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AGRONOMY

Impact of Phosphate Solubilizing Bacteria and Phosphorus Application on Forage Yield and Quality of Berseem in West Bengal

D.C. Roy[1], M. Ray[2], N. K. Tudu[3] and C.K. Kundu[2]

[1]Department of ILFC, WBUAFS, 37 K.B. Sarani, Kolkata-700037, India.
[2]Department of Agronomy, Bidhan Chandra Krishi Viswavidyalaya, Kalyani-741235, Nadia, West Bengal, India.
[3]Nadia Krishi Vigyan Kendra, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India.

Corresponding author: dcroy09@gmail.com

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Abstract

Phosphorus availability is increased through the use of phosphate solubilizing bacteria for enhancing the forage yield and improving the quality of berseem fodder. A field experiment was conducted in the fodder farm of West Bengal University of Animal and Fishery Sciences, Belgachia during *rabi*, season 2013-14. The crop was sown with inoculated (I_1) and un-inoculated (I_0) seeds of berseem (*Trifolium alexandrinum* L.) of variety warden and was given phosphorus fertilizer at the levels 40 (P_1), 60 (P_2), 80 (P_3) and 100 (P_4) kg P_2O_5 ha^{-1} in the form of SSP in RCBD with three replications in plots of net size 6 m X 2 m. PSB inoculation significantly increases green forage and dry matter yield by increasing plant height, no. of branches, leaf-stem ratio, etc. All the quality traits except ash content and dry matter percentage were significantly affected by PSB inoculation. Higher green forage yield (320.4 qt ha^{-1}), dry matter yield (39.2 qt ha^{-1}), crude protein yield (7.89 qt ha^{-1}), crude protein percentage (19.28%), ethyl extract (3.32%) and nitrogen free extract (41.75%) were recorded from the plots inoculated with PSB. Application of phosphorus fertilizers significantly influenced the green forage and dry matter yield of berseem, leaf-stem ratio, etc. though plant height and no. of branches were not-significant. All the quality parameters *i.e.* CP, CF, EE, NFE, except ash percentage were significantly influenced by phosphorus application. Best results were obtained with 80 kg P_2O_5 ha^{-1} in all the cases. Regarding interaction effect, higher yields of better quality green forage of berseem were obtained in I_1P_3 *i.e.* inoculation with PSB with phosphorus application of 80 kg P_2O_5 ha^{-1}.

Highlights

- Phosphate solubilizing bacteria enhances the forage yield and improve the quality
- Phosphorus application at the rate 80 kg P_2O_5 ha^{-1} gave better result

Keywords: PSB, phosphorus, berseem, crude protein, crude fibre

Fodder crops play a vital role in agriculture because, the supply of nutritious fodders in sufficient amount is a basic requirement for livestock to fulfill the increasing demand of milk, butter and other dairy byproducts for utilization by human beings. Due to ever increasing human population pressure, arable land mainly used for food and fodder production is limited only to 4.60% of the total cultivable land. At present, the country faces a net deficit of 35% green fodder, 10% dry crop residues and

Table 1. Effect of PSB and different levels of phosphorus on plant growth and forage yield of berseem

Treatment	Plant height (cm)	No. of branches per plant	Leaf stem ratio	Green forage yield (qt. ha^{-1})	Dry matter yield (qt. ha^{-1})
Inoculation					
I_0	51.60	8.78	0.88	302.5	36.8
I_1	52.30	9.52	1.09	320.4	39.2
S.Em (±)	0.15	0.13	0.03	2.38	0.22
C.D.	0.64	0.56	0.15	10.26	0.95
Phosphorus [P] (Kg ha^{-1})					
P1	51.93	9.11	0.87	297.1	37.0
P2	51.94	9.14	0.99	311.6	37.5
P3	51.98	9.19	1.08	321.7	39.4
P4	51.95	9.17	1.01	315.4	38.1
S.Em (±)	0.25	0.15	0.06	5.12	0.40
C.D.	NS	NS	0.14	12.54	0.98
I X P					
I_0P_1	51.58	8.72	0.74	288.5	36.6
I_0P_2	51.59	8.77	0.88	302.1	36.4
I_0P_3	51.63	8.82	0.98	312.6	38.1
I_0P_4	51.60	8.81	0.92	306.8	36.1
I_1P_1	52.28	9.48	1.00	305.7	37.8
I_1P_2	52.29	9.50	1.10	321.2	38.2
I_1P_3	52.33	9.56	1.17	330.8	40.7
I_1P_4	52.30	9.54	1.11	323.9	40.1
S.Em (±)	0.33	0.36	0.11	7.71	0.44
C.D.	NS	NS	0.26	18.87	1.08

