

Effect of phosphorus and bio-inoculants on root nodules, nutrient uptake, quality and economics of summer mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

A field experiment was conducted at Rajasthan College of Agriculture Udaipur, (MPUAT) (Rajasthan) during Summer season of 2016 to evaluate the effect of phosphorus and bio-inoculants on soil fertility and yield of Mungbean [*Vigna radiata* (L.) Wilczek] under factorial randomized block design with three replications and two factors likewise, four level of phosphorus Control ($0 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$), 20, 40 and $60 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ and four level of bio-inoculants (control, PSB, *Aspergillus awamori*, PSB + *Aspergillus awamori*) applied to the variety SML-668. The data were analyzed using standard statistical procedures. Results revealed that application of $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ significantly enhances number of total and effective nodules per plant flowering stage after 45 DAS, leaf area index, chlorophyll content, protein content in seed, as well as produces the higher net returns (62144 Rs /ha) and highest B:C ratio (3.86) which was statistically at par with $60 \text{ kg P}_2\text{O}_5 \text{ /ha}$. Inoculation of mungbean seeds with PSB + *Aspergillus awamori* significantly increased number of total and effective nodules at flowering after 45 DAS, LAI, chlorophyll content, nutrient content, protein content in seed, and gave highest net return $\text{₹}63187 \text{ /ha}$ as compared to control, PSB and *Aspergillus awamori*, whereas, B:C ratio of treatment PSB and *Aspergillus awamori* was highest 3.98 as compared to control.

Keywords: Mungbean, PSB, *Aspergillus awamori*, phosphorus

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most popular leguminous crops of arid and semi-arid regions of India. It is an important pulse crop due to its economic importance and an excellent source of 51 % carbohydrate, 26 % protein, 10 % moisture, per cent minerals, 3 % vitamins with high lysine content (460 mg g^{-1}) and tryptophan (60 mg g^{-1}) (Kaul, 1982 and Afzal *et al.* 2008). It also has remarkable quantity of ascorbic acid when sprouted and also bears riboflavin ($0.21 \text{ mg } 100 \text{ g}^{-1}$) and minerals ($3.84 \text{ g } 100 \text{ g}^{-1}$). Mungbean stands third after chickpea and pigeon pea among pulses in terms of production (Tamang *et al.*, 2015). Pulses have been justifiably described as 'The poor man's meat'. The 68th UN General Assembly declared 2016 as International Year of Pulses (IYP) nutritious food for a sustainable future (FAO, 2016). In India this is cultivated in three seasons i.e., *Kharif*, *Rabi* and *Summer*. During *Summer* season the crop is grown by a very limited number of farmers on account of inadequate water availability with them in this zone. The production potential of this crop during *Summer* can be fully explored using short duration and photo insensitive varieties. The crop in this

season is raised under controlled conditions and there is less infestation of insects, pests, diseases and even weeds. The sky remains clear and duration of sunshine hours is also more which may result in more photosynthetic efficiency of this crop, leading to subsequently more grain yield with good quality grains. During *Summer*, it can also be used as green manure crop. In India total area of mungbean was 4.5 m ha, production 2.5 mt and productivity was 548 kg/ha (DES, DAC&FW, ND,2020). Phosphorus (P) is one of the nutrients, limiting plant yield and productivity because of its important role in the process of flowering and seed formation. The application of P to mungbean has been reported to increase plant dry matter, seed yield and P intake (Shah *et al.*, 2006, Iqbal *et al.* 2012). Phosphorus is an important plant nutrient which is referred to as the master key element in crop production. It is associated with several vital functions including seed germination, cell division, flowering, fruiting and synthesis of fat and starch and in fact every biochemical activity. It also includes root proliferation, nodulation and nitrogen fixation. Hence pulses are generally considered as high P demanding crops for nitrogen fixation which is energy requiring process but it is a major constraint as nearly 98

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% soil in India has inadequate supply of P (Kanwar and Grewal., 1990). Only 20-25 % part of P applied to the soil is available to the crop in the year of its application and remaining part is converted into insoluble unavailable forms.

