

Integrated effect of phosphorus fertilizer in conjoint with organics and bioinoculant on growth, yield and quality parameters of blackgram in *Typic Chromustert*

Comment [SC1]: Need to simplify the title i.e. make it short

ABSTRACT

Aims: ~~An experiment on the u~~Use of phosphorus fertilizer along with farmyard manure, vermicompost and phosphorus solubilizing bacteria (*Bacillus megaterium*) in black calcareous soil was conducted to assess the impact on the growth, yield and quality parameters of blackgram variety VBN 8 during the year 2022.

Study design: ~~The experiment was laid out in~~ Randomized Block Design (RBD) with twelve treatments and three replications.

Place and duration of study: ~~Research trial was conducted at~~ Kalligudi block of Madurai district, representing the Southern part of Tamil Nadu during the summer season of 2022.

Methodology: Twelve treatments were followed with various combinations of organics viz., vermicompost and farmyard manure, bioinoculant and inorganic chemical fertilizer at different ratios. The impact of different treatments were recorded on shoot length, root volume, SPAD value, Leaf Area Index, grain yield, protein, cystine and methionine content.

Result: Among the treatments, application of 100% of P_2O_5 on Soil Test Crop Response (STCR) basis incubated for 30 days with vermicompost at 1:5 ratio + phosphorus solubilizing bacteria @ 2 kg ha^{-1} T₁₁ has improved the black gram shoot length (27.6 cm), root volume (2.43 cm^3), SPAD value (45.91), Leaf Area Index at 20 DAS (1.09) and 40 DAS (3.7), grain yield (936.1 kg ha^{-1}) protein content (23.21%), cystine content (1.97%) and methionine content (2.29%).

Conclusion: The integration of vermicompost and bioinoculant along with phosphorus fertilizer in black calcareous soil has improved the blackgram yield by 35.8% over the use of recommended dose of fertilizer alone. This result highlights the need of integrated application of phosphorus fertilizer along with organics and phosphorus solubilizing bacteria in black calcareous soil for sustainable production of blackgram.

Comment [SC2]: Need to include the detailed treatments in Methodology first. Unless it's not clear

Keyword: Calcareous soil, Bioinoculant, Phosphorus, Vermicompost

1. INTRODUCTION

Phosphorus is a critical plant nutrient that cannot be replaced by another element to sustain plant life. The use of phosphorus fertilizer is a crucial component for high yielding food crops, and the majority of the mineral P used annually at the global level is for food production. It plays a vital role in the molecular structure of crops and aids in the transformation of energy as well as the regulation of several enzymatic activities. Phosphorus is also required for energy production, nucleic acid synthesis, photosynthesis, glycolysis, respiration, redox reactions, membrane synthesis and integrity, enzymatic activation or inactivation, carbohydrate metabolism and signaling. Therefore, insufficiency of phosphorus levels in the soil will greatly affect the growth and development of crops [1].

The bioavailability of phosphorus is mainly affected due to its tendency to form a complex with soil mineral particles viz., calcium (Ca), magnesium (Mg), aluminum (Al) and iron (Fe) present in the soil causes significant crop yield losses [2]. The reaction of P in alkaline calcareous soil will be specific, that is calcium will react with the phosphorus which reduces its bioavailability due to the formation of calcium phosphate minerals which are less soluble at about pH 8. Important mechanisms of P retention in calcareous soil are surface adsorption and precipitation, which in turn reduces its availability. As a result of the low P solubility, fertilizer phosphorus efficiency is relatively low in alkaline calcareous soil. Adding P fertilizer at normal rates with conventional methods may not give optimal crop yield and quality.

