
Co-rhizobium inoculation and phosphate fertilizer management on nitrogen fixation and yield of mungbean

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Abstract TSP (triple superphosphate) applications combined with *Rhizobium* (SB2) produced the highest nodule dry weight at all stages. The highest levels of shoot dry weight were presented by TSP combined with SB2 at 10.0 g plant⁻¹ (R3.5 stage) and SBMix at 2.6 and 20.8 g plant⁻¹ (V4 and R7 stages), respectively. Moreover, TSP plus SBMix showed the maximum of total N and amount of nitrogen fixed at V4 (4.48%N and 4.9 kgN ha⁻¹) and R3.5 stage (3.66%N and 80 kgN ha⁻¹). At R7 stage, application TSP combined with SB2 provided the highest level of total N (3.70%N), amount of nitrogen fixed by mungbean (232 kgN ha⁻¹), thousand grains weight (76.4 g), number of seeds per pod (12.3 seeds pod⁻¹), and grain yield (1,138 kg ha⁻¹). In summary, phosphorus fertilizer combining inoculation rhizobium SB2 or SBMix could be recommended for increasing yield and nitrogen fixation of mungbean in the field.

Keywords: Rhizobia, Ureide technique, Legume plant, Mineral fertilizer

Introduction

Phosphorus is one of the essential elements for plants growth and metabolism (Abel *et al.*, 2002; Shenoy and Kalagudi, 2005). The phosphorus availability in soil solution has a limited effect on crop production including legume plants (Marschner, 1995). The fundamental practice of liming acid soils to the pH ranges from 6.5 to 7.0 is significant to the relationship between phosphorus and symbiotic nitrogen fixation process. Indeed, phosphorus influences nodule development, nodule dry weight and plants engaged in symbiotic nitrogen fixation (Robson, 1983; Jungk, 1998). However, legumes plantation grew in soil limited level phosphorus conditions leads to decrease nitrogenase activity, signal transduction, carbon partitioning, ATP substrate, nodule number, nodule mass, and plant nitrogen accumulation (Ribet and

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Drevon 1995; Al-Niemi *et al.*, 1997). In tropical soils, phosphorus deficiency is an important factor conducive to low nitrogen fixation, dry matter and yield of legumes. To improve growth of legumes can be solved by phosphorus fertilization applied (Mushtaq *et al.*, 1986; Ogata *et al.*, 1988; Thind *et al.*, 1990). Kumawat *et al.* (2010) report that nodule formation and microbial activities in the soil has a particular role in phosphorus management. Moreover, Hussain *et al.* (2010) and Prasad *et al.* (2014) also reported rhizobium inoculation along with phosphorus increased plant dry weight, nodulation, root growth, sufficient nitrogen fixation from atmospheric, phosphorus available in soil and enhancing productivity of green gram. To use rhizobium inoculation for mungbean under several phosphorus levels in Pakistan was noted that rhizobium inoculation had correlation with high phosphorus rate of application on fresh and dry weight of nodules, number of nodules, grain yield, percentage of relative ureide index, nitrogen and phosphorus uptake and nitrogen fixation (Tariq *et al.*, 2007; Ali *et al.*, 2010; Hussain *et al.*, 2012). Moreover, inoculation rhizobium along with phosphorus fertilizer improved soil fertility for sustainable agriculture (Fatima *et al.*, 2007).

Up to now, Maejo 3 (MJU3) is the new variety that is mass-selected from breeding lines VC3541B, originally selected by Asian Vegetable Research and Development Centre (AVRDC) in Taiwan. This variety was made by the single seed descent method (SSD) at Maejo University. These cultivars provided higher seed yield, drought tolerance and flood tolerance. Our study sites in central Thailand are characterized by low contents of available phosphorus and high levels of soil pH including high contents of calcium in topsoil. Iron and manganese deficiency symptoms in plants are also found in this area. The objective was to increase yields and enhance efficiency of plant nutrients in a field experiment which applied with varying amounts of *Rhizobium* inoculation and triple superphosphate to find the highest nitrogen fixation.

