Pak. J. Biotechnol. Vol. 13 (1) 67-71 (2016)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

RESPONSE OF DIFFERENT DOSES OF PHOSPHATE FERTILIZER AND PHOSPHATE SOLUBILIZING_BACTERIA AND MYCORRHIZA IN MUNGBEAN (*VIGNA RADIATA* L.)

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Article received 9.12.2016; Revised 11.3.2016; Accepted 15.3.2016

ABSTRACT

A field experiment was conducted in randomized complete block design (RCBD) with seven treatments in four replications at Nuclear Institute of Agriculture (NIA), Tando Jam. Five plants were selected from middle rows of the plot for yield parameters. Yield and yield components were recorded such as plant height (cm) number of pods/plant, single plant biomass weight (g), number of branches/plant, number of panicles/plant, single plant grain yield (g) biological yield kg/ha, grain yield (kg/ha), days to flowering, days to maturity of the crop, and 100-grain weight (g). Bacterial enumeration after sowing of one week and after harvesting of the crop were recorded. The results revealed that the all yield and yield components were highest in T2 (80 kg P/ha), whereas, the control was the lowest. In treatment *Bacillus megatherium* and 40 kg P/ha were identical with each other. T5 (*Bacillus megatherium*+40 kg P/ha), T6 (Mycorrhiza) and T7 (Mycorrhiza + 40 kg P/ha) were similar with each other but lower than T2, T3, T4 and higher than control. Biological yield and grain yield were highest in T2 (2541 kg/ha and 883 kg/ha). Same trend was found for bacterial enumeration after sowing of one week the crop and after harvesting of the crop at the depth of 0-15 and 15-30 cm in one gram of soil. It was observed that the addition of *Bacillus megatherium* increased the yield and other yield associated traits of mungbean. The results showed that the P solubilizing bacterial inocula alone may be used for obtaining reasonable yield in mungbean.

Key words: P**hosphate, Solubilizing, bacteria, mungbean**

INTRODUCTION

Mungbean (*Vigna radiata* L). is a well known pulse crop of Pakistan. It is a short duration crop and can be growth twice in a year. Being drought resistant, it can withstand adverse environmental conditions and is successfully cultivated in rainfed areas. This is digestible, high in protein (22-24%), (Malik,1994) and does not cause flatulence that many other legumes do moreover, it is rich in vitamins as A,B,C niacin, and minerals such as potassium, phosphorus and calcium which are necessary for human body (Rattanga wongsa, 1993). It is a good substitute of animal protein and forms a balanced diet when it is taken with cereals. Despite the high total soil P content, plant P availability is often reported to be limited, particularly in tropical soils (Collavino *et al*., 2010). Most soil P is usually present as insoluble metal chelates (Vassilev *et al*., 2006). Moreover, substantial amounts of applied chemical (P) fertilizer are also rapidly converted with insoluble phosphate source. This leads to regularly applying P fertilizers, which is not only costly, but also environmentally undesirable. In this context, microbial solubilization of soil insoluble phosphates with soluble forms is considered as an important process in natural and agricultural ecosystems. Several bacterial and fungal species are available with varied potential to solubilize inorganic phosphates have been found in rhizosphere of plants (Jain *et al*., 2012). Next two nitrogen, phosphorus is another important key element not only in plant metabolism but also present in soil microbiological processes, solubilization of bound phosphates by acid secretion, the mineralization of organic phosphatic compounds and the immobilization of phosphorus by which inorganic ion are incurporated in the microbial cell material. Organic matter rich soils are also rich in organic phosphorus derived from vegetation and protoplasm of microorganism which undergo decomposetion. Phosphorus is added to soil in the form of phosphatic fertilizer, part of which is utilized by the plant and the remaining is converted in the fixed and insoluble forms of phosphorus. Major portion of applied (P) in soil is converted in to non-available form, which is mostly due to high pH and activity of $CaCo_3$, (Sharif, 1985). Phosphate solubilising microorganisms are ubiquitons in soil and could play an important role in supplying p to plant in a more environment friendly and sustainable manner (Gyaneshwar *et al*., 2002). The microbial biomass in soil contains a significant amount of P and considerable pools are maintained even in soils considered to be P deficient for plant growth (Obserson *et al*., 2001). Phosphorus biofertilizers in the form of microorganism can help in increasing the avail-