I_0 - Seeds un-inoculated with PSB, I_1 - Seeds inoculated with PSB, P_1 – 40 kg P_2O_5 ha^{-1}, P_2-60 kg P_2O_5 ha^{-1}, P_3 – 80 kg P_2O_5 ha^{-1}, and P_4 - 100 kg P_2O_5 ha^{-1},

33% feeds (Planning Commission of India 2012; Handbook of Agriculture 2009). Berseem (Trifolium alexandrinum L.) is one of the most important rabi fodder crop. It is considered as the most potential crop from productivity as well as maintenance of soil fertility. It contains about 20-24% protein at green stages (Barik and Tiwari 1998). This nutritious, succulent and palatable fodder is available for fairly long period during winter, spring and early summer (Chaterjee and Das 1989).

In India, though the soil and climatic conditions are favorable for berseem production, but its per hectare yield of fodder is very low. There are many constraints for low yield of berseem, but improper fertilizer application is considered to be a major limiting factor. The correct and judicious fertilizer application can enhance yield up to 50% (Zia *et al.* 1991) and can also improve the quality of fodder (Keshwa and Singh, 1992). In a plant, phosphorus is a common component of many organic compounds. Phosphorus deficiency however significantly reduce the plant growth (Marschner 1997). Phosphorus plays an important role in photosynthesis and

Table 2. Effect of PSB and different levels of phosphorus on various quality parameters of berseem

Treatment	Dry matter (%)	Crude Protein yield (qt. ha^{-1})	Crude Protein (%)	Crude Fibre (%)	Ash (%)	Ethyl Extract (%)	Nitrogen Free Extract (%)
Inoculation							
I_0	12.18	6.57	18.59	22.92	14.35	3.29	40.85
I_1	12.30	7.89	19.28	21.03	14.62	3.32	41.75
S.Em (±)	0.09	0.13	0.14	0.15	0.11	0.005	0.18
C.D.	NS	0.57	0.60	0.65	NS	0.020	0.77
Phosphorus [P] (Kg ha^{-1})							
P1	12.17	7.02	18.66	22.83	14.45	3.20	40.87
P2	12.19	7.12	18.74	21.99	14.46	3.28	41.54
P3	12.32	7.60	19.33	21.03	14.53	3.41	41.72
P4	12.28	7.19	19.02	22.06	14.51	3.35	41.08
S.Em (±)	0.05	0.20	0.21	0.37	0.13	0.011	0.24
C.D.	0.13	0.48	0.52	0.91	NS	0.028	0.58
I X P							
I_0P_1	12.11	6.35	18.28	23.80	14.30	3.17	40.45
I_0P_2	12.13	6.46	18.41	22.92	14.33	3.27	41.07
I_0P_3	12.25	6.89	19.01	21.97	14.39	3.39	41.24
I_0P_4	12.23	6.58	18.66	22.99	14.38	3.33	40.64
I_1P_1	12.24	7.69	19.03	21.86	14.60	3.22	41.29
I_1P_2	12.24	7.79	19.07	21.06	14.59	3.28	42.00
I_1P_3	12.39	8.25	19.65	20.08	14.66	3.41	42.20
I_1P_4	12.33	7.83	19.37	21.12	14.63	3.37	41.51
S.Em (±)	0.13	0.54	0.49	0.63	0.16	0.13	0.38
C.D.	NS	1.32	1.21	1.55	NS	NS	0.93