Materials and methods

A field experiment was carried out at Prince Chakrabandh Pensiri Center for Plant Development, Saraburi Province. The study consisted of 8 treatments and 4 replications with split-plot in the randomized complete block design. Triple superphosphate (TSP) at 125 kg ha⁻¹ and without TSP fertilizer application were used as main plots factor; *Rhizobium* strains (without *Rhizobium* (-Rh), *Bradyrhizobium elkanii* (SB2), *Rhizobium* mix strain (*Bradyrhizobium* sp. (SB1), *Bradyrhizobium elkanii* (SB2) and *Rhizobium* sp. (SB3)) (SBMix) (Dechjiraratthanasiri *et al.*, 2021) and *Rhizobium* from the department of agriculture (DOA) were used as the subplot factor. Mungbean

MJU 3 variety was grown in a 6 x 6 m plot with 25 x 50 cm plant spacing. Sprinkler irrigation technique was applied during mungbean cultivation. Rhizobium culture preparation was grown in yeast mannitol broth (YMB) at 125 rpm min⁻¹ for 7 days at 30 °C, to gain a maximum number in excess of 10⁸ CFU g⁻¹ for peat moss sterile carrier-based inoculants. Thereafter, 200 g inoculants were mixed with 5 kg mung bean seeds. Soil chemical properties before experiment were analysed both topsoil (0-15 cm) and subsoil (15-30 cm). Both of soil levels were clay in texture, neutral in reaction (pH 7.72 and 7.77) measured in a 1:1 slurry with deionized water, organic matter: 2.20 and 1.80% by the method of Walkley and Black, extractable phosphorus by CAL-method: 23.8 and 22.5 mgP kg⁻¹ (Schüller, 1969), extractable potassium: 186 and 161 mgK kg⁻¹ respectively (Motsara and Roy, 2008). The amount of soil extraction of calcium and magnesium were shown from 6,135 to 6,200 mgCa kg⁻¹ and 350 to 399 mgMg kg⁻¹ in top and subsoil, respectively.

Plant biomass and nodules were collected to estimate the number of nodules, nitrogen content, dry weight at the V4 (fourth node), R3.5 (beginning seed) and R7 stages (harvesting) (Pookpakdi *et al.*, 1992). All plant samples were oven-dried at 65 °C for 48 hours and ground. The nitrogen content in the shoot was also determined by Kjeldahl method. Xylem sap samples were collected (10 plants sample⁻¹) of each stage for nitrogen fixation analysis. Ureide technique was used to measure for nitrogen fixation. The percentage of relative ureide index (%RUI) was calculated from the molar concentration of ureides by allantoin and allantoic acid, amino by colorimetrically with ninhydrin and nitrate portion by the salicylic acid method using the following equation: Percentage of relative ureide index (RUI) = 4 x ureide / 4 x ureide + amino + nitrate x 100 (Peoples *et al.*, 1989; Herridge and Peoples, 1990).

The percentage of nitrogen derived from the atmosphere (%Ndfa) was estimated from %RUI using the following equations: %Ndfa = 1.5 (%RUI - 21.3) at V4 stage (Peoples *et al.*, 1989) and %Ndfa = 1.33 (%RUI - 11.47) at R3.5 and R7 stages (Herridge and Peoples, 2002). The total nitrogen accumulation or shoot nitrogen uptake was calculated by (dry matter (g)) x (%N) / 100. The amount of nitrogen fixed by mungbean was estimated from total nitrogen accumulation x %Ndfa x 1.5* (*1.5 is a factor that is estimated for contribution by below-ground N (Peoples *et al.*, 1989)). Pod yield (1 m²) was amassed at harvesting stage. Grains weight of randomized 1,000 seeds was taken from each treatment. Number of seeds per pod was calculated on randomly selected 20 pods.

The data were organised for statistical significance using the Statistix 10 software analysis of variance (ANOVA). Means of the treatment were

compared by using the least significant difference (LSD) test at the significance level of $P < 0.05$.

