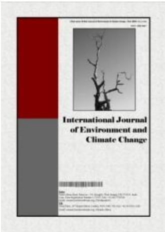

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Effect of Phosphorous Levels and Phosphorous Solubilizing Bacteria (PSB) on Growth, Quality Parameters and Profitability of Berseem (*Trifolium alexandrinum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted at Research farm of R.A.K., college of Agriculture, Sehore, Madhya Pradesh during 2020-21. The experiment was laid out using Randomized Block Design with different levels of Phosphorus with or without PSB application (T_1- 45 kg P_2O_5/ha, T_2- 60 kg P_2O_5/ha, T_3- 75 kg P_2O_5/ha, T_4-90 kg P_2O_5/ha, T_5 - 45 kg P_2O_5/ha + PSB @ 5g/kg seed, T_6 - 60 kg P_2O_5/ha + PSB @ 5g/kg seed, T_7 - 75 kg P_2O_5/ha + PSB @ 5g/kg seed, T_8 - 90 kg P_2O_5/ha + PSB @ 5g/kg seed). Treatment T_7 found to be best for yield and yield attributes of berseem. Maximum plant height (54.8, 71.5 and 65.5 cm), number of branches/plant (5.44, 6.72 and 8.53), dry matter accumulation (4.90, 6.64 and 5.00 g/plant) at 1st cutting, 2nd cutting and at harvest respectively, dry

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matter (13.01 %), crude protein (19.99 %), crude protein yield (420.21 kg/ha), Gross return (87,660 ₹/ha), net return (59,412 ₹/ha) and B:C ratio (3.03). While minimum recorded with treatment T_1 (45kg P_2O_5/ha without PSB application).

Keywords: Phosphorous levels; PSB; growth; quality.

1. INTRODUCTION

Berseem (*Trifolium alexandrinum* L.) is an important winter legume forage crop of irrigated area. It has become popular owing to rapid growth, multi-cut nature and, good recovery after cuttings, higher yield, and long period of fodder supply, high nutritive value and excellent palatability besides enrichment of the soil through biological nitrogen fixation [1].

Berseem or Egyptian clover is regarded as "King of fodder". It is a nitrogen-fixing annual leguminous crop which is highly adaptable to the semi-arid climate of Northern India. It's also a nutritious and high-yielding leguminous fodder crop. It has a cosmopolitan adaptability and diverse quality. It increases the amount of milk produced by cows and buffaloes.

On an average berseem fodder has 20-21% crude protein, 25.9% crude fibre, 40.7 percent nitrogen free extract, 14.16 percent ash, 1.92 percent calcium, 0.28 percent phosphorus, 70-72 percent dry matter digestibility and is calcium-rich. Berseem productivity is quite poor in India. There are a number of factors that contribute to low berseem yields, but inappropriate fertilizer application is one of the most significant. Correct and judicious fertilizer application can increase production by up to 50% [2] while also improving fodder quality [3].

The availability of adequate quality and quantity fodder is linked to the low productivity of Indian livestock. It's been a while seen as a big stumbling block in maximizing the livestock's potential [4,5].

Berseem is grown in India, Pakistan, Turkey, Egypt and countries of Mediterranean region. In India, it is grown mainly in irrigated area of Northern India and Western part of country. The main state growing this crop are Punjab, Haryana, Delhi, Rajasthan, Uttar Pradesh, Gujarat, and some parts of Bihar, Maharashtra, and Andhra Pradesh, [6].

Phosphorus plays an important role as a structural component of cell constituents and metabolically active compounds. The protein synthesis is controlled by the supply of phosphorus in plants. Phosphorus has an important role in the process of photosynthesis in plants [7]. It is an essential constituent of nucleic acid, phytin, phospholipids and enzymes and is responsible for root development and seed formation [8]. Moreover, P is an essential nutrient for growth and development which gives rapid and vigour start to plant and stimulate flowering and also help in nutrient uptake of N [9]. Phosphorus plays important role in legume crops. The effect of P is known to activate microbial population responsible for nodulation. Phosphorus in soil increases concentration of P ions of soil solution and ultimately affects formation of more nodules, Vigorous root development, better nitrogen fixation and overall better development of plants. Efficient nodulation due to P fertilization enhances N-fixation to be utilized by plants and ultimately increases protein content. Being a leguminous crop, it enriches the soil by fixing atmospheric N with the association of *Rhizobmm trifoli* [10].