Phosphorus solubilising microorganisms (bacteria and fungi) enable P to become available for plant uptake after solubilization. Several soil bacteria, particularly those belonging to the genera *Bacillus* and *Pseudomonas*, and fungi belonging to the genera *Aspergillus* and *Penicillium* possess the ability to bring insoluble phosphates in soil into soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric, and succinic acids. These acids lower the pH and bring about the dissolution of bound forms of phosphates. The phosphate solubilizing microorganisms improved P uptake over control with and without chemical fertilizers. The goal of the current study was to determine the effect of bio-inoculants with phosphatic fertilizers for optimizing nutrient content, uptake, quality and economics of the moongbean.

MATERIALS AND METHODS

A field experiment was conducted during *summer* season of 2016, at the Agronomy farm, Rajasthan College of Agriculture Udaipur, (Rajasthan) at an altitude of 582.17 metre above mean sea level and at 24° 35' N latitude and 73° 42' E longitude. Daily meteorological parameters during cropping season were recorded at meteorological observatory situated at same campus. The region falls under Agro-climatic Zone IV a (Sub- humid Southern Plain and Aravalli Hills) of Rajasthan. The mean annual rainfall of Udaipur is 610.2 mm, and zone IV a range between 582-620 mm, most of which is contributed by south west monsoon. Maximum and minimum temperatures ranged between 28.2 to 41.7 °C and 17.9 to 35.9 °C, respectively during *Summer*, 2016. The soil of research site (before *Kharif* 2016) was clay loam in texture, slightly alkaline in reaction. The soil was medium in available nitrogen, low in phosphorus while high in potassium, and sufficient in DTPA extractable micronutrients with pH 8.1, EC (0.81 dSm⁻¹ at 25 °C), organic carbon (0.58%), available nitrogen (203.30 kg ha⁻¹), available P₂O₅ (15.39 kg ha⁻¹), available K₂O (367.60 kg ha⁻¹), CEC (21.63 C mol (p⁺) kg⁻¹ soil). The

experiment comprised four level of phosphorus Control (0 kg P₂O₅ ha⁻¹), 20, 40 and 60 kg P₂O₅ ha⁻¹ and four level of bio-inoculants (control, PSB, *Aspergillus awamori*, PSB + *Aspergillus awamori*). The sixteen treatments combination were laid out in randomized block design and replicate thrice. Each experimental unit consist of 4m×3m plots. Experimental field was ploughed by tractor driven mould board plough followed by two cross harrowing and planking. A uniform basal dose of 25 kg N ha⁻¹ was applied through urea to the soil. The whole quantity of P was applied through SSP as per treatment details prior to sowing and incorporated manually in top 15 cm of soil. As per treatments, the seeds were inoculated with PSB, *Aspergillus awamori* and PSB + *Aspergillus awamori* before sowing using standard method and dried in shade (Paul *et al.* 1971). Thereafter, sowing was done in each marked plot. The SML-668 variety of *summer* mungbean was shown on 2 April 2016 using seed rate 20 kg ha⁻¹ with spacing of 30 cm × 10 cm. Intercultural operations like weeding and hoeing were done manually 25 days after sowing to facilitate aeration and removing the weeds. The crop was harvested manually on 18 June 2016. The borderline plants were harvested first and were removed from experimental area. Previously five selected plants of net area were tagged and harvested separately for recording post-harvest observations and their seed yield was added to final net plot yield. Then the plants of net area were harvested simultaneously and the produce was sun dried. After satisfactory drying, the plot wise threshing was done manually by beating the dried pods and winnowing and cleaning operations were done manually. Clean seeds were weighed to record seed yield. The straw yield was computed by subtracting the weight of seed yield from biological yield of each plot.

At flowering stage five plants were tagged, plants uprooted and counted for total and effective nodules per plant and average was calculated. For counting the number of root nodules per plant, five plants in each plot were randomly selected from crop row and were removed carefully after wetting the soil and taking the soil up to 30 cm deep. The plants were removed with soil from the plot and the soil mass embodying the root of mass was washed out with a jet of water. The nodules were removed with the help of forceps, counted and

the mean of five plants nodules was recorded as number of nodules per plant. Effective nodules were pinkish in colour whereas, rest of nodules were having black and pale white colour. For LAI five tagged plants were uprooted from sampling row of each plot at pre flowering stage, leaves were detached from the shoot and graded into large, medium and small types, leaf area (cm²) was measured with the help of leaf area meter (model 3100 area meter, USA). The leaf area for each sample was recorded and averaged to give area per plant. Land area per plant was used to compute LAI at each stage. The chlorophyll content at flowering was estimated by the method advocated by Arnon (1949) by taking 50 mg fresh leaf material. Samples were homogenized in 80% acetone, centrifuged for 10 minutes at 2000 rpm and made final volume to 10 ml. Absorbance of clear supernatant solution was measured by Spectronic-20 at wavelength of 652 nm.