Results

Co-rhizobium inoculation and phosphate fertilizer management on nodules and shoot dry weights at V4, R3.5 and R7 stages

Nodules were collected for evaluating the dry weight at the V4, R3.5 and R7 stages. At V4 and R7 stages, the nodule dry weight was not significantly different by TSP management. The highest dry weight of nodule was dramatically increased by +TSP at $0.121 \text{ g plant}^{-1}$ at R3.5 stage ($P < 0.05$). The results inoculated with rhizobium SB2 showed the highest nodule dry weight at $0.140 \text{ g plant}^{-1}$ at R3.5 stage ($P < 0.05$). It was found that the data of nodule dry weight at R7 stage, SB2 provided the nodule dry weights higher than SBMix and DOA treatments. Non-inoculated still was detected with the lowest values of nodule weights. On the other hand, as shown in Table 1, the interaction between management and rhizobium were not found to be significantly affected by treatments in all the stages. Generally, shoot dry weight at the vegetative growth (V4), there was not significant difference both by effects of phosphorus fertilizer and rhizobium inoculation. It can be seen that +TSP provided higher shoot dry weight of mungbean biomass than -TSP treatment at 9.7 g plant^{-1} at R3.5 stage (Table 1). However, different rhizobium strains and non-inoculation were not significantly differed in the parameter at R 3.5 stage. Moreover, rhizobium SB2 showed the highest of the shoot dry weight at V4 (2.4 g plant^{-1}) and R3.5 (9.6 g plant^{-1}) stages. At the R7 stage, the highest shoot dry weight was found in +TSP treatment ($20.3 \text{ g plant}^{-1}$) ($P < 0.05$). Clearly, inoculation rhizobium strains with mungbean was significantly presented in the values of shoot dry weight higher than non-inoculation plants. Although there was no interaction of shoot dry biomass between management of triple superphosphate and rhizobium strains at all of stages.

Co-rhizobium inoculation and phosphate fertilizer management on total nitrogen and amount of nitrogen fixed by mungbean

The %Ndfa and total nitrogen at V4 stage were not significantly differed by management of phosphorus fertilizer. However, the %Ndfa had shown beneficial effects on rhizobium strains inoculation. SBMix gave the highest percentages of Ndfa at 11.20% ($P < 0.05$) but it was not significant difference with rhizobium of DOA at 9.87%. Comparatively, the non-inoculated led to the

lowest %Ndfa at 5.83%. Differences in the total nitrogen from shoot of mungbean at V4 stage were not statistically significant for both TSP management and rhizobium inoculation. However, application of TSP and rhizobium inoculate tended to increase the percentage of total nitrogen compared with control treatment. Overall, the data of all parameters at vegetative stage had no interaction between management and rhizobium inoculation. Addition of triple superphosphate provided higher %Ndfa than -TSP treatment. Application TSP showed better performance on those parameters than without TSP at 53.08 %Ndfa at R3.5 stage ($P<0.05$). Similarly, the rhizobium inoculation had significantly on %Ndfa. SBMix showed the highest amount of %Ndfa at 54.28 %Ndfa ($P<0.05$), but it was not significantly different from SB2 and DOA. Whereas, the rhizobium inoculation resulted to the value of total nitrogen at R3.5 and R7 stages. The highest total nitrogen was found in the treatment of inoculated with SBMix at 3.58 %N at R3.5 stage and 3.52 %N by SB2 at R 7 stage ($P<0.05$) respectively.

Table 1. Influence of phosphate fertilizer and rhizobia inoculation on nodules and shoot dry weights at V4, R3.5 and R7 stages

Stages	Treatment	Nodule dry weight (g plant ⁻¹)			Shoot dry weight (g plant ⁻¹)		
		V4	R3.5	R7	V4	R3.5	R7
Manage							
	-TSP	0.036	0.104b	0.062	2.2	8.9b	18.9a
	+TSP	0.042	0.121a	0.065	2.3	9.7a	20.3a
	F-test ^{1/}	ns	*	ns	ns	*	*
Rhizobium							
	-Rh	0.010b	0.089b	0.036c	2.2	8.9	18.4b
	SB2	0.053a	0.140a	0.084a	2.4	9.6	19.8a
	SBMix	0.049a	0.114ab	0.073ab	2.4	9.4	20.3a
	DOA	0.044a	0.109ab	0.061b	2.0	9.3	20.0a
	F-test	*	*	*	ns	ns	*
Manage*Rhizobium							
	-TSP						
	-Rh	0.010	0.085	0.035	2.1	8.7	17.1
	SB2	0.048	0.130	0.080	2.3	9.2	19.3
	SBMix	0.045	0.108	0.073	2.2	8.8	19.8
	DOA	0.040	0.095	0.060	2.2	8.9	19.3
	+TSP						
	-Rh	0.010	0.093	0.038	2.2	9.1	19.6
	SB2	0.058	0.150	0.088	2.4	10.0	20.2
	SBMix	0.053	0.120	0.073	2.6	9.9	20.8
	DOA	0.048	0.123	0.063	1.8	9.7	20.7
	F-test	ns	ns	ns	ns	ns	ns