The microorganisms that are involved in phosphorus solubilization are looking for soluble phosphorus and promoting the effectiveness of biological nitrogen fixation, which results in greater availability to other trace minerals by developing plant growth promoting substances, and ultimately plant growth is improved [11]. PSB creates organic acids such as lactate, oxalate, succinate, tartarate, acetate, gluconate, glycolate, citrate, ketogluconate, and others [12]. Phosphate-solubilizing microorganisms can create and release organic acids and protons in their environment, lowering the pH and thereby solubilizing calcium-phosphorus complexes. PO_4^{-2} is swapped by acid anion or chelated as a result of these organic acids, and therefore forms dissolved mineral phosphate [13].

2. MATERIALS AND METHODS

The study was conducted during rabi season of 2020-21 at experimental block of Research farm, R.A.K. College of Agriculture, Sehore, Madhya Pradesh, India. The Farm is situated in the Eastern part of Vindhyan Plateau in subtropical zone at the latitude of 23^0 12’ North and longitude of 77^0 05’ East at an altitude of 498.77 m from mean sea level in Madhya Pradesh, India. The crop sown with inoculated and un-inoculated seeds of berseem (var. Bar-Bar) was given phosphorus fertilizer at the levels 45, 60, 75 and 90 kg $P_2 O_5$ ha^{-1} in the form of single super phosphate (SSP). The experiment was laid out in randomized block design with three replications measuring a net plot size of 4 m X 5 m the seeds of berseem var. Bar-Bar 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 40 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 150 DAS sowing at pod formation. The growth parameters like plant height, number of branches per plant were recorded by randomly selecting five 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. 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) of the samples were determined according to Association of the Official Analytical Chemist (AOAC) [14]. 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 observations on growth, quality parameters and economics were calculated using SPSS software, IBM Inc. 2009 and least significant was computed at $p \leq 0.05$ as described in Gomez and Gomez [15].

3. RESULTS

3.1 Growth Parameters

3.1.1 Plant height (cm)

The data on account plant height, presented in Table 1 revealed that different treatments had significantly influenced the plant height. Maximum plant height (54.8, 71.5 and 65.5 cm) during first cutting, second cutting and at harvest, respectively was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). Although, the treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,* 53.7, 69.5 and 64.1 cm and T_3 (75 kg P_2O_5/ha) *i.e.,* 52.6, 68.9 and 63.4 cm during first cutting, second cutting and at harvest, respectively was *at par* with the treatment T_7. Although, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum plant height *i.e.,* 47.0, 60.2 and 58.1 cm during first cutting, second cutting and at harvest, respectively.

3.2 Number of Branches/Plant

The data on account number of branches/plant, presented in Table 1 revealed that different treatments had significantly influenced the number of branches/plant. Maximum number of branches/plant (5.44, 6.72 and 8.53) during first cutting, second cutting and at harvest, respectively was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed).Although, the treatmentT_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,* 5.21, 6.65 and 8.52 and T_3 (60 kg P_2O_5/ha) *i.e.,* 5.11, 6.45 and 8.34 during first cutting, second cutting and at harvest, respectively was *at par* with the treatment T_7. While, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum number of branches/plant *i.e.,* 4.21, 5.05 and 6.08 during first cutting, second cutting and at harvest, respectively.

3.3 Dry Matter Accumulation (g/plant)

The data on account dry matter accumulation/plant, presented in Table 1 revealed that different treatments had significantly influenced the dry matter accumulation (g/plant). Maximum dry matter accumulation (4.90, 6.64 and 5.00 g/plant) during first cutting, second cutting and at harvest, respectively was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). Although, the treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,*4.72, 6.53 and 4.91 g/plant during first cutting, second cutting and at harvest, respectively and T_3(60 kg P_2O_5/ha) *i.e.,* 4.67, 6.45 and 4.91 g/plant during first cutting, second cutting and at harvest, respectively, was *at par* with the treatment T_7. While, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum dry matter accumulation *i.e.,* 3.63, 3.90 and 3.89 g/plant during first cutting, second cutting and at harvest, respectively.

Table 1. Effect of Phosphorous levels and Phosphorous Solubilizing Bacteria (PSB) on Growth, Quality Parameters of Berseem (*Trifolium alexandrinum* L.)