Several P fertilizer management strategies have been found to improve the P nutrition for plants grown in alkaline calcareous soil. Phosphorus application, combined with organic manures and phosphorus-solubilizing bacteria, effectively supplies P to growing plants in alkaline calcareous soil. Long-term manure application to alkaline calcareous soil had revealed that there is a significant amount of P accumulation, with 50 to 66 percent in plant available forms. The affinity constants and sorption capacities of soils for P are reduced by organic amendments, especially manure. This could be due to the production of organic acids by manure application competing for P fixation sites and the complexation of exchangeable cations by manure components [3]. PSB can be beneficial because these bacteria will secrete some phenolic compounds, protons, organic and mineral acids into the soil, causing soil acidification and resulting in P release from calcium phosphate. PSB secreted organic acids chelate with some of the cations such as Ca^{2+} , Al^{3+} , and Fe^{3+} which may increase bioavailable P [4]. However, the potential of PSB in soils of a calcareous nature and with an alkaline reaction has not been well documented. Considering the crop phosphorus requirements and its availability in calcareous soil, this study was conducted to manage phosphorus availability by the integration of organic, phosphorus solubilizing bacteria and inorganic sources for optimum blackgram production in calcareous soils.

2. Materials and Methods

Comment [SC3]: Reference??

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The present experimental study was conducted at Kalligudi block of Madurai district, representing Southern part of Tamil Nadu during summer season 2022 with black gram variety VBN 8. Climatic conditions of experimental site were 37.7°C and 20.3°C average maximum and minimum temperature respectively with a mean rainfall of 840 mm. The soil texture of the experimental site is clay loam belonging to the Vertisol soil order. The experiment was laid out in Randomized Block Design (RBD) with twelve treatments and three replications, as mentioned in Table 1.

Comment [SC7]: Reference?

Table 1. Treatment schedule for field experiment

Treatments	Details
T ₁	Absolute control
T ₂	Recommended N, P ₂ O ₅ , K ₂ O @ 25:50:25 kg ha ⁻¹
T ₃	100% of P ₂ O ₅ on STCR basis alone
T ₄	100% of P ₂ O ₅ on STCR basis incubated for 30 days with farm yard manure at 1:10 ratio
T ₅	75% of P ₂ O ₅ on STCR basis incubated for 30 days with farm yard manure at 1:10 ratio
T ₆	100% of P ₂ O ₅ on STCR basis incubated for 30 days with vermicompost at 1:5 ratio
T ₇	75% of P ₂ O ₅ on STCR basis incubated with vermicompost for 30 days at 1:5 ratio
T ₈	100% of P ₂ O ₅ on STCR basis + PSB @ 2 kg ha ⁻¹
T ₉	T ₄ + PSB @ 2 kg ha ⁻¹
T ₁₀	T ₅ + PSB @ 2 kg ha ⁻¹
T ₁₁	T ₆ + PSB @ 2 kg ha ⁻¹
T ₁₂	T ₇ + PSB @ 2 kg ha ⁻¹

The enriched phosphorus fertilizer was prepared by mixing two different sources of organic manures with inorganic phosphorus fertilizer (single super phosphate) at different combinations viz., 100 per cent and 75 per cent of P₂O₅ on STCR (Soil Test Crop Response) basis mixed with farmyard manure (1:10 ratio) and vermicompost (1:5 ratio). These combination were prepared and kept for incubation at 60 per cent moisture content in grow bag over a period of one month. Nitrogen and potassium were applied as urea and MOP on STCR basis in all the treatments except T₁, T₂ and T₈. T₁ was the absolute control, T₂ received recommended dose of fertilizer (N, P₂O₅, K₂O @ 25:50:25 kg ha⁻¹) whereas T₈ was an application of phosphorus on STCR basis with PSB.

The observations viz, shoot length, root volume, SPAD value and seed yield were measured at the harvest stage in each plot. Leaf Area Index was measured at 20 and 40 days after sowing (DAS). SPAD value was measured by using MC - 100 chlorophyll concentration meter. The quality parameters were estimated by standard procedure viz, protein content by Lowry's method [5], cystine content by Goa procedure [6] and methionine content by sodium nitroprusside method [7]. The data collected were analyzed by using AGRES software [8].

Comment [SC8]: how measured?