^{1/}: Value in the same column followed by different letters were significantly different by LSD, *=0.05.

Inoculation rhizobium SB2 was significantly increased in the percentage of Ndfa at 81.81 % ($P < 0.05$) at R7 stage. Moreover, there were not significant differences among rhizobium strains (SB2, SBMix and DOA) on those parameters. Likewise, interaction between the management of TSP and rhizobium inoculation was not significantly differed on the values of %Ndfa and total nitrogen. The treatment was applied by +TSP mixed SB2 gave the peak of the %Ndfa and total nitrogen at 86.33 %Ndfa and 3.70 %N (Table 2).

Amount of nitrogen fixation was measured by the amount of nitrogen accumulated and percentage of nitrogen derived from the atmosphere. The amount of nitrogen fixation rapidly climbed up from vegetative stage (V4) to reproductive stage (R3.5 and R7) as shown in Table 2. Moreover, there was not significant difference in the amount of nitrogen fixed among TSP managements both in V4 and R3.5 stages. Unlike the large differences that occurred in the R7 stage, the application of TSP gave the amount of nitrogen fixed at 205 kg N ha⁻¹ higher than without TSP ($P < 0.05$). SBMix had the effect of nitrogen fixation higher than the other treatments at 4.8 kg N ha⁻¹ (V4 stage) and 69 kg N ha⁻¹ (R3.5 stage) ($P < 0.05$). Nonetheless, at the R7 stage, SB2 provided the highest amount of nitrogen fixation at 206 kg N ha⁻¹ but it was not significantly different from SBMix and DOA. Interestingly, inoculation with rhizobium strains gave more nitrogen fixed than non-inoculation ranging from 61 to 145% at V4 stage, 22-42% at R3.5 stage and 57-69% R7 stage, respectively. The interaction effect of management phosphorus and rhizobium inoculation was not statistically significant on the amount of nitrogen fixation at all stages of growth. Application of TSP with SBMix provided the highest amount of nitrogen fixation by 80 kg N ha⁻¹ at R3.5 stage. In contrast, added TSP with SB2 showed the peak of nitrogen fixation at R7 stage (232 kg N ha⁻¹).

Co-rhizobium inoculation and phosphate fertilizer management on grain weight, number of seeds pod⁻¹ and grain yield

Management of triple superphosphate rose was significantly differed in one thousand grain weight, number of seeds pod⁻¹ and grain yield ($P < 0.05$). Applying TSP led to higher thousand grains weight (75.3 g), number of seeds pod⁻¹ (12.1 seeds) and yield (1,044 kg ha⁻¹) than without TSP. Inoculation with SBMix gave the highest thousand grains weight at 75.6 g, but not significantly different when compared with SB2 and DOA. The control (-Rh) was found the lowest value of thousand grains weight at 72.6 g. Number of seed pod⁻¹ was not significantly differed among rhizobium strains inoculation. Rhizobium SB2 showed the peak of number of seeds pod⁻¹ at 12.1 seeds. The highest grain yield reached by SBMix was 1,056 kg ha⁻¹ but it was not significant compared to

SB2. The control treatment was shown to be the lowest yield at 844 kg ha⁻¹ (P<0.05) (Table 3). Though, the interaction of thousand grains weight, number of seeds pod⁻¹ and grain yield were not statistically significant. Likewise, applying TSP combined with rhizobium SB2 led to the highest thousand grains weight, number of seeds pod⁻¹ and grain yield at 76.4 g, 12.3 seed pot⁻¹ and 1,138 kg ha⁻¹, respectively.