I_0 - Seeds un-inoculated with PSB, I_1 - Seeds inoculated with PSB, P_1 – 40 kg P_2O_5 ha^{-1}, P_2-60 kg P_2O_5 ha^{-1}, P_3 – 80 kg P_2O_5 ha^{-1}, and P_4 - 100 kg P_2O_5 ha^{-1},

the synthesis of nucleic acids, lipids, proteins and other important compounds (Guinn 1984). The phosphorus is immobile in the soil and due to its immobility the unused phosphorus applied as fertilizer that remains in the soil becomes available for the next crops (Read *et al.* 1973). According to Hamid and Sarwar (1977), the crop uses only 15-33% of the applied P and the rest results in the buildup of residual P. At present, domestic production of P fertilizers is insufficient to fulfill the country's demand, hence large amount of P fertilizers needed to be imported at the expenditure of massive foreign exchange. Moreover, world resources of economical P (Rock phosphate) may be washed-out by 2050 (Vance et .al., 2003). There is an increasing trend to replace the chemical fertilizers by a microbial self-propagating source of essential plant nutrients. The practice of microbial inoculation is becoming popular throughout the world due to being simple to use and have no side effects.

The role of microorganisms in solubilizing the inorganic phosphate was known as early as 1903 (Kuecey *et al.* 1989). Phosphorus unavailability is one of the important factors limiting yield of the crops. Phosphorus availability is increased through the use of phosphate solubilizing bacteria (PSB), and it may enhance the forage yield and improve the quality of berseem. The microorganism, involved in solubilizing phosphorus are searching soluble phosphorus and promotes the efficiency of biological nitrogen fixation which results in greater accessibility to other trace elements by producing plant growth promoting substances and ultimately plant growth is enhanced (Gyaneshwar et al., 2002). Phosphate solubilizing bacteria (PSB) produces organic acids like lactate, oxalate, succinate, tartarate, acetate, gluconate, glycolate, citrate, ketogluconate etc. (Gyaneshwar *et al.* 1998; Puente *et al.* 2004). The microorganism capable of solubilizng phosphate can produce and release organic acids and protons in their surroundings that decrease the pH in that area and thus solubilize calcium-phosphorus complexes. Due to these organic acids, PO_4^{-2} is exchanged by acid anion or is chelated and thus becomes dissolved mineral phosphate (Bajpal and Rao 1971).

The reports on interactive effects of chemical phosphorus applied, and phosphate solubilizing bacteria (PSB) on berseem is lacking in our zone. Therefore, the experiment was designed to evaluate the impact of phosphate solubilizing bacteria (PSB) inoculation and different levels of phosphate fertilizer on growth, forage yield and forage quality of berseem in West Bengal condition.

