. 498.
8. Marr IL and Cresser MS 1983, The lithosphere. *In*: Environmental Chemical Analysis. Blackie and Son, UK.pp.155-182.
9. Murphy J and Riley JP 1962, A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **27** 31-36.
10. Tinsley J 1967, Determination of cation exchange capacity (CEC) *In*: Soil Science Manual of Experiments. Department of Plant and Soil Science, University of Aberdeen, UK.pp. 72-73.
11. Piper CS 1944, Mechanical analysis. *In*: Soil and Plant Analysis. The University of Adelaide, Australia.pp. 47-79.
12. Cresser MS and Parsons JW 1979, Sulphuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. *Anal. Chim. Acta* **109** 431-436.
13. Koske RE and Gemma JN 1989, A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res.* **92** 486-488.
14. Giovannetti M and Mosseae B 1980, An evaluation of techniques for measuring vesiculars- arbuscular mycorrhizal infection in roots. *New Phytol.* **84** 489-500.
15. Ganry F, Diem HG, Wey J and Dommergues YR 1982, Effect of inoculation with Glomus *mosseae* on nitrogen fixation by field grown soybeans. *Plant Soil* **68** 321-329.
16. Hayman DS 1980, Mycorrhiza and crop production. *Nature* (London) **287** 487-488.
17. Abbot LK and Robson AD 1985, The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus. Aust.J. Soil Res.* **23** 253-261.
18. Osonubi O 1994, Coparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and phosphorus uptake of maize (*Zea mays* **L.**) *Biol. Fertil.* Soils **18** 55-59.