Table 2. Influence of phosphate fertilizer and rhizobia inoculation on total N, percentage of nitrogen derived from the atmosphere (%Ndfa) and amount of nitrogen fixed by mungbean

Treatment	Total N (%)			%Ndfa			amount of nitrogen fixed by mungbean (kg N ha ⁻¹)			
	V4	R3.5	R7	V4	R3.5	R7	V4	R3.5	R7	
Manage										
-TSP	4.26	3.40	3.24b	9.48	44.91b	68.76b	8.6	54	154b	
+TSP	4.38	3.47	3.46a	8.38	53.08a	80.13a	8.3	65	205a	
F-test ^{1/}	ns	ns	*	ns	*	*	ns	ns	*	
Rhizobium										
-Rh	4.18	3.22b	3.01b	5.83c	39.54b	60.49b	1.9b (100)	49b (100)	122b (100) ^{2/}	
SB2	4.33	3.49a	3.52a	8.81b	52.18a	81.81a	3.1b (161)	59ab (122)	206a (169)	
SBMix	4.38	3.58a	3.42a	11.20a	54.28a	79.18a	4.8a (245)	69a (142)	199a (163)	
DOA	4.39	3.44a	3.44a	9.87ab	49.99a	76.28a	3.1b (161)	61a (126)	191a (157)	
F-test	ns	*	*	*	*	*	*	*	*	
Manage*Rhizobium										
-TSP	-Rh	4.17	3.20	2.99	5.91	37.2	53.18	1.9	48	98
	SB2	4.25	3.46	3.35	9.44	47.58	77.30	3.3	59	180
	SBMix	4.28	3.49	3.32	12.68	48.30	73.48	4.6	58	174
	DOA	4.32	3.43	3.30	9.89	46.50	71.08	3.3	53	164
+TSP	-Rh	4.18	3.23	3.04	5.75	41.80	67.80	2.0	49	146
	SB2	4.41	3.53	3.70	8.18	56.78	86.33	2.9	60	232
	SBMix	4.48	3.66	3.53	9.72	60.25	84.88	4.9	80	224
	DOA	4.46	3.46	3.59	9.86	53.48	81.50	2.9	70	218
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	

^{1/}: Value in the same column followed by different letters were significantly different by LSD, *=0.05.

^{2/}: the number in parenthesis were compared to the control

Table 3. Influence of phosphate fertilizer and rhizobia inoculation on thousand grains weight, number of seeds pod⁻¹ and grain yield

Treatment	1,000 grains weight (g)	Number of seeds pod ⁻¹	Grain yield (kg ha ⁻¹)
Manage			
-TSP	73.8b	11.7b	881b
+TSP	75.3a	12.1a	1,044a
F-test ^{1/}	*	*	*
Rhizobium			
-Rh	72.6c	11.6	844c
SB2	75.4a	12.1	1,025a
SBMix	75.6a	12.0	1,056a
DOA	74.5ab	11.9	931b
F-test	*	ns	*
Manage*Rhizobium			
-TSP -Rh	71.8	11.3	769
SB2	74.5	11.9	906
SBMix	75.3	11.8	988
DOA	73.6	11.8	856
+TSP -Rh	73.3	11.9	919
SB2	76.4	12.3	1,138
SBMix	75.9	12.1	1,125
DOA	75.5	12.0	1,006
F-test	ns	ns	ns

^{1/}Value in the same column followed by different letters were significantly different by LSD, *=0.05.

Discussion

The study was focused on the raised by phosphorus management and rhizobium inoculation on nitrogen fixation and yield of mungbean in field experiment. The nodule weight was increased in the treatment that added TSP and rhizobium strains. Furthermore, the inoculation with rhizobium provided the nodule dry weight more than non-inoculated. Similar reports by Chaudhary *et al.* (2008) that P deficiency in soil decreased the nodule activity on mungbean, mash bean and soybean plantations. On the other hand, Fening and Danso (2002) reported that the amount and formation of nodule could vary on the environment. The result of this experiment related with Venkatarao *et al.* (2017) that studied phosphorus fertilizer and biofertilizers on mungbean plantation. They found that applied phosphorus up to 40 kg P₂O₅ ha⁻¹ increased the number of nodules per plant, plant height, leaf area index, total chlorophyll content and grain yield. Moreover, the nodule weight was raised up by the