ability of accumulated phosphates for plant growth by solubilisation (Goldstein, 1986; Gyaneshwar *et al*., 2002). About 20-30 percent of the soil microorganisms such as bacteria and fungi have been reported to solubilize the insoluble phosphates of soils in to soluble forms by secreting organic acid and lowering PH(Cunningham and Kirliack, 1992; Nahas, 1996) and are hence considered important for agriculture production (Motsara *et al*: 1995). Plant P-content increased only when *Penicillium simplicissimum* was inoculated. (Illmer *et al.*, 1995). Some fungi are able to release phosphate from insoluble inorganic phosphates, for example, species of *Aspergillus, Fusarium, Penicillium* and *Sclerotim* produce organic acids and /or chelating components which act as solubilising agents (Beever and Burns, 1980; wainwright, 1988). Such microbes act as bio-inoculant which increase plant growth and yield and reduce the chemical fertilizer requirement. There are several reports of phosphate solubilizing bacteria (PSB) which reported the increase growth and biomass of several crops. (Fernandez *et al*., 2007; Mittal *et al*: 2008; Vikram and Hamzehzarghani 2008; Hariprasad and Niranjana, 2009; Jain *et al*: 2010). Some fungi, along with some bacteria and actionmycetes are able to solubilize phosphate through secretion of organic acids (Khan and Bhatnager 1977; Kucey, 1987). The aim of these studies was to isolate the bacterial and fungal species which can enhance the mungbean production and to increase the solubilization of soil (P).

MATERIALS AND METHODS

A field study was conducted at Nuclear Institute of Agriculture (NIA) experimental farm Tandojam, Sindh Pakistan. Phosphate solubilizing bacteria and mycorrhizae were previously isolated from the maize crop at (NIA) Tandojam experimental farm. The phosphate solubilizing bacteria such as *Bacillus megatherium* and mycorrhizae (*Glomus fasciculatum)* were cultured on Pikovskaya's medium for conformation their halo zones formation. After confirmation the nutrient broth was prepared individualy then the both organisms were inoculated into broth containing flask 25 ml of each organism than the bacterial concentration at the rate of 221 x 10^3 /ml (25 ml/kg of mungbean seed) was inoculated and mycorrhizae at the rate of 108 x 10^2 spores /ml (25 ml/kg of mungbean seed) was inoculated. The experiment was conducted in randomized complete block design (RCBD) with three replications and 07 treatments.

T_1= control, T_2= 80 kg P/ha, T_3= *Bacillus megatherium* at the concentration of 221x10^3 /ml 25 ml /kg of mungbean seed, T_4=40 kg P/ha, T_5= bacteria *Bacillus megatherium* at the concentration 221 x 10^3/ml + 40kg P /ha, T_6= Mycorrhhizae (*Glomus fasciculatum)* at the concentration 108 x 10^2 spores /ml 25ml /kg of mungbean seed, and T_7=mycorrhizae same concentration as T_6 + 40 kg P/ha. During the study the days to flowering and maturity of the crop according to each treatment along with replication were noted.

At the maturity of the crop, five plants were randomly selected from middle two rows and harvested for data collection on plant height (cm), number of pods/plant, single plant dry biomass weight(g), number of branches/plant, number of panicles/plant, single plant grain yield (g), 100-grain weight (g), biological yield and grain yield (kg/ha). On microbial data, bacterial counting /gram of soil (pore plate method) were done after sowing of one week and after harvesting of the crop. The collected data was subjected to analysis of variance and DMR test for comparison of means.