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{A(652) \times 29 \times \text{Total volume (ml)}}{\alpha \times 1000 \times \text{Weight of samples}}$$

Where,

- α is the path length = 1 cm
- A (625) is Absorption at 652 nm
- Protein content (%) was estimated by

Angelo and Mann method (1973). Chlorophyll content Estimated by spectronic-20. After threshing, winnowing and cleaning the produce of each plot was weighed separately and converted in terms of yield in kg ha⁻¹. All data recorded were analyzed with the help of analysis of variance (ANOVA) technique (Gomez and Gomez 1984) for randomized block design. The least significant test was used to decipher the main and interaction effect of treatments at 5 % level of significance (P<0.05).

RESULTS AND DISCUSSION

Growth parameters

Total and effective number of nodules per plant at flowering stage

A perusal of data revealed that with the application of P, there was a significant increase in total and effective number of nodules per plant

at flowering 45 DAS on mungbean as compared to control 0 kg P₂O₅ ha⁻¹ (P₀) and 20 kg P₂O₅ ha⁻¹ (P₁). However, the increase in total and effective nodules per plant at flowering 45 DAS with 60 kg P₂O₅ ha⁻¹ (P₃) treatment was statistically at par with 40 kg P₂O₅ ha⁻¹ (P₂) treatment. Inoculation of mungbean seed with PSB, *Aspergillus awamori* and PSB + *Aspergillus awamori* were having a significant increase in total and effective number of nodules on mungbean as comparison to the control. Further, the increase in total and effective number of nodules with PSB + *Aspergillus awamori* was also found significantly higher over PSB as well as over *Aspergillus awamori* inoculations. However, the increase in total and effective number of nodules with inoculation of PSB was statistically at par with that of *Aspergillus awamori*. Phosphorus is an indispensable, constituent of nucleic acid, ADP and ATP. It has beneficial effects on nodulation, root development, growth and also hastens maturity as well as improves quality of crop produce (Choudhary *et al.* 2015).

Leaf area index and total chlorophyll content at flowering stage

Leaf area index and total chlorophyll content at flowering stage 45 DSA increased significantly with the application of P levels and different strains of bio-inoculants at flowering stage as compared to control. Data presented in table -1 revealed that LAI and total chlorophyll content significantly found highest with treatment 40 Kg P₂O₅ ha⁻¹ over control and 20 kg P₂O₅ ha⁻¹ which was statistically at par with 60 Kg P₂O₅ ha⁻¹. The increase in LAI of mungbean was to the extent of 74.37, 23.92 and 20.90% higher as compared to control, PSB and *Aspergillus awamori*, respectively. Dual inoculation with PSB + *Aspergillus awamori* significantly increased the total chlorophyll content at flowering by 24.06, 11.20 and 9.97 % in comparison to control, PSB and *Aspergillus awamori*, respectively. However application of PSB and *Aspergillus awamori* remained at par with each other. Phosphorus encourage formation of new cells, promote plant vigour and hastens leaf development, which help in harvesting more solar energy and better utilization of nitrogen, which help towards higher growth attributes. These findings are found relevant to Escalante *et al.* (2014) and Rathour *et al.* (2015).

Nutrient content, uptake and quality

Application of graded levels of P upto 40 kg P₂O₅ ha⁻¹ significantly increased the N, P and K content and uptake in seed and straw in mungbean over preceding levels and statistically at par with 60 kg P₂O₅ ha⁻¹. The application of 40 kg P₂O₅ ha⁻¹ significantly increased the nitrogen content to the extent 21.57 and 6.28 % in seed

and 30.85 and 13.80 % in straw, as compared to control and 20kg P₂O₅ ha⁻¹, respectively. Seed inoculation with PSB + *Aspergillus awamori* significantly increased the nitrogen content in seed by 27.73, 11.34, 8.74 % and 40.03, 19.88, 12.28 % in straw, as compared to control, PSB and *Aspergillus awamori*, respectively.