stages of mungbean that similarly finding reported by Luangmaka *et al.* (2013) on soybean cultivation in northern part of Thailand. In addition, the shoot dry weight at R3.5 and R7 stages were significant in the treatment of triple superphosphate applied. The results are similar with Kumaga and Ofori (2004), who also reported that accumulate of shoot weight could increase when apply fertilizer and inoculating with rhizobium. Furthermore, Schweiger *et al.* (2012) have also reported that rhizobium inoculation could promote more plant biomass than non-inoculation on soybean production in central Europe.

The concentrations of ureide, amino and nitrate at V4, R3.5 and R7 stages were determined to calculate the relative ureide index (%RUI) and percentage of nitrogen derived from the atmosphere (%Ndfa). The percentage of nitrogen derived from the atmosphere (%Ndfa) was recorded at the highest level at the R7 stage, ranging from 53.18-85.33 %Ndfa. The %Ndfa of all stages were associated with phosphorus fertilization and rhizobium inoculation. Similar results were also found by Tariq *et al.* (2007) and Hayat *et al.* (2008). Herridge *et al.* (2005) reported that the average of %Ndfa of mungbean in Gunnedah-Manilla, New South Wales was analysed from 15-72 %Ndfa by ureide method. Total nitrogen in this study decreased when mung beans grew up, ranging from 3.01 to 4.48%. The results are similar with Delić *et al.* (2011), who reported that the average of total N in mungbean was found at 3.62-4.82%.

The 1,000 grains weight, number of seeds per pod and grain yield were recorded the highest by the treatment of triple superphosphate and rhizobium management as similar results to Yedegari *et al.* (2008) and Ghanem and Abbas (2009). They also showed a remarkable increase in the number of pods, number of nodules, 1000 seed weight and seed yield in mungbean plantation by the combined application of rhizobacteria strains and fertilizer. Hossain *et al.* (2011) concluded that rhizobium inoculation combined with recommended fertilizer might increase mungbean yield, number of pods, number of seed, number of nodules and 1,000-seed weight. Nevertheless, the maximum grain yield in this experiment (1,138 kg ha⁻¹) also nearly reported by Verma *et al.* (2017) who found the yield of mungbean at 1,235 kg ha⁻¹ in the treatment of rhizobium inoculation + phosphate solubilizing bacteria + 60 kg P₂O₅ ha⁻¹.

In summary, triple superphosphate (TSP) applications combined with rhizobium SB2 produced the highest nodule dry weight at all stages. Shoot dry weight was found at the highest level by TSP combined with rhizobium SB2 (R3.5 stage) and SBMix (V4 and R7 stages). Moreover, the treatment of TSP with SBMix showed maximum positive on total N and amount of nitrogen fixed by mungbean at V4 and R3.5 stage. Application TSP with SB2 provided the highest level of total N, amount of nitrogen fixed by mungbean, thousand grains weight, number of seeds per pod, and grain yield especially at R7 stage.

The results of this research could be recommended for phosphorus fertilizer combining inoculation rhizobium SB2 or SBMix for increasing mungbean yield in the field of central region of Thailand.