RESULTS AND DISCUSSION

The results revealed that the plant height (cm) was significantly highest in T2= 80 kgP/ha as compared to all other treatments. Whereas, T1, T5, T6 and T7 showed lowest plant height. Whereas, T3 and T4 showed similarity in plant height (cm). The maximum number of pods/ plant were noded in T2= 80 kg P/h. Treatments T3 and T4 produced similar number of pods/ plant. Non significant difference was found in T5, T6 and T7. About 20-30 percent of soil microorganisms such as bacteria and fungi have been reported to solubilize the insoluble phosphates of soil in to soluble forms by secreting organic acids and lowering pH (Cunningam and Kuiack, 1992; Nahas, 1996) and are hence considered important for agriculture production (Motsara *et al*; 1995). The highest single plant biomass weight (g) was recorded in T2 = 80 kg P/ha, whereas, the T3 and T4 showed similar results in biomass weight (g). The lowest dry biomass/plant weight (g) was found in T1 = control. The maximum number of ranches/plant were recorded in T2 = 80 kg P/ha. Treatment T3 and T4 showed non significantly difference for number of branches /plant. Whereas, control = T1 produced minimum number of branches

/plant. Significantly the highest number of panicles /plant were recorded in T2 = 80 kg P/ha, Whereas, control could produce minimum number of panicles /plant. T3 and T4 produced similar number of panicles /plant followed by T5, T6 and T7 (Table 01). The highest single plant grain yield (g) was recorded in T2=80 kg P/ha, whereas, T3 and T4 showed similar single plant grain yield (g) followed by T5, T6 and T7. The control T1 was found as lowest in single plant grain yield (g). The result showed that 100- grain weight (g) was maximum in T2, Whereas, lowest was recorded in control. T3 and T4 showed same 100-grain weight (g) followed by T5, T6 and T7. Biological yield was recorded maximum in T2 while T3, T4 were identical with each other followed by T5, T6 and T7. T1 = control gave minimum biological yield kg/ha. The data revealed that grain yield was highest in T2. T3 and T4 showed same results followed by T5, T6 and T7. The control showed lowest grain yield kg/ha (Table- 2).

Table- 1: Influence of microbial different concentration on grain yield and yield associated characteristics of mungbean variety AEM-96

Treatments	Plant height (cm)	No:of pods/plant	Single plant bio-mass weight (g)	No.of branches /plant	No. of Panicles/ Plant
T1=Control	31 c	10 d	8.45 d	01 d	3 d
T2=80 kg P/ha	37 a	15 a	13.64 a	05 a	6 a
T3=*Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed)	34 b	14 b	10.29 b	04 b	5 b
T4=40 kgP/ha	34 b	14 b	10.33 b	04 b	5 b
T5= *Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed) +40 kgP/ha	33 c	12 c	9.31 c	03 c	4 c
T6=Mycorrhiza 108 x 10^2 /ml (25 ml /kg of seed)	33 c	12 c	9.31 c	03 c	4 c
T7=Myco+40kgP/ha	33 c	12 c	9.22 c	03 c	4 c

Table -2: Effect of synthetic P fertilizer and microbial inoculants on mungbean .

Treatments	Single plant grain yield (g)	100-Grain weight (g)	Biological yield (kg/ha)	Grain yield (kg/ha)
T1=Control	2.67 d	1.81 d	1391 d	381 d
T2=80 kg P/ha	3.94 a	4.81 a	2541 a	883 a
T3=*Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed)	3.50 b	3.71 b	2391 b	772 b
T4=40 kgP/ha	3.46 b	3.72 b	2376 b	777 b
T5= *Bacillus megatherium* 221x10^3 /ml (25ml/kg of seed) +40 kgP /ha	3.02 c	2.91 c	2112 c	581 c
T6=Mycorrhiza 108 x 10^2 /ml (25 ml /kg of seed)	3.23 c	2.90 c	2113 c	580 c
T7=Myco+40kgP/ha	3.12 c	2.92 c	2110 c	579 c