Table 1: Effect of phosphorus and bio-inoculants on growth & yield attributes, chlorophyll content, protein content and economics

Treatments	Total nodules	Effective nodules	Leaf area index	Chlorophyll content (mg g ⁻¹)	Protein content (%)	Net returns (Rs ha ⁻¹)	B:C ratio
Phosphorus levels							
P ₀ (Control)	26.81	18.02	2.06	3.11	18.25	29730	2.12
P ₁ (20 kg P ₂ O ₅ ha ⁻¹)	29.22	20.97	2.74	3.52	20.88	48572	3.23
P ₂ (40 kg P ₂ O ₅ ha ⁻¹)	31.51	23.02	3.11	3.81	22.19	62144	3.86
P ₃ (60 kg P ₂ O ₅ ha ⁻¹)	32.00	24.17	3.22	3.91	22.56	65424	3.82
SEm±	0.79	0.66	0.07	0.09	0.40	1729	0.11
CD (P = 0.05)	2.27	1.91	0.21	0.26	1.15	4992	0.32
Bio-inoculants							
B ₀ (Control)	26.17	18.25	1.99	3.20	18.23	31061	1.97
B ₁ (PSB)	29.93	20.99	2.80	3.57	20.92	54106	3.43
B ₂ (<i>Aspergillus awamori</i>)	30.14	22.40	2.87	3.61	21.42	57515	3.65
B ₃ (PSB + <i>Aspergillus awamori</i>)	33.30	24.53	3.47	3.97	23.30	63187	3.98
SEm±	0.79	0.66	0.07	0.09	0.40	1729	0.11
CD (P = 0.05)	2.27	1.91	0.21	0.26	1.15	4992	0.32

It is apparent from the data that, significantly highest N uptake (47.22 kg ha⁻¹ by seed and 49.15 kg ha⁻¹ by straw) at harvest of the crop were recorded under the treatment 40 kg P₂O₅ ha⁻¹, while minimum N uptake (21.29 kg ha⁻¹ by seed and 22.02 kg ha⁻¹ by straw) was recorded under Control (0 kg P₂O₅ ha⁻¹). The significantly maximum N uptake of seed was recorded as 49.68 kg ha⁻¹ and 54.49 kg ha⁻¹ in straw under the treatment PSB + *Aspergillus awamori* while minimum N uptake were recorded as 23.35 kg N ha⁻¹ in seed and 21.45 kg ha⁻¹ in straw under control (0 kg P₂O₅ ha⁻¹), respectively. Application of P @ 40 kg P₂O₅ ha⁻¹ increased the P content in seed by 28.82 and 11.42 % whereas in straw it was 25.65 and 8.10 % higher over control and 20 kg P₂O₅ ha⁻¹, respectively. Application of 40 kg P₂O₅ ha⁻¹ represented an increase of 135.80 and 40.44 % higher P uptake in seed over control and 20 kg P₂O₅ ha⁻¹, respectively. While in straw, P application @ 40 kg P₂O₅ ha⁻¹ reported an increase of 113.95 and 25.38 % higher P uptake over control and 20 kg P₂O₅ ha⁻¹. The bio-inoculants inoculation produced significant

impact on P uptake by seed and straw. The increase in P uptake of mungbean was registered as 124.53, 29.61, 20.07 % higher by seed and 144.47, 29.79, 21.33 % higher by straw over control, PSB and *Aspergillus awamori*, respectively. However, the P uptake by seed and straw with inoculation of PSB was statistically at par with that of *Aspergillus awamori*. Application of P @ 40 kg P₂O₅ ha⁻¹ being at par with 60 kg ha⁻¹ indicated an increase of 35.80, 14.54 % in seed and 17.05, 7.37 % higher in straw over control and 20 kg P₂O₅ ha⁻¹, respectively. An increase of 35.80, 14.54 % K content in seed and 17.05, 7.37 % higher in straw over control and 20 kg P₂O₅ ha⁻¹, respectively. The treatment 40 kg P₂O₅ ha⁻¹ remained at par with treatment 60 kg P₂O₅ ha⁻¹. However, inoculation with PSB + *Aspergillus awamori* had recorded significantly maximum K content 0.573 % in seed and 0.986 % in straw. Application of P increased total biomass (seed and straw) and the nutrient (N, P and K) content in mungbean by providing balanced nutritional environment inside the plant and higher photosynthetic efficiency. This might be due to

readily available N and P applied through chemical fertilizers with *Rhizobium* and also the ability of PSB and *Aspergillus awamori* to transform insoluble phosphate in soil into soluble forms by secreting organic acids and supplying

to plants through the hyphae of fungi. These findings are found similar with Rathore *et al.* (2010), Bhatt *et al.* (2013), Malik *et al.* (2013), Singh *et al.* (2013).