Table 2. Initial physico-chemical properties of the experimental soil

Particulars		Values	Methods
A.	Mechanical composition		
	Clay (%)	34.76	International pipette method [9]
	Silt (%)	20.41	
	Fine sand (%)	17.01	
	Coarse sand (%)	25.68	
	Texture class	Clay loam	
B.	Physical properties		
	Bulk density (Mg m^{-3})	2.50	Core sampler method [10]
	Particle density (Mg m^{-3})	1.34	
	Porosity (%)	41	
C.	Physico-chemical properties		
	pH	8.32	Potentiometry [11]
	EC (dSm^{-1})	0.18	Conductometry [11]
D.	Chemical properties		
	Free CaCO_3 (%)	7.63	Rapid titration method [9]
	Organic carbon (g kg^{-1})	3.80	Dichromate wet digestion method [12]
	Alkaline KMnO_4 – Nitrogen (kg ha^{-1})	234	Alkaline permanganate method [13]
	Olsen -Phosphorus (kg ha^{-1})	10.34	Olsen method [14]
	NH_4OAc – Potassium (kg ha^{-1})	214.24	Neutral normal NH_4OAc method [15]

3. RESULTS AND DISCUSSION

3.1 Effect of phosphorus fertilizer integrated with organics and PSB on growth attributes of blackgram

3.1.1 Shoot length

The perusal of the data (Table 3) shows that the growth parameters were significantly affected by the application of inorganic phosphorus fertilizer along with organics and PSB. A higher shoot length of 27.6 cm at the harvest stage was recorded in T_{11} (100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1}) which was followed by T_{12} (75% of P_2O_5 on STCR basis incubated with vermicompost for 30 days at 1:5 ratio + PSB @ 2 kg ha^{-1}) of 25.9 cm. The lowest shoot length was recorded in absolute control T_1 (13.07 cm) which was on par with the application of inorganic phosphorus fertilizer alone T_3 (13.57 cm). The treatment which received the recommended dose of fertilizer T_2 alone recorded comparatively lower shoot length (17.07 cm) than the conjoint application of inorganic fertilizer with organics and PSB. A positive response from the combined application of

vermicompost, PSB and inorganic fertilizer may be due to their specific role in shoot morphology, cell division, cell wall plasticity, energy metabolism, and improved root development, which may promote better shoot growth. Phosphate solubilizing bacteria and organics application along with phosphorus fertilizer might increase the bioavailability of insoluble soil phosphorus for plant use by producing organic acids [16]. Because of the mineralization, solubilization, and translocation of phosphorus through the production of organic acid and proton extrusion by the use of biofertilizer and organics have improved the phosphorus availability to plants, which in turn improves the plant shoot growth [17].

3.1.2 Root volume

The root volume was also enhanced by the application of enriched phosphorus with vermicompost and PSB. The application of 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha^{-1} achieved a higher root volume at the harvest stage (2.43 cm^3) which was followed by T_{12} (2.29 cm^3) whereas the treatment which received 100% of P_2O_5 on STCR basis incubated for 30 days with vermicompost at 1:5 ratio without PSB T_6 recorded comparatively lower root volume (1.93 cm^3). The lowest root volume was recorded in absolute control T_1 (1.22 cm^3). The positive influence of phosphorus, organics and bioinoculant on the formation, multiplication, and elongation of root cells could have resulted in the growth of the voluminous root system in blackgram. Combined application of PSB and organics along with inorganic fertilizer leads to higher Indole Acetic Acid production, a hormone produced by plants that regulate the growth and elongation of roots, which was likely the cause of improvements in seedlings' root morphological features [18].