Mungbean seed inoculated with *rhizobium* strain of bacteria and phosphate solubilizing bacteria (PSB) increased rate of nodulation and seed yield as compared to control (Chatterjee and Bhatterjee 2002). Another study showed that the mungbean seed inoculated with *Rhizobium* strain significantly increased the number of nodules / plant, dry matter accumulation in the shoot, crop growth and plant height. Bio-fertilizer supply nitrogen and phosphorus improve growth of several crop plant was observed (Maruwaha, 1995). The data showed that the T2= 80 kg P/ha showed the significantly lowest days to flowering followed by T3 and T4. There was non significant difference found in T5, T6 and T7. The maximum days for flowering of crop were recorded in control. The maximum days to maturity were recorded in control and the lowest days were recorded in T2= 80 kg P /ha. Similar days to maturity were recorded in T3, T4, T5, T6 and T7 (Table 03). The maximum bacteria at the depth of 0-15 cm in after sowing of 01 week ($200x10^6$) were found /g of soil in T2. Whereas, minimum bacterial counting was recorded in control = T1. The treatment such as T3 and T4 showed non significant difference among each other followed by T5, T6 and T7.

At the depth of 15-30 cm. The bacterial counting in T2 = 80 kg P /ha showed highest counting /g of soil. Whereas, control= T1 showed the lowest number of bacteria/g of soil. Treatment such as T3, T4 showed same bacterial counting/g of soil followed by T5, T6 and T7 (Table 4).

Table-3: Phonological trails of mungbean as affected by various treatments phosphorus and phosphor-bacterial and mycorrhizal inoculums.

Treatments	Days to flower	Days to mature
T1=Control	52 a	72 a
T2=80 kg P/ha	40 d	62 d
T3=*Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed)	42 c	65 c
T4=40 kgP/ha	42 c	65 c
T5= *Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed) +40 kgP/ha	46 b	67 b
T6=Mycorrhiza 108 x 10^2 /ml (25 ml /kg of seed)	46 b	67 b
T7=Myco+40kgP/ha	46 b	67 b

Table-4: Bacterial enumeration/g of soil after one week of sowing at the depth of 0-15 cm and 15-30 cm in different treatments.

Treatments	0-15 cm	15-30 cm
T1=Control	130x10^3 d	100x10^2 d
T2=80 kg P/ha	200x10^6 a	187x10^5 a
T3=*Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed)	190x10^5 b	140x10^4 b
T4=40 kgP/ha	191x10^5 b	141x10^4 b
T5= *Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed) +40 kgP/ha	166x10^5 c	130x10^4 c
T6=Mycorrhiza 108 x 10^2 /ml (25 ml /kg of seed)	166x10^5 c	130x10^4 c
T7=Myco+40kgP/ha	166x10^5 c	129x10^4 c

At the depth of 0-15 cm after harvesting of the crop: The highest bacterial counting /g of soil were recorded in T2.Whereas, T3 and T4 showed similar number of bacteria /g of soil followed by T5, T6 and T7 same trend was found at the depth of 15-30 cm. (Table 05). The various studies showed that the application of phosphate solubilizing bacteria (PSB) increased yield of maize, legumes and potatoes (Dubey and Billore 1992).

Table -5: Bacterial counting/g in soil after one week of harvesting at the depth of 0-15 cm and 15-30 cm in different treatments.

Treatments	0-15 cms	15-30 cms
T1=Control	110x10^2d	98 x10^2 d
T2=80 kg P/ha	198x10^4a	152 x10^3 a
T3=*Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed)	150x10^3b	140x10^2 b
T4=40 kgP/ha	150 x10^3b	141x10^2 b
T5= *Bacillus megatherium* 221 x 10^3 /ml (25 ml /kg of seed) +40 kgP/ha	140 x10^2c	130x10^1 c
T6=Mycorrhiza 108 x 10^2 /ml (25 ml/kg of seed)	141 x10^2c	131x10^1 c
T7=Myco+40kgP/ha	140x10^2c	130x10^1 c

REFERENCES

Beever, R.E. and D.J.W.Burns, Phosphorus uptake, storage and utilization by fungi. Advances in Botanical Research **8**: 128-220 (1980).

Chatterjee, A. and P.Bhattarjee, Influence of combined inoculation with *Rhizobium* and Phosphobacteria on mungbean in field. J. Mycopathological Res. **40**(2):201-203 (2002).

Collavina, M.M., P.A.Sasberro, L.A Mroginski, and O.M. Aguilar. Comparison of in vitro solubilization in activity of diverse phosphate solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biology and fertility of soils **46**: 727-7383 (2010)

Cunningham, J.E. and C.Kuiack, Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaji*. Applied and Environ. Microbial, **36**: 81-92 (1992).