Materials and Methods

A field experiment to study the impact of PSB inoculation and phosphorus application of berseem was conducted in the fodder farm of West Bengal University of Animal and Fishery Sciences, Belgachia fodder farm during rabi, season 2013-14. The crop sown with inoculated (I_1) and un-inoculated (I_0) seeds of berseem (var. warden) was given phosphorus fertilizer at the levels 40 (P_1), 60 (P_2), 80 (P_3) and 100 (P_4) kg P_2O_5 ha^{-1} in the form of single super phosphate (SSP). The experiment was laid out in randomized complete block design (RCBD) with three replications measuring a net plot size of 6 m X 2 m. the seeds of berseem var. warden were inoculated with PSB peat mixer and broadcasted in a well prepared seed bed in middle of November. A basal dose of 20 kg N ha^{-1} and 30 kg K ha^{-1} were given during land preparation. All the other cultural practices were kept normal and uniform for all the treatments. The crop was harvested 70 days a sowing at pod formation. The growth parameters like plant height, leaf stem ratio and number of branches per plant were recorded by randomly selecting ten plants from each plot. The plant height was measured with the help of measuring tape from ground level to highest leaf tip. For dry matter percentage, the sample was dried in shade and dried to electric oven at 70°C up to a period till constant weight was achieved. The drymatter percentage was used as tool for measuring the total dry matter yield. A fraction of dry mass was taken and grinded and then it was preserved in polythene bags for quality analysis. Quality parameters like dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) total ash (TA) and nitrogen free extract (NFE) of the samples were determined according to Association of the Official Analytical Chemist (AOAC) (Anonymous 1990). The dry matter was determined by drying the samples at 80°C till constant weight. Crude protein was estimated by micro 'Kjeldhal' method. The percent of nitrogen indicated the estimation of CP. The ether extract in a sample was determined by extracting with diethyl ether at 60°C in 'Soxhlet's apparatus'. For crude fibre, sample was reflexed first with 1.25% H_2SO_4 and subsequently with 1.25% NaOH for 30 min each to dissolve the acid and alkali soluble component present in it. The residue containing CF was dried to a constant weight, and the dried residue was ignited in muffle furnace, loss of weight on ignition was calculated to express it as CF. For ash, sample was ignited in muffle furnace at 550°C to burn all the organic matter and leftover was weighed as ash. The Nitrogen Free Extract (NFE) was calculated by subtracting the sum of CP, EE, CF and ash from the sample weight on dry matter basis. The observations on growth, yield and quality parameters were calculated using SPSS software, IBM Inc. 2009 and least significant was computed at $p \leq 0.05$ as described in Gomez and Gomez (1984).

Results and Discussion

Growth parameters and yields

It is clear from the table 1 that PSB inoculation has significant effect on growth parameters like plant height, number of branches per plant and leaf stem ratio. PSB inoculated plots recorded significantly higher plant height (52.30 cm); no. of branches per plant (9.52) and leaf stem ratio (1.09). Similar effect of PSB on cluster bean was reported by Ayub *et al*. (2013). Chintapalli *et al*. (2012) recorded the significant effect of Rhizobium + PSB on plant height of berseem. El-Gizawy and Mehasan (2004) also reported significant increase in plant height and number of branches per plant of chickpea by PSB inoculation. Ramana *et al.* (2010) reported that the inoculation with PSB increased leaf area of french bean significantly. However, non-significant difference was found for phosphorus on the plant height and number of branches per plant of berseem (Table 1). The possible argument may be due to less competition of the plants for nutrients particularly phosphorus. The results are in close agreements with that of Saeed *et al.* (2011). However, application of phosphorus resulted significant effect on leaf stem ratio of berseem (Table 1) and better results (1.08) obtained with phosphorus application of 80 kg ha^{-1} P_2O_5. Ayub *et al*. (2012) also reported a significant effect of phosphorus on leaf stem ratio of cluster bean.