3.1.3 SPAD value

The data on the SPAD value in various treatments revealed that the higher SPAD value of blackgram at the harvest stage was significantly affected by the different combinations of inorganic phosphorus fertilizer, farmyard manure, vermicompost and PSB. The SPAD value was higher in the treatment where phosphorus fertilizer was integrated with vermicompost and PSB T_{11} (45.91) as compared to the application of organics, PSB and phosphorus fertilizer alone. Whereas, the treatment which received organic and inorganic fertilizer without PSB T_6 recorded comparatively lowest SPAD value of 39.01. The lowest SPAD value was observed in absolute control T_1 (29.16) which was on par with T_3 (29.76). This occurred mostly as a result of PSB having the ability to enhance nitrogen fixation, which can encourage plant uptake of N and leaf chlorophyll content [19]. For instance, one of the most important parameters measured as an indicator of leaf nitrogen content, chloroplast development, general plant health, and photosynthetic capacity is the chlorophyll content of leaf tissue [20].

3.1.4 Leaf Area Index

The Leaf Area Index at 20 and 40 DAS was significantly higher in the T₁₁ treatment with 100% of P₂O₅ on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha⁻¹ (1.09 and 3.70) followed by T₁₂ (1.01 and 3.43) as compared to absolute control and use of inorganic fertilizer alone. The lowest Leaf Area Index at 20 and 40 DAS was noticed in absolute control T₁ (0.4 and 1.83) which was on par with the application of inorganic phosphorus fertilizer alone T₃ (0.42 and 1.85) which might be due to the greater supply of nitrogen by this treatment which in turn increased the leaf size, thereby resulting in maximization of LAI and light interception [21].

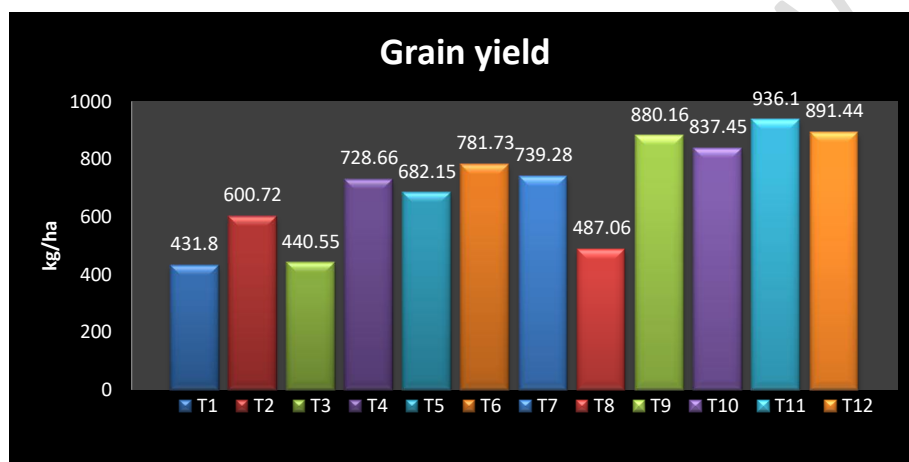
Table 3. Integrated effect of phosphorus in conjoint with organics and bioinoculant on growth parameters of blackgram

Treatments	Shoot length (cm)	Root volume (cm ³)	SPAD value	Leaf Area Index	
				20 DAS	40 DAS
T ₁	13.07	1.22	29.16	0.40	1.83
T ₂	17.07	1.51	33.37	0.56	2.39
T ₃	13.57	1.27	29.76	0.42	1.85
T ₄	19.63	1.76	36.69	0.70	2.73
T ₅	18.40	1.65	35.07	0.64	2.57
T ₆	21.60	1.93	39.01	0.82	3.01
T ₇	20.23	1.80	37.45	0.75	2.74
T ₈	14.87	1.38	31.15	0.47	2.06
T ₉	25.30	2.23	43.03	0.97	3.39
T ₁₀	23.70	2.10	41.21	0.90	3.21
T ₁₁	27.60	2.43	45.91	1.09	3.70
T ₁₂	25.90	2.29	43.92	1.01	3.43
S.Ed	0.40	0.04	0.66	0.01	0.05
C.D (p=0.05)	0.84	0.09	1.38	0.02	0.12