Dubey, S.K. and S.D. Billore, Phosphate solubilizing microorganisms inoculants and their role in augmenting crop productivity in India: a review. crop Research **5**: 11-14 (1992).

Fernandez, L.A., P. Zalba, M.A Gomez, and M.A. Sagardoy, Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under green house conditions. Biology and fertility of soils **43**: 805-809 (2007).

Goldstein, A.G., Bacterial Phosphate Solubilization. Histrical perspective and future prospects. Am. J. Alt. Agric.1: 57-65 (1986).

Gyaneshwar, P., G.N. Kumar, I.J. Parekh and P.S. Pool, Role of soil microorganisms in improving P nutrition of plants. Plant and soil **245**: 83-93 (2002).

Hariprasad, P., and S.R. Niranjana, Isolation and characterization of phosphate solubilizing

rhizobacteria to improve plant health of tomato. Plant and soil **316**: 13-24 (2009).

Illmer P, A. Barbato and Fschinner, Solubilization of hardly-soluble $ALPO_4$ with P-solubilizing microorganism. Soil Biology and Biochemistry 27(3): 265-270 (1995).

Jain,R., J.Saxena and V.Sharma, The evaluation of free and encapsulated *Aspergillus awamori* 529 on Mungbean (*Vigna radiata* cv RMG 492) growth. Folia Microbiological **57**: 533-541 (2010).

Jain, R., J. Saxena and V. Sharma, Effect of Phosphate solubilizing fungi *Aspergillus avamori* 829 on Mungbean (*Vigna radiata* L. cv. RMG 492) growth. Folia Microbiologica **57**: 533-541 (2012).

Khan, J.A. and R.M. Bhatnagar, Studies on solubilization of insoluble phosphates by microorganism. 1. solubilization of Indian phosphate rocks by *Aspergillus niger* and *Penicillium* Species. Fertilizer Technology **14**: 329-333 (1977).

Kucey, R.M.N., Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrient from soil by wheat. Can. J. Soil/ Review. Can.Sci.Sol. 68(2): 261-270 (1987).

Malik, B.A., Grain Legumes In Nazir, Ms(ed), Crop production, National Book Foundation, Islamabad Pp. 301(1994).

Maruwaha B.C., Biofertilizer. A supplementary source of plant nutrient. Fer. News **40**: 39-50 (1995).

Mittal, V., O. Singh, H. Nayyar, J.Kaur and R. Tewari, Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and P*enicillium citrinum*) on yield of chickpea (*Cicer arietinum* L. Cv. GPF_2). Soil Biol.Bioch, **40**: 718-727 (2008).

Motsara, M. R., P. Bhattacharyya and B. Srivastava, Biofertilizer Technology, Marketing and usage fertilizer Development and consultaion organization, New Dehli, India, Pp. 184 (1995).

Nahas, E., Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J. Microbial. Biotechnol. **12**: 567-572 (1996).

Oberson, A., D.K.Friesen, I.M. Rao, S. Buhler and E. Frosserd, Kinetics of microbial phosphorus up take in cultivated soils. Biol. Fertil. Soil. **34**: 32-41 (2001).

Rattanawongsa, N., The 19th International Mungbean Nursery Trial ARC-AVRD training report (1993).

Sharif, M., Improvement of phosphate fertilizer efficiency, Proc. Int. Seminar on fertilizer use Efficiency, Fauji fertilizer company, Lahore, Pakistan, PP.106-110 (1985).

Vassilev, N., M. Vassileva and I. Nikolaeva, Simultanious P- solubilizing and control activity of microorganism: potential and future trends. Applied Microbiology and Biotechnology **71**: 137-144 (2006).

Vikram, A., and H. Hamzehzarghani, Effect of phosphate solubilizing bacteria on nodulation and growth parameters of green gram (*Vigna radiata*). Res. Journal of Microbiol. **3**: 62-72 (2008).

Wainwright, M., Metabolic diversity of fungi in relation to growth and mineral cycling in soil- a review. Transaction of the British Mycological Society **90**: 159-170 (1988).