Seed Yield Treatments		Plant height (cm)			Number of branches/plant			Dry matter accumulation (g/plant)			Dry matter (%)	Crude protein (%)	Crude protein yield (kg/ha)
		70 DAS	110 DAS	At Harvest	70 DAS	110 DAS	At Harvest	70 DAS	110 DAS	At Harvest			
T_1	45 kg P_2O_5/ha	47.0	60.2	58.1	4.21	5.05	6.08	3.63	3.90	3.89	11.83	15.25	273.93
T_2	60 kg P_2O_5/ha	48.0	62.2	58.3	4.43	5.16	6.17	3.75	5.13	4.15	12.17	16.75	305.49
T_3	75 kg P_2O_5/ha	52.6	68.9	63.4	5.11	6.45	8.34	4.67	6.45	4.91	12.60	18.37	377.37
T_4	90 kg P_2O_5/ha	50.6	67.9	62.3	4.96	6.17	7.64	4.57	6.19	4.86	12.51	18.10	349.08
T_5	45 kg P_2O_5/ha + PSB @ 5 g/kg seed	48.2	62.6	58.4	4.47	5.84	6.64	4.19	5.71	4.24	12.38	16.85	323.27
T_6	60 kg P_2O_5/ha + PSB @ 5 g/kg seed	49.3	66.5	61.4	4.88	5.94	7.19	4.19	5.90	4.56	12.47	17.66	342.90
T_7	75 kg P_2O_5/ha + PSB @ 5 g/kg seed	54.8	71.5	65.5	5.44	6.72	8.53	4.90	6.64	5.00	13.01	19.99	420.21
T_8	90kg P_2O_5/ha + PSB @ 5 g/kg seed	53.7	69.5	64.1	5.21	6.65	8.52	4.72	6.53	4.91	12.90	19.27	409.49
S.Em±		**0.79**	**0.90**	**0.70**	**0.15**	**0.10**	**0.21**	**0.11**	**0.14**	**0.07**	**0.15**	**0.08**	**7.53**
CD (5%)		**2.37**	**2.70**	**2.10**	**0.45**	**0.29**	**0.63**	**0.32**	**0.41**	**0.21**	**0.45**	**0.24**	**22.54**

Table 2. Effect of Phosphorous levels and Phosphorous Solubilizing Bacteria (PSB) on Economics of Berseem (*Trifolium alexandrinum* L.)

Treatments		Cost of Cultivation (₹/ha)	Gross Monetary Return (₹/ha)	Net Monetary Return (₹/ha)	B:C Ratio
T_1	45 kg P_2O_5/ha	27,567	67,093	39,526	2.43
T_2	60 kg P_2O_5/ha	28,223	70,380	42,157	2.49
T_3	75 kg P_2O_5/ha	28,879	81,207	51,646	2.81
T_4	90 kg P_2O_5/ha	29,536	79,253	50,374	2.68
T_5	45 kg P_2O_5/ha + PSB @ 5 g/kg seed	27,592	74,424	44,888	2.69
T_6	60 kg P_2O_5/ha + PSB @ 5 g/kg seed	28,248	76,617	49,025	2.71
T_7	75 kg P_2O_5/ha + PSB @ 5 g/kg seed	28,904	87,660	59,412	3.03
T_8	90kg P_2O_5/ha + PSB @ 5 g/kg seed	29,560	84,759	55,855	2.86

3.4 Quality Parameters

3.4.1 Dry matter (%)

The data on account dry matter (%), presented in Table 1 revealed that different treatments had significantly influenced the dry matter (%). Maximum dry matter (13.01 %) was observed with the treatmentT_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). Although, the treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,* 12.90 % and T_3 (60 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,* 12.60 % at harvest, respectively, was *at par* with the treatment T_7. While, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum dry matter *i.e.,* 11.83 %.

3.4.2 Crude protein (%)

The data on account crude protein (%), presented in Table 1 revealed that different treatments had significantly influenced the crude protein (%). Maximum crude protein (19.99 %) was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). While, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum crude protein *i.e.,* 15.25 %.

3.4.3 Crude protein yield (kg/ha)

The data on account of crude protein yield (kg/ha), presented in Table 1, revealed that different treatments had significantly influenced the crude protein yield (kg/ha). Maximum crude protein yield (420.21 kg/ha) was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). Although, the treatmentT_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.,* 409.49 kg/ha was *at par* with the treatment T_7. While, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum crude protein yield *i.e.,* 273.93 kg/ha.