Significantly higher green forage yield (320.4 qt ha^{-1}) and dry matter yield (39.2 qt ha^{-1}) were recorded from the plots, inoculated with PSB (Table 1) and that was mainly due to greater values of plant height, no. of branches per plant and leaf stem ratio. Similar effect of PSB on green forage and dry matter yield of cluster bean had been observed by Ayub *et al*. (2013). Chintapalli *et al*. (2012) recorded maximum green forage yield of berseem when the crop inoculated with Rhizobium and PSB along with FYM. Effect of different levels of phosphorus application on green forage yield, as well as dry matter yield, was also found significant. Higher green forage yield (321.7 qt ha^{-1}) and dry matter yield (39.4 qt ha^{-1}) were obtained with phosphorus application of 80 kg ha^{-1}. Increase in P levels from 40 kg ha^{-1} to 80 kg ha^{-1} gradually increased the green forage and dry matter yield of berseem. Increase of P level beyond 80 kg ha^{-1} adversely affects the green forage and dry matter yield and the same were statistically at par with those achieved with the application of 80 kg P_2O_5 ha^{-1}. Maximum green forage and dry matter production at 80 kg P_2O_5 ha^{-1} might be the results of higher plant height, no. of branches per plant, leaf stem ratio and of course, the better root development which provides a better habitat for the activity of biological nitrogen fixing bacteria. The significant effect on dry matter yield of cluster bean at various levels of P had also been reported by Ayub *et al*. (2012) and Chauhan and Bajpal (1979). Interaction effect of PSB and phosphorus on plant height and no. of branches per plant were found not significant. Significantly higher green forage yield (330.8 qt. ha^{-1}), dry matter yield (40.7 qt. ha^{-1}) and leaf stem ratio (1.17) were obtained from the plots inoculated with PSB and having phosphorus application of 80 kg ha^{-1} might be due to better crop growth, higher plant height and more no. of branches per plant. Ayub *et al*. (2013) also reported significant effect of PSB inoculation and P application on green forage and dry matter yield in cluster bean.

Quality parameters

All the quality traits except ash content and dry matter percentage were significantly affected by PSB inoculation. PSB inoculation exhibited higher values for crude protein yield (7.89 qt. ha^{-1}), crude protein percentage (19.28%), ethyl extract (3.32), nitrogen free extract (41.75) and lowest crude fibre (21.03), than uninoculation (Table 2). Ayub *et al*. (2013) recorded higher CP and EE from the PSB inoculation crops of cluster bean. Rugheim and Abdelganj (2012) also reported the significantly higher values of CP and NFE in PSB inoculation in fava bean.

Application of phosphorus was significant for all quality parameters except the ash content. Increase in P levels from 40 kg ha^{-1} to 80 kg ha^{-1} gradually increased the dry matter percentage, crude protein yield, crude protein percentage, ethyl extract and nitrogen free extract of berseem and increase of P level beyond 80 kg ha^{-1} adversely affected those quality traits. However, the effect of P application from 40 kg ha^{-1} to 80 kg ha^{-1} on crude fibre percentage of berseem was just opposite. Higher dry matter percentage (12.32), crude protein yield (7.60 qt. ha^{-1}), crude protein percentage (19.33%), ethyl extract (3.41%) and nitrogen free extract (41.72%) were obtained with P application of 80 kg ha^{-1} (Table 2). However, significantly lowest crude fibre percentage (21.03%) was recorded with 80 kg P_2O_5 ha^{-1} and highest (22.83%) with 40 kg P_2O_5 ha^{-1}. Turk *et al.* (2007) also reported a decrease in crude fibre content with increase the levels of phosphorus in vetch crops. Ayub *et al*. (2013) reported the significant effect of P application on dry matter percentage and crude fibre content of cluster bean. Grewal *et al.* (2004) and Yadav *et al*. (2011) also recorded significant effect of P on crude protein percentage of cluster bean. Better quality green forage produced with 80 kg P ha^{-1} might be the results of better root development which provides a better habitat for the activity of biological nitrogen fixing bacteria. The higher root mass exploits the soil from surrounding more effectively and improves the nutrients availability for plants.

Interaction effect of PSB and phosphorus on dry matter percentage, ash content and ethyl extract was found not significant. Significantly higher crude protein yield (8.25 qt. ha^{-1}), crude protein percentage (19.65%), nitrogen free extract (42.20%) and lowest crude fibre (20.08%) were obtained from the plots, inoculated with PSB and having phosphorus application of 80 kg ha^{-1} (Table 2).

Conclusion

From the results of the present study, it can be concluded that phosphorus solubilizing bacterial inoculation along with phosphorus application are essential for obtaining higher yield of good quality fodder of berseem. For obtaining higher yield of good quality fodder of berseem, it seems better to inoculate its seed with phosphorus solubilizing bacteria (PSB) and apply phosphorus at the rate of 80 kg ha^{-1} under West Bengal condition.

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