Table 2: Effect of phosphorus and bio-inoculants on N content and uptake of N, P and K by seed and straw

Treatments	N content (%)		N uptake (kg ha ⁻¹)		P content (%)		P uptake (kg ha ⁻¹)		K content (%)		K uptake (kg ha ⁻¹)	
	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw
Phosphorus levels												
P ₀ (Control)	2.92	1.222	21.29	22.02	0.333	0.191	2.43	3.44	0.377	0.821	2.77	14.65
P ₁ (20 kg P ₂ O ₅ ha ⁻¹)	3.34	1.405	35.32	37.26	0.385	0.222	4.08	5.87	0.447	0.895	4.76	23.49
P ₂ (40 kg P ₂ O ₅ ha ⁻¹)	3.55	1.599	47.22	49.15	0.429	0.240	5.73	7.36	0.512	0.961	6.88	29.19
P ₃ (60 kg P ₂ O ₅ ha ⁻¹)	3.61	1.620	50.66	52.59	0.439	0.245	6.18	7.93	0.532	0.972	7.55	31.18
SEm±	0.05	0.033	1.86	1.91	0.007	0.004	0.22	0.27	0.016	0.015	0.32	0.98
CD (P = 0.05)	0.15	0.096	5.36	5.53	0.019	0.011	0.65	0.78	0.046	0.044	0.92	2.83
Bio-inoculants												
B ₀ (Control)	2.92	1.214	23.35	21.45	0.334	0.190	2.69	3.35	0.374	0.834	3.02	14.62
B ₁ (PSB)	3.35	1.418	39.30	40.11	0.395	0.224	4.66	6.31	0.441	0.907	5.23	25.45
B ₂ (<i>A. awamori</i>)	3.43	1.514	42.16	44.97	0.407	0.228	5.03	6.75	0.480	0.921	5.97	27.14
B ₃ (PSB + <i>A. awamori</i>)	3.73	1.700	49.68	54.49	0.450	0.257	6.04	8.19	0.573	0.986	7.74	31.30
SEm±	0.05	0.033	1.86	1.91	0.007	0.004	0.22	0.27	0.016	0.015	0.32	0.98
CD (P = 0.05)	0.15	0.096	5.36	5.53	0.019	0.011	0.65	0.78	0.046	0.044	0.92	2.83

Protein content

Protein content in seed of crop was found significantly highest 22.19 % under the treatment P₂ which was 21.58 and 6.27 % higher over control (18.25) and 20 kg P₂O₅ ha⁻¹ (20.88), respectively. The significantly maximum protein content in seed was recorded as 23.30 % under the treatment B₃. The protein content in seed was increased by 27.81, 11.37 and 8.77 % over B₀, B₁ and B₂ treatments, respectively. An increase in seed protein content might be due to enhanced uptake and translocation of nitrates which provide N for amino acid synthesis. Moreover, P is involved in the synthesis of ATP that is required in nitrogen uptake and protein synthesis. Higher seed protein content in mungbean in response to N and P applications have also been reported by Dewangan *et al.*

(1992) and Nazir (1993). The similar findings were also reported by Khan *et al.* (2015), Senthil kumar and Sivagurunathan (2012).

Economics

The application of P @ 40 kg P₂O₅ ha⁻¹ and dual inoculation of PSB + *Aspergillus awamori* gave maximum net return and B:C ratio as compared to the rest of the treatments. Application of 40 kg P₂O₅ ha⁻¹ gave net return (₹ 62144) and B: C ratio (3.86) and dual inoculation of PSB + *Aspergillus awamori* gave maximum net return (₹63187) and B:C ratio (3.98). This might be due to higher seed and straw yield. Higher marginal rate of return with P (40 kg P₂O₅ ha⁻¹) and bio-inoculants (PSB + *Aspergillus awamori*) indicated higher output per unit of P₂O₅ and bio-inoculants input.

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