3.5 Economics

The maximum gross monetary return was recorded with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.*, 87,660₹/ha. Although, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum gross monetary return *i.e.,* 67,093 ₹/ha.

The maximum net monetary return was recorded with the treatmentT_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.*, 59,412 ₹ /ha. Although, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum net monetary return *i.e.,* 39,526 ₹/ha.

The maximum B:C ratio of berseem was recorded with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) *i.e.*, 3.03 per ₹ invested. Although, the treatment T_1 (45 kg P_2O_5/ha) recorded minimum B:C ratio*i.e.,*2.43 per ₹.

4. DISCUSSION

4.1 Growth Parameters

Maximum plant height and number of branches/plant was observed with the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) and minimum plant height and number of branches/plant was observed with the treatment T_1 (45 kg P_2O_5/ha). The possible reasons may be attributed to PSB inoculation. Application of PSB might have solubilize the phosphate by the micro-organisms through the production of organic acids, citric acids and ketogluconic acids act as powerful chelator of Ca while humic acid and fulvic acid form stable complexes with iron and aluminum phosphate and thus make increased quantity of phosphorus available to plants. Similar effect of PSB on cluster bean was reported by Ayub et al. [16]. Chintapalli et al. [17] recorded the significant effect of Rhizobium + PSB on plant height of berseem. El-Gizawy and Mehasan [18] also reported significant increase in plant height and number of branches per plant of chickpea by PSB inoculation.

Increase in plant height with higher rate of P is attributed to its favourable effect on cell division and multiplication [19,20]. Since P plays an important role in extensive root development and translocation of photosynthates and phospholipids, its application increase different growth parameters [21], and hence plant height of oat has increased with P application under the present investigation. The potent role of P increasing plant height of berseem has been reported by many workers like Hussain et al. [22], Sardana and Narwal [23]. Inferior growth in height of plant under lower rate of P might be due to the fact that under phosphate deficiency, the synthesis of protein is adversely affected because of the accumulation of arginine in the tissues of leguminous plants which results in restricted plant growth [24].Increase of P level beyond 80 kg ha^{-1} adversely affects the plant growth and the same were statistically at par with those achieved with the application of 80 kg P_2O_5 ha^{-1} [25].

Maximum dry matter accumulation was observed with the treatment T_6 (@ 60 kg P_2O_5/ha + PSB @ 5 g/kg seed). It might be due to taller plants height, more tillers/plant and number of leaves [26]. The capacity of plant to accumulate dry matter is determined by its rate of CO_2 fixation, photosynthetic area, and duration of crop, tillers/plant and environmental factor besides management practices [27].

4.2 Quality Parameters

Application of phosphorus was significant for all quality parameters except the ash content. Higher dry matter percentage, crude protein percentage and crude protein yield were obtained with P application of treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed). However, significantly lowest dry matter percentage, crude protein percentage and crude protein yield with 45 kg P_2O_5 ha^{-1}. It might be due to smaller plants height, less tillers/plant and number of branches with low level of Phosphorus [26]. Ayub et al. [16] reported the significant effect of P application on dry matter percentage and crude fibre content of cluster bean. Grewal et al. [28] and Yadav et al. [29] also recorded significant effect of P on crude protein percentage of cluster bean. Better quality green forage produced with 75 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 [25].

4.3 Economics

Among different treatments, application of treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) gave maximum gross returns (Rs. 87,660/ha), net returns (Rs. 59,412/ha) and B:C ratio (3.03) closely followed by treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg). The result confirms the findings of Godara et al. [30].

5. CONCLUSION

On the basis of results obtained in present investigation it is concluded that treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) was found to be the best and closely followed by treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed) and treatment T_3 (75 kg P_2O_5/ha) for morphological (plant height, no. of branches/plant, dry matter accumulation/plant) and quality (dry matter, crude protein, crude protein yield) parameters. Although, for profitability in berseem cultivation the treatment T_7 (75 kg P_2O_5/ha + PSB 5 g/kg seed) was also found to be the best, which was closely followed by treatment T_8 (90 kg P_2O_5/ha + PSB 5 g/kg seed), while the minimum profitability recorded with treatment T_1 (45 kg P_2O_5/ha).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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