3.2 Effect of phosphorus fertilizer integrated with organics and PSB on yield of blackgram

The grain yield of blackgram recorded in the treatment supplied with 100% of P₂O₅ on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha⁻¹ (936.1 kg ha⁻¹) was significantly higher than the control and other treatments (Fig.1). The grain yield of absolute control T₁ (431.8 kg ha⁻¹) was on par with the treatment received inorganic ph

osphorus fertilizer alone T₈ (487.06 kg ha⁻¹) without organic manure and PSB. Likewise, there was no significant difference in the grain yield of the treatments received 100% of P₂O₅ on STCR basis incubated for 30 days with farm yard manure at 1:10 ratio T₄ (728.66 kg ha⁻¹) and 75% of P₂O₅ on STCR basis incubated with vermicompost for 30 days at 1:5 ratio T₇ (739.28 kg ha⁻¹). However, yield increase was more prominent in PSB inoculated and organics applied treatments compared to un-inoculated. This could be attributed to the release of unavailable P into the mobile pool by rapid mineralization of organic P and solubilization of Ca-P through acidifying and chelating mechanisms [22].



Comment [SC9]: please revise the figure and make simple and attractive. Need to add error bars and LSD levels.

Fig 1. Integrated effect of phosphorus in conjoint with organics and bioinoculant on yield of blackgram

3.3 Effect of phosphorus fertilizer integrated with organics and PSB on quality parameters of blackgram

The data depicted in Table 4. revealed that the integrated application of chemical fertilizer with organics and bioinoculant has a significant effect on protein, cystine and methionine content. Addition of 100% of P₂O₅ on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha⁻¹ recorded higher protein (23.21 %), cystine (1.97 g/16g N) and methionine content (2.29 g/16g N) which was on par with T₁₂ (22.28 % and 1.84 and 2 g/16g N). The lowest protein, cystine and methionine content were recorded with absolute control T₁ which is statistically on par with T₈. As PSB plays a key role in N fixation and this nitrogen would have helped in the synthesis of amino acids. Besides phosphate would have replaced the sulphate from the colloidal complex and increased the absorption of sulphate by the black gram. The protein content is dependent on plant growth and nutritional composition. Increased protein content in grain may be due to increased nitrate uptake and translocation, which provides nitrogen for

amino acid synthesis [23]. The highest percentage of crude protein content in seeds of blackgram with vermicompost and PSB applied treatment might be due to more availability of nitrogen. PSB may enhance both the rhizosphere's nutrient environment and its use in the plant system.

Table 4. Integrated effect of phosphorus in conjoint with organics and bioinoculant on quality parameters of blackgram

Treatment	Protein content (%)	Cystine content (g/16g N)	Methionine content (g/16g N)
T ₁	14.23	0.89	0.68
T ₂	16.51	1.22	1.08
T ₃	14.50	0.94	0.75
T ₄	18.64	1.41	1.37
T ₅	17.75	1.32	1.24
T ₆	19.87	1.58	1.71
T ₇	18.88	1.45	1.44
T ₈	15.41	1.06	0.90
T ₉	21.97	1.79	2.02
T ₁₀	21.08	1.70	1.88
T ₁₁	23.21	1.97	2.29
T ₁₂	22.28	1.84	2.00
S.Ed	0.30	0.03	0.03
C.D (p=0.05)	0.63	0.07	0.07

4. Conclusion

In black calcareous soil, it is possible to attain higher growth, yield and quality of blackgram by integrated use of phosphorus fertilizer along with vermicompost and phosphorus solubilizing bacteria. Our results confirmed that application of 100 % of P₂O₅ on STCR basis incubated for 30 days with vermicompost at 1:5 ratio + PSB @ 2 kg ha⁻¹ with nitrogen and potassium on STCR basis performs well in black

calcareous soil by reducing the phosphorus fixation and also solubilizes the fixed phosphorus as calcium phosphate by the production of various organic acids which in turn increases the phosphorus availability, growth and yield and quality of crops.

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