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References

- Abel, S., Ticconi, C. A and Delatorre, C. A. (2002). Phosphorus sensing in higher plants. *Physiologia Plantarum*, 115:1-8.
- Al-Niemi, T. S., Kahn, M. L. and McDermott, T. R. (1997). P metabolism in the bean-Rhizobium tropici symbiosis. *Plant Physiology*, 113:1233-1242.
- Ali, M. A., Abbas, G., Mohy-ud-Din, Q., Ullah, K., Abbas, G. and Aslam, M. (2010). Response of mungbean (*Vigna radiata*) to phosphatic fertilizer under arid climate. *The Journal of Animal and Plant Sciences*, 20:83-86.
- Chaudhary, M. I., Adu-Gyamfi, J. J., Saneoka, H., Nguyen, N. T., Suwa, R., Kanai, S., Shemy, H. A. El- Lightfoot, D. A. and Fujita, K. (2008). The effect of phosphorus deficiency on nutrient uptake, nitrogen fixation and photosynthetic rate in mashbean, mungbean and soybean. *Acta Physiologiae Plantarum*, 30:537-544.
- Dechjiraratthanasiri, C., Boonmee, P., Inthasan, J. and Santasup, C. (2021). Identification and characterization of native rhizobia from three mungbean varieties. *Malaysian Journal of Microbiology*, 17:121-129.
- Delić, D., Stajković-Srbinić, O., Kuzmanović, D., Rasulić, N., Mrvić, V., Andjelović, S. and Knežević-Vukčević, J. (2011). Effect of bradyrhizobial inoculation on growth and seed yield of mungbean in Fluvisol and Humofluvisol. *African Journal of Microbiology Research*, 5:3946-3957.
- Fatima, Z., Zia, M. and Chaudhary, M.F. (2007). Interactive effect of Rhizobium strains and P on soybean yield, nitrogen fixation and soil fertility. *Pakistan Journal of Botany*, 39:255-264.
- Fening, J. O. and Danso, S. K. A. (2002). Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Applied Soil Ecology*, 21:23-29.
- Ghanem, K. H. M. and Abbas, E. L. (2009). Improvement of mungbean growth and productivity in salinity affected soil after seed inoculation with phosphorus dissolving bacteria. VIIIth African Crop Science Conference Proceedings, 9:385-389.
- Hayat, R., Ali, S., Ijaz, S. S., Siddique, M. T. and Chatha, T. H. (2008). Biological nitrogen fixation of summer legumes and their residual effects on subsequent rainfed wheat yield. *Pakistan Journal of Botany*, 40:711-722.
- Herridge, D. F. and Peoples, M. B. (1990). Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by ¹⁵N methods. *Plant Physiology*, 93:495-503.
- Herridge, D. F. and Peoples, M. B. (2002). Timing of xylem sampling for ureide analysis of nitrogen fixation. *Plant Soil*, 238:57-67.
- Herridge, D. F., Robertson, M. J., Cocks, B., Peoples, M. B., Holland, J. F. and Heuke, L. (2005). Low nodulation and nitrogen fixation of mungbean reduce biomass and grain yields. *Australian Journal of Experimental Agriculture*, 45:269-277.

- Hossain, M. S., Karim, M. F., Biswas, P. K., Kawochar, M. A. and Islam, M. S. (2011). Effect of rhizobium inoculation and chemical fertilization on the yield and yield components of mungbean. *Journal of Experimental Biosciences*, 2:69-74.
- Hussain, A., Ali, A., Akhtar, J. and Yasin, M. (2010). Effect of Phosphorus in combination with Rhizobium inoculation on growth and yield parameters of mungbean (*Vigna radiata* L.). *Crop and Environment*, 1:53-56.
- Hussain, A., Ali, A. and Noorka, I. R. (2012). Effect of phosphorus with and without rhizobium inoculation on nitrogen and phosphorus concentration and uptake by mungbean (*Vigna radiata* L.). *Journal of Agricultural Research*, 50:49-57.
- Jungk, A. O. (1998). Dynamics of nutrient movement at the soil-root interface. In: *Plant Roots: the Hidden Half*. Y. Waisel, A. Eshel and U. Kafkafi. (eds.). Marcel Dekker, New York, pp. 529-556.
- Kumaga, F. K. and Ofori, K. (2004). Response of soybean (*Glycine max* (L.) Merrill) to Bradyrhizobia inoculation and phosphorus application. *International Journal of Agriculture and Biology (Pakistan)*, 6:324-327.
- Kumawat, N., Sharma, O. P. and Kumar, R. (2010). Effect of organic manures, PSB and phosphorus fertilization on yield and economics of mungbean *Vigna radiata* (L.) Wilczek. *Environment and Ecology*, 27:5-7.
- Luangmaka, N., Ongprasert, S. and Inthasan, J. (2013). Effect of organic fertilizer residue from rice production on nitrogen fixation of soybean (*Glycine max* L. Merrill) Chiang Mai 60 variety. *Maejo International Journal of Science and Technology*, 7:377-384.
- Marschner, H. (1995). Mineral nutrition of higher plants. 2nd (ed.). In: *Society for general microbiology*. Academic Press, San Diego, pp.201-228.
- Motsara, M. R. and Roy, R. N. (2008). *Guide to Laboratory Establishment for Plant Nutrient Analysis*. FAO Fertilizer and Plant Nutrition Bulletin No. 19. FAO, Rome. pp.1-103.
- Mushtaq, M., Shah, P., Jan, S. and Sattar, A. (1986). Effect of different levels of phosphorus and potash on the emergence, plant height and straw yield of mungbean (*Vigna radiata* (L.) Wilczek). *Sarhad Journal of Agriculture*, 2:467-472.
- Ogata, S., Adu-Gyamfi, J. and Fujita, K. (1988). Effect of phosphorus and pH on dry matter production, dinitrogen fixation and critical phosphorus concentration in pigeon pea (*Cajanus cajan* (L.) Millsp.). *Soil Science and Plant Nutrition*, 34:55-64.
- Peoples, M. B., Faizah, A. W., Rekasem, B. and Herridge, D. F. (1989). *Methods for evaluating Nitrogen Fixation by Nodulated Legumes in the Field*. Australian Centre for International Agricultural Research, Canberra. 81 p.
- Pookpakdi, A., Promkham, V. and Chuangpetchinda, C. (1992). Growth stage identification in mungbean (*Vigna radiata* (L.) Wilczek). *Agricultural Science Journal*, 26:75-80.
- Prasad, S. K., Singh, M. K. and Singh, J. (2014). Response of rhizobium inoculation and phosphorus levels on mungbean (*Vigna radiata* L.) under guava-based agri-horti system. *The Bioscan*, 9:557-560.
- Robson, A.D. (1983). Mineral nutrition. In: *Nitrogen Fixation of Legumes*. W.J. Broughton, (ed.). Oxford, UK: Clarendon Press, UK, pp.36-55.
- Ribet, J. and Drevon, J. J. (1995). Phosphorus deficiency increases the acetylene-induced decline in nitrogenase activity in soy-bean (*Glycine max* (L.) Merr.). *Journal of Experimental Botany*, 46:1479-1486.
- Schüller, H. (1969). Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphors im Boden. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 123:48-63.

- Schweiger, P., Hofer, M., Hartl, W., Wanek, W. and Vollmann, J. (2012). N₂ fixation by organically grown soybean in central Europe: method of quantification and agronomic effects. *European Journal of Agronomy*, 41:11-17.
- Shenoy, V. V. and Kalagudi, G. M. (2005). Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnology Advances*, 23:501-513.
- Tariq, S., Ali, S. and Ijaz, S. S. (2007). Improving nitrogen fixation capacity and yield of mungbean and mashbean by phosphorus management in Pothowar. *Sarhad Journal of Agriculture*, 23:1027-1032.
- Thind, S. S., Rishi, A. K. and Goswami, N. N. (1990). Utilization of applied phosphorus by green gram (*Vigna radiata* L. Wilczek), Bengal gram (*Cicer arietinum* L.) and cowpea (*Vigna unguiculata* L. Walp) in soils of Delhi. *Journal of Nuclear Agriculture and Biology*, 19:152-156.
- Venkatarao, C. H. V., Naga, S. R., Yadav, B. L., Koli, D. K. and Rao, I. J. (2017). Effect of phosphorus and biofertilizers on growth and yield of mungbean (*Vigna radiata* (L.) Wilczek). *International Journal of Current Microbiology and Applied Sciences*, 6:3992-3997.
- Verma, G., Singh, M., Morya, J. and Kumawat, N. (2017). Effect of N, P and biofertilizers on growth attributes and yields of mungbean (*Vigna radiata* (L.) Wilczek) under semi-arid tract of central India. *International Archive of Applied Sciences and Technology*, 8:31-34.
- Yedegari, M., Rahmani, A.H., Noormohammadi, G. and Ayneband, A. (2008). Evaluation of bean (*Phaseolus vulgaris*) seed' inoculation with *Rhizobium phaseoli* and plant growth promoting Rhizobacteria (PGPR) on yield and yield components. *Pakistan Journal of Biological Sciences*, 11:1935-1939.

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